Structure-based drug discovery in biotech and pharma

Michael Hennig on behalf of the leadXpro team and all collaborators
17/05/2023

leadXpro

leadXpro enables structure-based drug discovery on membrane proteins

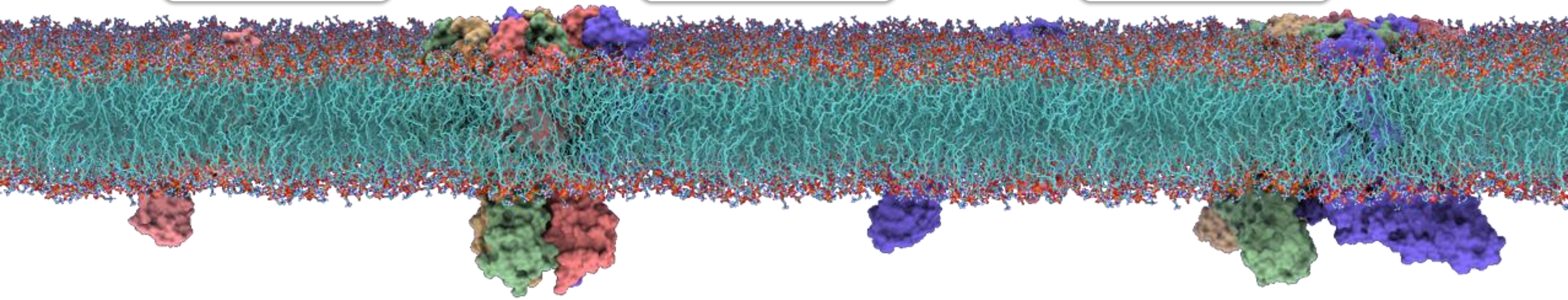
GPCR
Transporter
Ion channel

→

Structure-based drug design

→

New medicine



- Founded in **2015**
- Spin-out from the Paul Scherrer Institute (ETH)
- Utilize pioneering technologies (**Membrane protein science, Cryo-EM** and **XFEL**)
- Unmet proprietary access to large Swiss research facilities (SLS, SwissFEL and cryo-EM at Uni Basel)

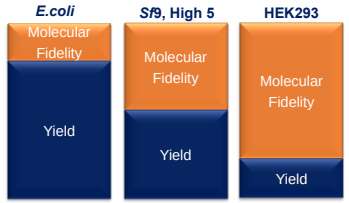
Michael.Hennig@leadXpro.com

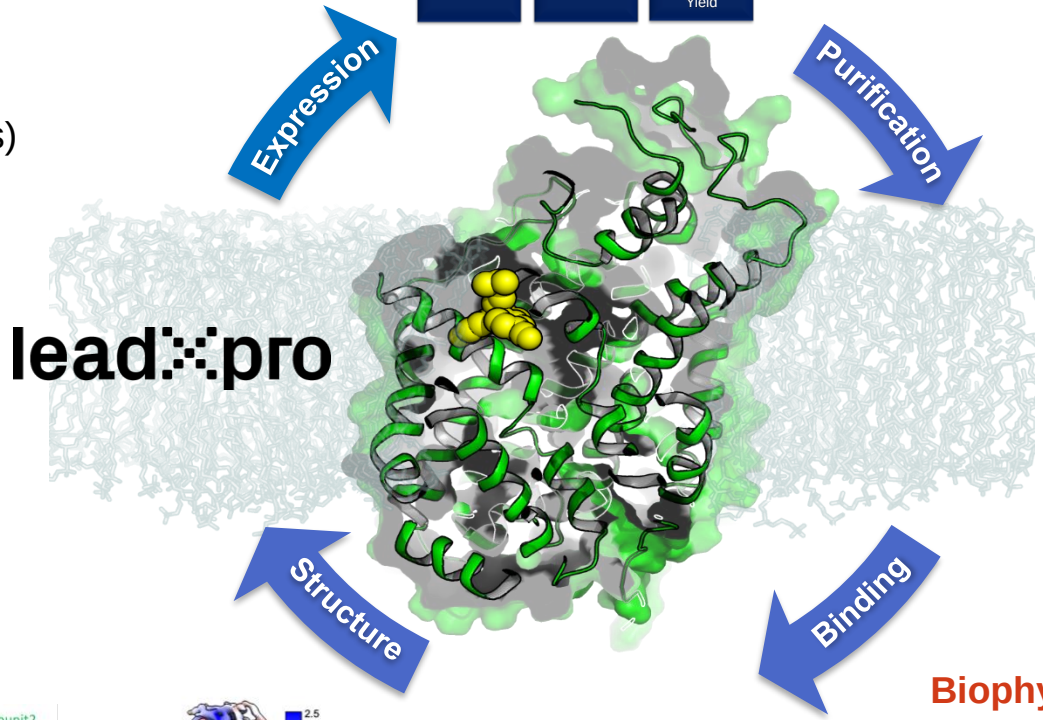
Co-location with Paul Scherrer Institute (Switzerland)

proprietary access to large academic research facilities

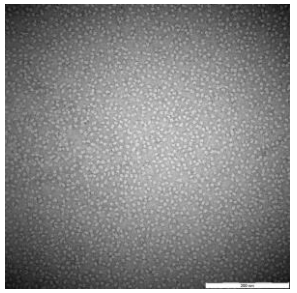
leadXpro's "Gene to Structure" Work flow

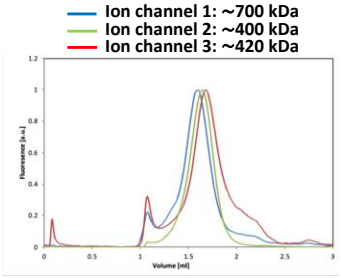
- Choice of the expression system (Bacterial, insect cells, mammalian cells)
- Plasmids optimization
- **Constructs design**
- Use of viral transduction for large proteins (e.g. Ion channels)

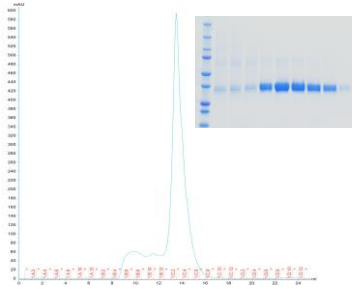




- **Protein** extraction
- Constructs and condition, detergent screening, protein formulation
- Purification and quality check



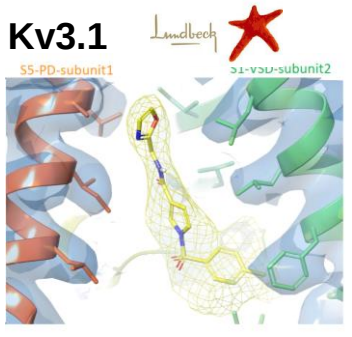


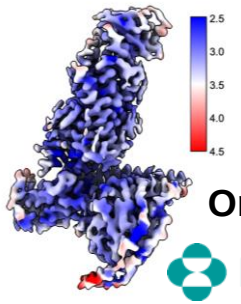


- **X-ray** crystallography
- Serial crystallography at SwissFEL and SLS
- Cryo-electron microscopy (**cryo-EM**)

LPTDE

Kv3.1

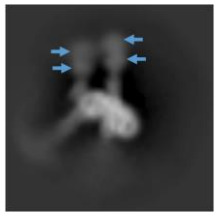




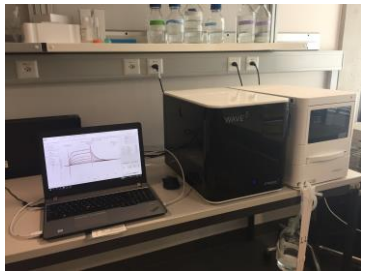
Orexin

MERCK

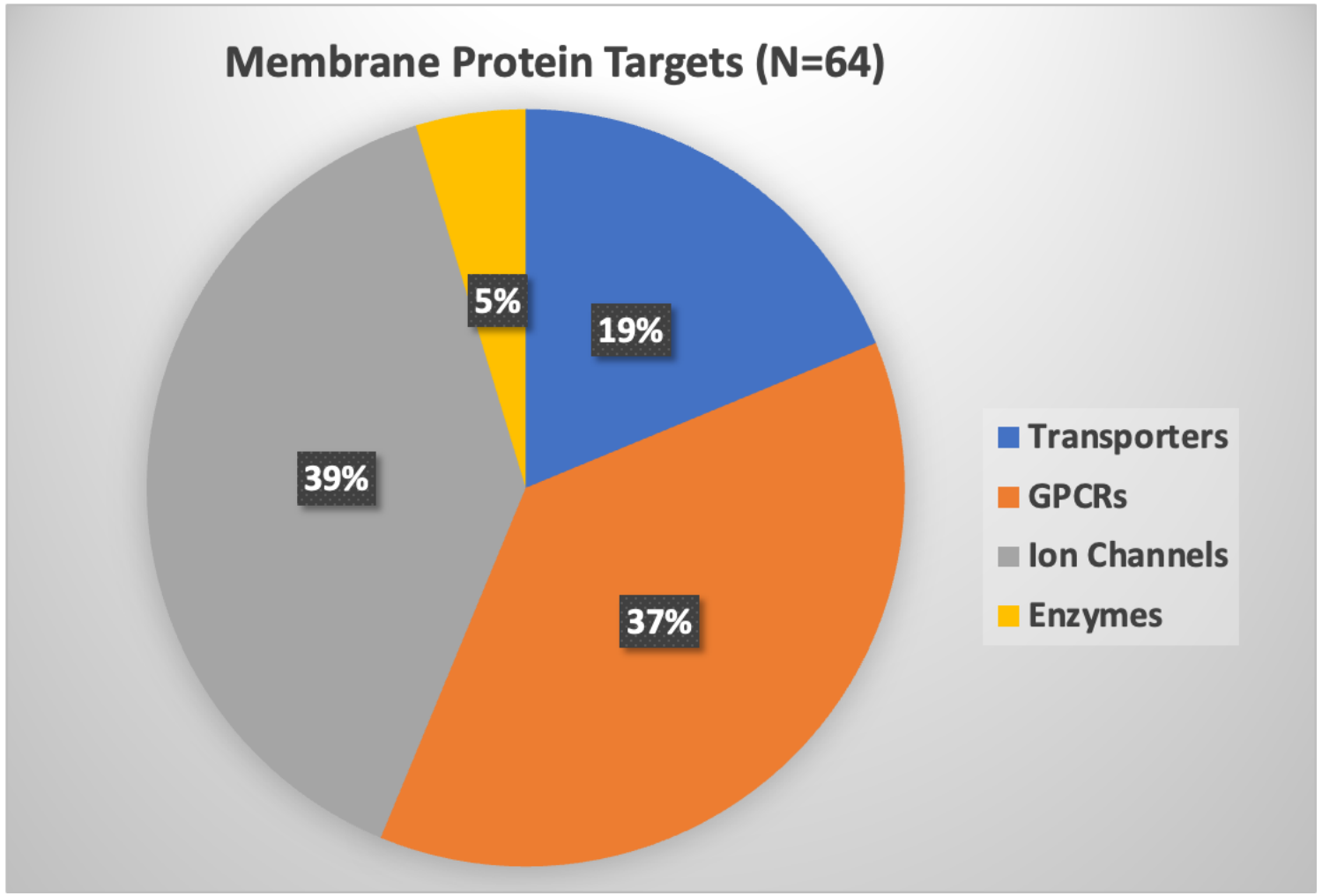
Nanobodies
Pro-Macrobody
(PMb)



Biophysical methods:
Grating-Coupled Interferometry (GCI), Thermal shift, Fluorescence based technologies



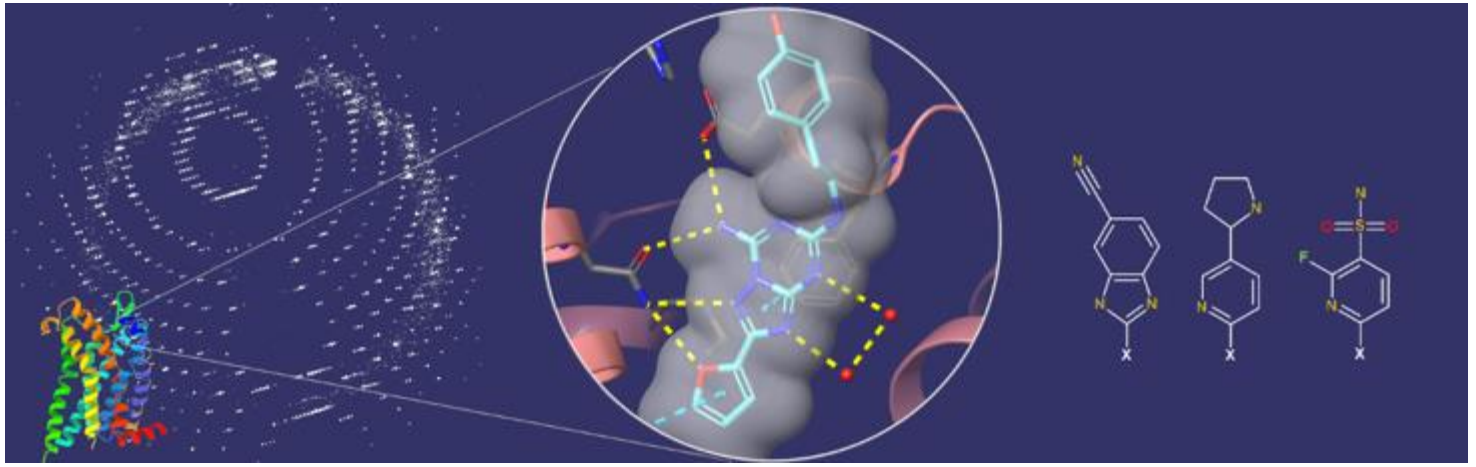
leadXpro portfolio of membrane protein drug targets



Drug Targets structural enabled to investigate ligand complex structures:

X-ray:	**11**
Cryo-EM:	**22**

Key Benefits of structure information for chemists



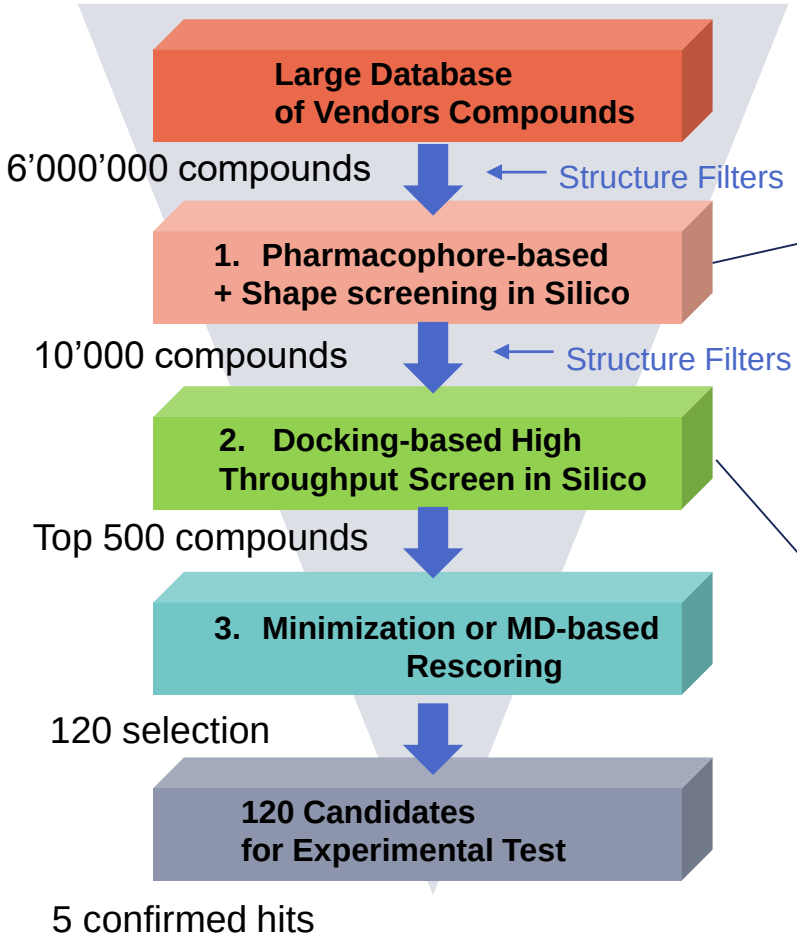
Hit finding and validation (LI)

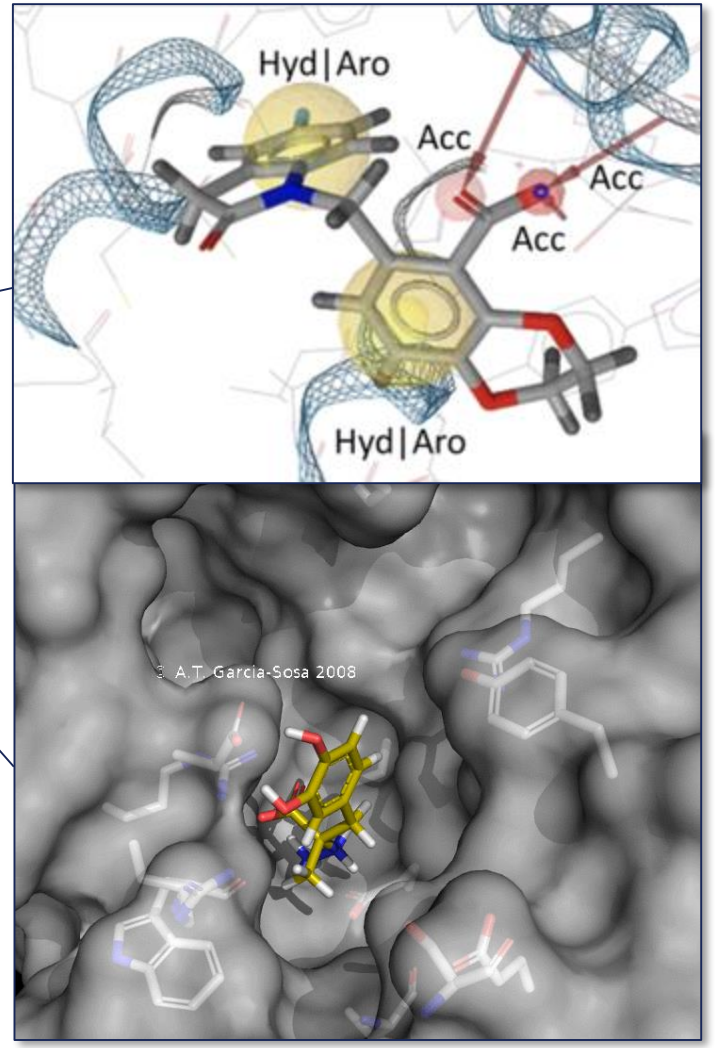
- Biophysical methods to confirm target binding (target engagement)
- Determination of affinity and binding kinetics (Creoptix), prioritize HTS hits
- In-silico screening of large libraries
- Identification of binding site, analysis of interaction, build SAR

Lead optimization (LO)

- Improve potency of binding
- Target specificity, reduce off-target binding
- Scaffold hopping to create novel IP on compounds
- Optimization of drug-like properties
- Get inspired by overlay of different chemical compound classes and their combination

Use of structure information – Virtual screening





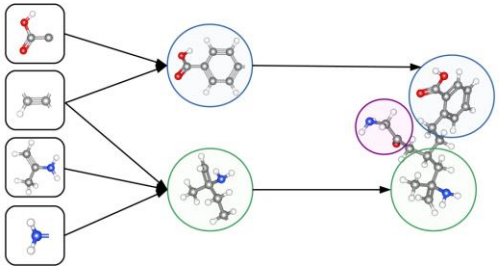
Much improved hit rate compared to HTS with random compound library

From structures to novel lead compounds

Computational chemistry to facilitate drug discovery

Ligand selection and derisking

Ligand properties predictions

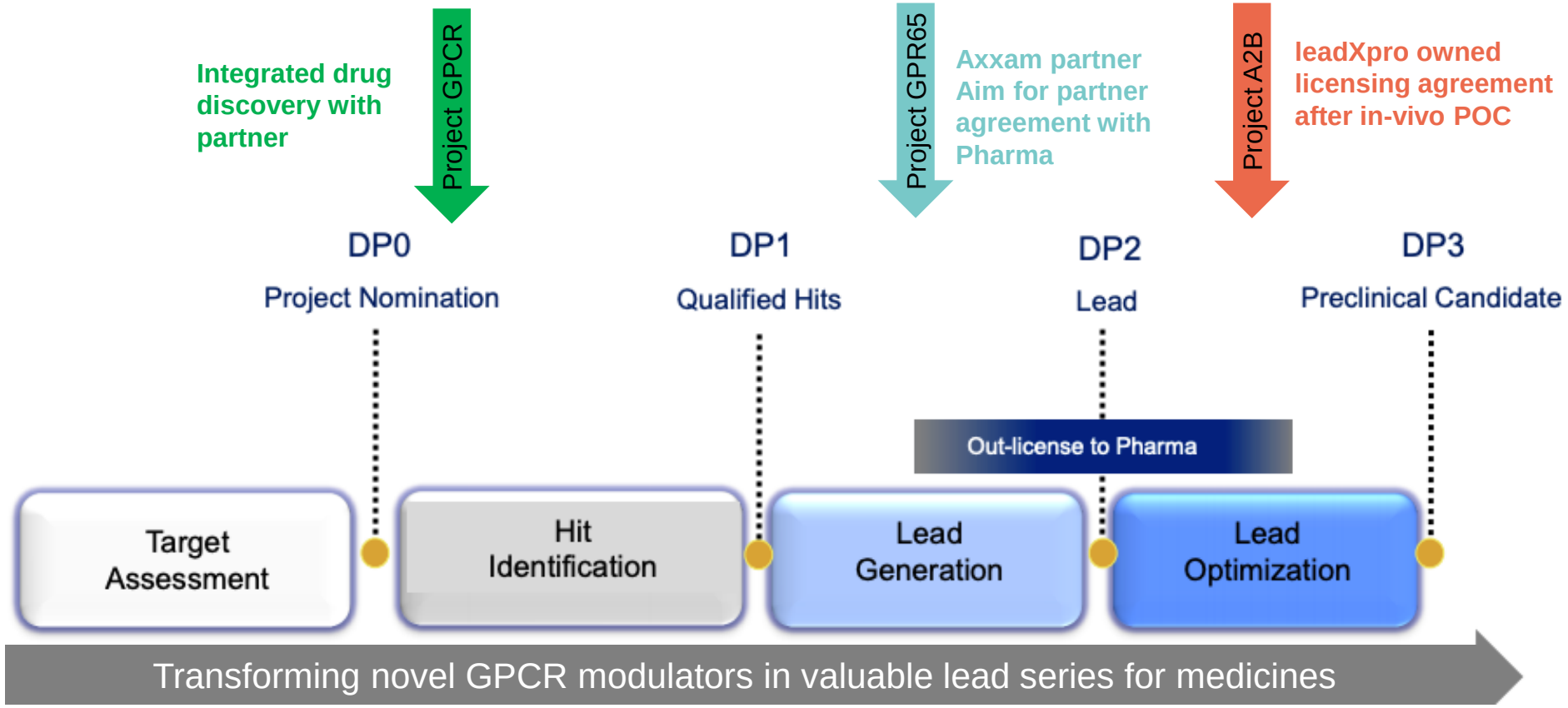


Pharmacokinetics predictions

Toxicity predictions / Artificial Intelligence

- Reduce time and costs for profiling of lead compounds
- Reduce animal experiments
- Increase the success of experiments

leadXpro internal drug discovery portfolio



Orphan GPCR - GPR65 drug target validation

Background

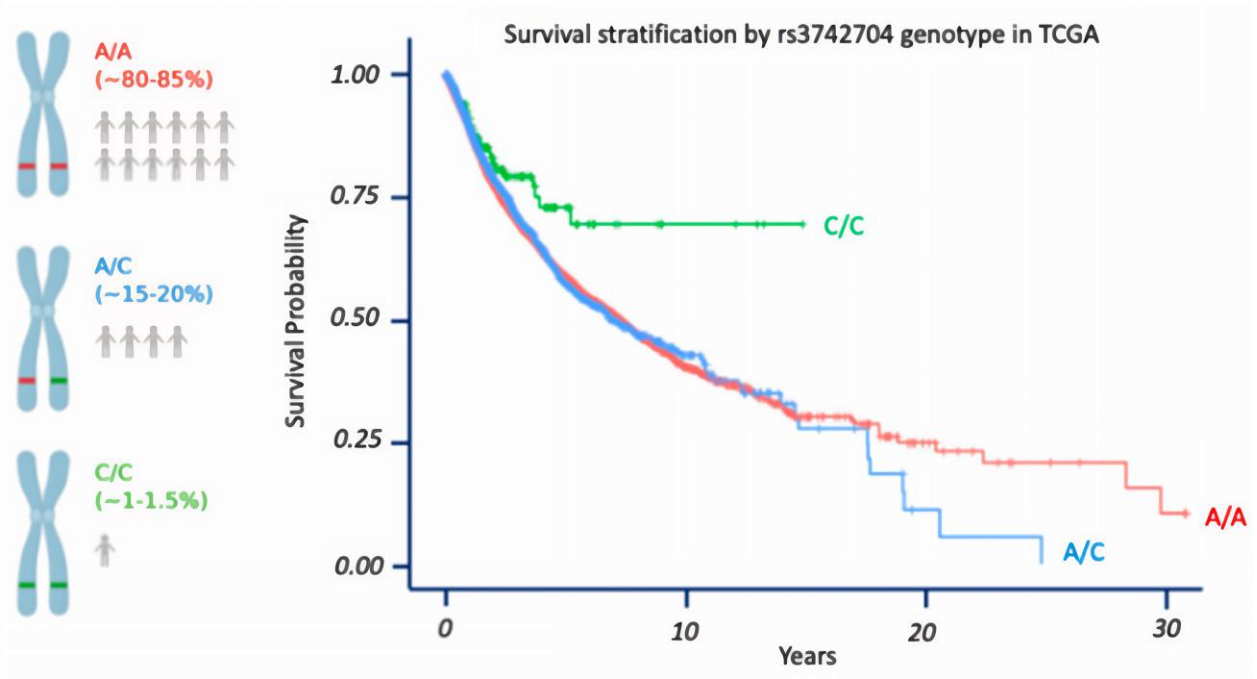
- Family of proton sensing GPCR's (GPR4, GPR65, GPR68, GPR132) sensing the acidic tumor or infammatory tissue environment

Aim

– Identification of GPR65 activity modulators

Therapeutic Application

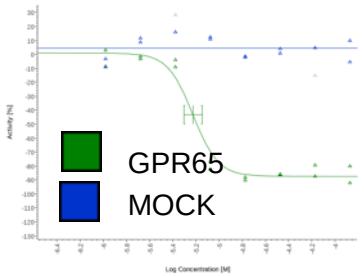
– Agonist/PAM: inflammatory bowel disease (IBD), neuroinflammation



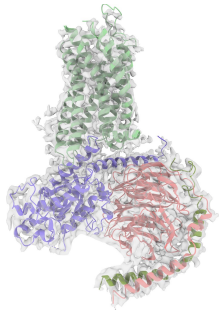
– Antagonist/NAM: cancer, immunosuppressive signalling in tumour-associated macrophages (TAMs), **loss of function I231L mutant** exhibit **improved survival** across multiple cancers

GPR65: Classical “HTS-driven” Discovery – no prior ligand information

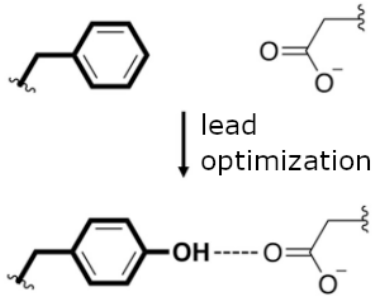
Axxam Screening facility



Selection of lead series



Binding site information



Optimization of lead compounds

Hit Finding

- Cellular assay for screening (275k compounds)
- Counter screen (mock cells)

Hit Validation

- Hit family clustering
- Validation at CRO & internal biophysics validation (binding, thermal shift assay)

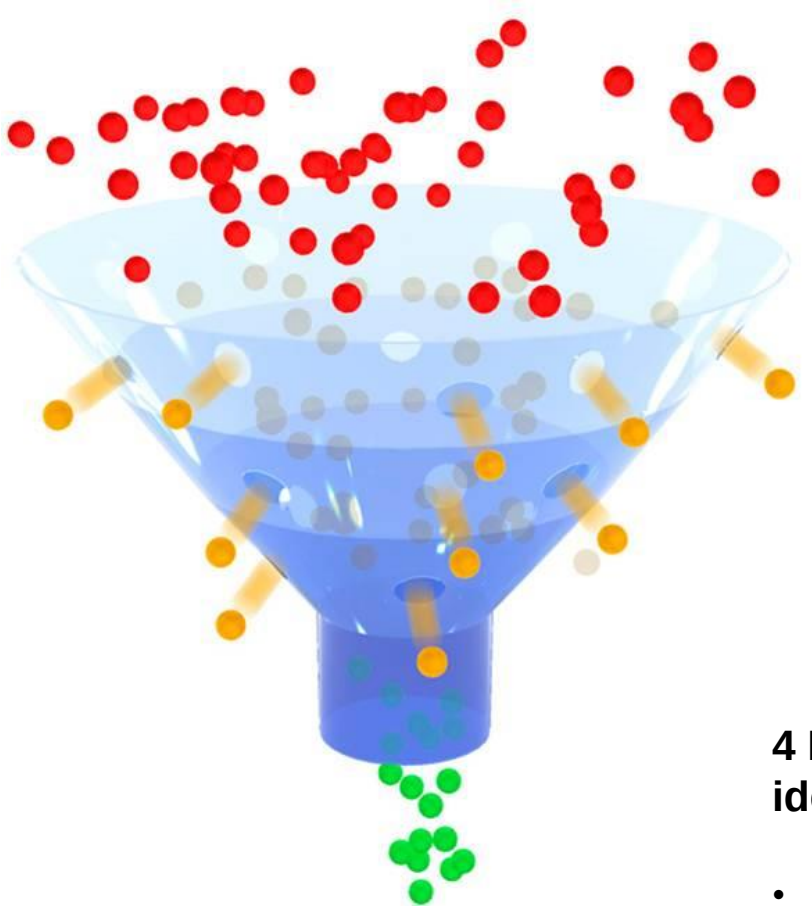
Structure

- Cryo-EM structure at 2.8 Ang, helped by protein engineering
- World-first structure of a proton sensing (H-GPCR)

Lead Optimization

- Structure-based design (SBDD) ongoing
- Structure activity relationship (SAR) to reach optimal properties

GPR65 screening cascade

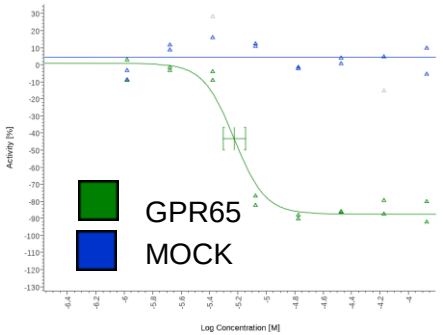


Extensive hit validation performed

- **Missense LoF variant assay:** human GPR65-I231L
- **Binding assay:** thermal shift, GCI human GPR65 (LXP)
- **Orthologue assay:** mouse GPR65
- **Selectivity assays:** GPR4 and GPR68
- Target engagement confimed by biophysical methods
- Structure determination

4 PAM and 4 Antagonist chemical classes identified and SAR exploration performed

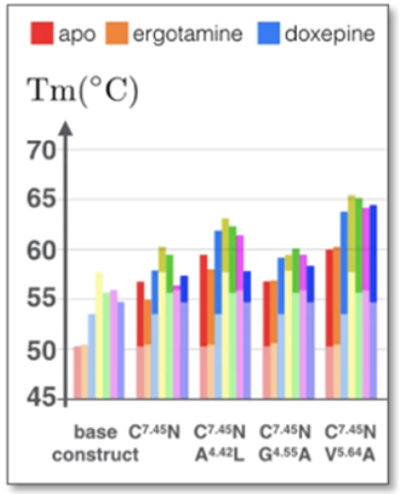
- 4 promising PAM series, optimisation in progress
- 5 promising Antagonist series



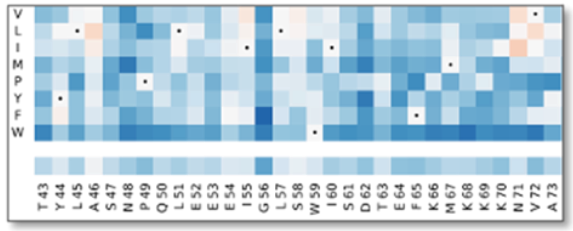
Protein engineering: Selection of stabilizing mutations for GPCRs

Alphafold, Rosetta
for protein design

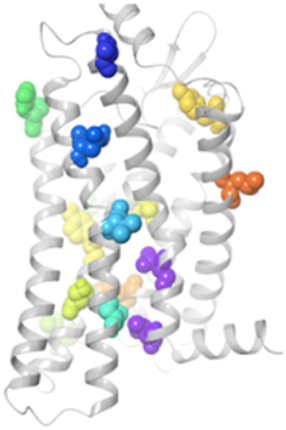
Machine Learning



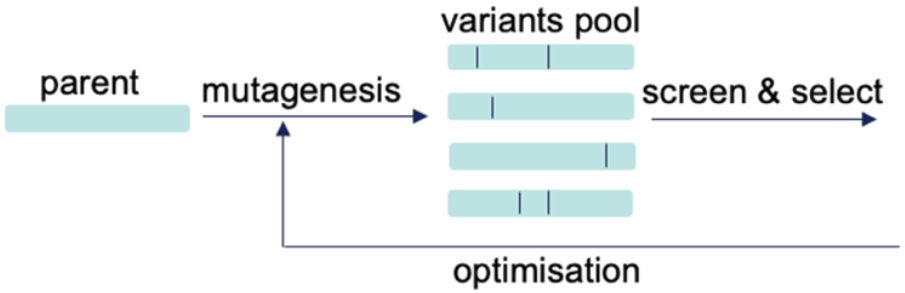
Sequence Alignment and Co-evolution



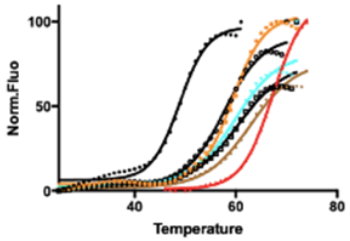
Model-based
Structural Predictions



Rational Construct Design



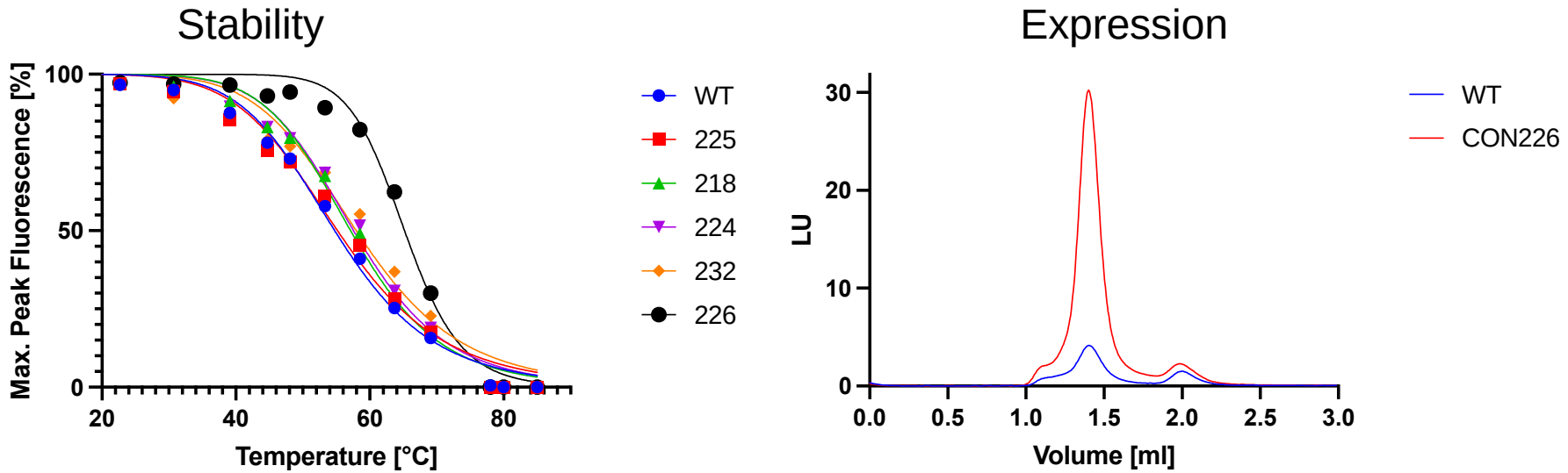
FSEC-TS



Engineering increases the chances of optimal protein for biophysics and structure work.
Experience based on solving >20 GPCR projects.

GPR65 - 250 constructs made to achieve Stability & Expression

Wild type (WT) hGPR65 showed very poor expression and stability.
Multiple mutations needed, (+truncation, fusion). Detergent screening.



Construct 226 showed +10°C thermal stability and a significant improvement (>6-fold) in expression.

Engineered mutations provided a path forward to biophysics & structure

The High sensitivity critical for membrane proteins

Creoptix
a Malvern Panalytical brand

High sensitivity

- Featuring GCI technology for kinetic rate determination
- Work with low immobilization levels
- Compatible with large ligand-to-analyte molecular weight ratios

Innovative microfluidic cartridge

- No-clogging with crude samples
- Fast transitions enabling widest k_d range
- Compatible with harsh solvents

WAVEdelta system with autosampler and waveRAPID software.

Temperature controlled autosampler

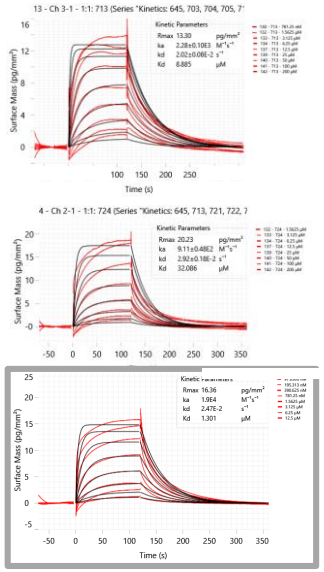
- No sample evaporation
- 120h of unattended operation
- Sample capacity of 96 or 384 well plates

Automated and intuitive software

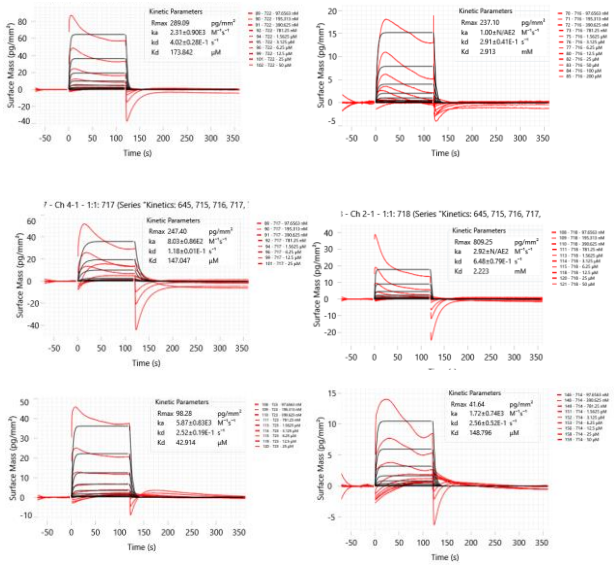
- **waveRAPID** – full kinetics (k_a, k_d, K_D and Rmax) from a single well
- Direct Kinetics – automated, evidence-based data evaluation

GCI measurements on membrane proteins

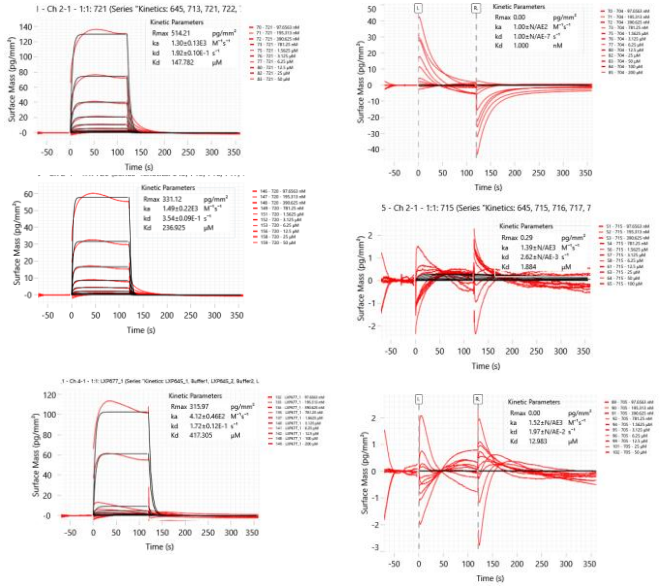
GOOD

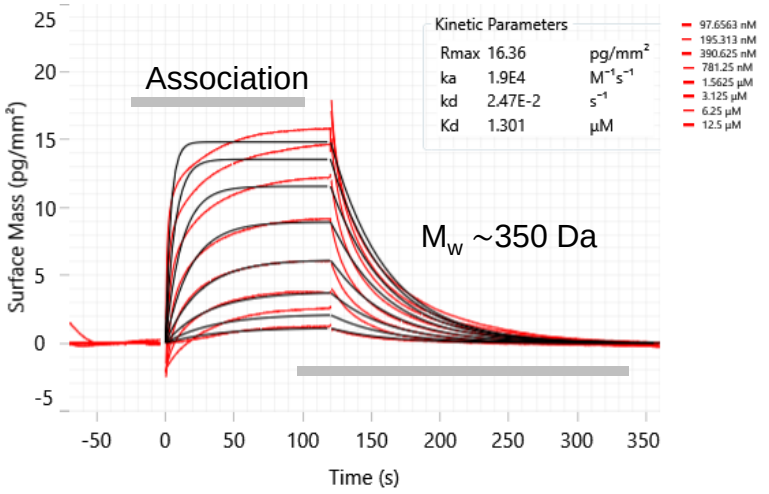


BAD

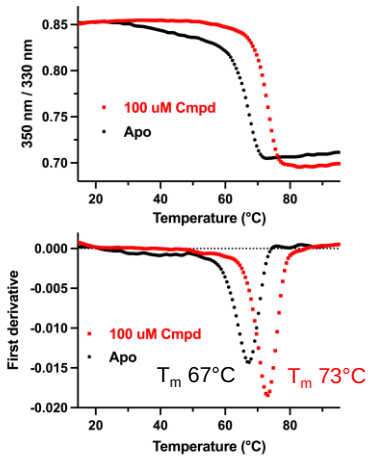


UGLY





nanoDSF
(Prometheus Panta)



GCI and **nanoDSF** are routinely used to identify and prioritize ligands for structure research.

leadXpro Cryo-EM strategy

Mastering all steps of the analysis

All protein science, grid freezing and grid quality control screening @leadXpro inhouse

Leica Pluncher for conventional blotting

Vitrojet for pin-printing of grids

Talos grid screening

GPU-based server cluster

cryoSPARC

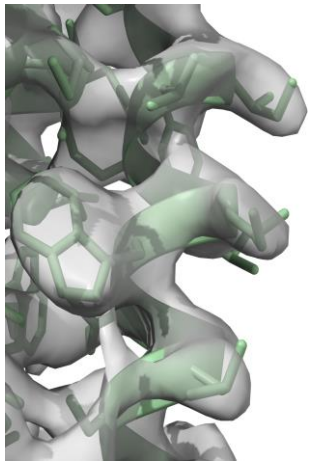
300kV Titan Krios G4 with Cold-FEG, RF-Cavity, Cs Probe Corrector, Falcon IVi and custom hybrid pixel detector camera

@DCI Lausanne
University of Basel

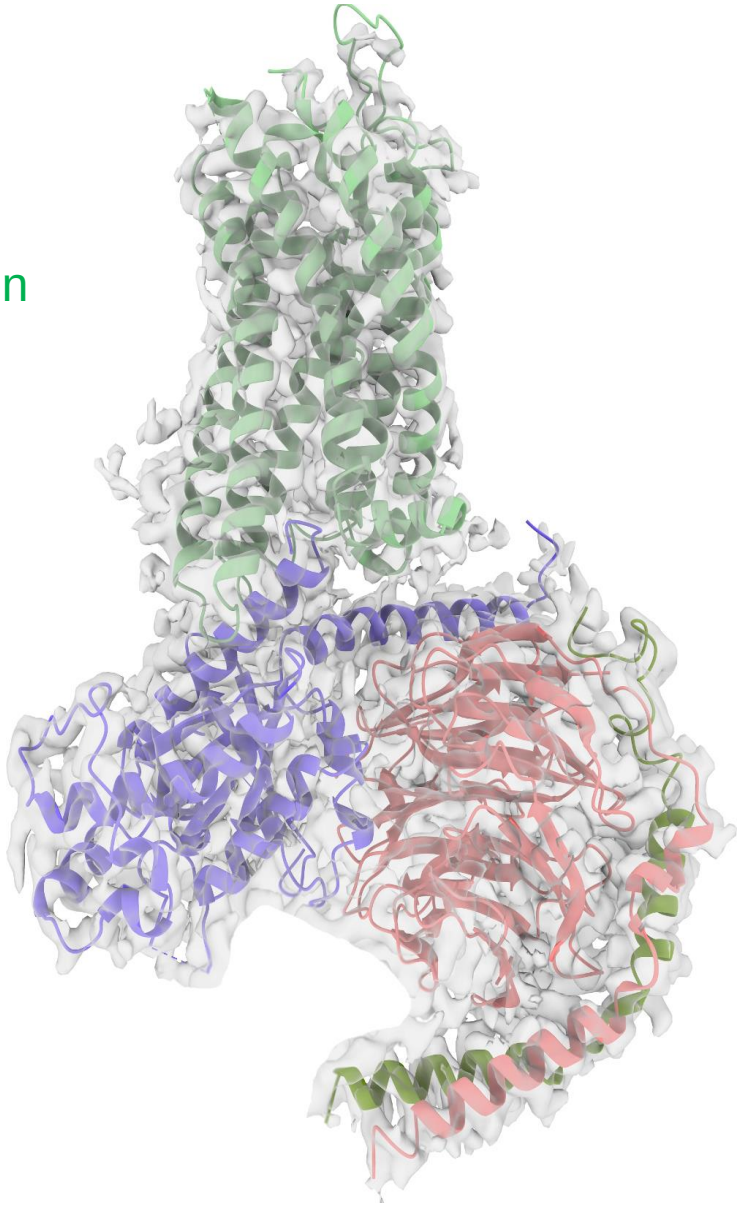
High resolution cryo-EM structure (2.8 A) of GPR65/G-protein complex to support drug discovery

GPR65 receptor in green

View showing the resolution close to ligand binding area



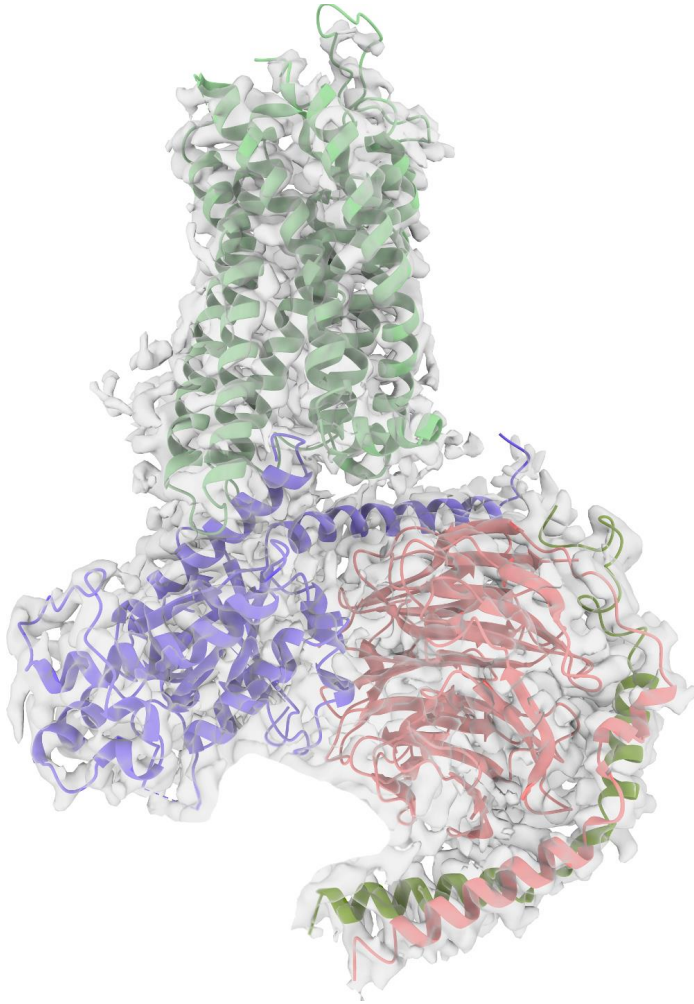
G-protein



GPR65 program summary

Status & Assets

- HTS completed, cAMP: several hit classes identified (PAMs, Antagonists)
- Hit-to-lead performed on three series (chemistry by leadXpro, biology by Axxam), initial SAR established.
- Primary and secondary functional cellular assays (loss of function GPR65-I231L mutant, selectivity, mouse).
- Purified proteins for biophysical methods, and world first cryo-EM structure of proton-sensing GPCR available to support drug discovery.
- Unique combination of assets and expertise to advance lead series.



X-ray

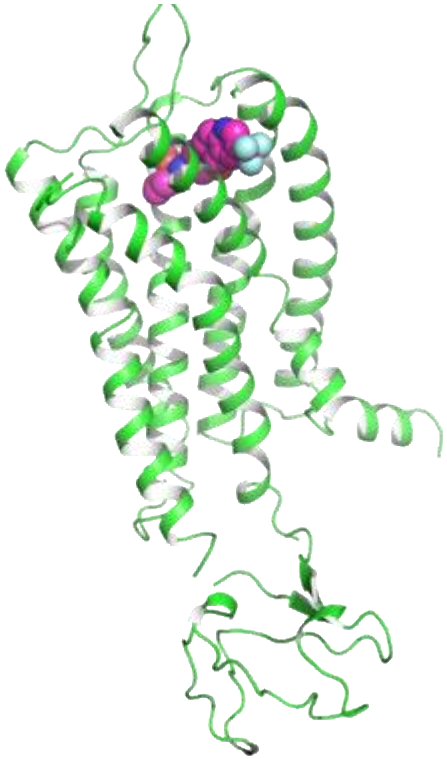
(with some emphasis on serial X-ray crystallography)

GPCR structure from scratch CCR2A

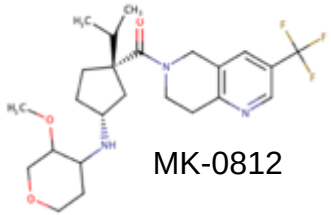
Collaboration with:

Boehringer Ingelheim

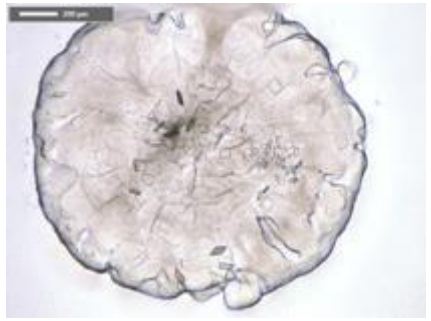
- 5 mutations: N14Q, C70$^{1.60}$Y, G175$^{4.60}$N, A241$^{6.33}$D, K311$^{8.49}$E
- Rubredoxin in ICL3
- Protein crystallized in LCP in high PEG (>40%) condition
- 3.3Å dataset from a single crystal with orthosteric compound MK-0812



Orthosteric antagonist



MK-0812



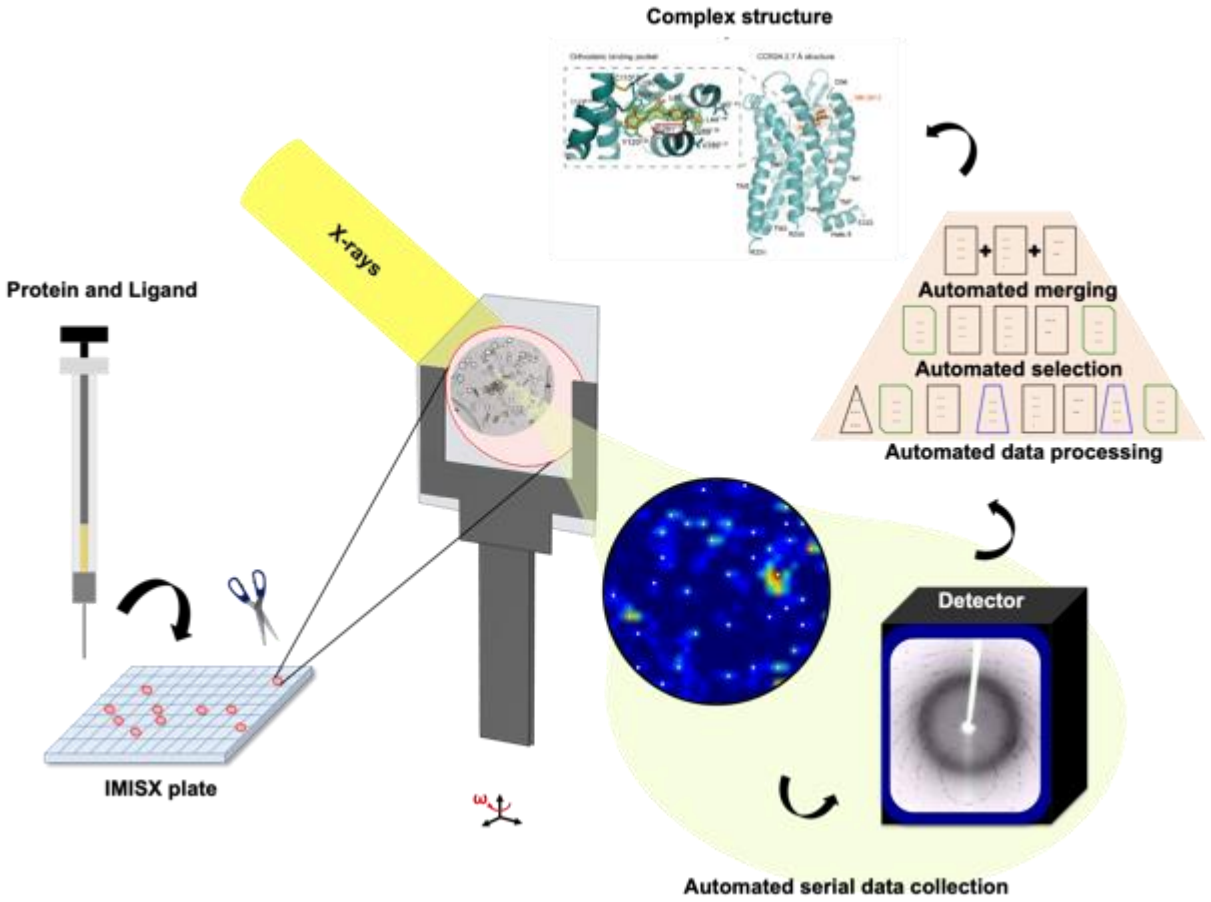
	CCR2A
Number of crystal	1
Spaceroup	$P2_1$
Cell dimensions	
a, b, c (Å)	38.50, 61.20, 123.57
α, β, γ (º)	90.00, 97.65, 90.00
No. of reflections	26000
Resolution (Å)	43.29–3.30 (3.57–3.30)
R_{merge}	0.134 (1.056)
$CC_{1/2}$	0.995 (0.479)
I/σI	5.7 (0.9)
Completeness (%)	95 (96.4)
Redundancy	3.1 (3.0)
R_{work}/R_{free}	0.243/0.296

Apel A. Structure et al. 2019
Cheng, K.Y. et al. 2019

CCR2A – Example for In-Meso In-Situ Serial X-ray (IMISX)

Collaboration with:

Boehringer Ingelheim



- To address sensitivity of crystal manipulation and freezing
- Diffraction test after freezing indicated freezing not seemed to be an issue
- Samples were measured at SLS, PXII
  <10° mini-dataset from 77 crystals using CY+ GUI interface (Wojdyla et al., 2018)

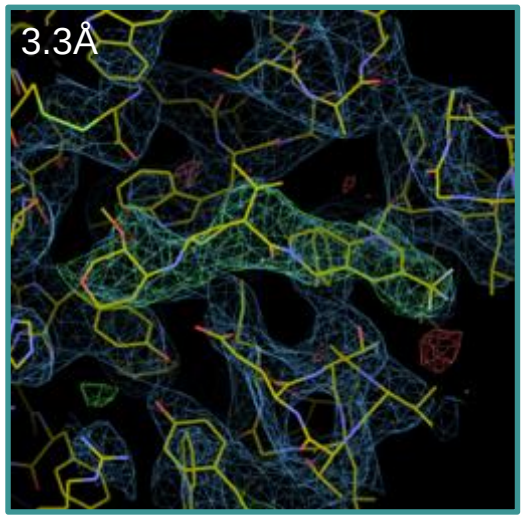
Apel A. et al. 2019
Cheng, R. et al. 2019

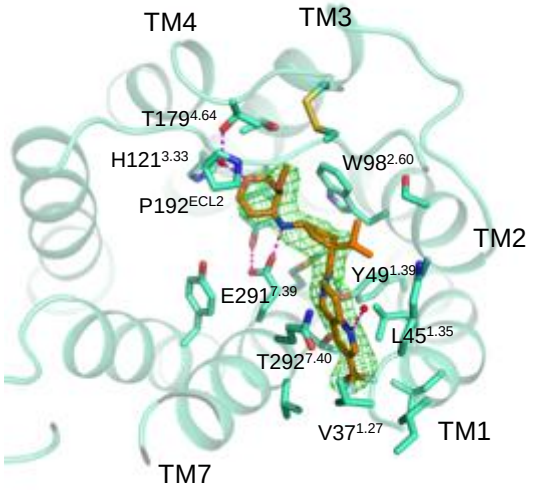
CCR2A – conventional and IMISX technology

Collaboration with:

Boehringer Ingelheim

	CCR2A (3.3Å)	CCR2A (2.7Å)
Crystals (dataset)	1	77
Spaceroup	$P2_1$	$P2_1$
Cell dimensions		
a, b, c (Å)	38.50, 61.20, 123.57	54.57, 64.55, 131.20
α, β, γ (º)	90.00, 97.65, 90.00	90.00, 91.18, 90.00
Resolution (Å)	43.29–3.30 (3.57–3.30)	**50–2.7 (2.77–2.70)**
R_{merge}	0.134 (1.056)	0.55 (0.863)
$CC_{1/2}$	0.995 (0.479)	0.993 (0.656)
I/σI	5.7 (0.9)	6.93 (1.17)
Completeness (%)	95 (96.4)	**100 (100)**
Redundancy	3.1 (3.0)	**15.8 (14.8)**





MK-0812
BMS-681

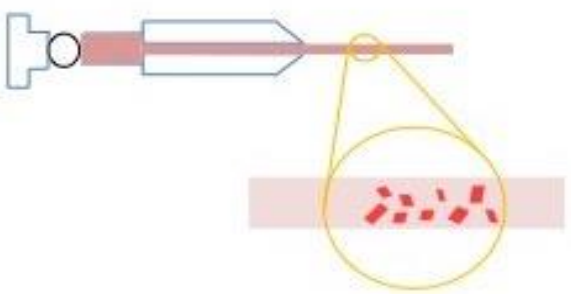
- Structure guided optimization of selectivity for CCR2 against other CCR receptors

Apel A. Structure et al. 2019
Cheng, R. et al. 2019

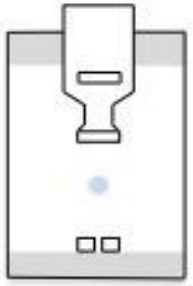
Serial Crystallography @ leadXpro = LCP only

sample delivery is key to success

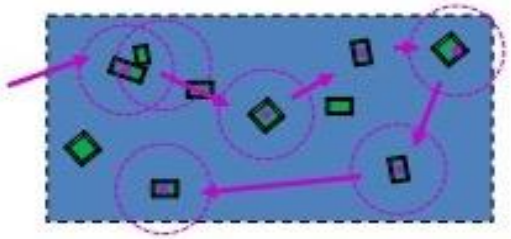
Injector



Acoustic levitation



Fixed-target

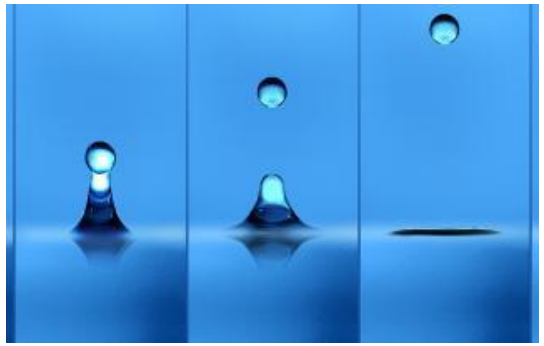


+ main delivery method now + LCP/Vapour feasible + Room temperature - Sample consumption (0.2-x. mg/data set) - Hit rate/efficacy - Jet flow needs optimization	+ low sample consumption + less sample handling + ligand soaking automation + Room temperature - Requires larger crystals - No routine application	+ low sample consumption + Room temperature AND freezing + minimum sample handling + maximum sample hit rate - Higher background - Automation challenges

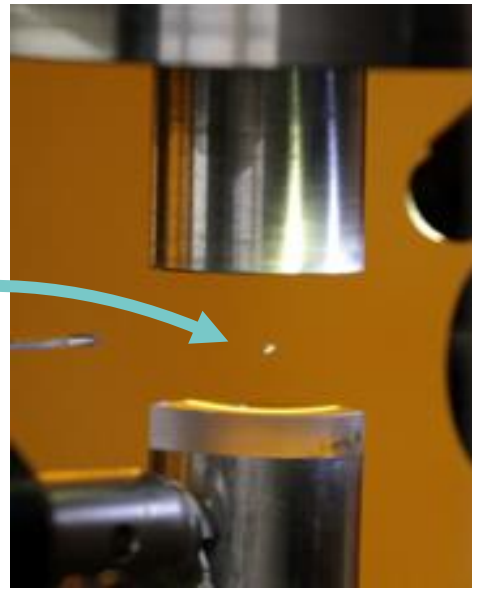
- - - Optimization of crystallisation for each protein required

Serial Crystallography

Sample delivery by acoustic dispensing & levitation



Acoustic dispensing
(well plate with crystal suspension)

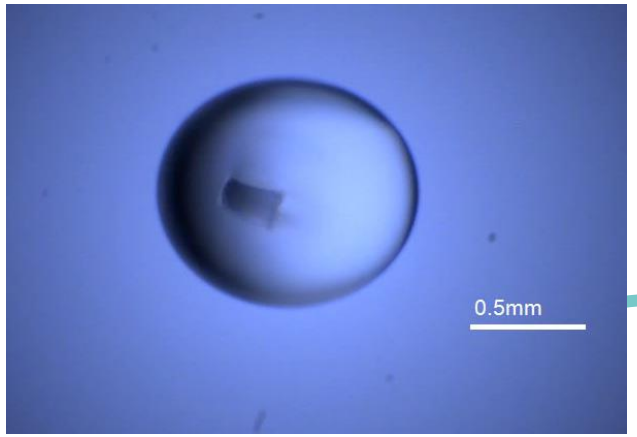


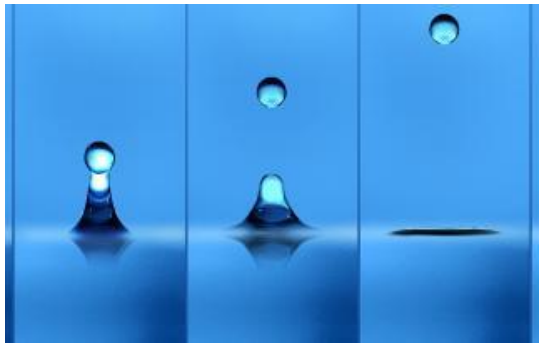
Levitator

CTI/Innosuisse funded technology project of PSI & leadXpro
T. Tomizaki, S. Tsujino, J. Standfuss

Serial Crystallography

Sample delivery by levitation





Acoustic dispensing
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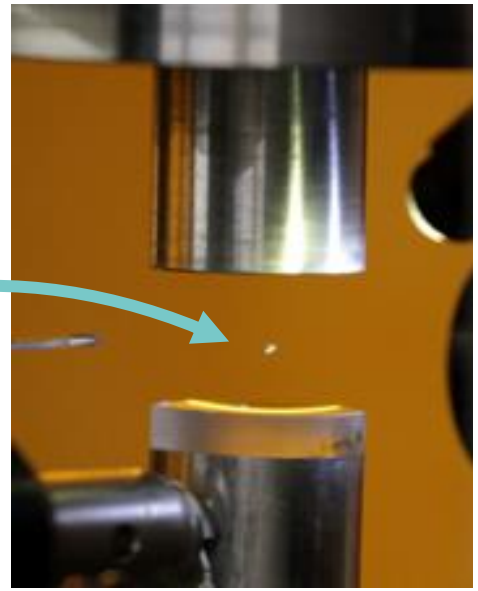
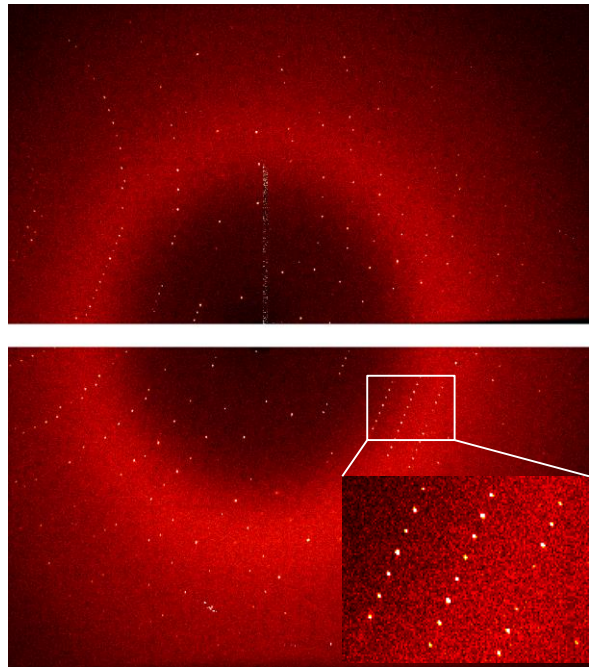


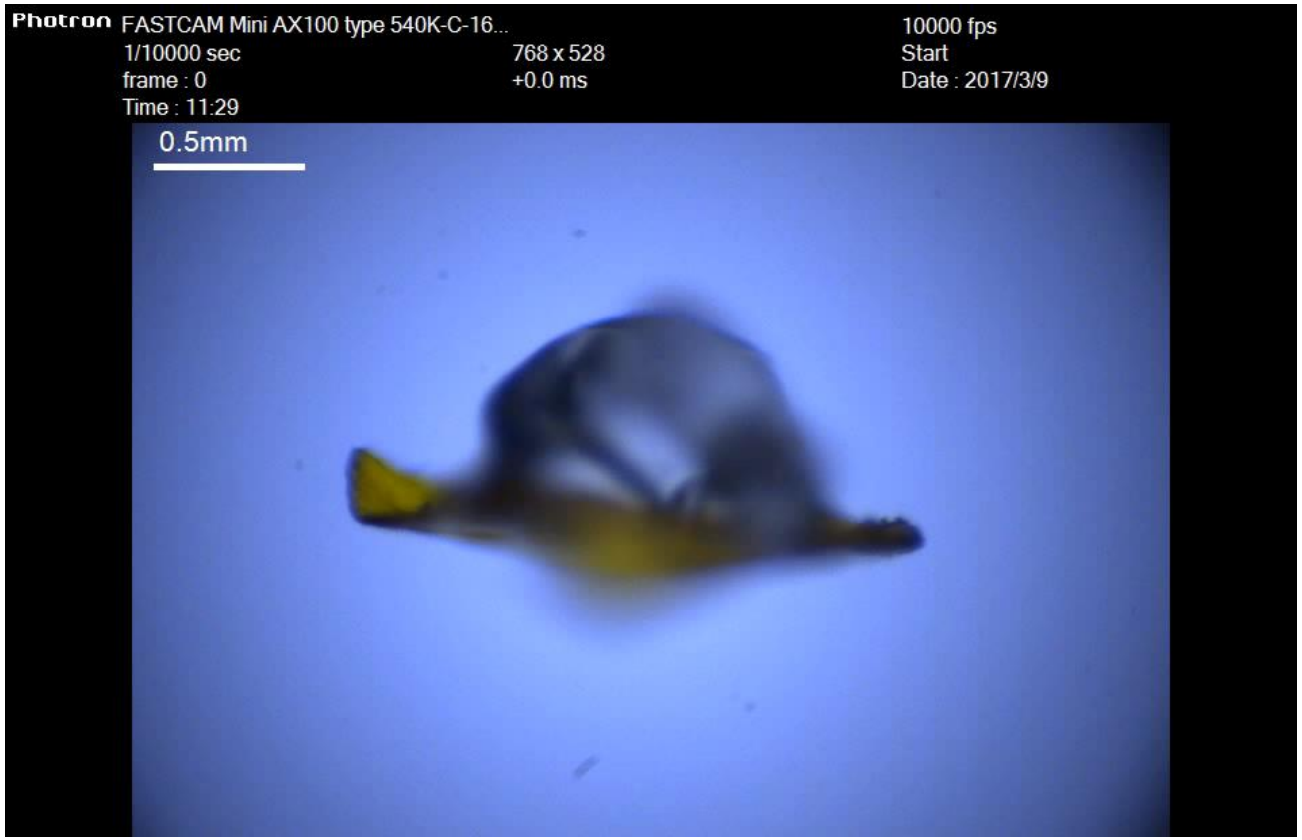
Levitator

Eiger read-out 1-3 kHz



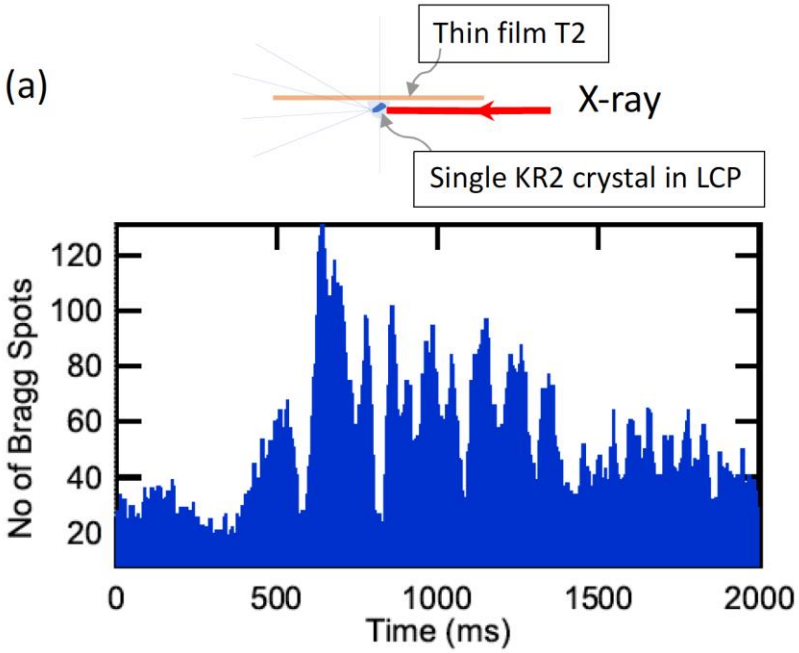
CTI/Innosuisse funded technology project of PSI & leadXpro
T. Tomizaki, S. Tsujino, J. Standfuss

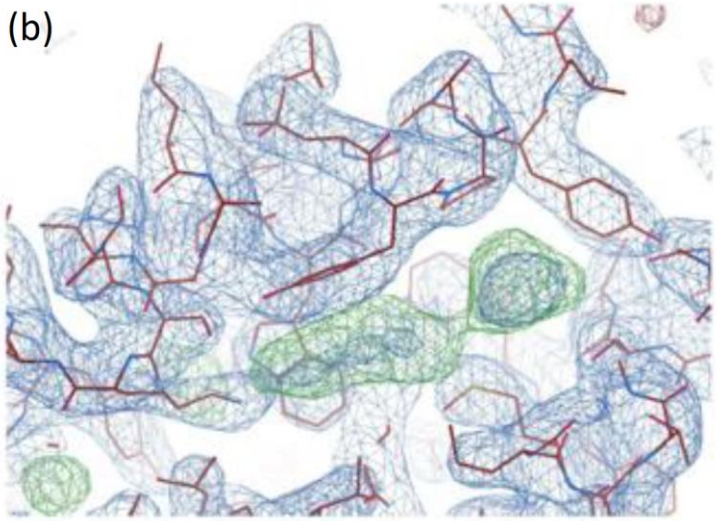
Serial Crystallography
LCP - *Sample delivery by levitation*



CTI/Innosuisse funded technology project of PSI & leadXpro
T. Tomizaki, S. Tsujino, J. Standfuss

Serial Crystallography
LCP - *Sample delivery by levitation*





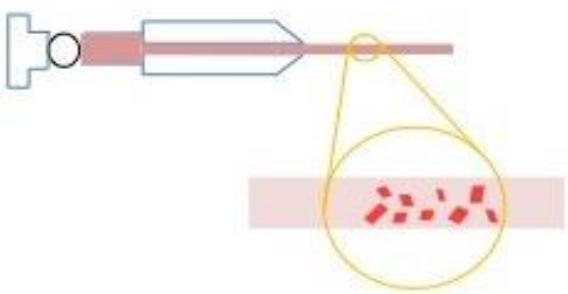
2.5A resolution structure
of KR2 membrane protein

Kepa et al., Scientific Reports | (2022) 12:5349

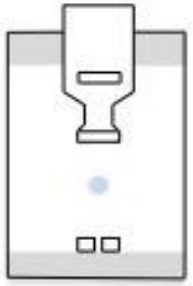
Serial Crystallography @ leadXpro = LCP only

sample delivery is key to success

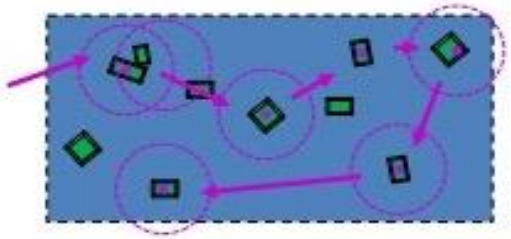
Injector



Acoustic levitation



Fixed-target



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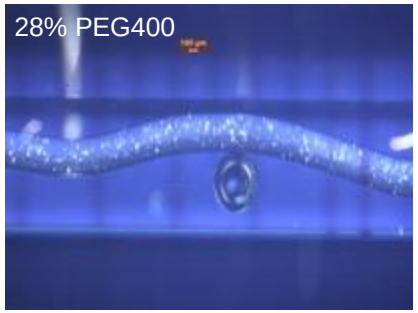
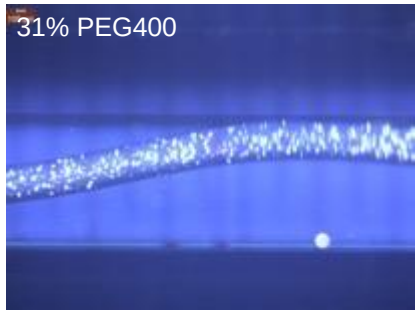
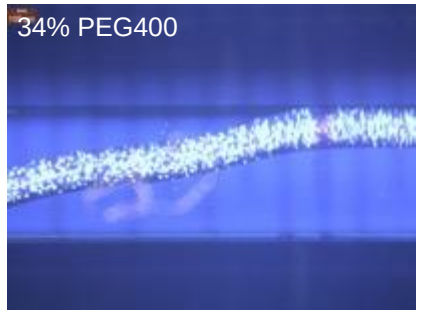
- - - Optimization of crystallisation for each protein required

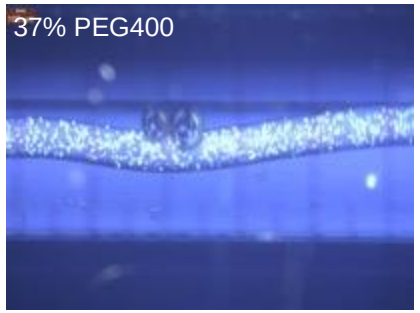
Serial Crystallography using LCP jet

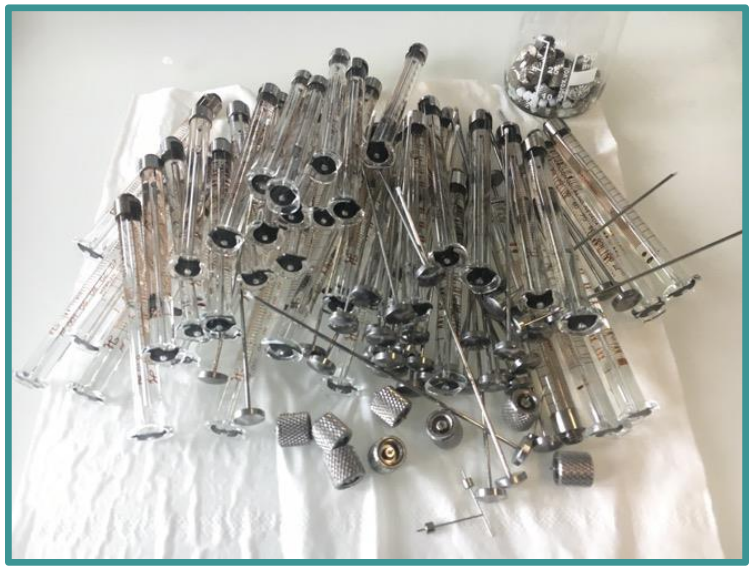
- A_{2A} receptor as membrane protein model system
- Crystallisation in syringes. ~30-40µm crystals, high density of crystals
- Injector jetting tested before beamtime



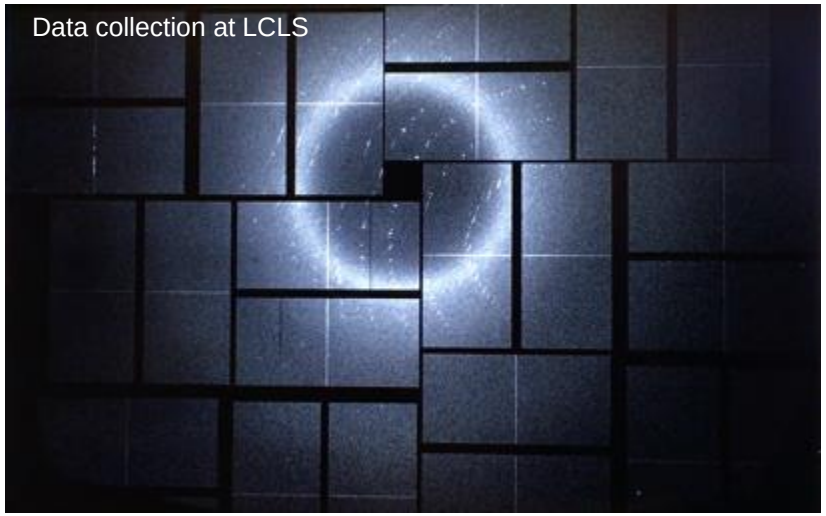








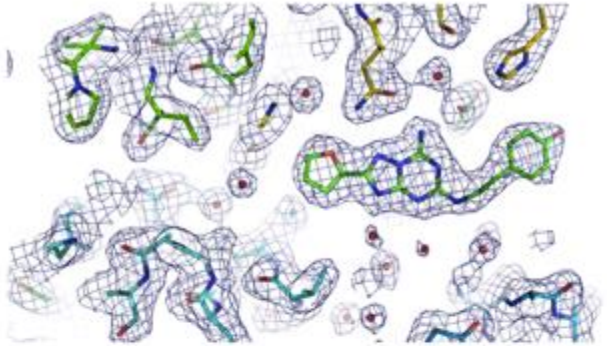
Serial Crystallography on A_{2A}



	Synchrotron, SLS, room Temperature Serial X-ray	X-FEL, LCLS Room temperature, Serial X-ray	Synchrotron, Diamond Conventional X-ray from 21 xtals
	SMX	**SFX**	**Cryo**
Energy (keV)	12.4	9.5	12.4
Measurement time (h)	6.6	0.36	4
Beam size (uM)	20x5	1x1	20x5
Collected images	**1,180,705**	**155,241**	**3500**
Indexed patterns	128,086	3563	3500
Indexed %	10.8	2.3	100
Resolution	**2.14**	**1.7**	**1.95**
Redundancy	**1007.4**	**23**	**9**

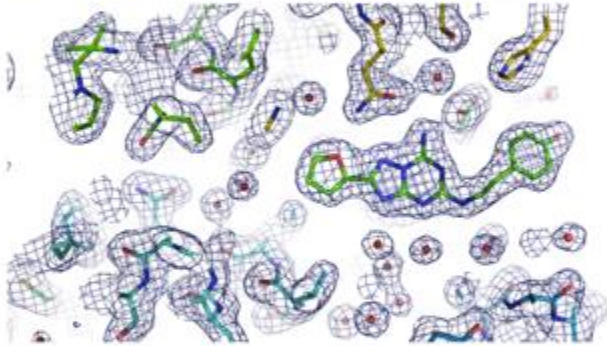
SMX

- Serial X-ray
- SLS
- Room Temperature



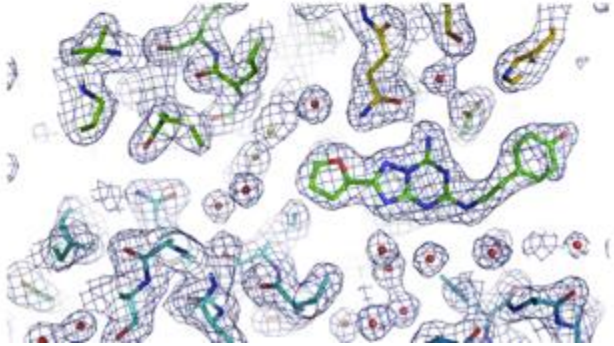
SFX

- Serial X-ray
- LCLS
- Room Temperature



Cryo

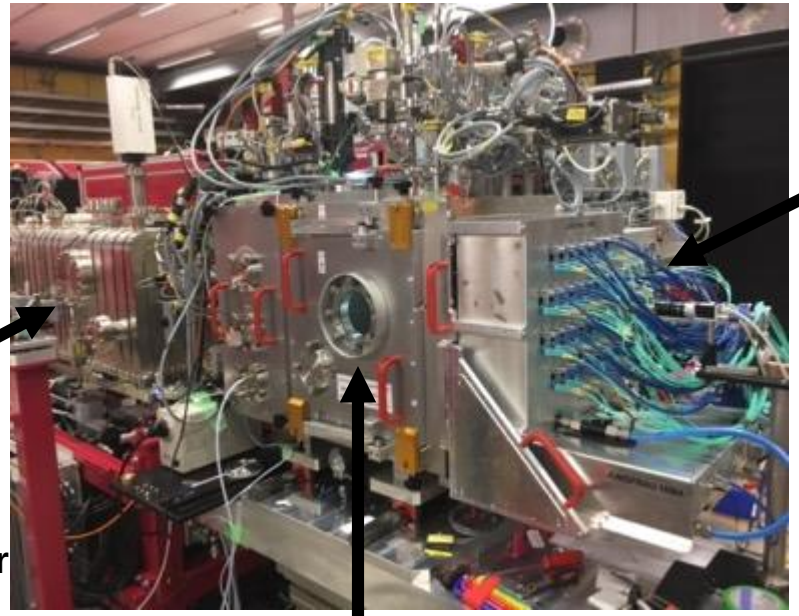
- Conventional Cryo X-ray
- Diamond
- 21 xtals



Weinert, T. et al. 2017

SwissFEL

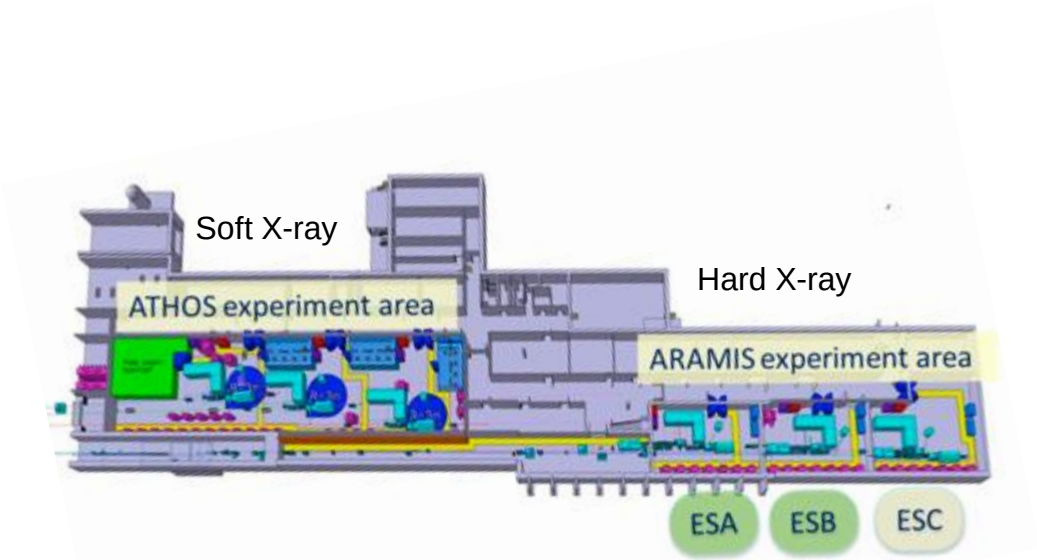
ALVRA (ESA) measurement station at ARAMIS



Jungfrau 16M detector, charge sensitive and high dynamic range

Collimator enabling focus at 1.5 μm diameter

Measurement chamber equiped with LCP jet

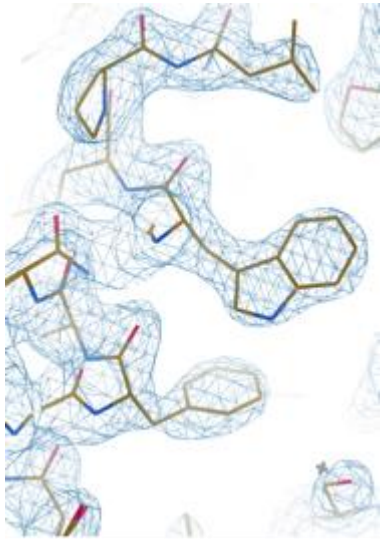


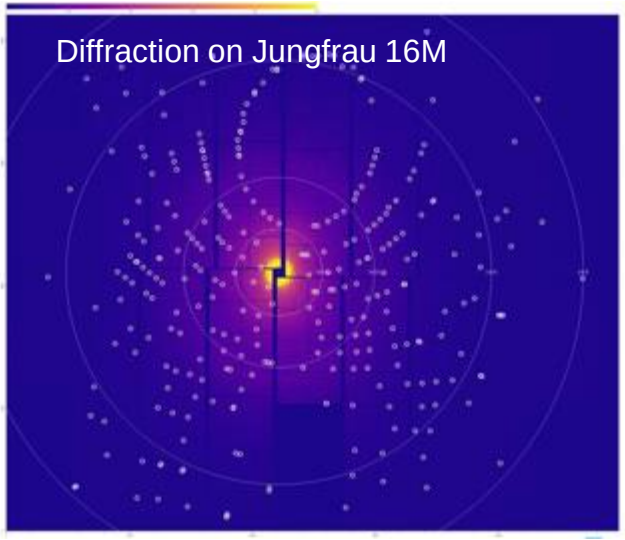
SwissFEL – Alvra (ESA)

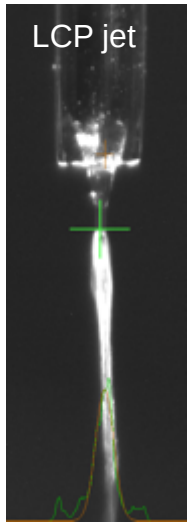
leadXpro with priority access to Free Electron Laser (SwissFEL)

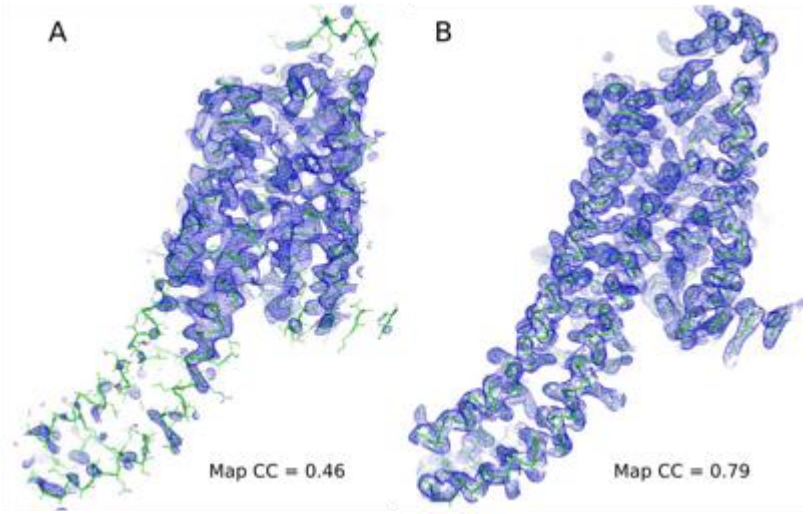
- **Pilot SFX experiment on GPCR**
- LCP Jet
- Native S-SAD phasing using long wavelength (2.713 Å, 4.57 keV)
- Much less data required than previous works at XFEL (Batyuk A. et al., 2016) and synchrotron (Weinert et al., 2017)
- 25Hz, 10um beam, 6e11 photons/pulse
- 2.6Å resolution

Final density



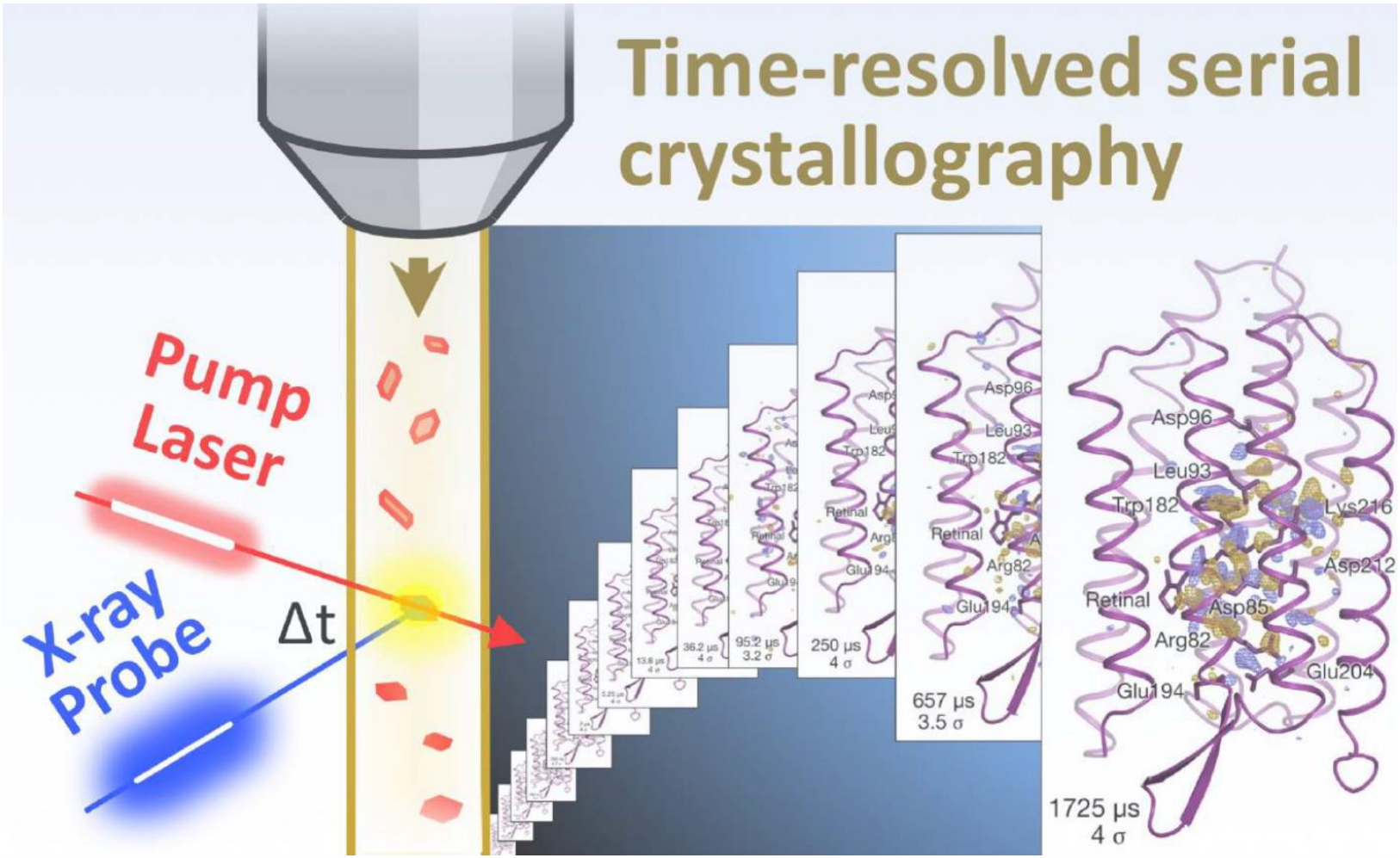






Nass, K. et al. IUCR, Volume 7| Part 6| November 2020| Pages 965-975, 2020

The future of Structure-based drug discovery is dynamic!

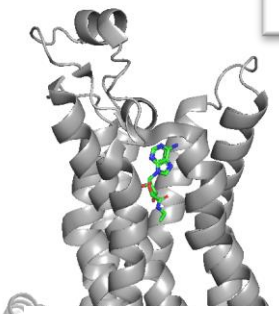


leadXpro – PSI Jörg Standfuss Group collaboration

3 GPCR targets with therapeutic value for pharma industry:

POSTER !!!

Adenosine A2 receptor



Synthetic Photoswitches to Study the Structural Dynamics of Ligand Dissociation in the Human A_{2A} Receptor

Hannah Glover[1], Torben Sassmanshausen[2], Quentin Bertrand[1], Robin Stipp[1], Matilde Trabuco[3], Robert Cheng[3], Florian Dworkowski[1], Michael Hennig[3], Jörg Standfuss[1]

We would like to thank the support at the Swiss Light Source and SwissFEL

[1] Paul Scherrer Institut (PSI), CH-5232 Villigen PSI, Switzerland

[2] Goethe University, Institute of Physical and Theoretical Chemistry, D-60438 Frankfurt am Main, Germany

[3] leadXpro, CH-5234 Villigen, Switzerland

- Protein production
- Photoswitch
- Crystal
- Time-resolved data

β2 adrenergic receptor

PAUL SCHERRER INSTITUT PSI

Investigation of beta-adrenergic receptor ligand unbinding dynamics using synthetic photoswitches

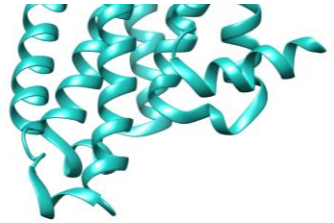
Robin Stipp, Quentin Bertrand, Yasushi Kondo, Hannah Glover, Fabienne Stierli, Steffen Brünle, Jörg Standfuss

Paul Scherrer Institut (PSI), Department of Biology and Chemistry, Laboratory of Biomolecular Research

Univeristy of Zurich (UZH): J. Ruf, P. J. Heckmeier, P. Hamm

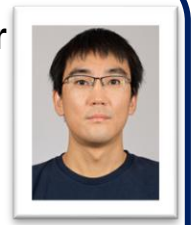
IQAC-CSIC: A. Duran-Corbera, X. Rovira, A. Llebaria

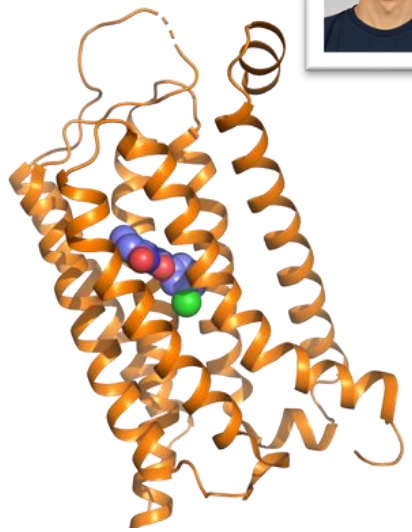
LeadXpro: M. Trabuco, R. Cheng, M. Hennig



- Protein production
- Photoswitch
- Crystal
- Time-resolved data

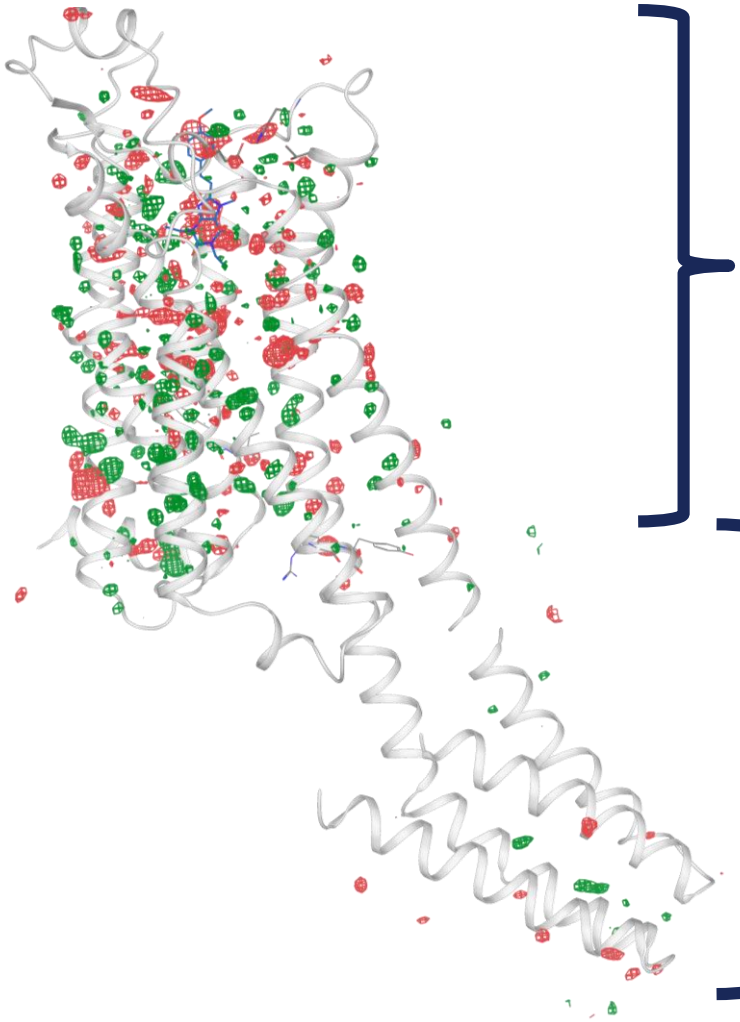
glutamate receptor mGlu5





- Protein production
- Photoswitch
- Crystal
- Time-resolved data

A2a time resolved data

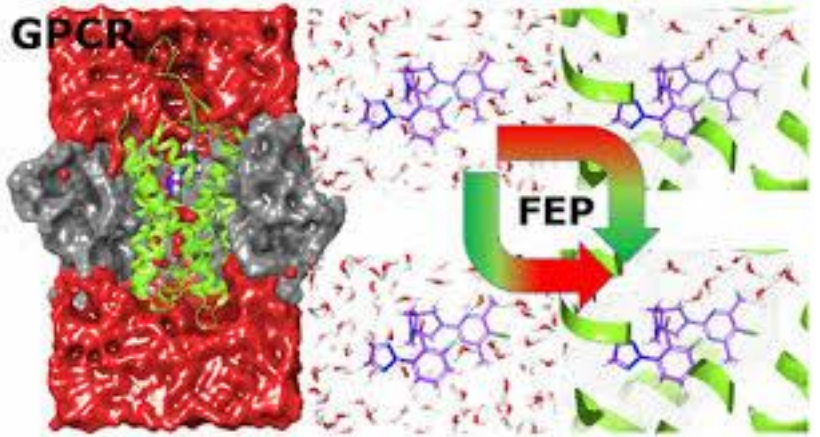


Receptor region with extensive changes in electron density suggesting major structural adaptation to the antagonist ligand escape form binding site

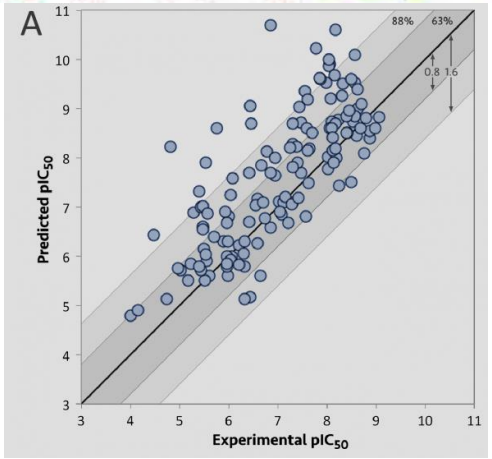
BRIL fusion region (introduced in the ICL3 loop to facilitate crystallisation) shows little or no positive or negative difference electron density suggesting no structural change

Impact of structure data of time resolved compound (un)binding to drug discovery

- **Understanding of protein mechanics and flexibility**
  - Understanding GPCR dynamics and mode of action
  - Analysis of ligand activity, selectivity & ligand binding kinetics
  - Investigation of induced fit ligand binding,
  - Ensemble of structures for in-silico screening approaches (apo, intermediate and ligand bond structures)



- **Enhance the impact of computational methods:**
  - Virtual Screening with information on structural flexibility of ligand binding site
  - New/Improved Quantitative Structure Activity Relationship (QSAR) models, FEP (free energy perturbation) calculation to calculate ligand affinities

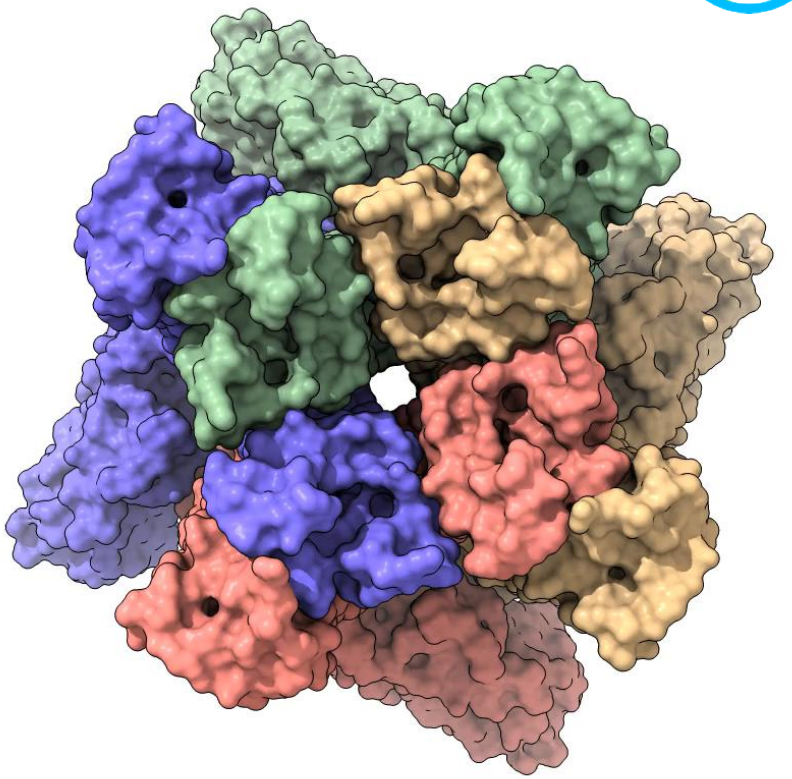


- **Development of photo-activated drug molecules (Amadeu Lleberia talk)**

leadXpro Vision:

«Visualize experimentally ligand binding induced molecular dynamics for the use in the design of novel medicines»

- **EM** for large conformational changes (ion channel, transporter activation states)



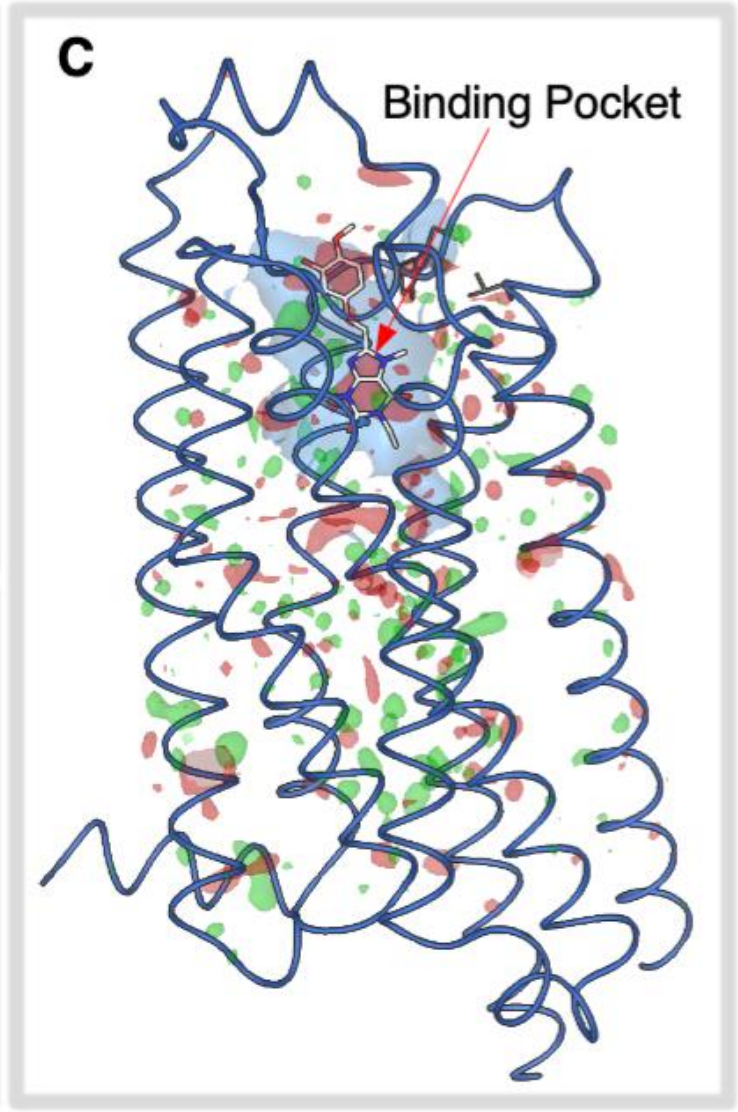
TRPV4
apo closed and agonist activated/open structure

https://doi.org/10.1101/2020.10.13.334797

leadXpro Vision:

«Visualize experimentally ligand binding induced molecular dynamics in the design of novel medicines»

- **EM** for large conformational changes (ion channel, transporter activation states)
- **Synchrotron** for up to μs, ms timescale
- **XFEL** for up to fs timescale to visualize small and fast structural changes (induced fit, water structure changes)



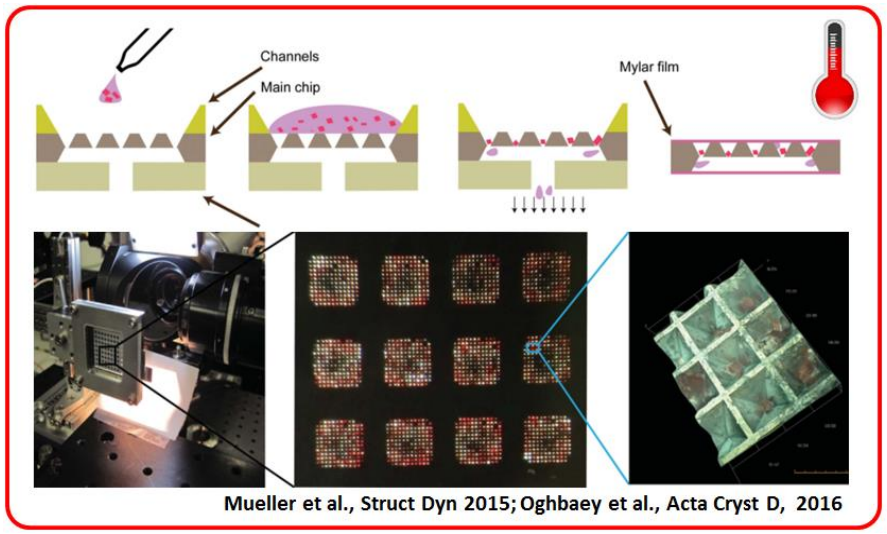
Areas to address for a bright future of time resolved X-ray

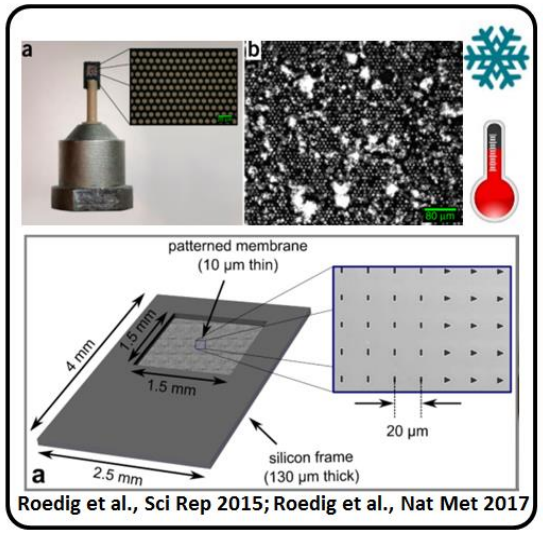
- **Sample delivery** systems to **reduce sample consumption**, efficiency and accuracy of data collection
- **Improved software**, experimental control, data management and analysis taking the variation of the properties of X-ray pulses (wavelength and flux density) into account
- Efficient design and synthesis of optimized **photo-switchable compounds** for time-resolved experiments (robust other methods to initiate structural changes)
- **Better accessibility** of XFEL facilities

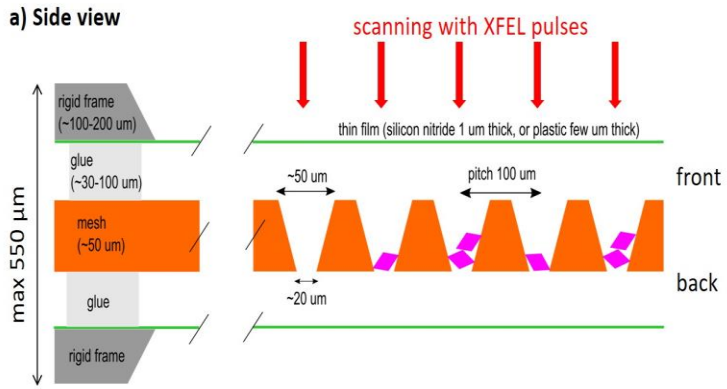
Backup

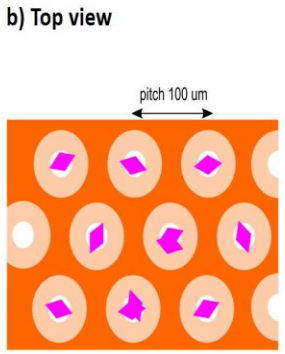
Serial Crystallography

Sample delivery by solid support









….we look forward to the use of SwissFEL-Cristallina end station dedicated to solid support measurements.