MIPT
MOSCOW INSTITUTE OF PHYSICS AND TECHNOLOGY

Research Center for MOLECULAR MECHANISMS of Aging and Age-Related Diseases

A CASE STUDY OF STRUCTURE-BASED DRUG DESIGN WITH CYSTEINYL LEUKOTRIENE G-PROTEIN COUPLED RECEPTORS

Dr. Alexey Mishin

Laboratory for structural biology of GPCRs

Moscow Institute of Physics and Technology

Research Center for MOLECULAR MECHANISMS of Aging and Age-Related Diseases

- 10 laboratories with >100 staff
- 9 advanced research platforms
- Educational chair of Biophysics: ~20 students/year
- 2 master programs

JÜLICH FORSCHUNGSZENTRUM

mpibp max-planck-institut für biophysik

University of Southern California 1880

HEINRICH HEINE UNIVERSITÄT DÜSSELDORF

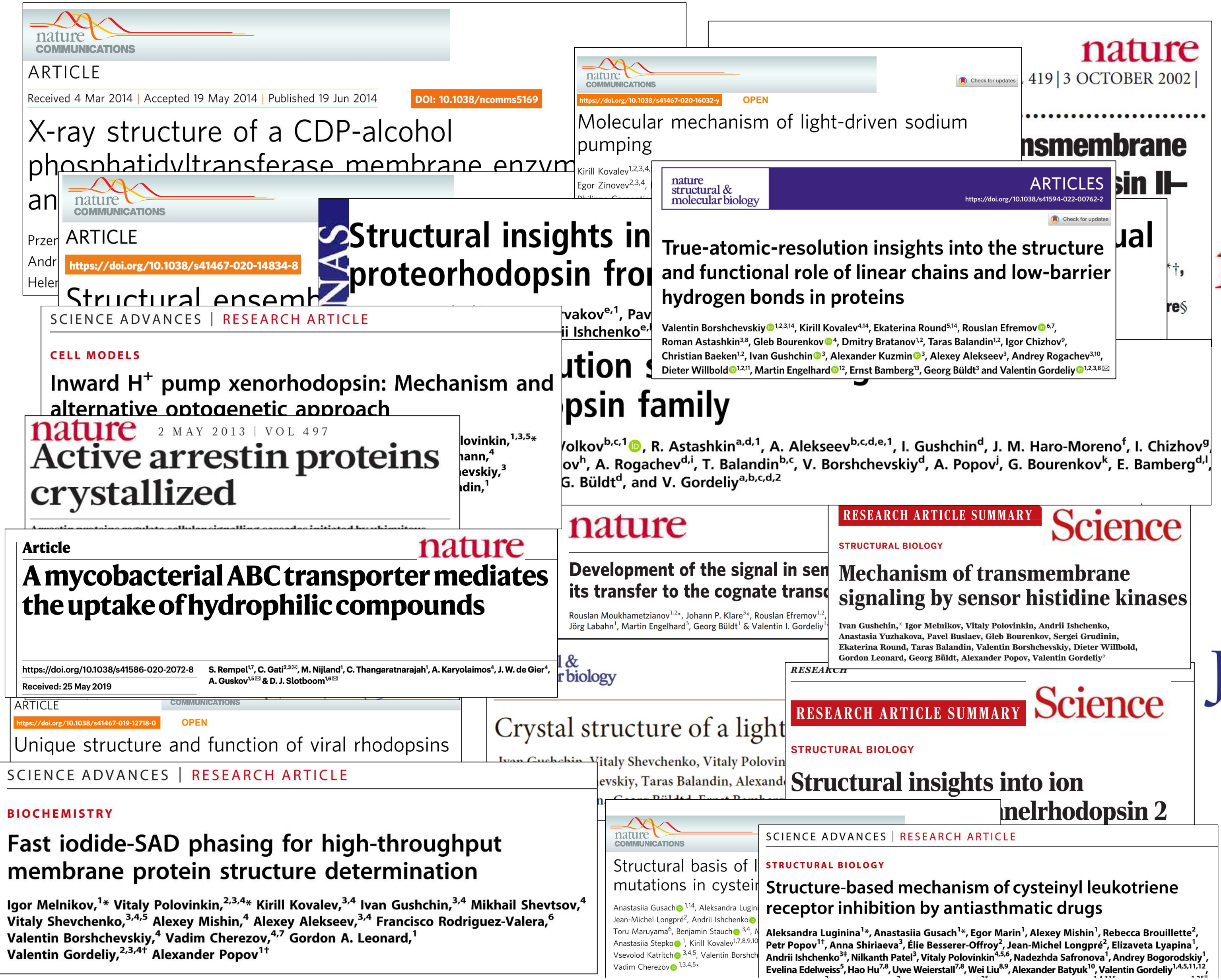
iHuman INSTITUTE

ibs

ESRF The European Synchrotron

KU LEUVEN

ИБХ

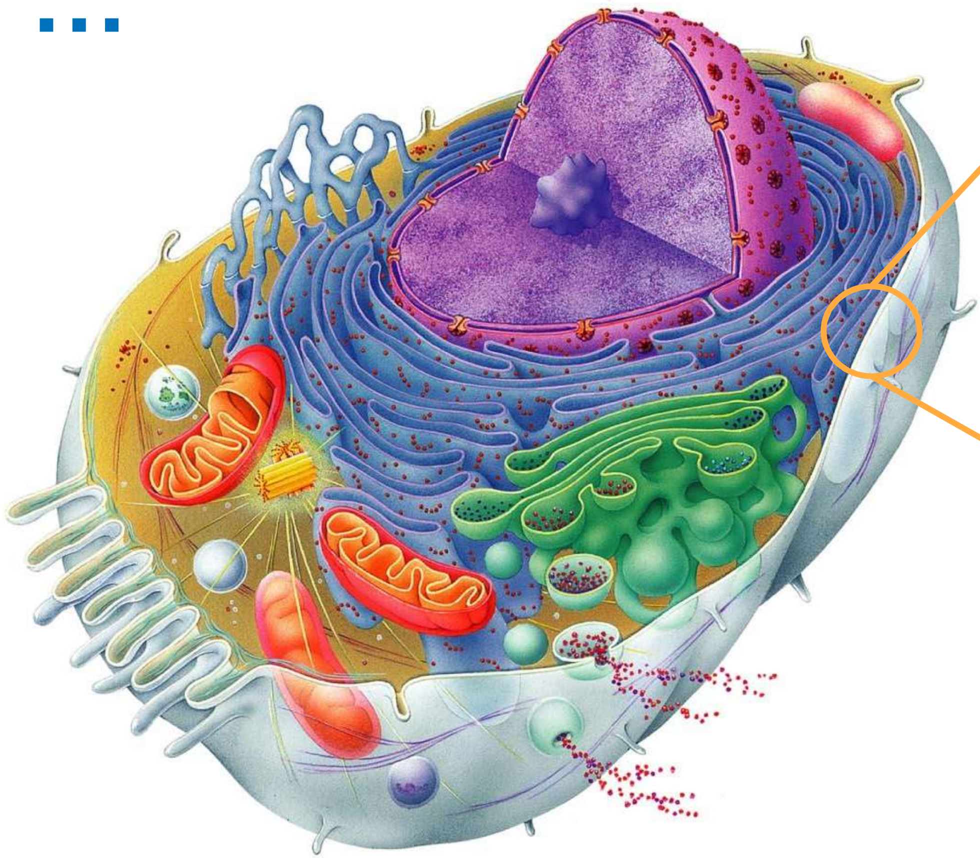


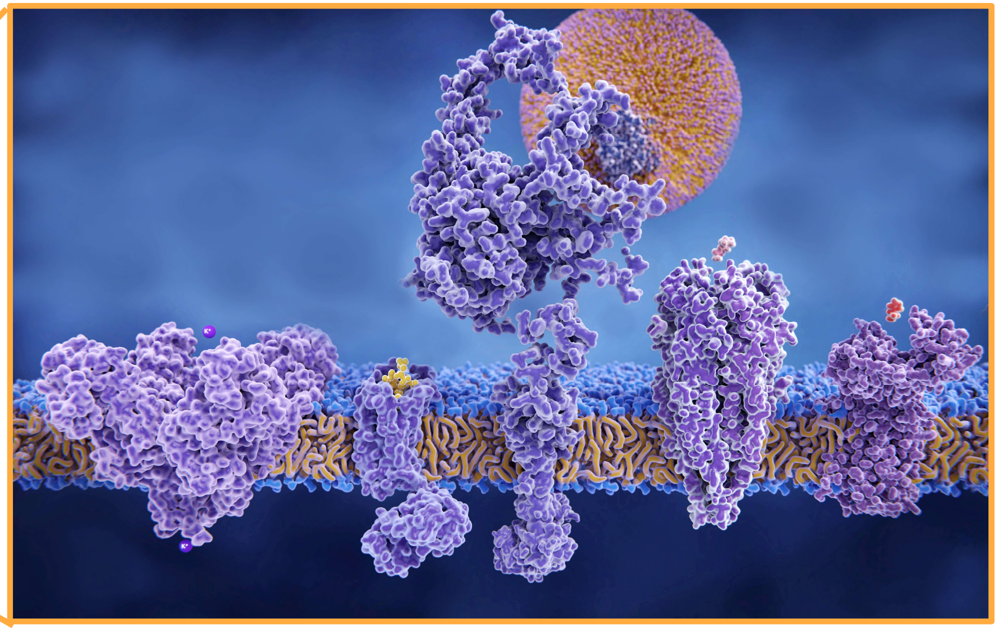
> 350 publications since 2011

- Nature – 2013, 2020
- Science – 2017, 2017
- PNAS – 2020×2, 2017, 2013, 2011
- Nature Communications –2022, 2020×2, 2019×2, 2014
- SciAdv – 2022, 2020, 2019×2, 2017×2, 2016
- JACS – 2021×2 , 2016, 2013
- Angewandte Chemie – 2021×2
- Nature Chemical Biology – 2022, 2015
- Nature Structural & Molecular Biology – 2023, 2022, 2015
- Chemical Reviews – 2021, 2015

Membrane proteins

…



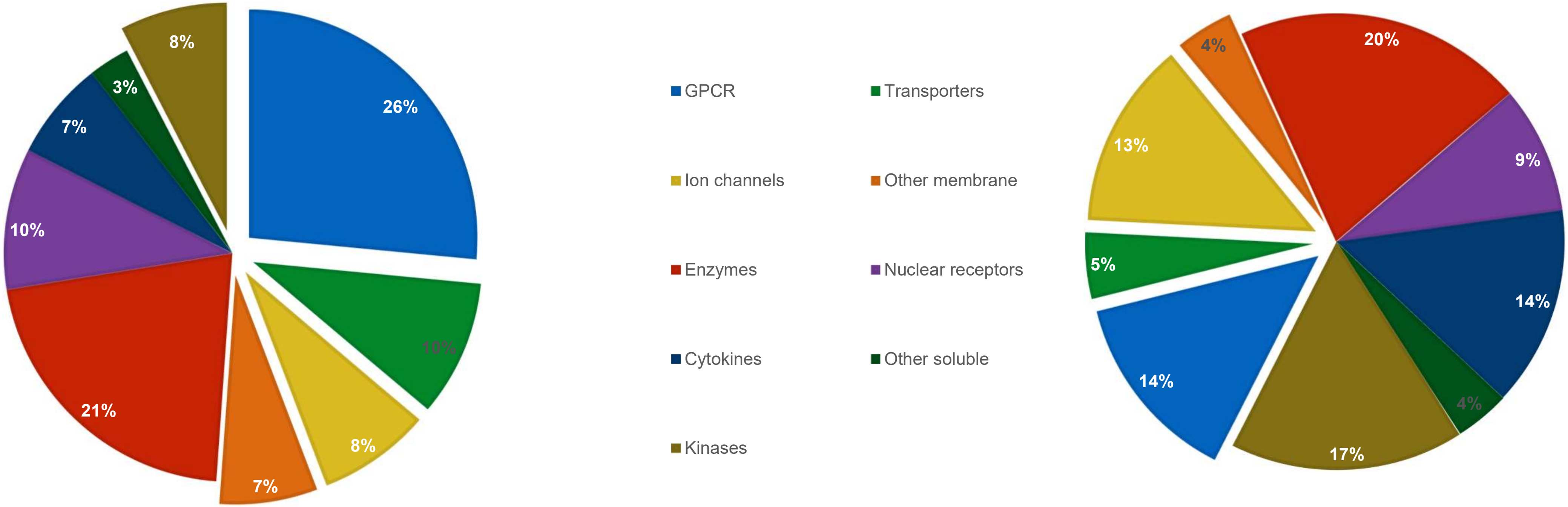


are major functional elements of cells

Global sales and research support

$3.5 tril. 2011-2015 according IQVIA

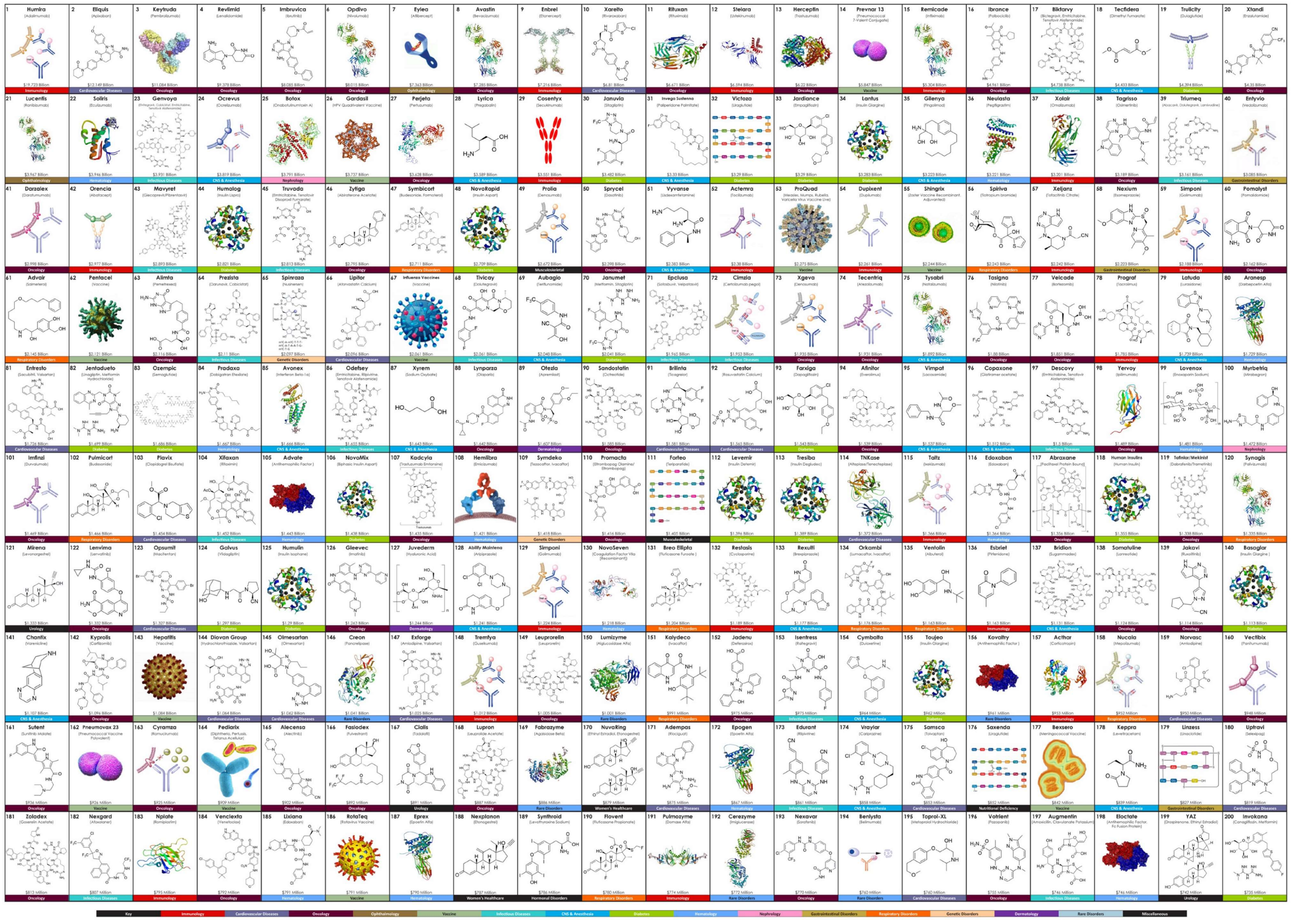
$4.2 bln. 2011-2015 гг. according NIH



Unexplored therapeutic opportunities in the human genome, (2018) Nat. Rev. Drug Discov.

Top 200 Pharmaceuticals by Retail Sales in 2019

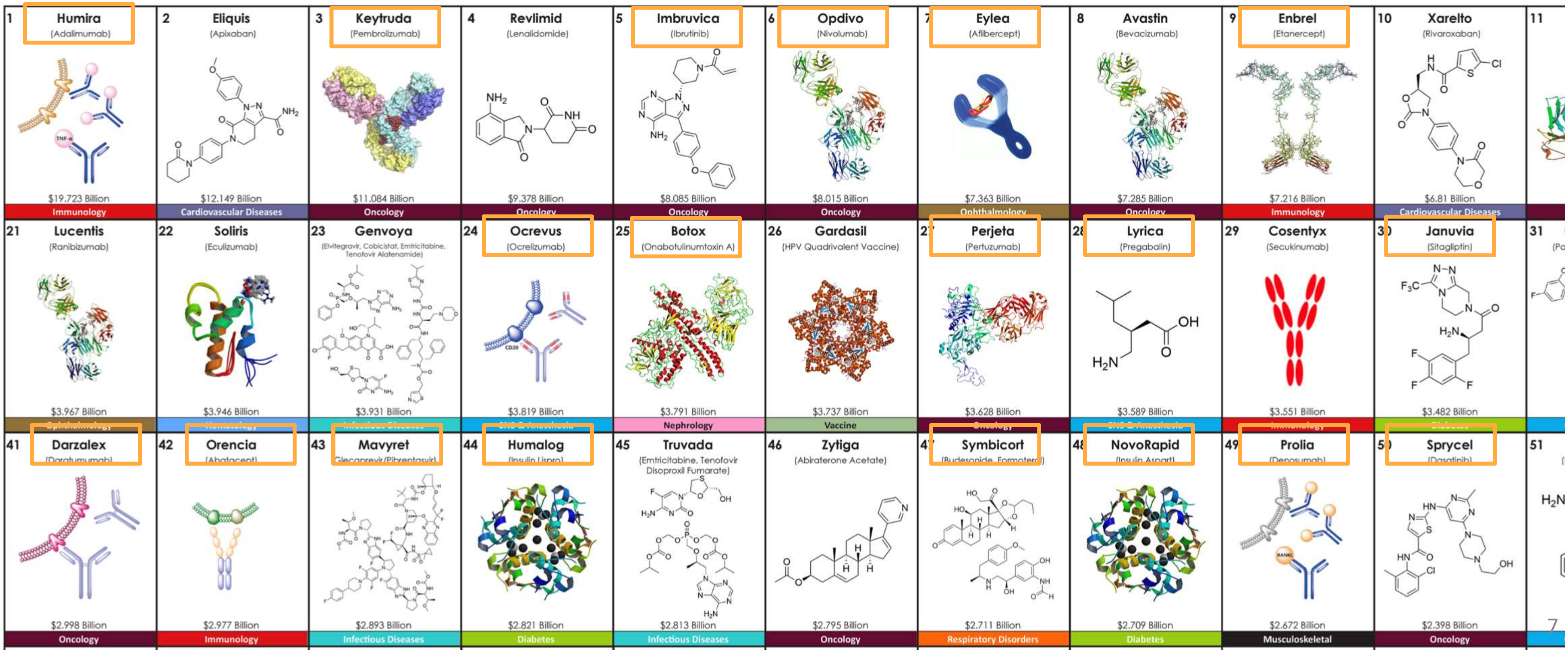
Compiled and Produced by the Njarðarson Group (The University of Arizona)



Top 200 Pharmaceuticals

Compiled and Produced by the Njarðarso

- targeted to membrane proteins



GPCR signalling: diversity of ligands

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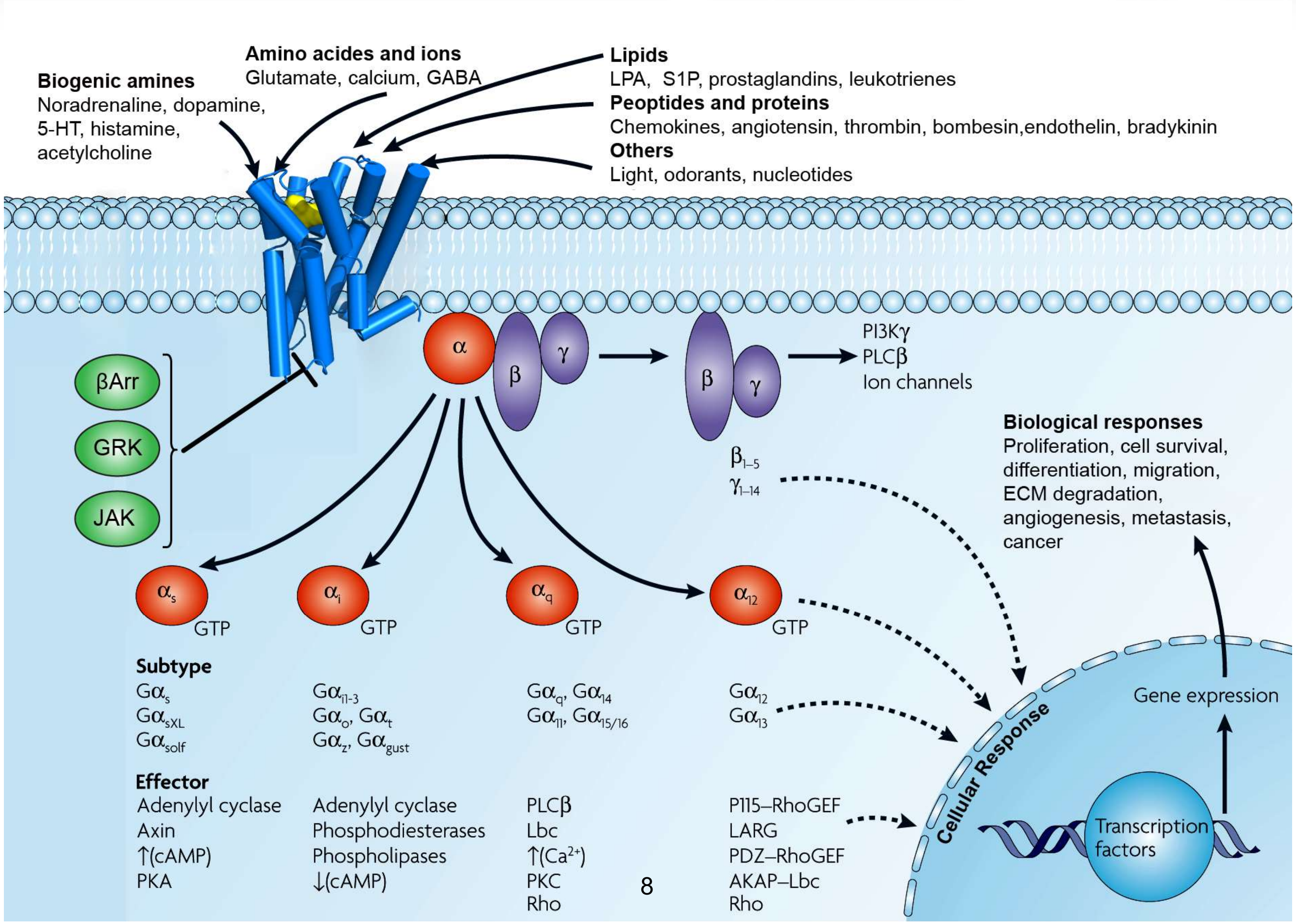
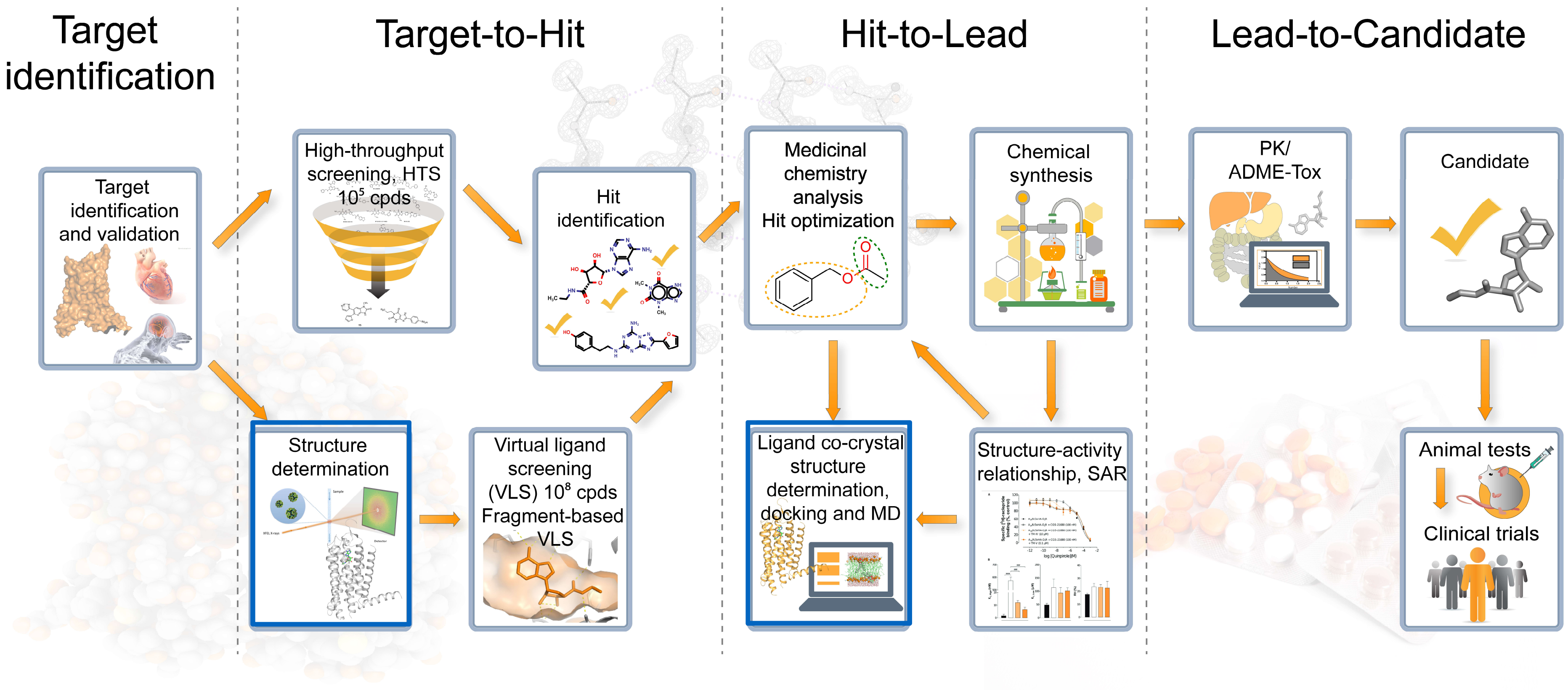


Drug discovery pipeline



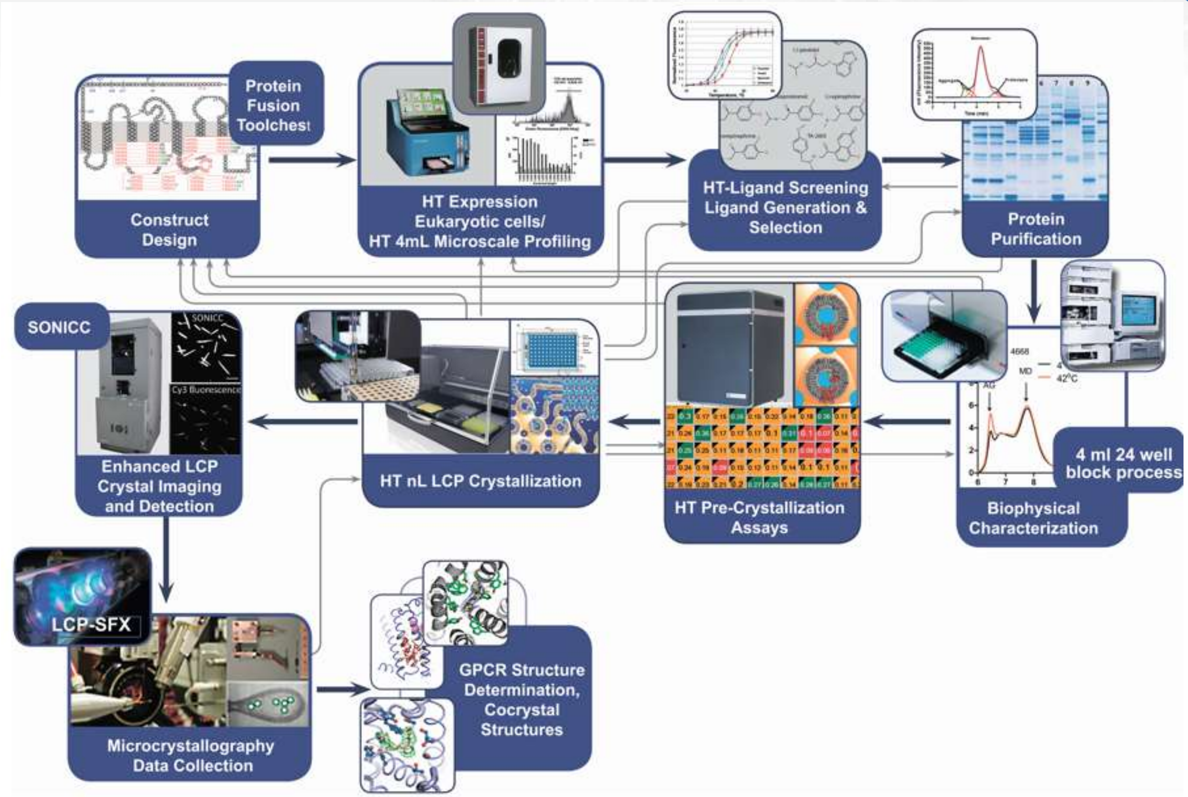
- 10-15 years
- >$2 bln.

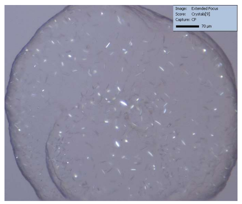
Blue frames shows steps that makes drug development cheaper and faster by 30-50%

Mishin et al. (2019) Expert Opinion on Drug Discovery

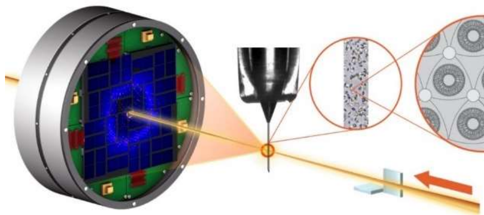
Structure Determination Pipeline

Research Center for MOLECULAR MECHANISMS of Aging and Age-Related Diseases

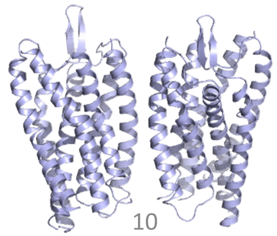




Protein crystals of GPCR related to inflammation

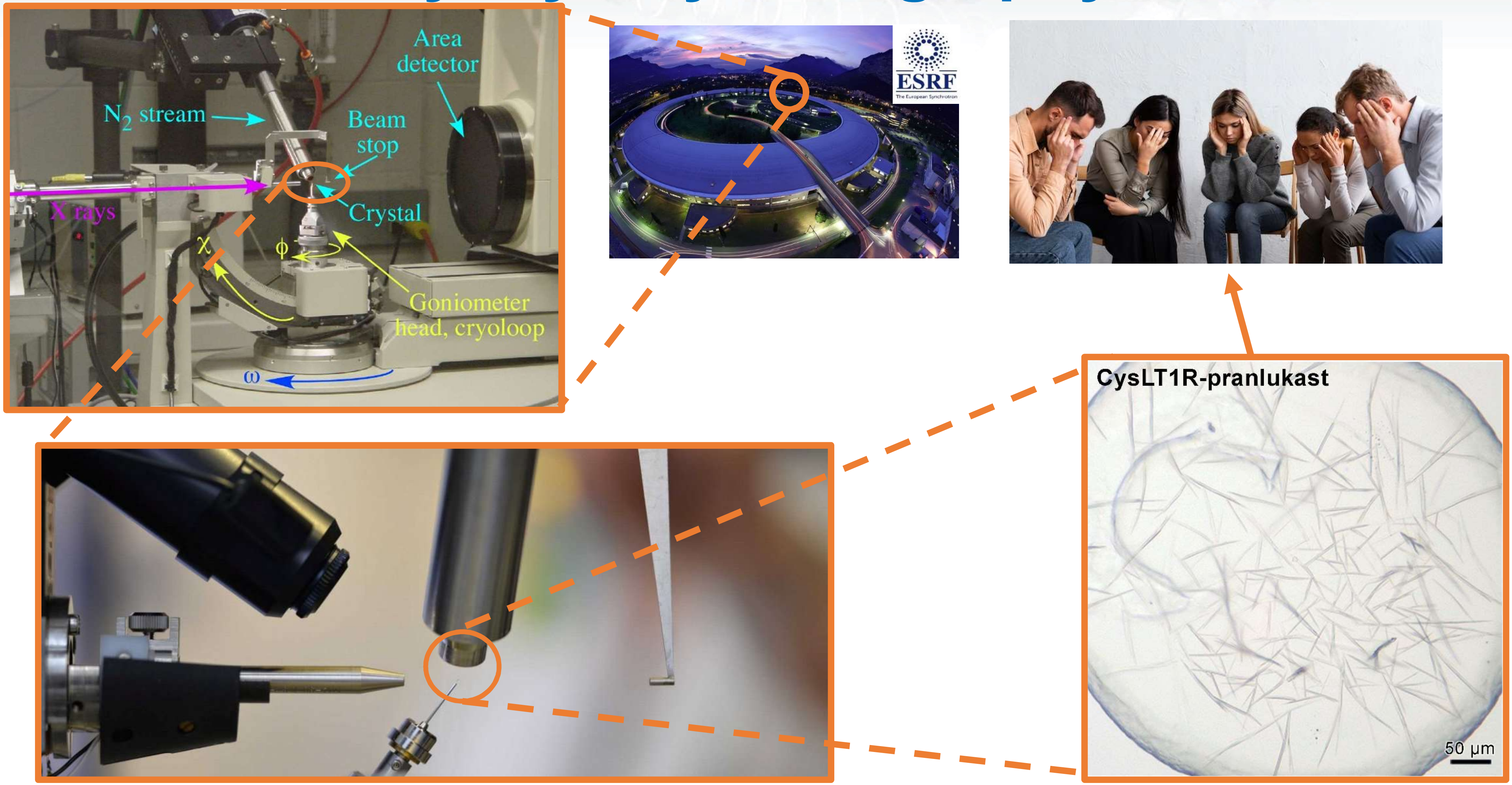


Studies at X-ray free electron laser

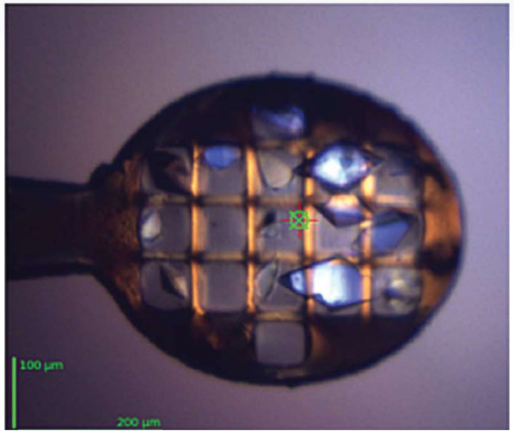


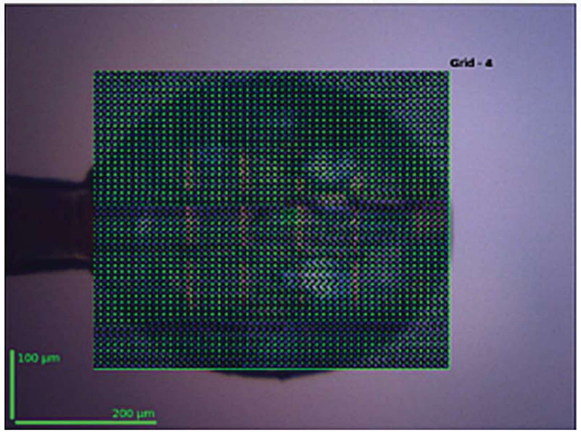
Structure of GPCR

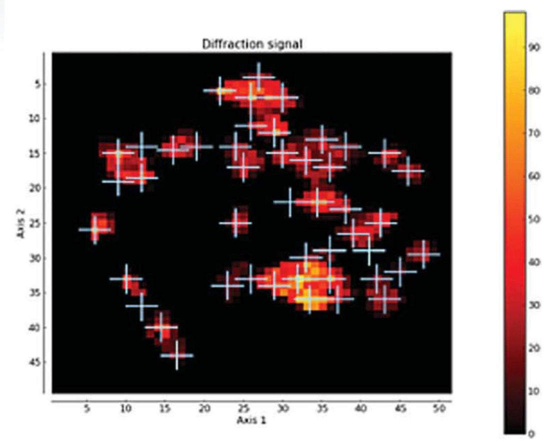
Classical X-ray cryocrystallography

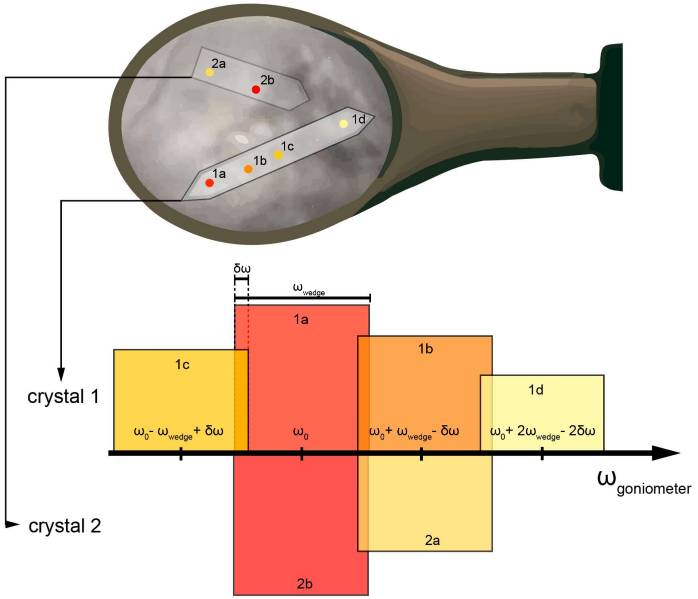


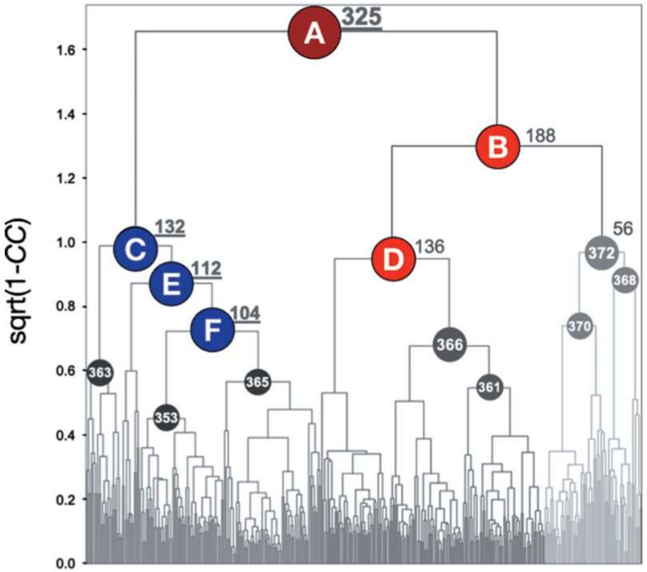
Small-wedge crystallography

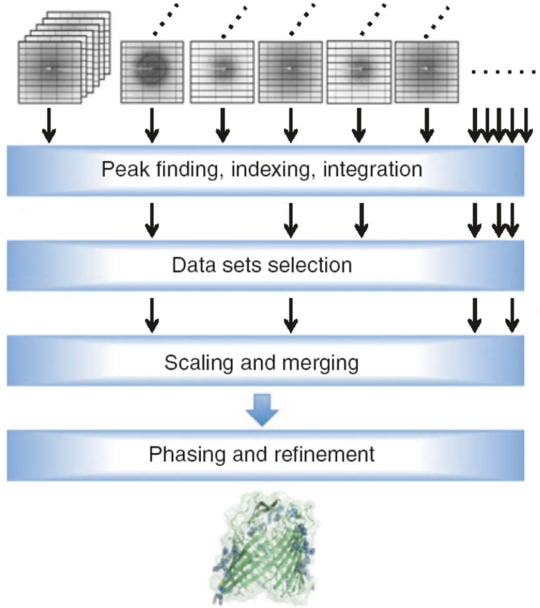




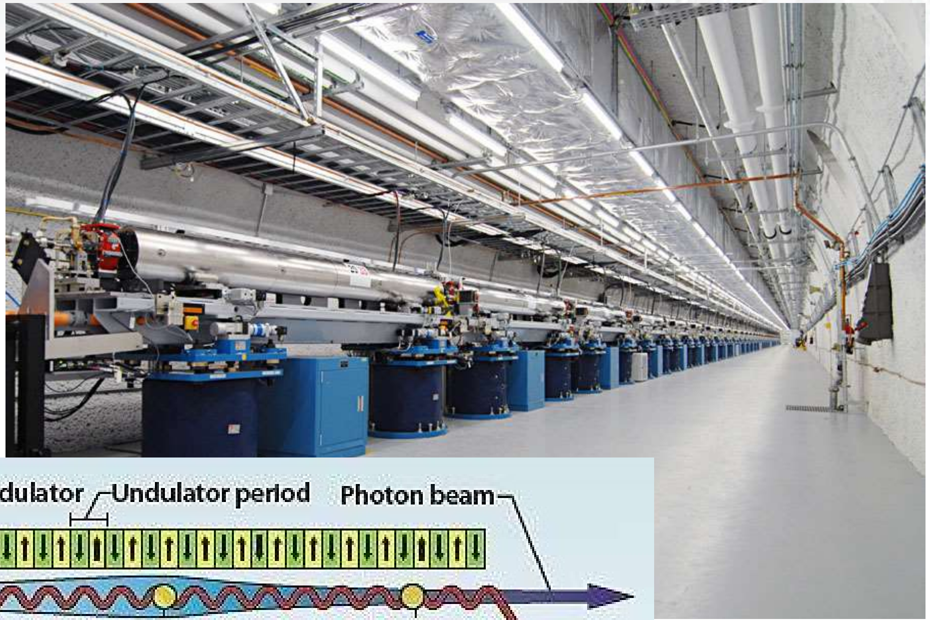


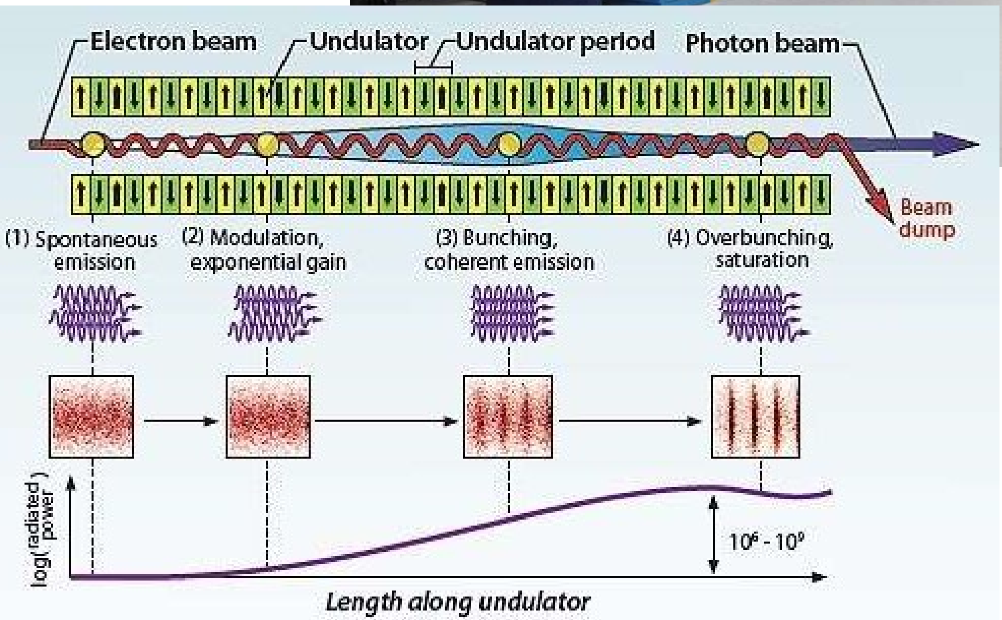




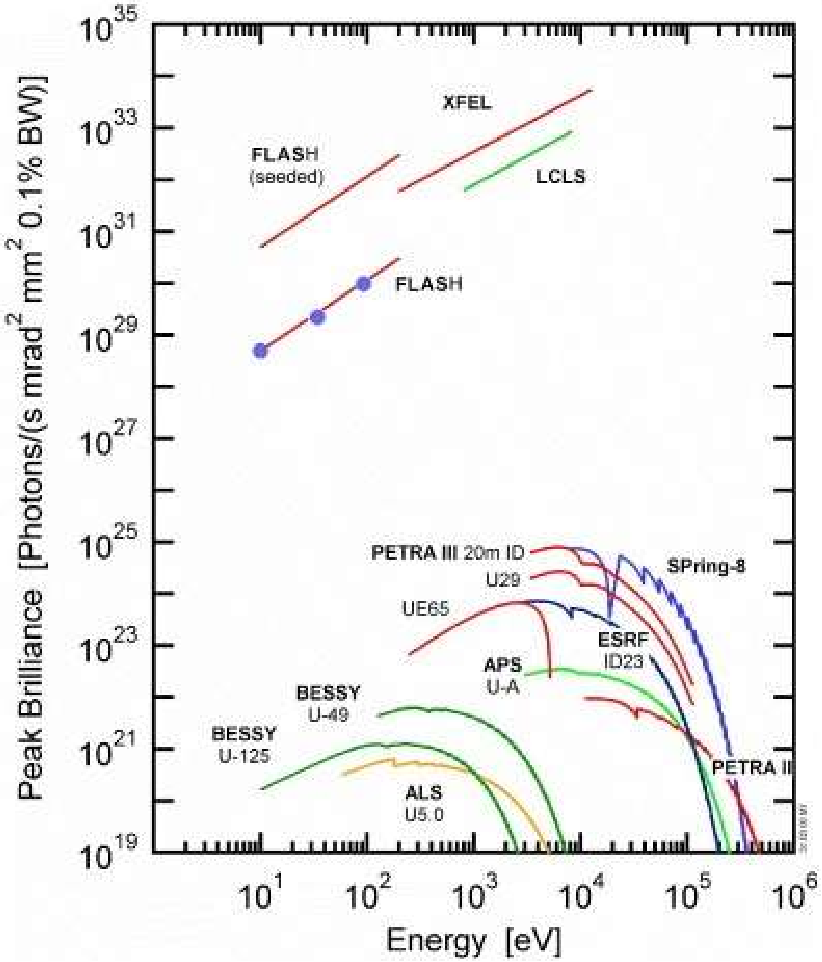


X-ray Free Electron Laser (XFEL)





~N ~N^2



Ultrabright and **ultrashort** pulses of **coherent** X-rays

Serial Femtosecond Crystallography: Diffraction before destruction

Potential for biomolecular imaging with femtosecond X-ray pulses

Richard Neutze*, Remco Wouts*, David van der Spoel*, Edgar Weckert†‡ & Janos Hajdu*

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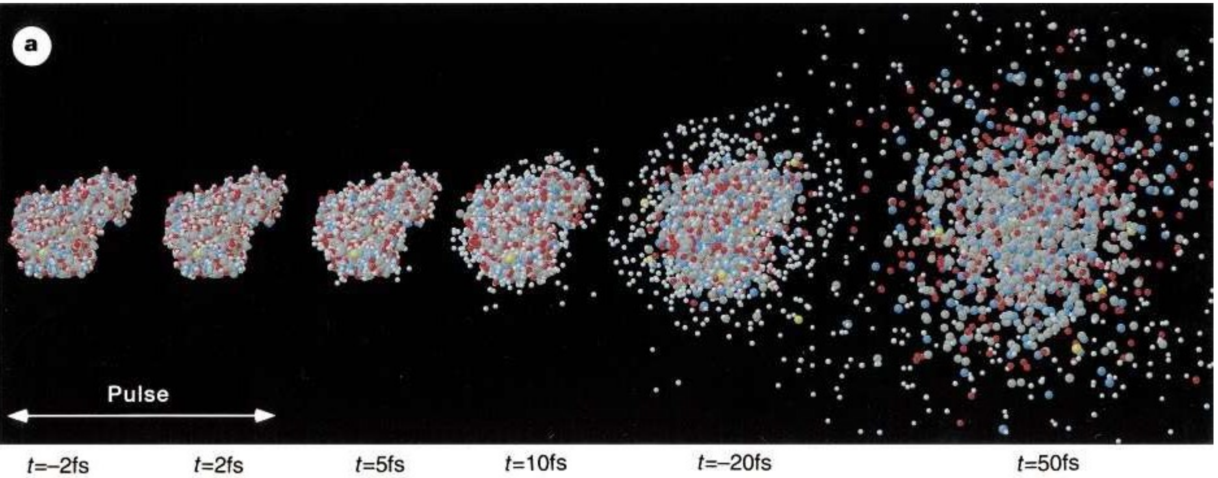
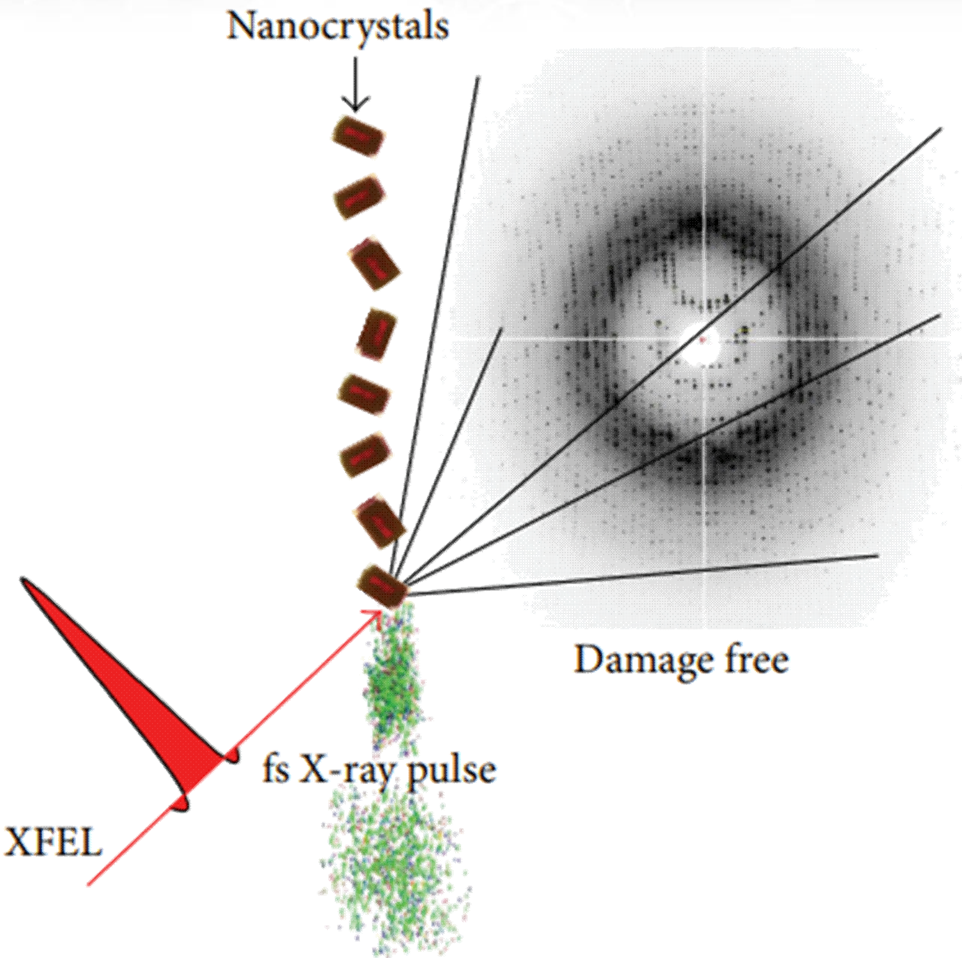
† Institut für Kristallographie, Universität Karlsruhe, Kaiserstrasse 12, D-76128 , Germany

NATURE | VOL 406 | 17 AUGUST 2000





Benefits of serial femtosecond crystallography at XFELs


Expert Opinion on Drug Discovery >
Volume 14, 2019 - Issue 9
Enter keywords, authors, D
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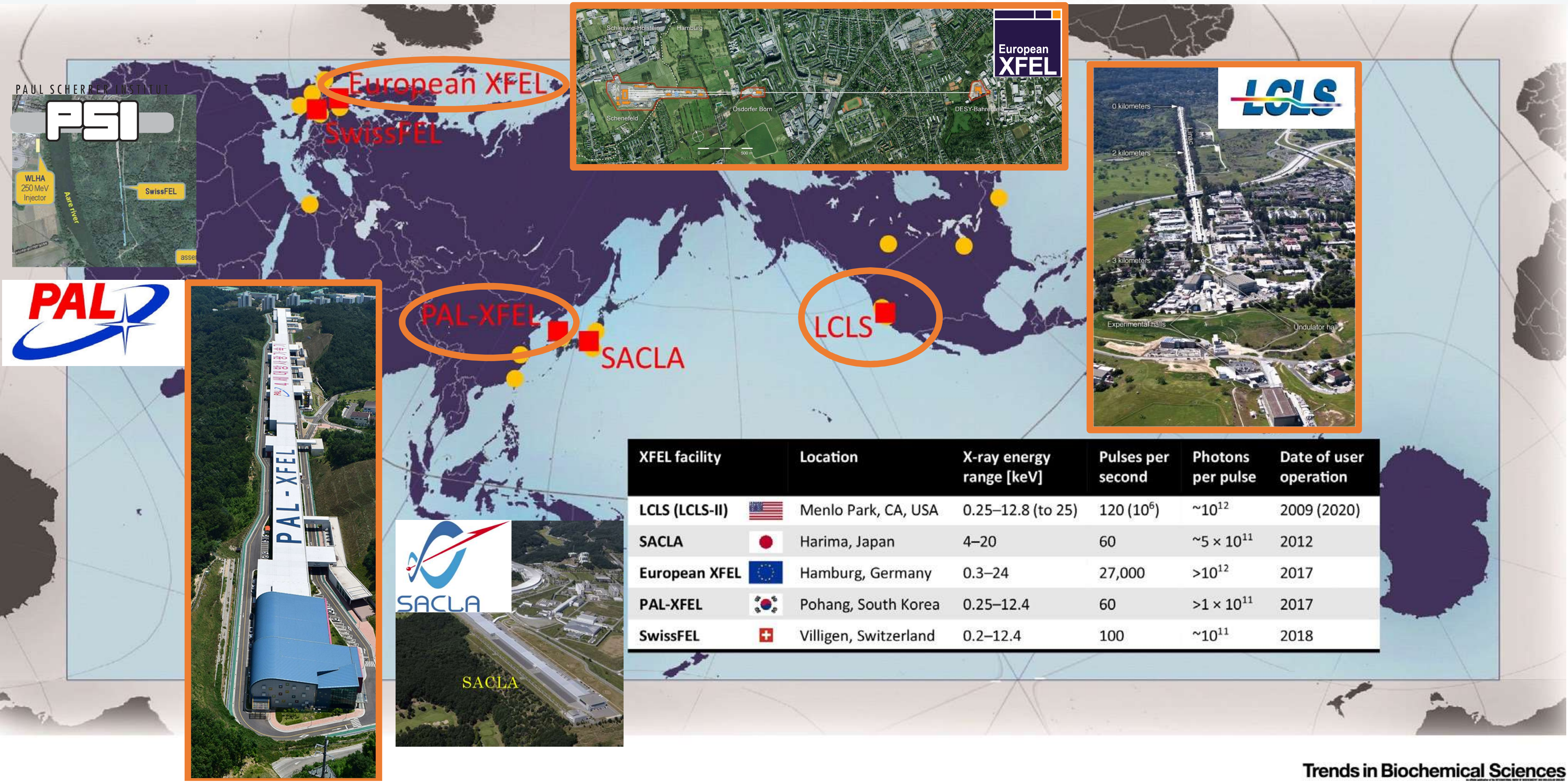
Journal homepage
Review
An outlook on using serial femtosecond crystallography in drug discovery
Alexey Mishin, Anastasiia Gusach, Aleksandra Luginina, Egor Marin, Valentin Borshchevskiy & Vadim Cherezov
Pages 933-945 | Received 26 Feb 2019, Accepted 30 May 2019, Published online: 11 Jun 2019
Download citation
https://doi.org/10.1080/17460441.2019.1626822
Check for updates

- The ability to work with challenging targets that have low expression and are difficult to crystallize.
- Small crystal size often translates into faster crystal optimization, lower mosaicity, and better diffraction quality, and can facilitate ligand soaking and exchange
- SFX obviates laborious crystal harvesting and necessity for cryo-protection, lending itself suitable for automation
- Room temperature: better water molecules distribution

Hard XFELs around the globe



XFEL facility	Location	X-ray energy range [keV]	Pulses per second	Photons per pulse	Date of user operation
LCLS (LCLS-II)	Menlo Park, CA, USA	0.25–12.8 (to 25)	120 (10^6)	~10^{12}	2009 (2020)
SACLA	Harima, Japan	4–20	60	~5×10^{11}	2012
European XFEL	Hamburg, Germany	0.3–24	27,000	>10^{12}	2017
PAL-XFEL	Pohang, South Korea	0.25–12.4	60	>1×10^{11}	2017
SwissFEL	Villigen, Switzerland	0.2–12.4	100	~10^{11}	2018

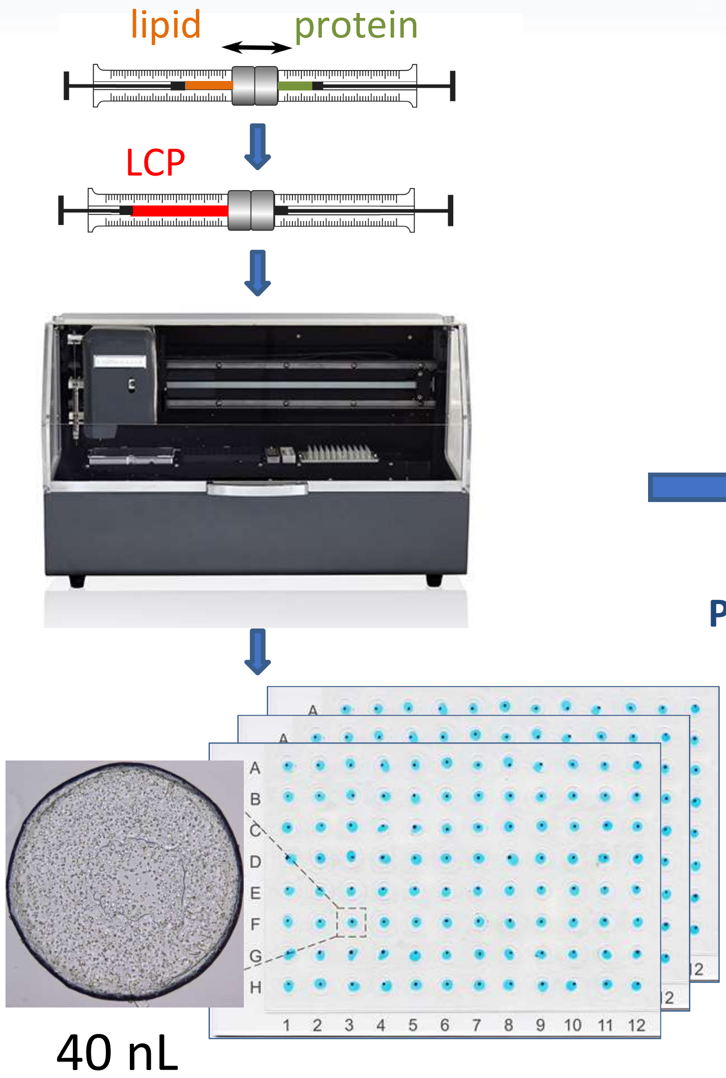
Trends in Biochemical Sciences

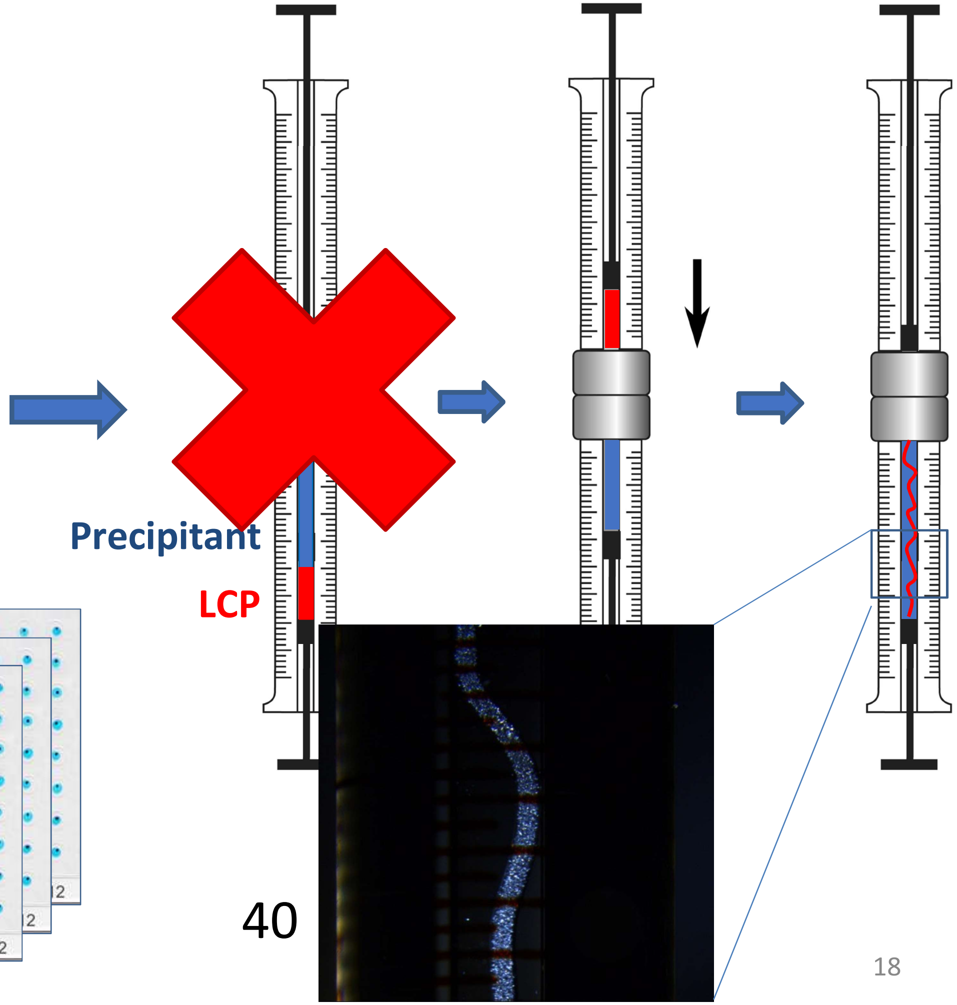
Experiments at PAL-XFEL

Lasing	
Wavelength range	2-20.4 keV (6.2-1.0 A)
Pulse length	45-60 fs
Repetition rate	1-60 Hz (30 Hz detector limited during beamtime)
Flux	3e11 at 9.7 keV (during beamtime)
Spot size	H:V 2x3 mm (FWHM), KB mirrors
Detector	MX225-HS (Rayonix)
Readout rate & binning	40 frames/second, 1440x1440; 75 frames/second, 960x960
Geometry	Single panel
Sample environment	LCP-jet/GDVN: He environment; Fixed target: vacuum
Processing	32 cores during beamtime, cluster after beamtime

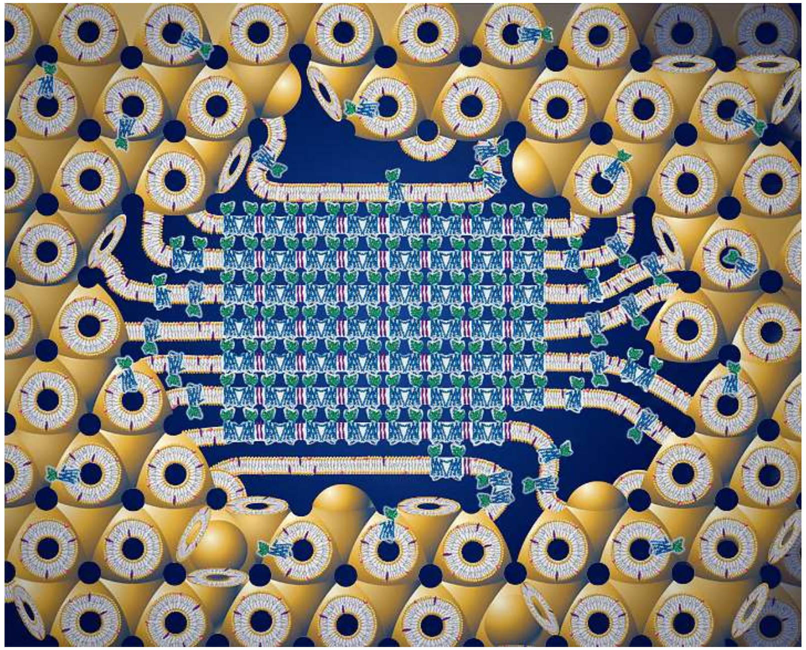
Sample preparation for LCP-SFX

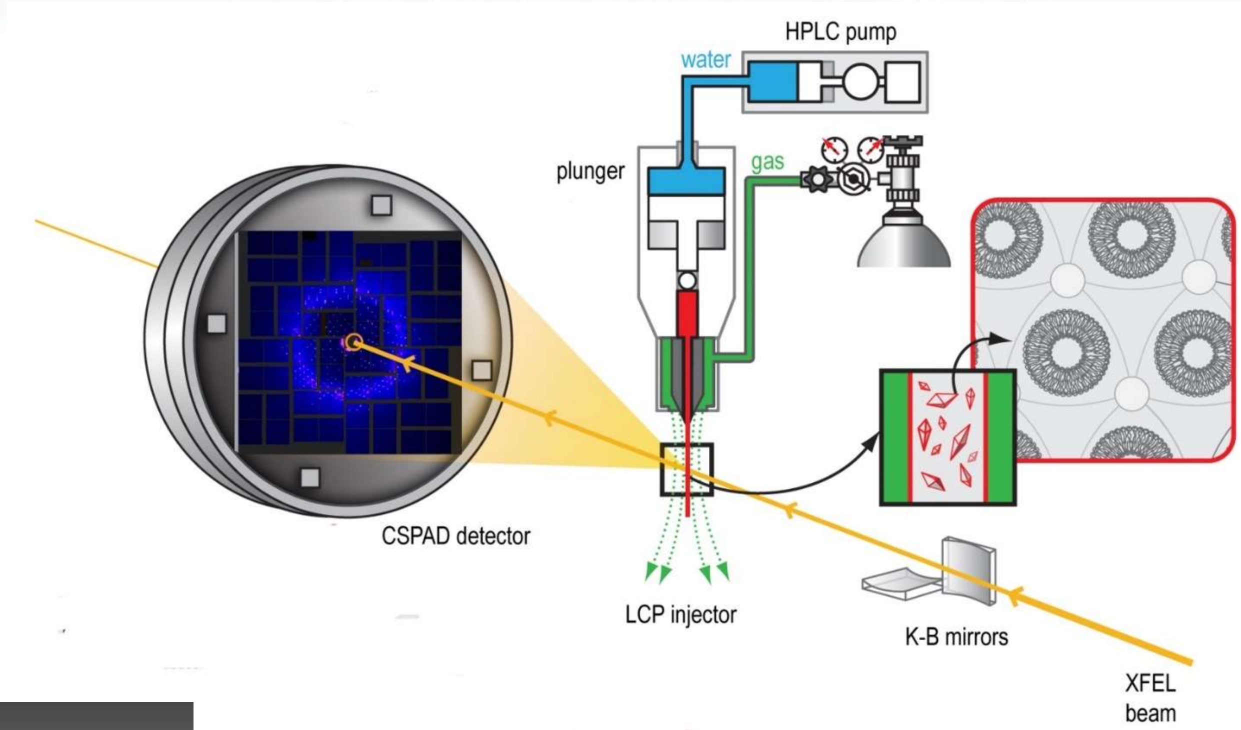
Liu et al. **2014.** *Nat Protoc* 9, 2123
Ishchenko et al. **2016.** *J Vis Exp 115*, e54463

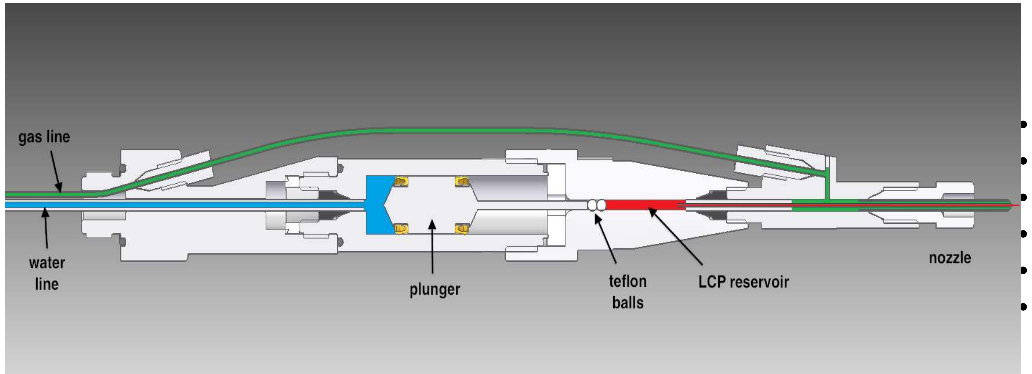




Crystal delivery: Lipidic cubic phase injection







- LCP as crystallization and crystal delivery medium
- Very small crystals (<10 µm)
- Room temperature structures
- No radiation damage
- No crystal harvesting
- Low protein consumption (<0.3 mg)

SFX Data Processing

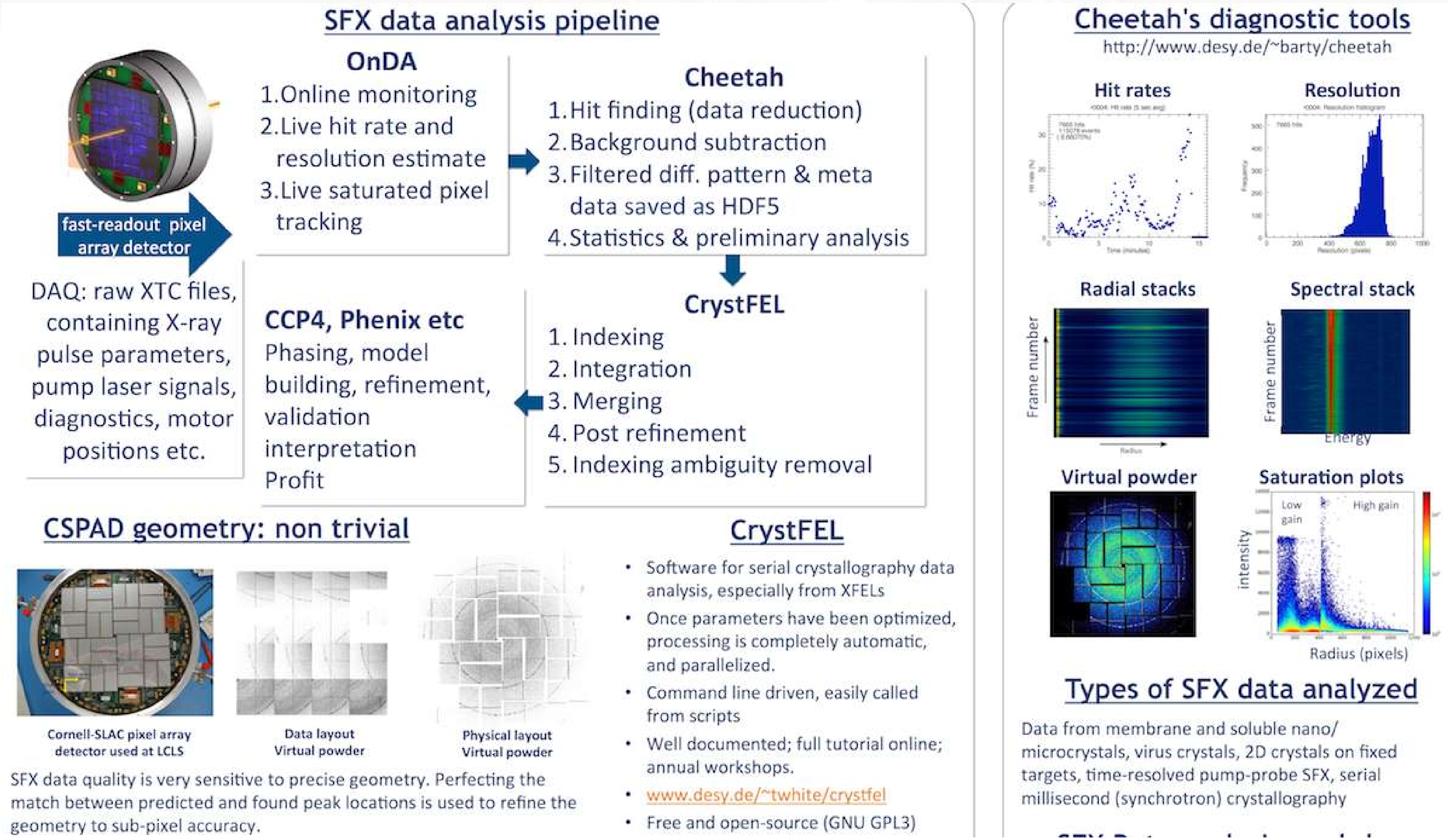
Large amount of data:
- LCLS – 2 TB/hour
- euXFEL – 400 TB/hour

Modular detector geometry

Partial reflections

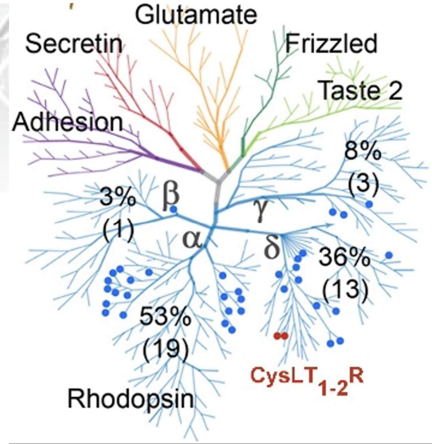
Variations in
- crystal size,
- mosaicity,
- quality,
- pulse intensity,
- spectrum, etc.

Indexing ambiguity



Cysteinyl leukotriene GPCR
– essential targets for asthma treatment

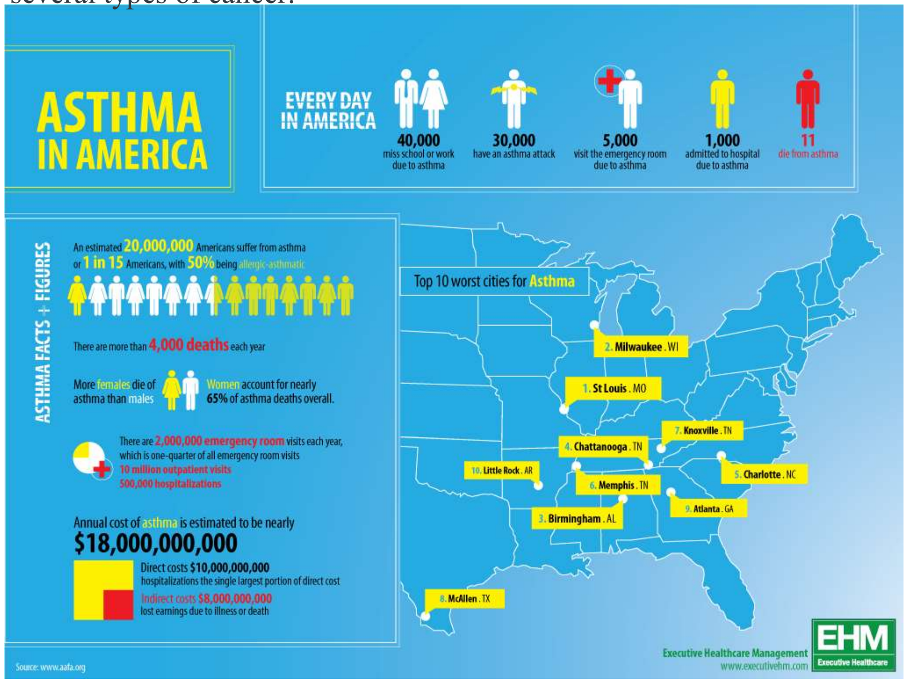
CysLT1R and CysLT2R are G Protein Coupled receptors (GPCRs) activated by cysteinyl leukotrienes. They are key inflammatory mediators in the human body and stimulate bronchial muscle constriction as well as immune cells migration, mucus secretion and edema formation, thus playing an important role in various inflammation-related disorders, such as asthma, allergic rhinitis and urticaria. Additionally through the immune response mediation CysLT1-2Rs are involved in cardiovascular and neurodegenerative diseases and several types of cancer.

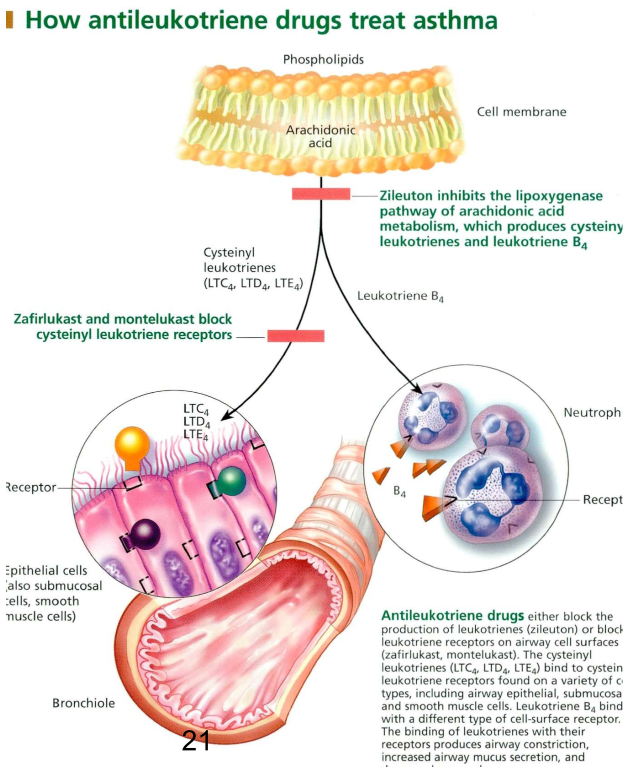


In Russia up to 5—7 %

of people suffer from asthma

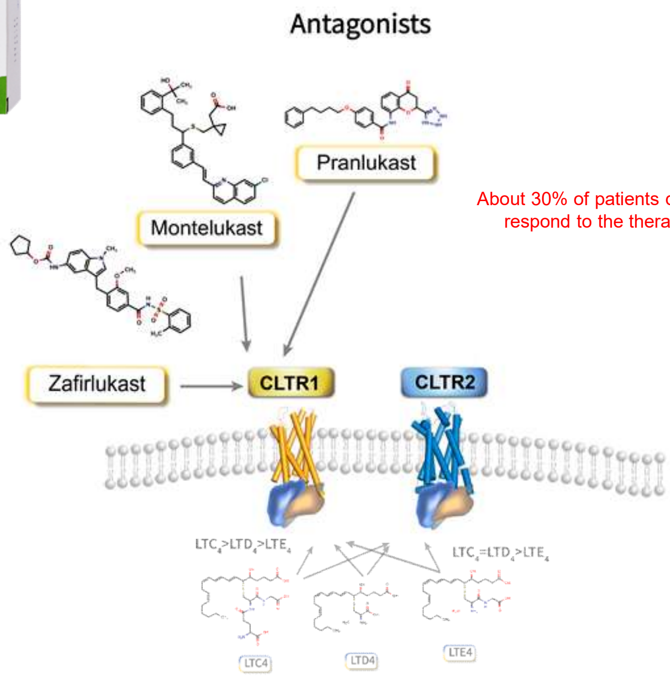
10% of children





Cysteinyl Leukotriene Receptors 1 and 2

CysLT1R antagonists: Anti-asthma oral treatment



About 30% of patients do not respond to the therapy

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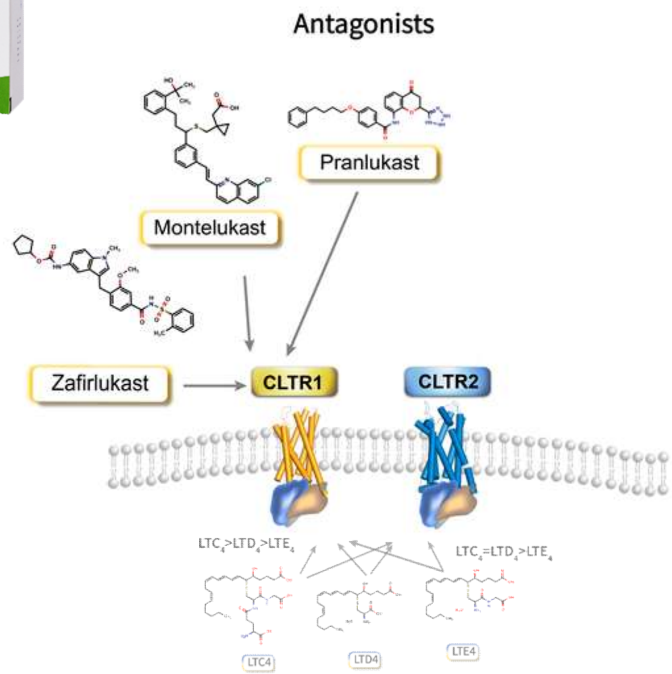
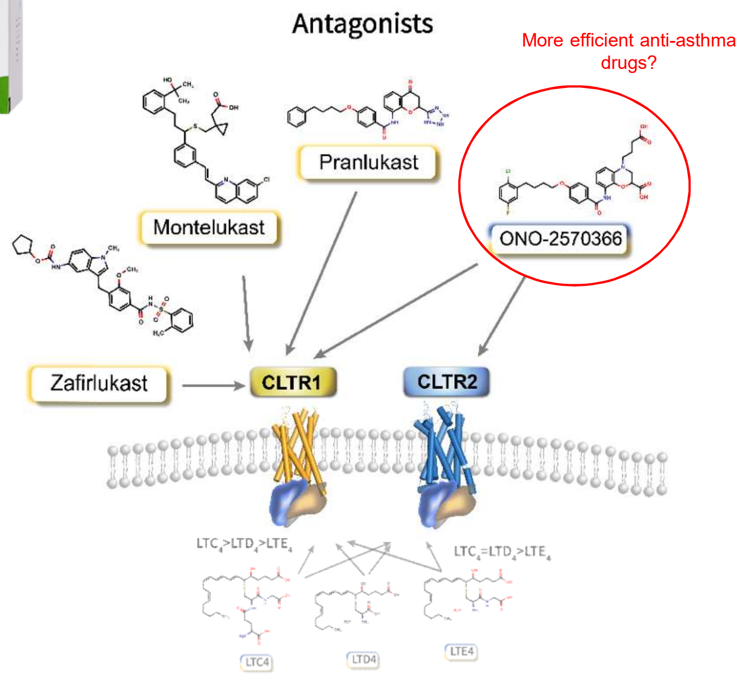
Cysteinyl Leukotriene Receptors 1 and 2

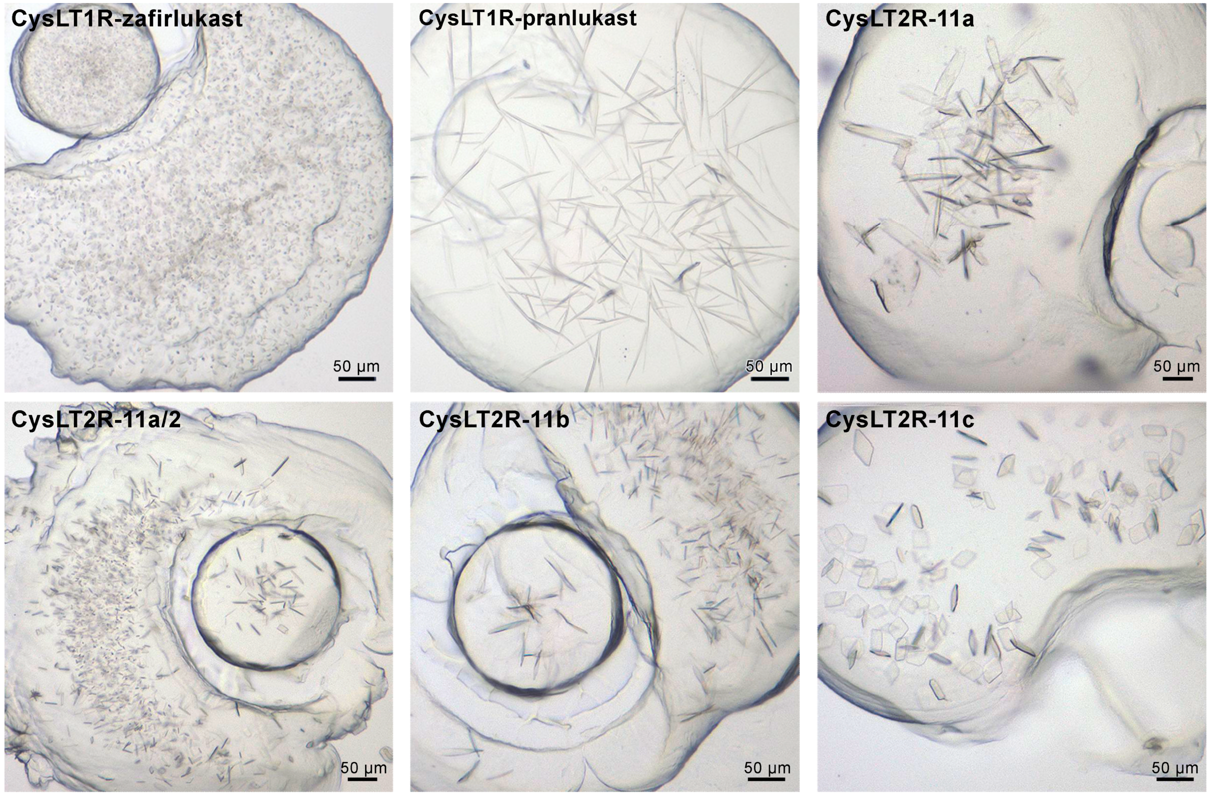
CysLT1R antagonists: Anti-asthma oral treatment



Cysteinyl Leukotriene Receptors 1 and 2

CysLT1R antagonists: Anti-asthma oral treatment




CysLT1R-zafirlukast
50 μm
CysLT1R-pranlukast
50 μm
CysLT2R-11a
50 μm
CysLT2R-11a/2
50 μm
CysLT2R-11b
50 μm
CysLT2R-11c
50 μm

Structural studies of cysteinyl leukotriene receptors as drug targets

Obtained structures for rational design

CysLT1 receptor stuctures: complexes with antiasthmatic drugs. Distinct features for the delta-branch receptors from class A GPCRs

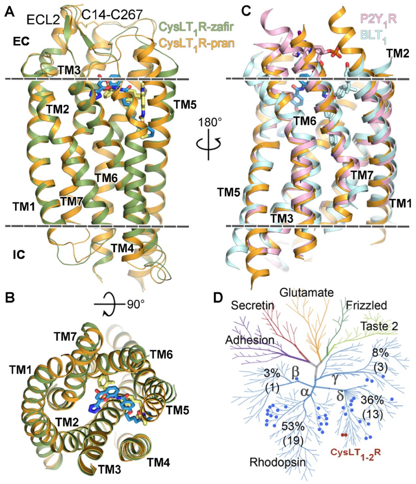


Fig. 1. Overall Structure of CysLT1R and its comparison with other receptors.

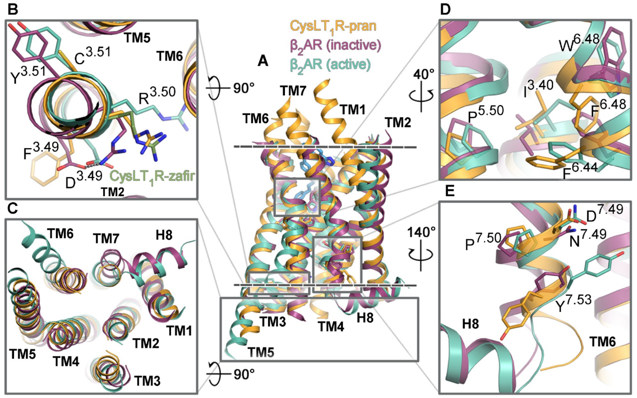


Fig. 2. Functional motifs of CysLT1R show unusual inactive state features. (A) Superposition of CysLT1R-pran (orange) with β2AR in inactive (PDB ID 2RH1, violet) and active (PDB ID 3SN6, teal) conformations. (B-E) Zoom in on functional elements: DRY motif (B), intracellular region (C), P-I-F motif (D), NPxxY motif (E).

CysLT2 receptor stuctures: Selectivity rationale for receptor subtypes and desease-related mutations mapping

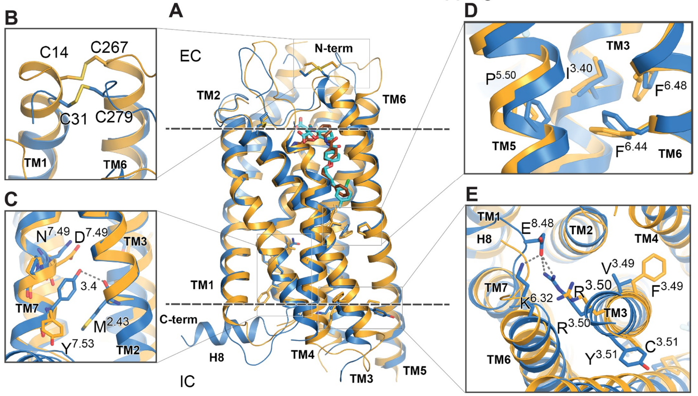


Fig. 3. Structure of CysLT2R. (A) Structural superposition of CysLT2R-11a (blue) with CysLT1R-pranlukast (yellow). (B) Comparison of disulfide bridges between CysLT1R (yellow) and CysLT2R (blue). (C-E) Comparison of functional motifs: NPxxY (d) P-I-F (e) and DRY (f).

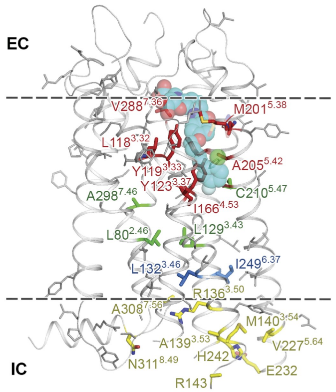


Fig. 4. SNVs from the ExAC database and L1293.43, colored according to their location: ligand-binding pocket (red), microswitches (blue), sodium site (green), G= protein and β-arrestin-binding interface (yellow).

SCIENCE ADVANCES | RESEARCH ARTICLE

STRUCTURAL BIOLOGY

Structure-based mechanism of cysteinyl leukotriene receptor inhibition by antiasthmatic drugs

Aleksandra Luginina[1]*, Anastasiia Gusach[1]*, Egor Marin[1], Alexey Mishin[1], Rebecca Brouillette[2], Petr Popov[1†], Anna Shiriaeva[3], Élie Besserer-Offroy[2], Jean-Michel Longpré[2], Elizaveta Lyapina[1], Andrii Ishchenko[3‡], Nilkanth Patel[3], Vitaly Polovinkin[4,5,6], Nadezhda Safronova[1], Andrey Bogorodskiy[1], Evelina Edelweiss[5], Hao Hu[7,8], Uwe Weierstall[7,8], Wei Liu[8,9], Alexander Batyuk[10], Valentin Gordeliy[1,4,5,11,12], Gye Won Han[3], Philippe Sarret[2], Vsevolod Katritch[3§], Valentin Borshchevskiy[1,4,11§], Vadim Cherezov[1,3§||]

nature COMMUNICATIONS

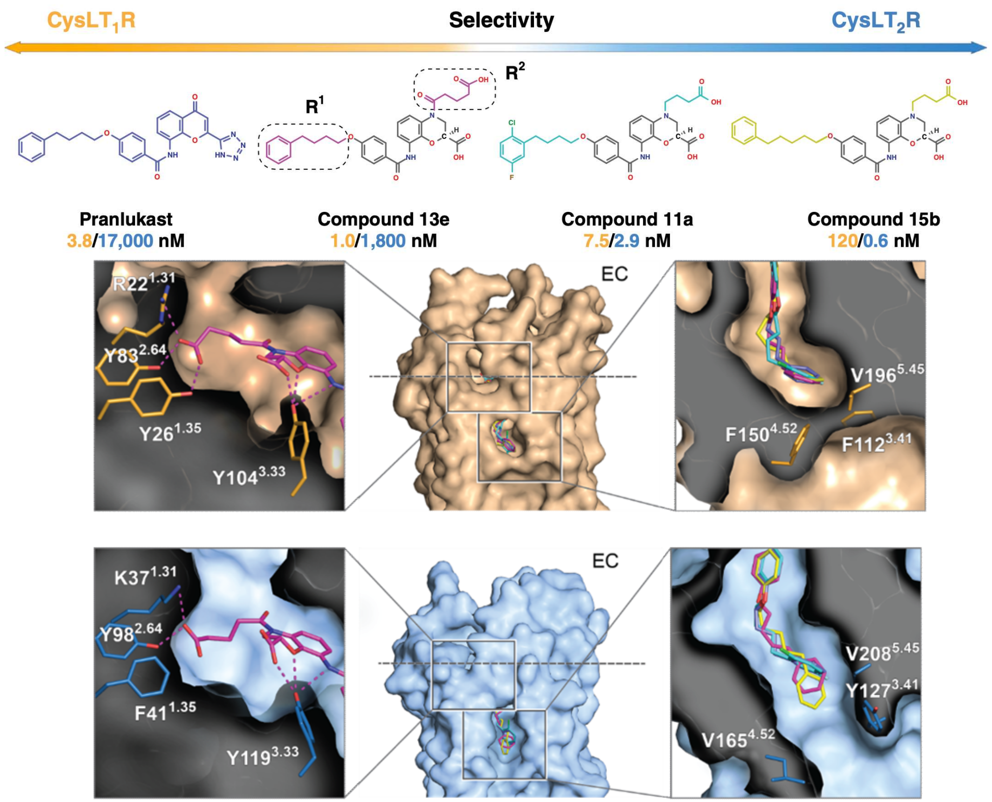
ARTICLE

https://doi.org/10.1038/s41467-019-13348-2 OPEN

Structural basis of ligand selectivity and disease mutations in cysteinyl leukotriene receptors

Anastasiia Gusach[1,14], Aleksandra Luginina[1,14], Egor Marin[1], Rebecca L. Brouillette[2], Élie Besserer-Offroy[2], Jean-Michel Longpré[2], Andrii Ishchenko[3,4,11], Petr Popov[1,12], Nilkanth Patel[3,5], Taku Fujimoto[6], Toru Maruyama[6], Benjamin Stauch[3,4], Margarita Ergasheva[1], Daria Romanovskaia[1,13], Anastasiia Stepko[1], Kirill Kovalev[1,7,8,9,10], Mikhail Shevtsov[1], Valentin Gordeliy[1,7,8,9,10], Gye Won Han[3,4], Vsevolod Katritch[3,4,5], Valentin Borshchevskiy[1,7,9], Philippe Sarret[2*], Alexey Mishin[1*] & Vadim Cherezov[1,3,4,5*]

CysLT1R-pranlukast vs CysLT2R structures

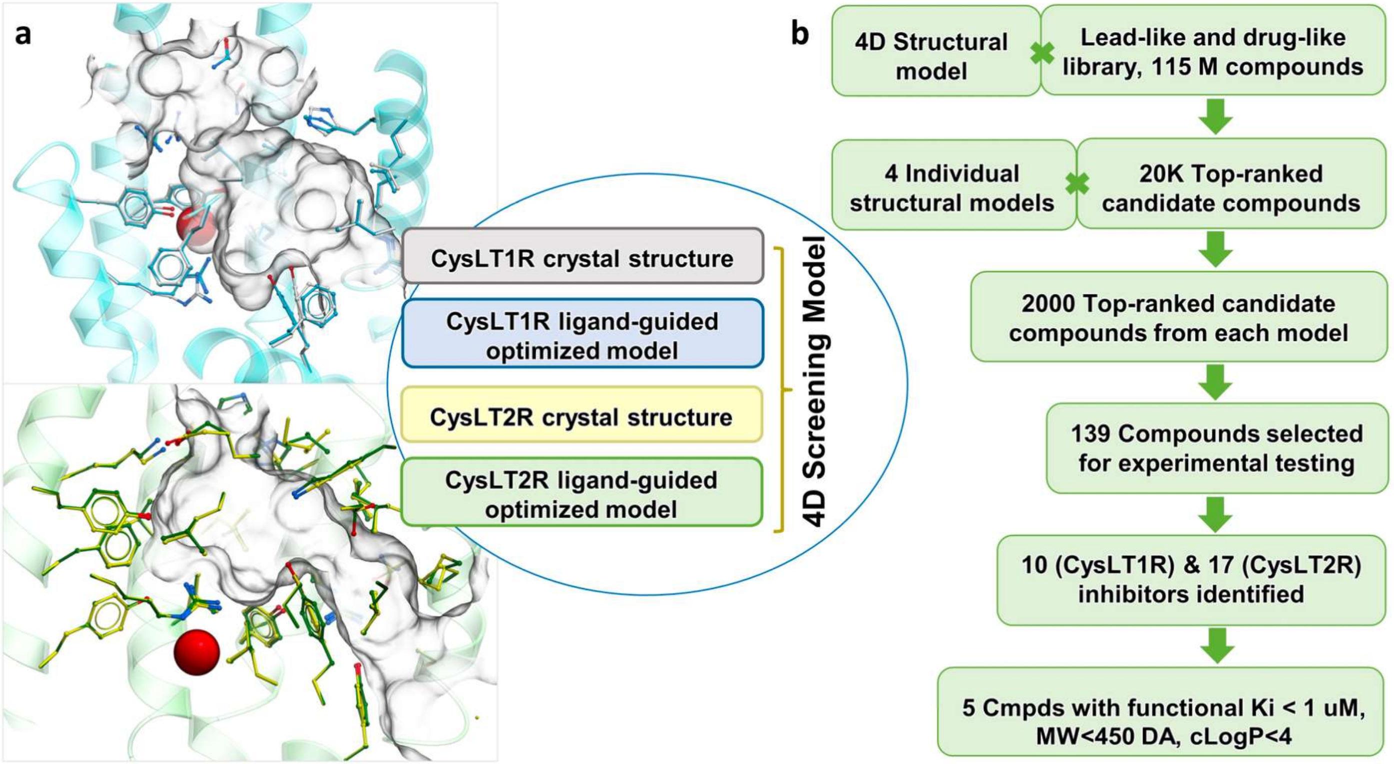


Overview of the ligand-binding pocket with the docked ligands for CysLT1R (beige) and CysLT2R (light blue). Inserts show docking poses and details of ligand interactions with CysLT1R and CysLT2R.

We identified the binding poses for ligands in both receptors and found out what are their structural properties, which may be responsible for binding specificity.

With this information we can find the dual ligand for both receptors.

Structure-based drug design: Virtual ligand screening



(a) Optimized ligand pockets for CysLT1R
(b) Flowchart of screening and ligand optimized pocket of CysLT1R and CysLT2R.

L129Q SNP: oncogenic mutation in CysLT2R Leading to Uveal Melanona.
An aggressive orphan cancer

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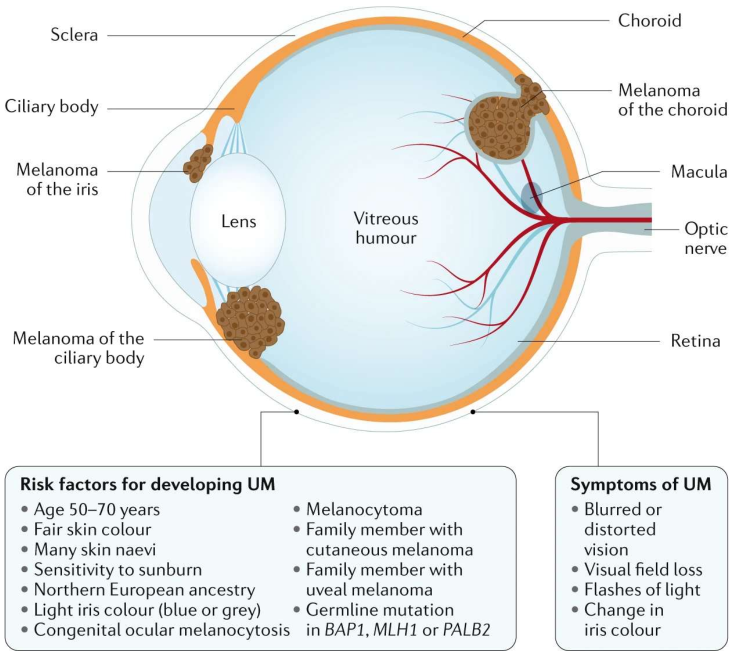
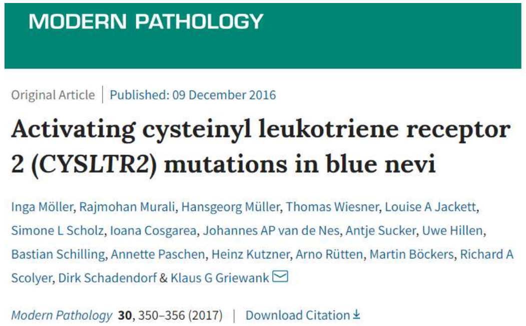




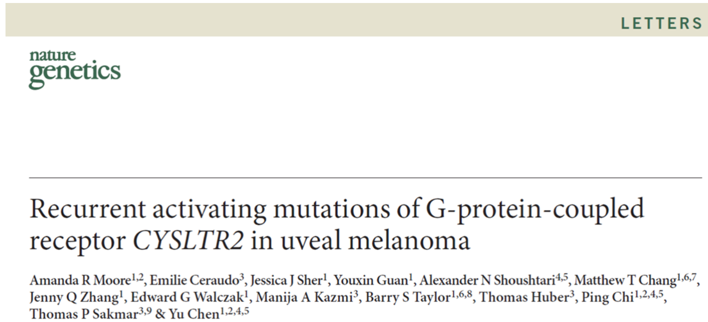
MODERN PATHOLOGY

Original Article | Published: 09 December 2016

Activating cysteinyl leukotriene receptor 2 (CYSLTR2) mutations in blue nevi

Inga Möller, Rajmohan Murali, Hansgeorg Müller, Thomas Wiesner, Louise A Jackett, Simone L Scholz, Ioana Cosgarea, Johannes AP van de Nes, Antje Sucker, Uwe Hillen, Bastian Schilling, Annette Paschen, Heinz Kutzner, Arno Rütten, Martin Böckers, Richard A Scolyer, Dirk Schadendorf & Klaus G Griewank

Modern Pathology 30, 350–356 (2017) | Download Citation



LETTERS

nature genetics

Recurrent activating mutations of G-protein-coupled receptor *CYSLTR2* in uveal melanoma

Amanda R Moore[1,2], Emilie Ceraudo[3], Jessica J Sher[1], Youxin Guan[1], Alexander N Shoushtari[4,5], Matthew T Chang[1,6,7], Jenny Q Zhang[1], Edward G Walczak[1], Manija A Kazmi[3], Barry S Taylor[1,6,8], Thomas Huber[3], Ping Chi[1,2,4,5], Thomas P Sakmar[3,9] & Yu Chen[1,2,4,5]

About 3% of all uveal melanomas -> several hundreds new cases each year

No specific theraphеutic treatment available

L129Q – oncogenic mutation in CysLT2R Leading to Uveal Melanona: constitutive activity

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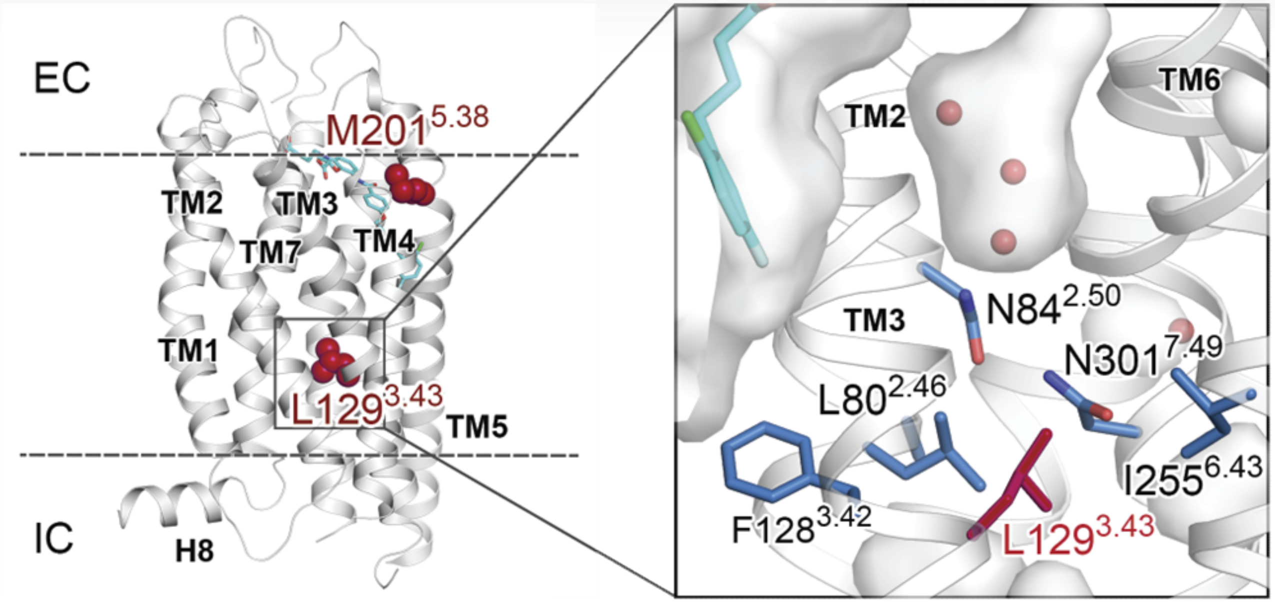

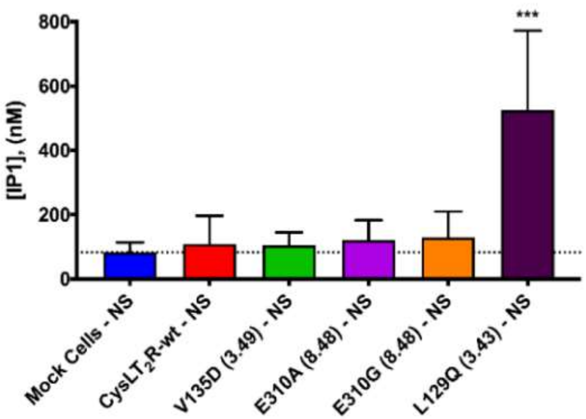

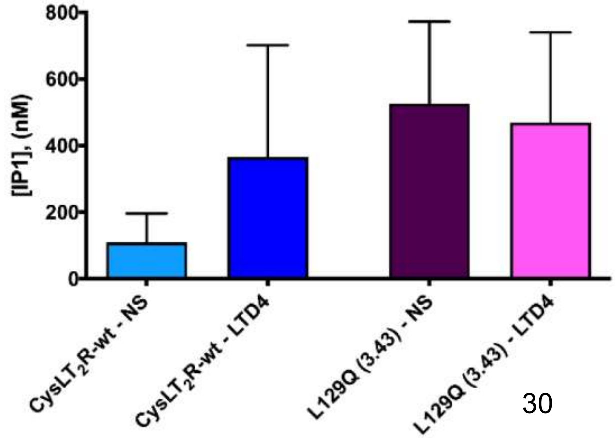

The project started in order to get new-generation anti-asthmatic drugs but we got also
Inverse agonists for oncogenic L129Q CysLT2R as a hit compound

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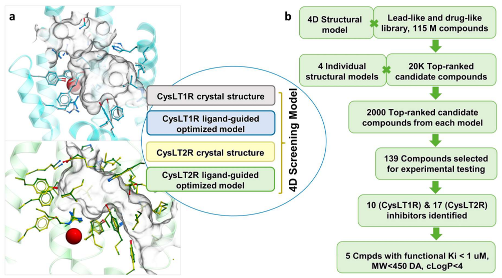
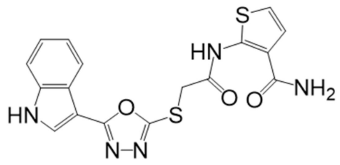
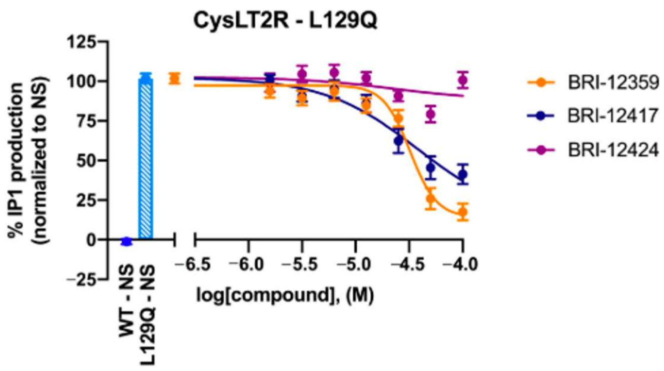
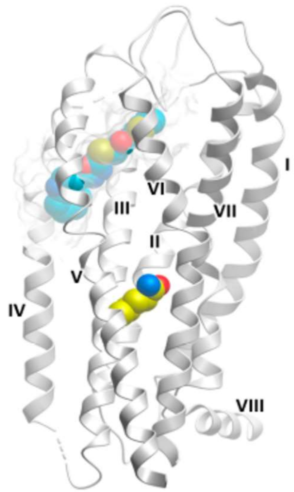




BRI-12359



BRI-ID	IC_{50} ± SEM (µM)	% max inhibition ± SEM
BRI-12359	32 ± 2	82 ± 11
BRI-12417	38 ± 10	59 ± 12
BRI-12424	N/C	21 ± 10



Current Project strategy

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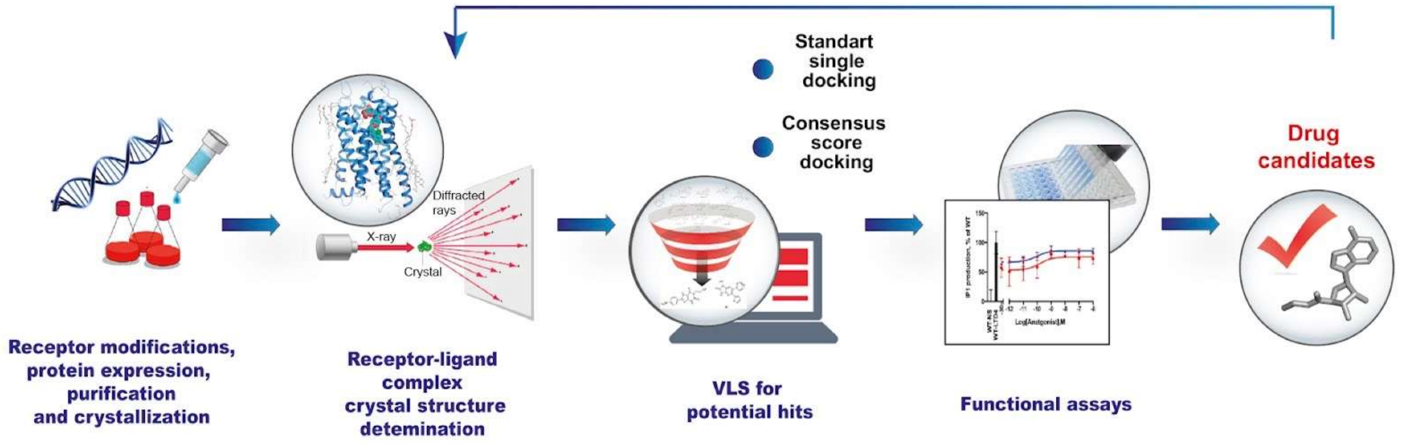

Off-target activity testing (reverse agonists)

Design of stabilized L129Q mutant of CysLT2 for crystallization in “constitutively active” conformation.

Co-crystallization CysLT2-L129Q with available high-affinity ligand for future VLS round

Future drug development

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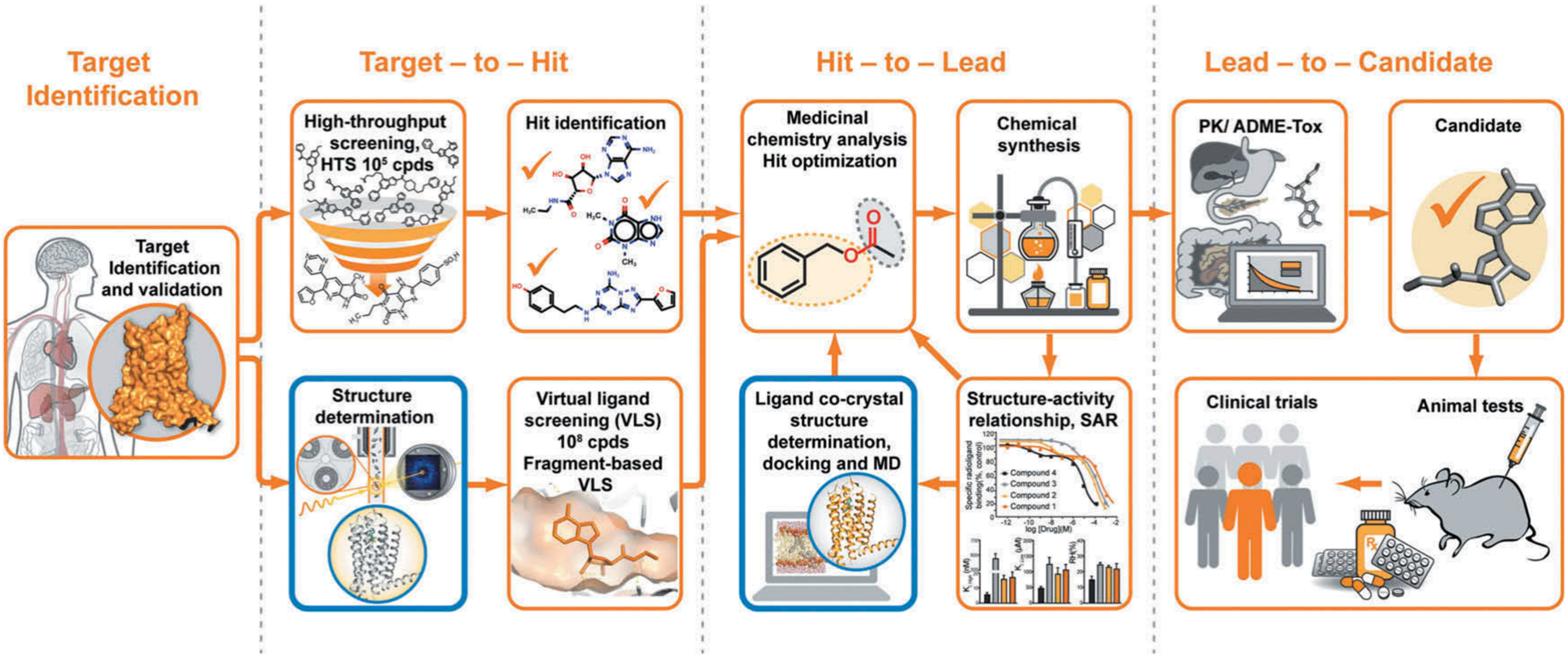

Alexey Mishin, Anastasiia Gusach, Aleksandra Luginina, Egor Marin, Valentin Borshchevskiy & Vadim Cherezov (2019):
An outlook on using serial femtosecond crystallography in drug discovery,
Expert Opinion on Drug Discovery

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