PCF2025
13 - 14 October
Basel, Switzerland

Hosted by Roche

http://www.physchem.org.uk/

22nd PhysChem Forum

Advancements and Challenges in Physicochemical Profiling for Emerging Modalities in Drug Research

Contents

Word from the organizing committee

Dear Participants,

Welcome to the 22nd PhysChem Forum 2025 hosted by F. Hoffmann-La Roche, Basel, Switzerland.

Since 2005, the PhysChem Forum has hosted an annual two-day meeting covering a variety of topics around the themes of physical chemistry (PhysChem) and absorption, distribution, metabolism, excretion and toxicity (ADMET; http://physchem.org.uk/index.html).

The focus of the 22nd PhysChem Forum is on ***Advancements and Challenges in Physicochemical Profiling for Emerging Modalities in Drug Research***.

Measuring **PhysChem props** of new modalities in the pharmaceutical industry presents significant challenges. Unlike the more traditional small molecules, new modalities such as protacs, peptides, oligonucleotides, antibody-drug conjugates, and other large or complex structures often defy traditional measurement techniques. Their size, properties, structural diversity, and instability can hinder accurate determination of properties like solubility, lipophilicity, permeability, and ionization. In addition, many new modalities have heterogeneous compositions or dynamic conformations that complicate standardization of assays. This complexity often requires the development or adaptation of specialized analytical methods and instrumentation, as well as novel computational approaches for prediction and interpretation. As these modalities become increasingly important in drug discovery and development, the ability to reliably characterize their physicochemical properties remains a critical—and evolving—scientific hurdle.

Despite several high-quality international meetings held covering various aspects on this field there has been limited scope for bench scientists to meet in a more informal setting allowing them to present current work, discuss challenges that were overcome and deliberate those yet to be faced. Furthermore, those meetings have generally been expensive, thus precluding multiple attendees from smaller departments. The PhysChem Forum Symposium event series is attempting to address those specific aspects by an adapted, yet very successful proven format. The PhysChem Forum is totally free, and its successful organisation relies on the passion and engagement of many people who dedicate their time and effort to make the PhysChem Forum a reality.

We would especially like to thank our sponsors for their kind support, so please visit their booths. Also big thanks to the speakers who are of course the heart of this forum and agree to share their knowledge with the rest of us receiving no compensation. And lastly, but not least, a big, big, special thanks to *Roche* for the local support in hosting this event!
Our goal is to generate an environment for informed debate and discussion, in a non-commercial setting, with excellent networking opportunities.

Kind regards,

The PCF2025 Organizing Committee

Antonio Llinas, AstraZeneca
Bjoern Wagner, F.Hoffmann-La Roche
Changhui Chen, Syngenta
Jonathan Burley, University of Nottingham
Karl Box, Pion
Linette Ruston, Seda Pharm. Dev.
Magdalena Kierkowicz, UCB
Shenaz Bunally, GSK
Sonia Espinosa, Almirall
Stephane Rodde, Novartis

PS: To subscribe to our mailing list and receive notifications about upcoming meetings, please e-mail webmaster at mailshot@physchem.org.uk and follow us on LinkedIn and physchem.org.uk

Programme

Monday 13th October 2025

At the Radisson Blu Hotel, Steinentorstrasse 25, Basel, Switzerland. Map

Chair: Antonio Llinas (AstraZeneca, Sweden)

14.00 - 15.30 Drinks reception and late registration
15.30 - 15.40 Welcome remarks and introduction
Antonio Llinas *(AstraZeneca, Sweden)*
15.40 - 16.20 How to deliver oral targeted modalities: experiences from protein kinase inhibitors and PROTACS
Hans Lennernäs *(Uppsala University, Sweden)*
16.20 - 17.00 Closing the design-make-test-analyze loop: interplay between experiments and predictions drives PROTACs bioavailability
Karolina Kwapień and Johan Wernevik *(AstraZeneca, Sweden)*
17.00 - 17.30 Tea break
17.30 - 18.30 Keynote: Navigating the shifting sands: tackling the evolving drug landscape
Paul Dickinson *(SEDA, UK)*
18.30 - 19.30 Social break
19.30 - 22.00 Symposium dinner

Tuesday 14th October 2025

At the F. Hoffmann-La Roche, Grenzacherstrasse 124, Basel, Switzerland. Map

Chair: Antonio Llinas (AstraZeneca, Sweden)

8.30 - 9.00 Late registration and poster set-up
9.00 - 9.50 Welcome introduction. The History of Roche
Alexander Bieri *(Roche, Basel)*
9.50 - 10.30 Use of cyclic ion mobility mass spectrometry to understand conformational space of BRo5 molecules and its impact on permeability
Jehan Claessens *(UCB, Belgium)*
10.30 - 11.10 Coffee break and poster viewing

Chair: Linette Ruston (Seda, UK)

11.10 - 11.50 How the interplay between shape and polarity can determine the cell permeability of PROTACs
Jan Kihlberg *(Uppsala University, Sweden)*

11.50 - 12.10 Flash poster session (poster authors introduce their posters, 2 minutes each)
12.10 - 13.40 Lunch and poster viewing / 30-minutes Roche Research Center tour (two groups in a row) / Group picture (photo session)

Chair: Shenaz Bunally (GSK)

13.40 - 14.20 Dynamic logP - Novel LC/MS method to measure chameleonicity of bRo5 macrocyclic peptides
Christian Weinmann *(Roche, Switzerland)*

14.20 - 15.00 Leveraging machine learning for predicting ADME properties of targeted protein degraders Raquel Rodriguez-Perez *(Novartis, Switzerland)*

15.00 - 15.40 Poster prize award followed by tea/coffee break and poster viewing

Chair: Magdalena Kierkowicz (UCB, UK)

15.40 - 16.20 A biological strategy for the oral delivery of poorly absorbed therapeutics
Randy Mrsny *(University of Bath, UK)*

16.20 - 17.00 Next-generation physicochemical assays to characterize new modalities
Stephane Rodde *(Novartis, Switzerland)*

17.00 - 17.10 Wrap-up and depart

Biosketches and Abstracts

Hans Lennernäs

Full professor in biopharmaceutics, Uppsala University, Sweden

Email: hans.lennernas@uu.se

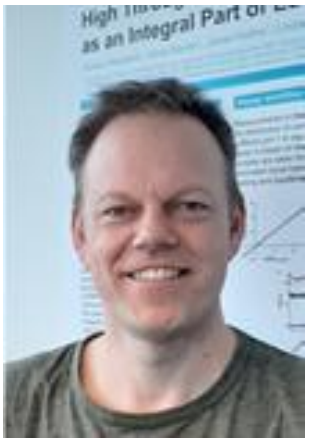
Hans Lennernäs is a full professor in biopharmaceutics at Uppsala University, ranked 104th among 150,000 researchers in Pharmacology-Pharmacy by the 2024 Stanford/Elsevier ranking. He was an adjunct professor at Copenhagen University from 2000 to 2012. His research aims to improve drug targeting and delivery for diseases like metabolic disorders, endocrinological conditions, and cancer.
From 1992 to 2000, Lennernäs was the Principal Investigator in a collaborative project with the FDA, the University of Michigan, and the Swedish Medical Products Agency, leading to the creation of the FDA's Biopharmaceutics Classification System (BCS). BCS is now a globally adopted tool in pharmaceutical research. He also established a human pharmacokinetic database with jejunal permeability values for 45 drug compounds, widely used in academia and industry.
Prof. Lennernäs has chaired numerous international conferences and reviewed for many scientific journals. He has over 270 peer-reviewed publications, 350 invited lectures, and 350 scientific presentations, with over 20,500 citations and an H-index of 68. He has supervised 29 doctoral theses and secured research grants from bodies like the Swedish Research Council and the EU's Innovative Medicine Initiative.
His awards include the Glaxo Wellcome Achievement Award (1997), the EUFEPS New Safe Medicine Faster Award (2008), and the Humboldt Research Award (2022). He managed an EU-grant of 24.5 million Euros from 2012 to 2018 and received the highest research rank at Uppsala University in 2011. Currently, his research focuses on treatments for hepatocellular carcinoma, prostate cancer, endocrinological disorders, and gastrointestinal diseases. He holds over 23 patents and co-founded seven start-ups, including one that developed Plenadren® for Addison's disease. His latest venture, ENDORIZ AB, focuses on circadian oral hormone treatment for hypothyroidism. Lennernäs has served on the boards of several companies, including PULS AB and Recipharm Pharmaceutical AB.

How to deliver oral targeted modalities: experiences from protein kinase inhibitors and PROTACS

In this presentation biopharmaceutical data for Proteolysis Targeting Chimeras (PROTACs) and protein kinase inhibitors be discussed. One part of this presentation aims to mechanistically elucidate the processes governing the intestinal absorption and bioavailability of PROTACs using ARV-110 (bavdeglutamide) and ARV-471 (vepdegestrant) as model compounds. The respective studies are focused on the characterization of key biopharmaceutical properties, such as solubility in relevant media, intestinal permeability with efflux transporter interactions, dissolution, supersaturation and precipitation mechanisms, luminal degradation as well as distribution and elimination pathways. Based on these insights, optimized drug delivery strategies will be explored to overcome barriers limiting PROTAC oral delivery.

Protein kinase inhibitors (PKIs) are known for their high potency but are often negatively affected by high rates of intolerance and drug resistance, making their outcomes highly sensitive to intra- and interindividual variability in systemic exposure. One promising solution to improve *in vivo* performance by reducing variability and increase precision in dosing is through advancements in biopharmaceutical properties. A novel amorphous solid dispersion (ASD)-based formulation with dasatinib or other PKIs represents a notable pharmaceutical innovation in the oral delivery of dasatinib for chronic myeloid leukemia (CML). It addresses longstanding challenges such as gastrointestinal absorption variability, drug-drug interactions with acid reducing agents, and tolerability. It ensures pH-independent solubility, enhanced bioavailability, and sustained systemic exposure, even under conditions like hypochlorhydria due to gastritis or concomitant antiretroviral therapy (ARA). These features are crucial given the high variability observed with conventional crystalline dasatinib formulations, where subtherapeutic exposures can compromise efficacy.

Johan Wernevik
Associate Director, AstraZeneca, Sweden



Email: johan.wernevik@astrazeneca.com

Johan Wernevik is an Associate Director in the Assays, Profiling & Cell Sciences department within Discovery Sciences. With 26 years of experience in bioanalytical chemistry, he specializes in quantitative LCMS across various phases of drug development. Over the past decade, he has focused on early DMPK profiling, developing miniaturized, high-capacity assays for logD, solubility, plasma protein binding, and Clint measurements in human hepatocytes. More recently, he has led his team to introduce chromatographic methodologies to support physicochemical profiling of new drug modalities, including PROTACs, peptides, and compounds beyond the Rule of 5 (bRo5). Johan brings decades of drug discovery expertise within AstraZeneca and currently co-leads the Center of Excellence for Mass Spectrometry at the Gothenburg site.

Karolina Kwapień
Associate Principal Scientist in Computational Chemistry, AstraZeneca, Sweden

Email: karolina.kwapien@astrazeneca.com

Karolina Kwapień is a computational medicinal chemist at the AstraZeneca In-Silico Center of Excellence. She supports multiple drug discovery projects within Respiratory and Immunology

portfolio. In her medicinal chemistry work, she combines approaches from physics-based methods, quantum chemistry, and artificial intelligence. Her research interests focus on PROTACs and the physicochemical properties that influence their bioavailability. Advancing our understanding of the features and properties that enhance oral absorption, along with implementing predictive modelling to prioritize compounds, would greatly reduce the number of poor compounds synthesized and tested in vivo.

Closing the design-make-test-analyze loop: interplay between experiments and predictions drives PROTACs bioavailability

The drug development landscape is expanding to include drug modalities such as PROteolysis-TArgeting Chimeras (PROTACs) and peptides, offering possibilities for previously intractable biological targets. However, with their size and chemical nature, they diverge from established frameworks for the prediction of oral bioavailability. This evolution to larger and more complex molecules necessitates new methodologies and prediction models to continuously expand on bioavailability guidelines. Herein, we describe the methods for standardized chromatographic logD (ChromLogD) and experimental polar surface area (EPSA). These two high-capacity assays are utilized to continuously refine internal ML models, which in turn direct compound synthesis decisions in the molecular design phase. Based on our in-house data for over 1400 PROTACs we confirm a sweet spot for oral bioavailability at log D values higher than the norm for small molecules. We show how interplay between experimental data and prediction models synergize to effectively drive chemistry optimization.

Paul Dickinson
Founder and Chief Scientist, SEDA, UK

Email: paul.dickinson@sedapds.com

Paul A Dickinson is a prominent pharmaceutical scientist known for his expertise in biopharmaceutics and clinical pharmacology. He is Founder and Chief Scientist at Seda, a company focussed on delivering clinical pharmacology and CMC services.
An internationally recognized dynamic multi-disciplinary scientist with a track record of leading, developing and applying innovative applied science for project benefit. Extensive early and late-stage regulatory experience include written, teleconference and face to face regulatory interactions. This experience includes the delivery of many products through approval. Paul's contributions his scientific fields have been instrumental in shaping modern pharmaceutical practices, ensuring that drug development is more efficient, scientifically grounded, and patient-centric.

Navigating the shifting sands: tackling the evolving drug landscape

As drug development expands from small molecules to degraders, bRo5 compounds, peptides, proteins, and ADCs, the complexity of delivery and characterization grows. In a dynamic biotech–pharma ecosystem, drug developers must remain agile—leveraging advanced analytics, flexible API strategies, and predictive modeling to guide decisions. Navigating this landscape requires a clear map and compass: BioRAM provides the strategic framework, while PK exposure serves as the guiding metric. This talk explores how these tools support dose selection, First-in-Human (FiH) studies, and beyond, enabling smarter, faster development across emerging modalities.

Alexander Lukas Bieri

Curator The Roche Historical Collection and Archive, F. Hoffmann-La Roche, Basel, Switzerland

Email: alexander.bieri@roche.com.

Alexander Lukas Bieri is the curator of Roche's Historical Collections and Archives. He has been in that position since 2000. Prior to that, he was responsible for the introduction of biopharmaceuticals. A Roche veteran for over 30 years, his responsibilities include the in-house publishing company Editions Roche and the responsibility for keeping Roche's company culture alive and tangible for every new generation of stakeholders.

Alexander's presentation will focus on the legacy of the company and how it became a powerhouse in biopharmaceuticals. Touching upon the history of the founding families, he will explain how Roche overcame times of crisis by reinventing itself from one technology to the next.

Jehan Claessens
Principal Scientist analytical sciences, UCB, Belgium

Email: jehan.claessens@ucb.com

Jehan Claessens is an analytical chemist based in Belgium, with deep expertise in mass spectrometry, liquid chromatography, purification techniques, and NMR spectroscopy. With over two decades of experience at UCB, he has played a pivotal role in advancing analytical sciences within the company. His contributions include the development of robust analytical methods for the identification, isolation, quantification, and structural characterization of a wide range of molecules—from small compounds to complex protein assemblies. Currently serving as Principal Scientist in Analytical Sciences at UCB, Jehan leads the Research Analytical Sciences laboratory, where he oversees cutting-edge instrumentation such as cyclic ion mobility systems, high-field NMR, and milligram-scale purification platforms. His work directly supports drug discovery efforts through the development of native MS techniques and innovative approaches to molecular characterization.
Jehan holds a bachelor's degree in chemistry from the Haute École Léonard de Vinci. He recently joined the Belgian Society for Mass Spectrometry Committee as an active and engaged member, contributing to the advancement of the field and fostering collaboration within the scientific community.

Use of cyclic ion mobility mass spectrometry to understand conformational space of Bro5 molecules and its impact on permeability

The ability of drug candidates to permeate the blood-brain barrier (BBB) is a critical determinant in the development of central nervous system (CNS) therapies. Traditional evaluation methods — spanning in vitro, in vivo, and in silico approaches — often yield inconsistent results and present ethical or methodological limitations. To address this, structure-derived molecular descriptors have emerged as essential tools for early pharmacokinetic assessment. Among these, the collision cross section (CCS), obtained via ion mobility mass spectrometry, has recently gained attention as a promising predictor of BBB permeability. CCS encapsulates key molecular features such as mass, volume, and flexibility—properties known to influence drug transport across biological membranes. Notably, the maximum molecular size inferred from CCS values for BBB-permeable compounds aligns with the physical

dimensions of BBB pores, suggesting a potential threshold for passive diffusion. To facilitate rapid and accurate CCS determination, we propose the use of cyclic ion mobility spectrometry (cIMS), which offers enhanced resolving power through extended ion path lengths. This high-resolution technique enables precise separation of isomeric species and provides deeper insights into molecular conformation. In particular, for Bro5 compounds, ion mobility allows the identification of distinct conformational states that may influence their interaction with the BBB. By resolving these conformers, cIMS contributes to a more nuanced understanding of structure-permeability relationships, potentially guiding the design of CNS-active molecules with optimized transport properties. Complementary statistical evaluation of computed CCS values further supports its utility. Our internal development demonstrates that integrating accurate CCS measurements with computational modelling can significantly improve early-stage screening of CNS-active compounds.

Jan Kihlberg
Professor, Department of Chemistry - BMC, Uppsala University, Sweden

Email: jan.kihlberg@kemi.uu.se

In 2013 Jan Kihlberg obtained a chair in Organic Chemistry at Uppsala University, where he is now professor emeritus. His key research interests are to understand what properties convey cell permeability, aqueous solubility and target binding to drugs such as macrocycles and PROTACs in the beyond rule of 5 space, and to translate this knowledge into guidelines for design. He has published more than 210 peer–reviewed publications and book chapters. He established his independent research group at Lund University in 1991, became full professor in Bioorganic Chemistry at Umeå University in 1996, then moved to AstraZeneca R&D in Gothenburg in 2003 while maintaining a research group at Umeå University. At AstraZeneca Kihlberg held the role as Director, Head of Medicinal Chemistry for seven years.

How the interplay between shape and polarity can determine the cell permeability of PROTACs
The discovery of cell permeable and orally bioavailable PROTACs is challenging as their structures locate them close to or beyond the outer limits of oral druggable space, with VHL PROTACs carrying a higher risk than CRBN PROTACs. We have studied series of CRBN and VHL PROTACs for which the linker has a profound impact on passive cell permeability using NMR spectroscopy and MD simulations. Determination of the solution ensembles in a nonpolar solvent revealed that high permeability was correlated to the ability of the PROTACs to adopt folded conformations that have a low solvent accessible 3D polar surface area for both classes of PROTACs. Highly permeable PROTACs were molecular chameleons or adopted congruent conformations in polar and nonpolar environments. Interestingly, two of the VHL PROTACs that differ only by the replacement of two methylene groups in the linker by oxygen atoms displayed vast differences in their cell permeability. This unexpected difference was traced to hydrophobic interactions, which were crucial for conformations adopted by the PROTAC having an alkyl linker both in nonpolar and in polar media. Our results provide mechanistic insight into the impact on the linker for PROTAC cell permeability and suggest that the design of cell permeable VHL PROTACs could focus on linkers that facilitate shielding of polar surface area in the VHL ligand in a nonpolar but not in a polar environment. In addition, MD simulations showed promise for the de novo design of cell permeable PROTACs.

Christian Weinmann
Scientist, Roche, Switzerland

Email: christian.weinmann@roche.com

During his PhD in Molecular Pharmacology at ETH Zürich, he worked on developing in-vitro models for cardiovascular diseases, gaining expertise in CRISPR/Cas, bioassay development, and high-throughput screening methods. Building on a strong foundation in organic and analytical chemistry, established through a Bachelor's degree in Chemistry from the University of Stuttgart and a Master's degree in Chemistry from the University of Freiburg, his interdisciplinary background enables him to tackle complex challenges in drug discovery.
Within his current position as a Phys-Chem Scientist at Roche Pharma Research and Early Development (pRED) in Basel, Switzerland, in the ADME (Absorption, Distribution, Metabolism, and Excretion) chapter, his work focuses on assay development and compound optimization, contributing to the discovery and advancement of innovative therapies.

Dynamic logP - Novel LC/MS method to measure chameleonicity of bRo5 macrocyclic peptides
Macrocyclic peptides (MCPs) are an upcoming modality in drug development as they are able to tackle difficult to target diseases. They can inhibit protein-protein interactions by binding to a larger protein surface without a defined binding pocket that is needed for small molecules. In addition, MCPs can pass membranes and address intracellular targets unlike very large modalities like antibodies. Nevertheless, MCPs are often in the beyond rule-of-five (bRo5) chemical space due to their size or high lipophilicity making it difficult to balance their physico-chemical properties. To overcome these challenges, large bRo5 MCPs need the ability to adapt to their environment to become orally bioavailable which is described as chameleonicity. We present a high-throughput reverse-phase LC/MS method to quantify chameleonicity of bRo5 macrocyclic peptides supported by molecular dynamics calculations of Cyclosporine A, a confirmed chameleon.

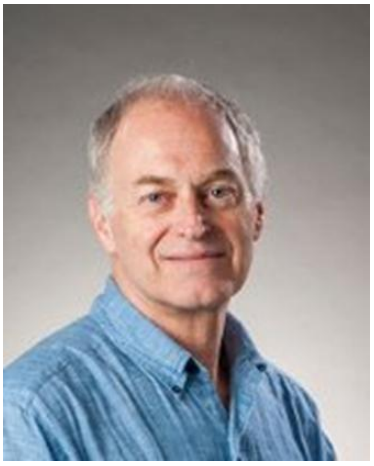
Raquel Rodriguez-Perez
Associate Director & Senior Principal Data Science, Novartis, Switzerland

e-mail: raquel.rodriguez_perez@novartis.com

Dr. Raquel Rodríguez-Pérez is Associate Director & Senior Principal Data Scientist at Novartis Biomedical Research. She leads data science initiatives specializing in AI for pharmacokinetics and drug design. Raquel broadly contributes to enabling data-driven decision-making through the integration of machine learning in early drug discovery at Novartis. She is a biomedical engineer with a PhD in Computational Life Sciences from the University of Bonn, and before working at Novartis she was a Marie Sklodowska-Curie fellow and data scientist at Boehringer Ingelheim. Raquel has authored over 40 peer-reviewed publications and serves as Associate Editor for the journal Artificial Intelligence in the Life Sciences. Overall, her research has focused on predictive modeling and pattern recognition for diverse applications in chemistry and life sciences

Leveraging machine learning for predicting ADME properties of targeted protein degraders
Targeted protein degraders (TPDs) represent a novel therapeutic modality with unique structural and mechanistic features that challenge traditional pharmacokinetic (PK) prediction paradigms. This talk discusses how machine learning (ML) models can be applied to predict key absorption, distribution, metabolism and excretion (ADME) and physicochemical properties of TPDs. I will discuss the modeling strategies used, the challenges posed by TPD submodalities such as molecular glues and heterobifunctionals, and how methodologies such as transfer learning can enhance predictive performance. Taken together, this talk highlights the potential of ML to support TPD design and accelerate drug discovery.

Randy Mrsny
Professor, University of Bath, United Kingdom



Email: rjm37@bath.ac.uk

Randy holds a Professorship in Epithelial Cell Biology where his research explores mechanisms at these anatomical sites that regulate health and disease; he also examines events at interfaces between a delivered drug formulation and the human body. Randy received his undergraduate training in Biochemistry and Biophysics at the University of California at Davis and stayed on at the medical school at that campus as an NIH predoctoral fellow to obtain a PhD in Human Anatomy and Cell Biology. He was awarded a 4-yr NIH postdoctoral fellow that allowed him to focus his studies on Membrane Biophysics at the Institute of Molecular Biology at the University of Oregon. He led the peptide biology group at ALZA for three years and subsequently the drug delivery/biology group at Genentech for 12 years before leaving to start the biotech companies Trinity Biosystems and Applied Molecular Transport while simultaneously returning to academics, initially at Cardiff University before moving to his current post at Bath.

Biological Strategies for the Oral Delivery of Poorly Absorbed Therapeutics

The intestinal epithelium provides the critical limiting barrier for the uptake of therapeutics administered to the small intestine. We have identified endogenous mechanisms to enhance oral bioavailability, one being appropriate for drugs in the size range of peptides and the other better suited for larger therapeutics agents such as proteins. We designed a series of small, stable, membrane-permeable inhibitor of myosin light chain phosphatase (PIP) peptides that dynamically activate the paracellular pathway used by mammals to absorb incompletely digested dietary peptides and saccharides. We show how administration of a PIP peptide to the luminal surface of the small intestine of rats greatly enhances the systemic delivery of a simultaneously dosed therapeutic peptide or poorly absorbed small molecule. The other technology that will be described is the vesicular transcytosis pathway used by exotoxin proteins secreted by certain bacteria, some of which are commonly resident in the human intestine. A non-toxic, poorly immunogenic element of the cholix (Chx) exotoxin from *Vibrio cholera* has been shown to safely and efficiently deliver a conjoined cytokine (interleukin 10) to the intestinal lamina propria in several Phase II studies. Use of Chx to deliver a protein therapeutic, however, has been severely limited by the lack of an efficient mechanism to separate the Chx carrier from a conjoined cargo. We recently demonstrated how inclusion of the C_H2 element of human IgG1 to the Chx carrier solves this issue when using a consensus furin cleavable sequence scission strategy,

and that this approach can be used for some of the other known exotoxins. Finally, we use our understanding of events involved in exotoxin transcytosis to design a ”humanized” format that can access the pathway co-opted by exotoxins to produce a promising oral protein delivery technology that, due to characteristics of vesicles involved in this transcellular trafficking, should also be able to deliver monoclonal antibodies and RNA-based drugs.

Stephane Rodde

Principal Scientist, Biomedical Research, Global Discovery Chemistry, Analytics and Separation, Novartis (Switzerland)

Email: stephane.rodde@novartis.com

Stephane obtained his BSc degree in Analytical Chemistry from the University of Paris XI in France. His journey as physchem expert started in Bernard Faller's lab part of the Metabolism and Pharmacokinetics group where he was mainly focused on measuring ionization constants supporting all drug discovery projects from Novartis in Basel. His scope of action expanded to all classical physchem measurements, and he was co-author of multiple papers, partnering with CADD experts in building models and validating QM-based methods.
Currently Principal Scientist, Stephane is leading the PhysChem lab in Basel where he has been working for more than 16 years. His main goal primarily focused on applying the solubility diagnosis concept to support projects in identifying the main factor impacting the solubility of their chemical series. For doing this, he focused on automating and optimizing the sample preparation as well as the data analysis, using LC-HRMS. More recently, new modalities came with new challenges and together with his team, he is deploying alternative approaches to characterize these molecules.

Next-generation physicochemical assays to characterize new modalities
Modern medicines are becoming more complex, moving beyond traditional small molecules to include new types like PROTACs, peptides, and radioligand therapies. These innovative treatments offer exciting possibilities for tackling diseases that were once considered "undruggable." However, their complexity also brings new challenges: the standard laboratory tests used to measure properties like solubility, acidity, and how easily a drug dissolves in fat or water often don't work well for these next-generation medicines. This presentation explains why scientists need to rethink and redesign these tests. It highlights real-world examples such as PROTACs and radioligand therapies where traditional methods fall short. It describes new approaches that are being developed to better understand and measure these advanced drugs. These include improved chromatography techniques, new ways to explore how molecules behave in the body, and more flexible testing protocols.

Posters

Number	Poster
1	Adelaide Savoy **Using Forward Linear Scattering for Highly Material-Saving Solubility Measurements**
2	Andrew Chervenak, Laurence Philippe-Venec **Evolution of Lipophilicity Assays within the BRo5 Space**
3	Andrius Sazanovas **A Comprehensive Evaluation of ACD/LogD v2025 on Pharmaceutical Compound Set**
4	Andrius Andrius Sazanovas **Quantitative Model of P-glycoprotein Substrate Specificity And Its Augmentig Effect on Prediction of Oral Bioavailability for bRo5 Compounds**
5	Antonio Viayna **Insights from SAMPL9 Blind Challenge: Tolune/Water Partition Coefficient (log P) Prediction Using ML and IEF-PCM/MST Models** Caroline Carrie
6	Apostolos Tsoumanis **Bioactive Peptides from Invasive Alien Fish: Structural Characterization and Antioxidant Potential**
7	Caroline Carrie **Structure-Property Relationships in Cryosprayed Liraglutide Microparticles**
8	Christian Weinmann **Dynamic logP - Novel LC/MS Method to Measure Chameleonicity of BRo5 Macrocyclic Peptides**
9	Chrysanthos Stergiopoulos **Stabilization and Oral Delivery of Oxytocin via PLA–BSA Core and Methacrylic Acid Co-polymer Coating**
10	Elisabet Fuguet **Development and Characterization of a Biomimetic HPLC Column with Immobilized MALT1 for Drug Discovery Applications**
11	Francesco Castagnini **Chromatographic Methods for Physico-Chemical Evaluation and Permeability Prediction in Early Drug discovery**
12	Huy Nguyen **Automating pKa Measurements - Both Hardware and Analytics**
13	Jan Brummund **Nanoforming - The Single Pill Technology: High Drug Load Nanocrystalline Tablets Enabling Patient-Centric Therapies**
14	Janis Niessen **The Effect of Drug-Rich Colloids on the Intestinal Apsorption of Two Proteolysis Targeting Chimeras (PROTACs) in Rats**
15	Jitka Kalasova **Second Harmonic Scattering in Hight-Throughput Evaluation of Solubility, Supersatiration and Self-Assembly Phenomenon in Aqueous Drug System**

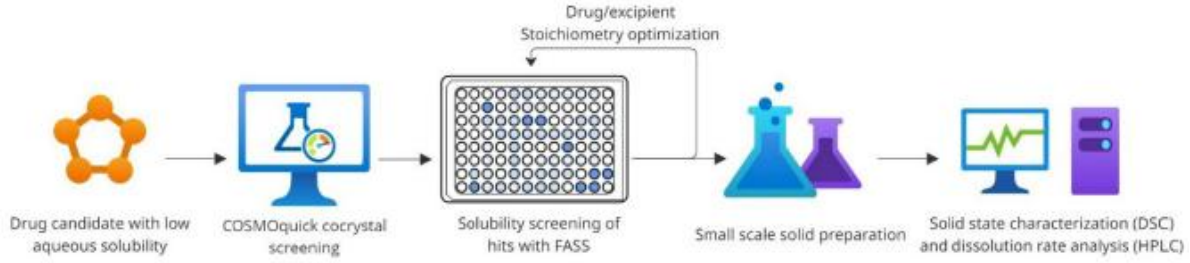
Number	Poster
16	Juliia Kuziv **Assesing PROTAC Permeability and Protein Binding: Challenges and Assay Optimization**
17	Katy Barnes **Measuring Plasma Protein Binding in Diverse Modalities – Key Considerations**
18	Krzesimir Ciura **Machine-Learning Driven Prediction of Phospholipid Affinity Based on IAM-HPLC Data**
19	Malte Mildner **In-situ Setup for Drug Permeation Screening by NMR Spectroscopy**
20	Nele-Johanna Hempel-Hojgaard **To Be or Not to Be a Peptide? Properties of Small Cyclic Peptides**
21	Nora Kern, Maximilian Koy **From Big Pharma to CRO PhysChem Lab: Assays Prediction and New Technologies**
22	Paula Vidal **Biomimetic Liposome Frameworks for Drug-Membrane Interaction Studies**
23	Petra Misetic **Plasma Protein Binding of PROTACs by Biomimetic Chromatography**
24	Rafal Bachorz **Expanding ADMET Predictor's Chemical Space: Enhanced bRo5 and Chameleon Molecule Predictions for HTPK**
25	Rebeca Ruiz **Study of the Influence of Biorelevant Media on the Solubility of Benzathiazide, Isoxicam and Piroxicam in the Presence of Several Excipients**
26	Sina Simon **Can We Predict the Unbound Fraction in Tissue from Physicochemical and Binding Parameters**
27	Susana Amézqueta **Interactions of antidepressants with human serum albumin**
28	Magda Swedrowska **Advancing Oral PROTAC Delivery: Biopharmaceutic Challenges and Opportunities**

Adelaide Savoy (adelaide.savoy@fhnw.ch)

Using Forward Linear Scattering for highly material-saving solubility measurements.

A. Savoy[1], M. Kuentz[1]
[1]*Institute of Pharma Technology and Biotechnology, FHNW, 4123 Muttenz*

Introduction: A novel pulsed ultrafast laser-based technology has been developed by a inno-suisse project (EASY) that will be commercialized by Oryl Photonics. This patented technology combines Second Harmonic Light Scattering (SHS) with Forward Linear Light Scattering (FLS). SHS leverages the solvent redistribution method [1] to provide structural insights into aggregates present in solution. FLS is distinct from traditional linear and dynamic light scattering techniques and detects light in-line with the laser path through a narrow iris. Preliminary results suggest a high sensitivity and further investigations and benchmarks are being conducted within the Fast and Accurate Solubility for Sustainability (FASS) project, a european collaboration funded by a HORIZON grant.
Aim: We aim to find applications within the pharmaceutical and chemical industry for this promising technology, more specifically in pre-formulation, where studies rely on limited quantities of compound. In this specific field, there is a lack of early-screening methods for the selection of suitable excipients in the development of co-crystals or co-amorphous solids [2] with enhanced properties, such as increased dissolution rate, prolonged shelf life or increased solubility in aqueous media. We aim to demonstrate that, by combining computational predictions and efficient screening tools, the pre-formulation of co-crystal and co-amorphous solids will become more sustainable, fast and thorough.
Methods: Combination of computational (COSMOquick) and experimental methods. The novel laser-based instrument is based on forward linear light scattering. The established tools are HPLC and DSC.
Results: We are proposing and studying the relevance and reliability of a new workflow for the screening of excipients for the preparation of co-crystals and co-amorphous solid dispersion. The first step is an optional computational screening. COSMOquick was chosen, as it has been demonstrated to yield accurate prediction for co-crystal screening [3]. The identified promising excipients then undergo further experimental screening. The miniaturized FLS technology would have a high potential as a first experimental screening method for excipient selection and optimization (see Figure below). Preliminary results are promising but it is essential to establish the limitations and restrictions of this novel screening tool. Following steps are the established experimental screening steps, namely DSC analysis and dissolution rate measurement by HPLC from the prepared co-crystal or co-amorphous solid dispersion.



Conclusion: Screening of excipients with FLS requires a minimal amount of drug (as little as 2µL of a stock solution per replica) with a library of excipients that can be readily available as stock solutions. It would also enable a faster and more thorough screening of excipients. This workflow could, down the line, be automated with technologies that are compatible with the well plate format.
Keywords: Workflow, Screening, Pre-formulation, Excipient, Solubility, Laser-based analytics.
References:
[1] Oryl Photonics - Solvent redistribution method, https://orylphotonics.com/category/knowledge/?data-id=279
[2] J. Liu, H. Grohganz , K. Löbmann , T. Rades, N.-J. Hempel, Pharmaceutics, 2021, 13, 389.
[3] M. Guidetti, R. Hilfiker, M. Kuentz, A. Bauer-Brandl, F. Blatter, Crystal Growth Design, 2023, 23, 842−852

Andrew Chervenak (achervenak@analiza.com),
Laurence Philippe-Venec (laurence@pic-analytics.com)

Evolution of Lipophilicity Assays within the BRo5 Space

Andrew Chervenak*, Lindsay Depenti*, Caylie Desmarteau*, Justin Longenberger*, Steve Placko*, Laurence Philippe-Venec**
**Analiza Inc, 3615 Superior Avenue, #4407B, Cleveland, OH, 44114*
***PIC Analytics, P.O. Box 192, Dexter, MI, 48130*

The lipophilicity of large molecules is becoming of increasing relevance and interest as a descriptor of permeability. Permeability assays for these molecules encounter significant challenges, including initial solubility, non-specific binding and size of the studied entities. These factors often lead to poor recovery and low reliability of the data obtained from such assays.

• The gold standard Shake-Flask method for lipophilicity measurement at pH 7.4 relies heavily on compound solubility, as it quantifies the concentrations in each non-miscible solvent, and its accuracy can be compromised in case of high lipophilicity.

• The ElogD assay, using HPLC/UV detection, has been developed by Franco Lombardo(1) to address the solubility issues and to expand the range of measurable lipophilicity. This chromatographic assay is based on hydrophobic interactions, described by retention times, between solutes and an amide-C16 support in different contents of organic solvent such as methanol. The resulting logk'w in aqueous buffer at pH 7.4 leads to the determination of final lipophilicity data against a calibration curve of known standards.

• The ElogD assay has furthermore been optimized into the alphalogD(2) assay, taking advantage of a stationary phase utilizing Semi-Porous Particle technology and allowing the use of a less complex mobile phase, enhancing throughput while being "friendlier" on the analytical instrumentation.

The comparison of the two chromatographic assays highlights different behaviors of some large molecules that can show aggregation according to the mobile phase. The discussion will also develop the notion of chameleonicity, or change of conformation, of the large molecule, as well as the competition between hydrophobic interactions and solubility in organic solvent in case of very high lipophilicity.

Finally, the alphalogD assay is broadly applicable to basic, neutral and weak acidic compounds. Can this assay be applied to measure lipophilicity of large acidic molecules?

(1) F. Lombardo, M.Y. Shalaeva, K.A. Tupper, F. Gao, "ElogDoct: A tool for lipophilicity determination in drug discovery. 2. Basic and neutral compounds", *J. Med. Chem., 2001, 44, 2490-2497.*
(2) D. Katz, K. Fike, J. Longenberger, S. Placko, L. Philippe-Venec, A. Chervenak, "alphalogD determination: an optimized reversed-phase liquid chromatography method to measure lipophilicity on neutral and basic small and beyond-rue-of-five compounds", *J. Chrom. A, 2002, 1674, 463146.*

Andrius Sazanovas (andrius.sazonovas@acdlabs.com)

A Comprehensive Evaluation of ACD/LogD v2025 on a Pharmaceutical Compound Set

A. Sazonovas 1,2, K. Lanevskij 1,2, R. Didziapetris 1,2
1 VšĮ „Aukštieji algoritmai“, A.Mickevičiaus 29, LT-08117 Vilnius, Lithuania
2 ACD/Labs, Inc., 8 King Street East, Toronto, Ontario, M5C 1B5, Canada

Lipophilicity, which is often expressed in terms of 1-octanol/water partitioning coefficient log*P*, or the corresponding pH-dependent distribution coefficient log*D*, is one of the key physicochemical characteristics of any new drug candidates, as it has a major influence on a variety of the compounds' properties constituting their ADME, pharmacokinetic, and drug safety profiles. Widely available *in silico* tools for predicting these properties are mostly based on experimental data for simple organic chemicals and marketed drugs. Consequently, as drug discovery projects are moving to increasingly novel regions of chemical space, utility of existing methods becomes more and more questionable. In several previously published evaluation studies, the mean log*P* prediction error for *in house* compound libraries of pharmaceutical companies was shown to exceed 1 log unit by almost all methods. Prediction of log*D* is even more challenging, as it requires accurate knowledge of both log*P* of neutral form and distribution of ionic forms of the compound in the relevant pH range.

The main objectives of the current study were:

(1) Collecting a data set of experimental log*D* values from recent publications dealing with novel congeneric compound series from drug discovery projects;
(2) Evaluating the performance of ACD/LogD predictor for the newly collected molecules using different combinations of available log*P* and p*K*a calculation algorithms;
(3) Investigating the potential for improving prediction accuracy for unknown compound classes by application of automated model training.

The compiled data set consisted of >1000 log*D* values measured at physiological pH conditions. According to the initial validation results, the highest accuracy of predictions based on the models employing only built-in compound libraries can be achieved using a combination of ACD/LogP Consensus and ACD/pKa Classic algorithms, yielding RMSE of about 1 log unit. However, utilizing the automatic training feature of ACD/LogP GALAS algorithm by the means of stepwise addition of collected data to the model self-training library allowed decreasing the RMSE of predictions for the reserved validation set to as low as 0.75 log units. These results demonstrate that performing experimental measurements for some selected representatives of a novel chemical series can be used to gradually adapt ACD/LogP and ACD/LogD predictors to provide reliable property estimates for the entire new class of compounds.

Andrius Sazanovas (andrius.sazonovas@acdlabs.com)

Quantitative Model of P-glycoprotein Substrate Specificity and Its Augmenting Effect on Prediction of Oral Bioavailability for bRo5 Compounds

K. Lanevskij 1,2, R. Didziapetris 1,2, A. Sazonovas 1,2
1 VšĮ „Aukštieji algoritmai“, A.Mickevičiaus 29, LT-08117 Vilnius, Lithuania
2 ACD/Labs, Inc., 8 King Street East, Toronto, Ontario, M5C 1B5, Canada

In silico studies of P-glycoprotein (P-gp) mediated efflux of pharmaceuticals usually treat it as a binary endpoint and only attempt to classify molecules as P-gp substrates or non-substrates. However, recently we have adopted a more advanced statistical approach that can circumvent the lack of accurate quantitative data by employing censored regression-based machine learning technique and can make use of experimental measurements recorded as open-ended intervals, or so called censored data points. Such models, parameterized using a minimal set of relevant physicochemical descriptors (lipophilicity, ionization, molecular size and topology), are capable of producing predictions in the form of numerical Efflux Ratio (*ER*) values, *i.e.*, the ratios of bidirectional permeation rates observed in polarized transport assays.

The proposed approach can also be extended by applying an estimate of passive permeability in Caco-2 cells to split measured ER values into the contributions of passive and active transport routes, and subsequently fitting the model to represent pure P-gp efflux effect. Both model types achieve similar predictive power on the qualitative classification task (> 75% overall accuracy at a threshold of *ER* > 2 for substrates), while providing a clear basis for mechanistic interpretation. Practical utility of quantitative predictions is demonstrated by using calculated *ER* values to augment predictions of passive intestinal permeability for a set of high molecular weight ligand-directed degrader molecules. The resulting approach was able to classify the compounds into high and low bioavailability categories with AUC = 0.87, illustrating that the employed mechanistic calculations are applicable even to complex molecules belonging to beyond rule-of-five chemical space.

Antonio Viayna (toniviayna@ub.edu)

Insights from the SAMPL9 Blind Challenge: Toluene/Water Partition Coefficient (log P) Prediction Using ML and IEF-PCM/MST models

A. Viayna, Barcelona/ES, W. J. Zamora, San José/CR, S. Pinheiro, San José/CR, C. Curutchet, Barcelona/ES, L. Bisbal, Barcelona/ES, R. Ruiz, Forest Row/UK, C. Ràfols, Barcelona/ES, F. J. Luque, Barcelona/ES,
Dr. Antonio Viayna, Department of Pharmacy, Pharmaceutical Technology and Physical Chemistry, Faculty of Pharmacy and Food Sciences, Universitat de Barcelona, Av. Joan XXIII, 27–31, 08028 Barcelona

Lipophilicity is a key property in drug design, influencing permeability, distribution, and bioavailability. While the octanol/water partition coefficient (logP) is widely used, the toluene/water system (logPtol/w) is more sensitive to conformational and intramolecular hydrogen bonding effects. [1] Within the SAMPL9 blind prediction challenge, we evaluated 16 small molecules using two complementary approaches to predict logPtol/w.

The first approach involved machine learning (multiple linear regression (MLR) and random forest (RFR), trained on 252 experimental logPtol/w values. Over 450 molecular descriptors were computed and statistically filtered to select 11 optimal variables. Both models showed strong correlation ($r^2 > 0.9$) and RMSE < 1 logP unit on externalpredictions.

The second strategy used a quantum chemistry-based solvation model (IEF-PCM/MST) with DFT (B3LYP/6-31G(d)) to estimate solvation free energies in toluene and water. [2] Conformer sampling and Boltzmann weighting were applied, with extended parametrization for underrepresented atom types. Though computationally demanding, this method offered deeper physical insight into solvent–solute interactions.

Both approaches performed competitively compared to empirical models like LSER.[3] Machine learning provided a rapid screening tool, while quantum solvation enabled mechanistic interpretation. Together, they represent a promising hybrid strategy for accurately predicting partitioning behavior in drug discovery. [4]

Literature:
[1] G. Caron, M. Vallaro, G. Ermondi, Drug Discov. Today Technol. 2018, 27, 65–70
[2] F. J. Luque, et al., Phys. Chem. Chem. Phys. 2003, 5, 3827.
[3] A.H. Abraham, H.S. Chadha, G.S. Whiting, R.C. Mitchell, J. Pharm. Sci. 1994, 83, 1085–100
[4] W. J. Zamora, et al., Phys. Chem. Chem. Phys. 2023, 25, 17952

Apostolos Tsoumanis (apostolostsoumanis@gmail.com)

Bioactive Peptides from Invasive Alien Fish: Structural Characterization and Antioxidant Potential

Apostolos TSOUMANIS, Chrysanthos STERGIOPOULOS, Ioanna BILALI, Maria AVGOUSTINAKI, Nikoletta TRICHA, Sofia PAPADAKI, Magdalini KROKIDA
PRINSUS IKE Technoblastos, Iroon Polytechneiou 9, 157 80 Athens, Greece

Introduction:
Invasive alien fish species pose serious ecological risks but represent an underexplored protein source. Their valorization into bioactive peptides offers a sustainable route to generate high-value molecules with defined physicochemical and biological properties.

Aims:
This study assessed the potential of invasive fish proteins as precursors of antioxidant peptides, focusing on their physicochemical profiling, stability, and molecular characteristics relevant to functional and health-promoting applications.

Materials and Methods:
Proteins were enzymatically hydrolysed using alcalase under controlled pH and temperature. Hydrolysates were fractionated via molecular weight cut-off membranes, and peptide profiles were evaluated by SDS-PAGE and HPLC-DAD. Antioxidant capacity was quantified using radical scavenging assays (DPPH, ABTS) and the ferric reducing antioxidant power (FRAP) assay. Solubility, stability, and size distribution of peptide fractions were also assessed to establish their physicochemical behaviour.

Results:
Hydrolysates demonstrated significant antioxidant activity, with low-molecular-weight fractions (<3 kDa) showing the highest radical scavenging capacity (DPPH inhibition >70%). Peptide profiling indicated distinct solubility and stability patterns, highlighting the potential for controlled bioactivity.

Conclusion:
The biomass of invasive alien fish can be transformed into peptide-based bioactives with defined physicochemical signatures and strong antioxidant potential. These findings highlight opportunities for sustainable valorization in food, nutraceutical, and feed applications, while providing insights into peptide profiling relevant to bioavailability and functional performance.

Keywords: invasive fish, peptide profiling, antioxidant peptides, physicochemical characterization, sustainable valorization

Acknowledgment: This research has received funding from the European Union's Horizon Europe Framework Programme (HORIZON) under the Marie Skłodowska-Curie grant agreement No 101131441-VALIAS.

Caroline Carrie (ccarrica80@alumnes.ub.edu)

Structure-Property Relationships in Cryosprayed Liraglutide Microparticles

Caroline Carrie1,2, Pierandrea Esposito2, Rafel Prohens1, Yolanda Cajal3, Francesc Rabanal4, Hana Danan2
1Departament de Farmacologia, Toxicologia i Química Terapèutica, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona (UB), Barcelona, Spain
2Sitec Pharmabio, Barcelona, Spain
3Departament de Farmàcia i Tecnologia Farmacèutica, i Fisicoquímica, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona (UB), Barcelona, Spain
4Secció de Química Orgànica, Dept. de Química Inorgànica i Orgànica, Facultat de Química, Universitat de Barcelona (UB), Barcelona, Spain

Oral delivery of peptides represents a major challenge in drug development, mostly because of molecules instability, sensitivity to pH and to proteolytic enzymes, which collectively result in poor bioavailability.

This work presents part of the physico-chemical characterization of lipid-based microparticles containing liraglutide, prepared by a cryospraying process based on liquid CO2 expansion. In particular, we focused on physicochemical characterization including surface tension experiments, monolayer experiments with excipients, XPS surface analysis of microparticles and microscopy images. The latter showed that liraglutide interacts differently with excipients monolayers and, although the images showed a homogeneous matrix composition, liraglutide amount on the surface of microparticles varies amongst formulations. In vitro release experiments at intestinal pH 6.8 and gastric pH 1.2 showed a very low release at gastric pH and a faster and complete release at intestinal pH where release profiles varied greatly between formulations. Surface tension studies showed that liraglutide micellar concentration is situated at approximately 0.2 µM and these tensioactive properties seem to impact interaction with excipients. XPS analysis showed that liraglutide is present in a greater proportion on the surface of L017 microparticles compared to formulations L009 and L019. As a result, release studies showed that liraglutide release profile from formulations L009, L017 and L019 differ greatly at intestinal pH 6.8. Consequently, excipient combination can affect surface and bulk properties of microparticles, that may modify peptide release in different physiological conditions.

These results may guide the development of optimized lipid-based liraglutide microparticles with enhanced bioavailability.

Christian Weinmann (christian.weinmann@roche.com)

Dynamic logP - Novel LC/MS method to measure chameleonicity of bRo5 macrocyclic peptides

Christian Weinmann1, Franz Waibl1,2, Silvan Kaeser1, Fabian Dey1, Björn Wagner1 and Janneke Keemink1
1 Roche Pharma Research and Early Development, Pharmaceutical Sciences, Roche Innovation Center Basel
2 Department of Chemistry and Applied Biosciences, ETH Zürich

Macrocyclic peptides (MCPs) are an upcoming modality in drug development as they can tackle difficult-to-drug diseases. They can inhibit protein-protein interactions by binding to a larger protein surface without a defined binding pocket that is needed for small molecules. In addition, MCPs can pass membranes and address intracellular targets unlike very large modalities like antibodies.

Nevertheless, MCPs are often in the beyond rule-of-five (bRo5) chemical space due to their size or high lipophilicity making it difficult to balance their physico-chemical properties. [1] To overcome these challenges, large bRo5 MCPs need the ability to adapt to their environment to become orally bioavailable which is described as chameleonicity. [2,3]

We use RP-LC/MS and measure the retention time of non-chameleonic small molecule standards with a known shake-flask logPow to determine the logP of bRo5 MCPs in two environments. Therefore, we vary the water:acetonitrile ratio of the mobile phase favoring H-bonding with the solvent (30:70, logP70) or intramolecular H-bonding (5:95, logP95). The difference between the two environments ΔlogP95-70 is a measure of the chameleonicity. The known chameleon Cyclosporine A shows a temperature dependent increase in lipophilicity in environments favoring intramolecular H-bonding while the lipophilicity of non-chameleonic small molecules is environment independent.

This novel RP-LC/MS assay provides a framework to characterize the dynamic lipophilicity of MCPs in different environments and measuring chameleonicity as ΔlogP95-70. The method aims to improve the understanding of structure-chameleonicity relationships, predict ADME parameters, and intentionally design chameleonic bRo5 macrocyclic peptides using machine learning to improve oral bioavailability.

References:

[1] V. Poongavanam et. al. Nature Reviews Chemistry 2024, 8, 45-60
[2] A. Whitty et al., Drug Discovery Today 2016, 21, 5, 712-717
[3] M. Mizuno-Kaneko et al., ACS Med. Chem. Lett. 2023, 14, 1174−1178

Chrysanthos Stergiopoulos (chrisxp3@hotmail.com)

Stabilization and Oral Delivery of Oxytocin via PLA–BSA Core and Methacrylic Acid Co-polymer Coating

C. Stergiopoulos*, M. Stramarkou, G. Frakolaki, A. Tsoumanis, N. Tricha, S. Papadaki, M. Krokida
PRINSUS IKE Technovlastos, Iroon Polytechneiou 9, 157 80 Athens, Greece

The stabilization and controlled release of bioactive peptides in harsh gastrointestinal (GI) environments remain critical challenges for oral delivery systems. Peptides such as oxytocin are prone to rapid degradation through acidic hydrolysis and enzymatic activity in the stomach, which significantly reduces their bioavailability [1]. To address this, rationally designed polymeric carriers with tailored physicochemical properties are required to protect peptides during GI transit and ensure targeted intestinal release. In this study, oxytocin was selected as a model compound due to its therapeutic and nutraceutical potential, as well as its known instability under gastric conditions [2, 3]. A coaxial electrospinning approach was employed to produce core–shell nanofibers, with poly(lactic acid) (PLA) serving as the hydrophobic shell and bovine serum albumin (BSA) acting as the core stabilizer. PLA provided a hydrophobic diffusion barrier to limit solvent penetration, while BSA contributed molecular affinity toward oxytocin, reducing peptide unfolding and aggregation. A design of experiments (DoE) methodology was employed to optimize polymer concentration and flow parameters, thereby ensuring systematic control over encapsulation efficiency (EE%) and fiber morphology. The optimized formulation yielded smooth, defect-free fibers with diameters below 400 nm and encapsulation efficiency exceeding 80%. The amphiphilic PLA–BSA environment promoted consistent oxytocin loading (~0.35 mg/run) and enhanced stability. To further improve functionality, the fibers were coated with a methacrylic acid copolymer (Eudragit®), introducing pH-responsive release properties. At gastric pH, the polymer remained protonated and insoluble, effectively suppressing premature peptide release. Under intestinal pH, ionization triggered hydration and diffusion, enabling controlled release. In vitro digestion assays confirmed this mechanism: uncoated fibers released ~45% of oxytocin under gastric conditions, whereas coated fibers restricted release to <10%. Upon transfer to intestinal conditions, Eudragit-coated fibers achieved a cumulative release of more than 70% within 4 hours, demonstrating selective and efficient delivery. This dual protection strategy-hydrophobic shell stabilization, protein-assisted encapsulation, and methacrylic copolymer coating-synergistically modulates release kinetics while maintaining peptide integrity. The platform offers a robust physicochemical framework for oral peptide delivery and has broad potential for stabilizing other labile biomolecules in pharmaceutical, nutraceutical, and functional food applications.

Literature

[1] Chen, G., 2025. Advances in the oral delivery of protein and peptide drugs. *Pharmaceutics*, 17, p.616.
[2] Zada, M.H., Rottenberg, Y. & Domb, A.J., 2022. Peptide-loaded polymeric nanoparticles by non-aqueous nanoprecipitation. *Journal of Colloid and Interface Science*, 622, pp.904–913.
[3] Adediran, E., Vijayanand, S., Kale, A., Gulani, M., Wong, J.C., Escayg, A., Murnane, K.S., & D'Souza, M.J., 2025. Microfluidics-assisted formulation of polymeric oxytocin nanoparticles for targeted brain delivery. *Pharmaceutics*, 17, p.452.

Elisabet Fuguet (elifuguetj@ub.edu)

Development and Characterization of a Biomimetic HPLC Column with Immobilized MALT1 for Drug Discovery Applications

Elisabet Fuguet[1,4], Olivera S. Marković[1,2], Anna Delgado[1], Antonio Llinàs[3], Martí Rosés[1], Clara Ràfols[1],
[1]*Departament d'Enginyeria Química i Química Analítica, Institut de Biomedicina de la Universitat de Barcelona (IBUB), Universitat de Barcelona, Martí i Franquès 1-11, 08028 Barcelona, Spain*
[2]*Department of Chemistry, University of Belgrade – Institute of Chemistry, Technology and Metallurgy – National Institute of the Republic of Serbia, Njegoševa 12, 11000 Belgrade, Serbia*
[3]*DMPK Department, Respiratory and Immunology, R&D BioPharmaceuticals, AstraZeneca, Mölndal, SE-43183, Sweden*
[4]*Serra Húnter Programm, Generalitat de Catalunya, Spain*

In recent years, there has been growing interest in the development and analysis of biomimetic HPLC systems capable of predicting specific biological properties relevant to drug discovery. Such systems rely on the principle of attaching biologically derived stationary phases—such as lipids, membranes, or proteins—to chromatographic columns, thereby mimicking biological environments. As a result, retention measurements in these systems can provide valuable insights into how compounds interact with biological targets. A major advantage of this approach is its versatility, as different biologically relevant stationary phases can be tailored to reproduce specific molecular interactions of interest.

One particularly relevant target for such applications is the mucosa-associated lymphoid tissue lymphoma translocation protein-1 (MALT1), a human paracaspase that plays a central role in immune responses. In addition to its proteolytic activity, MALT1 also acts as a scaffolding protein. Due to these functions, small-molecule MALT1 inhibitors are considered promising therapeutic candidates for the treatment of conditions such as allergic inflammation and autoimmune diseases.

The objective of this study was to design and characterize a Chromolith WP 300 Epoxy HPLC column with immobilized MALT1. Protein immobilization was achieved using the epoxy method. Initially, the column was equilibrated with an immobilization solution, after which MALT1 was dynamically immobilized by circulating the protein solution through the column for 24 hours. The column was then washed to deactivate any remaining epoxide groups.

The resulting column was evaluated for reproducibility, compatibility with various organic solvent concentrations, and its linear dynamic range. Furthermore, known MALT1 inhibitors were injected to investigate the correlation between their biological activity and chromatographic retention. The column's performance was also assessed using the Abraham solvation parameter model.

These findings provide a foundation for the further optimization of MALT1-immobilized columns. The development and characterization of biomimetic HPLC columns containing proteins associated with specific diseases could significantly influence drug discovery, offering numerous potential applications.

Francesco Castagnini (francesco.castagnini@unipr.it)

Chromatographic methods for physico-chemical evaluation and permeability prediction in early drug discovery

Introduction&Aim

Physico-chemical profiling of new chemical entities (NCEs) enables the early identification of promising compounds and the exclusion of those with unfavorable properties for further development. Among these properties, lipophilicity and polarity play a central role in ADME processes. In early drug discovery, the implementation of fast and reliable methods for physico-chemical characterization is essential to accelerate workflows, increase success rates, and reduce late-stage attrition.

This project evaluated two high-throughput chromatographic methods for the assessment of lipophilicity and polarity for 544 Chiesi's compounds belonging to three main projects (75% Ro5 and 25% bRo5). Since these parameters strongly influence passive permeability, the aim was to test the ability of Experimental Polar Surface Area (EPSA) [1,2] and Chromatographic Hydrophobicity Index (CHI) Log *D*7.4 [3,4] to predict permeability measured in the Caco-2 cell culture.

Methods

EPSA: Chirex 3014 column (50×4.6 mm, 5 µm), 40°C; mobile phases: gradient of CO_2 supercritical/20 mM ammonium formate in methanol; flow rate: 4 mL/min; detection: DAD 210–400 nm; instruments: Jasco SFC-4000 HPLC and Advion Expression MS.

CHI Log D7.4: Luna C18 column (30×2 mm, 3 µm), 40°C; mobile phases: gradient 10 mM ammonium acetate/ACN; flow rate: 0.5 mL/min; detection: DAD 210-400 nm; Waters Acquity UPLC coupled to Acquity QDa MS.

Results

There is a non-uniform compounds distribution in the phys-chem space, with well-defined clusters of class 1 enrichment (permeability > 50 nm/s), while Lipinski's rule fails to discriminate effectively across the dataset. Logistic regression provides a reliable interpolation of the empirical trends, enabling the development of predictive probability models. For EPSA there is an inverse relationship with class 1 probability (permeability > 50 nm/s): values below 100 Å² are associated with moderate-high permeability, whereas values exceeding 130-140 Å² notably decrease permeability, underscoring the detrimental impact of elevated exposed polarity. For CHI Log *D*7.4, the probability increases between 1.5 and 3.5, with a local maximum around 3.5, indicating that moderate lipophilicity is associated with higher permeability, whereas lower or higher values are not as strongly correlated.

Conclusions

EPSA and CHI Log *D*7.4 are confirmed as valuable descriptors to estimate passive permeability and to distinguish permeable from non-permeable compounds, especially for bRo5 compounds. Future work will focus on tailoring predictive models to different chemical classes and defining series-specific thresholds, thereby improving predictive accuracy. The results provide a solid basis for the development of iterative, data-driven models to be further validated in upcoming studies.

References

1. Yue-Ting Wang, Edward Price, Mei Feng, Jason Hulen, Stella Doktor, David M. Stresser, Estelle M. Maes, Qin C. Ji, and Gary J. Jenkins, *High-Throughput SFC-MS/MS Method to Measure EPSA and Predict Human Permeability,* Journal of Medicinal Chemistry 2024 67 (16), 13765-13777, DOI: 10.1021/acs.jmedchem.4c00571

2. Gilles H. Goetz, Laurence Philippe, and Michael J. Shapiro, *EPSA: A Novel Supercritical Fluid Chromatography Technique Enabling the Design of Permeable Cyclic Peptides,* ACS Medicinal Chemistry Letters 2014 5 (10), 1167-1172, DOI: 10.1021/ml500239m

3. Valko, Klara. (2019), *Application of biomimetic HPLC to estimate in-vivo behavior of early drug discovery compounds,* Future Drug Discovery, 1, DOI:10.4155/fdd-2019-0004

4. Klára L. Valkó, *Lipophilicity and biomimetic properties measured by HPLC to support drug discovery*, Journal of Pharmaceutical and Biomedical Analysis, Volume 130, 2016, Pages 35-54, ISSN 0731-7085, DOI.org/10.1016/j.jpba.2016.04.009

Huy Nguyen (nguyen.huy_quoc@gene.com)

Automating pKa measurements – both hardware and analytics

1Huy Q. Nguyen, 1Newton Wu, 2Dhireshan Gadiagellan
1 Discovery Chemistry, Analytical Research, Genentech, 1 DNA Way, South San Francisco, CA, USA
2 UK Robotics, Manchester, UK

Acquiring pKa measurements is quintessential in the drug discovery process to optimize molecular design and tune for specific activity. Certainly, there are commercially available models to predict pKa, but these can fall short given how fast the molecular space can change. Giving a clear need to generate data to feed these pKa models for improved predictions. Which leads to running titration experiments to actually measure pKa values. However, the throughput can be slow and equipment maintenance can be challenging. Here we propose a novel robotic system built from readily available components to run titration experiments and measure pKa using a UV-metric method. In addition, we propose a machine learning/AI method for directly extracting pKa data from the titration experiments.

Jan Brummund (jan.brummund@nanoform.com)

Nanoforming – the single pill technology: High drug load nanocrystalline tablets enabling patient-centric therapies

Authors: J. Brummund1, C.P. Worrall1, T. Solymosi1, P. Noppari1, S. Ikävalko1, M. Kaasalainen1, B. Derham1, P. Räsänen1, E. Hæggström1
Affiliation : *1Nanoform Finland Oyj, Helsinki, Finland*

Purpose
Enzalutamide and apalutamide have transformed treatment of advanced prostate cancer but are poorly soluble in their bulk crystalline forms and thus marketed as amorphous solid dispersions (ASDs). While ASDs improve solubility, they sacrifice drug loading, resulting in large tablets and high pill burden.

Method
Controlled Expansion of Supercritical Solutions (CESS®) is a nanocrystallization technology using supercritical CO_2 without additional solvents. It enables precise control of particle size, morphology, and crystalline form. [1-3] Atovaquone was used as a model compound to demonstrate tunable polymorphs. Enzalutamide and apalutamide were selected for comparison against marketed ASD products.

Results
- Nanocrystals produced via CESS achieved pharmacokinetic profiles equivalent to ASDs with fewer excipients.
- Atovaquone polymorphic form could be tuned via process parameters without excipients.
- Nanoenzalutamide tablets achieved > 3x higher drug loading vs Xtandi®, enabling a single 160 mg tablet instead of four 40 mg tablets.
- Nanoapalutamide tablets were up to 60% smaller than Erleada®, reducing pill burden.

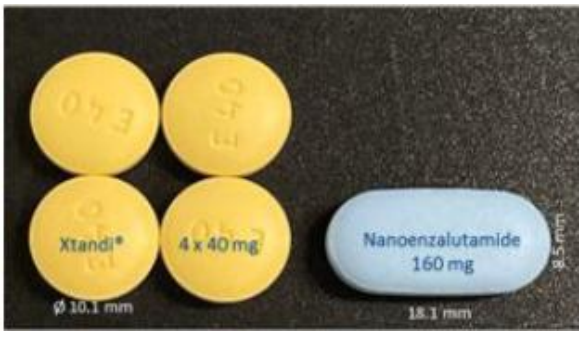


Figure 1: Comparison of Xtandi® (commercial ASD, left) and Nanoenzalutamide (right) tablets.

Conclusions
CESS® nanoforming is a clinically validated tool for poorly soluble APIs. It enables high-drug-load nanocrystalline tablets that reduce pill size and burden, supporting single-pill therapies and improved patient adherence.

References
1 Pessi et al, ***J Pharm Sci***, 2016, DOI: 10.1016/j.xphs.2016.05.022
2 Kauppinen et al, ***J Pharm Biomed Anal***, 2021, DOI: 10.1016/j.jpba.2022.115169
3 Lakio et al, ***Pharm Res***, 2023, DOI: 10.1007/s11095-023-03624-8

Janis Niessen (janis.niessen@uu.se)

The effect of drug-rich colloids on the intestinal absorption of two proteolysis targeting chimeras (PROTACs) in rats

Janis Niessen[a], Hans Lennernäs[a], Mikael Hedeland[b], Thomas Borchardt[c], Mirko Koziolek[d], David Dahlgren[a]
[a]*Department of Pharmaceutical Bioscience, Translational Drug Discovery and Development, Uppsala University, Sweden;*
[b]*Department of Medicinal Chemistry, Analytical Pharmaceutical Chemistry, Uppsala University, Sweden;*
[c]*Product Development Science & Technology, AbbVie Inc., North Chicago, United States;*
[d]*Synthetic Molecules CMC R&D, AbbVie Deutschland GmbH & Co. KG, Ludwigshafen am Rhein, Germany*

Aim: PROTACs exhibit a unique and promising pharmacology. However, this comes with physicochemical properties exceeding the 'drug-like' chemical space, often resulting in limited oral bioavailability due to solubility, dissolution, and/or permeability limitations. This study aimed to address the current lack of mechanistic understanding of the biopharmaceutic processes governing PROTAC intestinal absorption, which impedes the rational development of orally bioavailable PROTACs. We moreover evaluated the *in vivo* potential of particle-generating formulations to enhance the intestinal uptake of two PROTACs, ARV-110 (812 Da, LogP 3.6) and ARV-471 (724 Da, LogP 5.9).

Methods: Dose-escalation studies with particle-generating formulations were conducted to investigate the dose-dependent intestinal absorption and bioavailability of the model PROTACs ARV-110 and ARV-471 in rats. Data on systemic exposure after intraduodenal bolus administration were complemented by dose-dependent *in vitro* determinations of amorphous solubility in relevant media, solubilization dynamics, and characterization of the size and solid state of emerging drug particles. Numerical deconvolution enabled mechanistic analysis of *in vivo* absorptive flux in relation to particle concentration, size, and solid state of emerging particles. To directly test the proposed hypothesis that in situ-formed, drug-rich colloids enhance absorption of poorly soluble drugs, a colloid-forming formulation was compared with a non-colloidal counterpart with identical composition and free drug concentration.

Results and discussion: The two PROTACs consistently formed luminally stable, amorphous drug-rich colloids once their amorphous solubility was exceeded *in vivo*. These colloids enhanced absorptive flux up to 7-fold and bioavailability 2.4-fold compared with a non-colloidal formulation. Dose escalation revealed sustained, dose-proportional absorption up to ~15-fold above amorphous solubility, driven by particle drifting across the aqueous boundary layer. At higher doses, the effect was either capped by the saturation of the amorphous solubility in the aqueous boundary layer of the less soluble ARV-110 or diminished by the formation of larger, less mobile colloids of ARV-471.

Conclusion: Together, these findings advance the mechanistic understanding of PROTAC intestinal absorption and highlight that nanoparticle-generating formulations, such as amorphous solid dispersions, can be a viable formulation strategy for overcoming solubility limitations and substantially enhancing oral bioavailability.

Jitka Kalasova (jitka.kalasova@fhnw.ch)

Second harmonic scattering in high-throughput evaluation of solubility, supersat-uration, and self-assembly phenomenon in aqueous drug systems

Jitka Kalasová1,2, Orly Tarun3, Yevhen Shynkarenko4, Martin Kuentz2
1 University of Basel, Department of Pharmaceutical Sciences, Klingelbergstrasse 50 4052, Basel, Switzerland
2 University of Applied Sciences and Arts Northwest. Switzerland, School of Life Sciences, Institute for Pharma Tech-nology and Biotechnology, Hofackerstr. 30 4132 Muttenz, Switzerland
3 Oryl Photonics SA, 1066, Route de la Corniche 5B, Epalinges, Switzerland
4 Swiss Center for Electronics and Microtechnology (CSEM), 7302 Landquart, Switzerland

Aqueous solubility and supersaturation are key factors governing oral drug absorption and bioavailability. Traditional solubility assays often face trade-offs between sensitivity, throughput, and material consumption. To address these limitations, we present a novel plate-based method using non-resonant second harmonic scattering (SHS), which probes interfacial water ordering around solutes in a label-free and calibration-free manner.

In this study, SHS was applied to a set of poorly water-soluble drugs to determine apparent solubility, assess supersaturation behaviour over time, and explore self-assembly phenomena. Solubility values obtained by SHS showed strong agreement with reference chromatographic method, while supersaturation propensity could be linked to physicochemical properties (glass-forming ability, glass transition temperature, amorphous solubility). In addition, the platform enabled detection of self-assembly processes, with sodium lauryl sulfate serving as a benchmark for micellization. Several amphiphilic drugs exhibited similar concentration-dependent aggregation.

Overall, SHS enabled high-throughput solubility profiling with minimal material requirements, while simultaneously providing mechanistic insights into supersaturation kinetics and self-assembly. This versatile approach has the potential to complement conventional techniques and accelerate formulation development for poorly water-soluble drugs**.**

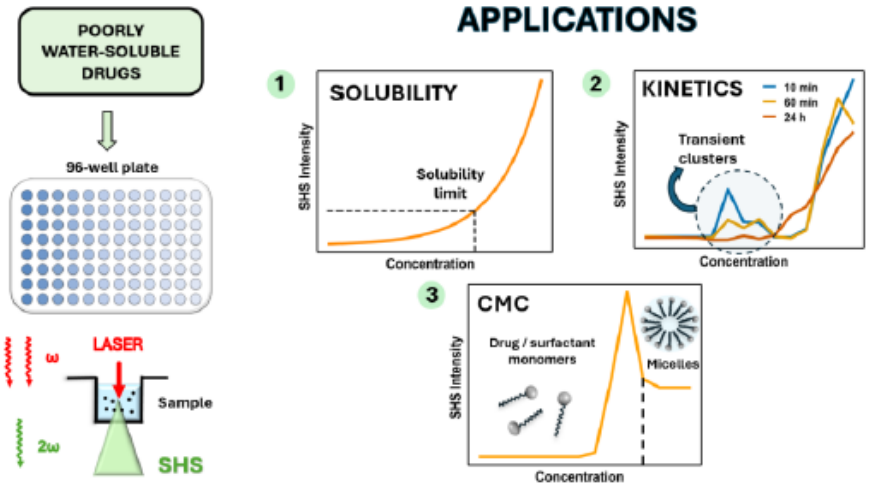


Figure adapted from: Kalasová J, Tarun O, Shynkarenko Y, Kuentz M. High-throughput analysis of aqueous drug solubility, super-saturation, and aggregation behaviour using second harmonic light scattering. *Int J Pharm.* 2025;660:126200. doi:10.1016/j.ijpharm.2025.126200

Juliia Kuziv (y.kuziv@enamine.net)

Assessing PROTAC Permeability and Protein Binding: Challenges and Assay Optimization

Yuliia Kuziv, Anzhela Rodnichenko, Natalia Chyhrynova, Nadiia Diyuk, Mariia Pavlichenko, Olesia Volovenko, Yuliia Holota, Petro Borysko
Enamine Ltd., Winston Churchill Street 78, 02094 Kyiv, Ukraine

The development of heterobifunctional degraders, or PROTACs, holds significant promise for targeted protein degradation therapies, particularly in oncology. However, their complex physicochemical properties – such as high molecular weight, large polar surface area, and low solubility and permeability – pose considerable challenges for preclinical DMPK assessment, especially permeability and plasma protein binding (PPB) studies. In this study, multiple PROTACs were tested for protein binding using equilibrium dialysis in 10% mouse plasma over 24 hours. Most compounds exhibited poor stability in plasma under these conditions. The addition of sodium fluoride (NaF) and protease inhibitors improved stability for certain compounds (e.g., dTAG-7 and dBET57), though others (e.g., ARV-110) remained unstable. Moreover, both additives influenced the observed binding levels, indicating potential interactions with assay components. Additional evaluation of chemical stability in phosphate-buffered saline (PBS), commonly used in PPB protocols, revealed that some PROTACs had limited stability (~20% recovery), highlighting the need for stability-aware interpretation of PPB data.

For Caco-2 permeability evaluation of selected PROTACs (dTAG-7, dBET57, ARV-110), different incubation conditions were investigated. Standard bidirectional Caco-2 protocols yielded low compound recovery, particularly after extended incubation (2 h). The addition of 0.5% bovine serum albumin (BSA) to the transport buffer improved recovery for dBET57 and ARV-110, while modulating efflux ratios for dTAG-7 and dBET57. Titration of BSA (0.25–2%) in the basolateral or both compartments revealed that 0.25% BSA provided the best balance between compound recovery and efflux behaviour and was selected as the optimal condition for further assay refinement.

Additionally, to support high-throughput prediction of human permeability for PROTACs, the use of supercritical fluid chromatography-mass spectrometry (SFC-MS) was explored to determine experimental polar surface area (EPSA). This approach provides a rapid and informative parameter that may enhance permeability modelling for this challenging compound class.

Katy Barnes (k.barnes@cyprotex.com)

Measuring Plasma Protein Binding in Diverse Modalities – Key Considerations

Katy Barnes, Emma Poole, Alec Spowage and Mark C. Wenlock
Cyprotex Discovery Ltd., No.24 Mereside, Alderley Park, Macclesfield, Cheshire, SK10 4TG, United Kingdom

Introduction

Plasma protein binding (PPB) is a key parameter in drug development, influencing its distribution, clearance, and therapeutic efficacy. While equilibrium dialysis remains the gold standard for measuring the fraction unbound (fu), emerging drug modalities—such as PROTACs, ADCs, macrocycles, and peptides—pose unique challenges due to their size, solubility, and membrane permeability. These complex molecules demand a re-evaluation of traditional PPB assay conditions to ensure accurate and reproducible results.

Summary

This study outlines key considerations to be taken into account when designing PPB assays for diverse and complex modalities, and possible adaptations that may be required to accommodate. It highlights how poor aqueous solubility, slow membrane permeation, and non-specific binding can compromise equilibrium and distort fu measurements if not fully understood and accounted for. Experimental data from six commercially available PROTACs demonstrate that generic protocols may not be suitable for such complex modalities. By modifying assay conditions—such as extending incubation times, —more reliable fu values can be obtained. However, assay modifications should be carefully considered to ensure they do not give rise to further problems, such as compound stability. These findings underscore the importance of tailoring PPB methodologies to the physicochemical properties of novel therapeutics, ensuring robust pharmacokinetic predictions and informed drug development decisions.

References

(1) M. J. Blanco et al., ACS Medicinal Chemistry Letters, 2022, 13, 1691-1698,
(2) L. Di et al., Journal of Pharmaceutical Sciences, 2012, 101, 1327-1335,
(3) Riccardi et al., Journal of Pharmaceutical Sciences, 2015, 104, 2627-2636,
(4) Zhang et al. Drug Discovery Today, 2012, 17, 475-485

Krzesimir Ciura (krzesimir.ciura@gumed.edu.pl)

Machine learning-driven prediction of phospholipid affinity based on IAM-HPLC data

Krzesimir Ciura1*, Wiktor Nisterenko1, Alicja Różycka1, Yash Raj Singh1, Katarzyna Ewa Greber1
1 Department of Physical Chemistry, Medical University of Gdansk, Al. Gen. Hallera 107, 80-416 Gdansk, Poland
** Correspondence: krzesimir.ciura@gumed.edu.pl*

Quantitative Structure–Retention Relationships (QSRR) remain an essential framework for elucidating the interactions between molecular structures and chromatographic systems. While recent advances in machine learning (ML) have substantially improved the predictive accuracy of QSRR models, this has often occurred at the expense of mechanistic interpretability. The present study addresses this challenge by integrating predictive performance with molecular-level insights for immobilized artificial membrane (IAM) chromatography.

A comprehensive dataset comprising over 2000 pharmaceutical agents, environmental toxicants, and drug candidates was tested using Valko's gradient IAM-HPLC methodology. For each compound, a broad spectrum of theoretical molecular descriptors was computed using the Chemicalize platform, encompassing lipophilicity indices (logP, LogD7.4), charge-related parameters, hydrogen-bonding capacities, polar surface area, molecular size and shape indices, geometrical and topological features, as well as descriptors reflecting molecular polarizability and refractivity.

An extensive screening of 40 regression algorithms identified the GradientBoostingRegressor as the most robust model (Q^2 = 0.81 on the test set), balancing predictive performance with mechanistic interpretability. SHAP (SHapley Additive exPlanations) analysis highlighted lipophilicity, isoelectric point, molecular polarizability, and charge distribution as dominant factors influencing IAM retention. Complementary t-SNE visualization revealed meaningful clustering within the chemical space, whereas applicability domain analysis ensured the reliability of predictions.

This study demonstrates that descriptor-based ML models can provide predictive accuracy comparable to advanced deep learning methods while preserving mechanistic interpretability, thereby offering a powerful tool for drug discovery, toxicological assessment, and chromatographic method optimization.

Acknowledgements:
This work was supported by the National Science Centre, Poland (grant no. 2022/47/D/NZ7/01043)

Malte Mildner (Malte.mildner@uni-wuerzburg.de)

In-situ setup for drug permeation screening by NMR spectroscopy

Malte Mildner[a], Franz Moser[b], Marcus Gutmann[c], Christoph Keßler[c], Josef Kehrein[c], Tomasz Jüngst[b], Lorenz Meinel[c], Ann Christin Pöppler[a]
a Institute of Organic Chemistry, University of Würzburg, Am Hubland, 97074 Würzburg, Germany
b Institute of Functional Materials and Biofabrication, University of Würzburg, Pleicherwall 2, Würzburg 97070, Germany
c Institute of Pharmacy and Food Chemistry, University of Würzburg, Am Hubland, Würzburg 97074, Germany

Traditional setups to investigate the passive diffusion of drugs utilize a diffusion cell,[1] where two chambers are separated by a membrane and at defined time intervals, a solution of defined volume is collected and the amount of drug determined via HPLC or UV/Vis spectroscopy.[2] In the last decades it has been shown that NMR spectroscopy is a versatile tool to be used in many different areas, from quality control during synthesis,[3] over anti-doping tests,[4] to automated screening procedures during drug development.[5] In this work, we demonstrate a method for conducting permeation experiments of different drugs inside an NMR tube, while taking full advantage of automation capabilities. The accuracy of the NMR-based concentration determination under the measurement conditions has already been previouusly verified via HPLC.[6] The new setup is very versatile, allowing the usage of different multi-layered membranes or even Caco-2 cells, combined with simulating intestinal fluids such as FaSSIF and FeSSIF, and the unique benefits of analyzing the same molecule through different nuclei. The results obtained with our new setup offer more insights on a molecular level, allowing us to observe molecular interactions, while showing the same trends with only small deviations in the apparent permeabilities to the results achieved with the traditional side-by-side setup, but without the need for multiple different devices and user interference for measuring and analysis.

[1] S. F. Ng, J. J. Rouse, F. D. Sanderson, V. Meidan, G. M. Eccleston, *AAPS PharmSciTech* 2010, *11*, 1432-1441.
[2] D. Sironi, M. Christensen, J. Rosenberg, A. Bauer-Brandl, M. Brandl, *Int. J. Pharm.* 2017, *522*, 50-57.
[3] U. Holzgrabe, *Prog. Nucl. Magn. Reson. Spectrosc.* 2010, *57*, 229-240.
[4] J. Zhao, M. Wang, B. Avula, I. A. Khan, *J. Pharm. Biomed. Anal.* 2018, *151*, 347-355.
[5] M. Bon, A. Bilsland, J. Bower, K. McAulay, *Mol. Oncol.* 2022, *16*, 3761-3777.
[6] M. Mildner, S. Hanio, S. Endres, L. Scheller, B. Engel, L. Castanar, L. Meinel, A. C. Pöppler, *Anal. Methods* 2024, *16*, 1468-1472.

Nele-Johanna Hempel-Hojgaard (nljh@novonordisk.com)

To be or not to be a peptide? Properties of small cyclic peptides

Nele-Johanna Hempel-Højgaard1
1Therapeutics Discovery, Research & Development, Novo Nordisk A/S, 2760 Måløv, Denmark

Introduction:
Cyclic peptides and cyclic peptidomimetics are an upcoming modality containing <= 12 AAs with MK-0616 and LUNA-18 being prominent examples for this modality class. Cyclic peptides have a higher molecular weight and a larger surface area than small molecules and are therefore suita-ble for targets with large binding sites or protein-protein interactions. Furthermore, compared to their linear counterparts, cyclic peptides are to a higher degree proteolytically stable, can have higher binding affinity and be membrane permeable, which enables targeting intracellular or oral delivery.

Aim:
As cyclic peptides take a target space and property space between small molecules and biologics, the aim of our studies was to identify if cyclic peptides should be discovered and developed as small molecules or as peptides.

Methods:
For a range of commercially available cyclic peptides, ADME properties, such as stability, solubil-ity, permeability and plasma protein binding were determined.

Results:
Based on the ADME properties, cyclic peptides are generally poorly soluble, and stable in intesti-nal fluid and plasma. Cyclic peptides are cleared by hepatocytes. Permeability was determined in various *in vitro* assays and most cyclic peptides are permeable, albeit to a lower extend than small molecules. Cyclic peptides are efflux transporter substrates.

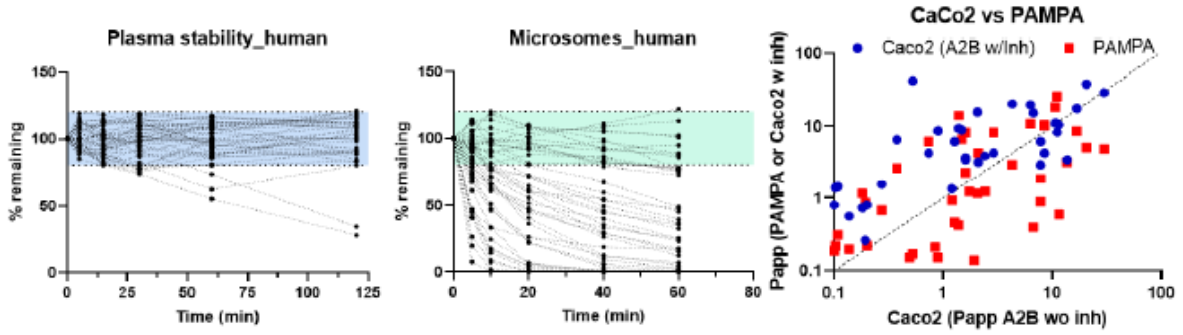


Figure 1: Stability in human plasma (left); Clearance in human microsomes (middle); Permeability data (right).

Conclusion:
Cyclic peptides were found to behave like small molecules and should therefore be developed as such after the initial hit finding phase. Within cyclic peptide hit discovery, both peptide and small molecule discovery platforms are suitable.

Nora Kern (nora.kern@nuvisan.com)
Maximilian Koy (maximilian.koy@nuvisan.com)

From Big Pharma to a CRO PhysChem Lab: Assays, Prediction and New Technologies

Nora Kern*, Maximilian Koy*
Nuvisan ICB GmbH, Muellerstr. 178, 13353 Berlin, Germany

In 2020, Nuvisan acquired the largest part of the Bayer Pharma Drug Discovery Unit in Berlin, including its PhysChem laboratory. During the transition, the PhysChem lab became part of a CRO business model and started offering important services for the characterisation and profiling of exploratory compounds in drug discovery.

A large variety of relevant PhysChem parameters such as various solubility endpoints, logD and all kinds of stability parameters (e.g. pH, heat and nucleophiles) are determined in a (semi-)high-throughput format. In addition, Nuvisan has access to a substantial set of preclinical data, therefore enabling the construction of robust prediction models for solubility and partition coefficients (and others) for early prioritization. The poster presents the performance in the context of compound evaluation in the Beyond-Rule-of-5 field.

Lastly, we established a high-throughput EPSA assay incorporating mass spectrometry to evaluate both classical small molecules and Beyond-Rule-of-5 compounds, including PROTACs and peptides. Those EPSA and logD measurements can be combined with Nuvisan's Direct-to-Biology platform to accelerate the synthesis and evaluation of large libraries in a sustainable way.

References:

[1] G. H. Goetz, L. Philippe, M. J. Shapiro, ACS Med. Chem. Lett. 2014, 5, 1167−1172.
[2] Y.-T. Wang, E. Price, M. Feng, J. Hulen, S. Doktor, D. M. Stresser, E. M. Maes, Q. C. Ji, G J. Jenkins, J. Med. Chem. 2024, 67, 13765−13777.

- Established assays: Solubility, logD, stability
- Experimental determination and strong prediction models

- New assay development: High Throughput EPSA
- Useful for small molecules, bRo5, crude plate-based reactions

Paula Vidal (paulavidaljuaneda2001@gmail.com)

Biomimetic liposome frameworks for drug-membrane interaction studies

Paula Vidal[1], Samia Arshad[1], Elisabet Fuguet[1,2], Martí Rosés[1]
[1]*Departament d'Enginyeria Química i Química Analítica, Institut de Biomedicina de la Universitat de Barcelona (IBUB), Universitat de Barcelona, Martí i Franquès 1-11, 08028 Barcelona, Spain*
[2]*Serra Húnter Programm, Generalitat de Catalunya, Spain*

In drug discovery, understanding the affinity of bioactive compounds to biological membranes is crucial, since it affects key pharmacokinetic properties including absorption, distribution, metabolism, excretion, and toxicity. Traditional membrane affinity assays typically depend on biological systems, which are not only costly but also time-consuming and ethically challenging. In response, this study introduces an alternative approach based on synthetic liposomes with defined compositions designed to mimic different biological membranes.

Drug-liposome interactions are analyzed using liposome electrokinetic chromatography (LEKC), where the liposomes serve as a chromatographic pseudostationary phase. The resulting systems are characterized by the Abraham solvation model, which expresses the retention factor (k) as a function of solute properties including polarity and polarizability, hydrogen-bonding and molecular volume:

$$log\ k = c + eE + sS + aA + bB + vV$$

The regression coefficients derived from the linear correlation elucidate the characteristics of solute–liposome interactions. In this work, two different liposomal systems were studied: the first consists of 49% phosphatidylcholine, 8% cholesterol, and 43% palmitic acid; the second contains 21% phosphatidylcholine, 29% phosphatidylethanolamine, 8% phosphatidylinositol, and 42% palmitic acid. In both linear correlations, hydrogen-bond basicity and molecular volume are the primary solute descriptors influencing partition into these two systems. However, the two systems differ in polarizability, which is higher in the liposomes containing phosphatidylethanolamine and phosphatidylinositol.

Using Euclidean distance and principal component analysis of the normalized system constants, the studied liposomes were compared with different *in vitro* biological partition models. The study suggests that both liposomal systems are good surrogates of blood–lung drug distribution. However, mimicking partitioning into other membranes, such as the blood–brain barrier or the blood–liver interface, would require compositional adjustments, especially in terms of the hydrogen bond acidity and basicity of the systems.

Petra Misetic (petra.misetic@selvita.com)

Plasma protein binding of PROTACs by biomimetic chromatography

Petra Mišetić1,*, Marija Marošević1, Marina Ilijaš2, Astrid Milić1, Jasna Padovan1
1Drug Metabolism and Pharmacokinetics, Selvita, Hondlova 2-12, 10000, Zagreb, Croatia;
2Zagreb, Croatia

Plasma protein binding has been recognized as one of the important ADME parameters that influence pharmacokinetic, but also pharmacodynamic parameters (1,2). Thus, plasma protein binding is closely monitored during the drug discovery process.

Proteolysis-targeting chimeras (PROTACs) are an emerging therapeutic modality that breach commonly accepted rule-of-thumb requirements that traditional small molecule drugs follow (3), resulting in the need to use tailored methods for ADME testing in this chemical space (4).

PROTACs often present experimental challenges when their plasma protein binding (PPB) is assessed by equilibrium dialysis (ED), including prolonged equilibration times, nonspecific binding or plasma instability (5). The aim of this study was to explore the possibility of using biomimetic methods as surrogates to investigate the binding of PROTACs to plasma proteins. Two HPLC-based chromatographic methods using columns containing stationary phases of immobilized human serum albumin (HSA) or α-1-acid glycoprotein (AGP) were explored (6).

Results obtained with chromatographic methods correlate well with the ED data for both HSA and AGP, ranking the compounds in the same manner. While no experimental challenges were observed with the chromatographic methods, more than one-third of results obtained with ED methods could not be interpreted due to low recovery values. These findings demonstrate the potential use of biomimetic methods for investigating PROTAC interactions with proteins and the ability to rank compounds consistently with ED.

Keywords: Plasma protein biding; PROTAC; Biomimetic chromatography.

References:

1. Rowland M, Tozer T. Clinical pharmacokinetics and pharmacodynamics: concepts and applications. 5th ed. Philadelphia: Lippincott; 2019.
2. Olson RE, Christ DD. Plasma protein binding of drugs. Annu Rep Med Chem. 1996;31:327-36.
3. Testa A, Hughes SJ, Lucas X, Wright JE, Ciulli A. Rational control of molecular properties is mandatory to exploit the potential of PROTACs as oral drugs. ACS Med Chem Lett. 2020;11(11):2114-23.
4. Apprato G, Ermondi G, Caron G. The quest for oral PROTAC drugs: evaluating the weaknesses of the screening pipeline. ACS Med Chem Lett. 2023;14(7):879-83.
5. Cantrill C, Chaturvedi P, Rynn C, Petrig-Schaffland J, Walter I, Wittwer MB. Fundamental aspects of DMPK optimization of targeted protein degraders. Drug Discov Today. 2020;25(6):969-82.
6. Valko K. Lipophilicity and biomimetic properties measured by HPLC to support drug discovery. J Pharm Biomed Anal. 2016;130:35-54.

Rafal Bachorz (rafal.bachorz@simulations-plus.com)

Expanding ADMET Predictor™'s Chemical Space: Enhanced bRo5 and Chameleon Molecule Predictions for HTPK

Jeremy Jones, Rafał A. Bachorz, Vladimir Chupakchin, Michael Lawless, and Robert Fraczkiewicz
Simulations Plus, Inc., Research Triangle Park, P.O. Box 12317, Durham, NC 27709, USA

ADMET Predictor has been enhanced to accurately predict properties of beyond Rule-of-Five (bRo5) molecules, including macrocycles and PROTACs. We introduce new descriptors for molecular chameleonicity and three specialized models: EPSA1 (Experimental Polar Surface Area), ChromLogD2, and ChameLogK3 (Chromatographic Chameleonicity), which serve as advanced molecular features for core ADMET predictions. These new descriptors improve predictive capabilities for various HTPK-input models (Hight Throughput Pharmacokinetics), such as liver microsome/hepatocyte clearance, fraction unbound in plasma, and blood-to-plasma ratio. This subsequently enhances overall HTPK modeling accuracy. Validation on novel molecules demonstrates significant performance improvements for bRo5 compounds. Case studies reveal substantial improvements in predicting key in vivo endpoints for challenging chemical space, supporting modern drug discovery.

References:

1Goetz GH, et al., J Med Chem. 2014;57(7):2920-2929. doi:10.1021/jm401859b
2Koch G, et al., J Med Chem. 2024;67(21):19612-19622. doi: 10.1021/acs.jmedchem.4c01956
3Garcia Jimenez D, et al., J Med Chem. 2023;66(15):10681-10693. doi: 10.1021/acs.jmedchem.3c00823

Rebeca Ruiz (rruiz@pion-inc.com)

Study of the influence of the biorelevant media on the solubility of benzthiazide, isoxicam, and piroxicam in the presence of several excipients

Rebeca Ruiz1, Elisabet Fuguet2, Clara Ràfols2
1Pion Inc., Forest Row, East Sussex, UK
2Institute of Biomedicine (IBUB) and Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, Martí i Franquès 1-11, 08028 Barcelona, Spain.

The bioavailability of a drug depends, among other parameters, on solubility. Only a drug in a solution can be absorbed through the gastrointestinal tract; however, a high number of drug candidates are poorly soluble not only in water itself but also in the presence of gastrointestinal medium components. One of the strategies used to enhance the solubility of sparingly soluble drugs is the use of excipients, as the excipients can interact with the drug by increasing its solubility and/or stabilizing supersaturated solutions. However, to have a better predictive bioavailability of the drug, solubility measurements must mimic *in vivo* conditions. Due to this, biorelevant media are used to simulate gastrointestinal fluids in fasted and fed states.

In this work the influence of the biorelevant media (fasted and fed states) on the solubility of three pharmaceutical compounds (benzthiazide, isoxicam, and piroxicam) is evaluated in the presence of five excipients: two cyclodextrins (Captisol and Cavasol) and three hydrophilic polymers (Klucel, Kollidon, and Plasdone-S630). Additionally, the intrinsic solubility (S0) and the capacity of the drugs to form supersaturated solutions were compared with aqueous conditions.

The CheqSol method was used for the study, and it was observed different behaviors depending on the excipient in solution. For instance, in the presence of cyclodextrins, there was an increase in the solubility for benzthiazide in SiF media but not a significant effect on the oxicam drugs. In the case of hydrophilic excipients, it is observed the influence of the SiF media on benzthiazide enhancing the solubility and increasing the Cmax of supersaturation solutions decreasing the duration of the supersaturation. On the contrary, it was not observed a significant influence of the biorelevant media on the oxicam compounds, just a slight increase for piroxicam in FeSSiF media.

Reference: Influence of Ionization and the Addition of Cyclodextrins and Hydrophilic Excipients on the Solubility of Benzthiazide, Isoxicam, and Piroxicam. Diego Lucero-Borja, Rebeca Ruiz, Elisabet Fuguet and Clara Ràfols. Pharmaceutics 2025, 17, 571.

Sina Simon (sina.simon@roche.com)

Can we predict the Unbound Fraction in Tissue from Physicochemical and Binding Parameters?

Sina Simon1, Victor Dudal1, Yann-Stanislas Barral1 , Björn Wagner1 and Janneke Keemink1
1 Roche Pharma Research and Early Development, Pharmaceutical Sciences, Roche Innovation Center Basel

Background:
The drug's free concentration at the target site is an important determinant of its biological activity and is crucial to understand the exposure-effect relationship [1]. This parameter is often referred to as fu,tissue (unbound fraction in tissue). Tissue binding appears to be driven mainly by non-specific binding to lipids and was shown to be similar across species (i.e. a single species can be taken as surrogate) [1,2].

Objective:
The goal of this project was to investigate, whether the fu,tissue in various tissues (including brain, heart, lung, liver, kidney, gut, skeletal muscle and adipose) can be predicted from a combination of physicochemical and binding parameters, which are routinely determined in early drug discovery.

Methodology:
For a set of 31 commercial small molecules (mainly within the rule of 5 space), fu,tissue in homogenised rat tissues was experimentally measured using rapid equilibrium dialysis (RED) by dialysis against a phosphate buffer for 4 hours. 25/31 compounds were then used for training of an *in silico* model, and 6/31 for its validation. Training data points were weighted based on the recovery fraction, attributing a lower weight to points outside 80–120% recovery. Log-transformation was applied to the descriptors, when it increased predictivity. The correlation between predictions and experimental data was quantified using the coefficient of determination (r^2) and percentage predicted within 2-fold (P2). Most predictive inputs were selected as maximising P2.

Results:
Using a combination of two descriptors, such as logD, fu in LIMBA and fu in plasma proteins as inputs, fu,tissue could be reasonably predicted in brain (P2=81%), heart (P2=83%), kidney (P2=78%) and liver (P2=80%). Similar predictivity was observed with training and validation compounds for these tissues. Predictivity for fu in adipose, gut, lung, and skeletal muscle was limited. Using fu, liver in combination with one descriptor further increased the model's predictivity (P2=79–90%, and P2=81% in skeletal muscle), but lower predictivity for fu in adipose, gut, and lung remained.

Conclusion:
The data has demonstrated that fu, tissue in rats' brain, heart, liver, kidney, and eventually skeletal muscle can be predicted within the 2-fold range using two descriptors. This enables an estimation of fu, tissue during the early discovery phase. As more data will be generated, especially "beyond the rule of 5 space'', continuous model refinement will increase confidence in extrapolation of descriptors to fu, tissue.

References:
[1] Ryu, S. et al.(2020). Evaluation of Fraction Unbound Across 7 Tissues of 5 Species. Journal of pharmaceutical sciences, 109(2), 1178–1190;
[2] Di, L. et al. (2011). Species independence in brain tissue binding using brain homogenates. Drug metabolism and disposition: the biological fate of chemicals, 39(7), 1270–1277.

Susana Amézqueta (samezqueta@ub.edu)

Interactions of antidepressants with human serum albumin

Susana Amézqueta1, Rama Haddad1, Clara Ràfols1
1Departament d'Enginyeria Química i Química Analítica and Institut de Biomedicina (IBUB), Universitat de Barcelona, Martí i Franquès 1-11, 08028 Barcelona, Spain

The binding of drugs to plasma proteins, particularly human serum albumin (HSA), plays a crucial role in modulating pharmacological activity, drug distribution, and potential adverse effects [1]. While protein binding facilitates drug transport and stabilizes plasma concentrations, extremely strong binding may restrict drug elimination, reduce its distribution to target sites, and require higher therapeutic doses. HSA, the predominant plasma protein, contains multiple ligand-binding sites, most notably in subdomains IIA and IIIA, making it a key target in drug-binding studies [2].

Here, the binding of three antidepressants (trazodone, imipramine and maprotiline) with HSA has been investigated using two complementary techniques: biomimetic chromatography and fluorescence spectroscopy. These heterocyclic compounds with basic characteristics are able to be transported through the bloodstream, mediated by protein transport, cross the blood-brain barrier and act at central nervous system level.

Biomimetic chromatography is an HPLC technique that employs a column with an immobilized protein as the stationary phase and a buffer at pH 7.4 as a part of the mobile phase to simulate the binding process in the human body. When an HSA column is used, it can mimic the in vivo interactions of drug molecules with HSA. The drugs are eluted according to their affinity for HSA and so, their retention times (tR) have a relationship with their binding affinities. In the case of fluorescence spectroscopy method, the fluorescence intensity decay upon the addition of consecutive volumes of the drug to a solution containing HSA is measured and related to the binding constant through previously described mathematical models.

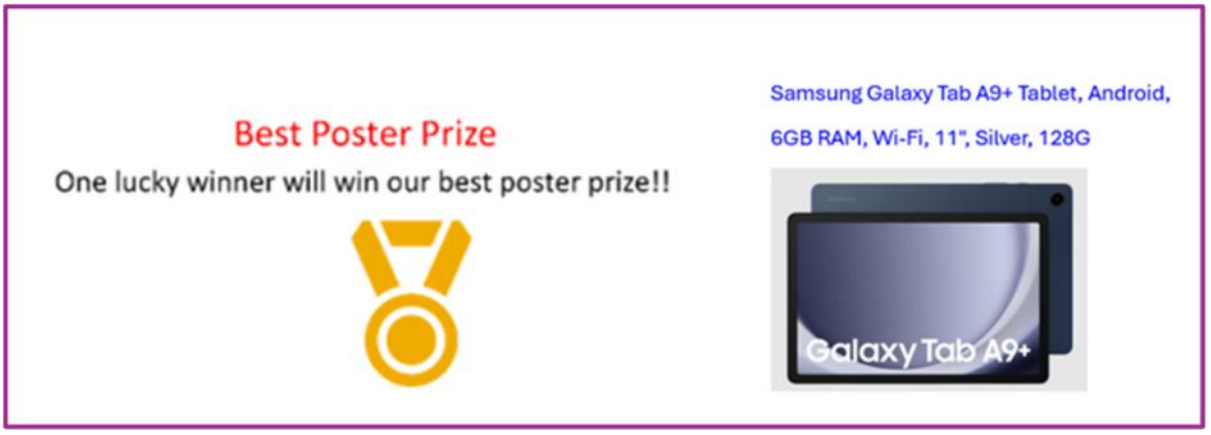
Results have shown that imipramine and trazodone have a binding of 90% and 93%, respectively. In the case of maprotiline, no binding has been observed by fluorescence quenching while biomimetic chromatography has shown 83% binding. The drug-HSA interaction in imipramine and trazodone would occur in a point near a fluorophore (Trp or Tyr residues) while in the case of maprotiline the position would be different.

The findings emphasize the importance of integrating complementary analytical methods to improve the reliability and depth of drug–protein binding studies.

[1] G. Fanali, A. Di Masi, V. Trezza, M. Marino, M. Fasano, P. Ascenzi, Human serum albumin: From bench to bedside, Mol Aspects Med 33 (2012) 209–290. https://doi.org/10.1016/j.mam.2011.12.002.
[2] J. Ghuman, P.A. Zunszain, I. Petitpas, A.A. Bhattacharya, M. Otagiri, S. Curry, Structural basis of the drug-binding specificity of human serum albumin, J Mol Biol 353 (2005) 38–52. https://doi.org/10.1016/j.jmb.2005.07.075.

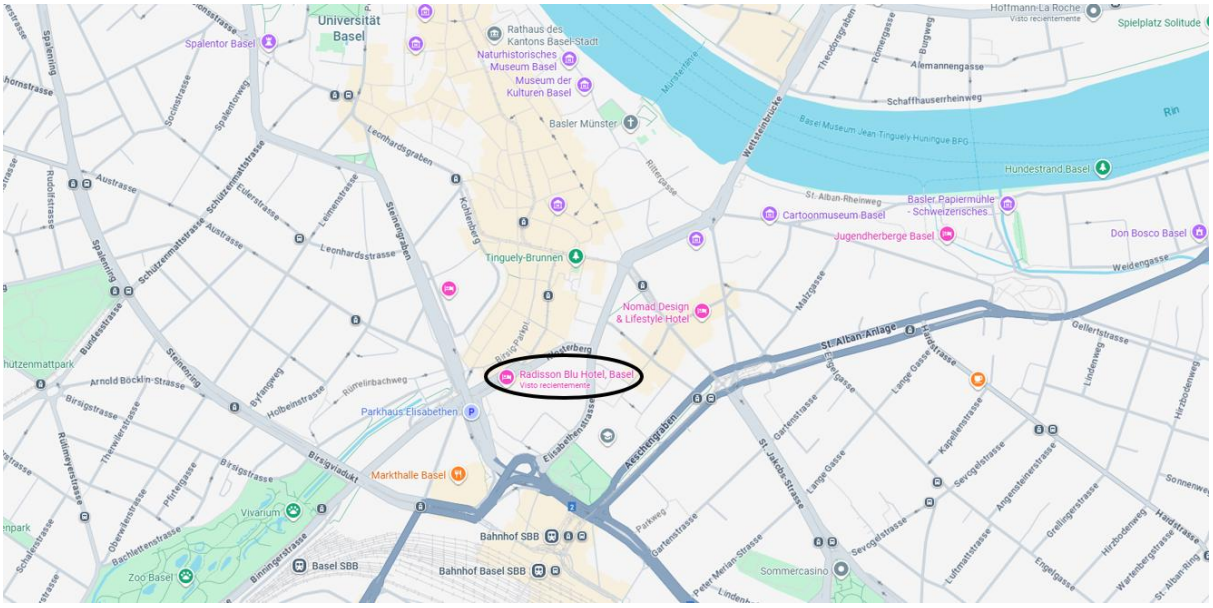
Magda Swedrowska (magda.swedrowska@astrazeneca.com)

Advancing Oral PROTAC Delivery: Biopharmaceutic Challenges and Opportunities
Abstract not available

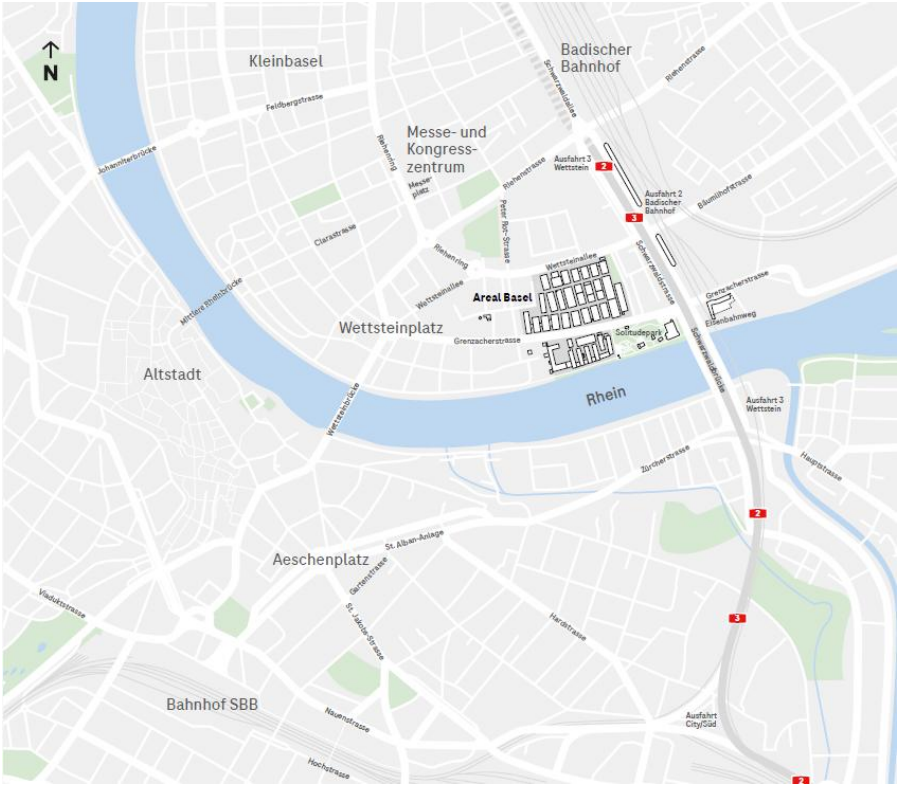


Maps

Radisson Blu Hotel, Steinentorstrasse 25, Basel, Switzerland. Map



F. Hoffmann-La Roche, Grenzacherstrasse 124, Basel, Switzerland. Map

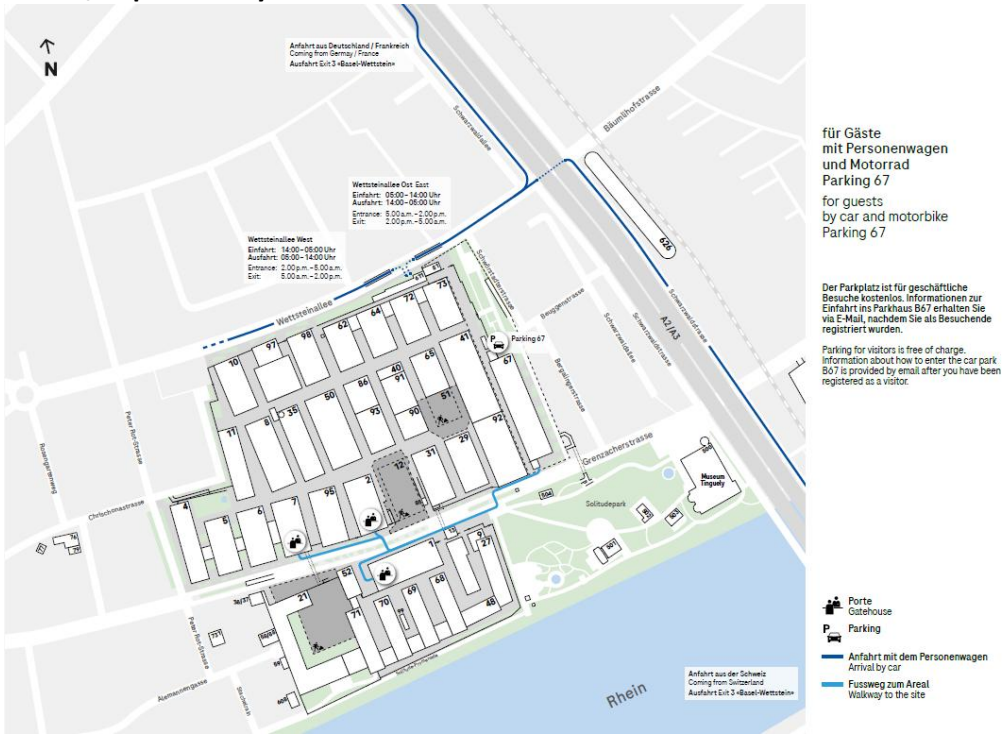


Visitor Arrival Instructions for F. Hoffmann-La Roche

The registration desk will be located at the entrance of building 7. Please follow the blue arrows displayed in the maps

(1) Arrival/Departure: on foot, via public transport, by bike

(2) Arrival/Departure: By car



Key Reminders

Thank you for joining us for the Phys Chem Forum, here are a few reminders:

Network and password

- Radison Blu Hotel: please select the network “Radisson_Meeting”; Password: Radisson4545!
- F. Hoffmann-La Roche: Please select the network 'guests' and accept the conditions.

Food and drink

- Upon your arrival, please feel free to help yourself to tea, coffee, and biscuits to refresh and relax.
- For those who pre-booked dinner when they registered, dinner will be served at the hotel at 19:30.
- Dinner costs CHF 59 for a 3-course menu and is billed together with your overnight stay (CHF 189 for bed and breakfast). If you are not staying at the hotel, please pay the bill by card after dinner.
- During the dinner, the following drink will be served: one bottle of red wine and one bottle of white wine per table of six people, water and one voucher per person for a soft drink will be provided.

Travel

Please read carefully of the arrival instructions on the booklet:

- Please be aware that hotel guests staying overnight will receive a travelcard, valid for both days of the event. Furthermore, if any attendees have not received their public transport ticket, they can simply present their hotel booking confirmation as proof of eligibility.
- Remember to wear your visitor badge at all times while on site for easy identification and security purposes.
- If you need a taxi after the event, please let us know upon arrival. A list is available for you to sign up for.

Site tour

- We have arranged a special F. Hoffmann-La Roche tour during the lunch break. As space is limited, please indicate upon arrival at the hotel or in the morning at F. Hoffmann-La Roche if you would like to join the tour.

Special Thanks

We would especially like to thank ACD/Labs, Agilent, ORYL, PHABIOC, Pion, SEDA, Selvita, SimulationsPlus and The Solubility Company for their kind support, so please visit their booths during lunch break.

Space for Notes