Poster Session 1 (Topic: Drug Discovery Technologies)

P76

Neuroprotection effects of the mitochondrial K-ATP channel opener, diazoxide, is mediated by BCL-2 gene expression upregulation

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Background: Even today there is no effective drug therapy to prevent neuronal loss after ischemic brain stroke. There is increasing evidence about the diverse functions of mitoKATP channels in the regulation of essential factors determining the outcome of ischemic stress on cellular function and survival. We studied the effects of the Mitochondrial K+ -ATP channel regulators on anti-death Bcl-2 gene expression, neuronal ultra structure, and neurological function after ischemia reperfusion in the rat.

Methods: Rats temporarily subjected to four vessels occlusion for 15 minutes followed by 24 hours reperfusion with or without K-ATP channel regulators (Diazoxide and Glibenclamide).

Results: The Bcl-2 expression significantly improved in the K-ATP channel opener (Diazoxide) treated ischemia-reperfusion group compared with the control group. Ultra structural changes and neurological scores were correlated to Bcl-2 gene expression in cells subject to ischemia and reperfusion treated with K-ATP channel regulators in vivo.

P77

Effects of nitric oxide on the prefrontal cortex in stressed rats

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Background: Nitric oxide (NO) exhibits both protective and detrimental effects in the central nervous system. Objective: To investigate the effect of NO on the prefrontal cortex in neonatal stressed rats.

Design, time and setting: A randomized, controlled, animal study was performed at the Anatomical Department of Iran University of Medical Sciences from May 2007 to August 2008.

Materials: Forty-eight male, Wistar rats were obtained from Pasteur’s Institute, Tehran, Iran.

Methods: Rat stress models were established by immobilization and randomly received intra-peritoneal injection of 2 mL physiological saline, L-arginine (200 mg/kg) as a NO precursor, N(G)-nitro-L-arginine methyl ester (20 mg/kg), or subcutaneous injection of 7-nitroindazole (25 mg/kg) as a NO synthase inhibitor.

Main outcome measures: After the rats were treated for 4 weeks, the frontal cortex was harvested for histological observation and NO detection.

Results: Subcutaneous administration of N(G)-nitro-L-arginine methyl ester or 7-nitroindazole resulted in significantly lower prefrontal cortex thickness and NO production compared with subcutaneous administration of L-arginine (P < 0.05). Prefrontal cortex thickness significantly increased in rats following L-arginine treatment, compared with physiological saline intervention (P < 0.05).

Conclusion: NO exhibited protective effects on the prefrontal cortex of stressed rats.

Key Words: nitric oxide; prefrontal cortex thickness; nerve cell; stress; rats; neural regeneration
P78

Quantitative structure-retention relationship of hydroxamic acids using partial least - squares modeling
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Hydroxamic acids have been recognized as compounds of pharmacological, toxicological and pathological importance. Taking the pharmacological potential of this class of compounds in account, a series of N-arylsubstituted hydroxamic acids with general formula $R_1NOH.R_2C(=O)$, where $R_1$ and $R_2$ are phenyl or substituted phenyl groups were synthesized. Lipophilicity is one of the most important physical property of biologically active compounds. Chromatography provides an easy, reliable and accurate way to measures the molecular hydrophobicity based on the retention factors of compounds. Knowledge of Quantitative Structure versus Retention Relationship (QSPRs) is important to predict the retention and its mechanism based on the structure of solute.

In the present investigation, retention factor, $k'$, of 15 hydroxamic acids have been measured by Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) with acetonitrile-water mixture as mobile phase and C-18 as stationary phase. By plotting the organic phase compositions and the corresponding log$k'$ values, the extrapolated lipophilicity, logkw values were obtained. The lipophilicity, logkw are also computed by applying mathematical model PLS, the multivariate least square projection to latent structure technique. This model is based on molecular descriptors which can be calculated for any compound utilizing only the knowledge of molecular structure. The PLS analysis resulted in a good predictive model for logkw values of hydroxamic acids with the statistics, $R_2 = 0.886$, $Q_2 = 0.745$, sd= 0.1052 and $F= 90.379$. The QSPR model developed reveals that the lipophilicity, logkw, values of hydroxamic acids are mainly governed by electronic, steric and hydrogen –bond parameters.

P79

Determination of aqueous solubility of hydroxamic acids use in drug design by PLS modeling
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The solubility of drugs in water is of central importance in the process of drug discovery and development from molecular design to pharmaceutical formation and bio-pharmacy. The ability to estimate the aqueous solubility and other properties of a promising lead compound affecting its pharmacokinetics is a prerequisite to rational drug design, although it has received much less attention than prediction of drug-receptor interactions. A drug must be soluble so that it can be absorbed across the biological membrane to reach the target organ or tissue. Solubility of a compound must be accurately determined to assess the concentrations that the drug will achieve in the target area, to establish the therapeutic level, and to prevent toxicity. Appropriate physicochemical properties (e.g. log P and solubility) together with pharmacokinetic properties and toxicity are the major determinants for progressing from a good lead to good drug. Hydroxamic acids are a group of weak organic acids having the general formula RC(=O)N(R')OH, show a wide spectrum of activities in analytical, agricultural, biological and medicinal fields.

Quantitative structure-property relationship (QSPR) for the solubility behavior, logSW, of N-arylhydroxamic acids is analysed using the molecular descriptors by partial least square (PLS) regression. The cross-validation $Q_2$ cum values for the optimal QSPR model of hydroxamic acids is 0.640 (remarkably higher than 0.500), indicating good predictive-abilities for logSW values of hydroxamic acids. The resulting QSPR model shows that logSW values of hydroxamic acids are mainly governed by excess molar refraction (XRM), energy GAP (EHOMO-ELUMO), and electronic parameter. These are the important determinants for predictive ability for aqueous solubility of hydroxamic acids.
P80

**The effects of simvastatin on functional recovery of rat reperfused sciatic nerve.**

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This study evaluated the effectiveness of simvastatin in protecting sciatic nerve from ischemia-reperfusion (I/R) injury using the model of experimental nerve ischemia. Sixty adult male Sprague-Dawley rats weighing 250-300 g were used. They were divided into ten groups (N = 6 per group). We used ischemia model in these groups. All ischemia groups were rendered ischemic for 3 h. Then followed by reperfusion durations of zero time (0 hR), 3 h (3 hR), 7 days (7 dR), 14 days (14 dR). The treatment group received intravenous simvastatin (1 mg kg(-1)) 1 h before ischemia, while the control group received an equal volume of intravenous vehicle at the same time schedule and route. Behavioral data were obtained immediately before euthanasia. The score was based on coordination, racing reflex, toe spread and reaction to pinch. In simvastatin treated I/R rats we had increase in functional recovery. In conclusion, pre-ischemic administration of simvastatin exhibits neuroprotective properties in I/R nerve injury.

P81

**The best in vitro tests to rule out low efficacy, high toxicity and adverse effects in the early stages**

*I. Gatlik*

*Gatlik Ltd. (Basel, CH)*

A major focus observed in recent years in drug development is to reduce the number of compound failures at a late stage. Such misfortune is frequently the result of unfavourable compound properties like low efficacy, high toxicity and adverse effects. To address this challenge, Gatlik Ltd has introduced an Electroactive Pharmaceutical Screening (EPS) System to allow more informed and accurate decisions about synthesis and lead selection in the early stages[1,2].

   "Oxidative and metabolic stability of drugs: assessment by a novel Electroactive Pharmaceutical Screening System"

2. A. Felix, 2009, PhD Thesis, University of Basel, Faculty of Science
   "Redox Potential and Metabolic Stability: Development of High Throughput Assays for Early Compound Profiling"
P82

Hepatoprotective effect of aqueous leaf extract of Pyrenacantha staundtii
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Liver disorders are a serious health problem. In allopathic medicinal practices, reliable liver protective drugs are not available, but herbs play an important role in the management of liver disorders. Traditional or herbal medicine is an evolutionary process as communities and individuals continue to discover new techniques that can transform practices in the field of medical sciences. Currently, traditional medicine and drug discovery using natural products is an important issue. According to the World Health Organization (WHO), about three-quarters of the world population depend on traditional remedies (mainly herbs) for the health care of its people. The aim of this study was to evaluate the hepatoprotective effects of Pyrenacantha staundtii. Hepatoprotective activity of aqueous extract of Pyrenacantha staundtii against paracetamol-induced hepatic damage in albino rats was evaluated. In the study, the effects of aqueous extract of Pyrenacantha staundtii on blood cells, liver enzymes and proteins namely alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (AP), serum total protein and albumin were analysed. Liver samples were observed for histopathological changes. It was observed that aqueous extract of Pyrenacantha staundtii has reversal effects on the levels of above-mentioned parameters except on packed cell volume and white blood cells in paracetamol-induced liver damage. The result obtained from the study showed that Pyrenacantha staundtii has hepatoprotective effect and this hepatoprotective activity may be due to normalization of impaired liver cell membrane function induced by paracetamol. It was therefore concluded that Pyrenacantha staundtii may be an alternative medicine to cure liver dysfunction.

P83

Monitoring the accumulation and clearance of exogenously introduced beta amyloid peptide in a cell-based model of Alzheimer’s disease by fluorescence microplate assay
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Recent studies have indicated that the liver may serve as a major source of neurotoxic amyloid beta-peptide (Abeta) that forms deposits in the brain characteristic of Alzheimer’s disease (AD) (Sutcliffe et al 2011, J. Neurosci. Res. 89:808–814). Peripherally-derived Abeta in circulating plasma appears to represent an important precursor pool for brain amyloid, crossing the blood–brain barrier (BBB) and entering cells through a poorly defined heterophagic mechanism (receptor-mediated endocytosis, pinocytosis and/or phagocytosis). A cell-based assay of Abeta accumulation was developed using the SK-N-SH neuroblastoma cell line, which is commonly used in cytotoxicity assays. A homogenous fluorescence-based microplate assay was devised to detect aggregated Abeta inside aggresomes and inclusion bodies within an authentic cellular context. The assay employs a novel red fluorescent molecular rotor dye, which is essentially nonfluorescent until it binds to structural features associated with the aggregated protein cargo. The assay does not require non-physiological protein mutations or genetically engineered cell lines. The cell-based assay generated Z’ factor values greater than 0.6 using a conventional fluorescence microplate reader. The assay was subsequently used to screen members of a focused compound library, containing well-characterized small molecules with defined modes of action relevant to autophagy. Several compounds that promoted protein aggregation were identified as well as one modulator that facilitates clearance of Abeta aggregate accumulation. The described cell-based screening workflow facilitates identification of inhibitors leading to aggresome/inclusion body formation as well as endo-lysosomal pathway activators which promote clearance of aggregated Abeta cargo.
Abstracts/Poster Presentations

P84

Robust nanoliter liquid handling for flexible assay miniaturisation
J. Jenkins*, B. Schenker, R. Lewis, C. Carter
TTP Labtech Ltd (Royston, UK)

Serial dilutions, plate replications and reformatting into higher density plates are important and often routine processes in drug discovery but, when assay volumes are miniaturised to nanolitre levels, there are some significant challenges to be met in the process of automating them. Miniaturised assays have important cost benefits in the reduction of precious compounds and reagents required, but it is paramount to maintain speed, accuracy and precision at these volumes or the efficiencies achieved become outweighed by the disadvantages. Miniaturising assays also allows users to draw on a wider range of high density plates for further efficiencies, but these often present challenges for liquid handling systems.

mosquito® HTS (TTP LabTech) is a nanolitre liquid handler flexible enough to automate the miniaturisation of all standard HTS assays without needing special labware or system set-up changes. It offers extremely fast and accurate plate replication and serial dilutions. It is also capable of reformatting between different plate types in the same protocol – even from standard 96-well formats direct to high density 1536-well plates.

P85

A fast and fully automated solution for Lipidic Cubic Phase (LCP) screening using mosquito LCP
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Membrane proteins, such as G-protein-coupled receptors, are known to be much more difficult to purify and crystallise than soluble proteins due to their native environment within the lipid bilayer of the cell membrane. The in meso (lipidic cubic phase or LCP) crystallisation technique has revolutionised the process of crystallising membrane proteins. However, there are a number of technical difficulties associated with the LCP method which makes this process difficult to perform and challenging to automate.

One problem is the viscous nature of the lipids which can be almost solid at room temperature. As a result the addition of protein to the lipid and subsequent reconstitution can be hard to achieve. In addition, the accurate dispensing of LCP, required for efficient miniaturisation, and the precise positioning of drops required for efficient imaging of membrane crystals present two other challenges.

TTP LabTech have solved this problem by developing mosquito® LCP, a dedicated instrument that offers a fully automated solution to LCP screening. Here we describe the benefits of the instrument and how the renowned and reliable mosquito positive displacement tip technology ensures that the LCP screening preparation is performed at fast throughput, high precision and unrivalled reproducibility.
P86

An integrated solution for automated nanoliter hit-picking at BioFocus
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(1)TTP Labtech Ltd (Royston, UK); (2)BioFocus (Allschwil, CH)

A prerequisite for efficient primary screening is rapid, automated selection of “hits” for confirmation and secondary profiling. The mosquito® X1 offers precision sampling of any individual well in 48-, 96-, 384- or 1536-well plates. This enables researchers to quickly select small volumes of hits from a variety of primary screening plates and transfer them directly to the next screening stage without further dilution. Mosquito X1’s disposable pipette tips guarantee zero cross-contamination, and their unique positive displacement pipetting technology ensures accurate and reproducible pipetting throughout the 25 nL -1.2 µL range.

This poster describes a section of BioFocus’s screening workflow where mosquito X1 is integrated with a RapidStak plate stacker (Thermo Scientific) using TTP LabTech’s CherryPicker software. This allows the mosquito X1 to work unattended for extended periods. The CherryPicker software drives the system automatically by converting pick lists provided by BioFocus’ LIMS system into mosquito protocols, and feeding appropriate plates via the RapidStak. The software optimises each protocol for efficient pipette use, and, if required, could also track the volumes of the source plates for subsequent reporting back to the LIMS system.

P87

The future of compound management
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TTP Labtech Ltd (Royston, UK)

Current compound management practices have evolved to support both primary and secondary screening projects from a centralized repository storing a combination of plates and tubes. Storage of both tube and plate formats is an inefficient design and adds to the complexity of these repositories, making them expensive to implement and more importantly difficult to expand or relocate. Also, new sample submission becomes a protracted process, which can lead to delays of several months until the samples are available for HTS.

Plates are considered the only way to support the throughput requirements of primary screening, but they also compromise sample integrity by introducing uncontrolled exposure to the environment and multiple freeze/thaw cycles. In addition, quality control of plate based material is almost impossible causing false positives and negatives, which are not picked up until the secondary screening stage.

Plates also limit the flexibility of a library, requiring the entire library to be screened in each HTS campaign. A more efficient approach to primary screening would be to only screen against library subsets that are chemically relevant to the target, but this would require ultra high throughput cherry-picking of tubes. In this poster we will describe an innovative large-scale tube-based compound management approach to maximize lab space; improve compound stability; enable rapid generation of custom screening sets; while providing background QC and real time library integration of new chemical entities.
Abstracts/Poster Presentations

P88

High-content analysis of signaling networks using protein fragment complementation assays (PCA)
(1)Odyssey Thera (San Ramon, US); (2)Lonza (Cologne, DE)

A major challenge for drug discovery remains the need for assays that capture diverse targets and pathways in their native state. We have created a cell-based assay platform that facilitates tackling a broad range of signaling pathways at almost any point of interest by analyzing protein-protein interactions. In our protein fragment complementation assays (PCAs) each protein partner of interest is fused to one fragment of a rationally dissected reporter protein. If the fusion proteins are co-expressed in a living cell, the fragments of the reporter protein can interact, re-fold, and generate a detectable signal only when the two test proteins form a complex. Using inherently fluorescent proteins as reporters, PCA can be combined with high-throughput automated fluorescence microscopy and image analysis. This strategy captures the dynamics of protein complexes and their sub-cellular location, as a result of cellular responses to drugs or genetic reagents. The PCA technology provides an unparalleled tool to directly focus on nearly all drug targets from various disease areas. It significantly broadens the spectrum of assays around well-known targets such as GPCRs enabling analysis of GPCR homo- and hetero-dimerization and biased signaling. PCAs also facilitate analysis of formerly “un-assay-able” processes like ubiquitylation and histone (de-)acetylation in a live-cell context. In this poster, we present data on several exemplary PCAs elucidating the universality of this technology.
P89

Revolutionary concept for drug discovery synthesis
A. Schnyder*
Chemspeed Technologies AG (Augst, CH)

In 2010 Chemspeed has made a breakthrough that has the potential to change how synthesis in Drug Discoveries are run. Chemspeed has developed a revolutionary approach called SMOLE / SMOLEFIN for chemical laboratories. SMOLE is a unique concept (2D-Matrix of coins for chemical synthesis) for compound handling and reaction preparation by providing pre-filled chemicals in various fixed molarities (e.g. 0.01, 0.02, 0.05, 0.10, 0.20, 0.50, 1.00, 2.00 and 5.00 mmol) in sealed and crushable ampoules named PINs (see figure 1). Tedious calculating, weighing, cleaning and handling procedures are hence eliminated or drastically decreased; the time to reaction is correspondingly reduced.

In Drug Discovery the screening of a wide variety of different substances is key. Having these chemicals ready in the needed stoichiometry reduces preparation time drastically. An example of a reaction is shown below (figure 2):

PINs can be simply chosen according to the needed molarity, picked with a special pistol and injected into a flask through a simple click. The glass PIN splits at the impact with the liquid surface and then delivers the chemical content.

Based on PINS of exact molarities, chemicals are now easily available in the correct amount - without calculation, weighing, cleaning and waste management. As shown in Figure 3, reaction preparation can be performed very easily and quickly.

The use of aggressive or toxic ingredients is also much safer, since the materials are not handled anymore openly. Another interesting advantage is the use of sensitive chemicals - these chemicals can easily be handled without precaution in the closed PINs.

Another key aspect in drug discovery is the scheduling of the reactions screened. Currently automated reactions are done mainly in sequential mode and so timing of the different reactions carried out are different. Due to the fact, that SMOLE PINs can be added in parallel to different reaction vessel and all reactions can be started in parallel, this issue is now also fully solved.

The SMOLE concept solves all technical challenges from drug discovery in a globally standardized way without changing the chemistry at all. SMOLE eliminates the challenges in experimental work and allows to flexibly and modularly build innovative, standardized, globally applicable libraries.
Abstracts/Poster Presentations

Figure 2: Wittig reaction with SMOLE

Classical procedure (individual functions, 60 mins)

Condensed Chemspeed Approach (Integral functions, 5 mins)

Replacing tedious, time-consuming, error-prone handling procedure... by a simple, exact and easy-to-use concept!

Figure 3: Simplification of reaction preparation with SMOLE approach
P90

**Automation of the western blot detection and analysis process using a new technology platform**

ProteinSimple (Santa Clara, US)

Western blot analysis of proteins is a widely used method for protein characterization. While improvements to individual aspects of SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Western methodologies have been developed in recent years, none has integrated the entire process onto a single platform. We have developed an automated protein sizing and quantitative immunodetection platform that can be used as an alternative to Western analysis. The platform integrates all manual operations associated with Western blotting including sample loading, SDS-denatured size based electrophoretic separation, membrane transfer, block and wash steps, primary and secondary antibody incubation, detection and analysis.

**Methods:** Denatured proteins in simple to complex matrixes are separated in-capillary and bound to the capillary wall via proprietary, photoactivated chemical capture. Target proteins are then identified using a primary antibody and subsequent immunodetection using a chemiluminescent substrate. Molecular weight and relative concentration for immunodetected proteins are automatically reported. Details of how the capillary-based assay is executed and example data illustrating performance will be presented.

**Conclusions:** The manual steps associated with Western blotting process can be time-intensive and lead to variability in results. Using an automated platform greatly improves time to result, increases overall data quality and provides more detailed characterization of proteins and cellular pathways.

P91

**A mix-and-read cell-based assay for antibody screening against epidermal growth factor receptor**

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Antibodies against a wide range of protein targets have been either approved or are currently under development for therapeutics. The conventional antibody screening assay based on antibody-antigen binding has been enzyme-linked immunosorbent assay (ELISA). While tedious and consuming, ELISA has proved sufficient for the identification of antibodies directed against secreted antigens. However, cell surface antigens (e.g. GPCRs) provide challenges for ELISA primarily due to the shortage of soluble antigens and high variability resulting from loss of cells during wash procedures. Mix-and-read assays – popularized as FMAT® assays – overcome such problems, while at the same time offering simplified workflows for automation.

We have developed a mix-and-read method for the screening of antibodies against cell surface proteins expressed on live cells. The method uses a high sensitivity microplate cytometer to quantify cellular fluorescence in cultures seeded in microplates. Here, we describe its use for the determination of human epidermal growth factor receptor (EGFR) antibody binding in A549 cells which are known to express high levels of EGFR. A549 cells were incubated with mouse anti-EGFR antibody and fluorescently-labelled anti-mouse IgG antibody. Without washing away unbound antibodies, plates were scanned and fluorescence of each cell quantified. Clear concentration-dependent antibody binding was observed with low assay variability. With its simple operation and high sensitivity, this new method is well-suited for high throughput antibody screening.
New tools for drug discovery: Monitoring intracellular second messengers in primary cells using high-throughput formats

(1)Lonza (Cologne, DE); (2)Rutgers University (Piscataway, US); (3)Promega Corporation (Madison, US)

Primary cells allow for a higher predictability of drug reactions in humans. These cells endogenously express relevant drug targets at physiological level and genuinely carry all the components required for specific signal transduction. They can be derived from the actual tissue of interest. These are significant advantages over immortalized cell lines, which may be derived from irrelevant tissue, be of non-human origin, and often express transfected drug targets at non-physiological levels. For these reasons, there is a growing demand for primary cells in drug screening and hit validation.

Here we show that Clonetics™ primary cells and Poietics™ stem cells can be used in high throughput formats (i.e. 96-well and 384-well plate) to monitor intracellular Ca2+ fluxes and changes of intracellular cAMP concentration.

The cells transiently expressing either Axxam’s luminescent calcium biosensor i-Photina® or Promega’s luminescent cAMP biosensor GloSensor™ can be cryopreserved without loss of functionality. Cells expressing i-Photina® can be loaded with coelenterazine, substrate for the calcium-sensitive photoprotein, prior to freezing and are therefore ready to use after recovery.

The functional expression of i-Photina™ was demonstrated through pore forming ionomycin causing Ca2+ influx, the functional expression of the GloSensor™ by forskolin directly activating the cAMP producing enzyme adenyl cyclase. Functionality and specificity of endogenously expressed G-protein coupled receptors triggering intracellular Ca2+ release or cAMP production was shown through receptor-binding agonists and antagonists in dose-dependent manner. EC50 and IC50 values nicely confirmed published data. Z’ values above 0.6 facilitate the use in high-throughput screenings. We also provide evidence for a versatile assay system, cells co-expressing calcium and cAMP biosensor. Both pathways can consecutively be monitored in the same sample.

These new technologies are non-toxic and non-destructive to the cells, and unlike with fluorescence-based assays, there is virtually no background signal and no interference from fluorescent compounds. These ready-to-use cell based assay systems are excellent tools to study drug effects on signaling in primary cells and will help open new roads for more predictable compound screening.
P93

The LAB2LAB™ Advantage
R. Wheatcroft* (1), B. Everatt (2), S. Tullett (1)
(1) TTP Labtech Ltd (Royston, UK); (2) Novartis (Horsham, UK)

Currently submission of chemical and biological samples to analytical equipment such as HPLC and GC/MS requires substantial manual investment. Typically samples are formulated and collated into racks before manually entering these into the analytical instrumentation. This can be a time consuming, error prone and inefficient process, time that scientists can better spend in the laboratory.

Lab2Lab from TTP LabTech is a novel approach to submitting and transporting samples for analysis across an entire site. Sample tubes are registered and methods selected, an ELN reference is assigned and the sample tube is placed into the “Sender”. The system then transports the samples using low pressure compressed air and directs them to the most appropriate analytical instrumentation available. The analytical results are then automatically returned to the originators ELN.

The system is unique in that it allows samples to be sent from anywhere within building, from disparate laboratories to collated or disparate analytical instrumentation. This poster will demonstrate how it is possible to rationalise expensive equipment, reducing the cost of maintenance and support whilst increasing availability.

In addition, the system has the capability to buffer submitted samples whilst the analytical equipment is busy or otherwise unavailable, meaning analyses can take place overnight.

This poster will describe the system recently installed at Novartis, Horsham (UK) as part of their “Open Access” initiative for the “Lab of the Future” and demonstrate the typical throughput capability and time savings this introduces to the users. It will show how the system can cope with failure of the analytical instrumentation by being able to redirect samples to working equipment. In addition, this poster will illustrate how the analytical system can be expanded to incorporate NMR and comPOUND libraries. This allows full follow up analysis of biochemical samples or 100% QC of samples pre and post HTS.

P94

High-throughput imaging of cellular models using an Acumen eX3
P. Wylie*, D. Onley
TTP Labtech (Royston, UK)

Microscope-based, high-content instruments are used for many cell based assays. However, these instruments require a trade-off to be made between using high optical resolution over a small area, and the robust data sets gained from viewing larger areas of the well but at the price of using low power objectives.

Most assays require the use of higher resolutions which entail lengthy read times, even without using multiple colours. Wherever possible, users are constrained to analyse only a small percentage of the total number of cells in a well to keep plate read times at a minimum.

The Acumen eX3 is the fastest imaging system available, collecting and simultaneously analysing over 40 images/second, covering the entire well, without the trade off of having to use lower resolution. Acumen is well established for cell-based high-content screening, but researchers have recently applied its large field of view to rapidly analyse complex cellular or animal models, such as angiogenic tube formation, C. elegans or drosophila larvae.

In addition to Acumen’s built in software, it offers the flexibility of exporting whole well open source TIFF images for batch processing by third party image analysis software packages. This new screening paradigm represents a major breakthrough in how microplate cytometers can be applied to complex cellular models since rapid cytometric analysis can now be combined with image-processing methodology.
Neutrophil Adhesion: A HCS compatible assay using the acumen eX3

P. Wylie*, D. Caracino
TTP Labtech Ltd (Royston, UK)

A variety of pathological conditions exist in which cellular adhesion events are key to the evolving disease state. These include myocardial infarction, Adult Respiratory Distress Syndrome (ARDS), and general trauma. Cell adhesion also plays a crucial role in the immune system and is an important factor in inflammatory diseases such as rheumatoid arthritis, asthma and psoriasis. The concepts involved in the cell adhesion process are a rapidly developing area of cell biology and over the last five to ten years it has become possible to work out, at a molecular level, how cells attach to each other and to extracellular matrix molecules. This will be important for future drug development. The importance of cell adhesion molecules in the homing of lymphocytes to lymphoid organs, in neutrophil localization in inflammation, and in the interaction of both lymphocytes and neutrophils with vascular endothelium suggests that defects in these molecules might have severe consequences.

The Acumen eX3 is the fastest imaging system available, collecting and simultaneously analysing over 40 images/second, covering the entire well, without the trade-off of having to use lower resolution. It offers up to three lasers from a range between 405nm to 633nm up to 4 channels of data collection per laser. The Acumen eX3 can be used to study the process of cellular adhesion, whereby adherent cells types specifically, endothelial cells can be grown to confluence in microtitre plate wells and other cells types e.g. neutrophils added. The neutrophils can be differentially stained with calcein AM and the adhesion profile monitored and quantified. Cell adhesion can be determined simply by correlating retained fluorescence with cell number. It is envisaged that this type of assay will be of use in the pharmaceutical screening of novel drugs directed against specific cellular adhesion molecules and in academia for the monitoring of the adhesion process in different disease states, such as myocardial infarction, Adult Respiratory Distress Syndrome (ARDS) and stroke in vitro. A simple, rapid, quantitative adhesion assay, routinely returning a signal to noise value of 6 to 1 has now been developed for the Acumen eX3 laser.
P96

A high-throughput colony formation assay for profiling novel compounds and RNAi reagents using the Acumen® eX3

P. Wylie*, A. Goulter, J. Mundin
TTP Labtech Ltd (Royston, UK)

Cell colony formation assays measure a cell's ability to grow unattached to a surface and have applications in a range of areas including hematopoietic stem cell research, cell transformation studies and the prediction of responses of tumors to chemotherapeutic agents. Traditionally, these assays have been carried out using a semisolid agarose bi-layer system in low density culture plates or petri-dishes with manual enumeration of colonies using a microscope. These methods are both low throughput and based upon subjective determination of the number of colonies thus making them unamenable to high throughput screening.

The objective of this study was to demonstrate that the Acumen platform could quickly and reproducibly quantify the effects of test compounds and RNAi reagents on their ability to affect cell colony formation, and therefore be used as a screening platform.

A549 cells, growing in a agarose gel were incubated for 8 days in the presence of staurosporine, RNAi reagents and respective controls. The assessment included studying the effect of varying initial cell number and output parameters on the quality of the data.

It was shown that colonies seeded at 1,000 cells per well and stained with propidium iodide generated robust data. Using TTP LabTech's proprietary spherical volume algorithm, the effects of the staurosporine on the colony formation showed a concentration dependent inhibition of cell colony formation; RNAi treatments 2, 3 and 4 caused a large inhibition compared to the control, RNAi 1, all of which were statistically significant from the respective controls.

The results of this study demonstrated that the Acumen eX3 can be used as a high-throughput platform for investigation of effects of test compounds and RNAi reagents on cell colony formation.

P97

Targeted integration of genes for cell based assays

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The development of cell-based assays for high-throughput screening (HTS) approaches often requires the generation of stable and isogenic cell lines expressing a gene of interest (GOI). However, these cell lines are essentially created by random integration with no control over the level and stability of gene expression. We developed a targeted integration system in CHO cells, called cellular Genome Positioning System (cGPS®), based on the stimulation of homologous gene targeting by highly specific endonucleases, the meganucleases. In particular, one potassium channel (hHCN4) and two GPCRs (hMT1 and hMT2) were knocked-in at the same locus in cGPS CHO-K1 cells. Further characterization revealed that the cGPS CHO-K1 system is more rapid (2-week protocol), efficient (all selected clones expressed the GOI), reproducible (GOI expression level variation of 12%), and stable over time (no change in GOI expression after 23 weeks of culture) than classical random integration. Moreover, in all cGPS CHO-K1 targeted clones, the recombinant protein was biologically active and it properties similar to the endogenous protein. This fast and robust method opens the door for creating large collections of cell lines expressing therapeutically relevant GOIs, enhancing the potential scope of HTS.
P98

**InfectX - systems biology of pathogen entry into human cells**

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InfectX is a consortium of 11 research groups, covering bacterial entry, viral entry, proteomics and modeling, plus a group of supporting functions. The goal of InfectX is to comprehensively identify the components of the human infectome for a set of important bacterial and viral pathogens and to develop new mathematical and computational methods with predictive power to reconstruct key signaling pathways controlling pathogen entry into human cells. The consortium uses state-of-the-art experimental approaches, i.e. genome-wide high-content RNAi screening and proteomics, to systematically identify the human infectome involved in pathogen entry; in particular the subset of human proteins of the model epithelial cell line HeLa that are required for cellular uptake of a representative set of bacterial and viral pathogens. In addition, a goal is to produce standards for data acquisition and analysis in order to produce reproducible and comparable analysis results. In order to reach these goals, open-source IT-infrastructure projects for image and data analysis, storage, and sharing have been initiated within the SystemsX and SyBit. In particular, OpenBIS (distributed system for managing biological information), iBrain2 (fully automated workflow processing manager), and CellClassifier (Data browser and machine learning tool) provide a seamless information flow from the automated microscopes, through image analysis on cluster computing, to the final statistical results. Novel multiparametric analysis and modeling approaches, including supervised and unsupervised machine learning techniques, are being integrated within the analysis workflow.

P99

**Quantification of cytokines on the SpectraMax® Paradigm® multi-mode microplate detection platform using alpha technology**

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Inflammation is accompanied by increased endothelial chemokine production and adhesion molecule expression, which may result in an extensive neutrophil infiltration. As such, the search for novel anti-inflammatory substances able to downregulate these parameters, as well as the tissue damage, holds therapeutic promise. In this poster, we describe how Alpha Technology, a bead-based, homogeneous assay for studying molecular interactions in a microplate format, has been used to detect anti-inflammatory metabolites from cyanobacteria in human endothelial cell-based in vitro assays. We show the quantification of cytokines (TNF-alpha) down to picogram levels with Molecular Devices’ SpectraMax® Paradigm® Modular Multi-Mode Reader. Alpha Technology can be performed on the SpectraMax Paradigm Reader in various plate formats ranging from 96-well to 1536-well.
P100

HMTs and histone demethylases – analysis of epigenetic targets on the LabChip3000 platform and in orthogonal assay formats
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BioFocus (Allschwil, CH)

Epigenetic targets have been recognized to be implicated in cancer, inflammatory, neurodegenerative and metabolic diseases. Histone methyl transferases (HMTs) and demethylases are two of several target classes of regulatory enzymes in the epigenetics field which are in the focus of drug discovery research.

Mobility shift assays (MSA) for the HMT G9a and the histone demethylase LSD1 were set up using the Caliper LabChip 3000 microfluidics system (Wigle et al). For assay validation, potencies of tool compounds (SAH, Sinefungin, BIX-01294 for G9a and Tranlycyptromine for LSD1) were determined. In parallel, orthogonal G9a and LSD1 assays were established - namely isotopic FlashPlate and fluorescence lifetime (FLT) assays for G9a and a formaldehyde detection assay with a fluorescence intensity read-out for LSD1. Although histone substrates differed significantly for all assays used, the IC50 values of the reference compounds indicated comparable assay sensitivities for all assay technologies.

Assay protocols were adapted to laboratory automation and compound subsets of the BioFocus library, which were specifically selected for both targets, were tested in Mini-Screens in both LC3000 assays and compound activities were compared to data from the orthogonal assays. These results support the usefulness of validating compounds in orthogonal assays already in the hit finding process and are in line with BioFocus’ plans to further add cellular assays and selectivity panels for hit identification of epigenetics targets.

P101

Using the Promega GloSensor™ cAMP technology on the FLIPR® Tetra system for live cell Gi- and Gs-coupled GPCR second messenger assays
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Detection of Gs- and Gi-coupled GPCR second messenger signal activity has traditionally been accomplished using endpoint assays such as radioactive ligand binding or assays that require cell lysis. These assays measure activity at a single point in the pathway and typically do not provide live-cell kinetic information. Alternate options use forced-coupling of Gs- and Gi- GPCRs to promiscuous G-proteins such as Galpha16, followed by fluorescence detection of calcium flux upon receptor activation. However, this assay is also sub-optimal as it does not signal through the biorelevant cAMP pathway.

In this poster we demonstrate the use of a genetically modified form of firefly luciferase containing a cAMP-binding protein moiety (Promega GloSensor™ Technology) on the FLIPR® Tetra system to enable real-time kinetic detection of cAMP mediated activity at Gs- and Gi- coupled receptors. These GPCR subtypes can now be evaluated in a live cell assay that measures direct changes in intracellular cAMP concentration, the relevant second messenger mechanism.

We demonstrate endogenous receptor activity in CHO K1 and HEK-293 cell lines stably expressing the GloSensor plasmid. In addition, transfected Gs- and Gi-coupled receptor activity will be shown from cell lines with stably transfected GPCR receptors and transient transfection of the GloSensor plasmid. Combined with the GloSensor™ cAMP Assay, the FLIPR® Tetra system delivers the complete solution for kinetic screening of all major classes of GPCR subtypes.
P102

A new nano dispenser for flexible assay development in 1536 well plate format

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A new, flexible, nano liquid dispenser Certus (Fritz Gyger AG) has been tested for assay development and possible integration in HTS robot systems. The flexible software handling enables assay development directly in 1536 well plates with very low reagent consumption. The dispenser technology bases on solenoid pressure fed microvalves. Data of elaborate biochemical and cellular experiments are shown.

P103

Neurotoxicity assays using iPS-derived neurons and high content imaging

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The nervous system is a target organ for the toxic effects of many substances. Neurotoxicity can damage the brain or peripheral nervous system and also lead to neurodegenerative diseases such as Alzheimer’s or Parkinson’s. Another cause of neurotoxicity is excessive activation of receptors in the brain during a pathological process called excitotoxicity, where nerve cells are damaged by neurotransmitters such as glutamate and similar substances. Accordingly, there is a great interest in developing more predictive, disease relevant cell-based models and efficient screening tools for assessing the neurotoxicity of chemical compounds, drug candidates and environmental agents.

Human neurons derived from induced pluripotent stem cells (iPS), such as iCells® Neurons, are very attractive for such studies because they exhibit functionality and behavior of mature neurons and are available in large quantities. We present here two models for assessment of neural toxicity. In one assay, cells are allowed to form neurite networks in 96 or 384 well plates and then cultured in the presence of toxic compounds for 72 hours. Neurites and neural networks are visualized with antibodies against beta-III tubulin, imaged with the ImageXpress® Micro system (Fig 1), and analyzed with MetaXpress® software using the Neurite Outgrowth and Cell Scoring Modules. Neuronal networks are characterized by length of neurite outgrowth, branching, and number of cells.

We have shown dose-dependent disintegration of networks and neuronal toxicity of kinase inhibitors and other cytotoxic compounds (Fig 2). However at present studies we were not able to detect sensitivity of iPS-derived neurons to excitotoxic substances glutamate or NDGA.

The second model assays mitochondria integrity in neurons. Mitochondrial depolarization has been shown to be an early signal for excitotoxicity, hypoxic damage or oxidative stress. We have monitored mitochondria membrane potential using the mitochondria active dye JC-10. Cells were grown in 384 well plates, treated with JC-10, exposed to antimycin A or valinomycin for 30 minutes, and imaged. The loss of mitochondria membrane potential and mitochondria damage was analyzed by using the software Granularity module (Fig 3). This assay can also be useful for modeling hypoxia and testing neuroprotective agents. The present study supports the use of human iPS-derived neurons for neurotoxicity assessment of compounds using high content imaging techniques.
**P104**

**Fluorescence lifetime assays - added value for drug discovery programs addressing epigenetic targets**  
(1)BioFocus (Allschwil, CH); (1)BioFocus (Saffron Walden, UK); (3)Almac Group (Edinburgh, UK)

Fluorescence lifetime (FLT) is considered an attractive assay technology in comparison to other approaches since assay interferences, caused by compounds or assay reagents, are minimised. Recent improvements in the field, namely the introduction of fluorophores with appropriately long lifetimes, the development of new approaches to measure enzyme activity through changes in fluorescence lifetime, and the availability of new FLT readers provided the setting for new kinase, phosphatase, protease, and, most recently, histone methyl transferase (HMT) FLT assays (Almac FLEXYTE assay platform). Here we describe  
i) assay development of a FLEXYTE FLT assay for the HMT G9a,  
ii) assay validation by potency determination of SAM (Sinefungin and SAH) and histone substrate binding competitors (BIX-01294), and  
iii) the impact of different histone substrates on assay sensitivity.  
A subset of compounds from the BioFocus compound library, which was specifically selected by virtual screening approaches, was tested against G9a in the FLEXYTE HMT FLT assay. Compound activities were compared to screening data that were obtained in G9a Caliper LC3000 mobility shift assay (MSA) and a radioactive FlashPlate assay. In summary, the results show FLT to be a highly attractive assay technology for drug discovery in the epigenetic field.

**P105**

**Creating readable protein SAR tables through consistent naming**  
JH Jensen*  
Biochemfusion ApS (Charlottenlund, DK)

Proteins are typically described via their sequences, or on an abstracted level by abbreviations or trivial names. In practical life, e.g. in protein SAR tables, full sequences are too detailed to be useful, while names and abbreviations are inconsistent, ambiguous, and often don't give a sufficiently precise description.  
We will present a pragmatic purely text-based approach for consistently naming proteins and peptides, resembling the IUPAC recommendations. "DerNot" (DERivatives NOTation) expressions address both the needs of end user researchers for simple, practical and yet precise naming as well as the needs for an email and document-friendly system that can integrate with current cheminformatics and bioinformatics systems.  
A DerNot expression lists the deletions, insertions, and substitutions necessary to get from protein A to protein B. If a protein B is created from a protein named "Ref" by deleting residue 3 and substituting residue 8 with arginine (Arg/R), the corresponding DerNot expression will be:  
des-(3) R(8) "Ref"  
DerNot expressions also handle D-form residues, chemically or post-translationally modified residues and terminals, and chain extensions.  
One application is to use DerNot expressions to produce SAR tables. In the example below, a database system stores the proteins as full sequences together with associated data on peak activity time T_max after subcutaneous injection. The system can then generate a SAR table that looks like this (see attached graphics for better layout):
DerNot expressions can also be used to simplify registration of protein derivatives. The end user researcher can register a new protein by entering e.g. des-(B30) "Human Insulin". The registration system can then expand this to a full protein entry and register it. An example of how DerNot expressions have been implemented in a system that integrates both cheminformatics and bioinformatics technologies will also be shown.

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<td>Aspart</td>
<td>D(B28) &quot;Human insulin&quot;</td>
<td>1 - 1.5</td>
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<tr>
<td>Glulisine</td>
<td>K(B3)E(B29) &quot;Human insulin&quot;</td>
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<td>Glargine</td>
<td>G(A21) &quot;Human insulin&quot; -RR-(B)</td>
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Whole-cell and target-based assays: two complementary strategies for antimicrobial drug discovery
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GlaxoSmithKline (Madrid, ES)

Historically, pharmaceutical drugs were discovered on the basis of their ability to produce a certain desired biological outcome. The idea of a drug interacting specifically with a suitable protein target to yield a positive effect caused a shift in drug discovery paradigm, placing the emphasis on first finding a suitable ‘druggable’ protein for a particular disease and then developing high-throughput screening assays to find compounds that interact with the isolated target. The “target-based” approach has been exhaustively exploited in recent years by the early drug discovery projects. Some of the major advantages of this strategy relies on its great productivity and its feasibility for the substructure search and the rationally guided new chemical synthesis of the series of interest [1]

Unfortunately, while most of the in vitro assays tend to yield small molecule hits, these ‘magic bullet’ leads often fail in immediate steps like their biological activity. With the biological relevance of such strategies now in question, whole-cell models based on phenotypic responses rather than simple binding assays has once again become popular for new drug development [2, 3]. This also applies to the work on the antimicrobial area. We illustrate here two examples of the dual strategy (whole cell & target based) applied in GSK in the antimicrobial arena: The Whole-cell BCG (Bacilo of Calmette-Gerin) against Micobacterium tuberculosis and the Plasmodium falciparum TrXR (Thioredoxine Reductase) against malaria. They both exemplify successful approaches that serve as basis for illustrating the main pros and cons of each individual strategy.

References:
P107

**Development of a panel of HTRF® assay reagents for epigenetic targets**

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Histone methylation is a post-translational modification that occurs on arginine and lysine residues. On histone H3, four lysines (at position 4, 9, 27 and 36) can be mono, di or tri-methylated and are of interest in the drug discovery field. Therefore, biochemical enzymatic assays able to detect a specific methylation state on histone H3 are useful tools for screening potential new drugs.

We describe here the generation of a panel of reagent tools using HTRF technology, based on TR-FRET between europium cryptate and d2 red acceptor.

Labelled anti-methylated histone H3 Ab can be combined with a labelled anti-histone H3 Ab to form a pair of reagents able to detect a specific methylation state on the histone substrate.

Several antibody pairs were tested out for their specificity towards a given methylation state, as well as for their ability to give a significant assay window. Our results demonstrate that a careful selection of specific antibodies and a straightforward assay optimization deliver robust HTRF-based solutions that can be easily implemented for HTS and investigation of histone methylation. This universal assay design is likely to be applied to other epigenetic targets providing specific bioprobes are available.

P108

**HTRF® cellular kinases assays: A simple way to study activated Erk1/2 and Akt in whole cells**

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Cell surface receptors can be investigated through their function and the assessment of signaling pathways. Phospho-Erk1/2, phospho-Akt (Ser473) and phospho-Akt (Thr308) assays use a straightforward MAb-based assay protocol and allow a robust cell based detection of phosphorylated ERK1/2 or Akt in a high throughput mode. Using a common simple protocol, the assays are a rapid alternative to traditional methods such as the Western blot or ELISA.

This poster presents the application of Phospho-Akt assay using various cell lines and shows that phosho-Erk assay enables agonist activation to be monitored equally well for G-alpha i, G-alpha s, G-alpha q protein coupled receptors and Receptor tyrosine kinases. Due to their high sensitivity, the assays are suitable for either over-expressed or endogeneous receptors and can be used in many types of cells including primary cells.
P109

Validation of 57 Tag-lite® ligand binding assays starter packs. Correlation of agonist and antagonist IC50s obtained with Tag-lite and radioactive ligand binding assays

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Cisbio Bioassays (Codolet, FR)

Cisbio Bioassays continues the expansion of cell-based ligand binding assays under its Tag-lite® platform. All these assays are available under a ready-to-use starter pack format containing the fluorescent ligand and the prelabeled frozen cells. At this stage, 57 fully validated packs are available. This poster presents how the characterization of Dopamine receptor family, ligand binding parameters are determined (Kd and IC50s) also show that the receptors functionality is not affected by the SNAP-tag. All together, more than 120 known agonist or antagonist were tested in 57 different Tag-lite ligand binding assays. The IC50s obtained with Tag-lite were correlated with the published IC50s of the same compounds measured in the equivalent radioactive ligand binding assays. The good correlation obtained confirms that Tag-lite ligand binding assays are an alternative to the existing radioactive assays.

P110

Fluorescence lifetime technology – an attractive tool for protease hit identification

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Fluorescence lifetime (FLT) has recently attracted new attention as a promising screening and profiling technology in drug discovery. In particular, the introduction of the NanoTaurus FLT plate reader (Edinburgh Instruments) compatible with high throughput screening (HTS) and the introduction of improved long lifetime reporters such as 9-aminoacridine (9AA) by Almac has provided the basis for new FLT assays for important target classes like kinases and proteases. The intrinsic nature of the FLT read-out ensures it is unaffected by light scattering, autofluorescence and inner filter effects, thus providing advantages over other screening methodologies. In addition, interference from fluorescent compounds is minimized.

At BioFocus we have developed a number of protease assays using the FLEXYTE FLT platform (Almac) for validation of the technology and to optimize the value of hit finding campaigns. Here, assay development results for a metalloprotease and HIV-1 protease are presented. A good correlation of IC50 values of active compounds against the metalloprotease was found when FLT was compared to an assay using fluorescence intensity (FI) readout. In addition FLT allowed the identification of additional metalloprotease inhibitors which were not pursued further using the FI assay only, because they were considered to be false negatives. The BioFocus panel of FLEXYTE protease assays has been further extended by an assay for identification of inhibitors of HIV-1 protease. The assay was validated with a number of reference compounds of known activity and literature values were confirmed.

These results show FLT to be a highly efficient and attractive hit finding and hit characterization technology for a target class that is known for yielding low primary hit rates in HTS campaigns.
Swelling effect and drug release studies from the hydrophilic matrices containing combination of different grades of hydroxyl propyl methylcellulose
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Devi Ahilya University (Indore, IN)

The current study examines the drug release from the hydrophilic matrices of zolpidem tartrate prepared using combination of different grades of hydroxyl propyl methylcellulose (HPMC), viz, HPMCK4M, HPMCK15M and HPMCK100M. The results indicate that swelling and release profiles were affected by concentration and viscosity grade of the polymer. The results of swelling studies are shown graphically in Double -Y plots showing dissolution profiles of zolpidem tartrate release and swelling from matrices containing HPMC K4M and K100M grades combinations, (formulation codes C1,C2,C3, Fig .(1a). The percent uptake swelling or water uptake plots are shown in Fig. (1b). Similar plots are shown in Fig (2a) and Fig (2b) for formulation codes D1, D2, D3, containing HPMC K4M and K15M combinations with different ratios and Fig (3a) and Fig (3b) for formulation codes E1, E2, E3, containing HPMC K15M and K100M combinations with different ratios. In order to elucidate the release mechanism, the data were fitted to equation described by Peppas and Korsmeyer (Mt/M ? Ktn). The value of release rate exponent (n) is a function of geometric shape of the drug delivery device. The results indicate that the mechanism of release is influenced greatly by the polymer concentration of the formulations as can be seen from the r² values and n was generally in accordance with these indications. The release is mainly determined by the Fickian diffusion which is also confirmed from the n values. Formulation C1 has n= 0.504, C2 has n=0.453 and C3 has n=0.444 indicating that the release mechanism is very close to Fickian transport i.e. belong to the Higuchi model. In this investigation it has been demonstrated that an inverse relationship exists between the drug release rate and matrix-swelling rate. When the amount of HPMC in the matrix is high, wetting improves and water uptake into matrices is enhanced. The higher amount of HPMC causes a greater degree of swelling. This in turn reduces the drug release, as the diffusional path length of drug is now longer. Conversely, reduction in the amount of HPMC reduces the degree of swelling and the thickness of gel layer. This enables faster drug release. Similar results are observed with the different viscosity grades of HPMC formulations, viz D1, D2, D3 and E1, E2, E3. HPMC of higher viscosity grades swells to greater extent and has greater intrinsic water uptake property than that of the lower viscosity grades.

Swelling studies reveals an inverse relationship between swelling and drug release. The rational combination of different grades of HPMC tends to provide quite regulated release of Zolpidem tartrate over an extended period of time.
P113

Homology modelling of beta- keto-acyl-acyl-carrier synthase I/II in plasmodium falciparum
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Devi Ahilya Vishwavidyalaya (Indore, IN)

Malaria imposes great socio-economic burden on humanity, with six other diseases (diarrhea, HIV/AIDS, tuberculosis, measles, hepatitis B, and pneumonia), accounts for 85% of global infectious disease burden. Malaria afflicts 90 countries and territories in the tropical and subtropical regions, and almost one half of them are in Africa, South of Sahara. About 36% of the world population (i.e., 2020 million) is exposed to the risk of contracting malaria. Of the four known human malaria parasites, Plasmodium falciparum is the predominant cause of mortality, with 120 million new cases and 1 million deaths per year globally. It is this particular species that has given rise to formidable drug-resistant strains, resulting in the urgent need for new chemotherapeutic agents. Recent discoveries reveal that Plasmodium synthesizes fatty acids in the apicoplast, which is a vestigial organelle thought to be derived from a chloroplast. Not surprisingly, Plasmodium fatty acid synthase (FAS) is a type II enzyme complex as found in plants and bacteria and, thus, differs markedly from human type I FAS. However, analysis of the recently published P. falciparum genome reveals just two different beta- keto-acyl-acyl-carrier protein synthase (KAS) enzymes, with the KAS I and KAS II of typical type II systems replaced by a single enzyme denoted by KAS I/II, and a separate KASIII. Objective of enzyme inhibition need structural characterization from its 3D structure. So it was worth while to derive 3D Structure of KAS I/II for the drug discovery paradigm. In present research work molecular modeling of beta-keto-acyl-acyl-carrier synthase I/II is achieved using in silico comparative modeling. Energy parameters were found to be thermodynamically stable. Packing quality of the model was assessed by anolea and empirical force field energy for each amino acid of the protein chain was evaluated by gromos. Further geometrical accuracy of structure was conferred by Ramachandran plot which signified the present work undertaken through conformational parameters phi and psi angles calculated from model with 85.1% residues in most favoured region. Root mean square distance of planarity found to be below 0.02. Bond angles and bond lengths present the qualitative part of work. With the help of 3D Structure of beta ketoacyl acyl-carrier-protein synthases many lead molecules can be identified from which drug for the treatment of malaria can be discovered which may be helpful for the mankind.
Identification and characterization of novel drugs for Alzheimer’s disease from a FRET calcium-imaging-based high-throughput compound screen

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Alzheimer’s disease (AD) is the most common form of dementia among elderly. Most of current AD drug discovery attempts target late events in the disease progression, e.g. removal of Abetapathology or reducing tangle formation. Alternatively, disrupted endoplasmic reticulum (ER) calcium homeostasis can be used as a target for drug development. Familial Alzheimer’s disease (FAD) mutations in presenilins (PS1 and PS2) disrupt ER calcium signaling in an early event during the AD pathogenesis. FAD presenilin mutations enhance the inositol 1,4,5-trisphosphate (IP3) receptor activity which presents a potential novel target for AD therapy. To perform a high-throughput drug screening, we established a fully automated FRET-based calcium imaging assay at a single-cell level on the Opera platform (PerkinElmer). We generated HEK293 cells co-expressing a disease causing mutated form of presenilin (FAD-PS1) and Yellow Cameleon 3.6 (YC3.6), a FRET-based calcium indicator. Agonist-induced IP3 production by carbachol (CCh) results in liberation of calcium from the ER. FAD-PS1 mutants show a bigger CCh calcium release compared to the wildtype controls. Using the described FAD-PS1/YC3.6 HEK293 cells, we screened a library of 20,000 small molecules. We identified hits which after treatment for 24 hours restored the potentiated CCh calcium release of FAD-PS1 cells in a dose dependent manner. Specificity of the hits was further confirmed by IP3-uncaging experiments. By determining the structure-activity relationships and performing cluster analysis, a hand full of novel lead structures were discovered. Interestingly, some of the identified lead structures also improved the mitochondrial function and lowered the Abeta42 levels in cell culture. Results from MTT assay showed no cytotoxicity, normal viability and proliferation after 48 hours treatment with the compounds. Overall, we performed a high-throughput calcium-imaging-based drug screening and identified promising lead structures which by simultaneously stabilizing the disrupted calcium signaling, ameliorating the mitochondrial function and lowering Abeta levels, present novel therapeutic agents targeting early events in AD progression before the disease becomes irreversible.
An integrated platform for data management and analysis in biologics R&D

S. Schlicker* (1), M. Wendt (1), J. Tupy (2), H.-P. Fischer (1)

(1) Genedata (Basel, CH); (2) Genedata (San Francisco, US)

Biologics-based therapeutics ("biopharmaceuticals") play an increasingly important role in the market for new medicines. There is a general trend in the pharmaceutical and the biotech industry to build-up or expand their biologics R&D programs, complementing “traditional” small-molecule drug discovery. However, the underlying discovery and development processes for biologics differ substantially from the R&D workflows for small-molecules, resulting in biologics-specific data management and analysis requirements.

Here, we present Genedata Biologics, an enterprise software platform that supports the entire biologics R&D process from library generation, antibody screening, protein engineering and molecular biology, to downstream protein expression and purification operations. Genedata Biologics is the result of a concerted four-year development program in close collaboration with leading international players in Biologics R&D. From a technical perspective, Genedata Biologics is based on a central Oracle database, with integrated tools enabling the analysis and visualization of biologics sequence, assay and analytics data. The system is open and provides a rich set of APIs for integration with existing corporate IT infrastructure. Its built-in business logic minimizes set-up, configuration and customization work.

The target audiences for Genedata Biologics are (1) established pharmaceutical companies which are in the process of building up internal biologics R&D operations to complement their existing small-molecule R&D processes; (2) established biotech companies with significant in-house legacy software which requires life cycle replacements to be able to address new requirements (e.g. next-generation antibody formats), or to overcome scalability bottlenecks; (3) small and medium biotechs and CROs which require commercial software to support their growth path in terms of scope and scalability; and (4) generics companies that need software to support their growing biosimilar product portfolios.

The main value of Genedata Biologics lies in automation, standardization and integration. It represents an open, scalable and modular enterprise software that make the overall biologics discovery and development processes more efficient and less error-prone.
P116

Combining the diversity of natural products and the power of yeast genetics to explore cellular pathways

Novartis Pharma (Basel, CH)

Introduction: Describing the metabolic and signaling pathways utilized by cells is fundamental to understanding basic biological processes. A description of these processes can then be used to understand the basis of human diseases and possibly how they might be treated. One approach to explore these basic pathways is to use compounds to disrupt these processes. Natural products constitute an enormous source, and diversity, of biologically active compounds with which to probe cellular functions. This presentation describes the use of natural product screening and yeast genetics to identify the target of selected natural products.

Materials and Methods: Biological activity, guided deconvolution of natural product extracts was used to identify compounds inhibiting proliferation of a mammalian cell line. Such a simple, phenotypic, screen allows the identification of potent compounds acting by any number of different pathways. Then using the tools of yeast haplo-insufficiency and over expression the mechanism of these novel compounds was explored.

Results and Conclusion: This report describes the identification of a number of compounds showing potent anti-proliferative effects. These compounds have been characterized by yeast genetics to generate target hypothesis for their possible mechanism of action. Compounds previously described in the literature will be used to illustrate how the mechanism of action of compounds can be explored.

P117

Identification of killer siRNA by cytotoxic assay screening

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For the last few years, siRNA screenings have been extensively used to characterize cellular pathways and to identify new. Despite this major interest, the output is still limited and results generated are often controversial. Among major drawback, siRNA inducing cell death is the most common. To identify killer siRNA, we designed a simple and cost effective cytotoxic screening assay based on Alamar Blue metabolism in a 384 well microplate format. Many pilot screens at the kinome level have been performed on different cell lines and with different siRNA collections. Except for PLK1, which induced high level of cell death in all cases (as expected and previously described), results clearly showed a poor overlap of killers between siRNA collections.
**P118**

**High content screening assays for cell death mechanisms: comparison of automated fluorescence microscopy and digital holographic**

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Phenotypic high content screening is widely used for investigating a variety of biological processes like cell death mechanisms, as well as for early toxicological profiling of compounds. The detection of cellular phenotypes induced by chemical compounds and/or by siRNAs can be obtained through fluorescence microscopy imaging. In the present study, we first described assays enabling to characterize cell death mode as apoptosis and autophagy for both chemicals and siRNA. Quantitative analysis of the images through open-source softwares (Cell Profiler and Cell Profiler Analyst) allowed us to generate high quality data. However, this approach required complex and time-consuming experimental protocols, including multiple labels staining and washing steps, as well as the use of expensive fluorescent probes and reagents. For overcoming these limitations, we explored the use of a non-invasive and label-free technology, the Digital Holographic Microscopy (DHM). The DHM is based on real time monitoring of the changes in cellular morphology. An automated method has been implemented for the analysis of the bright-field images generated by DHM, allowing the discrimination of living / dead phenotypes.

**P119**

**The FLEXYTE platform - expanding the scope of fluorescence lifetime assays to new target classes**

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We have recently developed the FLEXYTE assay platform to exploit the inherent benefits of fluorescence lifetime (FLT) for screening applications. As an intrinsic parameter of the fluorophore, FLT is independent of probe concentration and volume, thereby facilitating assay miniaturisation. Furthermore, FLT is unaffected by auto-fluorescence, light scattering and inner filter effects leading to more robust assays where interference from fluorescent compound libraries can be minimized. FLEXYTE FLT assay platforms have now been configured for a broad panel of drug targets including protein kinases (Ser/Thr/Tyr), phosphatases, and proteases. Key to the success of our technology has been the development and application of 9-aminoacridine (9AA) as the fluorescent reporter. The long FLT (17 ns) of 9AA is ideally suited for assay applications, enabling discrimination from interfering species which typically have lifetimes in the 1-5 ns range. Here we report the further application of the FLEXYTE platform to provide FLT assays for epigenetic enzyme targets. Homogeneous, antibody-free assays for histone methyltransferases and peptidylarginine deiminases have now been developed. These assays are configured using peptide substrates site-specifically labelled with 9AA, with a change in FLT of 9AA indicative of modification of the substrate by the target enzyme. This approach is applicable to other epi-enzyme classes, providing broad utility for screening this important target area. In addition, the ability of FLT assays to identify and mitigate against compound interference is also demonstrated. Given the benefits of FLT and the broad scope of the FLEXYTE technology, this platform offers an attractive and cost effective approach for drug screening and profiling applications.
P120

Chromobodies and the fluorescent 2-Hybrid (F2H) assay: new technologies for real time analysis of cellular components in live cells
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To follow the cell cycle is of fundamental importance to many areas of drug discovery. Analysing this essential process provides the opportunity to discover new therapeutic targets for anti-proliferative agents. In addition potential compounds and targets can be monitored for undesirable off-target effects on the cell cycle. Here we present our U2OS Cell Cycle Chromobody, a new screening cell line that dynamically reports the detailed stage of the complete cell cycle of individual cells in real time. This cell line is based on our Chromobody-Technology, which additionally allows easy acquisition of multiple readouts from one and the same cell in a single assay run. Chromobodies are a novel format of intracellular functional antibodies, which enable non-destructive visualization and tracing of endogenous components in live cells. Chromobodies can in principle detect any antigenic structure including posttranslational modifications and thereby dramatically expand the quality and quantity of information that can be gathered in High-Content Analysis (HCA).

In addition we present our Fluorescent 2-Hybrid (F2H)-Assay, a new assay to directly visualize protein-protein interactions (PPIs) in live cells. By tethering the fluorescent bait at a defined cellular structure we generate a positional protein-protein interaction biosensor (PPIB). In case of interaction the fluorescent prey proteins co-localizes at this PPIB. In a proof of principle screen we demonstrate the applicability of the F2H-Assay on the reversible interaction between p53 and Hdm2 respectively HdmX upon compound treatment. Automated image acquisition and analyses allowed us to identify molecules perturbing this specific PPI in the cellular environment in real time.

P121

The importance to control liquid handling parameters – Be sure that you really know what your robot is doing
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The usage of automated equipment in biology and chemistry labs has lead to relevant advances in testing capabilities over the past two decades. Automation has definitely led to increased numbers of experiments, as compared to manual testing, particularly for liquid volume transfers. Because of this advantage, automated liquid handling devices are used routinely even in small laboratories. However, in spite of all the advantages that a pipetting robot brings to a laboratory, it also brings a different set of commonly overlooked challenges. It may be considered that the biggest challenge presented by using a liquid handling robot is the potentially incorrect assumption that the robot is doing what it is “supposed” to be doing. The robot may in fact be doing exactly what the user told it to do, but is that really what the user wanted? One may say that the real question is, do you really know how your robot is behaving, and particularly, are you really sure that your robot is performing your assays in the correct way?

In this presentation we will present examples of the importance of monitoring various commonly employed tasks, which are likely considered mundane and often assumed to have little bearing on overall robot performance. Specific examples that will be presented include; 1) pre-wetting tips, 2) using a blowout volume after pre-wet, 3) protocol transfer from one robot to a sibling, 4) protocol differences between high volume and low volume dispenses, 5) etc. The examples presented herein will help users to think more about the specific tasks they are asking their robots to perform, and hopefully uncover certain steps that, if observed and controlled, will result in better performance.
P122

Finding new molecules actives against ALS: Discovery of new GSK-3 and CK-1 kinase inhibitors with antioxidants properties derived from natural products


Fundacion MEDINA (Armilla, ES)

Amyotrophic Lateral Sclerosis (ALS) is a rapidly progressive neurological disease of unknown origin which affects a particular part of the nervous system: motor neurons, responsible for controlling voluntary muscles and skeleton movements. The cause of ALS is currently unknown; there are only a few symptomatic treatments for this disease. However, recent discoveries of the effect of certain factors such as antioxidants, and the over-expression of kinases in patient’s tissues open up new possibilities in the fight against this disease. Oxidative stress has long been linked to neurodegeneration and it is known that accumulation of reactive oxygen species (ROS) causes cell death. MEDINA has developed and miniaturized an assay based on the ORAC (Oxygen Radical Absorbance Capacity) method. GSK-3 plays an important role in neurodegenerative diseases. The activity of this protein could be one of the pathogenic mechanisms of ALS, participating in neurofilament phosphorylation, axonal transport regulation and apoptotic pathways. The neuropathological signature of ALS is represented by the presence of hyperphosphorylated TAR DNA binding protein (TDP-43) in the cytoplasm of motor neurons. Protein kinase CK-1 has recently been recognized to be involved in this process. Discovering new inhibitors of these 2 protein kinases using the Kinase-Glo® Luminescent Assay with an additional antioxidant capacity would provide an opportunity to develop new treatments against ALS. This project aims to identify new molecules as an effective treatment for ALS. In particular, we propose to develop the following objectives:

1. A rational approach using a high throughput screening (HTS) against two targets involved in ALS: antioxidants, GSK-3 (Glycogen synthase kinase 3) and CK-1 (casein kinase 1) inhibitors, seeking new compounds using the FUNDACION MEDINA’s natural products collection.
2. Performance of functional assays that include Mode of Action studies, cell viability and High Content Screening technologies.
3. Characterization and evaluation of these compounds in ADME-Tox assays.

The discovery of new alternatives for the treatment of Amyotrophic Lateral Sclerosis (ALS) is a medical need since there is currently no treatment for this devastating disease, being microbial natural products a privileged source of new chemical structures.
Label-free, immobilization-free drug discovery using microscale thermophoresis

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The analysis of bio-molecular interactions, such as protein-protein, protein-nucleic acids or small molecule binding, not only helps to develop therapeutics or serves as diagnostic technique, but also provides novel insights into basic cellular processes. Techniques currently employed to interrogate these interactions typically require some type of labeling or rely on a surface-coupling of one binding partner by using surface plasmon resonance (SPR). The attachment of a binding partner to either a tag or to a surface may alter or inhibit the function of the binding characteristics. Furthermore, these preparations are time consuming and some biomolecules do not allow the necessary chemical reactions. So far, truly label-free interaction studies are mostly performed with isothermal titration calorimetry (ITC).

Here we show a new label-free and preparation-free technique to analyze the affinity of bio-molecular interactions based on microscale thermophoresis (MST).

Label-free MST uses the directed movement of molecules in optically generated microscopic temperature gradients. This thermophoretic movement is determined by the entropy of the hydration shell around the molecules. Almost all interactions between molecules and also virtually any biochemical process relating to a change in size, stability and conformation of molecules alter this hydration shell and can thus be determined and quantified. Up to now, reports on this technique rely on a fluorescent tag added to one of the binding partners. Here, we show the use of the intrinsic tryptophan fluorescence. Most proteins possess one or few tryptophan residues, offering a widespread use of this source of fluorescence. Label-free MST works by applying a small perturbation, a localized temperature increase in the sample, and recording the new steady state fluorescence under this disturbance. Hence, the observed fluorescence signals are relative to the undisturbed situation before applying the temperature rise. This facilitates the use of intrinsic fluorescence as uncertainties in the preparation of the sample due to for example pipetting errors cancel out.

In this talk we exemplify the technique by investigating the binding of ligands to membrane receptors, quantifying protein-DNA interactions and screen for small molecule binders to the kinase p38. All groups of interactions were readily accessible by label-free MST and the measured affinities confirmed values reported in the literature.
KINOMEscan™: a comprehensive biochemical screening solution that enables new paradigms for kinase inhibitor drug discovery
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The identification of selective inhibitors having minimal off-target activity across the highly conserved kinome continues to hamper kinase inhibitor drug discovery and development. KINOMEscan is the industry’s most comprehensive kinase-focused biochemical assay platform and is uniquely suited to address these selectivity bottlenecks at multiple stages of kinase inhibitor drug discovery and development. KINOMEscan is a quantitative assay platform that has created a new paradigm for kinase inhibitor discovery through a rich blend of key benefits, including breadth (>80% of the canonical human kinome represented), flexibility, speed, capacity, quantitative dynamic range, and sensitivity to multiple inhibitor types. In this new paradigm, inhibitor potency and selectivity are measured rigorously and continuously throughout the drug discovery process, where, traditionally, potency alone has been the primary endpoint. This KINOMEscan-driven process results in the efficient identification of potent inhibitors with appropriate kinome selectivity and annotates investigators’ valuable, but often underserved, chemical assets by revealing new target opportunities and facilitating “target hopping” strategies. Here, we describe KINOMEscan and illustrate several key features and benefits that support a critical role for this technology platform in the discovery and development of next generation kinase inhibitors.

A role for microRNAs in a new mechanism of resistance to TGF-beta; induced growth inhibition
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ETH (Zurich, CH)

In an effort to identify new RNA drug targets which have prominent roles in disease we are exploring the TGF-beta signaling pathway in cancer cell lines. Cancer cells usually secrete latent TGF-beta but are in fact resistant to TGF-beta induced growth inhibition. RNAi –mediated suppression of TGF-beta1 caused apoptosis in several cervical carcinoma cell lines and was accompanied by transient increases in phospho-SMAD2 and phospho-AKT. A microRNA expression profile of Hela cells after TGF-beta1 RNAi caused wide-scale oncomir repression. Two miRNAs, miR-18a and miR-24, which regulate TGF-beta processing factors thrombospondin-1 and furin, respectively were suppressed. Further experiments showed that latent TGF-beta1, thrombospondin-1 and furin participate in a miRNA-controlled regulatory feedback loop. For cells producing high levels of latent TGF-beta this loop possibly represents a general mechanism of escape from TGF-beta mediated growth inhibition (1). Targeting of TGF-beta1 mRNA using antisense or RNAi-based reagents may potentially be of value in diseases associated with loss of cytostatic TGF-beta signaling.

Fundación MEDINA’s Drug Discovery is focused on the search of novel therapeutic agents for infectious diseases (including tuberculosis, malaria and parasitic diseases), oncology and rare diseases. As part of this effort, Fundación MEDINA is developing screening programs to discover active molecules with potential application in amyotrophic lateral sclerosis (ALS).

Successful screening campaigns require the use of the broader chemical diversity of compound libraries for drug discovery. With this objective, Fundación MEDINA works continuously to create, improve and characterize a high quality Natural Products Extract Collection for HTS Drug Discovery.

Fundación MEDINA’s Collection of Natural Products, consisting of more than 100,000 extracts, covers an unexplored secondary metabolite broad chemical space resulting from the fermentation of filamentous fungi, actinomycetes and other bacteria. The microbial collection (more than 100,000 strains) is a unique and high valued resource currently used at Fundacion MEDINA as the starting point to create high quality and chemically diverse screening samples, by using semi-automated extraction methodologies and fractionation techniques:

- Differential extraction protocols based on the nature of starting materials: (i) Resin pre-adsorption of diluted, watery broths; (ii) Two-phase solvent extractions for lipid rich fungal strains; (iii) Aqueous-based extracts for actinomycetes.

- Automated tailor-made fractionation protocols for different starting materials: (i) Chromatography on highly retentive resins; (ii) Gel filtration; (iii) Regular reverse-phase separations.

- Bi-dimensional fractionation of crudes by using different physiochemical Properties: (i) Polarity; (ii) Size exclusion.

The High Throughput Screening (HTS) of the Collection Modules generated to discover active molecules against amyotrophic lateral sclerosis (ALS) has been developed on the basis of a rational approach involving two ALS-associated targets: i) Glycogen synthase kinase 3-beta (GSK3 beta) and Casein kinase 1 (CK-1) inhibition; ii) antioxidant capacities (see also Juan Cantizani et al contribution (poster)).

The subsequent tailor-made isolation, purification and structural elucidation of the active extracts have allowed the identification and evaluation of five families of molecules with potential therapeutic applicability against ALS.
P127

Discovery of novel, biased ligands using a suite of PathHunter® and HitHunter® GPCR screening platforms
DiscoveRx (Fremont, US)

As more is learned about the intricacies of GPCR signaling, the harder it becomes to accurately describe ligand activity using a single functional read-out. GPCR activation results in G-protein dependent as well as G-protein independent events such as arrestin activation and receptor internalization. Although these pathways are often modulated in concert, a number of compounds that have differential effects on the selected pathways have been reported. DiscoveRx has developed a suite of assays designed to detect GPCR signaling through second messenger activation, arrestin binding, and receptor internalization. These assays provide a fast and reliable means to rank order compounds and quantify the different aspects of GPCR activation in an HTS-friendly format. In order to gain further insight into the phenomenon of functional selectivity, we have initiated a systematic characterization of GPCR targets and their associated ligands using three read-outs: 2nd messenger signaling, arrestin activation, and receptor internalization. We find that functional selectivity is not an isolated event but is prevalent across receptor classes and ligand types. These studies provide a basis to evaluate ligand bias, and characterize compounds using empirically derived guidelines.

P128

Rapid quantification of bioactive ligands in serum and human plasma using PathHunter® eXpress beta-Arrestin GPCR assays
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Biomarkers are becoming an essential part of clinical GPCR drug discovery programs as indicators of drug efficacy and predictability of potentially failed compounds. Many naturally occurring bioactive peptides, hormones, and chemokines bind to and activate GPCR receptors and have been associated with specific disease states. Furthermore, cleaved fragments that result from intrinsic proteolytic processing in biological samples can show increased activity, act as antagonists or become functionally inactive. While classical methods of biomarker detection, such as mass spectrometry and immunoassays, can both detect and quantitate biomarkers, they cannot differentiate between biologically active and inactive fragments. In this study, we demonstrate that the PathHunter® eXpress beta-Arrestin GPCR assays are uniquely suited for this application due to their unparalleled sensitivity, specificity, and ease of use. The assays are tolerant to a wide range of serum and solvent concentrations. Moreover, the functional activity of the endogenous CCL5 chemokine ligand, as determined by the PathHunter eXpress beta-Arrestin assay, correlated with the concentration of the chemokine in biological samples, as determined by a standard immunoassay. Together, these data indicate that the PathHunter eXpress assays are ideal tools for quantification of biologically active peptides and peptide fragments in complex biological samples, such as human plasma.
**P129**

**Automation of a novel cell-based ELISA for cell signaling pathway analysis**  
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Monitoring and quantifying cell signaling pathways is critical for understanding the behavior of cell processes and many disease states. Protein kinases involved in these cellular cascades play many diverse biological roles including normal growth and development; their aberrant behavior is linked to a number of infirm states including cancer. A novel one wash step ELISA assay has been developed for detection of cell based signal transduction events. In addition to offering simplicity, speed, and flexibility, the technology is a highly sensitive technique that lends itself to automation at multiple levels including: seeding cells in microplates and stimulation; antagonist and agonist serial dilution and transfer steps; assay reagent additions; microplate washing; and, detection and data analysis. Assay performance studies were conducted using positive and negative controls to verify optimal automation performance for S/B, Z', and intra- and inter-assay precision. Kinetic data was generated to analyze the effects of HRP detection without the use of kit stop solution. Additionally, manual and automated data was examined for comparative validation. Using these optimized conditions, a model MCF-7/insulin system was developed to probe the phosphorylation of a number of protein kinases endogenously expressed. Pharmacology of stimulation and inhibition of the protein kinase phosphorylation event was assessed.

**P130**

**Screening of Mallotus philippinensis plant for its antifungal activity**  
*B. Raval*  
*K. J. College of Pharmacy (Vadasma, IN)*

The activity of plant extracts against fungi has been studied for years for its greater infections during cancer growth. Kamala is a common name for M. philippinensis, which is commonly used as anthelmentic. In the present investigation, first, ten pathogenic fungal strains constituting from Aspergillus, Cadida, Isatchenka and Cryptococcus families were obtained from Microbial type culture collection, Chandigarh. The freeze dried powders of the strains were sub cultured in their respective growth medium. These organisms were than used for the anti-fungal susceptibility test using NCCLS and EUCAST methods against methanolic and petroleum ether extracts of the plant followed by TLC and fractions were again utilized for screening. From one of the fraction Rottlerin was isolated and it was again screened for antifungal activity. Standard drug compared was Amphotericin B. Resultd revealed that that all the strains are susceptible to Rottlerin and Amphotericin B. EUCAST method showed that Aspergillus strains were inhibited at the concentration of 412.5 to 825 mg/L by Rottlerin while Candida species were susceptible at MIC range of 825 to 1650mg/L except Issatchenka orientalis (MTCC no. 3020) (MIC range: 412.5 – 825mg/L). Hence it may be concluded that Rottlerin is having good antifungal effect and can be of potential value for future therapies.
P131

Cytotoxic activity of thiazole and piperazine derivatives
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Objective of the undertaken study was to evaluate the cytotoxic activity of thiazole and piperazine derivatives against the panel of five different cancer cell lines, which were MCF-7(human breast cancer cell line), Hep-2(human larynx carcinoma cell line), U-937(human leukemic monocyte lymphoma cell line), HL60(human myeloid leukemia cell line), HeLa(human cervical carcinoma) and two different normal cell lines, which were HEK-293T(human erythrocyte kidney cell line), and Vero(african monkey kidney cell line).

For primary screening all thiazole & piperazine derivatives were screened at 100µM concentration against all cell lines with the use of XTT based cytotoxicity assays. Cells were seeded at a concentration of 5×10^4 cells/well in 100µl culture medium and various amounts of compounds were added (ranging from e.g. 100µM-0.005µM) into microplates. Culture were incubated for 24h at 37°C and 5% CO2. 50µl XTT mixture was added and a incubated for 18h at 37°C and 5% CO2. Absorbances of the samples for formazan product were measured using a microplate reader at 450 nm. Eligible candidates were screened for kinase inhibition assay to decide mechanism of action as well.

In case of Thiazole derivatives, compounds with 3,4 Dichlorophenyl substitution along with Piperidino group & Octyl group showed showed cytotoxicity against MCF7, HL60 and HEK293T cell line ranging from 2.043 to 6.07µM, compound with 3,4 Dichlorophenyl substitution along with NHCH2C6H5 group showed cytotoxicity against HL60 cell line at 5.074 µM, compound with 3,4 Dichlorophenyl substitution along with Morpholino group showed cytotoxicity against MCF7 & HL60 cell line ranging from 1.58 to 5.95 µM, compounds with 3,4 Dichlorophenyl & 2-Methylphenyl substitution along with Cyclohexyl & amino group showed cytotoxicity against MCF7 cell line ranging from 39.32 to 40.36µM. In case of Pipreazine derivatives, compound with -C6H5 substitution along with 4-Cl group showed cytotoxicity against MCF7, HeLa and Vero cell line ranging from 2.79 to 42.52µM.

3,4 Dichlorophenyl with Morpholino substituted piperazine derivative is found to be most potent compound with IC50 =1.589µM against HL60(human myeloid leukemia cell line). and also resulted in kinase inhibition. This compound can serve as an important lead for further optimization of anti-myeloid leukemia activity.
A decade ago the first drafts of the sequence of the human genome were published, accompanied by claims that genomics technologies would deliver better drugs through improved understanding of diseases. However, the tone of recent commentaries from opinion-makers of the pharma industry and academia reveals an overall disappointment with the apparent outcome. We argue that an objective discussion of this far-reaching subject for scientists and the public alike is hindered by an absence of clarity on what constitutes a genomics contribution and their inherently low visibility in drug discovery. Here we highlight a number of genomics drugs originating from the genomics sector for the treatment of several important diseases after interrogating a commercial database, listing more than 2000 drugs in Phases II-III. A Genomics Drug is so-defined if genomics data appears to have been essential for its discovery, i.e. in its absence the drug would likely not exist. Genomics data is contained typically in very large datasets which inform on broad sections of the genome, for example from sequencing during genome-wide association studies (GWAS), expression profiling, database mining etc. This working definition enabled us to assess the impact of genomics on drugs in late-stage clinical trials at an appropriate time-point given the 10-15 year timeline for drug discovery and development. Subjective judgment was often required in the analysis in cases where literature linked a target to the disease prior to genomics experiments. The genomics contributions represented in these examples delivered novel targets or alternative mechanisms to address atherosclerosis, asthma, cancer, lupus, osteoporosis and irritable bowel syndrome, in addition to several pertinent observations concerning the use of genomics technologies. We contend that these examples are the visible tip of the iceberg and therefore that the impact of genomics data in clinical medicine today is significant and rising.
P133

Towards highly standardized HCS cell-based assays: a sensitive cytoskeleton assay to detect toxic drug effects
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High Content Screening (HCS) is a powerful technology whereby complex cellular pathways and processes can be studied in individual cells. To date, HCS is carried out with adherent cell lines grown on homogeneously adhesive surfaces, resulting in a large variability of cell morphology, which in turn complicates data analysis and limits the potential of HCS. The innovative cell normalization technology based on adhesive micropatterns opens new avenues for HCS. By controlling cell adhesion geometry, micropatterns allow a highly reproducible and polarized internal cell organization, which translates in a significant reduction in cell-to-cell variability. Using L-shaped micropatterns (in a standard 96-well format) over which a cell builds a major stress fiber spanning the free edge of the triangle, we developed a cell-based assay which quantifies drug effects on the cytoskeleton. We selected a panel of drugs targeting directly (Cytochalasin D, Y27632) or indirectly (Nocodazole) the acto-myosin network, causing either a decrease or an increase in cell contractility. Acquisition and on-the-fly cell-by-cell analysis were performed on an ArrayScan HCS platform (Thermo Scientific). We first determined relevant cell morphology parameters (from the Morphology Explorer BioApplication) to efficiently quantify drug effects on actin at concentrations from 10 µM down to 10 nM and calculated the IC50. This case study demonstrates that drug-induced effects on the acto-myosin cytoskeleton can be measured reproducibly on a small number of cells (50 and less) from a normalized population and at very low doses. We show also that this approach can considerably simplify data management and storage. Finally, we introduce the concept of the Reference Cell(TM), a universal standard for comparability of cell based assays between platforms in both dose response assays and screens.

P134

Automated compound profiling applications for the assessment of lead compound off-target effects
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Lead compounds, identified during hit and hit to lead screening, are normally profiled to determine selectivity for the drug target. The procedure typically involves performing the test assay with a dilution series of the compound in order to ascertain the IC50 or EC50 value. Therefore, instrumentation is required that can serially titrate each compound, as well as dispense assay reagents to high density plates. Here we demonstrate the ability to perform automated compound profiling applications for two separate drug target families, known for selectivity issues. Kinases are one of the most diverse and highly studied enzyme families studied today. In this application, we used a luminescent assay chemistry which is able to detect small changes in ATP consumption from kinases having large ranges of ATPKm app values. Histone deacetylases (HDACs) are an emerging drug target family, and play a role in gene regulation. Here we incorporated a green-emitting fluorescent assay which is capable of detecting the activity and inhibition of multiple HDAC enzymes using the same set of reagents. Validation and pharmacology data confirm the ability to generate compound profiling data in an automated fashion.
P135

Protein thermal shift™ assay using applied biosystems’ real-time PCR instruments
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The Protein Thermal Shift™ (PTS) Assay is an excellent high-throughput screening method which enables researchers to rapidly monitor protein thermo-stability and to identify optimal conditions that favor protein stability, including the investigation of protein-ligand interactions and mutations in protein sequences. Protein Thermal Shift is based on temperature-induced protein denaturation, monitored using Applied Biosystems’ Protein Thermal Shift™ Dye. This assay does not require any prior knowledge of protein function or ligand activity. Applied Biosystems’ Real-time PCR Systems plot the fluorescence data throughout the thermal melt, generating a fluorescence profile specific to the protein of interest within the test buffer environment or in the presence of a test ligand. As a result, we developed a MATLAB based TmTool™ which utilizes the Boltzmann equation to calculate the Tm of the protein from the fluorescence melt plot. Comparisons can then be made between Tm values obtained using a range of buffer conditions, addition of different ligands, or protein sequence mutations that alter protein folding and stability. In this study, we demonstrate the utility of this assay in buffer and ligand screening for T4 DNA ligase, as its Tm shifts in the presence of ATP, making Protein Thermal Shift™ Assay an important new screening tool for X-ray crystallographers looking for conditions that promote the stability of their protein of interest. In addition, we demonstrate the utility of this PTS Assay to discriminate between point mutation variants of M-MLV, SuperScript®II and SuperScript®III Reverse-Transcriptase. Finally, we demonstrate that this PTS can effectively detect the binding of antibodies to its target protein.

P136

Recombinant protein based liposomal combination vaccine for intranasal immunization against hepatitis and influenza
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Dr. Harisingh Gour University (Sagar, IN)

The aim of present work is to investigate the prospective of recombinantly expressed influenza surface protein Heamgglutinin complexed liposomal construct for intranasal vaccine delivery. The impact of previous work on the life cycle of virus revealed that this protein mediates interaction of virus particle with mucosal surface (principally M cells) and pH dependent fusion of endosomal membrane, releasing its content into cytosol. Liposomes were prepared by reverse phase evaporation method and surface complexation of HA was performed with carbodiimide coupling method. The developed formulation was characterized for various in vitro parameters. Interaction of liposome with nasal mucosal surface was investigated by fluorescent microscopy of nasal tissue. Finally the developed formulation was administered in mice in order to study their feasibility as nasal vaccine carriers. A significant and perdurable immune response was obtained following in vivo administration of the developed formulation that was comparable with alum adsorbed HBsAg administered intramuscularly. However, alum-HBsAg vaccine did not elicit slgA in mucosal secretions as well as did not elicit cellular immune response as it was induced and measured in the case of nasal administration of HA complexed liposomal vaccines. Further, the HA complexed liposomes augment higher immune response as compared to plain liposomes that might be due to higher uptake of former as evidenced in microscopy study of nasal tissues. The significantly higher cellular response augmented by HA complexed liposomes might be due o characteristic pH dependent fusion property of HA protein. Theme of the present research work is presented in figure-1 and 2.

Key Words: Liposomes, Heamgglutinin, HBsAg, mucosal vaccine, M cells.
P137

Carbohydrate immobilized nanovehicles for delivery of an anti-cancer drug
A. Agarwal*, G. P. Agrawal
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The purpose of the present investigation was to evaluate the potential of surface engineered polypropylene imine (PPI) dendrimers as nanoscale drug delivery units for site-specific delivery of a model anti-cancer agent, doxorubicin hydrochloride (DOX). Dextran conjugated PPI dendrimers were synthesized, characterized and further loaded with DOX. The developed formulation was characterized by Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR) and transmission electron microscopic (TEM) studies. Dendrimer formulation was evaluated for in vitro drug release and haemolytic studies under various pH conditions. Cell uptake and cytotoxicity studies were performed on A549 cell lines using MTT cell proliferation assay. In vivo studies were conducted for evaluation of various pharmacokinetic parameters and tissue distribution pattern. In vitro, formulation displayed initial rapid release of the drug followed by rather slow release. Further, the dextran conjugated dendrimer formulation was found to be least haemolytic but more cytotoxic as compared to free drug. Cell uptake studies depicted that the formulation was preferably taken up by the tumor cells when compared to free drug. The conjugation of oxidized polyaldehyde dextran imparts macromolecular nature to the dendritic carrier, consequently the formulation was found to selectively enter highly porous mass of tumor cells at the same time precluding normal tissues. Thus it was concluded that the drug loaded dendrimer formulation would selectively localize in the tumor mass, increasing the therapeutic margin of safety while reducing the side effects associated with anti-cancer agents.
P138

Translational regulation by RNA secondary structures: New strategy for drug discovery
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Post-transcriptional regulation of gene expression is a complex process involving many different mechanisms allowing cells to rapidly modulate protein production. mRNA metabolism (maturation, localization, translation, degradation) involves trans-acting factors (proteins and other RNAs), and cis-acting elements present in the coding region and the 5' and 3'UTR of mRNAs. Long 5' and 3'UTR can form secondary structures, such as Internal Ribosome Entry Sites (IRES), hairpin loops or guanines quadruplexes (G-quartets) that can serve as sites for the control of translation in particular during initiation.

It is believed that IRES elements are used to favor cap-independent translation initiation for protein synthesis under stress conditions (e.g. starvation or hypoxia). Hairpin loops and G-quartets could regulate translation in specific cell states such differentiation. These structural RNA motifs (SRM) were identified in a number of disease relevant mRNAs implicated in the control of cell growth or death which are deregulated in some cancers (e.g. c-myc, FGF2 or VEGF).

This project is based on the hypothesis that gene expression may be modulated by SRM through environmental factors or low molecular weight compounds. Such regulators may act either directly by binding the SRM or indirectly by modulating the activity of factors interacting with the SRM.

To explore this hypothesis, cell lines expressing a mono- or bi-cistronic click beetle red and green luciferase reporter gene constructs directed by IRES elements were generated. After the assay conditions optimization, a screen of low molecular weight compounds was conducted to identify compounds that apparently modulate positively or negatively the reporter gene expression. Experiments are currently ongoing to investigate the mechanism of action of these compounds.
Miniaturized electrical impedance spectroscopy: the impedance pill

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A prototypic miniaturized electrical impedance spectroscope has been developed, based on a new robust algorithm method and system concept for real-time bio-impedance spectroscopy [1]. We envisage applying the novel technology for the design of endoscopic capsules as proposed in [3, 4]. For this purpose, the miniaturized impedance spectroscope development from the University of Applied Sciences Northwest Switzerland has already been successfully integrated into an existing capsule developed by the Bern University of Applied Sciences [2]. Recorded impedance data were transmitted in wireless mode from the capsule to a PC for data display. The material costs per capsule are estimated to approximately 20 CHF only.

The used methods can be applied for broadband bio-impedance spectroscopy, as well as for narrow band bio-impedance measurement. Impedance is determined by differences in concentration of ions in liquids, proteins concentration, geometry of the cell membrane (e.g. thickness, surface, shape...) and so on. The first results showed that the new methods can provide even more accurate results in a fraction of time.

The miniaturized impedance spectroscopy sensor is an innovative technology that opens new horizons for research for new drug delivery systems. The technology is a cutting edge tool for faster and cheaper manufacturing processes, especially when fast measurement with exact timing is required.

References:
P140

Pharmacological effects of rutin on metabolic parameters and body weights of rats
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Rutin, a major flavonoid existing in some medicinal plants like Hypericum perforatum, has been shown to possess antidepressant-like effect in several animal models. Antidepressant drugs effecting monoamine levels in the synapses, have been known to cause some changes on metabolic parameters, such as regulation of appetite and thirst and on body weights. Based on this information, putative effect of subacute rutin treatment on metabolic parameters such as food intake, water consumption, feces weight, urine volume and body weight of rats were aimed to evaluate in the present study. Rutin (5, 10, and 20 mg/kg) was administrated via intraperitoneal route for 14 days. Metabolic cage measurements pointed out the significant and dose-dependent decrease in the food intake, feces weight, water consumption and urine volumes of rats when compared to the control values. At the end of the second week, body weights of rats were also observed to be reduced significantly. Hypophagic, antidypsogenic and antidiuretic effects exhibited in the present study seem to be related with the rutin-mediated increase in the monoaminergic neurotransmission. Possible pharmacological mechanisms concerning to this idea were discussed.
P141

Standardized device integration
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In general a laboratory consists of medical devices for sample preparation and analysis. They are controlled by a central Process Management System (PMS). Standardisation in Lab Automation (SiLA) is an initiative that started 2009 to specify a standard communication protocol based on Ethernet between PMS and devices. Several companies are already realizing the standard. We want to support these efforts by a software library we created. It allows the user to rapidly integrate SiLA devices at runtime. Besides of discovery, which gains importance with the use of Ethernet, the library supports the execution of the command set that is provided by the devices.

The Figure shows that the library contains a factory. It can create instances of the class Device, which is a proxy class to control a device. It has a request handler that can send web service messages by means of the SoapBuilder. Additionally the RequestHandler registers the request ID, which is used to associate SiLA events to command calls. Such events are handled by the HttpWorker and forwarded over the Factory to the RequestManager. This has a list of devices available and can call an according method in the correct instance of Device.

We used our library already in different projects. Besides of customer projects we built a setup for fairs to show usages of our library, such as the control of a Thermo Fisher Multidrop on the MipTec 2009. To access the device via SiLA a converter box was used. With the user interface on a PMS only few options could be selected. These were a local reset of the dispenser, it could be primed and it allowed dispensing and shaking. This little demonstration shows that commands can be sent with our library over SiLA. Currently we are investigating whether a distributed laboratory control can be built up. We assume that a central PMS is not necessary, integrators are only used to it. This kind of setup came up based on interfaces, such as RS232 or USB. Using SiLA with Ethernet as communication interface, distributed control can be put in place.
Histone methyltransferases (HMTs) are highly interesting as drug targets, as it was discovered that they play an important role in epigenetic regulation. The methylation of histones affects their binding to DNA and thus causes inhibition or activation of transcription. This regulation of gene expression is linked to various diseases such as cancer, Alzheimer’s diseases, Parkinson’s diseases and multiple sclerosis.

HMTs are S-adenosylmethionine (SAM) dependent methyltransferases: The methyl group of the cofactor SAM acts as a donor for methylation of core histones by specific HMTs. Whereas detection of methylated histones requires dedicated assay development for each HMT, the common SAM reaction product S-adenosylhomocysteine offers the basis of a generic assay version for most HMTs. This generic readout system facilitates the adaptation for high throughput screening (HTS) and structure-activity relationship (SAR) measurements.

We compare two generic SAM activity assays, one in a fluorescence polarization (FP) and the other in luminescence format. Both readout formats are suitable for compound activity testing and convertible in miniaturized 1536-well format. A pilot screen of a 8800 compound library where the resulting hits were validated by dose response experiments displays robustness, assay quality, sensitivity and cost.
Abstracts/Poster Presentations

P143

Turning minus into plus: A universal gain-of-signal system for cellular assays
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High-throughput screening requires robust assays with low rates of false positive or negative signals. Often, cell-based assays rely on reporter gene expression regulated by changes of the promoter activity due to upstream events. Monitoring elevated reporter gene expression is mostly straightforward, whereas monitoring down-regulation of gene expression is error prone and difficult to adjust. Single cell readouts are even more problematic as they suffer from the intrinsic biological variability of the cellular system. Therefore, screening for negative events with a less error prone system is highly desirable.

We developed a reporter system capable of converting a negative event into a gain-of-signal readout. The system relies on the expression of two elements, 1) Tobacco Etch Virus (TEV) protease controlled by a promoter that is linked to the target of interest and 2) a dual reporter system consisting of a fusion of N-terminal red fluorescent protein (RFP) and C-terminal green fluorescent protein (GFP), fused via a TEV protease cleavage site. The GFP reporter contains at its N-terminal end a dormant protein destabilizing sequence ("N-degron").

In the default state (no active compounds present) TEV protease is expressed and the dual reporter system is cleaved, exposing the dormant N-degron on the green fluorescent protein which in turn leads to its proteasomal degradation. The N-terminal reporter gene (RFP) remains stable upon TEV protease cleavage and can be used for normalization. Compound-induced down-regulation of TEV protease expression prevents exposure of the dormant N-degron, thus leading to elevated levels of green fluorescence. Discrimination between positive and negative signals is simply achieved by calculating the ratio between green and red fluorescence.

This assay system can be used for plate reader-based readouts as well as for fluorescence-activated cell sorting (FACS)-based screenings. We focus on yeast based assays, but the TEV/N-degron system is versatile and can also be introduced into mammalian cells and other eukaryotic cell types.
P144

Ultra sensitive, 3456-1536-miniaturization-friendly chemiluminescent detection of PathHunter® cell-based GPCR and kinase assays  
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DiscoveRx’s PathHunter® platform is a simple, cell-based, chemiluminescent assay format that allows for screening large numbers of small molecules or hybridoma supernatants in a one-step, no-wash format making it both user-friendly and HTS-compatible. In this study, we demonstrate the 3456 and 1536 application of DiscoveRx’s PathHunter GPCR β-Arestin assays as well as PathHunter Receptor Tyrosine kinase assays using Echo® liquid handler for acoustic, non-contact compound and assay reagent dispensing to AURORA high performance 3456 plates. All assays are analyzed on the new highly sensitive BMG LABTECH PHERAstar FS microplate reader. Precise liquid handling and ultrasensitive instrumentation further enhances the speed of processing allowing a complete 3456 PathHunter assay plate to be read in less than 3 minutes. Robustness of the PathHunter assay is further enhanced on a BMG LABTECH instrument and in combination with the Echo liquid handler, it reduces assay variability, improves Z’ values as well as data quality. Performing a cell-based assay at < 5uL volume translates to very low compound requirement, fewer cells and low reagent usage. A highly miniaturized PathHunter assay in combination with the BMG instrument PHERAstar FS and provides pharma/biotech/academic consortia a faster, simpler, cost-effective and a more efficient way to screen large compound libraries thereby enabling cost effective drug discovery campaigns.

P145

Generation of stable cell lines using lentiviral vectors for drug discovery purposes  
H. Vergnault, R. Gayon, C. Duthoit, A. Iché, Y. Moal*, P. Bouillé  
Vectalys (Labège, FR)

The use of cell lines has increased dramatically not only because of their application as production tools but also because of their importance in a growing number research assays such as screening campaigns. Cell lines quality requirements include molecular characterization and selection of appropriate host cells. In parallel, testing different expression systems and most importantly functional cell line selection and characterization for a greater biological relevance need as well to be tested. Lentiviral vectors are useful tools for developing genetically modified stable cell lines over expressing an intracellular, transmembrane or secreted protein without any requirements of antibiotic selection and in a rapid timeline. As for classical transfection approaches, the challenge is to ensure an appropriate expression level in target cells for any transgenes and to offer the opportunity to observe a quantitative molecule effect linked to a differential gene expression level based on the use of controlled Multiplicity of Infection (MOI). Here, we present results showing the stability of expression among culture passages and the copy number determination in target cells using different transgenes. The key step is the vector efficient particles determination.
Highly purified and concentrated lentiviral vectors for drug discovery applications in neurosciences
H. Vergnault*, R. Gayon, C. Duthoit, A. Iché, Y. Moal, P. Bouillé
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Significant advancements in the use of experimental models in drug discovery have been recently reported for genetically mediated target discovery and validation schemes such as RNAi-mediated gene knockdown and gene overexpression studies. For in vitro studies, cell based assay development including immortalized cell lines, primary or stem cells is the clue of relevant gene validation or molecule screening. In the same time, preliminary in vivo experiments based on expression modulation into specific tissue of the animal become a key step for gene target validation. In both cases, lentiviral vectors offer the efficient tool to develop physiological screens of biology and functional genomic analysis for many reasons. First, they allow the stable expression of genes into the host cell genome without any antibiotic selection so that genes will have long term consequences including effects on resultant daughter cells. Second, lentiviral based vectors deliver genes efficiently in primary and stem cells both in vitro and in vivo. Here, we present first, efficient in vitro gene silencing and overexpression in CNS primary cells following cell transduction with lentiviral vectors. The second part is focused on in vivo gene validation with lentiviral vectors injection into mice brain by stereostatic injection.

A new platform for versatile high content analysis
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The wide variety of analysis samples in high-content analysis for drug discovery requires tools that could deal with a range of challenging goals without compromise on quality and speed. Our new IN Cell Analyzer 6000 is a high performance laser-based confocal imaging system, designed to provide a flexible approach to suit various samples and imaging conditions. It combines variable aperture design, next-generation low noise sCMOS-based detector technology and high power lasers. Together these elements of our new proprietary technology provides high sensitivity, effective background rejection, speed and image quality. We present here results from performance testing of IN Cell Analyzer 6000, demonstrating that this versatile system enables users to produce high quality images and extract quantitative, information rich data from a range of biological samples, including low signal and high background assays and 3D-objects.
**P148**

**Label-free optical-based functional assays for ion channel drug discovery**

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This poster described the development of a label-free cellular assay for the ionotropic purigenic receptor P2X1 using the Corning® Epic® technology. This technology is an optical-based and non-invasive high-throughput screening (HTS) system incorporating resonant waveguide grating biosensors to monitor cellular responses following receptor activation by target ligands. In this study, dose responses were evaluated to determine EC50 and IC50 values and rank order of potency for several P2X1 agonists and antagonists. The results showed that the compound pharmacology data obtained using the Epic technology was highly comparable to values previously reported using patch-clamp electrophysiology systems. The assay was easy to perform and robust with Z’ values of >0.5. The study demonstrated the utility of the Epic technology for cell-based HTS ion channel assays.

**P149**

**Applying fragment-based lead discovery, residence time optimization, and high throughput thermodynamics to epigenetic targets using reporter displacement assay technologies**

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Reporter displacement assay technologies applied to HDAC enzymes as an epigenetic target class reveal that minor changes in inhibitor structure suffice to cause large changes in the kinetic properties of these compounds, especially their residence times. Furthermore, our data show that significant kinetic selectivity can be achieved between different HDAC classes. Show cases within the HDAC family are presented that exemplify how the reporter displacement assay can be used for fragment-based lead discovery approaches to generate lead compounds against epigenetic targets.
P150

**On chip multiplexed label free bio-affinity analysis and MALDI-MS characterisation of bound analyte**

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The coupling of Surface Plasmon Resonance imaging (SPRi) and Matrix-Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-MS) is an innovative approach for biomarker discovery in biological fluids. Multiplexed SPRi analysis allows the direct visualization and thermodynamic analysis of molecular avidity, and is advantageously used for ligand fishing of captured biomolecules on multiple immobilized receptors on a SPRi-Biochip surface. MALDI-MS is a powerful tool for the identification and characterization of molecules by their molecular weight and peptide sequence. Therefore, the combination of SPRi and MS into one concerted procedure, using a unique dedicated surface, is of great interest for functional and structural analysis of bound molecules.

Results will be shown using the Lymphocyte Activation Gene 3 (LAG3) protein, a potential biomarker of breast cancer and tuberculosis. LAG3 was captured in human plasma by SPRi down to several femtomoles/mm². Then, after MS pre-processing, LAG3 was successfully identified by MALDI-MS directly on the SPRi biochip.

P151

**A beta-arrestin assay in 1536-w format to identify antagonists of a chemokine receptor**

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Evaluating GPCR activation in a high throughput screening (HTS) environment can be achieved today using a diverse set of technologies. Most of them are addressing the specific mode of coupling of the GPCR target. For instance, cAMP accumulation monitors Gs- or Gi-coupled receptors activation, whereas calcium mobilization and inositol-phosphate accumulation are mostly used with Gq and Galpha16-coupled receptors. New assay formats were recently developed that monitor beta-arrestin recruitment in a generic fashion regardless the GPCR coupling mode. In the case of the PathHunter (PH) approach, the GPCR of interest is linked to a small part of beta-galactosidase while beta-arrestin is fused to the complementary part of the enzyme. Upon activation by an agonist, beta-arrestin is recruited by the GPCR and both parts of the enzyme come to close proximity, allowing its functional complementation and leading to a detectable luminescent signal when applying the appropriate substrate. A PH assay based on CHOK1 cells expressing a chemokine receptor (CCRx) was developed in a 1536-well format with the objective of identifying antagonists. A full HTS campaign with more than 1.5 million compounds was completed and following a hit confirmation step, more than 3000 compounds were tested and their IC50 values were determined. Compound specificity was evaluated using a beta-2 adrenergic-PH CHOK1 cell line. The most potent and specific compounds were subsequently tested in a calcium assay with the aim of confirm putative specific antagonists using an alternative technology.

In this paper, the results of the screening campaign will be presented and the pros and cons of the beta-arrestin technology in HTS compared to more classical ones will be discussed.
P152

Automated production and processing of scaffold-free tumor and liver microtissues

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Although the advantages of organotypic 3D-cell culture models are known for years, complex production and readout processes impeded its industrial implementation. We present the development of a novel high throughput-compatible 96-well platform to produce organotypic, scaffold-free microtissues based on the hanging drop technology. In contrast to the classical hanging drop generation the plate allows for liquid top-loading similar to standard 96-well monolayer plates. This is achieved by a microfluidic channel connecting an inlet funnel at the top and an outlet funnel at the bottom of the plate, where a hanging drop is formed by a combination of capillary and surface-tension forces in which the tissue is formed. We present an implementation of this plate system on a Hamilton NIMBUS robotic liquid-handling device with a 96-multichannel pipette head whereas the volumetric precision was similar to standard multi-well plates. Based on this production technology a range of tumor microtissues as well as primary rat liver microtissues were produced. Using a colon carcinoma microtissue model, a dose response curves using four reference compounds were obtained and compared to classical monolayer cultures underlining the different biological response of both in vitro models. More physiological cell models in vitro will foster the predictivity of cell-based assays. However, implementation into an automated process is key for its industrial application. We present a novel technology to produce organotypic microtissues which can be implemented into an automated screening campaign using standard liquid-handling robotics to further improve the in vitro de-risking of drug candidates.
Poster Session 2 (All Topics)

P154

Nanocarriers based on galactosylated hydrophobic polyethylenimine for targeted delivery of plasmid DNA
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Hepatocytes are interesting targets for cancer gene therapy and several hepatocyte-directed gene delivery nanocarriers have been described. On the other hand, Polyethyleneimine (PEI) is a cationic macromolecule which is able to condense DNA into nanostructures, which are then amenable to cellular internalization via endocytosis. However, it is known that PEI cytotoxicity is mainly associated with a strong positive charge of this polycation. Therefore, modifications of polymeric backbone that reduce the positive charge of PEI might be useful in order to reduce the toxicity of the polymer.

In the present investigation, the first approach used was to alkyate high molecular weight PEI (25 KDa) with a series of Ohm-bromoalkylcarboxylates. In the second step, simple galactosyl residues coupled to hydrophobic polyethylenimine to produce efficient nanovector which selectively transfected hepatocytes via the asialoglycoprotein receptor-mediated endocytosis. Ethidium bromide dye exclusion was used to show the DNA binding ability of the polymers and the cytotoxicity of the free polymers and polymer/plasmid nanoparticles were evaluated in different cell lines such as N2a and HepG2. The results showed the ability of the modified polymer to condense plasmid DNA and form nanoparticles in the range of 70-150 nm. The modifications reduced polymer cytotoxicity significantly. Also galactose conjugation significantly increased the ability of nanostructured complexes to transfer plasmid DNA into hepatic cells. In conclusion, the hydrophobic galactosylated PEI obtained by this safe and inexpensive method can be considered as good candidates for transfer of genetic materials into hepatocytes in order to cancer gene therapy.
P155

Relation between molecular properties of drugs and their transport across the biological membrane
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Membranes are barriers which give cells the outer boundaries. The knowledge of transport properties of small molecules through the membrane enables to understand the structure-property relationship.

Hydroxamic acids are the neutral, polyfunctional biomolecules with general formula RC(=O)N(R')OH having interesting medicinal and biological potentiality. In the present investigation, the permeated amounts of three molecules viz., N-Phenylbenzohydroxamic acid (PBHA), N-o-Tolybenzohydroxamic acid (o-TBHA) and N-p-Tolybenzohydroxamic acid (p-TBHA) were measured by UV method to determine the diffusion coefficients at 303.15K for the biological membrane as controlling barrier. The values obtained are in the range from 2.38 to 3.16 m²/s and follow the sequence o-TBHA> p-TBHA> PBHA. It is well-known that drug lipophilicity plays an important role in transport across biological membranes. These data suggest that diffusion is related to the logP values and independent on molecular weight of molecules. The present approach is useful not only for the understanding of the therapeutic action of existing drugs, but also in the discovery process of new candidates.

Keywords: Hydroxamic acid, lipophilicity, membrane.

P156

Electron microscopic study of the effects of melatonin on dermal fibroblasts in wounded skin of aged mice
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Objective: Wound healing in aged skin is a complex process and has a slow rate in comparison with the young skin and is thought to involve decreased proliferative capacity of the cells. The pineal gland hormone melatonin used in a variety of age related diseases but reports of melatonin effects on wound healing are inconsistent. In this research the effects of melatonin on dermal fibroblasts were evaluated through light and electron microscopy.

Materials and Methods: 20 male white mice with the age of 16 months and 20-23 gr in weight were equally divided into control and experimental groups and under ether anesthesia the hair of their neck skin were shaved and an incision was made and then the incision was sutured. The experimental group was intraperitoneal injected with a daily single dose of 10mg/kg melatonin for 14 days. The control group received only saline. After the last injection all mice were killed under ether anesthesia and skin of the two groups were dissected in the region of the incision and processed for light and electron microscope observations. In morphometric study the thickness of epidermis and in dermis, the number of fibroblasts and the mean diameter of their nuclei and volume fraction (Vv) of euchromatin to nucleus of fibroblasts were evaluated.

Results: Melatonin administration to the experimental group increased the thickness of epidermis significantly (P<0.001). A significant increasing in the fibroblasts count was noted in dermis of the experimental group (P<0.001). Melatonin treated showed significant increasing of mean diameter of fibroblast nuclei compared to control group (P<0.001). Electron microscopic observation revealed that in experimental group the number of fibroblasts were increased and ultrastructural changes were found in the fibroblast nucleus compared to control group so that the Vv of euchromatin to the nucleus were increased significantly (P<0.001).

Conclusion: The results of this research indicated that melatonin enhances the proliferative capacity of the fibroblasts and activates them for collagen synthesis which finally help to repair wounded aged skin. Therefore these findings can be compared with human data and use in clinical application for rapid wound repair in aged skin.
P157

Computational fragment growing using the molecular invention engine muse
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Successful drug discovery often requires optimization against a set of biological and physical properties. We describe our work on multi-parameter approaches to structure-based molecular invention and studies that demonstrate its application in the field of computational fragment growing.

The software Muse is based on an evolutionary algorithm that operates on an initial population of structures to invent new structures with improved scores. The invention process uses chem-evolutionary operators and features customizable mutation probabilities and fragment databases, preservation of substructures during evolution and filtering of undesirable substructures. Muse is unique in that it has the ability to work with any user-defined scoring function that provides results in the form of a numerical evaluation of designed structures. The structure-based scoring function we present uses Surflex-Dock to pose and score invented structures in context of the protein and is combined with a number of popular “Lipinski-like” molecular properties to focus the design on medicinally relevant chemistries. With the ability of Surflex-Dock to start the docking process with a single or multiple placed fragments, this scoring function allows growing and optimizing attachments to fragments inside the target’s active site.

Several retrospective studies using the structure-based scoring function demonstrate the ability of the above mentioned approach to grow fragments generated from co-crystallized ligands into full-size molecules and generate novel ideas for various targets. Specific examples will be shown where the invented molecules present new molecular templates which are pose and shape similar to the co-crystallized ligands. The invented molecules further exhibited target specific hydrogen bond patterns although these were not specified as initial design criteria.

P158

An Insilico approach for the selection of aromatase inhibitor for breast cancer treatment
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Traditionally, drugs were discovered by testing compounds synthesized in time consuming multistep processes against a system of in vivo biological screens. Promising compounds were then further studied in development, where their pharmacokinetic properties, metabolism and potential toxicity were investigated. In current course of work a study on herbal lead compounds are mentioned and further their potential binding affinity to the effectors molecules of the disease breast cancer. Clinical studies demonstrate a positive correlation between the extent of production of estrogen from androstenediol after menopause and breast cancer through the release of epidermal growth factor (EGF). Therefore, identification of effective, well-tolerated aromatase inhibitors represents a rational chemo preventive strategy. Results investigated from the current study are showing the effects of naturally occurring piperine that significantly inhibits aromatase. Although other four compounds have also been investigated as better inhibitors in comparison of various drugs available in the market. Due to high ligand binding affinity of piperine towards aromatase introduces the prospect for their use in chemopreventive applications in addition as they are freely available natural compound i.e. from black pepper that can be safely used to prevent breast cancer.
Abstracts/Poster Presentations

P159

Screening of inhibitors of HIV-Protease by study of interactions and molecular dynamics - An Insilico approach
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Background: AIDS is a lethal disease of the immune system caused by the human immunodeficiency virus (HIV). This condition progressively reduces the effectiveness of the immune system and leaves individuals susceptible to opportunistic infections and tumors. HIV-1 protease (HIV PR) is a retroviral aspartyl protease that is essential for the life-cycle of HIV, the retrovirus that causes AIDS. HIV PR cleaves newly synthesized polyproteins at the appropriate places to create the mature protein components of an infectious HIV virion. HIV protease inhibitors are useful in the treatment of AIDS. The daunting ability of the virus to rapidly generate resistant mutants suggests that there is an ongoing need for new HIV protease inhibitors with superior pharmacokinetic and efficacy profiles.

Results: Our aim is to identify a therapeutically relevant drug candidate. Thus by homology modeling we modeled a 3-D structure for designing inhibitors of HIV protease and we have applied docking and molecular dynamics (by Gromacs) approach to study the binding of inhibitors to the active site of HIV protease and also to study the stability of the drug. By using modeler, we found Target 1.B99990001 of 3JUE has got 90.9% result. Further the docking results by Autodock4 shows that 3JUE when docked with ligand gives favorable docking scores and hydrogen bonds. To add to this speculation, molecular dynamics simulations reveal that tight binding conformation for 3JUE has high specificity for HIV protease active site. The graph (RMSD vs time) was generated for 60 ps and it showed stability after 30 ps. Thus its stability was relevant.

Conclusion: Thus, our work provides valuable insights on the ligands selected and their interaction with the target. This provides relevant ideas for future design of potent inhibitors and drugs.

P160

A new approach for in silico genotoxicity testing of impurities and degradants
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According to FDA Guidance for Industry, assessment of genotoxicity/carcinogenicity by computational methods is sufficient for impurities in drug products present at levels below the ICH qualification thresholds. This study presents a novel approach to aid this assessment based on a probabilistic predictor of Ames genotoxicity, and a knowledge-based system of structural alerts. The list of potentially hazardous structural fragments was compiled from various literature sources and refined by analyzing their performance on data from different assays detecting point mutational and/or clastogenic mechanisms of DNA damage (Ames test, in vitro chromosomal aberrations, micronucleus test, mouse lymphoma assay). Finally, the expert system was tested on the Carcinogenic Potency Database to ensure detection of common non-genotoxic carcinogens. Selected structural alerts achieved >90% sensitivity for recognizing positive compounds in Ames and Chromosomal Aberrations data sets showing that the absence of alerting groups is a reliable criterion for identifying impurities not posing significant genotoxic/carcinogenic risk.
Solid state NMR spectroscopy of boronic acid and organoboron compounds
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In recent years, boronic acid and organoboron compounds have been used in pharmaceutical industry as enzyme inhibitors, neutron capture agents for cancer therapy or antibody mimics that recognize biologically important saccharides as well as molecular sensor or receptors. Some 20 years ago, simple alkyl or arylboronic acids were used as a serine protease inhibitor. Since then, many boronic acid compounds with an appropriate peptide sequences have been designed and synthesized for the development of more potent and selective inhibitors. Next significant potential of boronic acid compounds lies in the development of feedback controlled delivery systems for insulin because boronic acids compound form reversible complexes with sugars. As a result, boronic acids and related molecules have now evolved as major players in synthetic and medicinal chemistry.

Very important, for finally using as a medicinal product, is precise description of structural homogeneity and purity. Excellent tool for structural analysis is solid state NMR spectroscopy. Organoboronic compounds description is possible by NMR measurements of included nuclei such as 13C, 11B etc. High sensitivity and abundance of boron nucleus is suitable for measurement and consequently analysis of NMR spectra. Quadrupolar character of boron atoms complicates detail analysis. However, the solution provides the MQ/MAS NMR experiment and using of relatively new techniques called biaxial shearing. This combination enhances the spectral resolution and thereby helps correctly analyze and describe structure units. In our contribution we will demonstrate our attempts to structure determination of organoboron compounds by 11B MQ/MAS spectroscopy. In particularly, applications of two-dimensional multiple-quantum experiments with biaxial shearing that provides detail information about local geometry of measured compounds and easier determine their structure arrangement.
Molecular modeling and docking supports target characterization of anti-fungal compounds


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The treatment of systemic life threatening fungal infections remains a significant unmet medical need. Phenotypic screening has been effective in discovering antibiotics. However, phenotypic screening does not provide any information about the target which is important for small molecule optimization studies. This report describes how molecular modeling and docking of compounds into a putative target can help support target validation. A combination of screening, genetic, and biochemical approaches were used for the identification of compounds with antifungal activity. These compounds were identified from a high throughput, whole cell, growth inhibition assay of yeast. Subsequent genetic analysis of this class of compounds suggested Erg11 as the putative target site. Interestingly, Erg11 is also known to be the target of antifungal azoles. Metabolic profiling studies performed for these compounds further confirmed the hypothesis presented by these genetic studies. A buildup of Erg11 substrate (lanosterol) was observed in the presence of these compounds. Besides Erg11, these compounds also inhibit mammalian cytochrome P450 (Cy450), which are closely related to Erg11. Since crystal structures of mammalian and bacterial cytochrome P450 are available, this structural information was used for homology modeling of Erg11. The Erg11 macromolecular model was used to dock the compounds to demonstrate their binding possibilities. In the absence of a crystal structure, this information will be useful for explaining the SAR for these compounds and propose site-directed mutants to explore the protein-ligand interaction.
Docking and 2D QSAR studies of BACE-1 inhibitors as anti-alzheimer agent
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In our work we carried out molecular docking for 130 analogous structurally of diverse beta-secretase inhibitors. 130 inhibitors were selected from protein data bank. The compounds collected for the known inhibitors of BACE1 were docked into the binding pocket of beta-secretase using the Autodock 4.0 program. AutoDock combines a rapid energy evaluation through precalculated grids of affinity potentials with a variety of search algorithms to find suitable binding positions for a ligand on a given macromolecule. Ligand, 2WF4 is best docked in the pockets of beta-secretase having interaction with ASP 94, GLY 292, ARG 190, TYR 260 and the best interaction is with ASP 94. The best conformations are chosen from the lowest docked energy solutions in the cluster populated by the highest number of conformations. Results has clearly indicated that the ligand complex 2WF4 have high binding affinity towards beta-secretase protein. The SAR analysis carried out to predict the IC50 value of those ligands which is not known in the dataset. We developed 2D QSAR equation t from the known beta-secretase inhibitors to predict the IC50 of 49 complex ligands . Multiple linear regression analyses were employed to correlate the variations of biological activities with the various physicochemical properties. The best-fit model for each series was identified on the basis of the leave-one-out cross validated correlation coefficient q2 and the correlation coefficient r2. These training sets were prepared to predict the value and by test set the validation of the equation were done. For this purpose we have taken out 130 beta-secretase inhibitors from the PDB. Out of which 81 inhibitors have known IC50 value and 49 doesn’t have. The SAR equation is developed on the basis of known 6 descriptors (M.W, Rot_Bond, logP, Hydrogen Bond Acceptor, Hydrogen Bond Donor and Docking score) and these descriptors used to predict the IC50 for unknown activity compounds. Our current study is useful for the chemist and biologist for in vitro study of BACE1 as Anti-Alzheimer’s agent.
P164

Identification of novel HIV-1 IN inhibitors by a shape based screening approach along with QSAR predictions and Docking studies

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HIV-1 IN has also been recognized as another lucrative target for inhibition of viral replication. Because it has no counterpart in the host cell, thereby selectively inhibit the viral replication. Till now, only two drugs Raltegravir[1] (US FDA approved) and Elvitegravir[2] (Phase III clinical trial) have been successfully tested for treatment of patients against HIV-1 IN. Therefore, discovery of new HIV-1 IN inhibitors with good inhibitory potency is the need of hour.

Pharmacophore mapping, docking, similarity and shape based searching are widely used approaches in the virtual screening field for the identification of new scaffold for target proteins. Among them, Shape based screening (SBS) has been recognized a powerful tool to identify shape similar molecules from drug databases. The basic assumption of SBS is that molecules having similar shape and chemical pattern with known active may bind similar manner into the active site as active molecule, hence probability to produce similar activity. Here, we have used SBS approach to screen the drug like databases. Combo and TanimotoShape scores ranked the molecules differently as both the scores have different parameter for screening. After in silico toxicity and ADME studies, 2D and 3D[3,4] QSAR models were used to predict in silico activity of screened molecules. The QSAR predictions from models, have no guarantee that predicted activity is reliable. Therefore, applicability domain[5,6] was performed. Most of the molecules occupied the same domain as training data, hence prediction was reliable. To explore the binding of screened molecules, docking studies were performed. The criteria for selection of best docking pose was used[4]. The best binding poses were considered as new potential hits for HIV-1 IN, but need to be verified to confirm their anti-HIV-1 IN activity by an in vitro assay. This approach resulted in identification of the novel molecules of same scaffold with different substituent's as well as completely diverse molecules. This approach can also be implemented for other proteins or targets.

Abstracts/Poster Presentations

Drug like molecules library

Screened molecules

Shape Based Screening

ADMET

• Toxicity and ADME studies

QSAR

• 2D QSAR model
• 3D QSAR model

Docking

• FlexX, AutoDock, DOCK Programs
• Best docking pose

New Hits
P165

**The development of data driven workflow tools to facilitate pathway screening.**

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In order to investigate the pathways that may be influencing a given biological phenotype it is very helpful to be able to select tools that disrupt the pathways that may interact with the phenotype. LMW compounds have a number of features that differentiate them from genetic tools such as siRNAs or cDNA clones. For example compounds can be used with cell lines or tissues that cannot be transfected, and they allow rapid and temporal control of gene function. Here we present the application of a set of tools which can be used by bench scientists to quickly get to their LMW compound of interest. Tools presented include databases enumerating known genes within a given pathway, databases of compounds reported to modulate or bind gene products, databases and links to chemical structures allowing the identification of available compounds, and workflow management systems combining these data sources for efficient and reproducible usage.

P166

**A solid-state NMR study of polymeric matrix influence on creation of solid dispersions of poorly water-soluble drugs**

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In this contribution a solid-state NMR study of structure and segmental dynamics of solid dispersions of active pharmaceutical ingredients (API) in polymer matrix is presented. In many clinical studies it has already been demonstrated that higher efficiency of APIs significantly reduces menace of many diseases. API has higher efficiency when has good bioavailability and ultimately good dissolution, solubility in human fluids and good permeability in gastrointestinal tract (GIT). Unfortunately a lot of pharmaceutical substances exhibit low solubility in water. That is why current pharmaceutical research focuses on increasing solubility and bioavailability of these substances. Among many procedures how to improve dissolution rates of poorly water-soluble drugs, the transformation from their crystalline state to more soluble amorphous, nanocrystalline solid dispersion and/or solid solution represents one of the most promising ways. In our work we focussed our attention on the study of structural properties of APIs in the prepared solid polymer dispersions exhibiting increased solubility and polymeric matrix influence (various molecular weight of polymer matrix) on the above-mentioned prepared systems. The acetylsalicylic acid was used as a model of APIs with low solubility. Several procedures were used to combine this model compound with polymeric nontoxic water soluble matrix (PEG, PVP, HPMA). In some cases the observed drug-polymer interaction significantly enhanced dissolution rates of the APIs. Structural reasons of the increased solubility in solid dispersions and polymeric matrix influence on these systems were extensively probed by a wide range of ssNMR experiments including 13C CP/MAS NMR, 1H DQ-BABA, 13C-1H HETCOR and relaxation experiments. The obtained results are comprehensively discussed in this contribution. In particular, the extent of mutual interaction is thoroughly examined.

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P167

**Genome-wide profiling of aging associated DNA methylation in normal colon of healthy population**

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Promoter methylation associated gene silencing at CpG sites is a prominent feature in colorectal cancer (CRC). Although, age is the main risk factor of CRC, interindividual methylation variations in normal ageing colorectal tissue are poorly characterized. Identifying their extent in the aging process is important for understanding fundamental dynamics of normal individual epigenomes and their contribution to CRC carcinogenesis. We performed comprehensive DNA methylation profiling of promoter regions of 41 human donors at age 50 and 75 using illumina infinium HumanMethylation27 BeadChip. Methylation profile classes derived from unsupervised modeling were significantly associated with age (P<0.0001). In total, 653 age-associated differentially methylated CpGs (proximal: 491 CpGs; distal 162 CpGs) were identified. Among them, we found highly significant CpG island–dependent correlations; loci in CpG islands gained methylation with age, loci not in CpG islands lost methylation with age (P<0.001), and this pattern was consistent in both colon segments. Lastly, in gene ontology analysis, we found age-associated differentially methylated CpG sites in physical proximity to genes involved in regulation of transcription. This suggests that specific age-related DNA methylation changes may have quite a broad impact on gene expression in the normal colon. We used pyrosequencing to quantitatively validate the microarray data and confirmed linear age-related methylation changes for all 10 genomic regions examined. This work provides insight into epigenetic deregulation by age-related methylation alterations. Our data will be useful for future investigations towards understanding the role of aging associated methylation changes in colorectal tumorigenesis. Furthermore, our data suggest the utility for the DNA methylation patterns in these genes as clinically useful surrogate markers in colon cancer, as well as new molecular pathways for further investigation as therapeutic targets.

P168

**Fluorine-protein recognition mechanism revealed by 19F NMR chemical shift**

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The 19F NMR isotropic chemical shift is a sensitive indicator of the covalent/electrostatic character of an F-X bond. A more shielded fluorine atom, according to the NMR shift (i.e., with increased electron density), imparts a less covalent and more electrostatic character to the C-F bond resulting in a larger dipole. A correlation between 19F NMR isotropic chemical shift and fluorine protein interactions was recently proposed [1]. Shielded fluorine atoms are found preferentially in close contact to hydrogen bond donors of the protein suggesting the possibility of intermolecular F…H hydrogen bond formation. Deshielded fluorines are found preferentially in close contact with hydrophobic side chains and with the carbon of carbonyl groups of the protein backbone. A mono-exponential function was derived for correlating the 19F NMR chemical shift with the strength of the intermolecular F…H hydrogen bond. Based on all these findings a "rule of shielding" was proposed for the judicious selection of appropriate fluorinated moieties to be inserted into a molecule for making the most favorable interactions with the receptor. We now present application of the "rule of shielding" to the design of several fluorinated scaffolds with the potential of recognizing different protein secondary structural elements. [1] C. Dalvit, A. Vulpetti, ChemMedChem  6, 104-114 (2011).
Antibacterial effects of peptide D28 and its new dimeric analogues

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Aims: The major aim of this study was synthesis and assay of the antimicrobial activity of peptide D28 and its new analogues as dimeric peptides.

Methods: Three antimicrobial peptides known as D28, Di-D28-Lys, Di-Cys-D28 including 20, 41, 42 residues were synthesized respectively. For peptide synthesis, solid phase peptide synthesis method using blocked amino acids with fluorenyl methoxy carbonyl group and for peptide purification HPLC were used. Peptides compositions were confirmed by amino acid analysis and electrophoresis. Antimicrobial tests against Staphylococcus aureus and Pseudomonas aeruginosa were performed by disk diffusion on plate as well as broth culture (Broth macrodilution) at different concentrations.

Results: The peptides (D28, Di-D28-Lys, Di-Cys-D28) were synthesized successfully. Although, all three peptides were effective against S. aureus, but on the contrary to other ones, Di-Cys-D28 had no antimicrobial activity against P. aeruginosa. The inhibitory activity of Di-D28-Lys against P. aeruginosa was more than that of D28 peptide.

Conclusion: Improvement of the antimicrobial activity of peptides through dimerization depends on the methods of dimerization and the strain of bacterium. In comparison, Di-D28-Lys peptide showed wide range and more antimicrobial activity than D28 and Di-Cys-D28. Therefore, Di-D28-Lys peptide could be a suitable antibiotic candidate for future studies.
P170

Flexible desktop software for genome assembly and variation analysis
DNASTAR (Madison, US)

The new biotechnology and biomedical frontiers opened up by next generation sequencing (NGS) technologies also pose significant hurdles in terms of data management, assembly and analysis. Most available software solutions require cobbling together disparate programs that typically require substantial computing resources and bioinformatics expertise. In addition, these systems may be fine tuned to a particular sequencing platform. At DNASTAR, we have developed an integrated suite of software programs for assembling and analyzing NGS data from all of the major platforms and supporting the key workflows on a desktop computer.

At the core of the suite is the SeqMan NGen assembler, which runs on Windows, Macintosh and Linux operating systems, and assembles single and paired end data from all the major NGS technologies: Illumina, Roche 454, Ion Torrent, Helicos and SOLiD. The assembler shows excellent performance on modestly priced desktop computers. For example, whole human genome assemblies of deep Illumina data (35x) take less than 24 hours on a $2500 desktop computer with 16GB of RAM while human exome assemblies (~150x coverage) take about three hours on the same computer. Probabilistic SNPs/small indels and genotype calls are included in the output with known variants identified by their dbSNP IDs.

The new assembler version expands on this foundation in two crucial areas. First, a structural variation report is included for identification of large indels, inversions and translocations. Second, multiplexed (e.g. MID-tagged) samples can be processed and assembled either as individual data sets or as one or more groups. These capabilities make SeqMan NGen a truly fully functional, high performance desktop assembler for discovering genetic variation from NGS data sets of any size or configuration.

Interactive alignment and strategy views within SeqMan Pro, together with tables for SNPs, structural variation, coverage, and features, allow for efficient navigation through even large projects such as human genome assemblies. New multi-sample views and tables provide for analysis of MID-tagged or indexed samples in a single project. For large multi-sample projects composed of hundreds of individual data sets, SeqMan NGen generated variation tables can now be exported to ArrayStar, which has tools for filtering, set comparison and clustering that support a number of different workflows, such as identifying candidate polymorphisms in a NGS-based association study.
MRL/lpr mice: a preclinical drug validation model of lupus-like autoimmunity and autoimmune tissue injury of skin, lung and kidney
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Introduction: Novel immunosuppressive and anti-inflammatory compounds require in vivo validation in suitable models of autoimmunity and autoimmune tissue injuries. MRL-MPJ Faslpr (MRL/lpr) mice are a prototypic murine model for lupus-like systemic autoimmunity. From 8-wks-of-age these animals develop polyclonal B cell activation, an expansion of autoreactive B and T cells that cause massive lymphoproliferation. By 12 to 16-wk-of-age MRL/lpr mice begin to produce antinuclear antibodies directed against RNA and DNA autoantigens. From 16 to 24 wk, MRL/lpr mice develop autoimmune pathology of the kidney (lupus nephritis), the lung (bronchial-associated lymphoid tissue), and the skin (cutaneous lupus). Kidney disease rapidly progresses to renal failure and death in 50% of the mice by 22-24-wk-of-age.

Methods: We intervened the disease in MRL/lpr mice with small molecule drugs and biopharmaceuticals targeting various pathomechanisms involved in lupus nephritis from 12 to 22 wks of age. Drug effects were evaluated by analysis of markers of systemic autoimmunity (serum cytokines and autoantibodies), immune dysregulation (FACS of splenocyte subtypes), skin disease (histology), lung disease (histology), kidney disease (serum creatinine/BUN, proteinuria/hematuria, histology), overall survival, drug toxicity (bone marrow FACS and CFU count), pharmacokinetics during renal failure.

Results: This poster will provide some examples like anti-inflammatory drugs (anti-Ccl2, chaperonin 10), immunoregulatory drugs (DHODH inhibitor), cell cycle regulators (nutlin-3) used in this model. We could identify the systemic and/or tissue specific effects of these drugs and how this model can be used to validate experimental compound for immunotherapy and how a sophisticated phenotype analysis can pin point their way of action.

Conclusion: MRL/lpr mice represent a good preclinical drug validation model of lupus-like autoimmunity and autoimmune tissue injury of skin, lung and kidney.

References:
2. Anti-Ccl2 Spiegelmer permits 75% dose reduction of cyclophosphamide to control diffuse proliferative lupus nephritis and pneumonitis in MRL-Fas(lpr) mice. Kulkarni et al. J Pharmacol Exp Ther. 2009 Feb;328(2):371-7
Uninephrectomy of db/db mice: a preclinical drug validation model of advanced diabetic nephropathy

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Introduction: Animal models remain an important tool for dissecting the molecular pathology of diabetic nephropathy (DN). While rodent models of DN are instrumental in studying the molecular mechanisms of early mesangial matrix remodelling and albuminuria, they have been less useful in studying the molecular mechanisms that mediate the progression to late stage DN. In humans late stage DN is characterized by severe glomerulosclerosis, reduced GFR, interstitial immune cell infiltrates, tubular atrophy, and interstitial fibrosis. Advanced DN develops over more than a decade in humans, hence it remains difficult to address in animal models. Experimental means that intend to accelerate the development of late stage DN in mice should enhance crucial pathomechanisms of DN, e.g. glomerular hyperfiltration. Experimentally, hyperfiltration can be induced by a reduction of renal mass, in which the extent of renal mass ablation should correlate with the extent of hyperfiltration and the progression of kidney disease. We therefore hypothesized that early uninephrectomy would accelerate the progression of DN in type 2 diabetic db/db mice.

Methods: We performed uninephrectomy on db/db mice at age of 5-6 week and housed until they show signs of renal damage (proteinuria, reduced GFR). Disease progression in these mice was then intervened by using various drugs modulating the factors associated with DN. Mice were sacrificed at week 24 to evaluate the effect by measuring GFR, albumin/creatinine ratio (renal parameters), histological analysis (T cells, macrophages infiltration, tubular morphometry), RT-PCR and serum cytokine analysis.

Results: We hypothesized that early uninephrectomy would accelerate the development of severe diabetic glomerulosclerosis, albuminuria, increased expression of chemokine and cytokines and subsequent tubulointerstitial damage in type 2 diabetic db/db mice. This poster will summarise how small molecule antagonist for chemokines (Anti-ccl2, CXCL2), chemokine receptors (CCR1, CCR2) attenuates the disease progression and how this mouse model could be useful to understand the mechanisms of the advanced diabetic nephropathy.

Conclusion: Uninephrectomy of db/db mice represent a preclinical drug validation model of advanced diabetic nephropathy.

References:
P173

Large-scale evaluation of CavBase for analyzing the polypharmacology of kinase inhibitors
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Binding site similarity analysis has been suggested to be useful in the prediction of selectivity profiles of kinase inhibitors. One of the few readily available methods for binding site analysis is CavBase, which is part of Relibase+ from Cambridge Crystallographic Data Centre. CavBase uses a simple scheme to encode the surface properties of all cavities in the protein structure to a set of descriptors called pseudocenters. Cavities are detected by a modified version of LigSite algorithm. Although CavBase has been available for some time, there are no published studies that investigate the impact of the various parameters of the method to the actual prediction accuracy. In this study, several different searching strategies of CavBase were tested on a kinase data set of 16 inhibitors. Different query definitions, as well as the accuracy of the all three scoring functions implemented in the software, were investigated. The data set was constructed by mapping the biological activity data from Ambit kinase panel to the structural information from the Protein Database (PDB). Data set consisted of 938 crystal structures for 131 kinases. An inhibitor was considered to be either active or inactive against a given kinase with threshold Kd of 10µM. Overall, CavBase demonstrated reasonable ranking accuracy with an average Receiver Operating Characteristic Area Under Curve (ROC AUC) value of 0.73. The default strategy of defining the query pocket by selecting pseudocenters using the distance of 4Å from inhibitor atoms and the use of original scoring function produced the best results. The study showed that it is possible to identify, on the basis of binding site similarities, those kinases that are likely to bind the same ligand, thus providing a useful tool for the rational design of targeted polypharmacology in kinase field. The low throughput of CavBase limits the applicability of the method for larger data sets (such as the whole PDB).
P174

Inhibitory activity of isomers of bexlosteride on human steroid 5-beta-reductase: Virtual screening & molecular docking study

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Human steroid 5beta-reductase (aldo-keto reductase (AKR) 1D1) catalyzes reduction of Delta (4)-ene double bonds in steroid hormones and bile acid precursors. 5beta-reductase is responsible for generating 5beta-pregnanes, which are natural ligands for the pregnane-X receptor (PXR) in the liver. PXR is involved in the induction of CYP3A4, which is responsible for the metabolism of a large proportion of drugs. Thus, 5beta-reductase is involved in the formation of potent ligands for nuclear receptors. We have attempted with the help of virtual screening and molecular docking approach using Lamarckian Genetic Algorithm to elucidate the extent of specificity of AKR1D1 towards isomers of Bexlosteride. The study of 3000 molecules also revealed the binding mode of AKR1D1 with different classes of Bexlosterides. The binding energy ranges from -9.82 kcal/mol to -4.36 kcal/mol. As of structure analysis 20 molecules showed better binding affinities with the active site pocket (comprising of TYR 58) of the AKR1D1 enzyme. AKRs much can be inferred about the structure-function of AKR1D1. The conserved AKR tetrad consists of Tyr, Lys, Asp, and His, where Tyr acts as the general acid/base. Our study gives an idea about the interaction between the active site residues and the substrate which is explained on the basis of size & hydrophobicity of the binding pocket.
Lead optimization for glycosidase inhibition: Computational based analysis

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Lead optimization is an important step in drug design and discovery because it causes most of the favorable and unfavorable effects of a molecule. The efficiency of most of the drug molecules is reduced due to its unwanted side effects, toxicity and poor ADME properties. Hence, it is necessary to consider these effects in the early stages of lead optimization and drug design. In this present investigation, we have used 50 different lead structures obtained from literature (marketed, clinical trials and active compounds from literatures) were used for the study. The computational analysis was performed using different software. The toxicity studies (hERG and LD50) on the molecules reveal that those compounds has flexible lead structure posses toxic activity at low concentrations while rigid structures shows activity at higher concentrations. Literatures show these compounds also possessed significant glycosidase inhibitory activities. The fragmental analysis result shows that the polar and aromatic substitutions on the lead can improve the glycosidase inhibitory activity. The QSAR, pharmacophore and other computational analysis on the compounds also explain that the polar, aromatic/hydrophobic properties on the vdW surface of the molecules are favorable for the inhibitory activity. But the flexibility of bonds in the molecule is important for all activities such as glycosidase inhibitory, hERG blocking, and other toxicity. The lead structure analysis illustrate that the fused aromatic rings (chromenone, andrographolide, xanthones, etc) are having less toxicity against the hERG target and have significant inhibitory activity on glycosidase targets. The fragment analysis provides information that a lead compound with less flexibility have less toxicity and substituted with aromatic or polar fragment can improve the glycosidase inhibitory activity. The active site environment analysis of glycosidase enzymes of different species by multiple alignment techniques shows that the enzymes exhibit polar and aromatic residues (especially histidine, glutamic acid and aspartic acid) which reveal that the aromatic (PHE and HIS) and polar residues can interact significantly with the enzymes. The metabolic prediction study shows that the olefinic double in the molecules cause epoxide formation that causes DNA damage. These promising results will helpful to design novel glycosidase moieties with less toxicity.
P176

From multi-omics research to drug R&D
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Pharmacogenetics is the study of the role of inherited and acquired genetic variation in drug response. Recently, the field of pharmacogenetics has evolved into “pharmacogenomics,” involving a shift from a focus on individual candidate genes to genomewide association studies. Using next generation sequencing technology such as Illumina, BGI could get an all-round view of pharmacogenomics at three levels including DNA (Genomics), RNA (Transcriptomics) and epigenetic (Epigenomics).

Genomics Sequencing based on a rapid scan of markers across the genome of persons affected by a particular disorder or drug-response phenotype and persons who are not affected, with tests for association that compare genetic variation in a case–control setting, can identify the mechanism of disease, to make more diverse and more neoteric drugs.

Pharmacogenomics facilitates the identification of biomarkers that can help physicians optimize drug selection, dose, and treatment duration and avert adverse drug reactions. We can use Transcriptomics Sequencing for evaluating the response of medical treatment to make more efficacious and safer drugs.

Epigenomics involves the study of changes in the regulation of gene activity and expression that are not dependent on the DNA sequence, which refers to a more global analysis of epigenetic changes across the entire genome. The Epigenomics Sequencing study of variable responses of humans to drugs and toxic agents may benefit the individual personalized medical therapy, to make more accurate and more appropriate drugs.

Pharmacoproteomics is also an important contribution to drug R&D as it is a more functional representation of patient-to-patient variation than that provided by sequencing. BGI can support the pharmacoproteomics study to be a complementary method to the above sequencing research of three levels.
P177

Retrospective analysis of the performance of lead-like screening libraries

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Lead-likeness has become an industry benchmark for hit identification in the early phases of drug discovery. Although numerous lead-like hits have been reported from high-throughput screening campaigns, a systematic evaluation of the quality of screening libraries used has nevertheless been scarce. The screening data for 15 enzyme assays from a diverse screening library (DSL) (59,443 compounds) and a focused kinase library (FKL) (3,287 compounds), both compiled using lead-like criteria,[1] were analyzed. A principal component analysis of the chemical space described by 15 physicochemical property descriptors suggested the active hits identified were spread across the entire chemical space represented within each library. We also examined the presence of pan assay interference (PAINS) compounds within each library. The application of a literature PAINS substructure filter [2] revealed a low percentage of PAINS in both libraries, at 2.9% and 1.5% for DSL and FKL respectively. Although some hits were found to contain structural motifs which could potentially display PAINS behavior, no individual compound was reported to be active in more than 50% of the assays screened. This indicates the literature filter may only apply to certain screening assay technologies. These analyses demonstrate that the entire chemical space represented by these lead-like screening libraries can be effectively utilized to identify hits for both diverse and target-focused screenings.


Pharmacodynamic parameters of sulfated cellulose from Gossipium cotton at intravenous administration to rabbits

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Researching of new anticoagulants (AK) of direct action among polysaccharides’ derivatives of bacterial, plant or animal origin is carrying out strongly in many countries. These AK along with antiaggregants, fibrinolytics and AK of indirect action are main drugs for prevention and treatment thromboembolism of human and mammals.

A sulfated cellulose (SC) obtained from Gossipium cotton with molecular weight (MW) 10-30 kDa, sulfation degree (SD) 1.8, antithrombin (aIIa) and antifactor Xa (aXa) activities 130±12 U/mg and 45±3 U/mg accordingly was investigated. Sixty four Chinchilla rabbits having weight 3.5-4.5 kg were used for experiments ex vivo. For analysis of AC’s pharmacodynamic parameters SC with doses 0.3-10 mg/kg in salt solution was administrated to ear’s regional vein of rabbits and samples of blood were collected in certain time period. Anticoagulant activity of rabbits plasma was investigated in coagulation assays activated partial thromboplastin time (aPTT) and Heptest, comparing it with control unfractioned heparin (UFH) (aIIa=160±15 U/mg, aXa=145±8 U/mg).

With increasing doses SC from 0.3 to 10 mg/kg clotting time (CT) of rabbits plasma in aPTT at the 5th minute after administration increased from 15.5±1.2 to 411.4 ± 71.7 seconds and more. Action of AC in doses 0.3-3 mg/kg was reliably over in 300 minutes after administration. At dose 5 mg/kg AC’s half-life was 255 minutes and lifetime was more 300 minutes. Required increasing of rabbits plasma’s CT in 2-2.5 times (comparing with control) and aIIa to therapeutic dose (0.35-0.7 U/mg) was observed at doses 3-5 mg/kg. In Heptest CT of experimental animals plasma increased with increasing doses from 0.3 to 10 mg/kg. In 5 minutes after intravenous administration SC in the smallest and highest doses CT was 18.6±1.2 and 241.2 ±31.6 seconds accordingly. Reliable increasing of anticoagulant effect in 2-2.5 times in comparison with control wasn’t observed at doses administration 0.3-3 mg/kg. However plasma CT increased in 10-14 times in 5-240 minutes after dose administration 5 mg/kg.

We observed increase of rabbits plasma CT in assays aPTT, Heptest and plasma’s aIIa, aXa at intravenous administration to rabbits with increasing doses (0.3-10 mg/kg) of SC from Gossipium cotton with MW=10-30 kDa, SD=1.8, aIIa=130±12 U/mg and aXa=45±3 U/mg. Preventive and therapeutic doses of SC at intravenous administration have the range of 3-5 mg/kg. The half-time and half-life of AC increase with increasing its doses.
P179

SpectraMax® microplate readers: A complete solution for Transcreener® assays
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Molecular Devices (Wokingham, UK)

Transcreener® ADP2 Assays are homogenous assays with fluorescent readouts that enable the detection and screening of established drug targets including protein and lipid kinases, as well as emerging targets such as carbohydrate kinases, triphosphatases, heat shock proteins and other ATPases. The assay is based on the immunodetection of ADP. Three detection modes are offered to accommodate users’ needs and detection format preferences: fluorescence polarization (FP), time-resolved Forster-resonance energy transfer (TR-FRET), and fluorescence intensity (FI).
This poster illustrates that Molecular Devices’ SpectraMax® Microplate Readers have been validated for Transcreener ADP2 assays and enable users to choose the detection format they prefer. The SpectraMax® Paradigm® Modular Multi-Mode Reader also offers user upgradeability so that new detection capabilities can be added as users’ screening needs evolve.

P180

Novel therapeutic targets in Shwachman-Diamond syndrome
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A growing number of disorders (known as ribosomopathies) associated with bone marrow failure and leukaemia predisposition are caused by defects in ribosome biogenesis. One such disorder is Shwachman-Diamond syndrome (SDS), an autosomal recessive disorder that is caused by mutations in the SBDS (Shwachman-Bodian-Diamond syndrome) gene. By combining the power of yeast genetics with conditional deletion of the SBDS gene in the mouse, we have demonstrated that SBDS is required during a late cytoplasmic step in the maturation of the 60S ribosomal subunit. Specifically, SBDS cooperates with the GTPase elongation factor-like 1 (EFL1) to catalyse the release of eukaryotic initiation factor 6 (eIF6) from nascent 60S ribosomal subunits. The removal of eIF6 is a prerequisite for the formation of mature, actively translating ribosomes as eIF6 acts as a ribosome anti-association factor by physically blocking inter-subunit bridge formation. As disease mutations in SDS impair eIF6 release, we propose that modulating the affinity of eIF6 for the ribosome with small molecules may provide a novel therapeutic strategy in SDS. We have therefore initiated a Fragment-based lead discovery (FBLD) approach using NMR spectroscopy to develop compounds that bypass loss-of-function mutations in SBDS by inhibiting the interaction between eIF6 and the ribosome.
P181

A lipid based system for the oral delivery of macromolecules
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The development of formulations for the oral administration of peptide and protein drugs is rendered difficult for several reasons. Peptides may be degraded pre-systemically in the gastrointestinal tract and the low fraction reaching the intestinal wall is mostly poorly absorbed due to the high molecular weight or hydrophilicity of peptides. Lipid based delivery systems may offer a promising approach to overcome these problems by protection of peptides/proteins and enhancement of their uptake. Unfortunately, most common liposomal formulations are unstable in the gastro-intestinal tract and their performance as bio-enhancers is not sufficient for peptide delivery. In this work, we combine classical phospholipids with bacteria derived lipids to stabilize preformed vesicles and to improve the absorption of incorporated peptide and protein drugs. The properties of the different formulations were investigated by photon correlation spectroscopy, zeta-potential measurements, differential scanning calorimetry and lipid analysis by HPLC. Promising formulations were selected for in-vitro stability assays simulating different gastro-intestinal fluids. Compared to carrier systems consisting of pure phospholipids a significantly improved stability could be achieved in gastric and intestinal fluids when bacteria derived lipids were incorporated. Promising candidates were selected for in-vivo studies and the bioavailability of octreotide and human growth hormone – 2 model peptides – was determined in rats after oral administration. For both peptides a dramatic increase of absolute bioavailability (up to 300-fold compared to the peptide in solution) could be achieved. Thus, this system offers a promising tool to increase the oral bioavailability of peptides and proteins, which can otherwise only be administered parenterally.

P182

Analytical performance of a higher throughput multi-volume microplate accessory for microplate spectrophotometer platforms
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Accurate determination of molecular concentrations is a prerequisite to the use of purified biomolecules for a multitude of downstream applications. Quantification is routinely accomplished by spectrophotometric analysis. A novel microplate accessory is described here which incorporates the ability to measure up to forty-eight low-volume (2µL) samples for direct quantification and two vertical 1 cm pathlength cells, in a standard microplate sized format. Its use allows a researcher to quantify samples without dilution while also allowing more dilute samples to be read in a standard 1 cm pathlength format. The analytical performance of the accessory will be discussed including limit of detection, dynamic range and accuracy for the quantification of total protein and isolated nucleic acids.
P183

Automation of a generic fluorescence methyltransferase activity assay

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Epigenetic processes are attracting considerable attention in drug discovery as their fundamental roles in controlling normal cell development and contributing to disease states become more clearly defined. Methylation is known to be a ubiquitous covalent modification involved in regulation of a diverse range of biomolecules. Histone methyltransferases (HMTs) are of particular interest as drug targets as histone methylation is linked to some disease states, including a wide variety of cancer types. An HTS-ready, universal methyltransferase activity assay was recently developed based on fluorescent immunodetection of AMP, formed from the MT reaction product S-adenosylhomocysteine in a dual enzyme coupling step. Here we demonstrate automation of the assay in a 384-well format suitable for HTS while examining assay performance and pharmacology of a HMT.

P184

Aflibercept-mediated early angiogenic changes in aggressive B-cell lymphoma

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Diffuse large B-cell lymphoma is the most common and one of the most aggressive lymphomas in adults. The standard, R-CHOP-based treatment needs improvement. There has been recent interest in anti-angiogenic therapy, but its cellular effects in lymphoma are little known. In five aggressive B-cell lymphoma patients, we analyzed the microvessel and lymphoma cell changes after Aflibercept, an angiogenic inhibitor. Under ultrasonography, we performed two biopsies, one before any treatment and one two hours after Aflibercept, before R-CHOP. Using ultrasonography, immunohistochemistry, double immuno-fluorescent staining, and electron microscopy, we compared the early changes induced by Aflibercept to the early changes induced by R-CHOP in three control patients. We identified microvessel damage in the five patients treated with Aflibercept but not in the three patients treated with R-CHOP. Two hours after Aflibercept, microvessel damage was focal, with severely damaged microvessel sections close to normal ones in the same area; different stages of microvessel damage were concomitantly found, with an increase in relative necrosis area in three cases. There was no difference in necrosis or relative microvessel area after R-CHOP. For lymphoma cells, the two biotherapies induced similar changes, with increase in apoptosis but not in proliferation. We identified focal microvascular damage, necrosis, and apoptosis of lymphoma cells in aggressive B-cell lymphoma as soon as 2 hours after Aflibercept. This suggests that there is more than one mechanism associated with the early effect of anti-angiogenic therapy in lymphoma.
P185

Ruggedness testing including an evaluation of automation of a cell-based bioluminescent TNF alpha blocker bioassay

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TNF alpha blockers represent an important and successful class of protein drugs used in the treatment of several autoimmune diseases, including rheumatoid arthritis, psoriasis and Crohn’s disease. The biologics Remicade, Enbrel and Humira are the most commonly prescribed TNF alpha blockers on the market and generated close to US$13 billion in 2010. The success of these marketed biologics is driving the discovery of new versions and new indications. Considerable biosimilar development is also evident, as the drugs come off patent protection soon. Bioassays are indispensible tools in biologic drug development and commercialization. Precision and accuracy of the bioassay are all-important in both drug discovery and development, and in manufactured lot release, yet many bioassays suffer from drawbacks such as complexity and variability of results. We developed a simple, homogeneous and robust bioluminescent TNF alpha blocker bioassay based on quantification of caspase 3 activity. The bioassay uses U937 (human) cells which exhibit rapid response to TNF alpha. By developing and using U937 cells in single-use frozen, thaw-and-use format, we were able to remove variability arising from continuous cell culture. Part of bioassay development also includes analysis of bioassay ruggedness, in which the influence of external factors on bioassay test results is measured. Our study here describes such an analysis of the TNF alpha blocker bioassay using a 96-well plate format. We include evaluation of automation in the analysis. The assay steps of antibody titration and of cell and reagent dispensing were automated using a simple, yet robust liquid handler. Validation of the use of instrumentation with the assay chemistry was part of the study. Ruggedness variables evaluated were (i) manual and automated pipetting, (ii) bioassay plate used, (iii) luminometer used, and (iv) bioassay run. Assessment of ruggedness was based on (a) variability around RLUs obtained in plate uniformity tests using a single dose of TNFalpha blocker, and (b) variability of inter-assay EC50 and Hill-slope generated in full dose-response TNF alpha blocker titrations.
Live cell beating assay using human iPS-derived cardiomyocytes for evaluation of drug efficacy and toxicity

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A large percentage of new drugs fail in clinical studies due to cardiac toxicity. Therefore development of highly predicative in vitro assays suitable for HTS is extremely important for drug development. Human cardiomyocytes derived from stem cell sources can greatly accelerate the development of new chemical entities and improve drug safety by offering more clinically relevant cell-based models than those presently available. iCell® Cardiomyocytes are especially attractive because they express ion channels and demonstrate spontaneous mechanical and electrical activity similar to native cardiac cells. Here we demonstrate cell based assays for measuring the impact of pharmacological compounds on the rate of beating cardiomyocytes with different assay platforms.

We developed methods for the ImageXpress® Micro system that enable image acquisition and determination of beating rate from time-lapse images. One protocol captures mechanical movement of cells; a second monitors changing in intensity of Ca2+ fluxes synchronous with beating (Fig. 1). Both methods allow monitoring of drug impact on the beat rate and amplitude in 96 or 384 well formats. The system allows saving data as a video, presenting intensity curves, and automatic analysis of beat rates.

A second method uses the FLIPR® Tetra system to monitor changes in intracellular Ca2+ fluxes associated with cardiomyocyte contractions. The FLIPR system allows automatic addition of reagents and compounds, simultaneously with reading from 96 or 384 wells. Temporal response curves can be acquired in ~ 2 min per plate (Fig. 2). This method is suitable for HTS screens of compound libraries.

We have demonstrated use of both systems for two important applications: screening compounds for cardiac toxicity, and pre-clinical testing of potential cardiac drug candidates. Cardiac toxicity can cause arrhythmias or heart failure. We have shown dose-dependent atypical patterns caused by several cardiotoxic compounds including cisarpide and tetrodotoxin. Cardioactive compounds are used in clinical treatment of heart failure, arrhythmia or other cardiac diseases. We have demonstrated effects of several positive (epinephrine, etc.) and negative (A and B blockers) chronotropic agents on cardiac rates and determined EC50s (Fig. 3). Both imaging and FLIPR methods in combination with iCell Cardiomyocytes are well suited for safety testing and can be used to estimate efficacy and dosing of drug candidates prior to clinical studies.
Abstracts/Poster Presentations

P187

New method for automated development of live cell assays and bioprotocols will enable efficient automated extraction and sharing of specific and reliable biological knowledge

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Bioprotocols are carefully timed, multi-step applications of defined stimuli (including alterations of the microenvironment) that can move a population of living cells from an initial physiological state (e.g., State A) through a series of intermediate physiological states (e.g., States B-K) to a final, desired physiological state (e.g., State L). Bioprotocols can reproducibly "move", "guide", or "manipulate" living cells into a desired physiological state. Development of bioprotocols adds to biological knowledge and assists in the development of biological models and mathematical simulations of biological processes.

We present a patented method (US 7546210) called Visual-Servoing Optical Microscopy (VSOM). VSOM is a method for the efficient automated extraction, sharing, and re-use of biological knowledge. VSOM™ Systems (comprised of an automated optical microscope, automated peripherals, and VSOM™ Application Software with access to a VSOM experiment knowledgebase) can be used to develop physiological state interrogation assays (a type of bioprotocol) useful for monitoring specific changes in the physiological states of specific types of living cells. These assays are called VSOM™ Interrogation Assays (VIAs). VSOM™ Systems utilize one or more previously developed VIAs as follows. One or more VIAs are used to "sense" (in real time) the physiological effects that applied stimuli are having on individual living cells. A VSOM™ System: (i) applies stimuli, (ii) interrogates cells via intelligent selection and application of one or more VIAs, (iii) interprets changes in physiological states resulting from applied stimuli, (iv) evaluates progress towards completion of an assigned task, (v) makes automated (software-based) decisions about what action to take next, and (vi) executes additional automated actions as necessary. A VSOM™ System repeats steps (i-vi) in a closed-loop manner as it attempts to complete its assigned task in an autonomous manner. Examples of assigned tasks include the development of new VIAs and the development of new bioprotocols.

We will present experimental results obtained with a third generation VSOM™ System currently undergoing testing at a commercial assay development facility (Murigenics, Inc., Vallejo, CA). We will also present software (developed in collaboration with ImpulseLogic, Inc., San Ramon, CA) suitable for the generation of expert rule sets that facilitate automated decision-making during real-time VSOM experiments.
Tag-lite® is a powerful solution for the cellular screening and characterization of therapeutic antibodies targeting receptor tyrosine kinases

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The development of promising new therapeutic strategies, such as monoclonal antibodies directed against Receptor Tyrosine kinase (RTK) has become a challenge in the treatment of cancers. Since the end of 1990’s, biotherapeutic antibodies have represented an increasing group of FDA-approved medicines mainly in the oncology and immunology therapeutic areas. The selection of potent and selective MAbs is therefore the first critical step in the process running from drug discovery to clinical trial phases. Here we present an innovative, universal and simple assay which allows the screening and the characterization of antibodies targeting cell-surface receptors. This method is based on the recently launched Tag-lite platform which combines HTRF detection with the fluorescent labeling of receptors using the SNAP-Tag technology. On living cells, the binding of specific antibodies to a receptor of interest is detected through a time-resolved energy transfer occurring between a HTRF donor-labeled receptor and a secondary anti-species antibody labeled with a HTRF acceptor. This assay was successfully applied to monitoring antibody binding to a large set of RTK. By performing competition experiments with natural RTK ligands, our approach determines whether the previously selected antibodies were directed or not against the orthosteric binding site. Moreover, it enables complete antibody characterization by determining its affinity (KD) and pharmacokinetic (Koff) constants. We demonstrate that Tag-lite® is a powerful, easy to use and efficient technology to screen and characterize antibodies targeting RTK on living cells. This flexible and radioisotopic- free method enables the detection of different and specific binder classes, as well as the determination of their pharmacological features and functional responses.
Cisbio provides a full cell-based platform for the investigation of therapeutic antibodies and small molecules action mechanisms: an EGFR1 case study
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Deregulated EGF receptor is involved in many cancers, particularly in brain tumors, colorectal or non small cell lung cancers. One of the most popular therapeutic strategies targeting EGFR aims at inhibiting its catalytic activity borne by the intracellular part of the receptor. This can be achieved through the use of either small molecule inhibitors like erlotinib, which bind the ATP pocket site located in the c-terminus of EGFR, or by compounds able to interfere with the ligand binding on the EGFR extracellular domain. The latter strategy is mainly based on the use of blocking antibodies, like Cetuximab. Despite encouraging therapeutic benefits for certain patients, some others are refractory to treatment or even develop resistance, as has seen for trastuzumab-treated breast cancer patients. Therefore the development of a potent and selective medicine is closely associated with a good understanding of the action mechanism of the drug toward its target in a relevant biological context. With this objective in mind, we have developed a full set of binding and functional assays (Tag-lite® HTRF) on EGFR to investigate the antibody action mechanisms. For extracellular binding events, we have generated and validated a Red fluorescent EGF ligand in binding and competition assays, on frozen labeled SNAP-EGFR expressing cells. These tools have enabled us to determine such pharmacological features as the KD and Ki of reference compounds, eg cetuximab. In addition, we have developed EGFR autophosphorylation assays to monitor EGFR activity modulation in response to drug treatment, either in recombinant or in native cellular backgrounds. Induced EGFR phosphorylation is quantified in a HTRF sandwich assay using an anti-total EGFR and an anti-phospho specific antibody. Finally, during this technological development, we revealed an intriguing behavior in one antibody. These new integrated HTRF cell-based assays have shown their worth 1) in the upstream screening process dedicated to the identification of biotherapeutics in the extracellular domain and of ATP competitive small molecule inhibitors 2) in the downstream process, oriented to fine pharmacological characterization.
A new cellular binding platform to measure the Fc gamma receptor (FcgammaR) / hIgG interaction based on the Tag-lite® technology

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Immunoglobulin G1 (IgG1) Fc receptors play a critical role in linking IgG1 antibody-mediated immune responses with cellular effector functions. Multiple FcγRs exist, which differ in ligand affinity, cellular distribution, and effector function.

The focus of this study is on CD16a, a low affinity receptor for monomeric IgG which has been exhaustively described to recruit effect cells in Fc-dependent cellular cytotoxicity events. This receptor exists under 2 different polymorphism variants differing at position 158 (Val/Phe). CD16a (Val 158) displays a higher affinity for the Fc fragment compared to CD16a (Phe 158). This difference in affinity has been directly correlated to the effector functions mediated by the ADCC (Antibody-Dependent Cellular Cytotoxicity) mechanism.

The design of new generations of improved antibodies for immunotherapy should aim at Fc optimization to increase the engagement of activating FcgammaR present on the surface of tumor-infiltrating effector cell populations.

With this goal in mind, we have developed a cellular binding assay to efficiently and precisely measure the binding of the different subclasses of IgG with the CD16a variants. We also evaluated the effect of antibody fucosylation on the FcgammaR interaction.
The NanoWizard® 3 represents the latest in AFM technology. The new Vortis™ controller series uses the latest FPGA architecture to guarantee highest digital performance. Fast signal acquisition and control, advanced feedback and analysis are key components of a modular and ultra flexible controller. The high-speed data acquisition makes the controller perfect for time resolved force spectroscopy, higher harmonics imaging or high frequency cantilever use. Cantilever calibration by thermal noise method up to 3.25 MHz is unique. HyperDrive™ is a soft sample imaging technique in liquid which provides sub-nanometer lateral resolution with minimal tip-sample interactions and works with off-the-shelf cantilevers. This is made possible by the new optics and electronics of the NanoWizard® 3 AFM head, which gives the lowest noise level in the cantilever deflection detection system available commercially. The NanoWizard® 3 maximizes stability, performance and ease of handling for samples in fluid and for full integration with optical microscopy. This enables the simultaneous acquisition of high quality AFM images with optical imaging, under physiological conditions. This is critical as for biological samples, whether cells or molecules, extra information from optical signals can be vital to interpreting the AFM images. This can be fluorescent markers of certain components, for instance, or structural information from optical phase contrast or DIC. The unique DirectOverlay™ software for the JPK NanoWizard® systems uses the tip location to calibrate accurately the optical images and integrate them into the AFM software for direct AFM navigation. In addition, exact, quantitative correlation of AFM and optical features is possible. With the NanoWizard® 3, AFM imaging and force spectroscopy can also be combined simultaneously with high end optical techniques such as confocal LSM, FCS, FRET, FRAP or TIRF. The Vortis™ controller allows direct photon counting for single-molecule experiments and extension to near-field techniques. Flexibility is critical, as the future of modern AFM is increasingly in combining different measurement techniques and flexibility in different experiments. The range of electrical experiments such as CAFM, STM, Piezo Response, KPM, SCM etc. has been widely extended by different accessories as the boundaries between scientific disciplines provide some of the most interesting applications.
P192

Overcoming challenges in data management and analysis in antibody engineering
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The engineering of antibodies is a critical step in the biologics R&D process that allow giving birth to vastly improved antibody therapeutic candidates that lead to better treatments. This step is accomplished by a variety of means, along with rapidly evolving technologies, all resulting in a collection of new biomolecules that each need to be expressed and evaluated. From small laboratories to large pharma organizations, this poses serious data management and analysis challenges.

Here, we discuss some of these challenges and present a novel data management and analysis platform for Antibody Engineering that addresses these. Genedata Biologics is a software platform resulting from a multi-year development project, designed and implemented together with leading pharma and biotech players in Biologics R&D.

Specific topics include storing the diverse biomolecule formats, tracking biomolecule genealogy, minimizing business-critical data and material handling errors, integrated sequence and assay data analyses, and coping with high-throughput technologies.

The resulting platform provides consistent storage and referencing of all relevant biomolecules and biomaterials that arise from antibody engineering activities. Biomolecules include standard (i.e. Fab, IgG) and next generation Ab formats (e.g. bispecifics, tribodies); Abs from different species (e.g. IgNAR, VHH), antibodies in various stages of engineering (e.g. humanized, germlined, Fc-engineered), and antibody-drug conjugates.

Downstream entities supporting production, characterization and evaluation of these engineered biomolecules include vectors, expression constructs, cell lines, and expression batches, based on a structure following the intrinsic biologics R&D process. It incorporates various assay and analytics results derived from Biacore, FACS, ELISA, SEC, among others.

Tools include automated sequence and assay data processing pipelines, automated molecule registration, and vector verification, among others.

The main value of the platform lies in integration, automation, and standardization. It greatly facilitates laboratory workflows as well as material and data hand-overs in a division-of-labor environment. It allows the collection and analysis of all relevant experimental data generated along the biologics R&D process, and in particular in antibody engineering.
An integrated platform for data management and analysis in biologics expression optimization, analytics and BioProcess evaluation

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The development of optimized protein expression and purification processes is a key step in the successful production of biomaterials for further biologics therapeutic evaluations and larger-scale manufacture. Various disciplines and laboratories including molecular biology, cell line engineering, assays and analytics development and bioprocess development work in conjunction with each other to achieve a common goal. This requires a different research IT infrastructure approach that (1) recognizes the differences of the types of data produced while maintaining the key backbone of biologically relevant relationships and (2) provide appropriate tools that encourage unified data submission.

Here, we present a novel data management and analysis platform for Protein Production. Genedata Biologics is a software platform resulting from a multi-year development project, designed and implemented together with leading pharma and biotech players in Biologics R&D.

A central database enables consistent storage and referencing of all relevant biomolecules and biomaterials that arise from protein production activities.

To aid in biologics expression optimization, the system provides automated bulk in-silico cloning tools and supporting highly organized vector storage that allow the production and management of in-silico constructs to record all vector optimization and cell line development activities. This supports the automated bulk registration of expression constructs resulting from the combinatorial complexity of investigating different transcription promoters, resistance genes, leader peptides, tags, etc. The system also supports specialized DNA synthesis workflows verify optimized DNA inserts and resulting constructs.

To further aid in bioprocess development, there is comprehensive tracking of all evaluated vectors and expression systems, as well as expression and purification products. The system incorporates various assay and analytics results derived from, e.g. SEC, Biacore, FACS, ELISA, that allow evaluation of protein production process success. For example, statistical analysis tools and visualizers are available to investigate the correlation of yields with specific cell lines or vector properties.

Genedata Biologics (1) helps make the overall biologics discovery and development, and supporting manufacturing processes more efficient, (2) ensures a consistently high quality standard across research groups, and (3) enables informed and rational decision-making.
P194

Comparative study on antioxidative status of lung tissue to two different forms of curcumin
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Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is a natural compound with antiproliferative properties. This study was carried out to determine the antioxidant response of mice lung tissue to different levels (0 to 200µm) of poly and monomer forms of curcumin after 24h incubation. Result showed inhibition effects on ascorbate dehydrogenase, superoxide dismutase and glutathione reductase activities in a dose dependent manner of both drugs. However polymer form was more effective than monomer form and the inhibition reached to 2.5 and about 3 at 25µmol concentration. The inhibition effect was considerable for SOD, GR and ASP respectively. Total anti oxidant capacity fall down in the presence of these drugs with enhancement effect of polymer construction form. Malondialdehyde and carbonyl protein as lipid and protein oxidative damage biomarkers increased in exposure of lung tissue to both types of curcumin forms. However, there was markedly increase in these biomarkers; however, polymer form was more effective as compared with monomer form.

As conclusion, polymer form of curcumin as a new form was more effective to inhibit antioxidant enzymes and increase oxidative damage biomarkers. These characteristics may relate to their antibacterial and anti inflammatory effects.

Keywords: curcumin, antioxidant enzyme, oxidative biomarkers.

P195

Pandora's tox: multiple mechanisms of toxicity in one assay
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Toxicity is a key reason for drug attrition. Identifying potential toxicity at an early stage in drug discovery can reduce the likelihood of late stage failure, saving both time and development costs. High Content Screening (HCS) is a powerful, well validated and flexible technology for quantifying and understanding the changes that occur in cells when they are exposed to potentially toxic xenobiotics. HCS allows the analysis of multi-parametric indicators of cellular toxicity, detecting cell death and mechanisms of cell death, and covering a wide spectrum of cytopathological changes. CellCiphr™ combines HCS technology with a unique database comprised of marketed drugs, FDA and EPA-submitted and pharmaceuticals that failed in preclinical and clinical studies (Figure 1). CellCiphr™ HCS endpoints probe the interacting network of processes that are involved in cellular function and cellular toxic response, and have been chosen for their relevance in predicting aspects of drug-induced injury. The data generated for each compound lays the foundation for understanding the mechanisms of toxicity induced by any given compound, and the potential for different organs to undergo different responses. We present and discuss relative toxicity rankings of a selection of results for HepG2 cells and rat hepatocytes (Figure 2), and also explore relationships between different CellCiphr™ endpoints.
P196

An Ex vivo high content imaging assay for phospholipidotic potential
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Within the cell, defects in phospholipid metabolism and/or trafficking can lead to the accumulation of phospholipids in lysosomally derived multi-lamellar bodies. This pathological phenomenon is commonly found in multiple tissues in the liver, lung macrophages, eye and kidneys. Phospholipidotic liability is associated strongly with cationic amphiphilic compounds. Several in silico and high content analysis (HCA) in vitro techniques are now used within drug discovery processes to screen out phospholipidotic liability before the Lead Optimisation phase. During in vivo pre-clinical study, phospholipidosis is detected using pathology scoring and confirmed using electron microscopy. HCA is an established tool to observe the response of multiple biological parameters following pharmacological or molecular intervention, and has been used for some time to enrich the data generated by biological assays following primary screening. Increasing the complexity of the biological models and combining this with a use of HCA delivers screens with improved physiological relevance and can provide better early predictive power of compound effects.

Here we describe the development of an ex vivo assay using immunohistochemical techniques and Definiens image analysis to detect phospholipidosis in compound treated, formalin fixed, paraffin embedded rat liver sections. Lysosome associated membrane protein 2 (Lamp2) is a membrane glycoprotein with an important role in lysosomal function. Immunohistochemical labelling of the lysosome membrane Lamp2 is used to detect changes to these organelles in response to phospholipidotic events. An HCA based ex vivo assay of phospholipidotic liability will aid in understanding the translation of in vitro assays into the pre-clinical environment and deliver detailed quantitative data to increase turnaround of pre-clinical phospholipid detection.
Introduction: The aim of the study is to develop non-invasive methods to investigate the effect of drugs and chemicals in a human three-dimensional (3D) liver cell model for pharmacology and hepatotoxicity studies.

Methods: The human hepatoma cell line HepaRG, able to differentiate into hepatocyte-like cells, was cultured in a 3D model. Cells were seeded on porous plasma activated Polystyrene (PS) scaffolds to supply more physiological culture conditions (3D). HepaRG cells were seeded at 1x10^6 cells per polystyrene sponge and cultured for one week. To differentiate, cells were treated with 1% DMSO for 28 days. Differentiation of HepaRG cells was induced by dimethyl sulfoxide. The effect of Acetaminophen (APAP) and chemicals (Ethanol, Sodium chloride, Tween, Glucose) on differentiated 3D hepatic cells were tested using two different non-invasive methods: reflection coefficient (dB) and impedance Z (Omega).

Results: Different concentrations of APAP were able to induce a dose-dependent response to transmission measured by reflection coefficient S11 on differentiated HepaRG 3D cell model. Furthermore, the monitoring of impedance on HepaRG 3D cell model with drug and different chemicals showed a short-term effect (5min) and a long-term effect (2.5h, 5h, 24h).

Discussion: The Usage of non-invasive methods, like reflection coefficient (dB) determination or/and impedance spectroscopy might be suitable for monitoring of drugs and chemicals on cell behaviour of 3D cell culture models under physiological conditions.

Keywords: 3D cell model, liver, scaffold, non-invasive method, impedance, reflection coefficient
Towards a genome-wide screen unveiling host pathways hijacked by Bartonella henselae
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Revealing virtually all host factors involved in pathogen entry is a powerful approach to get a global mechanistic picture of the host pathways hijacked during infection. To reach this ambitious goal, the InfectX consortium uses genome-wide siRNA-based high-throughput screens, modelling, proteomics and statistical analyses to study the entry of various viruses and bacteria. In this project, we focus on the entry of Bartonella henselae into HeLa cells. B. henselae is a vascular tumor-inducing pathogen that invades human cells by recruiting the actin cytoskeleton to the entry site where it forms a unique structure called the invasome. Invasome formation depends on the translocation of Bartonella effector proteins into the host cell where they interfere with integrin beta1-dependent signalling cascades as well as the Rho GTPases Rac1 and Cdc42. To generate optimal setup for genome-wide screens for B. henselae-triggered invasome formation, we ought to improve the existing experimental procedure as well as the automated invasome detection. For this, semi-automated cell seeding, siRNA transfection, bacterial infection and imaging processes were implemented. Automated computational Invasome detection was optimized by the introduction of a novel pattern recognition algorithm, implemented as a CellProfiler module. True positive detection has been optimized using an image analysis tool named CellClassifier. To test these developed procedures, we performed a human kinome screen where we automatically quantified invasome abundance in infected cells. Our data show the strengths and caveats of our setup and highlight the importance of the kinome screen as a pilot screen before proceeding to a genome-wide screen; especially in cases where the biological system and its readout are complex as B. henselae infection and the invasome detection are.
Screening of phenotypic genes and pathway modularization through whole-genome profiling, RNA-seq, and synthetic engineering of a family of radiation resistant organisms

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We have studied members of the radiation-resistant genus Deinococcus in an effort to understand the molecular basis for radiation resistance, with our eventual goal to create modular, transplantable cassettes of genetic components that confer radioresistance. We have sequenced the genomes of ten members of this genus and conducted RNA-seq transcriptional profiling over a time course of radiation exposure. With this information, we were able to derive information about the relationship of each organism to one another, define gene families that are upregulated after exposure to radiation, and isolate candidate genes most likely conferring radiation resistance for cloning into model organisms for further analysis. We have "biobricked" over 40 of the top candidate genes and here report initial progress in transplanting these targets into other organisms and the best combinations for recapitulating the phenotype of radioresistance.

We used classical RNA-seq data analysis tools such as TopHat and Cufflinks to map reads to and count transcripts, but have also employed Frequency Feature Profiling, an alignment-free genome comparison tool, to rapidly characterize new species and their response to radiation. Because this method does not require entire genomes to be aligned to estimate distances and gene families, we are not biased against large genomic rearrangements and repeats that are typically difficult to align. The advantage of our method is the ability to define significant genes without knowing the function, or even the rough ontology, of such genes. In our current study, the species with the most annotated genome has less than 50% of the genes identified with known function, yet we can account for the majority of its groupings and sequence clusters. Together, these methods show the promise of NGS tools applied to specific phenotypes that can be engineered with synthetic biology.
Abstracts/Poster Presentations

P200

A simple, robust automated multiplexed cell-based assay process for the assessment of mitochondrial function
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Mitochondrial perturbation is a common mechanism of drug-induced toxicity. Recent advances in mitochondrial study have revealed that numerous drugs that were withdrawn from the market demonstrate strong mitochondrial impairment in the liver or the heart. These include troglitazone (Rezulin), cerivastatin (Baycol), and nefazodone (Serzone). Therefore it is becoming increasingly important to focus on earlier identification of lead compounds that impact mitochondrial function during the discovery phase of drug development.

Here we demonstrate the utility of a multiplexed assay to assess cell membrane integrity changes (cytotoxicity), as well as mitochondrial function. Cytotoxicity is first assessed by measuring the presence or absence of a distinct protease activity associated with necrosis using a fluorogenic peptide substrate (bis-AAF-R110) to measure “dead cell protease activity”. The substrate cannot cross the intact membrane of live cells and therefore gives no signal with viable cells. Mitochondrial function is then measured by adding an ATP detection reagent, resulting in cell lysis and generation of a luminescent signal proportional to the amount of ATP present. The combination of the two readouts allows for distinguishing between compounds that exhibit mitochondrial toxicity and those that inhibit ATP production due to cytotoxic effects, such as primary necrosis. The entire process was automated, including cell dispense, compound titration and transfer, and reagent addition. Validation and pharmacology data demonstrate the utility of this automated, multiplexed assay to rapidly profile, compounds for effects on mitochondrial function.

P201

Effects of sample preparation on transcriptome sequencing
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High-throughput transcriptome sequencing has been herald as a ‘revolutionary tool for transcriptomics’. But beyond the hype and promise of gene discovery and absolute transcript quantification lies significant library construction biases. Here, we analyzed the effects of sample preparation methodology on transcriptome sequencing, utilizing synthetic RNA spike-ins from the External RNA Control Consortium. We showed that downstream discoveries and determinations are, in part, a function of library construction method, across different modalities of measurement (exon and gene expression, isoform detection, novel transcript identification, and variant calling). Specifically, we compared Epicentre ScriptSeq against Illumina TruSeq RNA-seq, amplification versus amplification-free libraries, and ribosomal reduction contra polyadenylate selection. We demonstrated the utility of synthetic RNA spike-ins to correct for some of the biases between different library constructions, enabling more concordant measurements. These results can inform future transcriptome studies on an individual basis, and highlight the importance of uniform library methods within a study for comparability, as well as the benefit of multiple methods for fuller characterization of a transcriptome.
**Abstracts/Poster Presentations**

**P202**

**Next gen cell engineering: The jump-in™ cell engineering platform**

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*Life Technologies (Madison, US)*

The stable introduction of genetic material into cell lines has become one of the most widely used methods since its introduction more than 3 decades ago. Cell engineering has become an indispensable tool for a wide range of areas in Life Science, such as cell biology, physiology, in vivo modeling, drug discovery and bioproduction.

However, most cell engineering strategies currently employed for the generation of stable cell lines require substantial commitment in respect to time, FTE hours and resources. In addition, most cell lines are generated by relying on random integration of genetic material into the genome, which typically results in a wide range of expression levels within a cell population. For these reasons the frequent generation of engineered cell lines is in general cumbersome and unpredictable.

Life Technologies recently developed and validated the Jump-In™ cell engineering platform, a novel gene targeting technology based on R4 integrase mediated site specific homologous recombination. This technology allows the targeted integration of genetic material into a specific pre-engineered site, which by design reduces the effort required for generation of stable cell lines compared to standard methods. Isogenic expression from a defined genomic locus provides the ideal solution for comparative analysis of gene families, isotypes or orthologs. This technology was applied to two widely used cellular backgrounds, HEK293 and CHO. We used these cellular backgrounds as case studies to demonstrate how this technology can be applied to rapidly generate engineered cell lines for a variety of cell based assay applications, including reporter assays for pathway analysis, GPCR activity assays and analysis of post-translational modifications.

In summary, Jump-In™ technology enables researchers to accelerate the development of cellular assays by greatly reducing the amount of time and resources required for the upfront cell engineering process.

**P203**

**Stimulation of the amino acid transporter SLC6A19 by JAK2**

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JAK2 (Janus kinase-2) is expressed in tumor cells and contributes to the survival of those cells. Moreover, the gain of function mutation V617FJAK2 mutant is found in the majority of myeloproliferative diseases. Tumor cell survival depends on availability of amino acids. Concentrative cellular amino acid uptake is in part accomplished by Na+ coupled amino acid transport through SLC6A19 (B(0)AT). The present study thus explored whether JAK2 activates SLC6A19. To this end, SLC6A19 was expressed in Xenopus oocytes with or without wild type JAK2, V617FJAK2 or inactive K882EJAK2 and electrogenic amino acid transport determined by dual electrode voltage clamp. In SLC6A19-expressing oocytes but not in oocytes injected with water or JAK2 alone, the addition of glutamine (2 mM) to the bath generated a current (Ig), which was significantly increased following coexpression of JAK2 or V617FJAK2, but not by coexpression of K882EJAK2. Coexpression of JAK2 enhanced the maximal transport rate without significantly modifying the affinity of the carrier. Exposure of the oocytes to the JAK2 inhibitor AG490 (40 µM) resulted in a gradual decline of Ig. According to chemiluminescence JAK2 increased enhanced the carrier protein abundance in the cell membrane. The decline of Ig following inhibition of carrier insertion by brefeldin A (5 µM) was similar in the absence and presence of JAK2 indicating that JAK2 stimulates carrier insertion into rather than inhibiting carrier retrieval from the cell membrane. In conclusion, JAK2 up-regulates SLC6A19 activity which may foster amino acid uptake into JAK2 expressing tumor cells.
Recombinant human interferon gamma (gamma immunex) in treatment of atopic dermatitis

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Background: Atopic dermatitis (AD) is a chronic, inflammatory skin disease which is characterized by severe pruritus and affects patients' quality of life. In recent years gamma interferon (IFN-gamma) has been accepted as a novel treatment for severe AD, however its mechanism of action is not clearly identified. Present study evaluated the effect of recombinant human interferon gamma (rIFN-gamma: Gamma Immunex, Exir Pharmaceutical Company, Iran) on severity of AD (SCORAD), dermatology life quality index (DLQI) as well as serum levels of IL-4, IgE and IL-6 in AD patients.

Methods: twenty AD patients were entered study in Baqiyatallah outpatient clinics and received rIFN-gamma (50 µg/m2 body area, 3 times per week, subcutaneously) for one month. SCORAD and DLQI assessed at beginning and end of treatment period. IL-4, IL-6 and IgE were measured in blood samples before and after one month treatment with rIFN-gamma.

Results: DLQI mean value before treatment was 20.80±3.95, which decreased to 8.20±2.14 after treatment (p<0.001). SCORAD-A (percentile of the body surface involved in AD), SCORAD-B (the severity of clinical features) and SCORAD-C (patients' scaling of itching and somnolence) significantly decreased after treatment (p<0.001). Total SCORAD at the end of treatment period was less than basal value (27.83±8.48 vs. 70.04±8.48; p<0.001). Treatment with rIFN-gamma decreased serum levels of IL-4 and IL-6 (p<0.001), but IgE remained unchanged.

Conclusions: results suggested the controlling effect of rIFN-gamma treatment on clinical symptoms of AD, which involves suppression of IL-4 but not IgE production.

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In each set of variables, 1= before treatment and 2= after treatment
P205

HTS assays for the identification of ligand-biased S1P1 receptor agonists for autoimmune diseases
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(Stevenage, UK)

This poster describes the first reported agonist 1536 High Throughput Screens on the human endothelial
differentiation Sphingolipid receptor 1 (S1P1) using both yeast reporter gene and TANGO beta-Arrestin
recruitment technology.
Recent reports have show the beta-Arrestin pathway selective activation as a new way for finding new
chemical structures as potential drugs. Therefore a broader and “multi-readout” approach to early stage drug
discovery efforts may open a wider breadth of possible chemical starting points.
Correlating activity data from both assays gives a subjective view on the feasibility and efficiency of a dual HTS
approach for ‘ligand-bias’ drug discovery. We conclude with recommendations for tackling future targets where
such pharmacological ligand profiles are of interest.

P206

Identification of compounds preventing statin-induced toxicity using mesodermal progenies derived
from human ES cells
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I-STEM (Evry, FR)

The mevalonate pathway or HMG-CoA reductase pathway is an important cellular metabolic pathway present
in all higher eukaryotes and many bacteria. It is important for the production of dimethylallyl pyrophosphate
(DMAPP) and isopentenyl pyrophosphate (IPP), which serve as the basis for the biosynthesis of molecules
used in processes as diverse as terpenoid synthesis, protein prenylation, cell membrane maintenance,
hormones, protein anchoring, and N-glycosylation. It is also a part of steroid biosynthesis.
Statins are a class of HMG-CoA reductase inhibitors used to lower serum cholesterol levels and effectively
prevent cardiovascular events. However, almost 15% of statin recipients developed adverse effects on skeletal
muscles, ranging from slight myalgia to severe rhabdomyolysis, including reduction of muscle contractility. One
of our research objectives is to identify drug capable of preventing statin specific muscle toxicity and more
largely drugs that can delay muscle atrophy.
We have developed a protocol for the reliable generation of mesodermal stem cells (MSCs) from human
pluripotent stem cells (embryonic or induced). Apart from their homogeneous, self-renewal, and bankable
properties, MSCs show significant dose response sensitivity to the statin toxicity, that can be prevented by a
mevalonate treatment. We validated the luminescent cell viability assay CellTiter-Glo (Promega) on the MSCs
model in 96 and 384 wells microplates.
Here we report a high throughput assay for the detection of small molecules that prevent statin-induced
toxicity. MSCs were pre-treated with 7000 molecules (Prestwick and Chem-X Infinity library) for 24h. They
were then treated with a constant concentration of simvastatine for 72h, before the viability test was performed.
Among the 30 primary hits that were retested, one compound from the Prestwick library, and two chemical
families present in the Chem-X Infinity library were identified as rescuers of the statin toxicity. They all show
dose response effect on MSC treated with simvastatin, EC50 values between 1-5µM, and a highest effect
comparable to the one induced by mevalonate.
P207

Measurement of antibody drug kinetics for native antigens on whole cells
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More recently, awareness is growing that affinity and binding kinetics of biopharmaceuticals, e.g., therapeutic antibodies, need to be tested on native membrane antigens, however until now the suitable tools have not been available. It would be desirable to study binding of biopharmaceuticals to membrane protein targets in their native environment within whole cells or vesicles, in order to avoid artifacts from purification and solubilization steps.

A label-free biosensor based on surface acoustic wave (SAW) technology is now used for the first time assessing antibody binding kinetics to their corresponding native membrane antigens on whole cells, in this example MCF-7 breast cancer cells.

Epithelial MCF-7 cancer cells in suspension were loaded onto a SAW-sensorchip through standard autosampler and fluidics on the SAW-biosensor device (sam®5, SAW-Instruments, Bonn, DE) that are used for injecting samples. For immobilization of cells, the dimerization of cell-surface E-Cadherin with E-Cadherin monomers presented as primary ligand on the sensorchip surface were used. Monomeric E-Cadherin was coupled by means of a self-assembled monolayer with Carboxyl-groups (SAM-COOH). Alternative ways of immobilizing cells involve the use of antibodies against cell surface antigens on the chip as primary ligands which conveys even higher sensitivity.

The bound cancer cells were used as target for characterization of antibody affinity against different surface antigens used as drug targets. The chip surface can be regenerated for repeated cell binding, enabling kinetic evaluation and comparative measurements.

The SAW biosensor can measure binding of antibodies to cell surface receptors and can clearly distinguish these based on abundance and specificity. Kinetics can then be evaluated as well as structural changes. Examples showing different antibody-membrane antigen pairs are presented and compared to the solubilized antigens.

P208

The pipeline pilot NGS collection: The application of graphical data pipelining to NGS data analysis
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Discussions of the NGS domain repeatedly focus on the exceptional volume of data generated by these technologies. However, a less frequently acknowledged but perhaps more significant challenge is posed by the rapid evolution of available algorithms and attendant computational best practices, and the need for techniques tailored to specific research goals across a wide swath of disciplines, including oncology, infectious disease and agricultural research. We discuss how Pipeline Pilot and the NGS Collection provide the agility, versatility, and scalability needed by computational teams to address these fundamental challenges.
**P209**

**Advanced methods for genome-wide methylation detection reveal novel epigenetic dynamics of leukemia**  
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DNA methylation is a critical epigenetic modification that regulates gene expression and mediates cellular differentiation states. Recently, methylation signatures have been utilized to stratify different sub-types of leukemia, which indicate their important relevance for clinical applications. Current high-throughput methods for examination of the epigenome, such as reduced representation bisulfite sequencing (RRBS), ameliorate the cost of sequencing after bisulfite treatment but limit the number of regions that can be examined. Here, we report biochemical and bioinformatics improvements in the RRBS method, creating a new technique called enhanced RRBS (eRRBS). We show extremely high reproducibility with this technique (R>0.96), even with very-low input levels (5ng vs. 1ug). Our method also shows an improved conversion efficiency of the non-methylated cytosines (>99.5%). We also find an increased number (11-61%) of CpG sites that can be detected and quantified in all areas: CpG islands, promoters, CpG shores, introns, and exons. Moreover, we can use this method to effectively determine differentially methylated regions (DMRs) and shores (DMSs) that reveal new regions of the genome that are altered in acute lymphoblastic leukemia.

**P210**

**Screening for novel compounds affecting chronological lifespan in S. pombe**  
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HTW Berlin (Berlin, DE)

The identification of compounds that affect lifespan and the characterization of their molecular targets is an important aspect of aging research. These studies lead to the discovery of new compounds and aging pathways and establish the foundation for treatments that might delay cellular aging or shorten lifespan of cancer cells. Simple organisms such as yeast have contributed a large part to the molecular fundamentals of aging. Studies in yeast elucidated several important molecular aging mechanisms and discovered novel longevity genes that proved to be conserved also in multicellular eukaryotes. The aim of the project is to identify compounds that affect cellular aging of the model organism S.pombe. Concretely, we are seeking to identify small molecules that affect the chronological lifespan of the yeast and are thus candidates for substances that influence the aging of post-mitotic cells. For this purpose, we established a cell based high-throughput screening assay which enables the detection of small molecules from large natural compound libraries that delay or accelerate the aging of stationary phase cells. In a next step, we will characterize the molecular targets of the identified compounds. For this purpose, we will narrow down their site of action by a yeast three hybrid screen, genetic and biochemical analyses. In first screens we have identified several compounds that affect chronological lifespan in S.pombe. Preliminary results will be presented.
P211

Growth of Campylobacter using BMG LABTECH’s FLUOstar Omega equipped with ACU
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Campylobacter are Gram negative microaerophilic organisms which are a common commensal of mammals and birds^1 and are one of the most frequently isolated causative agents of human bacterial enteritis worldwide^2. Infection in humans is frequently associated with the consumption of under cooked poultry^3 and is generally self limiting^1 though it can in a small number of cases result in severe complications such as Guillain-Barre syndrome^4. Though similar spiral shaped organisms had been observed in stool samples from enteritis patients for many years, Campylobacter jejuni was only properly linked with disease in the 1970's when new culture methodologies were developed.5 C. jejuni has quite stringent requirements for growth, namely; a rich nutrient broth, temperatures of 37-420C, and microaerophilic conditions, i.e. oxygen reduced to 5-8% and carbon dioxide elevated to 10%. It has been suggested that these conditions closely mimic those in the intestine of avian hosts to which the bacteria have become adapted in the wild. Described here is a comparison of C. jejuni growth studies performed using the traditional method of tubes shaking in a VAIN with that of a study completed in a 96 well microplate format using a FLUOstar Omega plate reader equipped with BMG LABTECH’s Atmospheric Control Unit (ACU) to independently control oxygen and carbon dioxide.