Room 1 & 2

Poster #1: Capacity limits of ASGPR mediated liver targeting and insight on dosing optimization

Charlotte Bon, Thomas Hofer, Mark R. Davies, Thierry Lavé, Ben-Fillippo Krippendorff
Hoffmann-La Roche

Efficient delivery of therapeutic molecules into the targeted tissues and cells remains a limiting factor for antibody based therapies and the wider use of new therapeutic technologies such as nucleic acid therapeutics and CRISPR mediated gene editing. The abundant cell surface asialoglycoprotein receptor (ASGPR) is a highly selective receptor found on hepatocytes that potentially can be exploited as a selective shuttle for delivery. Hence, various nucleic acid therapeutics that bind to this receptor are already in clinical development. However, this receptor mediated delivery mechanism can be saturated which will likely result in a reduced selectivity for the liver and therefore increase the likelihood for systemic adverse effects. Therefore, when aiming to utilize this mechanism, it is important to optimize both the administration protocol and the molecular properties. We here present a study using a novel ASGPR-targeted antibody aimed to estimate ASGPR expression, turnover and internalization rates in-vivo in mice. Using pharmacokinetic data (intravenous and subcutaneous dosing) and an in-silico target mediated drug disposition (TMDD) model we estimate an ASGPR expression level of 1.8 million molecules per hepatocyte. The half-life of the degradation of the receptor was found to be equal to 15 hours and the formed ligand-receptor complex is internalized with a half-life of 5 days. A biodistribution study was performed and confirmed the accuracy of the TMDD model predictions. The kinetics of the ASGPR shows that a saturation of the shuttle at therapeutic concentrations is possible, however simulation allows the dosing schedule to be optimized. This optimization can also be extended to different modalities, to incorporate the different pharmacokinetic properties (non ASGPR related). The developed TMDD model can be used to support the development of therapies that use the ASGPR as a shuttle into hepatocytes

Poster #2: Ligand binding kinetics for the H1 receptor-- properties of long binders

R. Bosma, S. Kuhne, A.J. Kooistra, I.J.P. de Esch, C. de Graaf, H.F. Vischer, R. Leurs
VU University Amsterdam

Ligand-receptor binding kinetics are considered better predictors then affinity for in vivo effects and could therefore improve drug design. Binding kinetics for H1 antihistamines were analyzed with radioligand binding studies. Data suggested that second generation antihistamines have longer complex lifetimes with the receptor then first generation antihistamines implying a possible relation between ligand-receptor binding kinetics and success in the clinic. Mutational analysis and analysis of structurally related compounds help to pinpoint binding sites and structural differences between H1 ligands which determine the kinetic profile of ligand binding to the H1 receptor. Results show how the different facets, ranging from radioligand binding to tailored in silico experiments, are a powerful way to mechanistically explain how GPCR ligands differ in binding kinetics for their receptor, paving the way for optimized ligand design.

Poster #3: Targeting protein-protein interactions with low molecular weight fragments

B.Carrington, M.Westwood, R. Taylor
UCB Celltech

In the quest for small molecule drug candidates there have been a number of different approaches used including: existing lead or drugs, natural products, high-throughput screening for drug discovery. More recently fragment-based screening has become established as an upfront screening approach rather than when other approaches have failed. This has led to screening >15 targets, the majority of which are PPI, against a 16000 fragment library. In this talk, I will discuss the development of the sensitive and robust SPR-based assays for small molecular fragment hit identification. I will give an overview of the key steps in this process, and main learning drawn from the screening campaigns undertaken at UCB.

Poster #4: Characterization of ERK2 allosteric inhibitors.

Juan Castro, Sharna Rich, Charlotte East, Valerio Verdini, Joe Coyle, Marc O'reilly, Caroline Richardson, Tom Heightman , Joanne Munck, Nicola Wallis.
ASTEX Pharmaceuticals

(ERK) are serine/threonine kinases that comprise a key component of the MAPK signalling pathway, which regulates cell survival and proliferation and is frequently upregulated in cancer. The MAPK signalling cascade is composed of RAS, RAF, MEK and ERK and is activated by extracellular stimulation of cell-surface receptor tyrosine kinases. Once activated, ERK phosphorylates more than 200 different nuclear and cytoplasmic substrates such as RSK, c-myc and ELK1. The MAPK pathway inhibition has been clinically validated by BRAF and MEK inhibitors, which are approved for the treatment of $BRAF^{V600E}$-mutant melanoma. However, these inhibitors have a short-lived clinical activity due to acquired drug resistance. One common characteristic of the RAF or MEK inhibitor resistance is the re-activation of ERK signalling, which drives cell survival and proliferation even in the presence of BRAF and MEK inhibitors. As ERK is the primary downstream effector of the MAPK pathway, the direct targeting of ERK is an attractive therapeutic approach to overcoming the limitations of RAF or MEK inhibitors.
Targeting allosteric sites of kinases may affect alternative signalling pathways to ATP competitive site inhibitors. It has been observed that the ERK inhibitor SCH772984 not only acts as an ATP competitive inhibitor of ERK catalytic activity, but also modulates the phosphorylation levels of ERK. The mechanism of this modulation is not completely understood and its characterization could help to design ERK inhibitors that would not only inhibit ERK catalytic activity by binding to the ATP site but additionally prevent the phosphorylation of ERK by MEK, which could overcome the increase in pERK levels commonly observed in response to MAPK inhibition. Using MEK-ERK biochemical cascade experiments and kinetic characterization of several ERK inhibitors, we confirmed the molecular basis for this phospho-ERK2 modulation in which the compounds inhibit the phosphorylation of ERK by MEK without directly inhibiting MEK. Additionally, we verified that this modulation is not driven by the slow inhibition properties of the compounds.

Poster #5: Structural and mechanistic basis of differentiated inhibitors of the acute pancreatitis target Kynurenine-3-monooxygenase

Chun-wa Chung, Jonathan P. Hutchinson, Paul Rowland, Mark Taylor, Erica M. Christodoulou, Carl Haslam, Clare M. Hobbs Duncan Holmes, Paul Homes, John Liddle, Damian J. Mole, Iain Uings, Ann Walker, Scott P. Webster, Christopher G. Mowat,
GlaxoSmithKline R&D

Kynurenine-3-monooxygenase (KMO) is a key FAD-dependent enzyme of tryptophan metabolism. In animal models, KMO inhibition has shown benefit in neurodegenerative disease such as Huntington's and Alzheimer's. Most recently it has been identified as a target for Acute Pancreatitis multiple organ dysfunction syndrome (AP-MODS); a devastating inflammatory condition with a mortality rate in excess of 20%.
Here we report and dissect the molecular mechanism of action of three classes of KMO inhibitors with differentiated binding modes and kinetics. Two novel inhibitor classes trap the catalytic flavin in a previously unobserved tilting conformation. This correlates with picomolar affinities, increased residence times and an absence of the peroxide production seen with previous substrate site inhibitors. These structural and mechanistic insights culminated in GSK065(C1) and GSK366(C2), molecules suitable for preclinical evaluation. Moreover, revising the repertoire of flavin dynamics in this enzyme class offers exciting new opportunities for inhibitor design.

Poster #6: Kinetic profiling of positive allosteric modulators of the metabotropic glutamate 2 receptor

Maarten L.J. Doornbos, Jose María Cid, Jordi Haubrich, Alexandro Nunes, Jasper W. van de Sande, Sophie Vermond, Hilde Lavreysen, Andrés A. Trabanco, Laura H. Heitman, Gary Tresadern, Adriaan P. IJzerman.
Leiden University

Allosteric modulation of the metabotropic glutamate 2 (mGlu2) receptor, a class C G protein-coupled receptor (GPCR), is considered a promising approach for treatment of various psychiatric and neurological disorders, like schizophrenia.
In recent years receptor binding kinetics is improving our understanding of the translational value of in vitro experiments to the clinic. Therefore, we aimed to evaluate this concept for the mGlu2 receptor, by studying a library of positive allosteric

modulators (PAMs).
A structurally diverse selection of both novel and reference mGlu2 PAMs were initially studied followed by a more detailed analysis of a novel series of 7-aryl-1,2,4-triazolo[4,3-a]pyridines. Full characterization of affinity and kinetics enabled evaluation of structure-affinity relationships (SAR) and structure-kinetics relationships (SKR). The mGlu2 PAMs showed various kinetic profiles; values for the association rate constant kon ranged over three orders of magnitude, whereas residence time (RT) values were within a smaller, 10-fold range. Further analysis revealed that kon was linearly correlated to affinity, while this was not the case for RT. Evaluation of the shortest, a medium and the longest RT compound in the whole cell label-free xCELLigence® assay revealed that the longest RT compound displayed a functional effect that was not easily washed out, whereas this was smaller or the other two compounds.
These results show that affinity-only driven selection would have resulted in mGlu2 PAMs with high values for kon, but not necessarily with optimized RT which over the entire dataset did not track with affinity. This further emphasizes the need to study target binding kinetics in drug discovery.

Poster #7: Inhibition of the fascin-actin interaction using a multidisciplinary fragment-based screening approach

Andrea Gohlke, Gillian Goodwin, Justin Bower and Alexander Schuettelkopf
CRUK Beatson institute

Fascin 1 is an actin bundling protein that cross-links filamentous actin into parallel bundles leading to the build-up of cellular protrusions (eg lamellipodia or filopodia) used during cell migration. Its expression is low in normal epithelia but is dramatically increased in a variety of metastatic cancers. Here, it has been reported to contribute to the formation of actin-rich finger-like protrusions (invadopodia) which are involved in the degradation of the extracellular matrix of cancer cell lines. Fascin 1 is currently used as an independent prognostic indicator of poor clinical outcome and its knockdown has been shown to reduce tumor cell invasion, proposing it as a valid drug target.
However, as the detailed interaction of fascin 1 with its binding partner actin is still unclear it remains a highly challenging target. Using a multidisciplinary approach of fragment-based screening, X-ray crystallography, computational chemistry, biophysical, biochemical and cell based assays, we have been able to identify and optimize novel fascin 1 inhibitors of sub-micromolar affinity.
Initially, from a fragment based screen using surface plasmon resonance screen (SPR) 53 hits were identified binding to fascin 1. Using these fragments in a co-crystallography study we were able to distinguish four independent ligand binding sites. Focussing on a deeply enclosed pocket between fascin domains 1 and 2, a number of fragment hits and analogues were found to bind and open a channel towards the surface of the protein. Virtual screening based on these early compounds finally identified a compound which binds with an SPR Kd of 29.7µM and an IC50 of 50 µM as revealed in a functional biochemical screening assay that measures fascin-mediated actin bundling. After structure-based optimization and co-crystallography compounds with > 100 fold increase in both binding affinity and functional activity were identified. Following this, we are currently working on the optimisation of these compounds exhibiting sub-micromolar affinity using isothermal calorimetry and fluorescence polarisation as additional biophysical methods. Using Saturation Transfer Difference (STD)-NMR we are also obtaining information about the binding epitope of our compounds confirming our crystal structures in solution.
Finding a strong inhibitor of the fascin-actin interaction will ultimately help to decrease the metastasis of various severe tumour forms

Poster #8: Assessment of coagulation Factor Xa inhibitors in functional, binding and in-vitro systems

Sheraz Gul, Gesa Witt, Chun-wa Chung, & Dymphy Huntjens
Fraunhofer

Coagulation Factor Xa is a serine protease of therapeutic importance for which a number of inhibitors have been approved for clinical use (e.g. fondaparinux, rivaroxaban, apixaban and edoxaban). These Factor Xa inhibitors are anticoagulants that block Factor Xa activity, thus preventing blood clots to develop.
Within the scope of the K4DD project encompassing WP1 (Molecular understanding), WP2 (Screening approaches) and WP3 (translation to in-vivo kinetics and drug effects), we have evaluated 55 compounds provided by the EFPIA partners (Bayer, Merck, Roche, and Sanofi) in target based functional (fluorescence assay using a peptide substrate) and binding (SPR) assays

and determined their potencies, kon and koff (and thus KD) values. A subset of compounds with diverse binding kinetic profiles were selected and evaluated in analogous assays for rabbit and rat coagulation Factor Xa and molecular simulations were used to explore binding mechanism.
The in-vitro results have correlated well in terms of rank order between the three species (human, rabbit and rat) for the pIC50 and KD values of the compounds at different temperatures. The analysis of the in-vitro drug effects for the selected compounds is currently ongoing which includes determination of the prothrombin time (PT) and activated partial thromboplastin time (aPTT) in plasma coagulation assays. The obtained data will further be used to predict the in-vivo anticoagulation effects using mathematical modelling.

Poster #9: Physiologically-based modeling approach to predict dopamine D2 receptor occupancy of antipsychotics in brain: translation from rat to human using PK-Sim/MoBi

Yin-Cheong Wong, Elizabeth C.M. de Lange
Leiden Academic Centre for Drug Research, Leiden University

Backgrounds: Receptor occupancy (RO) is a translational biomarker for assessing drug efficacy and safety, which could facilitate both dose optimization of existing drugs and evaluation of drug candidates.
Aims: We aimed to apply a physiologically-based pharmacokinetic (PBPK) modeling approach to predict the brain dopamine D2 RO of antipsychotics.
Methods: Clozapine and risperidone were modeled together with their active metabolites, norclozapine and paliperidone, using the PBPK models in PK-Sim® and MoBi®. These compounds have different binding kinetics (association and dissociation rates) to D2. The plasma PK models of rat and then human were constructed. Drug-D2 binding kinetics was incorporated to the plasma PK model to predict RO based on the simulated concentration in brain extracellular fluid.
Results: From an extensive literature search and data extraction, 29 and 25 datasets on plasma PK and RO, respectively, were prepared and compared with the model prediction. The rat PK model and RO model were first developed, which accurately predicted the observed data. System-specific parameters including the expression and transport kinetics of P-glycoprotein transporter at the blood-brain barrier were then adapted when translating the rat model into human model. The human model captured the plasma PK and the general trend of RO despite the large inter-individual and inter-study variability. The developed human model was successfully applied to predict the PK and RO changes observed after risperidone dose reduction in a clinical trial in schizophrenic patients.
Conclusion: Based on prior preclinical knowledge from rats, a human PBPK model was developed that could reasonably predict the plasma PK and RO of antipsychotics.

Poster #10: A covalent antagonist for the human adenosine A2A receptor

Xue Yang, Dong Guo, Thomas J.M. Michiels, Eelke B. Lenselink, Laura Heitman, Julien Louvel and Ad P. IJzerman
Leiden University

In a radioligand binding assay LUF7445 acted as a potent antagonist, with an apparent affinity for the hA2A receptor in the nanomolar range. Its apparent affinity increased with longer incubation time, suggesting an increasing level of covalent binding over time. An in silico A2A-structure-based docking model was used to study the binding mode of LUF7445. This led us to perform site-directed mutagenesis of the A2A receptor to probe and validate the target lysine amino acid K153 for covalent binding. Meanwhile, a functional assay combined with wash-out experiments was set up to investigate the efficacy of covalent binding of LUF7445. All these experiments led us to conclude LUF7445 is a valuable molecular tool for further investigating covalent interactions at this receptor. It may also serve as a prototype for a therapeutic approach in which a covalent antagonist may be needed to counteract prolonged and persistent presence of the endogenous ligand adenosine.

Poster #11: The relevance of calculating the Ki for bi-substrate enzyme inhibitors - A KAT8 case story

Hannah Wapenaar, Frank J. Dekker
University of Groningen

Histone acetyltransferases (HATs) are epigenetic enzymes involved in the regulation of gene transcription and are potential targets in disease. The development of bioactive molecules or molecular probes targeting these enzymes, such as inhibitors,

has met many challenges. One of these challenges is the lack of kinetic evaluation of the enzymes and their inhibitors. HATs catalyze the reaction between two substrates, which complicates the calculation of inhibitory potency values (Ki values) from their observed 50% inhibitory concentration (IC50) in biochemical assays. Since the IC50 values depend on assay conditions, it is not possible to use these values for reproducible determination of the potency and selectivity. Therefore, using biochemical and biophysical methods, the Ki values of two structurally different inhibitors for the HAT subtype lysine (K) acetyltransferase 8 (KAT8) were determined. The inhibitors showed different inhibitory mechanisms, resulting in a striking difference in potency that could not be revealed by the IC50. The Ki values additionally revealed that the mechanism of inhibition could have consequences for the inhibitor activity in-vivo. This shows that the IC50 does not give sufficient information on the inhibitory potency and that the Ki values should be determined in case of KAT8 and other bi-substrate enzymes.

Poster #12: Quantification of receptor dimerization on living cells by a novel kinetic proximity assay

Sina Bondza, Karl Andersson, Hanna Björkelund, Marika Nestor, and Jos Buijs
Ridgeview Instruments AB

We have developed a new cell-based assay to quantify the kinetics and affinity of two interactions that occur in close proximity. The assay relies on real-time interaction analysis on living cells with fluorescent quenching and is suitable for studying co-localization of cellular receptors, receptor interactions with multiple ligands, and the specificity of ligands. The method was validated by studying the simultaneous binding of two antibodies to the same cellular receptor. Co-localization was illustrated using the therapeutic antibodies Cetuximab and Trastuzumab that bind the tyrosine kinase receptors EGFR and HER2, respectively. A fluorescence signal, originating from FITC-Cetuximab, was reduced upon addition of Quench-Trastuzumab confirming the presence of EGFR-HER2 heterodimers on an ovarian cancer cell line. Gefitinib treatment resulted in increased heterodimer levels, whereas no significant differences in the binding characteristics of Cetuximab and Trastuzumab were found. Further applications of this assay are monitoring the on-set of drug induced dimerization or dimer disruption, as well as receptor clustering in real-time.

Poster #13: Application of fluorescent ligands to determine the ligand-receptor binding kinetics in the A3 receptor

M Bouzo-Lorenzo, L Xia, LA Stoddart, LH Heitman , AP IJzerman, SJ Briddon, SJ Hill
University of Nottingham

Currently, G protein-coupled receptors (GPCR) superfamily is the target of approximately 30% of the approved drugs (Santos et al., 2017). The adenosine A3 receptor (A3AR) belongs to a family of four G-protein coupled receptors (A1, A2A, A2B and A3) that all respond to adenosine. A3AR couples mainly to Gi/o proteins and is involved in a variety of intracellular signalling pathways and physiological functions. Its peripheral location allows it to mediate a sustained cardioprotective function during cardiac ischemia or being involved in the inhibition of neutrophil degranulation in neutrophil-mediated tissue injury. Additionally, it has also been implicated in neuroprotection and neurodegeneration processes. For all these reasons, A3AR has been extensively studied as a drug target.

The prediction of the in vivo efficacy of a drug nowadays is based mainly on steady-state metrics like affinity or potency values. However, the binding of a drug to its target is not static, is a dynamic and reversible process. Drugs with similar affinity can display markedly different binding kinetics. Furthermore, one crucial step to reduce the actual drug discovery attrition rates and translate in vitro results to clinical needs is optimize the acquisition of drug's binding kinetics parameters (Guo et al., 2016).

During the last years, Bioluminescence Resonance Energy Transfer (BRET) has been used to determine the ligand-binding kinetics of receptors in their natural cellular environment (Stoddart et al.,2015). The interaction of fluorescently labelled agonists and antagonist with NLuc (nanoluciferase) tagged receptors is monitored in real time. In this study, different fluorescent ligands for the adenosine receptors in combination with NLuc tagged A3 receptor were used to determine the kinetics of the ligand-receptor interactions in HEK 293 living cells using BRET. Additionally, the action of the fluorescent ligands has also been tested using the traditional radioligand binding assays.

Poster #14: Studying functional and binding properties of CXCR4 intracellular loop 1 pepducins by GloSensorTM and NanoBRET

B. Caspar, L. Stoddart, J. Goulding, S. Briddon, S. Hill
University of Nottingham

Introduction: Lipidated peptides, known as pepducins1, which are derived from the sequence of one of the internal loops of a GPCR, have been shown to act as either positive or negative allosteric modulators. Pepducins have been described for the chemokine receptor, CXCR4, which act as positive allosteric modulators and also show agonist behaviour in the absence of the endogenous ligand CXCL12.2, 3 Furthermore, a CXCR4 pepducin based on intracellular loop 3 acts as an antagonist.4 To date, their precise mode of action is unclear. In this study, we aimed to investigate the interaction of CXCR4 and intracellular loop 1 pepducins.
Results: Functional responses of CXCR4-expressing HEK cells in response to pepducins were studied in a GloSensorTM (Promega) cAMP assay5. Inhibition of forskolin stimulated cAMP production was observed upon addition of CXCL12 as well as pepducins. Control pepducins without a lipid tail showed reduced potency. The agonist action of both CXCL12 and the pepducins was antagonised by the CXCR4 selective antagonist AMD3100 (0.1 to 10 µM). No significant effects were observed for CXCL12 or the pepducins in native HEK cells.
To further study the interaction of the pepducins with CXCR4, the binding of fluorescently labelled ligands (CXCL12 or pepducins) was studied with a NanoBRET assay6. Using HEK cells expressing CXCR4 tagged with NanoLuc on its N-terminus (NL-CXCR4), the affinity of fluorescent CXCL12 (CXCL12-red) was determined (pKd = 7.61 ± 0.10 nM, n = 5). The binding of CXCL12 to CXCR4 could be inhibited by pre-incubation with the small molecule antagonists AMD3100, IT1t as well as pepducins. Control pepducins with no lipid tail showed no ability to disrupt the binding between CXCL12 and CXCR4.
Conclusion: Our data shows that receptor-dependent signalling pathways can be activated by pepducins and that the lipid tail plays a crucial role in the binding of pepducins to CXCR4. The NanoBRET studies suggest that pepducins have a direct influence on the binding of CXCL12 to CXCR4. Further work will be aimed at understanding their exact mechanism of action.

Poster #15: Using TR-FRET Methods to Determine the Kinetics of Inhaled Therapeutics

John P. Evans and Daniel A. Thomas
GlaxoSmithKline R&D

A drug candidate must accumulate at its target site at sufficient levels to exert its downstream consequences in a narrow window of time before it can be metabolized or transported away. Unsurprisingly a large number of commercialized drugs are characterized by long residence times. Long residence time also often equates with extreme potency of the binding interaction that precludes equilibrium measurements on a reasonable time scale, therefore kinetic off rate measurements can provide a more practical and accurate measure of intrinsic potency. New technologies, including more sensitive plate readers fitted with injectors, have facilitated the time resolved-fluorescence resonance energy transfer (TR-FRET) technique for measuring association and dissociation kinetic rate constants. We present the initial setup of TR-FRET methods for measuring compound kinetics for novel inhaled therapeutics, for which long target-site residence is desirable. Describing the kinetic characterization of the fluorescent probe ligands, competitive binding kinetics experiments to measure both the association and dissociation rate constants for unlabelled compounds to derive structural kinetic relationships, and then compare these constants with those measured by other means, e.g. SPR and steady state kinetic methods.

Poster #16: Biophysical screening towards the development of small molecule inhibitors of mutant GTPase KRAS

Gillian Goodwin, Andrea Gohlke, Justin Bower and Chris West
Beatson Institute for Cancer Research

The RAS family of proto-oncogenes (KRAS, HRAS and NRAS), are small GTPases that cycle between an active guanosine triphosphate (GTP)-bound state and an inactive guanosine diphosphate (GDP)-bound state. In the active GTP-bound state KRAS signals from the plasma membrane through a functionally diverse set of down-stream effector proteins including PI3K, RAF and RALGDS to pathways that control cellular growth, apoptosis, survival and differentiation.
The significance of KRAS mutation, keeping it in its active state and thereby driving and maintaining oncogenesis is well recognised and is associated with over 20% of all human cancers, especially pancreatic, colon and lung cancer.

In the presented study we therefore aim to find compounds that interrupt KRAS signalling in order to reduce tumour growth. The initial fragment screen against KRAS using surface plasmon resonance (SPR) and subsequent nuclear magnetic resonance (NMR) fragment screens has produced a number of different chemical starting points for further optimisation. The progression from there has been challenging, but by using in-house medicinal chemistry, SPR, NMR, X-ray crystallography, computational modelling, isothermal calorimetry (ITC), Ras/Raf pulldown and a biochemical assay nucleotide exchange assay, a number of new chemical entities have been synthesised with improved binding to KRAS, demonstrating functional effects in cell-free assays.
This cascade led us to our current compounds binding to KRAS-G12D in the GDP as well as in the GMPPnP nucleotide bound state with affinities in the sub-µM range.
Finding a competent inhibitor for K-RAS signalling will ultimately help to decrease tumour growth and therefore improve therapy for cancer patients.

Poster #17: Ligand induced conformational changes in proteins monitored time resolved and label free - towards a conformational activity screening for drug discovery

Jörn Güldenhaupt, Marta Amaral, Jonas Schartner, Carsten Kötting, Matthias Frech and Klaus Gerwert
Ruhr Universität Bochum

We report on time-resolved attenuated total reflection – Fourier Transform Infrared (ATR-FTIR) investigations of ligand induced conformational changes of immobilized proteins. Using ATR-FTIR as a detection technique we combine label-free ligand binding kinetic measurements with conformational change detection. Infrared spectroscopy monitors the amide vibrations of the protein backbone and can thereby detect small secondary structure transitions (2 - 5% residues involved). The investigated proteins were immobilized on germanium internal reflection elements (IREs) by multistep in situ surface modification methods. We generated NTA- and Streptavidin modified surfaces for immobilization of His-tagged and biotinylated proteins respectively. On these ATR surfaces a set of proteins, namely His-tagged HSP90 (WT and L107A mutant) and pregnane X receptor (PXR) and biotinylated AuroraA-, CDK2- and IFG1R- kinases were immobilized and ligand interaction experiments were performed.
Time resolved ATR-FTIR measurements on the ligand interaction of immobilized HSP90 were performed. All proteins were succesfully immobilized to a sufficient amount and stability on the ATR surface to allow for the ligand interaction experiment. However not for all of the examined proteins significant signals in the ligand induced difference spectra were detected.
In case of HSP90 the time resolved conformational activity of two types of compound classes, namely loop- and helix-binder compounds were characterized and successfully validated with independent experimental information. Structural/conformational change information was validated by comparison with X-ray structures, and SPR kinetics information was used for validation of conformational change kinetics. Additionally, the comparison of compound interaction spectra of HSP90 WT and L107A mutant for a subset of compounds clearly shows that the APO state of L107A is predominantly in helix conformation.

Poster #18: Structural understanding of the histamine H1 receptor

Gregory Verdon, Reggie Bosma, Sebastiaan Kuhne, Chai Gopalasingam, So Iwata, Rob Leurs, Konstantinos Beis
Imperial College London

The Histamine receptor H1 (H1R) belongs to the family of G-protein-coupled receptors (GPCRs).
Activation of this receptor increases vascular permeability causing fluid to escape from capillaries into the tissues, which leads to the common symptoms of allergic reactions. Antihistamines are inverse agonists of the H1R and many drugs in the market have been approved for H1R. The first-generation drug doxepine is an H1R antagonist but it also shows side effects such as sedation, dry mouth and arrhythmia, because it can bind to other aminergic GPCRs, monoamine transporters and cardiac ion channels. Second generation drugs such as olopatadine and acrivastine have better pharmokinetics and fewer side effects. The main difference between the doxepine and olopatadine drugs is the methyl-carboxyl substitution in one of the doxepine's benzene rings. Our group has previously published the structure of the human H1R in complex with doxepine. A novel feature of the H1R-doxepin complex is the existence of an anion-binding site (phosphate) at the entrance to the ligand binding pocket. In order to understand, why olopatadine and acrivastine show greater selectivity towards H1R, we have determined their structures in complex with H1R at 4 Å and 3.1 Å resolution, respectively. The structures show that olopatadine and acrivastine

have a different binding pose than doxepine despite their close structural similarity that explain the better pharmacokinetics and higher selectivity over first generation drugs.

Poster #19: Interactions between indomethacin and quercetin in binding to human serum albumin

Hrvoje Rimac, Željko Debeljak, Mirza Bojić
Faculty of Pharmacy and Biochemistry University of Zagreb

Indomethacin, as well as a large number of drugs, binds in the IIA subdomain of human serum albumin (HSA). The binding site located in the IIA subdomain of HSA is very spacious and able to bind two or more ligands at the same time. Certain flavonoids, such as quercetin, exhibit significant increase in fluorescence when they are bound to HSA, so their fluorescence can be used as a proof of their binding. In this study we measured quercetin fluorescence by steady-state fluorescence spectroscopy when a certain percentage of HSA binding sites in the IIA subdomain were occupied by indomethacin, which was coupled with docking studies of quercetin to indomethacin-bound HSA. Fluorescence results show that with the increasing occupation of the subdomain IIA by indomethacin, the intensity of quercetin fluorescence also increases. This is also confirmed by docking results, which show that indomethacin and quercetin bind in close vicinity of each other, suggesting additional steric constraints of quercetin molecule or even an energy transfer between indomethacin and quercetin, resulting in increased fluorescence intensity of the quercetin molecule.

Poster #20: Ligand binding kinetics: A case for the ENT1 transporter

A. Vlachodimou, K. Konstantinopoulou, E. Hemme, A. Emmanouil, L.H. Heitman, A.P. IJzerman
Leiden University

Many potential drugs showing promising in vitro results fail during in vivo experiments or are withdrawn from clinical trials due to lack of efficacy or safety. This indicates a translational gap between the two types of experiments that leads to loss of time and resources.
Within the last 10 years, receptor-ligand binding kinetics, i.e. association and dissociation of the ligand to and from its target over time, proved to be essential for drug discovery.1 For that reason, the traditional paradigm, emphasizing affinity and potency only is being complemented with a new one that accentuates ligand-receptor residence time (RT), i.e the duration that a ligand stays in complex with its target.2
Equilibrative Nucleoside Transporter-1 (ENT1) is the most abundant nucleoside transport protein and mediates the facilitative diffusion of nucleosides to their concentration gradients. Its (over)expression has been linked to a variety of cancers, and as a result, ENT1 inhibitors that diminish its activity are proposed as an add-on treatment. Moreover, ENT1 inhibitors can potentially be used in the treatment of ischemic heart disease, stroke, inflammatory diseases and viral infections.3, 4 Therefore, this project is focused on the synthesis of new high affinity ENT1 inhibitors and on the determination of their binding kinetics, in order to establish structure-kinetic relationship (SKR) in addition to the structure-affinity one (SAR).
Experimental approach. A library of compounds targeting ENT1 was obtained or synthesized. A radioligand displacement assay was developed and optimized for the human ENT1 using the radiolabeled inhibitor [3H]NBTI to determine the affinity of ENT1 inhibitors. In addition, a radioligand competition association assay was developed to determine binding kinetics.
Results. The affinity (Ki) and binding kinetics of more than 40 ENT1 inhibitors from four different scaffolds were determined, yielding a wide variety in values for both parameters. Three of the scaffolds presented good affinities and short to moderate RT (1 – 48 min). On the other hand, analogues of draflazine showed longer RT, with one of the analogues having the longest RT of all (500 min).
Conclusions. Our research is aimed at new drugs targeting the ENT1 transporter, by incorporating kinetic binding parameters next to affinity. More generally, this approach could inspire future drug discovery in the field of membrane transporters. *Hoffmann, C.; Castro, M.; Rink**.** **1**en, A.; Leurs, R.; Hill, S. J.; Vischer, H. F. Ligand Residence Time at G-protein-Coupled Receptors-Why We Should Take Our Time To Study It. Mol Pharmacol 2015, 88, 552-60. **2.** Guo, D.; Hillger, J. M.; IJzerman, A. P.; Heitman, L. H. Drug-Target Residence Time—A Case for G Protein-Coupled Receptors. Med. Res. Rev. 2014, 34, 856–892. **3**.Rehan, S.; Ashok, Y.; Nanekar, R.; Jaakola, V.-P. Thermodynamics and kinetics of inhibitor binding to human equilibrative nucleoside transporter subtype-1. Biochemical Pharmacology 2015, 98, 681-689. **4**. Lin, W.; Buolamwini, J. K. Synthesis, flow cytometric evaluation, and identification of highly potent dipyridamole analogues as equilibrative nucleoside transporter 1 inhibitors. J Med Chem 2007, 50, 3906-*

Room 4 & 5

Poster #21: Kinetic Analysis of Small-Molecule Binding with Label-Enhanced SPR

Anders Hanning
Episentum

Label-Enhanced SPR (LE-SPR) significantly improves both the sensitivity and the specificity of Surface Plasmon Resonance analysis. LE-SPR can be used directly on standard Biacore™ instruments without any modification of the existing instrument hardware.
LE-SPR is based on labelling of one interactant with specialized dye labels combined with software-based curve shape analysis of the entire SPR dip curve. In this way, a highly sensitive and fully specific measure of the binding of dye-labelled compound is obtained and plotted as an enhanced sensorgram or 'epigram'.
For the analysis of small molecules and fragments, LE-SPR offers a 100-fold sensitivity increase as compared to conventional SPR. Further, the high specificity allows removal of most sources of background signal, e.g. bulk disturbances like varying DMSO concentrations.
We will explain the basic theory of LE-SPR and show how LE-SPR can be used in competitive assays using Motulsky-Mahan theory. The advantage of the competitive mode is that the analyte itself need not be labelled, but only a competing species. Further, this one competing species can be utilized for the screening of a large number of unlabelled analytes.

Poster #22: Fluorescence Correlation Spectroscopy based approaches to measure binding kinetics and time resolved target occupancy

Stefan Hannus
Intana Bioscience GmbH

Fluorescence Correlation Spectroscopy (FCS) is a single molecule sensitive method to monitor molecular motion of fluorescent particles in solution. Intana Bioscience employs the versatile approach to measure dissociation- and rate constants of target inhibitor interaction to support our customers drug discovery programs. At Intana we believe that proteins never act alone: For this reason we determine affinities and kinetics under in vivo like conditions. FCS and the related FCCS (dual color cross correlation) technology have been successfully applied on long term binding kinetics, jump dilution measurements, rate constants of GPCR-Ligand interactions and time resolved target occupancy studies. Taken together, FCS is a powerful tool to investigate binding kinetics under physiological conditions.

Poster #23: Structure and biophysical methods based drug discovery to membrane proteins

Robert Cheng, Sandra Markovic-Müller, Mathieu Botte, Nicolas Bocquet and Michael Hennig
LeadXpro AG

Structure based drug discovery is well implemented in the the drug discovery engine of many pharmaceutical companies. Whereas soluble proteins are managed well within the project timelines and portfolio changes in pharmaceutical industry, transmembrane proteins still represent a significant challenge. LeadXpro combines expertise of drug discovery, excellence in membrane protein science and use of cutting edge X-ray data collection at synchrotron and FEL sources. In addition, high quality solubilized and purified membrane proteins can be used to apply biophysical methods including single particle cryo electron microscopy to facilitate the discovery and optimization of lead compounds. Strong relationship between leadXpro and Swiss large research facilities like PSI-SLS and SwissFEL as well as CINA enables advances in structure determination of challenging membrane protein drug targets that have not been feasible before. Knowledge of the drug candidate and protein target 3d-structure, together with the full characterization of its interaction and binding kinetics by biophysical binding and functional assays enables to generate novel and better lead molecules for medicines of the future.

Poster #24: Kinetic and Thermodynamic Characterization of Pi-Cation Interactions for Galectin-3 by various biophysical tools

Thomas Neumann, Emmanuel Klein, Dominique Roecklin, Christina Muller, Valerie Vivat, Cyrille Grandjean, Cédric Atmanene, Valérie Vivat Fabrice Ciesielski
NovAliX

Combination of biophysical and structural techniques allowed characterizing and uncovering the mechanisms underlying increased binding affinity of lactosamine derivatives for galectin 3. In particular, complementing information gathered from X-ray crystallography, native mass spectrometry, isothermal microcalorimetry, Biacore SPR and others is compared to each other.
Our studies showed favorable enthalpic contribution of cation-pi interaction between lactosamine aryl substitutions and arginine residues from the carbohydrate recognition domain, which resulted in two log increase in compound binding affinity. This incrementing strategy allowed individual contribution of galectin inhibitor moieties to be dissected. Altogether, our results suggest that core and substituents of these saccharide-based inhibitors can be optimized separately, providing valuable tools to study the role of galectins in diseases.

Poster #25: Flourimetric quenching titration methods for drug albumin binding kinetic study

Somaieh Soltani, Zhaleh Bahlouli, Golnaz parvizi, Mina shaban, Hossein Haghaei, Mostafa Zakariazadeh, Sara Asadi, Faezeh Emami, sheida Afkham, Jalal Hanaei, Mohamadreza Rashidi
Institut für Pharmazie, FÜB

Drug binding to human serum albumin is one of the main characteristics which affect both pharmacodynamic and pharmacokinetic properties. At the same time one of the most common drug-drug interaction can be occur via HSA binding. Due to albumin important role in drug efficacy, study of drug binding mechanism (mainly kinetic properties) are interested by researchers worldwide. Between the applied methods for such studies, application of flourimetric quenching titration methods is reported frequently. The advantages such as possibility of real time study, and in solution study, high sensitivity, etc, mentioned everywhere. Drug binding kinetic of 20 different drugs were studied using the flourimetric quenching titration studies and the related kinetic parameters were calculated. The binding kinetics were studied with molecular docking method for all drugs. The results were compared by both the predicted parameters for the same drugs using molecular docking methods and available experimental kinetic parameters. The correlation between the Flourimetric data were correlated with predicted and experimental data. The binding kinetic parameters of the studied drugs compared according to their molecular structure. The reliability of obtained data using flourimetric quenching titration methods and also its applicability as an index of binding kinetic parameters were discussed for each drug.

Poster #26: The Creoptix WAVEdelta: experience a new level of sensitivity and flexibility in kinetics analysis

Fabio Spiga, Thomas Raschle, Matyas Vegh and Kaspar Cottier
Creoptix sensors

By combining best-in-class sensitivity, crude sample robustness and a very fast transition capability, the Creoptix® WAVE system is revolutionizing the study of molecular interactions and changing the world of drug discovery.
Engineered around the proprietary waveguide interferometry technology and the powerful yet intuitive software, the WAVE system offers superior data quality across the broadest range of compounds and sample types. The Creoptix® WAVE accurately characterizes weak binder kinetics with off-rates of 5/sec as well as high affinity antibodies with several hours of dissociation. The microfluidics sustains pure blood serum/plasma, cell supernatant, larger particles and even crude membrane preparations enabling novel approaches especially in the field of membrane protein research.
Here we present data showing:
• The outstanding sensitivity of the Creoptix® WAVEdelta with resolution of kinetics from an interaction of a small molecule with a protein at very low immobilization densities resulting Rmax below 1pg/mm2 (equivalent to 1RU)
• The characterization of weak binders with a small molecule-protein interaction that dissociates with an off-rate of 2.8s-1
• The crude sample robustness by determining the binding kinetics of an antibody with its antigen in 90% (untreated) blood serum

• New possibilities in drug discovery with the interaction kinetics studies between a nanobody and a GPCR that was immobilized directly from a crude cell membrane extract (no detergents)
• The versatility of the Creoptix® WAVEdelta allowing the thermodynamic characterization of a DNA hybridization binding by performing kinetics measurements at different temperatures

Poster #27: High throughput SPR kinetic rate and thermodynamic analysis

Sven Malik, JoAnne Bruno Klaus Wiehler, Christopher Whalen
Sierra Sensors GmbH

Not available.

Poster #28: High-throughput time-resolved method for inhibitor-induced conformational changes in kinases

Katelyn Connell, R.V. Agafonov, S. Biswas, A. Temesgen, S. Kintz, J. Salafsky and D. Kern
Biodesy, Inc

Developing inhibitors that are highly selective towards a particular kinase is essential for targeted cancer treatment with minimal perturbation of target-independent signaling pathways and thus side-effects in the clinic. Although kinases are structurally homologous as seen in X-ray structures, they often adopt unique conformations upon ligand binding. Consequently, techniques capable of monitoring structural transitions upon target-drug binding will have a much higher likelihood of identifying selective compounds. Unfortunately, conventional biophysical techniques for investigating protein dynamics (e.g., NMR, stopped-flow kinetics, time-resolved x-ray crystallography) have a very low-throughput, preventing their use for screening. Here we present a novel technique that combines the speed of traditional high-throughput screens on 384-well plates with an ability to detect protein conformational changes in solution and in real time. The method is based on the phenomenon of non-linear optics known as “second harmonic generation” (SHG), and is capable of detecting the orientation of an SHG-active dye label, attached to the molecule of interest, which in turn is tethered to a surface. To illustrate power of the method, we used SHG to characterize the binding of a panel of compounds to human protein kinases Src and Abl, with both the full-length proteins and the catalytic domains.

Poster #29: Kinetic rates prediction of protein kinase inhibitors by enhanced molecular dynamics

Abdennour Braka, Samia Aci-Sèche, Stéphane Bourg, Norbert Garnier, Pascal Bonnet
University of Orleans

Historically, some drug design programs have focused on the optimization of drug candidates based on structure-activity relationships (SARs) as an approximation of in vivo efficacy. However, the efficacy of a ligand is not always adequately described by the SAR because it mainly depends on the lifetime of in vivo interactions between the ligand and its receptor. Today, structure-kinetic relationships (SKR) are of major interest for the discovery of new drugs, particularly in the early stage of optimization of molecules in order to better evaluate their safety and efficacy.
With the growing interest of SKRs, we propose a new methodology to understand the mechanism of the binding of a ligand with its biological target in order to predict its kinetic constants.
Our method begins by identifying the binding pathways of the molecules. The simulation of the binding process is carried out using a combination of enhanced molecular dynamics to drive the ligand from/to the binding site. Based on the transition state theory, the energy profile of the simulations will be used to predict the kinetic constants and to identify the structural determinants responsible for the energetic barriers detected during the simulations.

Poster #30: Finding the molecular interactions that govern ligand residence time with enhanced sampling algorithms for molecular dynamics

Alex Dickson & Samuel D. Lotz
Michigan State University

It has been reported on a set of applications that the binding kinetics is more predictive than the binding affinity of the efficacy of a drug molecule in vivo. Although the thermodynamics depends only on the endpoints of the binding pathway, the kinetics

depends on details of the binding process, particularly the ligand binding transition state. Details of the ligand binding transition state have proved difficult to study both experimentally – due to its transient nature and short lifetime – and by simulation – due to the long timescales involved in ligand binding and release. Fundamental outstanding questions remain: How broad is the transition state? How robust is the transition state with respect to small changes in the ligand? Can we use models of the transition state to rationally design ligands with faster or slower binding kinetics?
To address these questions, we have developed WExplore, an algorithm based on concurrent loosely-coupled trajectories. By measuring the trajectory flux into the unbound state, we directly compute ligand residence times without using a Markovian assumption that show excellent agreement with those determined experimentally. We report simulations of the release of ligands from soluble epoxide hydrolase (sEH), which have an experimentally determined residence times extending to tens of minutes. We obtain broad sampling of ligand exit pathways, involving distinct exit channels and significant coupled motions between the ligand and the bound receptor. These simulations allow us to quantify the breadth of the transition state ensemble, and identify the specific protein-ligand interactions that govern binding kinetics.

Poster #31: COMparative BINding Energy (COMBINE) analysis to predict drug-binding kinetics.

Gaurav Kumar Ganotra, Daria B Kokh, Sadiq Kashif, and Rebecca C Wade
Heidelberg Institute for Theoretical Studies (HITS), Heidelberg

Over the past few decades, many classical regression techniques have been developed and successfully applied to correlate the properties of a series of molecules with their biological activities to derive quantitative structure activity relationships (QSAR). With the availability of the three-dimensional structures of many macromolecular drug targets and data on the activities of a family of compounds, these approaches have been extended in three dimensions to derive 3D-QSARs. COMparative BINding Energy (COMBINE) analysis is one such approach that has been successfully applied to number of targets to derive target-specific scoring functions based on molecular mechanical calculations for binding affinity and to predict target selectivity. Unlike other 3D-QSAR methods, COMBINE makes full, simultaneous and systematic use of all the available information on 3D structures of receptor-ligand complexes and the measured bioactivities of compounds, by explicitly including information about the receptor-ligand interaction energies rather than only about the interaction properties of the ligands.
In this work, we have applied COMBINE analysis to derive quantitative structure-kinetics relationships (QSKRs) for the dissociation rates of inhibitors of HIV-1 protease and heat shock protein 90 (HSP90). In this approach, ligand-receptor interaction energies are computed using a molecular mechanics model. These energies are then partitioned and subjected to regression based methods such as Partial Least Squares (PLS) regression, to derive a statistical model which relates dissociation rates to weighted selected components of the drug-receptor interaction energy. We obtain predictive models for dissociation rate constants and insights into the structural features that contribute to the variance in binding kinetics.

Poster #32: Simulation Enhanced Estimation of Kinetic Rates (SEEKR): A Hybrid Molecular Dynamics, Brownian Dynamics, and Milestoning Approach for Calculating Protein-Ligand Binding Kinetics

Benjamin R. Jagger and Rommie E. Amaro
University of California San Diego

We present recent developments to our SEEKR software, an open-source collection of python scripts used for the calculation of kinetics rates. SEEKR is a multiscale simulation approach that combines fully atomistic molecular dynamics simulations and rigid body Brownian dynamics simulations with milestoning theory to calculate association and dissociation rates as well as binding free energies for protein ligand complexes. The advantage of this approach is its highly parallel nature resulting in significant speedup compared to conventional molecular dynamics simulations of association and dissociation events. In addition, the extensive sampling obtained through this approach in combination with milestoning theory provides a statistically robust framework for the calculation of kinetic parameters. We also describe recent successes including the calculation of association and dissociation rates for the well-studied system of trypsin with its inhibitor, benzamidine.

Poster #33: Binding kinetics survey of the drugged kinome

Victoria Georgi, Benedict-Tilman Berger, Felix Schiele, Amaury Fernández-Montalván
Bayer AG

In recent years binding kinetics has enjoyed increasing acceptance as optimization parameter in target-based drug discovery. The underlying idea proposes that compound's association (kon) and dissociation (koff) rates for primary- and off-targets are better predictors for efficacy and safety than steady-state affinity per se (KD = koff / kon). While appealing, this concept is mainly backed by studies performed with small and/or incomplete drug-target interaction datasets.
Here we present a comprehensive analysis of the binding kinetic and affinity parameters of 270 small-molecule kinase inhibitors against 40 clinically relevant kinases.
Surprisingly, for the wide spectrum of kinase-inhibitors profiled, our results reveal that affinity is mainly driven by on-rates. On the other hand, the proportion of slow off-rate interactions increases as compounds progress through clinical development phases, whereas the same does not apply for fast on-rates. We assess if target selectivity can be differently assessed from the equilibrium- or kinetic perspectives. Furthermore, we identify cases in which binding kinetics parameters could be effectively used to model the dynamics of target occupancy in vivo. Finally, we disclose structure-kinetic relationships which add to the existing structure-activity relationship knowledge and provide a rational basis for the prospective design of kinetic rate constants.
Our results will contribute to realize the potential of binding kinetics for drug discovery and represent a valuable resource for future studies in this field.

Poster #34: Novel approaches for the assessment of data from competitive binding kinetics assays

Victoria Georgi, Alexey Dubrovskiy, Margarita Shatalina, Solene Rolland, Reggie Bosma, Sebastian Räse, Stephan Heyse, Stephan Steigele and Amaury Fernández-Montalván
Bayer AG

Since the introduction of the drug-target residence time model 10 years ago, binding kinetics have become an important enhancement to "classical" drug discovery metrics (such as potency) when it comes to making compound progression and candidate selection decisions. This paradigm shift has been backed by retrospective analysis of recently approved drugs showing that nearly one third act via non-equilibrium mechanisms, and by studies suggesting that the incorporation of in vitro kinetic information in drug discovery programs could support better prediction and modeling of in vivo efficacy and PK/PD relationships. Thus, there is an increasing demand for assay technologies that enable measurements of drug–target association (kon) and dissociation (koff) rates.
The challenge in leveraging kinetic information in early drug discovery stages lies in how to cost - effectively obtain kon and koff parameters at sufficient throughput for systematic assessment of compounds. Recently, the kinetic Probe Competition Assay (kPCA) - a universal and scalable assay format relying on TR-FRET technology and the Motulsky-Mahan competitive binding kinetics theory - has been reported as possible solution to this challenge.
In this poster, we illustrate the principle of the kPCA assay, its application in a high-throughput setting, and the issues associated with the evaluation of large datasets generated with the method. We describe solutions implemented to cope with these problems, including 1) an automated analysis procedure based on the Motulsky-Mahan model implemented in the Genedata Screener® software, 2) modifications of the model intended to cope with fluorescence signal decay and 3) Monte Carlo analyses to simulate the pitfalls and boundaries of the model.
The theoretical knowledge and software tools resulting from this work have significantly enhanced our understanding of the Motulsky-Mahan model of competitive binding, and accelerated our kPCA analysis workflow from raw data assessment to results evaluation and reporting.

Poster #35: Structural and dynamic insights into the energetics of activation loop rearrangement in FGFR1 kinase

Tobias Klein, Navratna Vajpai, Jonathan J. Phillips, Gareth Davies Geoffrey A. Holdgate, Chris Phillips Julie A. Tucker, Richard A. Norman
Bayer AG

Protein tyrosine kinases differ widely in their propensity to undergo rearrangements of the N-terminal Asp–Phe–Gly (DFG) motif of the activation loop, with some, including FGFR1 kinase, appearing refractory to this so-called 'DFG flip'. Recent inhibitor-bound structures have unexpectedly revealed FGFR1 for the first time in a 'DFG-out' state. Here we use conformationally selective inhibitors as chemical probes for interrogation of the structural and dynamic features that appear to govern the DFG flip in FGFR1. Our detailed structural and biophysical insights identify contributions from altered dynamics in distal elements, including the αH helix, towards the outstanding stability of the DFG-out complex with the inhibitor ponatinib. We conclude that the αC-β4 loop and 'molecular brake' regions together impose a high-energy barrier for this conformational rearrangement, and that this may have significance for maintaining autoinhibition in the non-phosphorylated basal state of FGFR1.

Poster #36: In Silico Prediction of Relative Drug-Protein Residence Times

Daria B. Kokh, Marta Amaral, Joerg Bomke, Matthias Dreyer,Matthias Frech, Maryse Lowinski, Alexey Rak, Rebecca C. Wade
HITS gGmbH

The aim of this study in the K4DD project is to develop methods for the computational prediction of the relative residence times of protein-ligand complexes. These methods should aid the rational design of new drugs with desired kinetics. However, the computation of dissociation rate constants, which determine residence times, is extremely challenging because: (i) the residence times of protein-ligand complexes usually extend to times far beyond those that can be simulated by conventional molecular dynamics (MD) techniques, and (ii) the residence time is often determined by a transition state that is rarely accessible in MD simulations.
We therefore implemented an enhanced sampling MD approach for the prediction of relative dissociation rate constants and for obtaining insights into ligand unbinding pathways and mechanisms. The approach is based on the random acceleration molecular dynamics (RAMD) method. We evaluated the method on more than 50 diverse inhibitors of an important cancer target, heat shock protein 90 (HSP90). The computed relative residence times show a good correlation (R2~0.9) with the experimental values from surface plasmon resonance measurements for ~85% of the compounds. Though limitations of the standard molecular mechanics forcefield may cause systematic underestimation of the binding affinity for compounds with particular chemical groups, the proposed method correctly predicts trends and gives estimates of the change in relative residence time upon making small substitutions in a compound that affect the height of the transition barrier to dissociation. Furthermore, the proposed method provides information on the possible ligand dissociation pathways and gives insights into the nature of transition barrier.

Poster #36A: Hsp90 dynamic changes upon ligand binding

Macek P, Amaral M, Bomke J, Frech M, Boisbouvier J, Kerfah R.
NMR-Bio SAS, Grenoble, France

Hsp90 is a major cellular chaperone required to maintain cellular homeostasis and to buffer genetic variation. Hsp90 is a homodimer of 180kDa which stabilizes client proteins in an ATP dependent cycle. It was found to be important in stabilization of many signaling proteins such as kinases and nuclear hormone receptors, also implicated in cancerogenesis. Several pharmacophores were found to inhibit the hsp90 in an ATP competitive manner. The cellular efficacy of hsp90 inhibitors was found to be correlated with the inhibitor residence time, a lifetime of the drug-ligand complex introduced by Copeland et al. in 2006, which is related to the dissociation rate constant koff.
The molecular determinants of the protein-ligand half time are not fully understood yet. Beside the ligand physico-chemical properties, the conformational dynamics of both, protein and ligand, play a role in protein-ligand lifetime [1, 2]. In our work, we focus on the comparison of s-ms timescale dynamics of Hsp90-NTD in apo- and ligand bound state. We show that upon the binding of resorcinol based ligand with bulky substituents, the s-ms timescale dynamics close to the binding centre is decreased, while dynamics in the structurally distant regions is increased. We speculate that rigidification of

binding centre along with increased protein conformational entropy participate in increasing the half-life in comparison to ligands with small substituent. [1] Frauenfelder et al. (1991) [2] Uchida et al. (1997)

Hallway

Poster #37: Escaping from the Histamine Pocket

Albert J. Kooistra, Reggie Bosma, Sebastiaan Kuhne, Henry F. Vischer, Rebecca C. Wade, Tatsuro Shimamura, So Iwata, Iwan J.P. de Esch, Chris De Graaf, Rob Leurs
Vrije Universiteit Amsterdam

Obtaining ligands with a high affinity for a therapeutic protein target has been the main driving force of drug development programs. However, often this high affinity does not translate into the desired therapeutic effect when the experiments are moved from an in vitro to an in vivo setting.1 In the last decade, researchers have uncovered that the duration a drug molecule is bound to its protein target significantly correlates with its clinical efficacy.1 Unfortunately, a detailed understanding of the molecular determinants of the protein-ligand binding kinetics is lacking.
In this study, we performed MD simulations to study the dissociation of levocetirizine from the histamine H1 receptor (H1R). Using random acceleration MD2 (in which a small force is applied to the ligand in a random direction) in combination with conventional MD simulations the egress of levocetirizine from H1R was simulated. Fifty simulation replicates were subsequently analyzed based on the observed protein-ligand interactions3 (IFPs) and the distance of the ligand from its original binding mode. This analysis resulted in a residue-based interaction profile along the dissociation pathway of levocetirizine from H1R. These profiles validated experimental mutagenesis and binding kinetics studies and confirmed the role of K1915.39 as key residue in the extracellular vestibule for the egress of levocetirizine. Follow-up RAMD simulations on in silico mutated H1R structures unraveled a partial escape mechanism for the K1915.39A mutant. Further mutagenesis and binding kinetics studies designed based on the RAMD studies confirmed this mechanism.
The combination of enhanced MD simulations with molecular interaction fingerprint analyses3 allowed for the accurate description and prediction of molecular and structural determinants of ligand binding kinetics. The approach was experimentally validated by site-directed mutagenesis studies and is currently being used for structure-based design and synthesis of ligands with longer residence times. *(1)Copeland et al., Nat. Rev. Drug Discov., 2006, 5, 730.(2)Ludemann et al., J. Mol. Biol., 2000, 303, 797. (3)Vass et al., Curr. Opin. Pharmacol., 2016, 30, 59.*

Poster #38: Computing protein-ligand binding association rate constants by combining Brownian dynamics and conventional molecular dynamics simulations

S. Kashif Sadia, Daria Kokh, Gaurav Ganotra, Rebecca Wade
Heidelberg Institute for Theoretical Studies

Understanding the detailed molecular kinetics of protein-ligand association is of fundamental importance in biomedical research – increasingly so, as the kinetic characteristics of drugs become more widely appreciated as key to their efficacy. Advances in force fields, and the use of novel computer architecture paradigms and analysis methodologies have enabled conventional unbiased all-atom molecular dynamics (MD) simulations to accurately sample the millisecond timescale and allowed detailed kinetic properties of binding, conformational changes and protein folding to be reconstructed from large ensembles of simulations. However, it still remains a major computational hurdle to calculate protein-ligand binding kinetics for even moderately sized drugs and/or large receptor targets, especially if the binding pathway is coupled to conformational changes in either, or if the kinetics is too slow. Binding timescales for clinically relevant inhibitors usually extend far beyond those that can be simulated by conventional MD methods. Implicit solvent, rigid-body Brownian dynamics (BD) methods are promising because calculations are computationally feasible. However, whilst such methods have been successful at computing diffusional association rate constants, a rigid-body approach cannot capture the various ligand/protein conformations often involved along protein-ligand binding pathways. Here, we develop a multiscale method that integrates conventional MD with rigid-body BD that allows target-ligand flexibility to be integrated into calculations of association rate constants (k_on). Our approach involves pre-computing the conformational kinetics of the apo-protein ensemble using MD simulations and adaptive Markov state models and then integrating a set of kinetically distinct conformers within the framework of the BD calculations. Based on this approach, we compute k_on for two sets of inhibitors with known

experimental kinetics, that bind to the conformationally flexible proteins, HSP90 and HIV-1 protease, respectively. The method also allows us to compute and analyze ligand gating effects mediated by the major conformational changes in such proteins.

Poster #39: Molecular dynamics simulation to predict kinetic binding constants: application to a kinase protein.

Sonia Ziada, Samia Aci-Sèche, Eric Raimbaud, Pierre Ducrot, Pascal Bonnet
ICOA laboratory, Orléans, France

Continuous increase in time and cost of drug development leads the scientific community to minimize the causes of failure. Numerous studies show that a preclinical evaluation of the binding kinetic constants limits the failure rates of the drugs in Phase II clinical trials. Indeed, the dissociation constant is directly linked to the residence time, a key parameter to enhance the in vivo efficiency of the compound. Thus, kinetic constant study is the cornerstone of drug design strategies, but it remains a lack of theoretical protocols to estimate such a determinant.
We present here an in silico approach based on molecular dynamics simulations to predict the
dissociation process of an active molecule with its target, leading to the kinetic constant calculation. We developed a three-step protocol which starts by identifying the entry/exit pathway of the molecule to the binding site using a biased molecular dynamics method. Conformations of the system are then sampled to provide a statistical distribution of its states along the pathway. The free energy profile is estimated from these statistical distributions using the Weighted Histograms Analysis Method (WHAM) and binding kinetic constants are deduced. The proof of concept is performed on CDK8, an emerging therapeutic target involved in colorectal cancer.
The first results obtained are satisfying and provide a plausible exit pathway for all the molecules. The simulated exit times show a good classification of molecules spanning small, medium and long
residence times. This project presents the advantage of explaining the associated molecular mechanism and constitutes the first step of the quantitative study of structure-kinetic relationship.

Poster #40: Adiabatic bias/metadynamics approach on desmond gpu: high throughput residence time estimation

Davide Branduardi; Andrea Bortolato; Dmitry Lupyan; Francesca Deflorian; Victoria Feher
Schrodinger

The basis of drug action is strongly related to the thermodynamics of the ligand binding to the target and equilibrium conditions are generally assumed. However in recent years it has been recognized that often times this assumption does not hold in-vivo.[Copeland Future Med. Chem (2011) 2(12) 1491] Furthermore residence time (the inverse of koff) can be useful to further characterize the target selectivity and therefore is now often considered in SAR analysis in drug discovery programs. As a consequence, we observed a growing interest for its in-silico estimate and a number of methods have been proposed in the recent years.
Here we contribute to the field by presenting an augmented version of the Adiabatic bias/Metadynamics approach from Bortolato [Bortolato et al J. Chem. Inf. Model. (2015) 55, 1857] capitalizing on the speed of Desmond molecular dynamics code on GPU. Compared with standard metadynamics, this approach prevents the system from revisiting the old portion of phase space by virtue of an adiabatic bias and this accelerates considerably the exploration of the full unbinding path.
This approach is very empirical in its spirit but very appealing since it requires minimal human intervention and allows tens of compounds to be evaluated overnight which is particular interesting in a drug-discovery setting, where tight deadlines must be met. Here we outline the essential features of the protocol, the scoring function and show its correlation with residence time for few target of pharmaceutical interest.