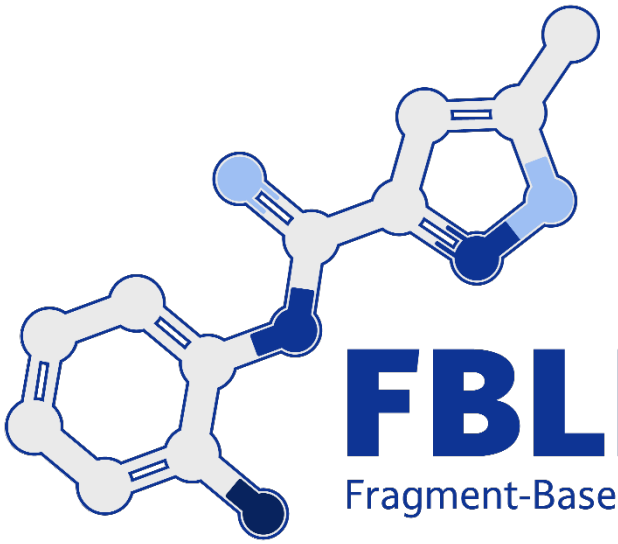


FBLD 2024

Fragment-Based Lead Discovery Conference

22 to 25 September 2024

George Sherman Union

Boston University, Boston, USA

CONFERENCE BOOKLET

Table of Contents

Acknowledgements

Sponsors

We proudly acknowledge the generous support of our 2024 sponsors

Committee Members and Volunteers

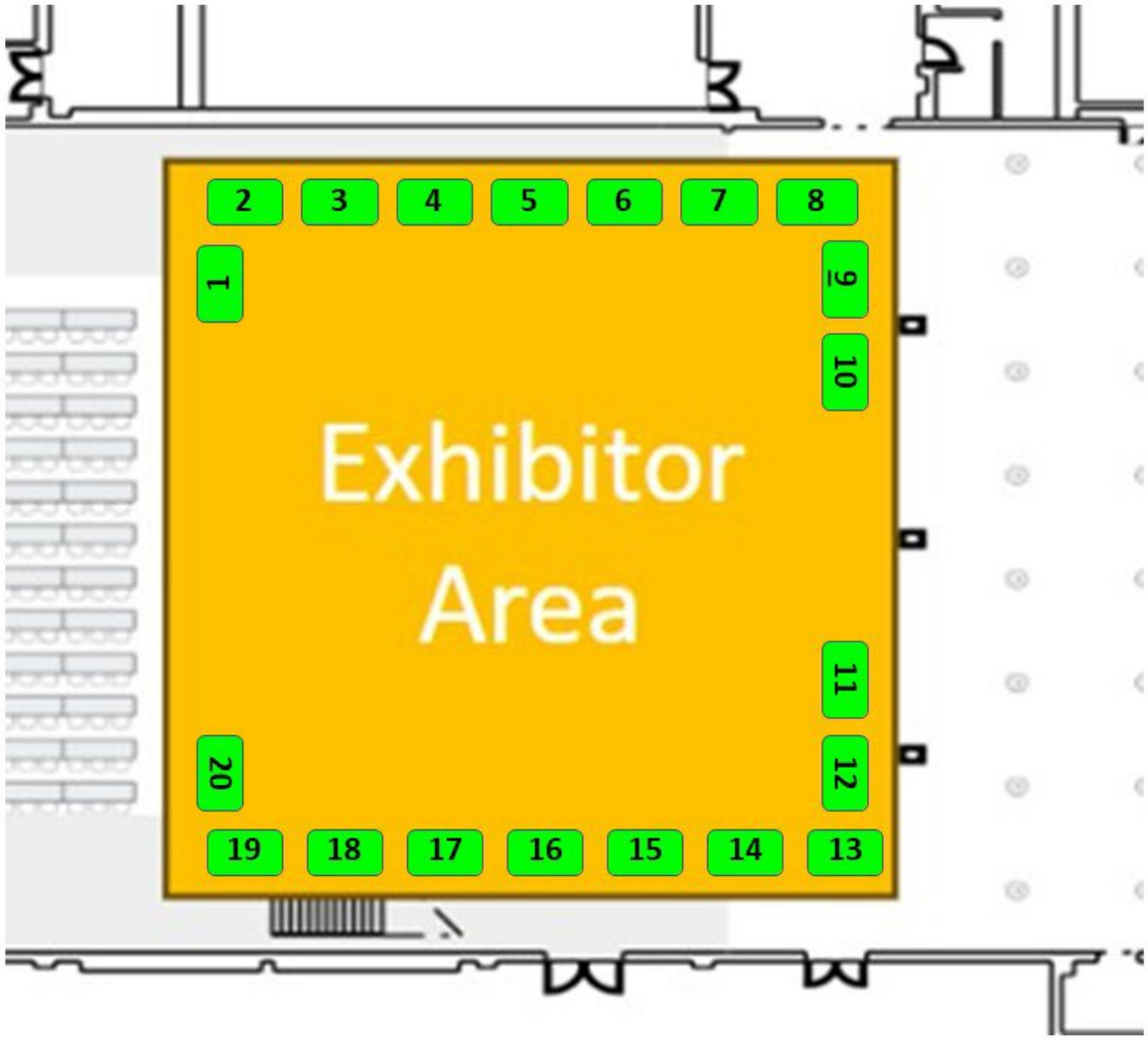
We want to also particularly thank the FBLD 2024 Committee Members for their invaluable contribution to this event:

Prof. Martin Scanlon, Monash University
Dr. Joe Patel, Nexo Therapeutics
Dr. Heike Schönherr, Relay Therapeutics
Dr. Dan Erlanson, Frontier Medicines
Dr. Daniel Wyss, Merck & Co., Inc.
Ms. Anne Meyer, Monash University

Many thanks to our volunteers on the day who help to run this event smoothly:
Dr. Sue Saalau, Photys Therapeutics

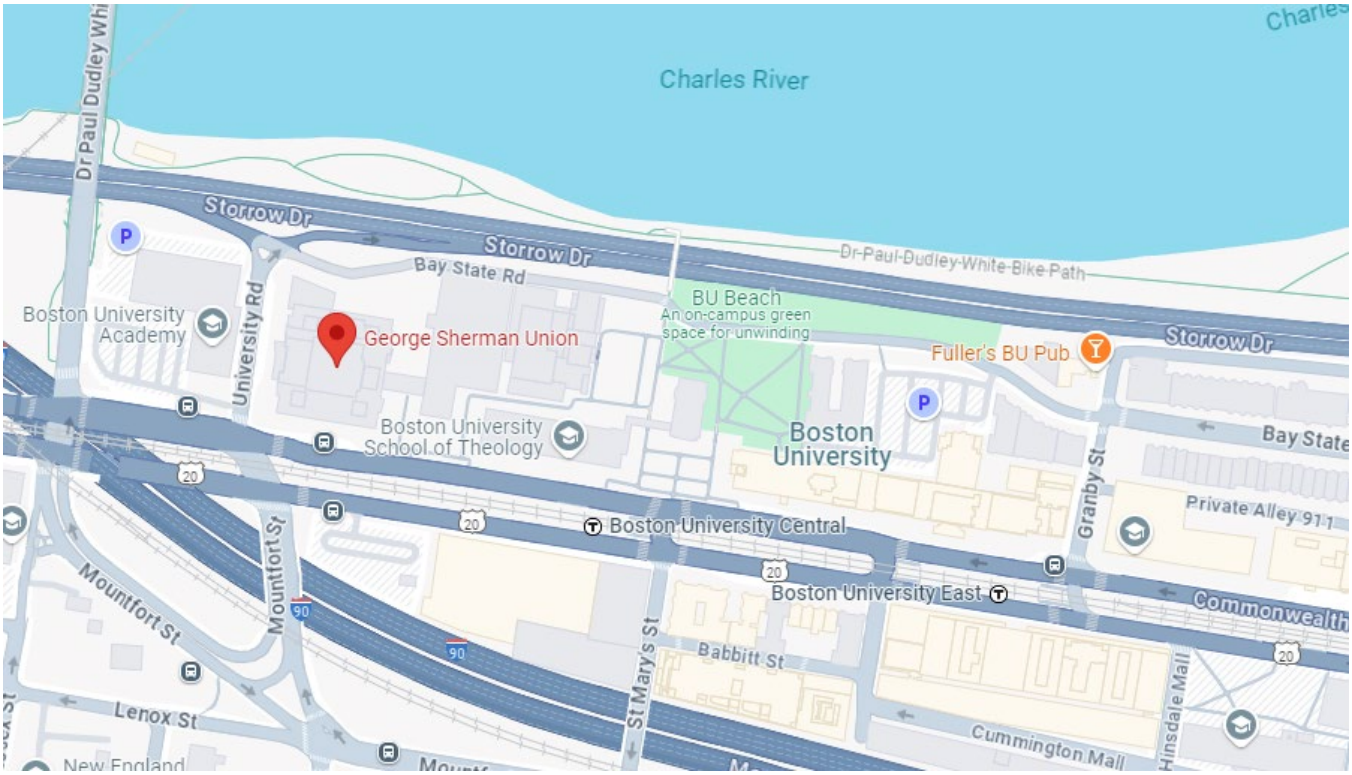
Exhibition Layout

Malvern Panalytical	1
Sygnature	2
CCG	3
Bruker	4
ALPX	5
Mitegen	6
Carterra	7
eMolecules	8
Viva Biotech	9
Key Organics	10
Vernalis	11
WuXi	12
TBD	13
SpiroChem	14
NMX	15
Biortus	16
XtalPi	17
Nuvisan	18
Enamine	19
Molox	20



Conference Information

Conference Venue
George Sherman Union
Boston University
775 Commonwealth Ave
Boston MA 02215
United States



The conference venue has three main meeting rooms:

1. Metcalf Hall for lectures and exhibition
2. Terrace Lounge for workshops
3. Ziskind Lounge for catering

Parking
Parking is available at various lots on campus for $25/day. Guests can pull a ticket upon entry and pay on exit. Parking is available at the following lots:

- Agganis Arena Garage (A): 925 Commonwealth Ave (entrance behind building complex)
- Langsam Garage (B): 142 Gardner St.
- Warren Towers Garage (K): 700 Commonwealth Ave (entrance to left on Hinsdale Mall)

Program

Schedule

22 September	23 September	24 September	25 September
1:30 pm Registration open:	8:00 am Registration open & poster breakfast	8:00 am Poster breakfast	8:00 am Poster breakfast
2:00 pm Workshop start	8:30 am Presentations start	8:45 am Presentations start	9:00 am Presentations start
5:00 pm Exhibition open	12:30 pm Lunchtime Workshop	5:30 pm Day 3 end	1:00 pm FBLD end
6:00 pm Networking Welcome Reception	5:30 pm Poster Session	7:00 pm Conference dinner	
8:30 pm Day 1 end	7:00 pm Day 2 end		

Sunday, 22 September 2024

1:30 PM **Registration opens**

2:00 PM **Workshop**
Introduction to Fragment-Based Design
Dr. Dan Erlanson, Frontier Medicines & Dr. Ben Davis, Vernalis Research

5:00 PM **Exhibition opens**

6:00 PM **Networking Welcome Reception**

We are delighted to invite you to join us for our Networking Welcome Reception in the Ziskind Lounge. This reception will be an excellent opportunity to mingle with fellow attendees, speakers, and industry leaders in a relaxed and informal setting. Please come and enjoy an evening of networking, drinks, and finger food as we kick off what promises to be an exciting conference.

8:30 PM End Day 1

Monday, 23 September 2024

8:00 AM Registration Opens & Poster Breakfast

Session 1 – Fragments Past and Present Chair: Dr. Joe Patel, Nexo Therapeutics
8:30 AM to 10:30 AM

8:30 AM Welcome Note
Dr. Joe Patel, Nexo Therapeutics

8:45 AM **Opening Keynote**
25 Years of Thinking Small
Dr David Rees, Astex Pharmaceuticals

9:30 AM **Invited Speaker**
Using Fragments to Answer Questions in Exploratory Drug Discovery
Prof. Rod Hubbard, University of York and Vernalis Research

10:00 AM *FBDD Approaches to Tackle Emerging Multi-Component Therapeutic Targets*
Dr. Juliet Morgan, Sygnature Discovery

10:30 AM	**Networking Break**

Session 2 – Methods Chair: Dr. Tony Giannetti, Carterra
11:00 AM to 12:15 PM

11:00 AM *Photoactivated Covalent Capture of DNA-Encoded Fragments to Identify Ligands for Challenging Targets*
Dr. Ben Davis, Vernalis Research

11:30 PM *Expanding Chemical Diversity in Drug Discovery Through Novel Amine-Acid Coupling*
Ruheng Zhao, University of Michigan

12:00 PM *Biophysical and Structural Biology Methods Enable Fragment-Based Ligand Discovery*
Dr. Moran Jerabek-Willemsen, WuXi AppTec

12:15 PM	**Networking Lunch**

12:30 PM Lunchtime Workshop:
Fragment-based Design: Scaffold Hopping, Fragment Growing and Bioisosteric Replacements
Dr. Michael Drummond, Chemical Computing Group

Session 3 – Crystallography Chair: Dr. Ben Davis, Vernalis Research
2:00 PM to 3:45 PM

2:00 PM **Invited Speaker**
High-Quality Hits from Xchem with Fast-Forward Fragments Combining HT Crystallography, Fragment Merging and Low-Cost Robotics
Prof. Frank von Delft, Oxford University/Diamond Lightsource

2:30 PM *Fast fragment hit-to-lead in drug discovery for novel drug targets*
Dr. Debanu Das, Xpose Therapeutics

3:00 PM *Crystallographic fragment screening identifies diverse chemical scaffolds for Zika virus NS2B-NS3 protease inhibitor development*
Dr. Xiaomin Ni, Oxford University

3:30 PM *Accelerating Drug Discovery: Advanced Structural Biology Solutions at ALPX*
Dr. Andrea Pica, ALPX

3:45 PM	Networking Break

Session 4 – Computational I Chair: Prof. Rod Hubbard, University of York
4:15 PM to 5:45 PM

4:15 PM *Invited Speaker*
AI-First Fragment-Based Drug Discovery at Isomorphic Labs
Dr. Rebecca Paul, Isomorphic Labs

4:45 PM *Computational Hot Spot Mapping for Fragment-Based Lead Discovery*
Prof. Diane Joseph-McCarthy, Boston University

5:15 PM *High-Throughput Activity-Based Fragment Screening with Ulysses™: Generating Robust Datasets for Machine Learning-Driven Drug Discovery*
Thomas Fleming, Spirochem/Actoris

5:30 PM	Poster Session

5:30 PM *Poster Flash Talks*

Join us for a drink and finger food during our poster and networking session. Make the most of the chance to talk to the speakers and fellow delegates. Discover more collaboration opportunities or get answers to your burning questions on fragment-based lead discovery that go beyond the topics discussed today.

7:00 PM End Day 2

Tuesday, 24 September 2024

8:00 AM **Poster Breakfast**

Session 5 – Success Stories Chair: Dr. Dan Erlanson, Frontier Medicines
8:45 AM to 10:15 AM

8:45 AM **Invited Speaker**
Fragment-Based Discovery of Novel and Potent STING Agonist
Dr. Chaohong Sun, Abbvie

9:15 AM *Fragment-Based Discovery of Allosteric Modulators of β-Glucocerebrosidase*
Dr. Puja Pathuri, Astex Pharmaceuticals

9:45 AM *Identification and Development of Fragment-Derived Chemical Matter in Previously Unknown Allosteric Sites of WRN*
Dr. Daniel Wyss, Merck & Co.

10:15 AM **Networking Break**

Session 6 – Computational II Chair: Prof. Diane Joseph-McCarthy, Boston University
11:00 AM to 12:30 PM

11:00 AM **Invited Speaker**
Combining Active Learning, Synthesis on Demand Libraries, and Fragment Screening in Early Drug Discovery
Dr. Pat Walters, Relay Therapeutics

11:30 AM *Marrying Structures with Computation for Augmented Fragment-Based Drug Discovery*
Dr. Paul Mortenson, Astex Pharmaceuticals

12:00 PM *Fragment-Based Identification and Analysis of Cryptic Ligand Binding Sites*
Dr. Sandor Vajda, Boston University

12:30 PM **Networking Lunch**

Session 7 – Fragments Against SARS-CoV-2 Chair: Dr. Daniel Wyss, Merck & Co.
1:45 PM to 3:15 PM

1:45 PM **Invited Speaker**
The SARS-CoV-2 Macrodomain and How to Inhibit it
Prof. James Fraser, University of California, San Francisco

2:15 PM *Utilizing FBDD Screening to Develop a Novel Class of Inhibitors for SARS-Cov-2 Papain-Like Protease*
Dr. Ashley Taylor, Vanderbilt University

2:45 PM *Fragment-Based Lead Discovery Against SARS-CoV-2 nsp3 Macrodomain*
Dr. Thomas Vargo, PostEra

3:15 PM **Networking Break**

Session 8 – SARS-CoV-2 and Beyond Chair: Prof. Martin Scanlon, Monash University
3:45 PM to 5:30 PM

3:45 PM *From Fragments to In-Vivo Optimized Leads for the SARS-CoV2 Nsp3 Macrodomain Mac1 – An Emerging Target in Antiviral Therapy*
Prof. Adam Renslo, University of California, San Francisco

4:15 PM *Development of EcDsbA Inhibitors as Antivirulence Agents*
Dr. Yildiz Tasdan, Monash University

4:45 PM *Accelerating Structure Enabled Antiviral Discovery Through Crystallographic Fragment Screening with The Xchem Platform*
Dr. Blake Balcomb, Oxford University/Diamond Lightsource

5:15 PM *Every Crystal Matters*
Dr. Holger von Möller, Molox GmbH

5:30 PM **End Day 2**

7:00 **Conference Dinner at Nightshift Brewing**

Wednesday, 25 September 2024

8:00 AM **Poster Breakfast**

Session 9 – Finding New Sites with Fragments Chair: Dr. Heike Schönherr, Relay Tx
9:00 AM to 10:15 AM

9:00 AM *Biological Validation of a Novel eIF4E Binding Site Identified by Fragment Screening Using Mutational Analysis and Targeted Protein Degradation*
Andrew Woodhead, Astex Pharmaceuticals

9:30 AM *Enabling NMR Fragment Screening and Hit-to-Lead Medicinal Chemistry via NMR for SAR*
Prof. Steven LaPlante, NMX Research and Solutions Inc

10:00 AM *Exploring the Power of Structural Biology on Degrader Discovery*
Larry Jin, Biortus Biosciences Co. Ltd.

10:15 AM *Award Ceremony & Announcements*
Dr. Heike Schönherr, Relay Therapeutics

10:30 AM **Networking Break**

Session 10 – Fragments in the Future Chair:
11:00 AM to 12:45 PM

11:00 AM **Invited Speaker**
Sample multiplexing combined with profiling covalent cysteine libraries for FBDD
Prof. Steven Gygi, Harvard University

11:30 AM *Fragment Screening of Adenosine A2a Receptor Using Native Lipid Nanodiscs*
Philip Rawlins, Domainex

12:00 PM **Closing Keynote**
Prospective Discovery of Molecular Glues Using Site-Directed Fragments
Prof. Michelle Arkin, University of California, San Francisco

12:45 PM *Closing remarks*
Joe Patel, Nexo Therapeutics

1:00 PM **End FBLD 2024**

Social Functions

Networking Reception – 22 September

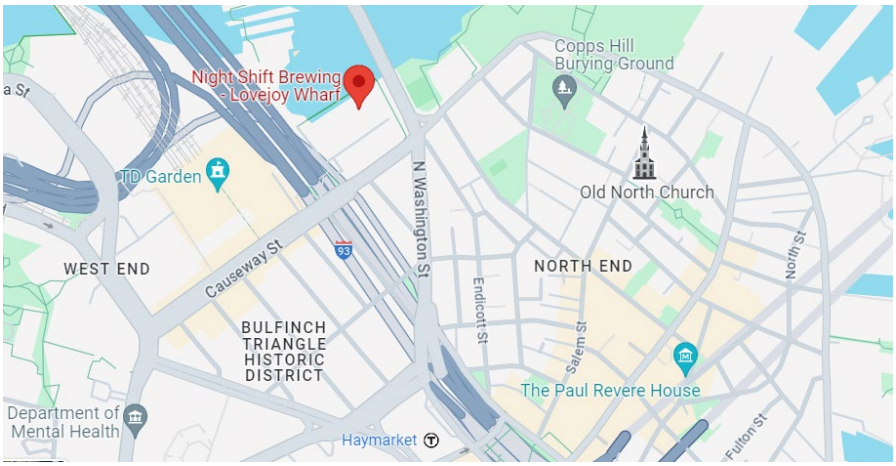
We are delighted to invite you to join us for our Networking Welcome Reception in the Ziskind Lounge. This reception will be an excellent opportunity to mingle with fellow attendees, speakers, and industry leaders in a relaxed and informal setting. Please come and enjoy an evening of networking, drinks, and delicious food as we kick off what promises to be an exciting conference.

Poster Session – 23 September

Join us for a drink and finger food during our poster and networking session. Make the most of the chance to talk to the speakers and fellow delegates. Discover more collaboration opportunities or get answers to your burning questions on fragment-based lead discovery that go beyond the topics discussed today.

Conference Dinner – 24 September

We invite you to attend the Conference Dinner on 24 September at the Nightshift Brewery. Join us for an unforgettable evening of networking and dinner at Boston's Lovejoy Wharf. Overlooking the Boston Harbour, Zakim Bridge and neighbouring Charlestown, this venue provides an excellent opportunity to delve further into today's topics. Immerse yourself in the vibrant atmosphere of this renowned craft brewery. Savour delicious local brews and gourmet bites while fostering your network. Whether you're forging new partnerships or exchanging ideas, the conference dinner promises to be an inspiring and engaging event.



Address:
1 Lovejoy Wharf #101
Boston, MA 0211

Start: 7:00 pm
End: 10:00 pm

List of Registrants

First Name	Last Name	Institution
Roman	Agafonov	C4 Therapeutics
Nader	Amin	JEOL USA
Ben	Apker	MiTeGen
Brent	Appleton	C4 Therapeutics
Michelle	Arkin	University of California San Francisco
Yann	Ayotte	NMX Research and Solutions
Yonghong	Bai	MoMa Therapeutics
Yulia	Bakanovych	Enamine
Blake	Balcomb	Diamond Lightsource
Lilia	Baldauf	University of California San Diego
Violla	Bassim	University of California San Francisco
Christian	Becke	Molox GmbH
Ayse	Bekar	Boston University
Kinga	Bercsenyi	Arctoris
Jay	Bertrand	Evotec US
Ray	Boffey	DomainEx
George	Bondar	Life Chemicals
Simon	Bury	Viva Biotech Ltd
Percy	Chen	MorphicTx
Ryan	Chen	XtalPi Inc.
Jay	Chodaparambil	Sanofi
Damien	Clavel	ALPX
Galen	Correy	University of California San Francisco
Debanu	Das	Accelero Biostructures Inc
Doug	Davies	UCB
Ben	Davis	Vernalis
Shane	Dawson	Harvard Medical School
Iwan	de Esch	Vrije Universiteit Amsterdam
Simon	Debrand	Servier
Tom	Dekker	Vrije Universiteit Amsterdam
Karuna	Dixit	Vertex Pharmaceuticals
Michael	Drummond	Chemical Computing Group
Josh	Eckman	Carterra
Megan	Egbert	Isomorphic Labs

First Name	Last Name	Institution
Dan	Erlanson	Frontier Medicines
Robert	Everley	Frontier Medicines
Yiting	Fan	StoneWise AI Ltd
Thomas	Fessard	SpiroChem AG
James	Féthière	Sygnature Discovery
Thomas	Fleming	Arctoris
Guillaume	Fortin	Chemical Computing Group
James	Fraser	University of California San Francisco
Jessica	Freeze	Conifer Point
Nathan	Fuller	Rectify Pharmaceuticals
Mingi	Gao	Biortus
Tony	Giannetti	Carterra
Christian	Giddens	Carterra
Serghei	Glinca	CrystalsFirst GmbH
Jack	Greisman	D. E. Shaw Research
Elizabeth	Griffith	St Jude Children's Research Hospital
Michael	Grillo	Jnana Therapeutics
Steven	Gygi	Harvard Medical School
Kenth	Hallberg	SARomics Biostructures
Gregory	Heffron	Harvard Medical School
Jacqueline	Hicks	Merck & Co., Inc.
Jeffrey	Holden	Vilya
Michael	Holliday	Relay Therapeutics
Justin	Holowachuk	BioSolveIT
Hongwei	Huang	Nexo Therapeutics
Rod	Hubbard	University of York and Vernalis Research
Iryna	Iavniuk	Enamine
Ishrat	Jalal	WuXi AppTec
Moran	Jerabek-Willemsen	WuXi AppTec
Larry	Jin	Biortus
Jan	Jiricek	eMolecules, Inc.
Catherine	Jorand	Nexo therapeutics
Diane	Joseph-McCarthy	Boston University
Camil	Joubran	JEOL USA
John	Kelly	Schrödinger Inc.
Ryan	Kelly	XtalPi Inc.

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Deren	Koseoglu	eMolecules, Inc.
Tobias	Krojer	MAX IV Laboratory
Matt	Labenski	Jnana Therapeutics
May Poh	Lai	Malvern Panalytical Inc
Steven	LaPlante	NMX Research and Solutions
Lucie	Laurin	NMX Research and Solutions
Maria	Lazou	Boston University
Richard	Lee	St Jude Children's Research Hospital
Guy	Lewy	Cambridge Molecular
Andrew	Lowerson	Key Organics Limited
Tom	Mastrangelo	Bruker BioSpin Corporation
Brian	McMillan	Jnana Therapeutics
Frank	Moffatt	NovAliX
Juliet	Morgan	Sygnature Discovery
Paul	Mortenson	Astex Pharmaceuticals
Gerhard	Muller	SpiroChem AG
Alan	Nafiev	Receptor.ai
Xiaomin	Ni	University of Oxofrd
Marc	Obiols	MAX IV Laboratory
Marc	O'Reilly	Astex Pharmaceuticals
Victoria	Ouroutzoglou	Viva Biotech Ltd
Juliette	Pabst	Nuvisan GmbH
Mackenzie	Parker	Kymera Therapeutics
Joe	Patel	Nexo Therapeutics
Puja	Pathuri	Astex Pharmaceuticals
Rebecca	Paul	Isomorphic Labs
Lewis	Pennington	Drug Hunter
Andrea	Pica	ALPX
Nikolay	Plotnikov	Treeline Biosciences
Emily	Podd	University of California San Diego
Chris	Przybylski	BioSolveIT
Philip	Rawlins	DomainEx
David	Rees	Astex Pharmaceuticals
Stephanie	Reeve	Scorpion Therapeutics

First Name	Last Name	Institution
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Justin	Rettenmaier	Jnana Therapeutics
Caroline	Richardson	Astex Pharmaceuticals
David	Robinson	BioAscent Discovery Limited
Joana	Rocha	ALPX
Nahomi	Rodriguez-Sastre	Biortus
Cheri	Ross	Relay Therapeutics
Sue	Saalau	Consultant
Clemente	San Felipe	University of California San Francisco
Martin	Scanlon	Monash University
Martina	Schaefer	Nuvisan GmbH
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Frank	von Delft	Diamond Lightsource
Holger	von Möller	Molox GmbH
Pat	Walters	Relay Therapeutics
Feng	Wang	Biortus
Rebecca	Whitehouse	Harvard Medical School
Andrew	Woodhead	Astex Pharmaceuticals
Haihong	Wu	AbbVie, Inc.
Jiaquan	Wu	Biortus
Zhicai	Wu	Merck & Co., Inc.

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Bing	Xia	Viva Biotech Ltd
Jun	Xian	Biortus
Linlong	Xue	Biogen Inc
Ruheng	Zhao	University of Michigan
Jeremy	Zhong	Biogen Inc
Yingjie	Zhu	Biortus
Quinn	Zona	WuXi AppTec

Oral Abstracts

SYSTEMATIC DISCOVERY OF MOLECULAR GLUES USING REVERSIBLE-COVALENT FRAGMENTS

Michelle Arkin

University of California, San Francisco

Molecular glues (MGs) – compounds that induce or stabilize protein-protein interactions – are fundamentally changing the way drug hunters think about targeting previously undruggable targets. Natural and synthetic MGs can induce non-native (neomorphic) interactions or further stabilize native complexes; they can also lead to pathway activation, inhibition, or even degradation of one of the target proteins. Creative cellular approaches have been developed to screen for MGs, particularly MG degraders. Nevertheless, there are few methods for prospective discovery of MGs for a particular protein-protein interaction. Our collaborative team has developed cell-active molecular glues for several proteins, including the kinase CRAF and transcription factors estrogen receptor and YAP, that bind to the phosphoprotein-chaperone 14-3-3. Our approach to systematic discovery of MGs uses the disulfide tethering and other reversible covalent libraries to screen for fragments that bind to the protein complex more strongly than to either of the individual proteins. By increasing residence time, covalency might increase the chance for both proteins and compound to form a ternary complex. Through screening and structure-guided optimization, we are developing the rules-of-thumb for designing molecular glues.

ACCELERATING STRUCTURE ENABLED ANTIVIRAL DISCOVERY THROUGH CRYSTALLOGRAPHIC FRAGMENT SCREENING WITH THE XCHEM PLATFORM

Balcomb, B.H. [1,3], Winokan, M.[1,3], Thompson, W.[1,3], Fieseler, K.[5,10], Wills, S.[5,10], Aschenbrenner, J.C.[1,3], Tomlinson, C.W.E.[1,3], Fairhead, M.5, Marples, P.G.[1,3], Koekemoer, L.[5], Lithgo, R.M.[1,3], Ni, X.[5], Chandran, A.C.[1,3], Wild, C.[1,3], Capkin, E.[1,3], Ferla, M.P.[5,10], Golding, M.[1,3], Godoy, A.S.[8], Barr, H.[4], Griffin, E.[6], Lee, A.2, Chodera, J.D.[9], von Delft, A.[5], Walsh, M. [1,3], Fearon, D.[1,3], von Delft, F.[1,3,5,7], and the ASAP Discovery Consortium

[1]Diamond Light Source Ltd, Harwell Science and Innovation Campus, UK, [2] PostEra, USA, [3] Research Complex at Harwell, Harwell Science and Innovation Campus, UK, [4]G-INCPM, The Weizmann Institute of Science, Israel, [5]Centre for Medicines Discovery, University of Oxford, UK, [6]MedChemica, UK, [7]Department of Biochemistry, University of Johannesburg, South Africa, [8]Institute of Physics of São Carlos, University of São Paulo, Brazil, [9]Memorial Sloan Kettering Cancer Center, New York, NY, [10]Department of Statistics, University of Oxford, UK.

As an effort towards pandemic preparedness, the National Institutes of Health (NIH) established nine Antiviral Drug Discovery (AViDD) Centers for Pathogens of Pandemic Concern. One of these Centers, the **A**I-driven **S**tructure-enabled **A**ntiviral **P**latform (ASAP), aims to accelerate structure-based open science antiviral drug discovery by building a robust global antiviral discovery pipeline. Crystallographic fragment screening and high-throughput structural biology, powered by the XChem facility at Diamond Light Source (Douangamath et al, 2021), is central to hit identification and lead optimisation within ASAP. Performing such large-scale structural biology experiments is relies on structurally enabling a target which is critical for efficient fragment hit-to-lead and lead optimisation. Literature surveys of known fragment-to-lead medicinal chemistry campaigns over the past eight years have shown that nearly 70-80% of all campaigns generated a structure for either hit or lead chemical matter. With 100% of all campaigns using structural information during lead optimisation (Woodhead et al, 2024). Beyond a crystallographic fragment screen, how one can reliably and efficiently progress fragment hits with the goal of on-scale potency, including retaining the shape and colour of the base compound in lead generation. Here we highlight the value of having structurally enabled drug discovery campaigns with a focus on how high-throughput structural biology and interaction-driven algorithmic methodologies can accelerate fragment progression strategies and ameliorate the design-make-test cycle in the ASAP consortium.

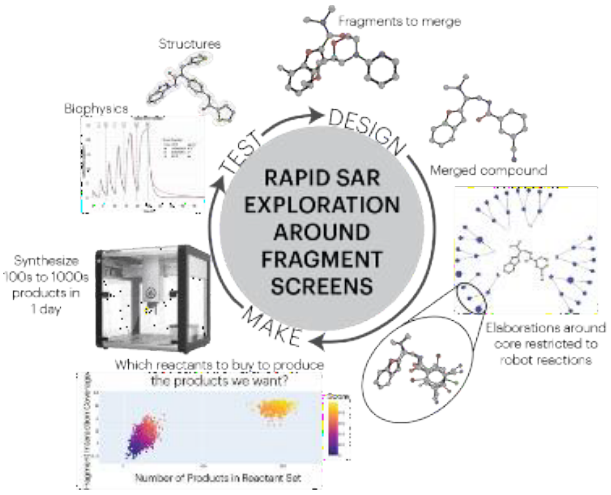

Figure 1: *Going beyond the crystallographic fragment screen: closing the loop on the design-make-test cycle.*

References

Douangamath, A. et al(2021), Achieving Efficient Fragment Screening at XChem Facility at Diamond Light Source, J Vis Exp May 29:(171). Woodhead, A. et al(2024), Fragment-to-Lead Medicinal Chemistry Publications in 2022,J. Med. Chem. 2024 67 (4).

FAST FRAGMENT HIT-TO-LEAD IN DRUG DISCOVERY FOR NOVEL DRUG TARGETS

Debanu Das[1,2], Ashley Deacon[1,2], Matthew Duncton[1]

[1]XPose Therapeutics Inc., CA, USA, [2]Accelero Biostructures Inc, CA, USA

Using state of the art protein X-ray crystallography-driven fragment screening and drug discovery platforms, in a Fragment-Based Drug Discovery approach, we have discovered and developed novel inhibitors against DNA Damage Response (DDR) proteins APE1 and POLH, which represent the first crystal structures of small molecules bound to these proteins, to enable these oncology therapeutics targets that have been refractory to targeting by other methods. Simultaneous production of both hits and crystal structures allowed us to implement a rapid hit-to-lead generation and lead optimization campaign. For APE1, our novel inhibitors exhibit IC50s of ~250 nM to ~10 uM in *in vitro* biochemical assays with robust activity in cell-based assays. The lead inhibitor, XPTx-91, and its compact cyclic relative XPTx-387, have biochemical IC50s of ~500 nM and ~250 nM, respectively. XPTx-91 is already 4x more potent (and XPTx-387 is 7x) than APE1 Inhibitor III, which is the leading APE1 inhibitor widely used in biochemical and cellular characterization of APE1. XPTx-91 enhances cytotoxicity of a common genotoxin, MMS, tested in a glioma cell line, in a concentration dependent manner, and this sensitization is observed at concentrations of XPTx-91 that are not cytotoxic. This indicates that our APE1 inhibitors will likely potentiate the activity of chemotherapeutic alkylators that promote AP site formation, without causing broad toxicity. XPTx-91 is selective and specific over other endonucleases, and selectivity results show that XPTx-91 is superior to APE1 Inhibitor III. This selectivity translates to observations in the glioma cell line assay that can measure broad cellular toxicity. A broad preliminary cancer cell line screening using XPTx-91 demonstrated an ~15% growth inhibition in CNS and ovarian cancer; ~18% in renal cancer; and ~10% in breast cancer. We anticipate these results to improve as we perform optimization of XPTx-91 and XPTx-0387. POLH (or POL Eta) is implicated in cisplatin resistant ovarian cancers. Our approach has yielded a POLH hit with IC50 ~220 uM in an *in vitro* assay, which we are also advancing similar to our APE1 efforts.

References

Fragment- and structure-based drug discovery for developing therapeutic agents targeting the DNA Damage Response. Wilson DM 3rd, Deacon AM, Duncton MAJ, Pellicena P, Georgiadis MM, Yeh AP, Arvai AS, Moiani D, Tainer JA, Das D. *Prog Biophys Mol Biol. 2021* Aug;163:130-142.

Early Drug Discovery and Development of Novel Cancer Therapeutics Targeting DNA Polymerase Eta (POLH). Wilson DM, Duncton MAJ, Chang C, Lee Luo C, Georgiadis TM, Pellicena P, Deacon AM, Gao Y, Das D. *Front Oncol. 2021* Nov 19;11:778925.

A New Drug Discovery Platform: Application to DNA Polymerase Eta and Apurinic/Apyrimidinic Endonuclease 1. Das D, Duncton MAJ, Georgiadis TM, Pellicena P, Clark J, Sobol RW, Georgiadis MM, King-Underwood J, Jobes DV, Chang C, Gao Y, Deacon AM, Wilson DM 3rd. Int J Mol Sci. 2023 Nov 23;24(23):16637.

PHOTOACTIVATED COVALENT CAPTURE OF DNA-ENCODED FRAGMENTS TO IDENTIFY LIGANDS FOR CHALLENGING TARGETS

Lucy Pluckrose[1], Huiyong Ma[2], Ben J. Davis[1], James B. Murray[1], Huadong Luo[2], Xuemin Cheng[2], Qiuxia Chen[2], Chao Song[2], Cong Duan[2], Ping Tan[2], Lifang Zhang[2], Jian Liu[2], Barry A. Morgan[2], Jin Li[2], Jinqiao Wan[2], Lisa M. Baker[1], William Finnie[1], Lucie Guetzoyan[1], Richard Harris[1], Nicole Hendrickson[1], Heather Simmonite[1], Julia Smith[1], Roderick E. Hubbard[1] and Guansai Liu[2]

1. *Vernalis, Granta Park, Cambridge UK*
2. *HitGen, Tianfu International Bio-Town, Chengdu, China*

We are developing new approaches for identification of hit compounds for challenging targets, combining the sensitivity of DNA-encoded libraries (DEL) with the chemical space coverage of fragments (FBLD).

We recently reported PAC-FragmentDEL (Ma *et al*, 2022), where photoactivation captures the fragment binding to the target, demonstrated for the protein kinase, PAK4 and the more challenging antibacterial target, 2-epimerase. The first part of this presentation will provide an update on the evolution of fragment hits to identify for the first time, potent 2-epimerase inhibitors with antibacterial activity.
The strategies we have developed to optimise the hit-id process will be discussed with examples focussed on challenging targets including protein-protein interaction, unliganded novel protein domains and targets with no known ligands.
We will also discuss advances in the PAC-FragmentDEL approach, including applications of the next generation libraries and the use of high throughput techniques – including crude-reaction mix screening and custom DEL libraries - to rapidly evolve fragments to sub M affinities. Importantly, these evolution strategies do not require structural guidance, and so can be applied generically.

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INTRODUCTION TO FRAGMENT-BASED DESIGN

Dr. Dan Erlanson[1] & Dr. Ben Davis[2]

[1]Frontier Medicines
[2]Vernalis Research

Are you new to fragment-based drug discovery (FBDD) and wondering what all the excitement is about? Or a long-time practitioner looking for the latest developments? Either way, join Ben Davis (Vernalis) and Dan Erlanson (Frontier Medicines) to discuss both theory and practice, including fragment screening, fragment validation and characterization, plus case studies in FBDD. Questions and comments are welcome!

HIGH-THROUGHPUT ACTIVITY-BASED FRAGMENT SCREENING WITH ULYSSES™: GENERATING ROBUST DATASETS FOR MACHINE LEARNING-DRIVEN DRUG DISCOVERY

Thomas Fleming[1], John Evans[1], Anthony Brown[1], Kinga Bercsenyi[1], Thomas Fessard[2], Gerhard Mueller[2]

[1]Arctoris Ltd., [2]Spirochem AG

The application of artificial intelligence (AI) in drug discovery is heavily dependent on the quality and reproducibility of training datasets. Robotics has emerged as a crucial enabler for generating large volumes of high-quality data to fuel machine learning (ML) algorithms. In this study, we present the development of a comprehensive technology platform that combines robotic assay platforms with automated analytics pipelines to streamline the data generation and analysis process for ML-driven drug discovery. Our platform, Ulysses™, achieved a throughput of 5,500 fragments tested against 12 distinct targets per day over a total of 65 protein targets, producing a uniquely rich dataset for ML model training. The initial predicted potencies exhibited a strong correlation with the confirmed potencies, demonstrating the reliability of the generated data. We highlight the importance of assay precision and quality to fully exploit the potential of ML in drug discovery. Furthermore, we showcase the advantages of activity-based profiling, which provides immediate indications of compound activity, in contrast to affinity-based methods such as surface plasmon resonance (SPR). Activity-based profiling facilitates the identification of functionally relevant hits, enhancing the efficiency of the drug discovery process. The integration of automated analytics pipelines enables rapid data processing, rigorous statistical treatment, generation of 'gold standard' ML-ready datasets and reduces the time and effort required for data analysis. Our findings emphasise the significance of data volume, quality, and automated analytics for successful ML applications in drug discovery, and the pivotal role of robotics in achieving these requirements. By leveraging high-throughput, high-quality data generation through robotic assay platforms and automated analytics pipelines, we can accelerate the development of novel therapeutics and optimise the drug discovery workflow.

THE SARS-COV-2 MACRODOMAIN AND HOW TO INHIBIT IT

James Fraser

University of California, San Francisco

The nonstructural protein 3 (NSP3) macrodomain of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Mac1) removes adenosine diphosphate (ADP) ribosylation posttranslational modifications, playing a key role in the immune evasion capabilities of the virus responsible for the coronavirus disease 2019 pandemic. Starting from a collaborative X-ray-based fragment screen, multiple computational strategies and structure-based design has facilitated the development of potent inhibitors.

SAMPLE MULTIPLEXING COMBINED WITH PROFILING COVALENT CYSTEINE LIBRARIES FOR FBDD

Steven Gygi[1]

[1]*Harvard Medical School, Dept. of Cell Biology*

Electrophilic compound libraries contain hundreds to thousands of members which can often target cysteine residues in target proteins. Sample multiplexing can be used to increase throughput and a new 32plex TMT reagent set is now available, making the profiling of entire libraries possible. In this presentation, I will show examples of cysteine profiling for electrophilic libraries in both targeted and untargeted assays. For untargeted assay, more than 20,000 cysteines can be examined for compound binding in a single run. In addition, we used the 32plex reagent set to quickly profile all compounds in our library containing a cyclic sulfone which has been shown to bind to the pocket of Pin1 and to cysteine 113. We identified pocket features that facilitate fragment binding.

USING FRAGMENTS TO ANSWER QUESTIONS IN EXPLORATORY DRUG DISCOVERY

Roderick E. Hubbard[1,2]

[1] *Vernalis (R&D) Ltd, Granta Park, Cambridge, CB21 6GB, UK*
[2] *YSBL, University of York, YO10 5DD, UK*

It is nearly 30 years since a team at Abbott first demonstrated fragment-based drug discovery and there will be many examples at this conference which illustrate how careful medicinal chemistry optimisation of fragment hits can lead to potent lead compounds.
In this presentation I will focus on another major application of fragments - aiding the initial stages of the drug discovery process. Many of the potential therapeutic targets being identified in modern drug discovery are novel with various challenges and questions – such as is there a "ligandable" but functionally relevant site for small molecules to bind?, which sites on the protein and mechanism of action is to be targeted?, is it possible to achieve selectivity? Various modalities of fragment screening can be used to ask these questions and, if successful, provide understanding of the chemical biology of the system as well as possible start points for evolving hit compounds potent enough to establish the platform for a drug discovery project.
Using examples from projects at Vernalis, I will start with a story that illustrates the main features of fragment-based discovery, before discussing exploratory work on various protein targets where different fragment screening approaches attempt to answer these questions.

BIOPHYSICAL AND STRUCTURAL BIOLOGY METHODS ENABLE FRAGMENT-BASED LIGAND DISCOVERY

Moran Jerabek-Willemsen

WuXi Apptec

Powerful biophysical and structural biology tools enable the study of large numbers of fragments and are opening up new possibilities in the treatment of various diseases. Here we report the results of a conventional and a covalent Fragment Screening and show how orthogonal biophysical and structural methods enable rapid identification, characterization, and optimization of fragments.

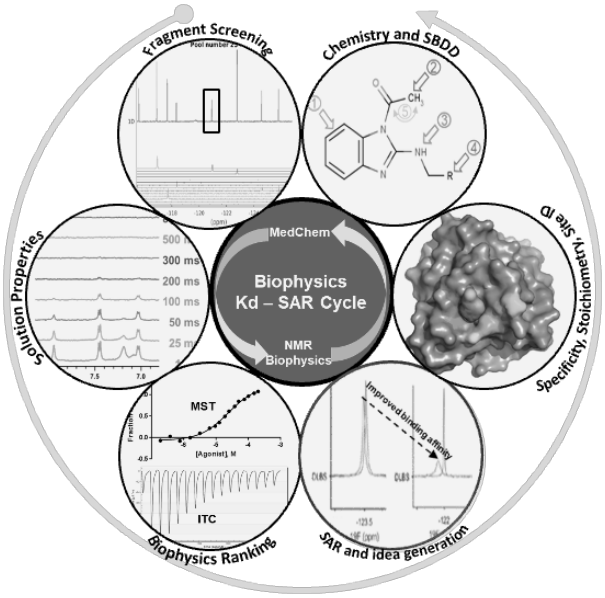
ENABLING NMR FRAGMENT SCREENING AND HIT-TO-LEAD MEDICINAL CHEMISTRY VIA NMR FOR SAR

Steven R. LaPlante,[1,2]

[1] *INRS, Laval, CANADA* [2] *NMX Research and Solutions Inc., Laval, Canada*

The discovery of small-molecule drugs remains crucial for combating diseases. This presentation will convey the critical role NMR plays in discovering hits and leads using fragment-based lead discovery (FBLD). We developed integrated strategies that involve the design and curation of new fragment libraries, the implementation of consensus NMR screening strategies, and the construction of in-house software for rapid data analyses purposes. The ensemble of our NMR and biophysics platforms are crucial for prioritizing fragment hits that have appropriate free and target-binding solution properties. Examples will be shown where our workflow supported the discovery of drug leads for many programs, along with the exposure of unexpected compound solution behaviors (solubility, aggregation), the revelation of target protein features (folding and changes), the determination of stoichiometric binding attributes, and the enablement of medicinal chemistry efforts by affinity-ranking analogues.

Overall, the ensemble of NMR and biophysics methods has been central for enabling drug discovery campaigns by deprioritizing hit artifacts (false positives and false negatives) and enabling medicinal chemistry efforts to establish structure-activity relationships and thus effectively reduce downstream expenditures of time and resources. This will be demonstrated by examples where high-quality micromolar binders can be generated from the initial millimolar fragment screening hits against the “undruggable” protein targets.



Acknowledgements
NMX team, INRS team, CQDM, MEIE, Mitacs, Gregory Heffron of HMS.

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COMPUTATIONAL HOT SPOT MAPPING FOR FRAGMENT-BASED LEAD DISCOVERY

Diane Joseph-McCarthy[1], Maria Lazou[1], Ayse Bekar-Cesaretli[2], Sandor Vajda[1,2]

[1]Boston University, Department of Biomedical Engineering,
[2]Boston University, Department of Chemistry

Identification of fragment-binding positions on the surface of macromolecules is a key to locating druggable sites, assessing the druggability of novel targets, and developing starting points for finding new chemical entities. Computational hot spot mapping was performed to identify binding sites across a set of known or potential drug targets using FTMove (Egbert et al., 2022), and a novel machine learning approach was employed to select the top druggable sites, including those at active and allosteric sites. Within this context, the utility of experimentally determined vs. AI-generated protein models obtained using AlphaFold (Jumper et al., 2021) was assessed (Bekar-Cesaretli et al., 2024).

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FBDD APPROACHES TO TACKLE EMERGING MULTI-COMPONENT THERAPEUTIC TARGETS

Juliet Morgan[1], Rachel Lawrence[1], Benoit Gourdet[1], Duncan Smith[1], Joshua Shaw[1], Louise Birch[1], Philip Addis[1], Roland Hjerpe[1], Stuart Thomson[1]

[1]Sygnature Discovery UK

In recent years there has been an increasing effort to tackle more challenging therapeutic targets. This has been driven by a combination of better biological knowledge to identify targets for difficult to treat diseases, as well as existing successes with many of the more tractable targets. We have observed within our work that there has been a significant increase in drug targets that involve the interaction of two or more components, which can make the discovery of new therapeutic molecules complex and challenging (Fitzgerald et al., 2024). Two key areas within this space include induced proximity therapeutics, such as bifunctional degraders and molecular glues, and the targeting of nucleic acid binding proteins, such as helicases and polymerases. Whilst different in nature, both classes involve interactions beyond the one-to-one binding of a drug to a target, whether that is inducing the formation of a complex, or drugging an activation state that is only available in the presence of a binding partner, such as a nucleic acid. We believe that a biophysical approach using fragments is ideally suited to both applications (and more), to deliver medicinal chemistry starting points where other methods do not.

We present here an approach for the rational identification and subsequent hit validation of potential molecular glues against two targets, SOS1 and SHP2, using the Sygnature Fragment Library. A molecular glue that stabilises the interaction of either of these targets with the wider E3 ligase complex would have the potential to lead to ubiquitination and degradation, that would limit RAS activation and the proliferation of cancer cells (You et al., 2018).

Acknowledgements
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MARRYING STRUCTURES WITH COMPUTATION FOR AUGMENTED FRAGMENT-BASED DRUG DISCOVERY

Paul Mortenson, Carl Poelking, Marcel Verdonk, Davide Branduardi

Astex Pharmaceuticals, 436 Cambridge Science Park, Cambridge, CB4 0QA, UK

The small size of fragments means that they typically require excellent complementarity with a protein binding site in order to bind detectably. As such, experimental protein- fragment structures are uniquely informative for designing high affinity ligands. Over the past 25 years, Astex has generated an unrivalled resource of several thousand such structures. In this talk I will discuss how we have used this rich dataset to develop and validate a range of computational approaches to facilitate the journey from fragment to candidate. These include both predictive and generative AI methods, along with cutting- edge physics-based approaches. I will also show how these technologies are deployed to complement the skills of our expert designers, in an approach we refer to as Augmented Interactive Design.

Acknowledgements
It is a pleasure to acknowledge the many past and present Astex scientists who generated the structural data that I will discuss here.

CRYSTALLOGRAPHIC FRAGMENT SCREENING IDENTIFIES DIVERSE CHEMICAL SCAFFOLDS FOR ZIKA VIRUS NS2B-NS3 PROTEASE INHIBITOR DEVELOPMENT

Xiaomin Ni[1,5], Andre Schutzer Godoy[2,5], Peter G. Marples[3,4,5] , Blake H. Balcomb[3,4,5], Matthew J Evans[6], Jenke Scheen[7], Matteo P. Ferla[1,5,8], Charles W.E. Tomlinson[3,4,5], Michael Fairhead[1,5], Siyi Wang[1,5], Jasmin C. Aschenbrenner[3,4,5], Ryan M. Lithgo[3,4,5], Max Winokan[3,4,5], Anu V. Chandran[3,4,5], Warren Thompson[3,4,5], Eleanor P. Williams[1,5], Martin Walsh[3,4,5], Daren Fearon[3,4,5], Lizbé Koekemoer[1,5], Frank von Delft *[1,3,4,5]

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Zika virus (ZIKV) infections cause microcephaly in new-borns (de Araujo et al, 2018) and Guillain-Barre syndrome in adults (Krauer, F. et al, 2017) raising a significant global public health concern, yet no vaccines or antiviral drugs have been developed to prevent or treat ZIKV infections. The viral protease NS3 and its co-factor NS2B are essential for the cleavage of the Zika polyprotein precursor into individual structural and non-structural proteins and is therefore an attractive drug target. Generation of a robust crystal system of co-expressed NS2B-NS3 protease has enabled us to perform a crystallographic fragment screening campaign with 1076 fragments. 48 binders with diverse chemical scaffolds were identified in the active site of the protease, with another 6 fragment hits observed in a potential allosteric binding site. Algorithmic merging of the fragment hits yields an extensive set of “merge hits”, defined as synthetically accessible compounds that recapitulate constellations of observed fragment-protein interactions. These moreover explore diverse binding opportunities identified from the many bound and unbound structures in the fragment screen, and thus yield robust starting points developing potent NS2B-NS3 protease inhibitors. We further characterise these opportunities by mapping them onto interacting residues fitness results from deep mutational scanning, allowing us to prioritise merge hits based on resistance risks.
We therefore assess this fragment screen to have been effective and generate a priority list of chemical matter that maximally explore the binding opportunities to NS2B-NS3, facilitating its inhibitor development.

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FRAGMENT-BASED DISCOVERY OF ALLOSTERIC MODULATORS OF β-GLUCOCEREBROSIDASE

Puja Pathuri

Astex Pharmaceuticals

β-Glucocerebrosidase (GBA / GCase) mutations found in Gaucher´s Disease, Parkinson's Disease and Dementia with Lewy Bodies lead to defective protein stability and intracellular trafficking, which contributes to endoplasmic reticulum (ER) stress and lysosomal dysfunction. The identification of small molecule pharmacological chaperones that can stabilize mutant GCase and increase its delivery to the lysosome is a strategy under active investigation. We applied fragment screening using X-ray crystallography and other biophysical methods that has enabled the identification of new, potentially druggable, sites on the GCase protein surface. Subsequent, structure-based, optimization of fragment hits led to the discovery of a series of compounds that bind GCase with nM potency. Multiple assays have been put in place to characterise the effect of compounds in cellular models.

AI-FIRST FRAGMENT-BASED DRUG DISCOVERY AT ISOMORPHIC LABS

Rebecca Paul[1]

[1]*Isomorphic Labs*

Isomorphic Labs is a digital biology company working to reimagine drug discovery with the power and pace of artificial intelligence. The company was founded with the belief that cutting edge computational and AI methods hold promise to improve upon traditional approaches to drug design. Through the application of AlphaFold 3 (Abramson et al, 2024), developed by Isomorphic Labs in collaboration with Google DeepMind, along with other breakthrough AI models developed at Isomorphic Labs, the company is able to better understand the underlying biological mechanisms related to therapeutic targets of interest and advance the rational design and optimisation of small molecules.

One thesis currently being explored at Isomorphic Labs is the potential to enable the rapid optimisation of fragments to leads. The use of AlphaFold 3 allows scientists at Isomorphic Labs to work on structure-based rational drug design without the need for experimental structure determination. In addition, the power of the company's generative AI in combination with its predictive models, enables rapid elaboration of fragment starting points. The combination of both these elements holds potential for fragment-based drug design at Isomorphic Labs and progress towards this approach will be presented.

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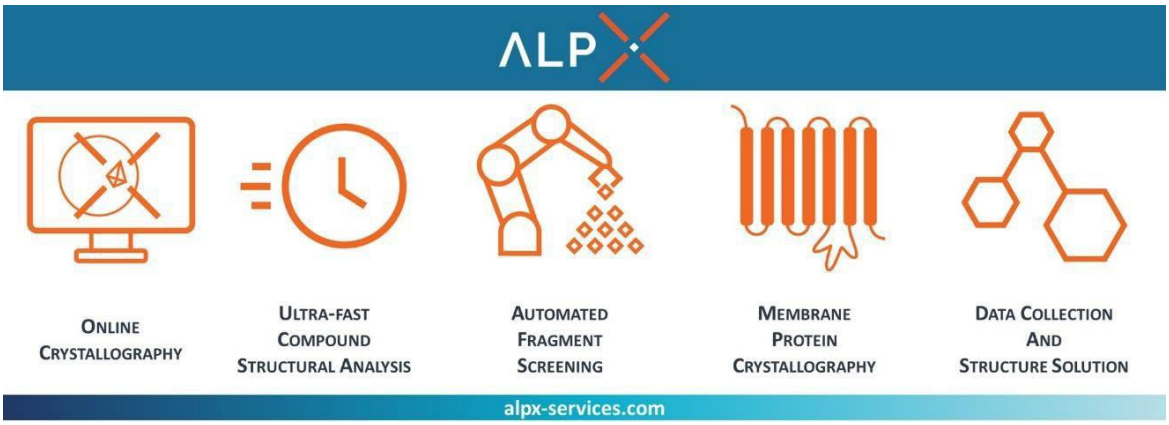
ACCELERATING DRUG DISCOVERY: ADVANCED STRUCTURAL BIOLOGY SOLUTIONS AT ALPX

Andrea Pica[1], Damien Clavel[1], Gavin Ferguson[1], Petra Drncova[1], Irina Cornaciu- Hoffmann[1]

[1]ALPX, 71 avenue des Martyrs, Grenoble, France

ALPX is a French CRO, a spin-off of the European Molecular Biology Laboratory, that excels in providing cutting-edge structural biology solutions to pharmaceutical and biotech companies. We harness the power of our unique CrystalDirect™ (Cipriani et al, 2012) and CRIMS technologies to support an automated, high-throughput protein-to-structure pipeline (Cornaciu et al, 2021) that significantly accelerates drug discovery. Thanks to our technology and expertise, we can handle even the most challenging targets, including large complexes, molecular glues, PROTACs, protein-antibodies, protein-DNA complexes, and membrane proteins (Healey et al, 2021).
ALPX services encompass structure determination, compound and fragment screening, data collection, and consultancy. The weekly access to synchrotron sources for data collection ensures rapid and reliable results, including for crystals generated by our clients. Our screening-by-crystallography capabilities make us highly effective and efficient, allowing the swift delivery of results with optimized resources. Additionally, via CRIMS, we provide complete access to both the results obtained and the resources used, making the road to success fully transparent. ALPX was recognized among the top 10 CROs for drug discovery in both 2022 and 2023.
In our talk, we will showcase how we can support fragment screening campaigns efficiently exploiting our advance pipeline, illustrating our advanced methodologies and the impactful role ALPX plays in accelerating structure-based drug discovery.



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FRAGMENT SCREENING OF ADENOSINE A2A RECEPTOR USING NATIVE LIPID NANODISCS

Philip Rawlins[1], James Craswell[1], Soumya Dhanavade[1], Anna Hopkins[1], Marieke Lamers[1], William Lee[1], Holly Madden[1], Jim Reid[1], Andrew Ratcliffe[1], Stefanie Reich[1] and Trevor Askwith[1]

[1]Domainex Ltd., Churchill Building, Chesterford Research Park, Saffron Walden, CB10 1XL, UK

Membrane proteins are important drug discovery targets but are challenging to purify in sufficient quantities for biophysical fragment screening. Detergent solubilization is commonly used for membrane protein purification but does not represent the natural membrane environment. Synthetic polymers (e.g. Styrene maleic anhydride) enable the formation of nanodiscs directly from the cell membrane and therefore allow extraction and purification of membrane proteins in their native environment. Despite this, there are few examples where synthetic polymers have been successfully applied to measuring the affinity of small molecules to membrane proteins.
At Domainex, we have developed a workflow to evaluate a range of Polymer Lipid Particles (PoLiPa) in parallel. This enables efficient identification of the optimal polymer for extracting soluble and affinity-purified membrane protein. Domainex has successfully applied this to a range of different membrane proteins including GPCRs, ion channels and solute carriers. A case study will be presented, which includes the development of a biophysical Spectral Shift (NanoTemper) assay for the adenosine A2a receptor. This assay was validated using several small molecule compounds known to bind to the adenosine A2a receptor and was then used to screen Domainex's fragment library. Several ligand efficient (>0.3) fragment hits were identified including analogues of theophylline. In addition, novel fragment hits were identified which were confirmed using orthogonal biophysical assays. We believe this is the first time a native lipid nanodisc has been used for fragment screening. Work is ongoing to generate X-ray crystal structures and explore the structure-activity-relationships (SAR) of these novel hits.

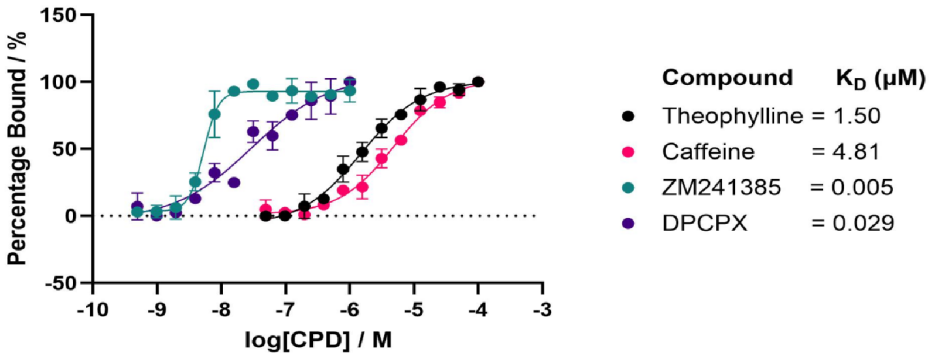

Figure 1. Affinity determination of four adenosine A2a receptor antagonists in the Spectral Shift (NanoTemper) assay.

25 YEARS OF THINKING SMALL

David Rees

Astex Pharmaceuticals, Cambridge, UK

FBDD) for over 20 years and has contributed to the discovery of three launched drugs for cancer patients, ribociclib (Astex Novartis collaboration), erdafitinib (Astex Newcastle University, then Astex Janssen collaboration), and capivasertib (Astex, AstraZeneca, and ICR collaborations). Astex have progressed several other projects from fragment-to-clinical trials. This lecture will describe some of these projects, the lessons learnt, and opportunities for the future as we investigate the potential for CryoEM to impact on FBDD.

FROM FRAGMENTS TO IN VIVO OPTIMIZED LEADS FOR THE SARS-COV2 NSP3 MACRODOMAIN MAC1, AN EMERGING TARGET IN ANTIVIRAL THERAPY

Adam Renslo, Priyadarshini Jaishankar, Moira Rachman, Galen Correy, Rahul Suryawanshi, Takaya Togo, Deepthi Ramesh, Yagmur Doruk, Maisie Stevens, Ryan Gonciarz, Eric Hantz, Zain Alam, Patrick O'Leary, Jeffrey Neitz, Danielle Swaney, Saumya Gopalkrishnan, Nevan Krogan, Alan Ashworth, Melanie Ott, Brian Shoichet, James Fraser

Quantitative Biosciences Institute Coronavirus Research Group (QCRG)
University of California, San Francisco, San Francisco, CA, USA

The COVID-19 pandemic highlighted the urgent need for novel targets and antiviral therapies to address current and future pandemics. The macrodomains of coronaviruses are erasers that remove ADP-ribose marks employed by the host cell for interferon signalling (Ferh, 2020). Recently, QCRG investigators showed that an engineered SARSCoV-2 virus bearing a catalytically inactivated Mac1 exhibited attenuated virulence in a mouse model infection (Taha, 2023) To identify *bona fide* drug leads that could also be used to validate Mac1 as an antiviral target, the QCRG team employed fragment-based screening by X-ray and computation (Schuller, 2021) followed by fragment growing and merging strategies to produce multiple lead-sized inhibitors with single-digit uM binding affinities for Mac1 and reasonable drug-like properties (Gahbauer, 2023). Subsequent structure-informed optimization of these scaffolds, with extensive in vitro ADME profiling, led to multiple advanced leads with potencies in the low to single-digit nM regime, and a favorable ADMET profile. Pharmacokinetic profiling in mice identified specific lead compounds for which unbound plasma concentrations significantly exceeded Mac1 biochemical IC50 values several hours following a single IP dose of 10 mg/kg. This talk will describe the path from fragments to advanced Mac1 leads, with a particular focus on the use of computationally-triaged X-ray fragment screening employed throughout the process of lead optimization.

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FRAGMENT-BASED DISCOVERY OF NOVEL AND POTENT STING AGONIST

Dr. Chaohong Sun

Abbvie

STING plays an essential role in the innate immune system and has been an attractive therapeutic target for drug discovery. In this talk, I will describe our fragment effort using complimentary biophysical approaches to identify and characterize fragment hits, 2D NMR as one of the primary SAR assays and applying iterative structural based drug design to deliver novel and potent STING agonists with robust *in vivo* efficacy. I will also discuss some key takeaways and lessons learned from our FBLD practice over the years.

DEVELOPMENT OF *EC*DSBA INHIBITORS AS ANTIVIRULENCE AGENTS

Yildiz Tasdan[a,b,c], Olga V. Ilyichova[a], Naureen Akhtar[a], Indu Chandrashekaran[a], Gautham.Balaji[a], Menachem Gunzburg[a], Lucie Guetzoyan[c], Daniel Maddox[c], James Davidson[c], Stephen Roughley[c], James Murray[c], Ben Capuano[a,b], Bradley C. Doak[a,b], Martin J. Scanlon[a,b]

[a] *Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Victoria, 3052, Australia;* [b] *ARC Industry Transformation Training Centre for Fragment Based Design (CFBD), Monash University, Parkville, Victoria, 3052, Australia;* [c] *Vernalis (R&D), Granta Park, Great Abington, Cambridge, CB21 6GP, UK.*

Bacterial resistance and the lack of novel classes of antibacterials has created an urgent need for novel antibacterial therapies. It is hypothesized that blocking bacterial virulence without causing the death of bacteria would lead to an effective antibacterial therapy. *Escherichia coli* DsbA (*Ec*DsbA) catalyses the formation of disulfide bonds and folding of multiple bacterial virulence factors making it a key regulator of virulence. Studies have shown that bacteria lacking functional DsbA are avirulent[1], making *Ec*DsbA a promising antibacterial target.
Multiple fragment screens have been conducted against *Ec*DsbA and provided fragments that bind within the substrate binding site of *Ec*DsbA. However, their development into high affinity binders is difficult due to the shallow, hydrophobic nature of the site which binds its peptide substrates with low specificity[2,3]. Recently our group identified and characterized a "cryptic" pocket that is enclosed within *Ec*DsbA through a combination of NMR dynamics and ^{15}N-^{1}H HSQC fragment screening. The cryptic pocket is opened through protein dynamics but once a ligand is bound becomes small and enclosed, currently limiting the development of high affinity binders. Here we focus on strategies to develop high affinity inhibitors that bind to both the cryptic pocket and hydrophobic groove simultaneously. Initially by identifying motifs that can open the cryptic pocket and provide a linker to the hydrophobic groove, and then by expansion into the hydrophobic groove. This strategy employed multiple biophysical methods including protein-detected NMR, SPR, X-ray crystallography, crude reaction mixture screening by SPR and ASMS, and automated/parallel synthesis[4,5]. We have successfully identified compounds that open the cryptic pocket and bind to the hydrophobic groove. To our knowledge, these are the highest affinity *Ec*DsbA inhibitors that have been identified to date.

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UTILIZING FBDD SCREENING TO DEVELOP A NOVEL CLASS OF INHIBITORS FOR SARS-COV-2 PAPAIN LIKE PROTEASE

Ashley J. Taylor[1], Qiangqiang Wei[1], Kevin Teuscher[1], Tyson A. Rietz[1], Kangsa Amporndanai[1], Taylor M. South[1], Mackenzie M. Crow[1], Chideraa Apakama[1], John L. Sensintaffar[1], Jason Phan[1], Taekyu Lee[1], Stephen W. Fesik [1]

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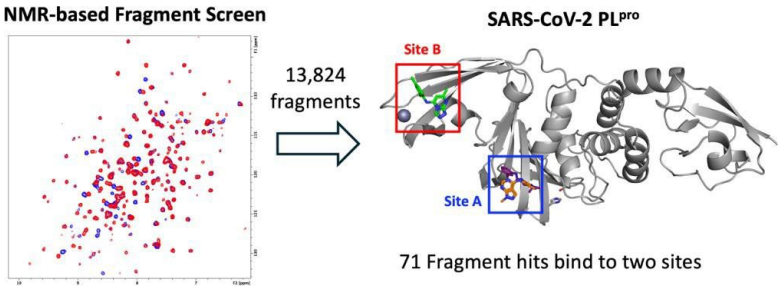
Coronaviruses have been responsible for numerous viral outbreaks in the past two decades due to the high transmission rate of this family of viruses. The deadliest outbreak is the recent Covid-19 pandemic which resulted in over 7 million deaths worldwide (WHO). Despite the threat that this viral family poses, only 3 drugs have been approved by the FDA for the treatment of SARS-CoV-2. Covid-19 research has been primarily focused on the development of RNA-dependent RNA polymerase (RdRp) and main protease (Mpro) inhibitors however, SARS-CoV-2 viral strains with resistance to these classes of inhibitors has already been observed (Iketani, Mohri et al. 2023). This highlights the need to develop small molecule inhibitors against new coronavirus targets with novel mechanisms of action. SARS-CoV-2 papain like protease (PL^{Pro}) plays a key role in both viral replication and host immune suppression and is highly conserved across the coronavirus family, making it an ideal drug target. Multiple drug discovery campaigns have been launched against PL^{Pro} however, all reported inhibitors are derived from GRL- 0617 -a PL^{Pro} inhibitor developed against SARS-CoV-1 in 2008 (Ratia, Pegan et al. 2008) – and no candidates have progressed to clinical trials. We have conducted a fragment screen against PL^{Pro} using protein detect NMR experiments, identifying several new classes of small molecule inhibitors. X-ray crystallography confirms fragment binding to two distinct regions of the target protein with the zinc finger using site not previously being reported in the literature. Subsequent hit to lead development of a key fragment series has yielded novel small molecules inhibitors of PL^{Pro} with sub μM activity in both biochemical and cellular inhibition assays.



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FRAGMENT BASED IDENTIFICATION AND ANALYSIS OF CRYPTIC LIGAND BINDING SITES

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Many proteins have cryptic sites that are not easily detectable in some ligand-free structures, and require a conformational change to become apparent (Sun, et al., 2020; Vajda, et al., 2018). We show that such sites have nearby binding hots spots, i.e., energetically favourable regions in the vicinity even without a well-defined pocket, and hence can be identified by protein mapping (Beglov, et al., 2018). The FTMap server maps the surface of a target protein using 16 fragments as molecular probes (Kozakov, et al., 2015). FTMap has been recently expanded to the FTMove server that finds consensus binding sites in multiple structures (Egbert, et al., 2022), and for fragment- based identification of pharmacophore regions near the FTMap-identified ligand binding sites (Khan, et al., 2024). It was shown that FTMap can find the hot spots in protein models generated by the AlphaFold2 program (Bekar-Cesaretli, et al., 2024). We also explored opening cryptic pockets using AlphaFold2 with reduced depth MSA, and found the results dependent on the conformational states of the ligand binding sites in the structures available in the PDB. Finally we report the results of druggability analysis showing that cryptic sites opening only by side chain conformational changes don't seem to provide better than high micromolar binding affinity. Better binding ligands can be found if the opening of the site involves movement of loops or secondary structure elements.

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FRAGMENT-BASED LEAD DISCOVERY AGAINST SARS-COV-2 NSP3 MACRODOMAIN

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[1]PostEra Inc.

Fragment based biophysical screens are a proven strategy for hit identification, with structural determination of fragment binding modes via x-ray crystallography providing additional support for hit validation and facilitating structure based optimization. SARS-CoV-2 nsp3-mac1 is a viral macrodomain with ADP-ribosylhydrolase activity which counteracts host immune response. Nonetheless, it is unclear whether chemical inhibition of nsp3-mac1 translates to antiviral activity against SARS-CoV-2. Here we report a fragment-based lead generation campaign guided by structural and machine-learning approaches. We discovered tool compounds with low nanomolar affinity, responsive structure-activity relationships, and drug-like properties. Our work serves as a robust starting point in the development of therapeutics targeting coronavirus nsp3-mac1.

COMBINING ACTIVE LEARNING, SYNTHESIS ON DEMAND LIBRARIES, AND FRAGMENT SCREENING IN EARLY DRUG DISCOVERY

Pat Walters

Relay Therapeutics

The introduction of ultra-large screening libraries has presented both opportunities and challenges for virtual screening. With incredibly vast collections like the Enamine REAL and WuXi GalaXi, brute-force screening is no longer a practical solution. To address this, computational groups are actively developing innovative approaches. One such approach is Thompson Sampling (TS), an active learning method that utilizes machine learning models as surrogates for more computationally intensive calculations. TS offers a rapid and accurate approach for the virtual screening of multi-billion molecule libraries. It seamlessly integrates with docking, similarity search, and other machine learning models. This presentation will showcase a real-world application of TS and highlight how it complements experimental data.

BIOLOGICAL VALIDATION OF A NOVEL EIF4E BINDING SITE IDENTIFIED BY FRAGMENT SCREENING USING MUTATIONAL ANALYSIS AND TARGETED PROTEIN DEGRADATION

Andrew J. Woodhead

on behalf of Astex Pharmaceuticals, Cambridge, UK and in collaboration with the Centre for Cancer Drug Discovery, Institute of Cancer Research, London UK

Directly targeting the translation initiation factor eIF4E has long been considered a promising anticancer strategy but it has remained 'undruggable'. Astex applied NMR and X-ray crystallographic fragment screening and subsequent structure-guided design to develop compounds that target eIF4E at a site of unknown functional relevance (site 2). The lead molecule had an affinity of 100 nM, disrupted the protein-protein interaction (PPI) between eIF4E and eIF4G in vitro, inhibited cap-dependent translation in cell lysates and demonstrated cellular target engagement, however, no functional cellular activity was observed. To further explore the functional relevance of site 2 we established a cell model where endogenous eIF4E was replaced by an eIF4E fusion protein that was sensitive to PROTAC mediated degradation. Degradation of this eIF4E fusion protein resulted in inhibition of cell growth and reduced expression of MCL1, which was rescued by re-expression of wild-type eIF4E. Using this system, we characterised the phenotype of various eIF4E mutants including those in site 2. Our results suggest it may be necessary to disrupt a larger PPI interface on eIF4E to drive a strong functional effect in cells and that the eIF4E:eIF4G interaction may be more complex than predicted by both in vitro and cell lysate assays. This approach demonstrates the power of coupling targeted protein degradation and genetic rescue approaches to explore novel pockets discovered through fragment-based lead discovery.

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Manuscript under review with Nature Communications

IDENTIFICATION AND DEVELOPMENT OF FRAGMENT DERIVED CHEMICAL MATTER IN PREVIOUSLY UNKNOWN ALLOSTERIC SITES OF WRN

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Werner Syndrome helicase (WRN) is a DNA helicase with a synthetic lethal phenotype in cancer cells with mismatch repair deficiency (MMRd), making it an attractive target for inhibition in the treatment of microsatellite instability-high (MSI-H) or MMRd tumours (Lieb et al, 2019; Kategya et al, 2019; Behan et al, 2019; Chan et al, 2019). Inhibitors targeting the ATP binding pocket are limiting both in size and in ability to specifically target WRN. Fragment-based screening efforts were carried out in a search for new inhibitory binding pockets and novel chemical matter.
Here we will describe the screening of an internal ^{19}F fragment library by NMR, followed by biophysical and biochemical fragment hit qualification, to prioritize a subset for x-ray structure determination. In this process we identified a novel allosteric fragment binding pocket along the cleft between the two ATPase domains where the two WRN RecA ATPase domains are rotated up to 180 degrees in relation to each other and ATP binding is abrogated by the movement of protein backbone into the ATP binding pocket.
Chemical progression of one such fragment hit will be described in more detail in this presentation to underscore some of the challenges encountered when targeting this conformationally dynamic helicase mechanoenzyme. Careful evaluation of emerging chemical matter by a combination of biochemical, biophysical, and structural tools were required to achieve functional inhibition of WRN during fragment hit expansion in this allosteric binding pocket.

Acknowledgements
Joey Methot, Aileen Soriano, Yan Hou, Xiaomei Chai, Minjia Zhang, My Sam Mansueto, Stuart Shumway.

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EXPANDING CHEMICAL DIVERSITY IN DRUG DISCOVERY THROUGH NOVEL AMINE-ACID COUPLING

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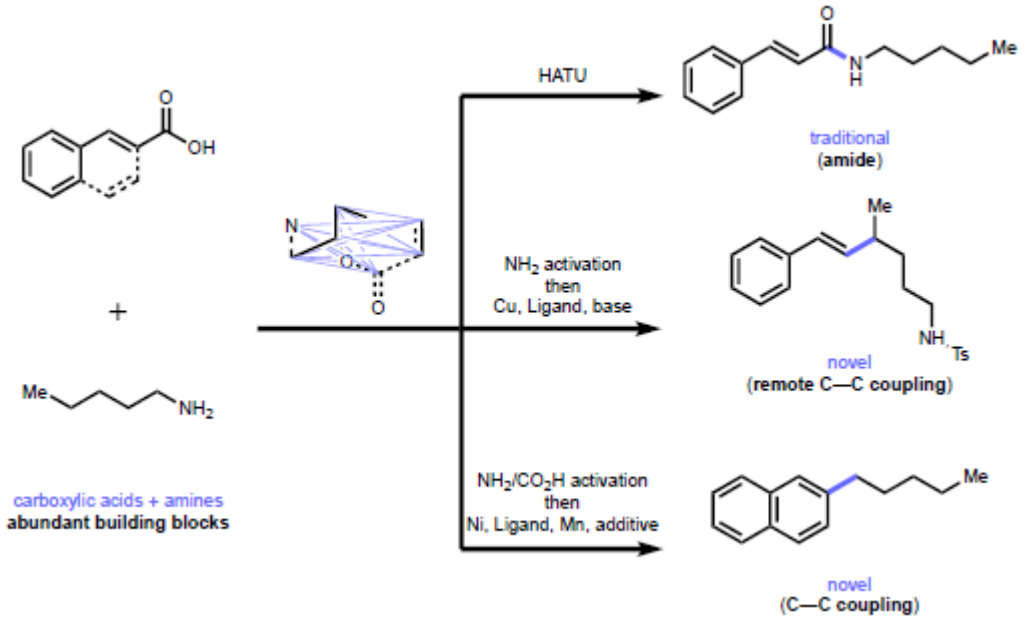
The current medicinal chemist's toolbox limits the chemical space available for optimization, often leading to structural homogeneity in drug molecules.1,2 Our research group focuses on repurposing amine-acid coupling methodologies to expand this toolbox.3-
6 Amine and acid functional groups are among the most abundant building blocks in bioactive molecules and drug molecules. Our advancements in their coupling reactions significantly enhance the chemical diversity accessible in the lead optimization process. In this talk, I will discuss our latest findings on novel amine-acid coupling methods. By repurposing sp2-acids and sp3-amines, we emphasize the formation of C–C bonds, the most frequent connections among all molecules, instead of traditional amide bonds. This chemistry discovery was facilitated by High-Throughput Experimentation (HTE), enabling rapid exploration of large chemical spaces. These novel methodologies have successfully been leveraged on late-stage functionalization of dozens of drug molecules and amino acids, demonstrating the methods' practical applications.
The chemical space accessed through these novel coupling paradigms imparts different physicochemical properties to target molecules, such as molecular weight, hydrogen bond donors, pKa, pharmacokinetics, and tissue distribution. It also introduces a new paradigm for fragment linking, which can lead to the development of more effective and innovative drug candidates. Our advancements not only push the boundaries of organic chemistry but also provide valuable tools and strategies, significantly impacting the field of drug discovery.



Acknowledgements
We thank all Cernak lab members for helpful discussions on developing the chemistry.

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Poster Abstracts

FRAGMENT-BASED LIGAND SCREENING IN JASON

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JASON Software Team, JEOL (UK) Ltd., 4 Bankside, Long Hanborough, OX29 8LJ, UK

JASON (JEOL Analytical SOftware Network) is a software suite designed for the processing, analysis, and reporting of NMR data. As a vendor-agnostic platform, JASON seamlessly handles data from all major NMR equipment providers, offering a powerful analysis and reporting package regardless of users' instrumentation.
Here we describe a new software plugin currently under development, for the analysis and reporting of fragment-based ligand screening (FBLS) data. The plugin is designed with an emphasis on a flexible and intuitive interface, addressing the common challenges faced in FBLS, such as the absence of a standardized system for organizing and defining fragment screening data. This lack of standardization often necessitates arbitrary file system structures or the use of custom scripts to make the data compatible with existing analytical software.
Our fragment screening plugin offers a powerful utility for defining and managing diverse screening experiments and sample types from any NMR vendor. It accommodates a variety of organizational structures, enabling users to easily define and arrange screening data into cocktails for analysis. The manual analysis of screening data is facilitated by JASON's unique canvas-based interface, which provides a user-friendly and efficient workflow, even for complex datasets.

X-RAY CRYSTALLOGRAPHY PLATFORM AT UCSF

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The X-ray Crystallography Facility at UCSF(1) offers an infrastructure designed to streamline and optimize protein crystallization and fragment-based screening using X-ray crystallography.
Our platform is equipped with nano-volume crystallization Mosquitos(2) that ensure high- precision pipetting, crucial for accurate and reproducible crystallization trials for soluble and membrane proteins. In addition to that, the Dragonfly Crystal(3) equipment facilitates precise preparation of crystallization gradients and optimization screens, even for the most viscous liquids. All the crystallization plates can be incubated and imaged using the Formulatrix Rockimager 1000(4) which allows a systematic and high-resolution monitoring for fragment soaking.
The SMDC facility at UCSF(5) provides an Echo 650 acoustic dispenser(6) for rapid and efficient transfer of small fragments into protein crystals, enhancing high-throughput capabilities.
Once the fragments are soaked in, the OLT Shifter (Wright ND at al, 2021) and Nanuq ensure an automated crystal harvesting with fast cryocooling and puck loading.
Our platform provides the tools needed to pursue a fragment-based screening using X- ray crystallography and has access to all the light sources for data collection.

Acknowledgements
Macromolecular Structure Group (MSG), UCSF Fraser lab, UCSF
Small Molecule Discovery Center (SMDC), Arkin lab, UCSF

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EXPLORING DRUGGABILITY THROUGH BINDING HOT SPOT ANALYSIS ACROSS STRUCTURAL ENSEMBLES OF PROTEIN TARGETS

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Druggability assessments remain a dynamic and significant venture in experimental and computational fields. One method is computational hot spot mapping, combining experimental fragment screening with binding hot spot identification. A pressing issue of hot spot mapping is choosing the appropriate protein structure to map. Large benchmark sets of proteins with high structural availability are required to investigate the implications of different structures on computational druggability assessments. Such studies can be computationally expensive and time-consuming, primarily when methods like molecular dynamics simulations are employed. In this study, we analyzed the binding hot spot properties across the structural ensemble for 20 proteins using FFT-based fragment probe docking over multiple structures. We used experimental ligand affinity data to reference true druggability. Analyzing hot spots across the structural ensemble for proteins where single-structure mapping disagrees with experimental druggability shows that ensemble mapping more accurately captures true druggability. Additionally, unbound structures offer better druggability insights druggability than ligand-bound structures. Ensemble mapping demonstrates that it is insufficient for a protein to occasionally adopt a rare but druggable conformation. Instead, a specific threshold exists for the number of druggable structures within the ensemble to render the target as druggable. Furthermore, our results emphasize evaluating all three fundamental binding hot spot characteristics of strength, connectivity/compactness, and size of the binding site. Ensemble mapping resolves selecting an unrepresentative structure, which is challenging to overcome with single- structure mapping methods. Our ensemble mapping methods provide a reliable, fast, and computationally affordable means to understand the druggability of a protein.

FRAGMENT SCREENING OF GPCRS USING GCI TECHNOLOGY

Edoardo Fabini[1], Sara Crespillo Torreno[1], Alicia Churchill-Angus[1], Anja Greule[1], Ami Miller[1], Florian Krieger[2], Cedric Fiez-Vandal[1], Jay Bertrand[3], James Errey[1]

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Membrane proteins such as G-protein-coupled receptors (GPCRs) account for roughly half of the known drug targets, but they have proven to be challenging for fragment-based methods. With this in mind, Evotec wanted to assess the suitability of the grated-coupled interferometry (GCI) technology for membrane protein fragment screening by running a pilot on the Creoptix DeltaWave. The GCI technology is similar in nature to the established surface plasmon resonance (SPR) and emerged as an attractive approach to fragment screening. The innovative microfluidic system paired with the high sensitivity and the RAPID injection method allows to determine affinity and kinetics of ligand from a single concentration, greatly reducing the time required to screen and rank large library of compounds. In the present study, a proof of principle was carried out by testing 704 fragments on the human adenosine 2a receptor (A2AR) using the RAPID injection. Hit calling criteria were devised to provide a robust method to identify genuine fragments directly from the primary screen. Using this criteria, 16 fragments were identified as genuine binders of hA2aR. Affinities of the fragments identified with this procedure were ranging from low to high µM, with reasonable kinetic profiles. The identified fragments were retested in a more traditional multi cycle kinetic approach as a follow up to the primary study. Overall, the process took less than a week to complete and consumed less than 80 µg of proteins.

SEARCH FOR TRANSGLUTAMINASE 2 (TG2) COVALENT INHIBITORS USING DOMAINEX'S COVALENT FRAGMENT LIBRARY, AND A LC-MS BASED COVALENT FRAGMENT SCREENING PLATFORM

Ray Boffey[1], Anna Hopkins[1], Gary Wu[1], Jason Pembroke[1], Megan Ashley[1], Andrew Ratcliffe[1] and Scott Martin[1]

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Introduction

Recent advances in the tools to support discovery/design of covalent inhibitor drugs and the success of block buster drugs such as Osimertinib and Ibrutinib has led to increased interest in 'electrophile-first' covalent drug discovery. Covalent fragment-based screening by intact protein mass spectrometry (MS) has been shown to be a powerful tool, demonstrated by the discovery of KRAS(G12C) inhibitors (Orgován et al, 2023). Additional assays to support covalent fragment-based screening, including assessing warhead reactivity via a GSH assay and binding site identification by proteolytic digestion and peptide mapping optimise hits. Covalent inhibition is time-dependent, so the preferred measure of potency is the second-order rate constant kinact/Ki, rather than IC50 (Li et al, 2022)

Here we describe a case study assessing covalent fragments against TG2 protein implicated in the pathogenesis of several diseases including cancer, fibrosis and neurodegenerative diseases (Szondy et al, 2017), which to our knowledge has not been done before.

Summary of Work

Domainex Internal covalent library was screened against TG2 protein utilising four LC-MS based assays; Intact mass covalent fragment screening to determine protein binding and stoichiometry followed by assessment of hits with glutathione covalent reactivity, binding site identification by proteolytic digestion/peptide mapping and Kinact/Ki determination to assess potency. Due to low signal to noise ratio seen for the full length TG2 protein by MS, a truncated version of TG2 was selected for this study. Binding experiments were conducted at a protein concentration of 2 µM and fragments were pooled into groups of 5 (at least 5 Da apart) and incubated with TG2 at a concentration of 20 µM (1:10 ratio) for 2 h at room temperature. Analysis was performed on a Waters G2-XS QToF, utilising the chromatography from a Waters ACQUITY UPLC Protein BEH C4 VanGuard Pre-column, 300Å, 1.7 µm, 2.1 mm X 5 mm on a Waters Acquity H-Class Plus Bio.

Results

Multiple hits were observed, strong binders (>80% binding) were selected for glutathione reactivity assessment. Fragments with a GSH reactivity t½ <100 minutes are unlikely to exhibit favourable properties and were rejected. All 10 strong binder hits had a ½ life >100 minutes (Table 1) and were taken forward for binding site identification by proteolyticdigestion, followed by peptide mapping. High quality MS/MS data confirmed binding to the target cysteine residue (Figure 2).

Conclusions

Domainex fast MS based covalent platform successfully identified several fragments as potential leads for chemistry optimisation against TG2

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NOVEL FRAGMENT LIBRARIES FROM LIFE CHEMICALS

George Bondar

Life Chemicals Inc.

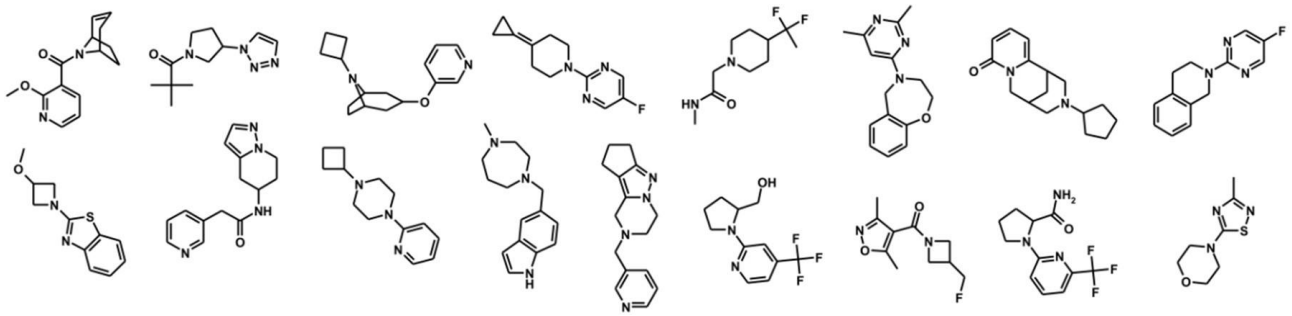
Fragment-based drug discovery (FBDD) is an important approach to design of potential lead compounds, which has started to deliver first approved drugs recently.

With over 36,000 Fragments in stock, Life Chemicals has designed exclusive Fragment Libraries that enable exploration of broad spectrum of new “difficult” pharmacological targets: Superior Fragment Library, Soluble Fragment Library, Fsp3 enriched Fragment Library, PPI Fragment Library, Fluorine-labelled Fragment Library, special set of 1,000 Fluorinated fragments offered as 100 Fluorine Fragment Cocktails, Bromine-labelled Fragment Library, Covalent Fragment Library and Natural Product-like Fragment Library. Advanced tuning of “Rule of three” parameters ensures selected Fragments to be perfect elements for generation of leads with excellent ADME profile.

Besides the Libraries generated on the basis of stock collection Life Chemicals proposes the database of over 5,000 synthetically feasible compounds, which is specially designed for fragment-based drug discovery. The database is generated by virtual coupling of carefully selected set of building blocks, which define the following features of the resulting library:

- High quality of the fragments defined by rigorous control of Phys-Chem properties
- Ro3 extension, PAINS and in-house developed structural filters are applied
- High fraction of sp3-carbons in the compounds
- Diversity of the library is kept optimal for even covering of chemical space
- High novelty of the chemical structures
- Rapid SAR generation potential

Over 800 compounds have been already synthesized in 100mg+ quantities.



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THE DESIGN AND PERFORMANCE OF 3D FRAGMENT LIBRARIES

T. Dekker[1], M. Wijtmans[2], I.J.P. de Esch[2]

[1]Division of Medicinal Chemistry, Amsterdam Institute of Molecular and Life Sciences (AIMMS), Vrije Universiteit Amsterdam, The Netherlands.

In recent years, increasing attention has been given to three-dimensional (3D) fragments. The increased complexity that is associated with such fragments is somewhat contradictory to the principle of FBDD (i.e., reducing complexity by using low-molecular weight screening compounds) and may result in lower hit rates when compared to their flat (2D) counterparts. Furthermore, the synthetic complexity is often also increased. However, the part of chemical space that is covered by 3D fragments also remains underutilized, as well as other potential benefits such as increased solubility and reduced promiscuity. Since a fragment hit typically represents the scaffold through hit-to-lead optimization, screening of 3D fragments will ensure that the three-dimensional character is introduced in an early stage of the drug discovery process, and that there is no need to introduce such character later on. We develop tools and synthetic strategies to design 3D fragment libraries, including for example an automated cheminformatics design workflow in the open-source platform KNIME.1 We also evaluate the performance of 3D fragments; Screening of a focused 3D library provided hits for several GPCR targets, from which we selected a cyclobutane-based hit for the histamine H1 receptor for further exploration. The efficient hit identification and optimization case study showcases how 3D fragments can provide useful starting points in a drug discovery campaign.

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A COMPUTATIONAL APPROACH FOR PERFORMING MEDICINAL CHEMISTRY TRANSFORMATIONS WITHIN A 3D ACTIVE SITE

Guillaume Fortin

Chemical Computing Group

In this work, MedChem Transformations, a modeling application for performing medicinal chemistry transformations in the context of the 3D receptor and ranking the resulting molecules is presented. The methodology is outlined and a test study using a PDE5A-Sildenafil complex is performed. The results demonstrate that including pocket atoms and preserving key interactions help generate promising candidates that are relevant to the PDE5A receptor as well as a known PDE5A ligand (Vardenafil) from the original Sildenafil molecule.

MASSIVELY PARALLEL SPR-BASED FRAGMENT SCREENING OF KINASE ARRAYS

Anthony Giannetti PhD, Rebecca L. Rich PhD, Noah T. Ditto, John Rosenfeld PhD, Adam Shutes PhD, Yusuke Kawase PhD

As a drug target class, kinases continue to provide a wealth of opportunities for addressing human disease, but often can be challenging to work with in vitro. Additionally, the ubiquitous nature of kinases across many critical pathways means therapeutic targeting of this class necessitates careful consideration regarding off- target profiles. Direct label-free approaches, such as SPR, can complement activity assays by providing the intrinsic affinity and real-time kinetics of interactions. Here we highlight the power of combining an extensive panel of ready-made biotinylated kinases with HT-SPR to generate a wealth of compound binding information. In three days over 125,000 interactions were measured between a panel of kinases and the Maybrdge 1000 fragment library. We also profiled a kinase-focused small molecule library an obtained more than 80,000 binding interactions in a three-day instrument run. Detailed kinetics were then subsequently obtained for hits of interest. Beyond simple yes/no reporting, this approach allows for nuanced kinetic profiling for up to hundreds of binding events in parallel, thereby enabling thoughtful discovery of safe and efficacious drug candidates.

A MAGNET FOR THE NEEDLE IN HAYSTACKS: UNLOCKING CHEMICAL MATTER USING “CRYSTAL STRUCTURE FIRST” FRAGMENT HITS

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The growing body of literature indicates that structural data in FBDD is still largely underutilized1, and pure affinity metrics bias the decision making in medicinal chemistry2. We have pioneered the “crystal structure first” approach and argue that the underutilization of structural data in FBDD is due to the unsystematic nature of the setup of soaking systems.

In a case study, we use four protein kinase A (PKA) small-molecule fragment complexes as starting points for a template-based docking screen without prior knowledge of affinity3. Here, the Enamine's multibillion REAL Space was utilized. Out of the 106 chosen compounds, 93 molecules in total were successfully synthesized. At least forty compounds showed activity in validation assays, with the most active follow-up exhibiting an affinity increase of 13,500-fold. Six of the most promising binders quickly had their crystal structures determined, confirming the binding mode. This innovative fragment-to-hit strategy achieved a 40% overall success rate in just 9 weeks.

Our current efforts are directed towards developing the “crystal structure first” approach for design of mono- and multi-specific binders, e.g. degraders.

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FRAGMENT SCREENING TO LIGAND C-DEGRON E3 LIGASE KLHDC2

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The use of proteolysis targeting chimeras (PROTACs) for targeted protein degradation (TPD) is a rapidly expanding technology for gaining therapeutic access to difficult targets. To date, work in this field has been restricted to the use of a few E3 ligases. In this work, we explore using the C-end degron receptor of the E3 ligase KLHDC2 (Scott et al.) for TPD by discovering small molecule ligands of this alternative receptor. A thermal shift screen was designed and performed against KLHDC2 using a St Jude screening set developed for biophysical screening, which produced tractable hits that were validated by ITC and SPR. Structure-guided optimization of ligands led to high-affinity binders and proof of concept PROTACs. The use of the biophysical approach proved advantageous and complementary to high throughput and virtual screening campaigns simultaneously ongoing against this target at St Jude.

Acknowledgements
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FRAGMENT SCREENING OF IL-23 BY WEAK AFFINITY CHROMATOGRAPHY

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Interleukin 23 (IL-23) is a heterodimeric inflammatory cytokine composed of an IL-12B (p40) and an IL-23A (p19) subunit. It signals through a receptor complex formed by IL- 12Rb1 and IL-23R, playing a critical role in the maintenance and expansion of T helper type 17 cells (Th17 cells). Aberrant Th17 activity is associated with multiple autoimmune conditions. Clinically, antagonist antibodies targeting IL-23, such as ustekinumab, have been approved for the treatment of autoimmune diseases. Additionally, an orally administered small-molecule inhibitor of the IL-23 pathway has the potential to provide significant benefits for patients suffering from autoimmune inflammatory disorders. However, identifying small-molecule inhibitors of the IL-23 pathway has proven to be a challenging process.

Fragment screening by weak affinity chromatography (WAC) has the advantage of detecting weak binders by screening at low concentrations (<5 μM) with immediate ranking of hits and is therefore well suited for PPIs.

Here we present the result of a WAC screen towards IL-23 with hit validation by NMR, TSA and X-ray crystallography.

BROMOFRAGLITES FOR ASSESSING FRAGMENT STRUCTURAL ENABLEMENT

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Fragment Based Lead Discovery (FBLD) is an established method for the generation of ligand efficient, small molecule therapeutics with demonstrated clinical impact. To maximize the impact of FBLD amongst multiple screening approaches, we developed an assessment tool to select targets for fragment-based screening. The approach assesses the probability of fragment structural enablement and the unique opportunity available from FBLD. The probability of fragment structural enablement was assessed for Tau- tubulin Kinase 1 (TTBK1) using a set of highly soluble, promiscuous, or halogenated fragments. The high hit rate observed from this set and robust nature of the crystal system indicated a high probability of structurally enabling fragment hits derived from biophysical screening. This result along with the need for ligand efficient, allosteric chemical matter for TTBK1 triggered a biophysical screen and FBLD campaign to identify selective TTBK1 inhibitors. The screening approach identified multiple allosteric binding sites within TTBK1 which were assessed for tractability and potential selectivity. The success of promiscuous and halogenated fragments in building an understanding of the likelihood of fragment structural enablement inspired the development of a small set of fragments for this purpose. Inspired by 'FragLites', we developed 'BromoFragLites' as a subset of our fragment library to understand target feasibility at an early stage. This poster will describe a method to assess targets for FBLD and the utility of small fragment subsets in this assessment.

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A PRACTICAL AND EFFICIENT METHOD FOR DETERMING k_{inact}/K_I OF COVALENT FRAGMENTS

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After the clinical success of covalent drugs such as ibrutinib and sotorasib, there is renewed interest in covalency for rational drug design. Covalent drugs offer extended engagement and the potential to target proteins traditionally considered undruggable by reversible small molecules. Instead of the conventional IC50, the preferred potency metric for covalent modifiers is the second-order rate constant of inactivation (*k*inact/*K*I). Existing methods for measuring *k*inact/*K*I are resource-intensive and involve complex data interpretation, limiting the routine use of *k*inact/*K*I for SAR. We propose an efficient mass- spectrometry-based method called diagonal dose-response time-course (dDRTC) to estimate *k*inact/*K*I. Our results demonstrate accurate determination of *k*inact/*K*I values across a wide potency range using covalent inhibitors of $KRAS^{G12C}$. The dDRTC method is high-throughput compatible, provides a broader coverage of dose-time space compared to a DR or TC curve, is applicable to multiple targets, and directly quantifies the potency of covalent modifiers to accelerate efficient SAR interpretation and lead discovery.

GUIDING FRAGMENT EXPANSION WITH E-FTMAP

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In fragment-based drug discovery, the binding mode of a fragment bound to a hot spot is expected to be conserved as it is optimized into a larger ligand (Kozakov et al, 2015). Therefore, predicting the locations of intermolecular interactions that are conserved in fragment-lead pairs is of great importance in the context of pharmacophore generation. Typically, these interactions can be identified through NMR or X-ray crystallography as regions on a receptor surface in which intermolecular interactions are conserved across a variety of fragment hits (Mattos et al, 1996; Hajduk et al, 2005). However, when there is insufficient structural data it is challenging to reliably identify pharmacophore features within a binding site. To aid in the identification of pharmacophore regions in ligand binding sites we have developed E-FTMap, a computational solvent mapping algorithm which exhaustively maps binding sites with dozens of small organic probes, and identifies important interaction sites as atomic consensus sites (ACSs) where similar chemical groups bind. We validate E-FTMap against a set of 109 experimentally derived structures of fragment-lead pairs, finding highly ranked pharmacophore features that overlap with corresponding atoms in both fragment and lead compounds. Additionally, we compare mapping results to pharmacophores derived from ensembles of bound ligands, revealing that E-FTMap results tend to sample highly conserved protein-ligand interactions (Khan et al, 2023). Furthermore, we apply E-FTMap to 18 druggable binding sites, demonstrating the utility of the method as a computational tool which can complement fragment optimization efforts in the absence of experimental data.

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THE FRAGMAX FACILITY FOR STRUCTURE-BASED DRUG DISCOVERY AT MAX IV LABORATORY

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The FragMAX facility supports structure-based drug and chemical tool compound discovery at MAX IV Laboratory. It was designed as a platform for crystal-based fragment screening, but the underlying workflows are applicable to all medium- to large-scale protein-ligand studies. The platform is comprised of four primary elements: (i) a high-throughput crystal preparation facility, (ii) a collection of fragment libraries, (iii) automated diffraction data collection at the BioMAX beamline, and (iv) software tools for large-scale data processing. In 2019, the FragMAX platform began providing services to external users and has since established an international user program that is accessible to academic and industrial research organizations. Access can be requested through the MAX IV user program and the MAX IV Industrial Relations Office.

The FragMAX crystal preparation facility is co-located with the Lund Protein Production Platform (LP3) and provides the complete set of instruments for protein crystallization and crystal optimization. In addition, it offers workflows that include liquid handling systems for automated crystal soaking and robot-assisted crystal mounting. The facility provides access to multiple fragment libraries, including the in-house developed FragMAXlib, and allows users to send their own compound collections or screening sets. FragMAX personnel can help with sample preparation, and users are also welcome on-site. Data collection is performed at the BioMAX beamline which offers a high-intensity X-ray beam, fast and reliable sample changer (464 crystals), state-of-the-art X-ray detector (Eiger2 16M CdTe) and fully automated operation. All steps of the experiment, from crystal preparation to structure refinement, are recorded in a transferable database system. In addition, FragMAX provides several freely accessible tools for accelerated structure modelling and refinement as well as PDB deposition support. FragMAX provides customized experiments and a modular experimental design, allowing users of varying levels of expertise to routinely obtain actionable screening hits for their targets.

BOLTZMANN MAPS: A POWERFUL WEB-BASED TOOL FOR EARLY-STAGE DRUG DISCOVERY

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Boltzmann Maps (BMaps) is a low-cost web application with a broad suite of capabilities designed to empower researchers in early-stage drug discovery. It provides tools to design and prioritize small molecules. By visualizing and quantitatively scoring how compounds interact with target proteins, researchers can discover high affinity and selective compounds.
BMaps is easy to use and accessible, employing structure-based and fragment-based design. Users can select proteins from a 500+ library with pre-computed water maps and chemical fragment maps or upload their own structures. New maps can be generated through a simple web interface for fragments from numerous standard libraries or for user-defined fragments. Druggable hot spots are pre-computed and displayed. Compounds can be imported or drawn and modified with an integrated 2D editor. The software allows researchers to explore and evaluate modifications to improve a compound's affinity using fragment maps or other systematic chemistry substitutions. The application integrates tightly with various other web services such as CDDVault, PubChem, Mcule, Reaxys, and Pharmit Screening. It has RDKit and OpenBabel built-in, and runs Autodock and DiffDock with a concise dialog.

BMaps recently added features that include GPU-supported OpenMM energy minimization and pre-selected sample compounds. Energy minimization allows for more accurate comparisons of compounds. Water maps enable accounting for the impact of tightly bound waters in chemistry design. Sample compounds available have been specifically docked against COVID-19 structures to aid researchers in structure-based design.

In conclusion, Boltzmann Maps is a unique, low-cost tool for researchers in early-stage drug discovery. It is easy to use, supports fragment-based design, and offers a wide variety of capabilities to aid in the design of high quality new chemical entities.

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HARNESSING THE BIOPHYSICAL FRAGMENT SCREENING SWEET SPOT TO DISCOVER NEW CHEMOTYPES FOR BRPF1 INHIBITION

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The efficient sampling of the chemical universe is at the heart of the fragment screening hit identification paradigm. The smaller the fragments, the more efficient the sampling. How small we can go is determined by our ability to detect very weak binding events between small molecules and target proteins. At Concept Life Sciences and Creoptix, we joined forces to develop a unique fragment screening platform combining a purpose-built collection efficiently sampling the biophysically accessible chemical universe with the cutting-edge Grating-Coupled Interferometry (GCI) technology. By operating in this biophysical "sweetspot", we aim to identify the most efficient fragment hits in a matter of days; thus providing highly desirable start points for fragment hit optimization campaigns, and a quicker path to candidate nomination. We report below our initial studies using our platform to identify new fragments efficiently binding the BRPF1 bromodomain.

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FRAGMENT-BASED COMPUTATIONAL MAPPING OF STRUCTURAL ENSEMBLES TO ASSESS DRUGGABILITY: APPLICATION TO SARS-COV-2

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Target identification and evaluation is a pivotal step in the drug discovery and development process. Although time-intensive and complex, the challenge becomes even more acute in the realm of infectious diseases, where the rapid emergence of new viruses, the swift mutation of existing targets and partial effectiveness of approved antivirals can lead to outbreaks of significant public health concern. The COVID-19 pandemic, caused by the SARS-CoV-2 virus, serves as a prime example of this. Despite substantial resources being allocated to drug development, the process remains lengthy, with Paxlovid being the only currently effective treatment for COVID-19, and even then, its efficacy is only partial causing COVID-19 rebound in some population (Wang et al., 2022). In this work we used computational fragment-based hot spot mapping techniques to identify regions on protein surfaces where drug molecules and other ligands are most likely to bind. Specifically, the computational hot spot mapping method, FTMove, was utilized to take into account protein dynamics and flexibility by analysing a set of structures or structural models for a given protein (Egbert et al., 2022). We further developed the approach through a comprehensive comparison with existing literature and the incorporation of machine learning to select the most druggable fragment-binding sites. FTMove is highly effective at rapidly identifying optimal starting points for fragment-based drug design, and is able to pinpoint positions for fragment expansion, making it a valuable tool in the early stages of drug discovery.

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PANK INHIBITORS IN REVERSE AND BACK AGAIN

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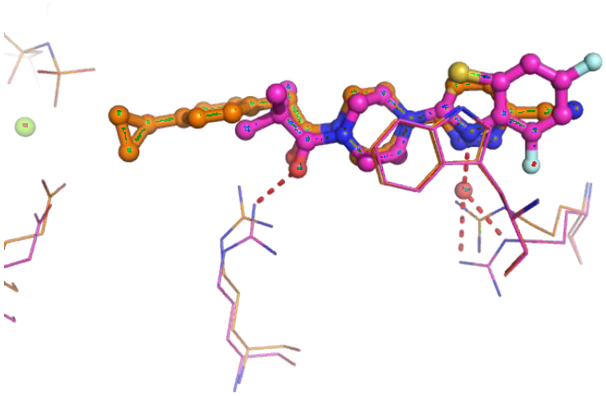
Pantothenate kinase (PANK) is a metabolic enzyme that regulates cellular coenzyme A (CoA) levels. Human cells express four closely related isoforms of PANKs (PANK1a, PANK1ß, PANK2, and PANK3), which regulate CoA levels in different cellular compartments, and their inactivation leads to various disease states. Our work has focused on discovering chemical probes of PANK enzymes, which ultimately yielded our clinical candidate BBP-671, a PANK activator (Subramanian et al., 2024). During its discovery, a prior preclinical lead, PZ-3022 (Sharma et al., 2018) and (Subramanian et al., 2021), was generated that failed pre-clinical toxicity testing. In this presentation, I will discuss our PZ-3022 backup program, in which alternative chemotypes were identified and advanced using fragment screening and merging approaches. These efforts have led to the discovery of ultra-potent PANK inhibitors with new cellular pharmacology, an important development in our research.



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TAILORED FRAGMENT SCREENING STRATEGIES FOR DIVERSE FRAGMENTS

Frank Moffatt

Novalix

Fragment-based drug discovery (FBDD) identifies low MW compounds that bind weakly to biological targets, but which can be optimized to give potent drugs. The approach is tailored to the class of fragments to be evaluated. For non-covalent interactions, surface plasmon resonance (SPR) and nuclear magnetic resonance (NMR) are the key hit-finding techniques. To find covalent binders (fragments typically linked to electrophilic warheads), a high-throughput automated MS-based solution that combines intact RPLC-MS and bottom-up proteomics is employed. Examples of each are presented.

FRAGMENT SCREENING OF ADENOSINE A2A RECEPTOR USING NATIVE LIPID NANODISCS

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Membrane proteins are important drug discovery targets but are challenging to purify in sufficient quantities for biophysical fragment screening. Detergent solubilization is commonly used for membrane protein purification but does not represent the natural membrane environment. Synthetic polymers (e.g. Styrene maleic anhydride) enable the formation of nanodiscs directly from the cell membrane and therefore allow extraction and purification of membrane proteins in their native environment. Despite this, there are few examples where synthetic polymers have been successfully applied to measuring the affinity of small molecules to membrane proteins.
At Domainex, we have developed a workflow to evaluate a range of Polymer Lipid Particles (PoLiPa) in parallel. This enables efficient identification of the optimal polymer for extracting soluble and affinity-purified membrane protein. Domainex has successfully applied this to a range of different membrane proteins including GPCRs, ion channels and solute carriers. A case study will be presented, which includes the development of a biophysical Spectral Shift (NanoTemper) assay for the adenosine A2a receptor. This assay was validated using several small molecule compounds known to bind to the adenosine A2a receptor and was then used to screen Domainex's fragment library. Several ligand efficient (>0.3) fragment hits were identified including analogues of theophylline. In addition, novel fragment hits were identified which were confirmed using orthogonal biophysical assays. We believe this is the first time a native lipid nanodisc has been used for fragment screening. Work is ongoing to generate X-ray crystal structures and explore the structure-activity-relationships (SAR) of these novel hits.
Figure 1. Affinity determination of four adenosine A2a receptor antagonists in the Spectral Shift (NanoTemper) assay.

DEVELOPING SMALL MOLECULES AGAINST THE INNATE IMMUNE ANTAGONIST ORF9B

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Open Reading Frame 9b (Orf9b), an accessory protein of SARS coronaviruses, is involved in innate immune suppression through its binding to the mitochondrial receptor Translocase of Outer Membrane 70 (Tom70)[5]. Structural studies of Orf9b in isolation revealed a beta sheet-rich homodimer[1], however, a structure of Orf9b in complex with Tom70 revealed a monomeric helical fold[2,3]. This suggests that Orf9b exists in an equilibrium between monomeric and dimeric forms of Orf9b, with the helical form necessary for suppressing the type 1 interferon response[2,4]. We hypothesized that a small molecule that biases the Orf9b equilibrium towards the dimer would improve innate immune activation. We previously identified the binding of a lipid-like molecule to the Orf9b homodimer at a hydrophobic channel that runs the length of the homodimer to have a strong stabilizing effect on the homodimer which dramatically slows the time it takes for equilibrium between Orf9b and Tom70 to be reached. As there are no known small molecules that directly target Orf9b, we performed a crystallographic fragment screen to develop small molecules targeting the Orf9b homodimer. We identified approximately 60 hits out of 390 fragments screened that bound to either the central channel entrances or to an external dimer interface. We have begun to improve upon our initial hits by developing more potent compounds that stabilize the homodimer. Together, our initial fragment hits serve as the starting points for developing small molecules that stabilize the Orf9b homodimer with potential downstream implications to interferon signaling.

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CRYSTALLOGRAPHIC FRAGMENT SCREENING: IDENTIFICATION OF PROMISING MOLECULES BINDING THE SARS-COV-2 PLPRO PROTEASE

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The Covid-19 pandemic has been successfully tackled by the quick development of mRNA vaccines. While vaccines are very effective in slowing the viral spreading, therapeutic drugs help deal with severe cases of infection. So far, only one drug has been developed against the SARS-CoV-2 Mpro. To combat the ever-increasing number of SARS-CoV-2 variants and prepare for future coronavirus outbreaks, addressing multiple viral proteins is has attracted considerable attention as a multi-target therapeutic strategy. One such example is the Papain-like Protease (PlPro) domain of the non-structural protein 3 (NSP3). NSP3 fulfils multiple crucial functions in viral replication. After the genome translation into two polyproteins, PlPro cleaves part of the polyprotein to release the functional NSPs 1-3. PlPro furthermore suppresses the human innate immune system by its deubiquitinating and de-ISGlating properties. Furthermore, NSP3 together with NSP4 forms so called double membrane vesicles (DMVs) with the ER-membrane, which are a hub for viral RNA replication.
Here, we describe a crystallographic fragment screen successfully performed on the target domain Ubl2-PlPro, measuring ~800 different fragments of Nuvisan's X-ray fragment library that resulted in multiple promising hits. Furthermore, three TSA fragment screens of Nuvisan's fragment library with ~2000 fragments were performed against Ubl2-PlPro and its neighbouring NAB domain as well as a Ubl2-PlPro-NAB. Multiple hits were identified for each domain. The most promising hits were analysed by Surface Plasmon Resonance (SPR) and showed binding with Kd values in the low 3-digit μM range.

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BIOPHYSICAL APPROACHES TO DEFINE THE MODE OF ACTION OF FRAGMENTS

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The mechanisms by which small molecules exert a pharmacologic effect have greatly diversified beyond active site inhibition. Allosteric inhibitors/activators, cryptic site binders, monofunctional degraders all require (not) binding at a specific site on the target. While it is possible to run a generic screen and sort through the hits for the desired properties, this is both inefficient and possibly misleading. A strategy to direct screening hits towards (or away from) a particular site on the target can be a significant advantage. Here we present a number of biophysics based approaches including SPR and NMR that we have successfully employed to develop an allosteric inhibitor of PI3Ka, a m6A RNA “reader” protein and a PPI inhibitor. We further show how the complementary application of biochemical assays and structural biology increases confidence in promising chemotypes or to find new ones in these 3 different systems. This approach has allowed us to discovery ligands selective for a biologically relevant, yet less ligandable site, in the presence of a far more ligandable small molecule binding site.