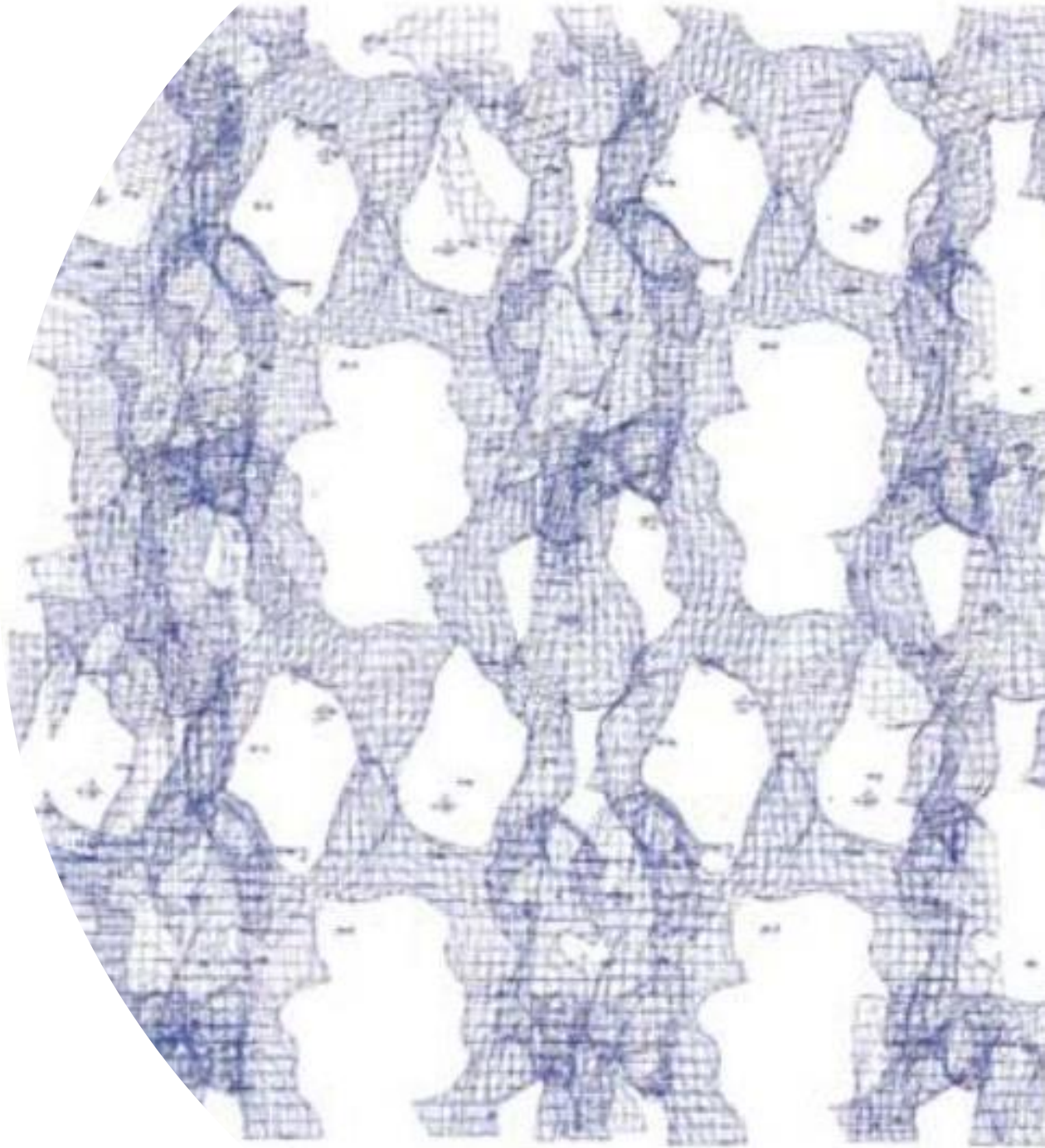
Membrane protein purification and application in drug discovery

LINX (Lund Institute of Advanced Neutron and X-ray Science) Membrane Protein Symposium

“Structural Resolution of Membrane Proteins: From Expression to Sample Preparation”

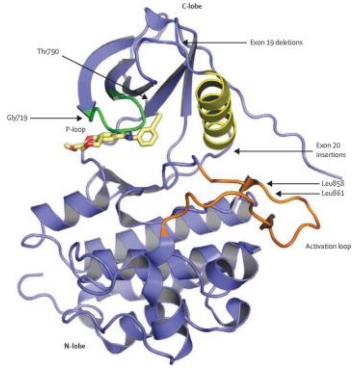
Arjan Snijder

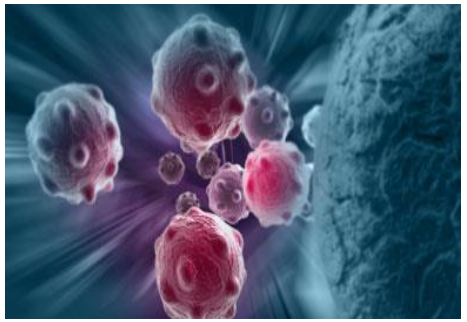
26th May 2021



Snijder, H. J., et al, (2003) *Journal of structural biology*

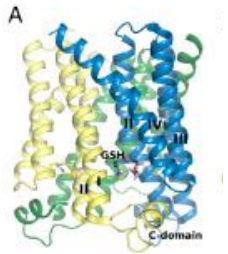
Protein Science in early drug discovery at AstraZeneca





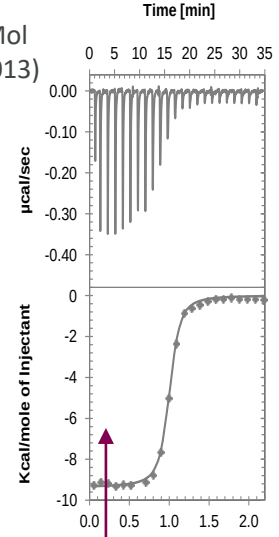
- Protein Science provides the critical building blocks for drug discovery projects aiming to develop medicines to improve the lives of patients all over the world
- The success of our projects is crucially dependent on the quality of the target proteins we produce to provide the right data to drive progress
- The provision of appropriate protein samples is often the first and rate-limiting step for new projects
- As protein scientists, we need to be fast, innovative, agile and productive in order to meet the needs of multiple projects across the AstraZeneca disease areas
- Applications: assay, affinity screening, HTS, Biophysics mode of action, structure, antibody generation, bio-analyte reference, *in vivo* protein PK etc.

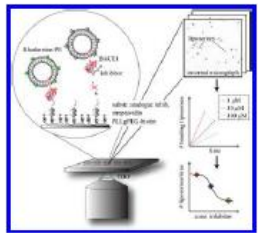
Membrane proteins @ Astrazeneca



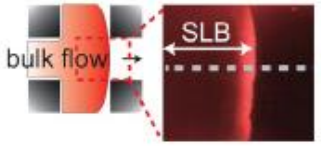
mPGES (Sjögren et al., PNAS 2013)

(Wöhri et al., Mol Membr Biol. 2013)



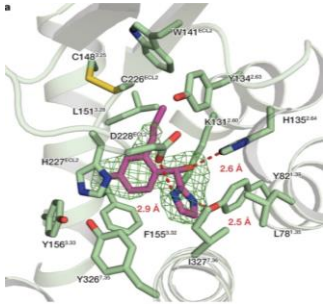


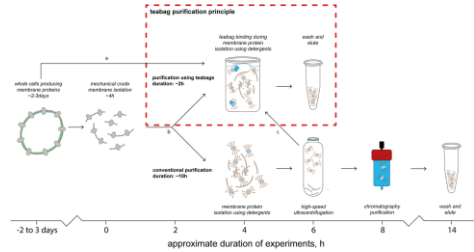
(Gunnarsson et al. Anal Chem. 2015)



(Gunnarsson et al. ChemistryOpen. 2016)

(R K Y Cheng *et al. Nature* 1–4 (2017) doi:10.1038/nature22309)

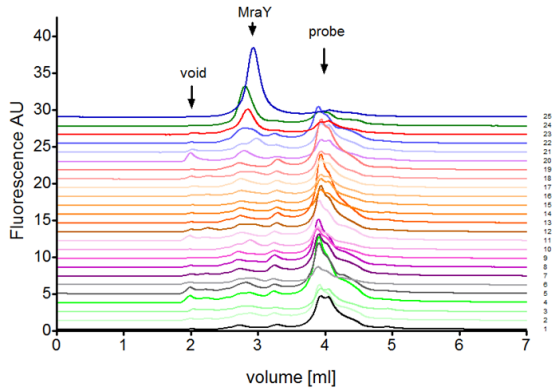


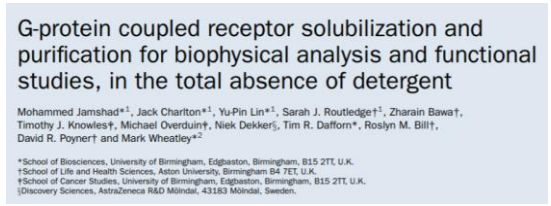


(Hering et al. Scientific Reports. 2020)

2005 first human recombinant membrane protein structure → FSEC His specific probe screening → Pichia pastoris & Biophysics ITC → Exploring SMALPS → Membrane proteins & single molecule spectroscopy → Structure determination of MraY in complex with tunicamycin → GPRC structures → DNA-encoded library screening → Fast purification teabag method

(Backmark et al., Protein, 2012)

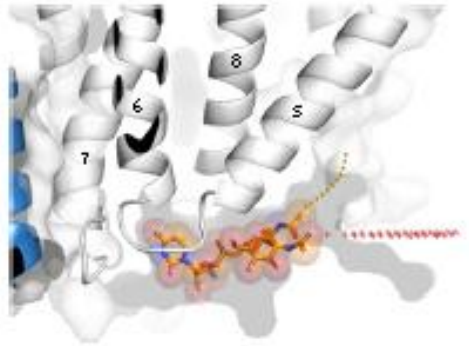



G-protein coupled receptor solubilization and purification for biophysical analysis and functional studies, in the total absence of detergent

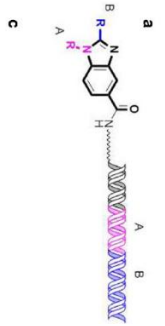
Mohammed Jamshad*†‡, Jack Charlton*†‡, Yu-Pin Lin*†‡, Sarah J. Routledge†‡, Zharain Bawa†, Timothy J. Knowles†, Michael Overduin†, Niek Dekker§, Tim R. Dafforn*, Roslyn M. Bill†, David R. Poyner† and Mark Wheatley*‡

*School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, U.K.
†School of Life and Health Sciences, Aston University, Birmingham B4 7ET, U.K.
‡School of Cancer Studies, University of Birmingham, Edgbaston, Birmingham, B15 2TT, U.K.
§Discovery Sciences, AstraZeneca R&D Mölndal, 43183 Mölndal, Sweden.

(Jamshad et al., Biosci Reports, 2015)



(Hakulinen et al., Nature Chem. Biol., 2017)

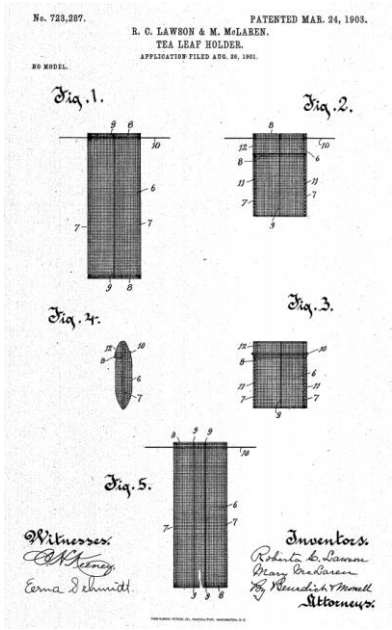


(Brown et al., SLAS Discovery, 2018)

(Re)-Invention of the teabag

Roberta Lawson & Mary McLaren filed a patent for a 'novel tea-holding pocket constructed of open-mesh woven fabric, inexpensively made of cotton thread'. US723287 A 1903

Thomas Sullivan (New York) 1908 accidentally re-invented the teabag and commercialized the teabag. Tea in sewn silk bags, customers using these directly to make a brew



Teabag, US patent, 723287 A from 1903

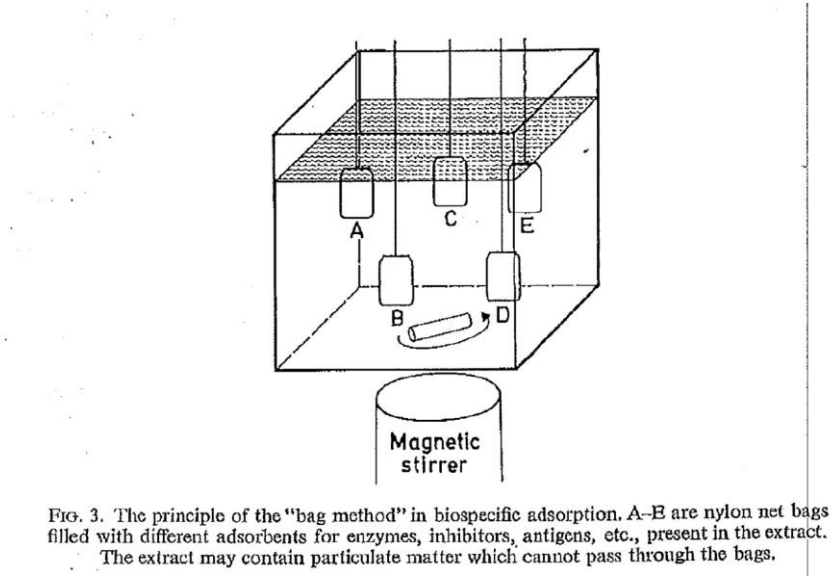
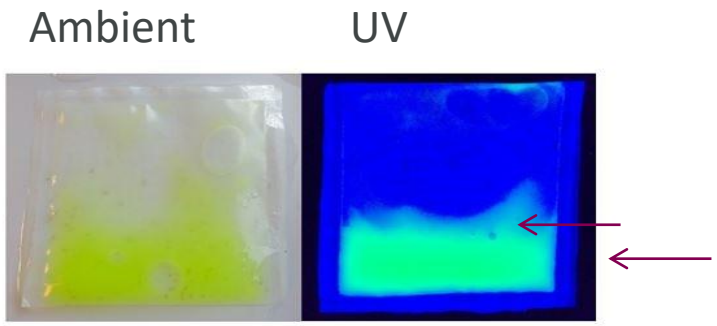


FIG. 3. The principle of the "bag method" in biospecific adsorption. A–E are nylon net bags filled with different adsorbents for enzymes, inhibitors, antigens, etc., present in the extract. The extract may contain particulate matter which cannot pass through the bags.

Porath and Sundberg, 1971



Ni-NTA Agarose
loaded with His-GFP

Castaldo et al., Scientific Reports 2015

Teabag materials/shapes/construction/use

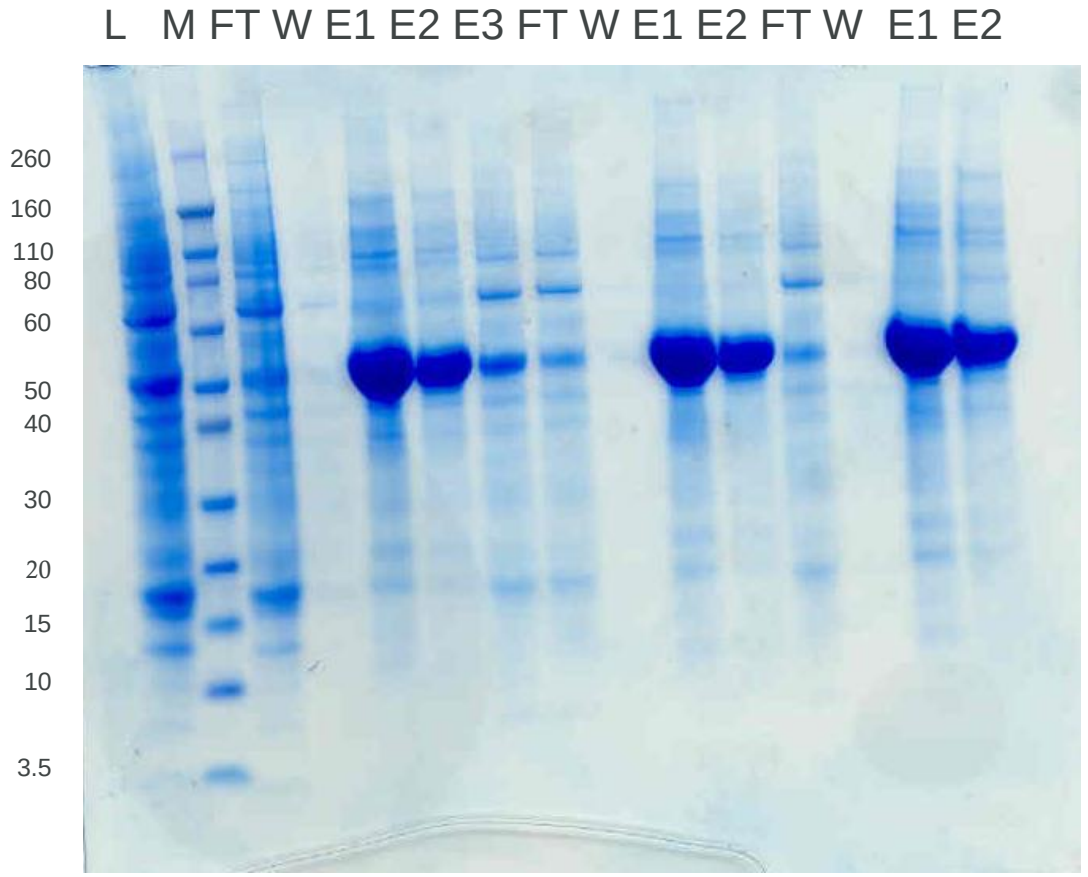
Material
Sefar Petex/Nitex, Precision woven, open mesh fabric, Controlled mesh size and consistent surface properties, low protein binding. Meshes 17, 25 and **40 µm**

Construction
Cut 4x8 cm of mesh, fold and heat seals along two edges to form a 4x4 cm bag. Fill with resin of choice and seal the bag

Shapes
Pyramids, tetrahedral, cylinders, **squares or rectangles**
With or **without** a string attached

Use
Equilibrate resin filled bag in buffer
Transfer to broth or cleared lysate, incubate with agitation; transfer to wash buffer and subsequently to elution buffer

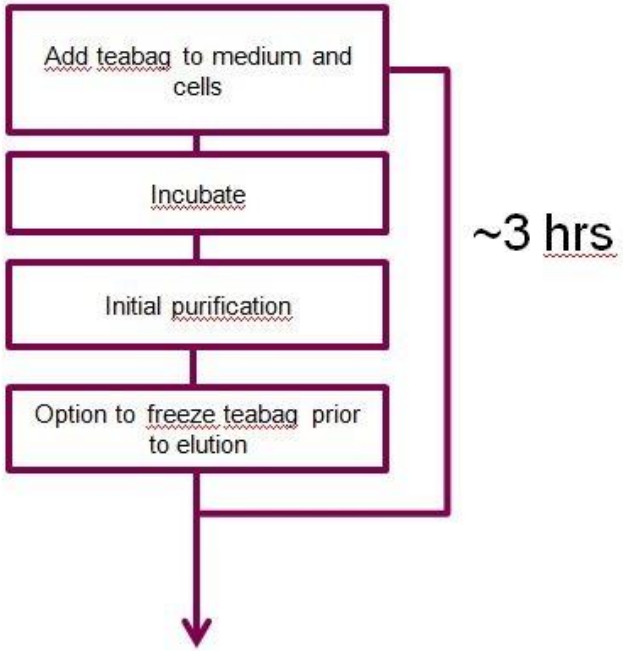
Purification of secreted His-tagged mPAI from HEK culture



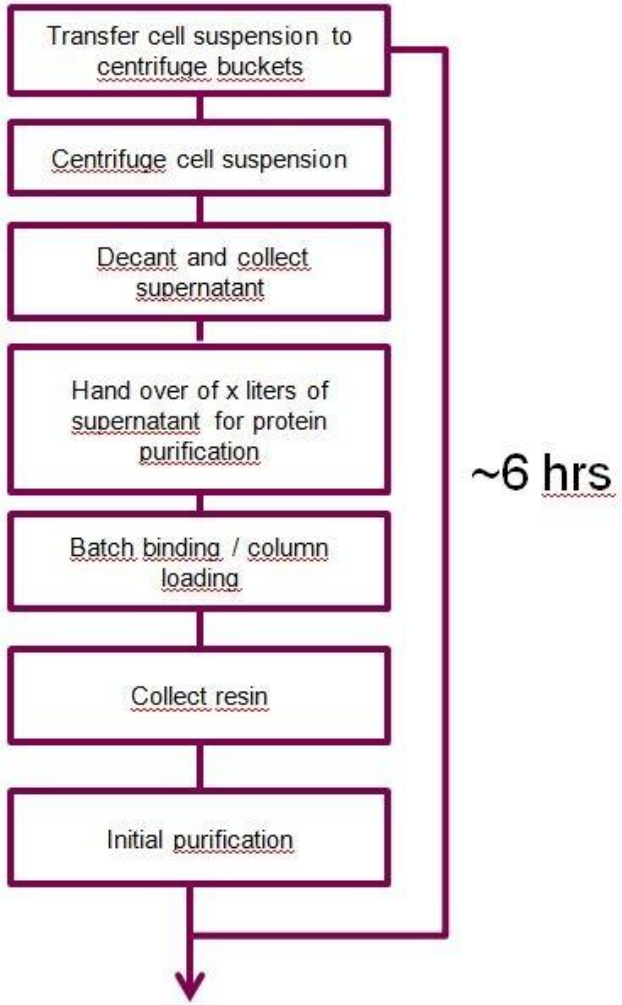
Target: mPAI
Culture volume: 100 ml
Resin: 1ml Ni sepharose excel
Bead size: 90 µm (median size)
Yield: 2 mg (20 mg/L)

Benefit: time savings

Teabag purification

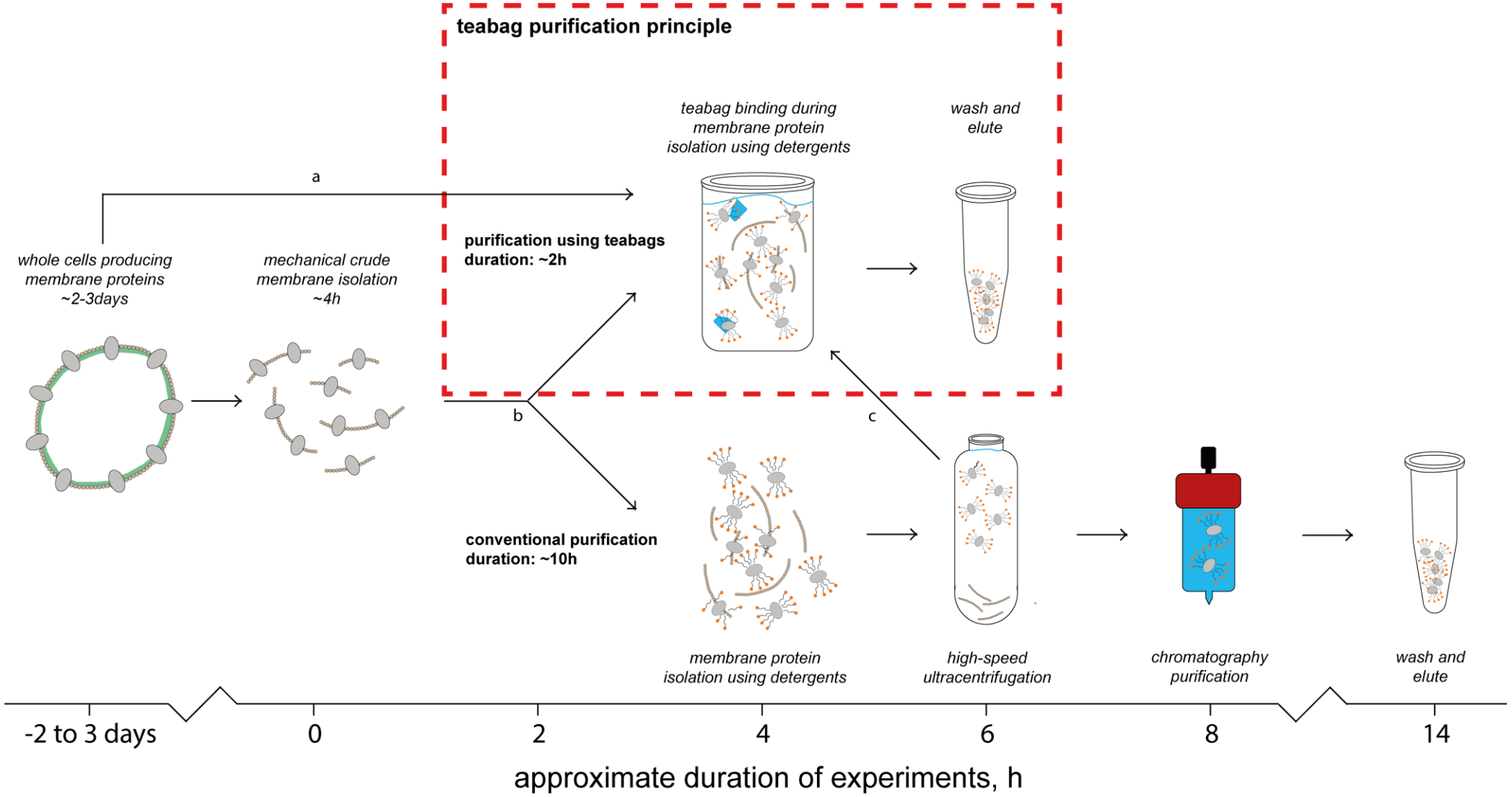


Conventional purification



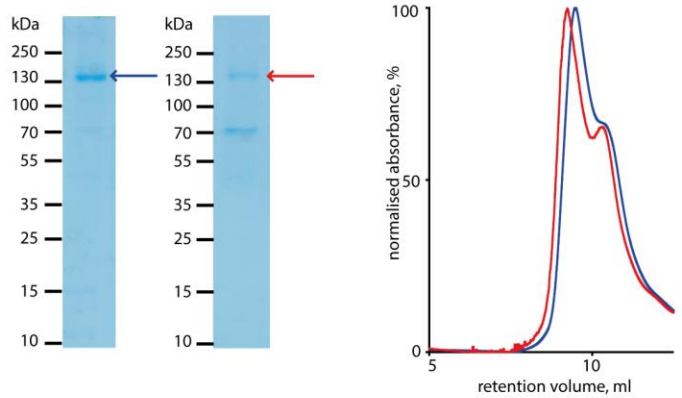
and …. anecdotical quality improvement for sensitive proteins

Teabag purification of membrane proteins

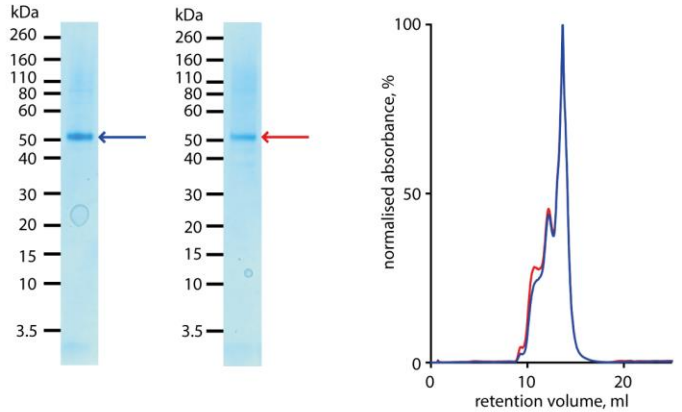


(Hering et al. Scientific Reports. 2020)

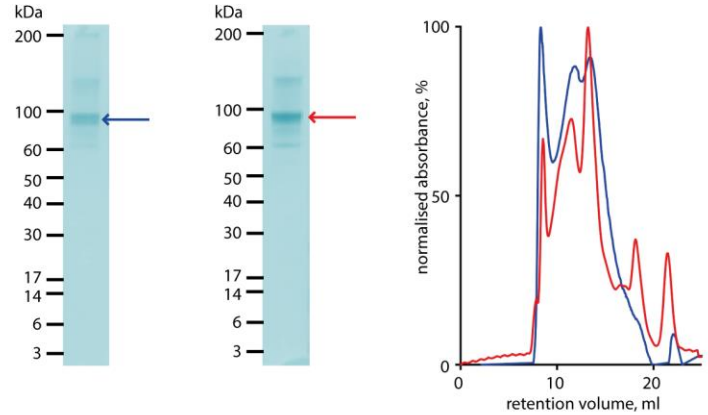
(a) CIC-1



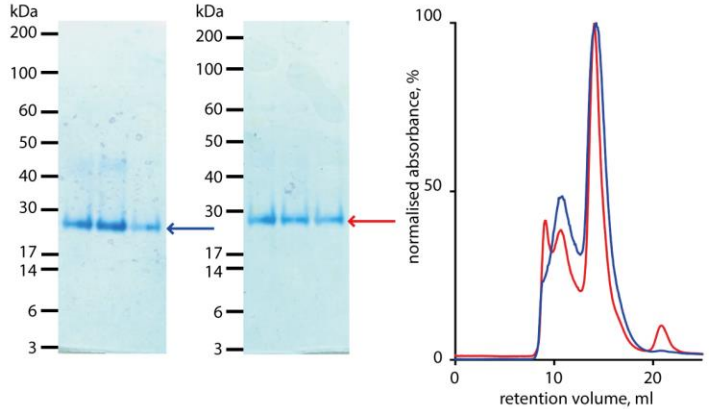
(b) PAR2



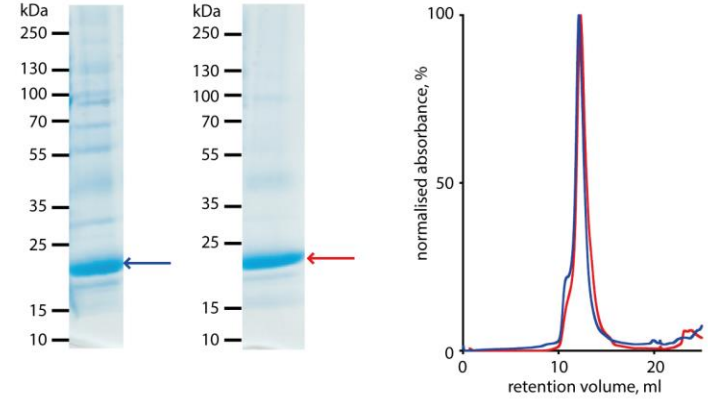
(c) KCC2



(d) MraY

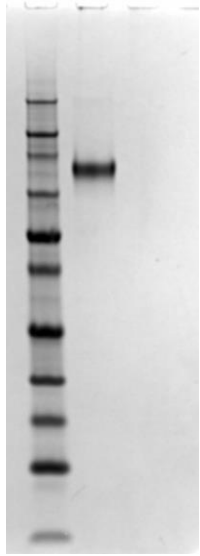


(e) AQP10



Teabag purification summary

- Reduced purification time
- Reduced hands-on time
- Quality equal or exceeds conventional methods
- Scalable & allows for simple parallelization
- Disadvantage: somewhat reduced yields



Teabag purification
of an orphan GPCR

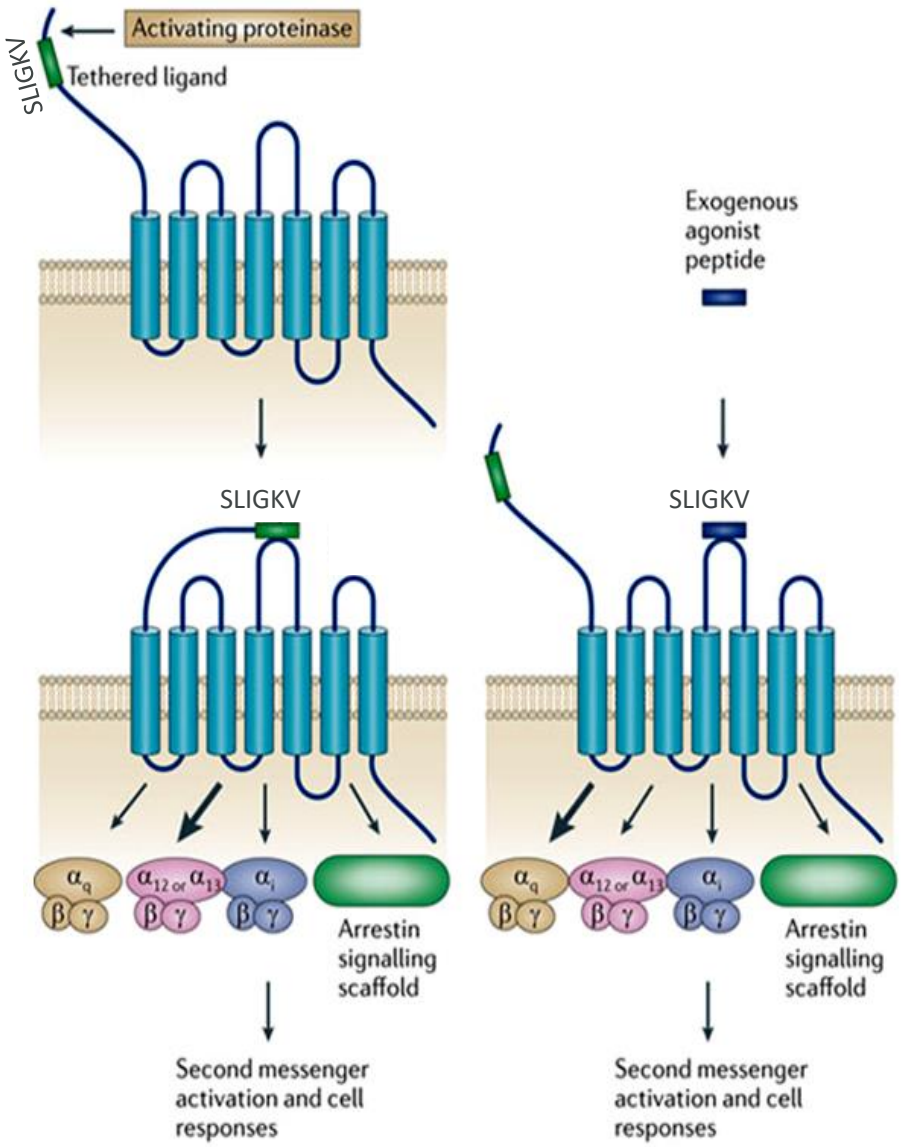
Protease activated receptors

Background

- The protease activated receptors (PARs) form a family of 4 class A G protein-coupled receptors (GPCRs)
- The receptors are irreversibly activated by proteolytic cleavage of the N-terminus, which unmasks the tethered peptide ligand
- PARs are implicated in a wide range of diseases including arthritis, asthma, neurodegenerative conditions, cancer and cardiovascular diseases

Protease activated receptor 2 (PAR2)

- PAR2 is predominantly activated by serine proteases, revealing the activating sequence SLIGKV (SLIGRL in rodents).

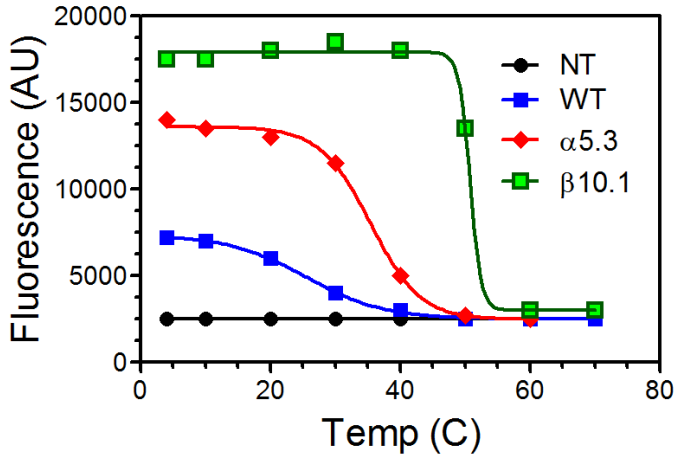


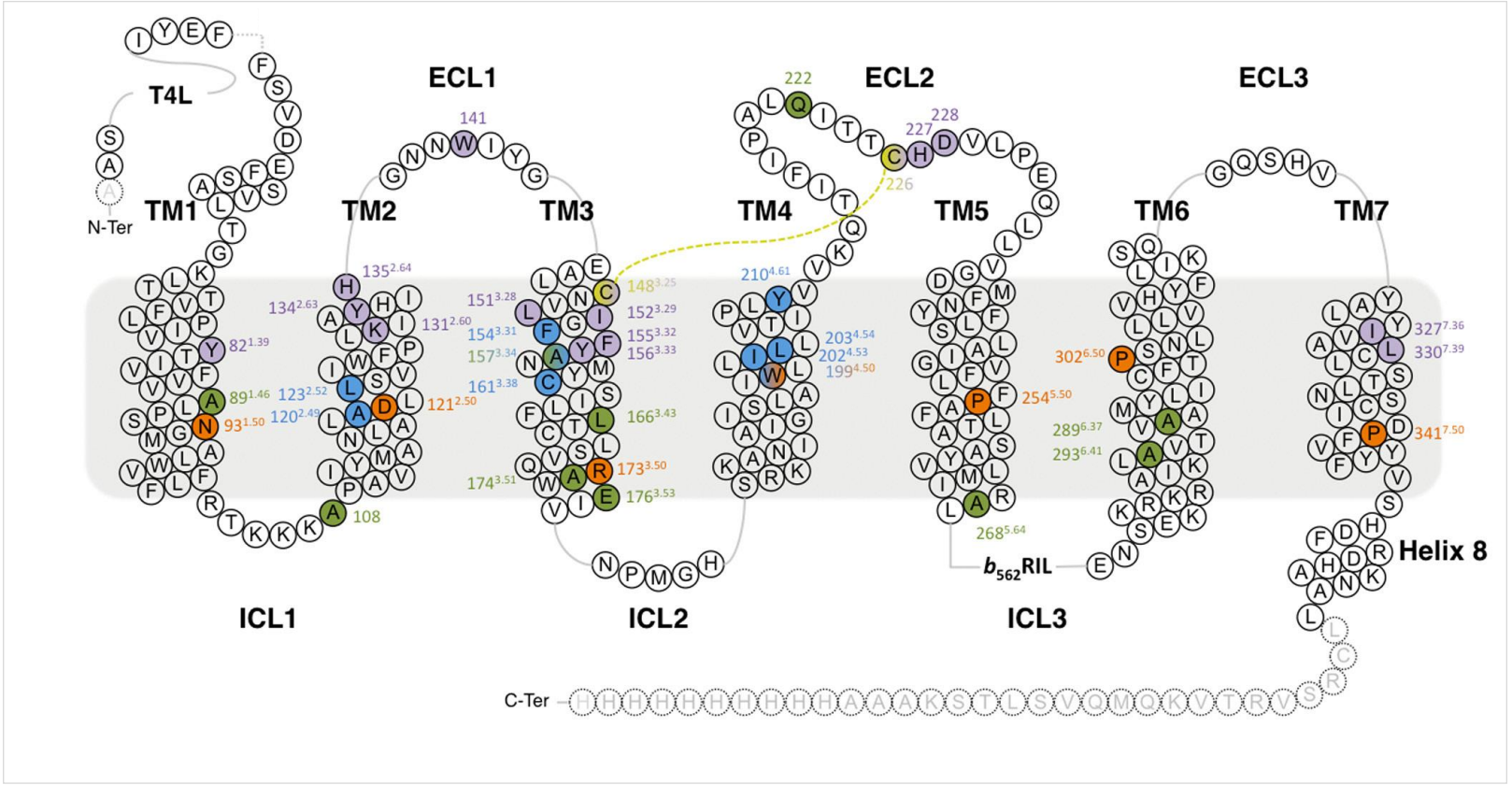
Ramachandran et al. *Nature Reviews Drug Discovery* **11**, 69-86 (2012).

Protein biochemistry

A StaR is born

- In 2011 AstraZeneca entered a collaboration with Sosei Heptares pharmaceuticals with the aim to generate Stabilised Receptors (**StaR**s) for drug discovery
  - Conformational selection
  - Mutagenesis
  - Thermostability
  - Pharmacology
  - Recombination

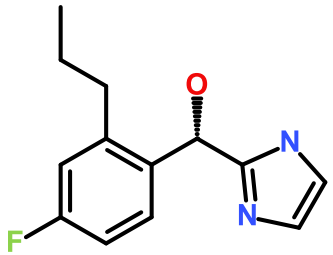




Cheng et al. *Nature* **545**, 112-115 (2017)

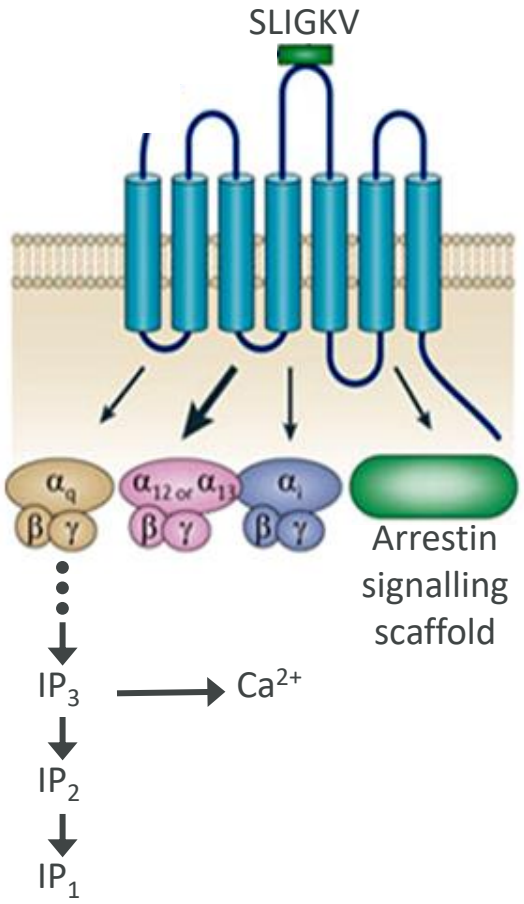
PAR2 in complex with AZ8838

- AZ8838 belongs to the imidazole series, originally identified by HTS (FLIPR Ca2+).



AZ8838

	AZ8838
IP-One (SLIGKV)	IC_{50} 1.5 µM
FLIPR (SLIGKV)	IC_{50} 2.3 µM
FLIPR (Trypsin)	IC_{50} 4.2 µM
β-Arrestin-2	IC_{50} 0.63 µM
SPR (K_d)	K_d125 nM



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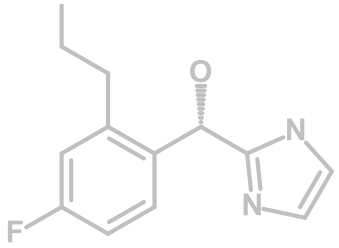

AZ8838

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IP-One (SLIGRL)	IC_{50} 1.5 µM
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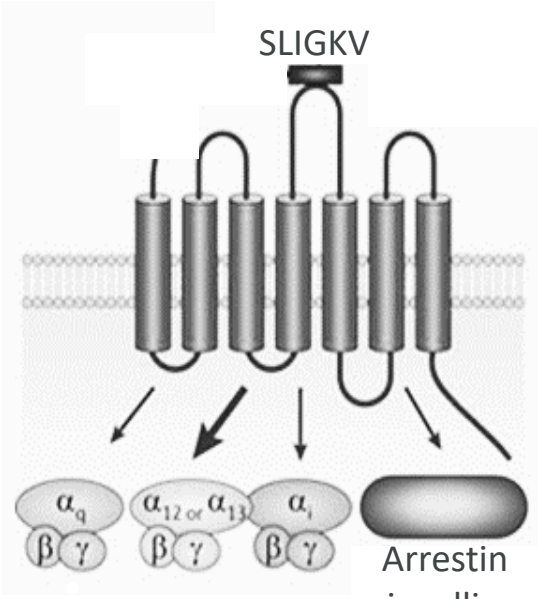
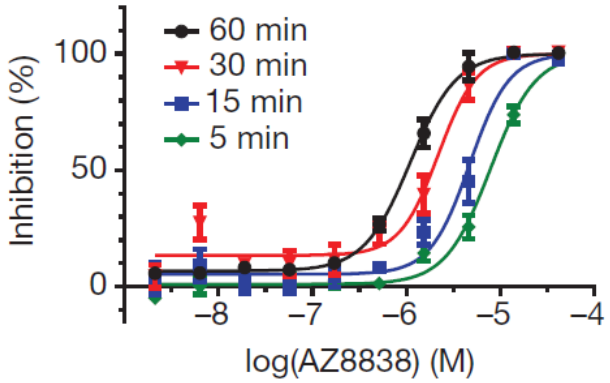
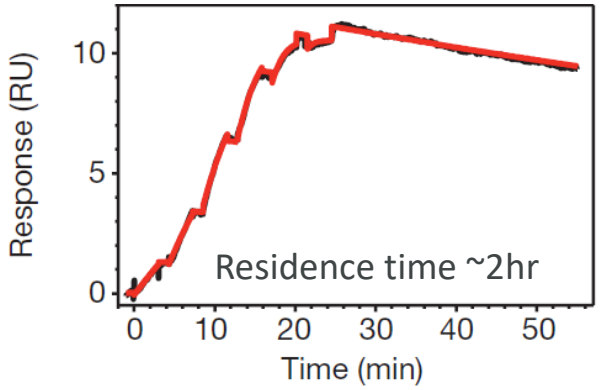


- AZ8838 activity is dependent on pre-incubation time.

FLIPR (SLIGKV)



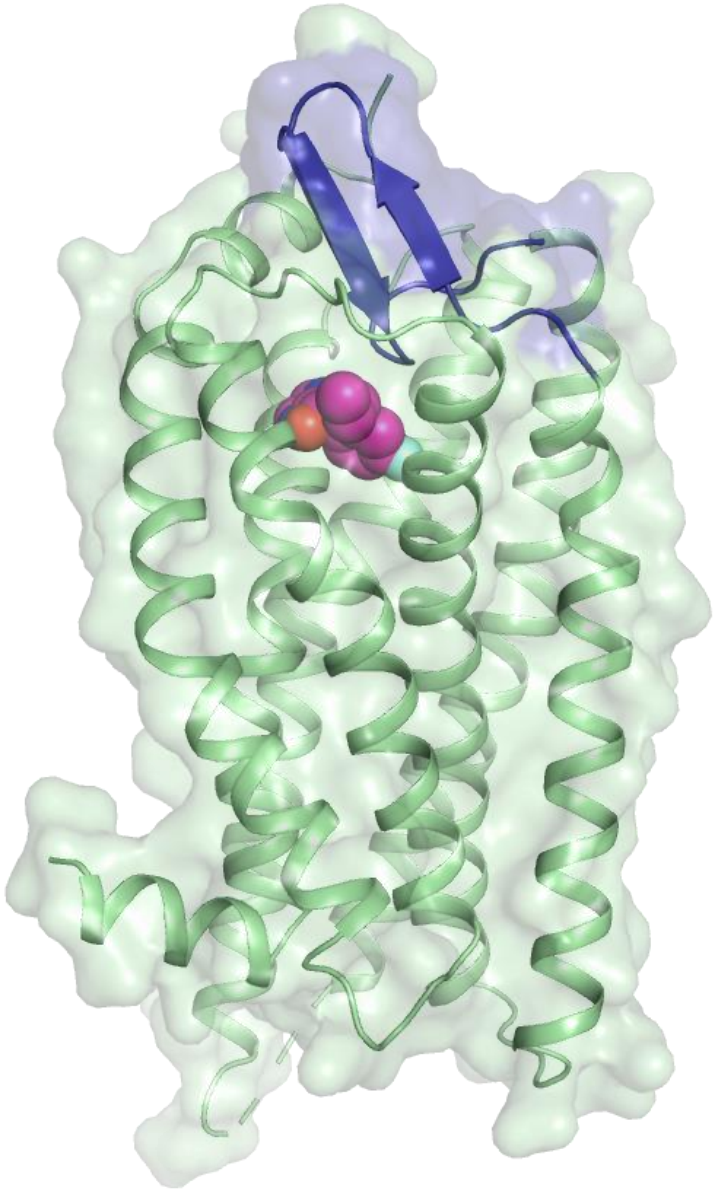
SPR

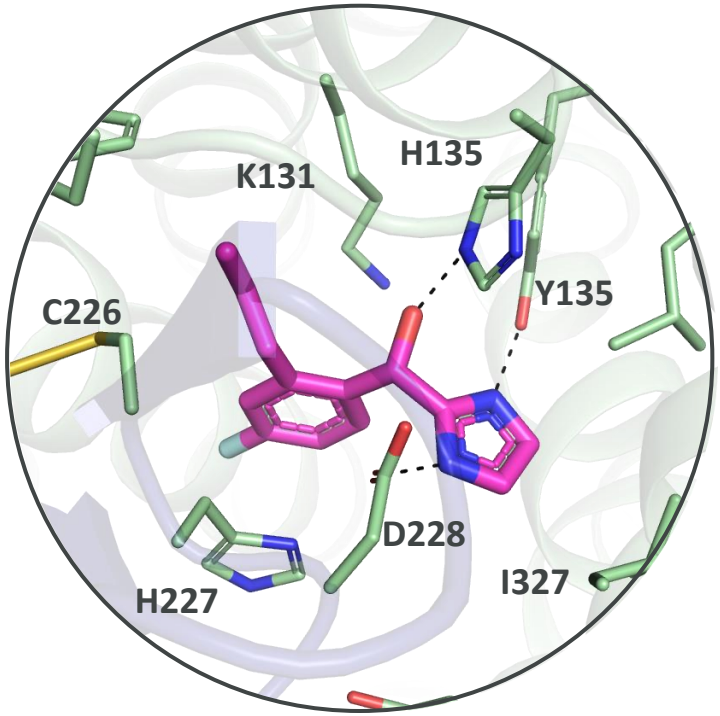


Cheng et al. *Nature* **545**, 112-115 (2017)

PAR2 in complex with AZ8838

- The PAR2:AZ8838 complex structure was solved to 2.8Å
- AZ8838 binds in a fully occluded pocket beneath ECL2
- AZ8838 pharmacophore matches the pocket properties very well
- Binding mode supported by mutations





AZ8838
PAR2
PAR2 ECL2

Cheng et al. *Nature* **545**, 112-115 (2017).

DNA-encoded library screening

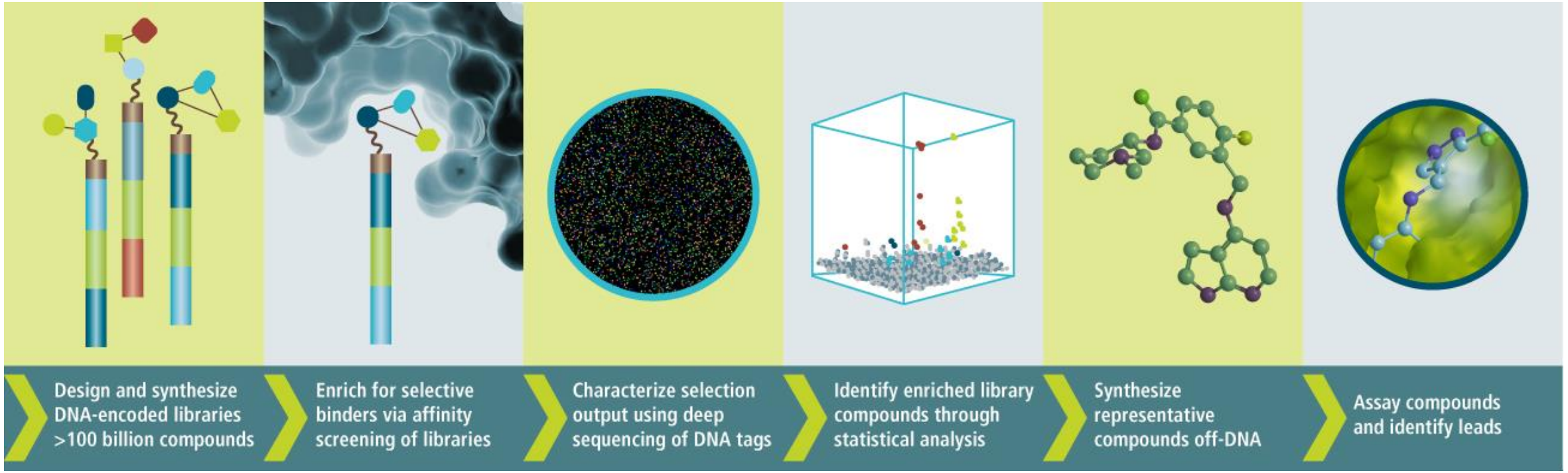
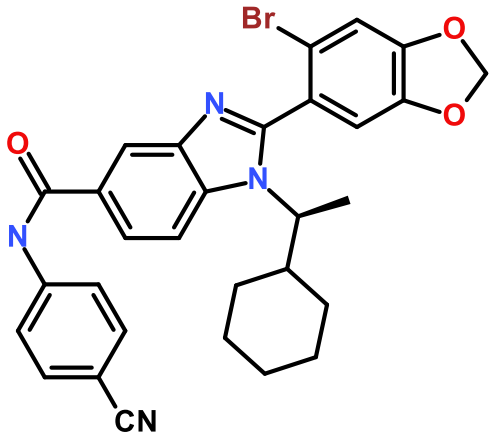


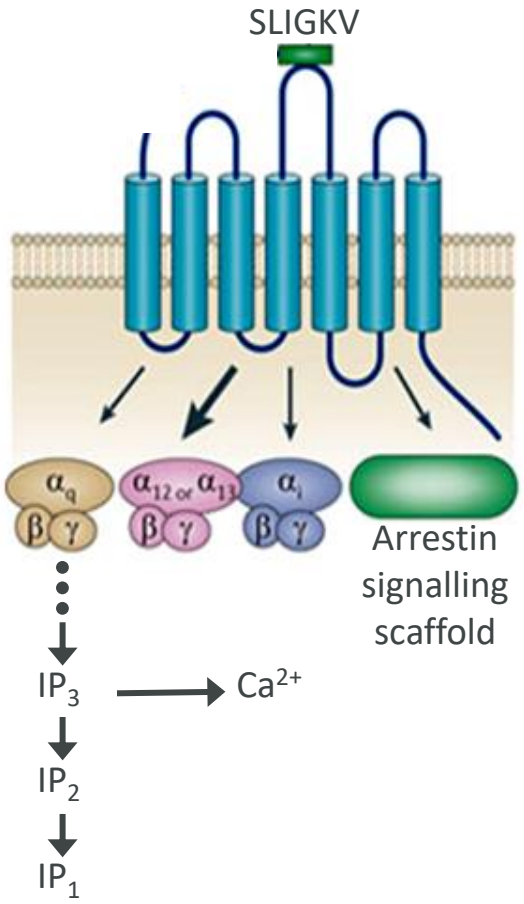
Image from XChem

PAR2 in complex with AZ3451

- AZ3451 belongs to the benzimidazole series, originally identified by DEL screening.



	AZ3451
IP-One (SLIGKV)	IC_{50} 23 nM
FLIPR (SLIGKV)	IC_{50} 5.4 nM
FLIPR (Trypsin)	IC_{50} 6.6 µM
β-Arrestin-2	IC_{50} <2.5 nM
SPR (K_d)	K_d 14 nM



Brown et al., SLAS Discovery, 2018 & Cheng et al. *Nature* **545**, 112-115 (2017)

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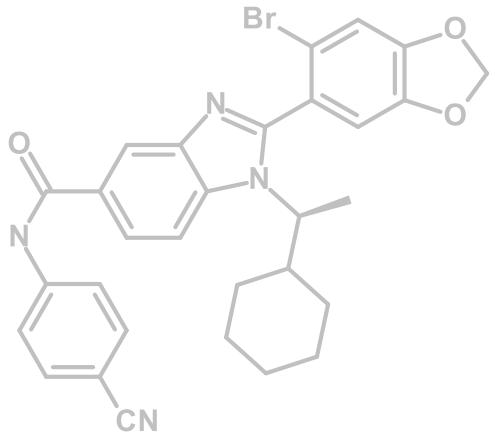

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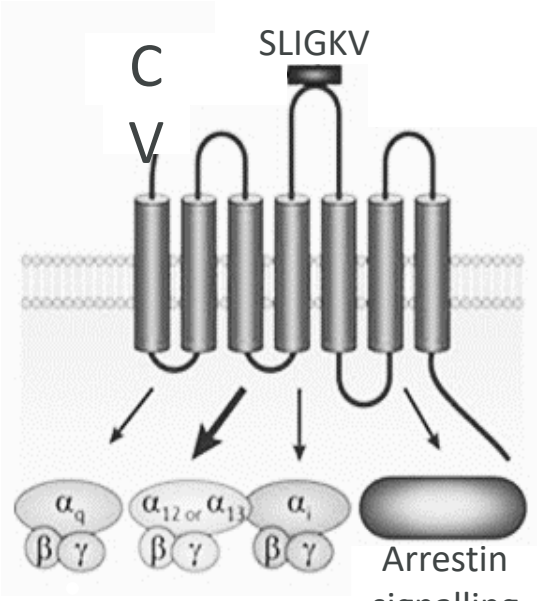
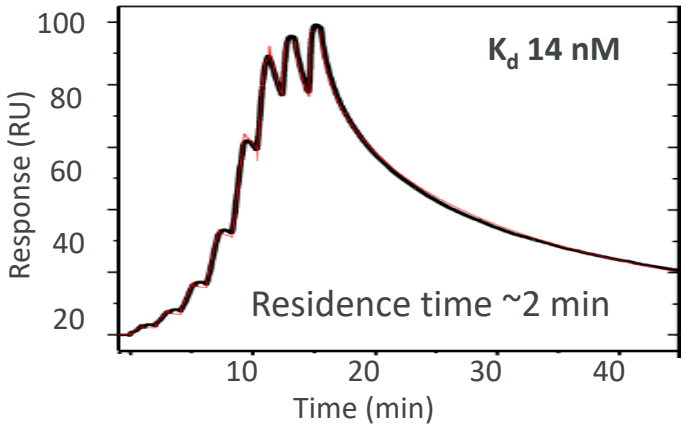
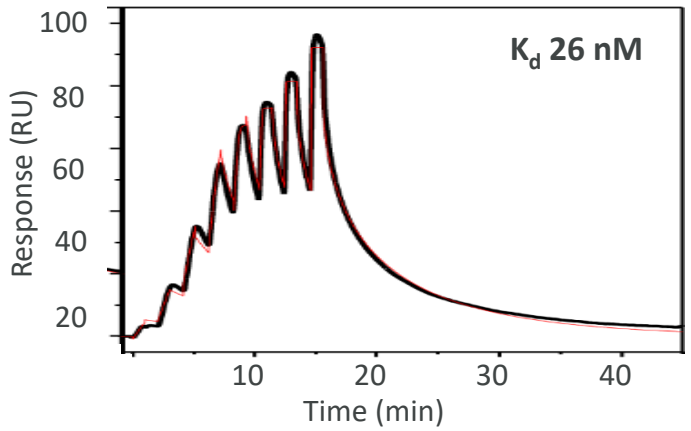
SPR (K_d)	K_d 14 nM



- AZ3451 exhibits conventional binding kinetics



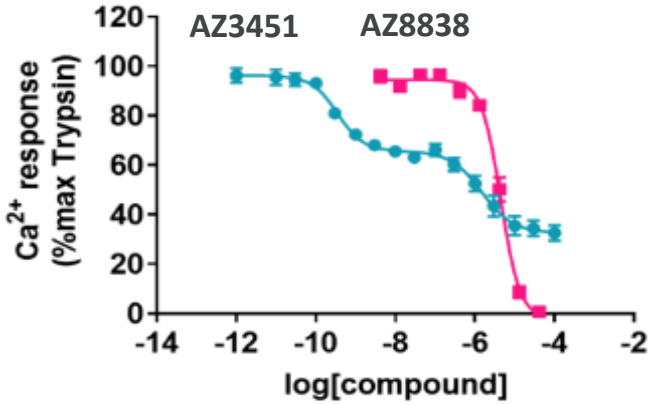
+ AZ8838



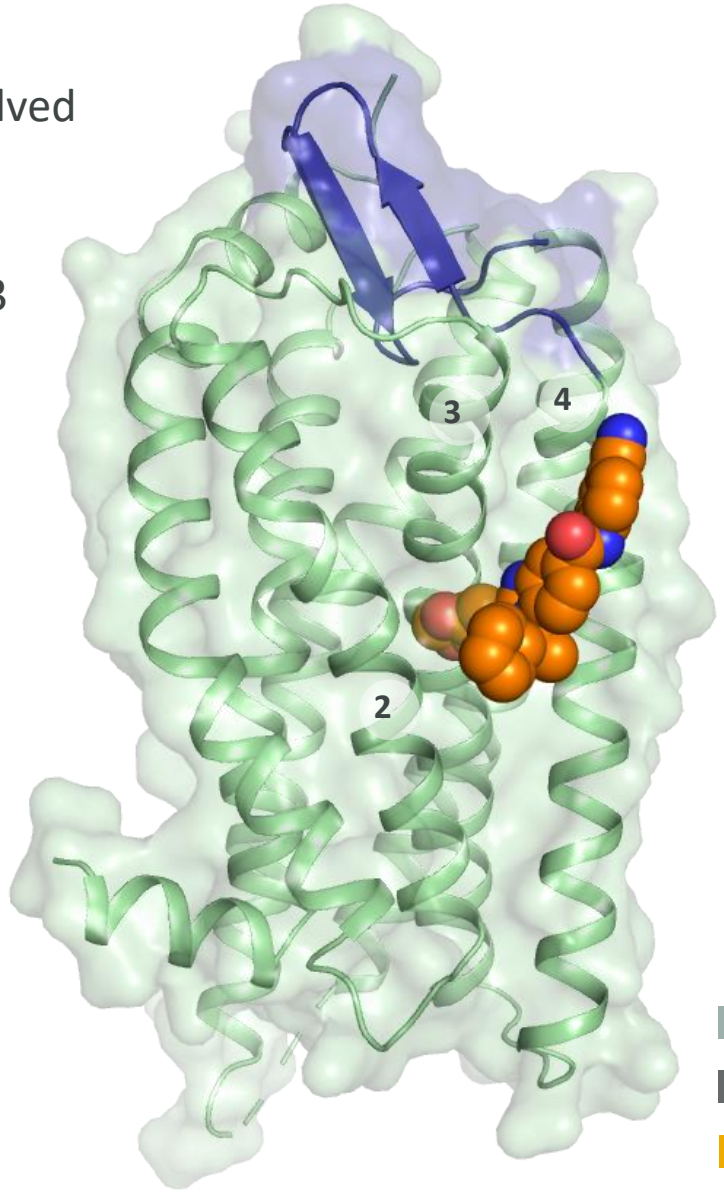
Brown et al., SLAS Discovery, 2018 & Cheng et al. *Nature* **545**, 112-115 (2017) & Kennedy et al. submitted

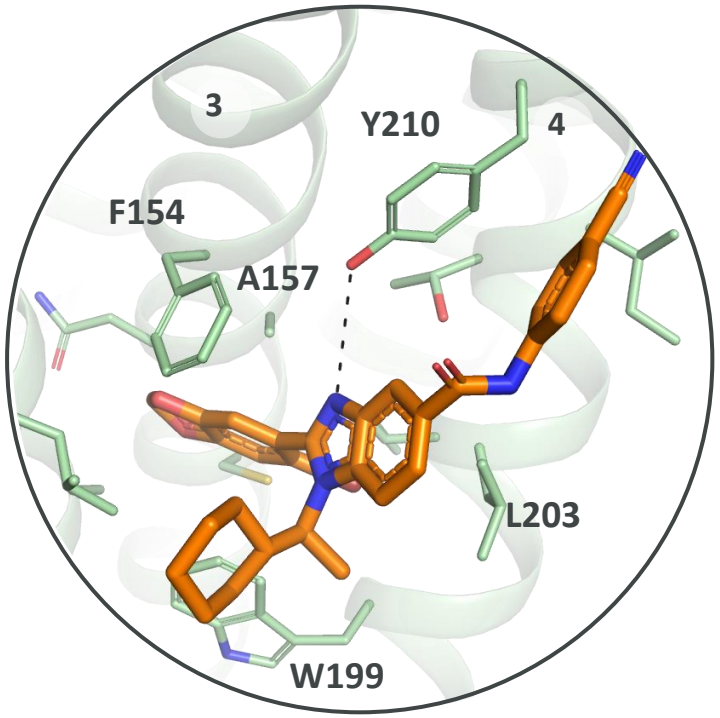
PAR2 in complex with AZ3451

- The PAR2:AZ3551 complex structure was solved to 3.6Å
- AZ3451 binds in a site outside the transmembrane domain bundle (helices 2, 3 and 4)
- Binding mode supported by mutations
- Trypsin activation gives biphasic functional assay profile



Kennedy, A. Jet al. (2020) *Communications Biology* ***3, 782***



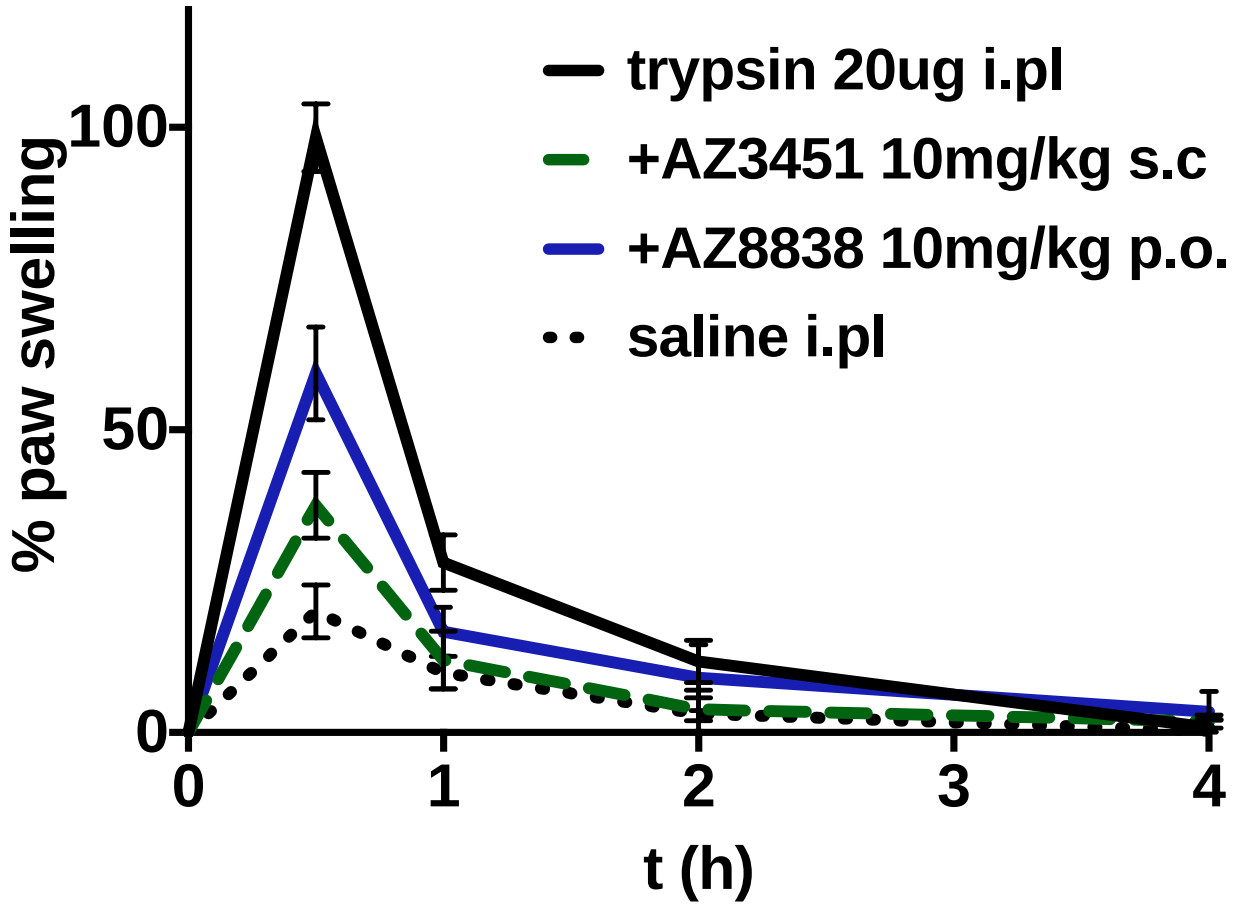


AZ3451
PAR2
PAR2 ECL2

Cheng et al. *Nature* **545**, 112-115 (2017)

Anti-inflammatory effects in a rat paw oedema model

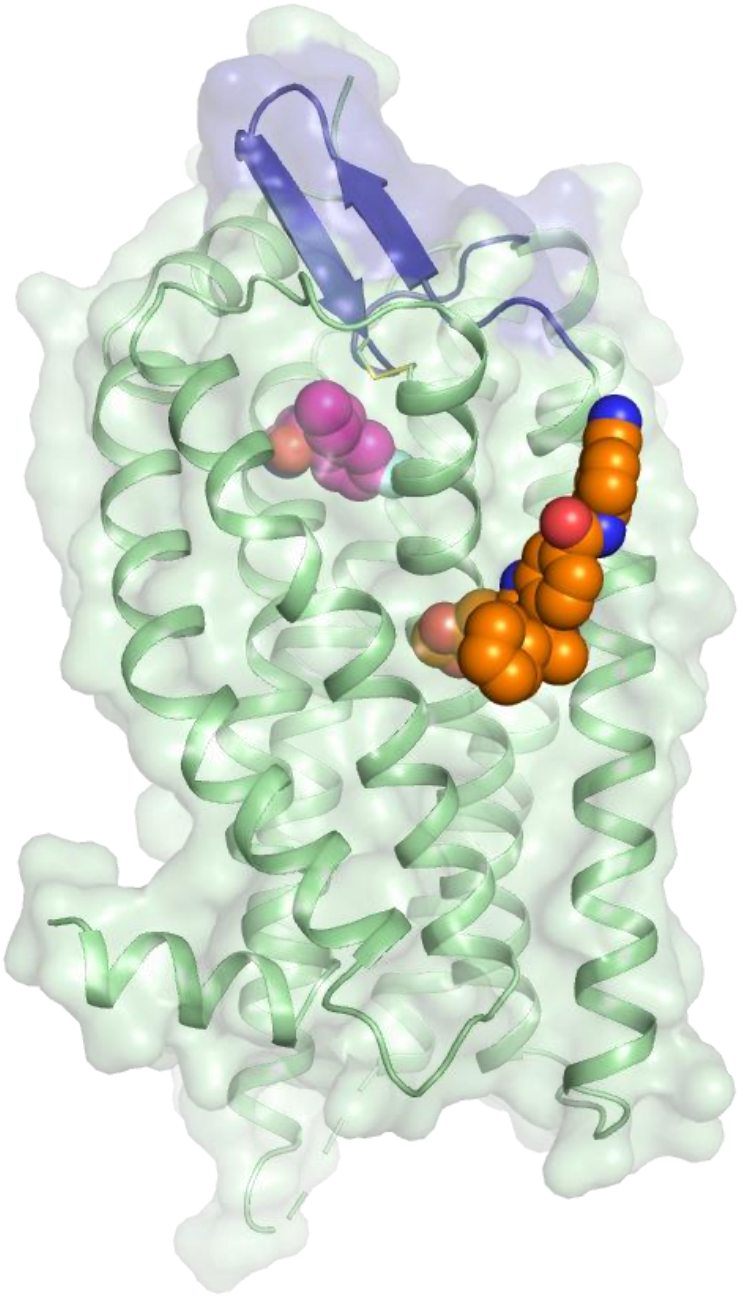
- Sub-plantar injection of protease (or activation peptide 2fLIGRL-NH2) triggers acute paw swelling in male Wistar rats
- Pre-treatment with AZ3451 (s.c.) or AZ8838 (p.o.) effectively reduce the swelling
- Histological analysis showed a reduction of activated mast cells and neutrophils in pretreated animals (not shown)

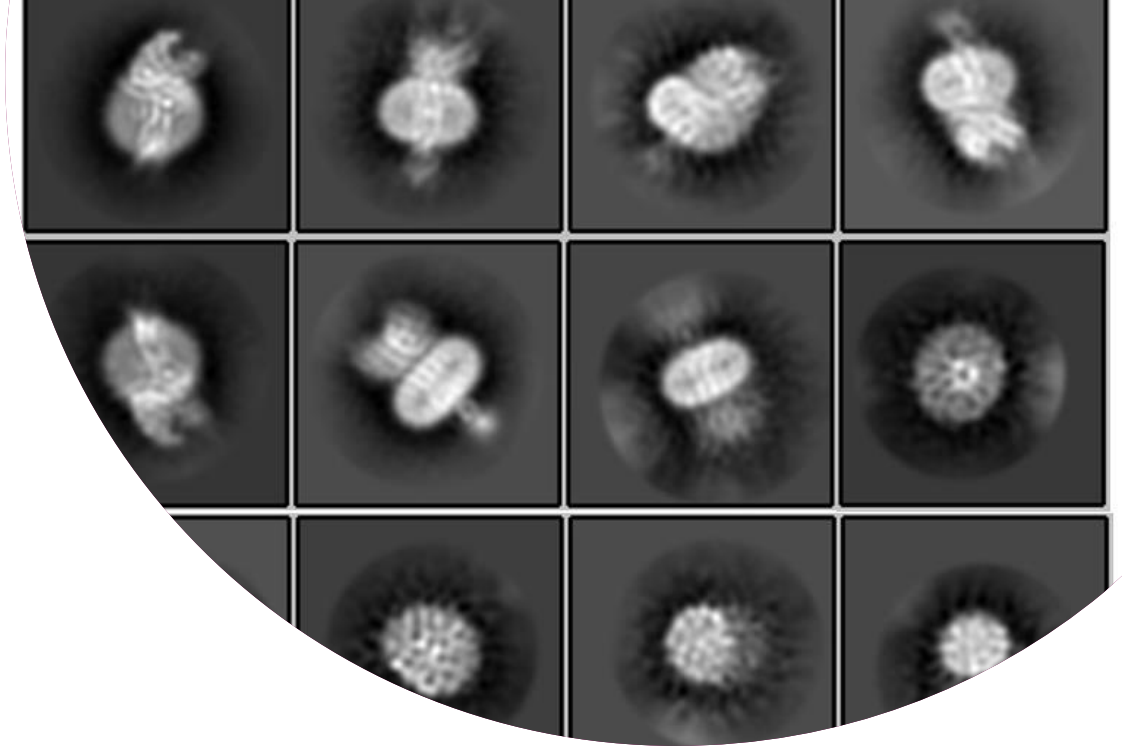


Kennedy, A. Jet al. (2020) *Communications Biology* ***3, 782***

Summary

- Purified stabilized PAR2 StaR enabled DNA encoded library screening, structural and biophysical studies
- Structural system was successfully transferred to AZ
    - 40 different protein batches produced (protein is co-purified with ligand)
    - 47040 crystallization drops setup
    - 2 additional complex structures determined
- Structural information was key for design in both imidazole and benzimidazole series
- AZ8838 and AZ3451:
    - bind in distinct pockets
    - are effective antagonists of G-protein and G-protein independent pathways
    - effectively reduce paw swelling in a rat paw oedema model





Looking ahead

- Increasing complexity of targets and targets with little precedence
- Further application of DNA-encoded library screening as rapid route for tool and hit-finding
- Riding the waves of the cryo-EM revolution

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Questions & discussion

Identifying new partnerships through our Open Innovation portal

+1,000

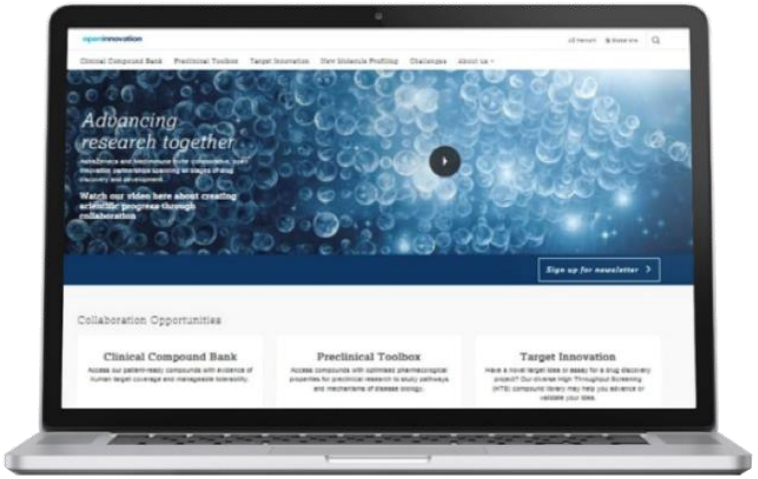
Proposals received from 40 countries on 6 continents

425

Pre-clinical studies and 35 clinical trials ongoing / planned

250,000

Our Open Innovation programme has over 250,000 compounds available from our screening library



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