

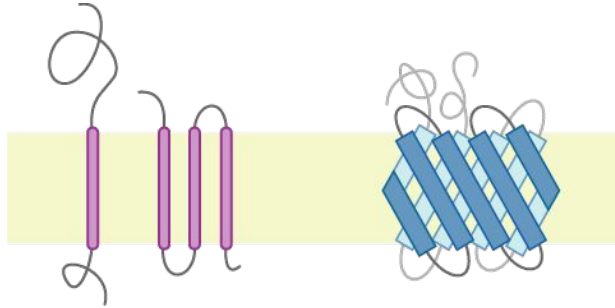


Membrane Proteins and Small-Angle Scattering

Marie Lycksell

Membrane proteins have a range of topologies and functions

Transmembrane proteins



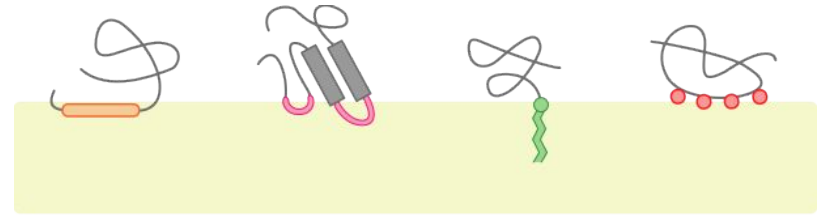
Proteins with an embedded section that **spans the full membrane**

Are permanently attached to a biological membrane → *Integral membrane proteins*

Functions include:

transport, channels, signaling, enzyme activity, structural anchoring to membranes, cell adhesion, ...

Peripheral membrane proteins



Membrane associated proteins that **do not span the full membrane**

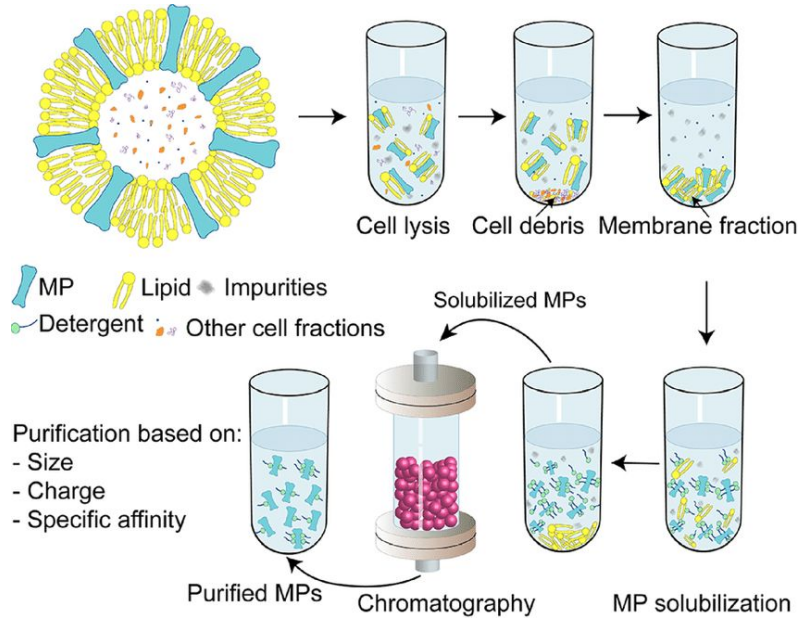
May be permanently attached (integral) or temporarily adhered (not integral)

Functions include:

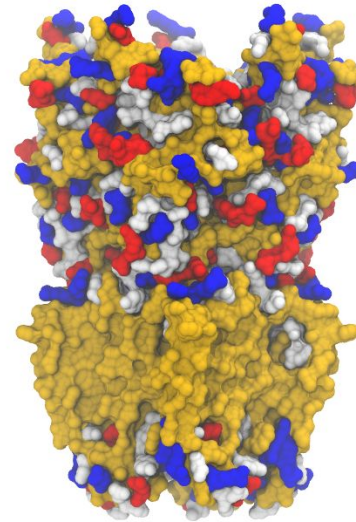
enzyme activity, structural anchoring to membranes, membrane targeting, carriers, toxins, ...

Membrane proteins are often challenging to work with

Example of typical membrane protein purification protocol:

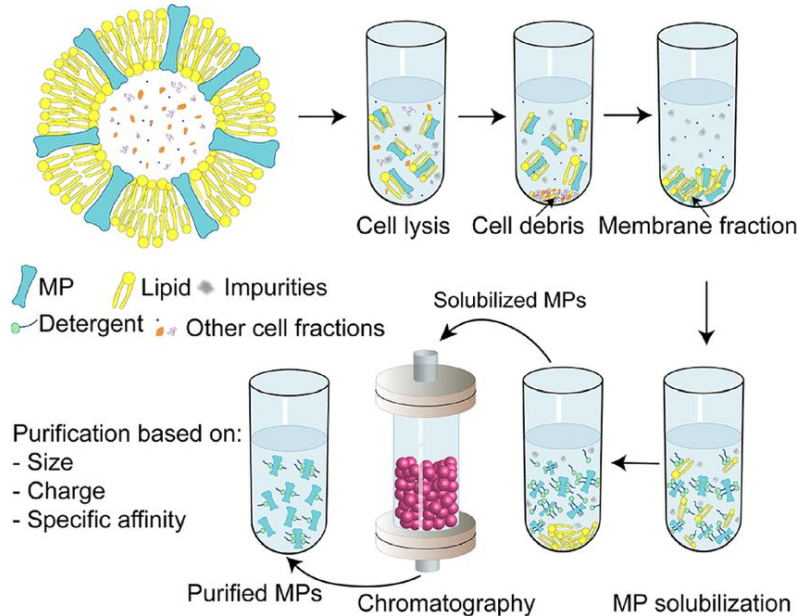


- Yield is often lower than for soluble proteins
- Often aggregation prone due to large hydrophobic surfaces

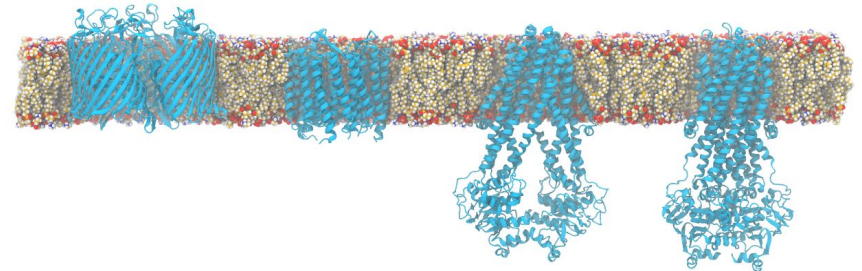


Membrane proteins are often challenging to work with

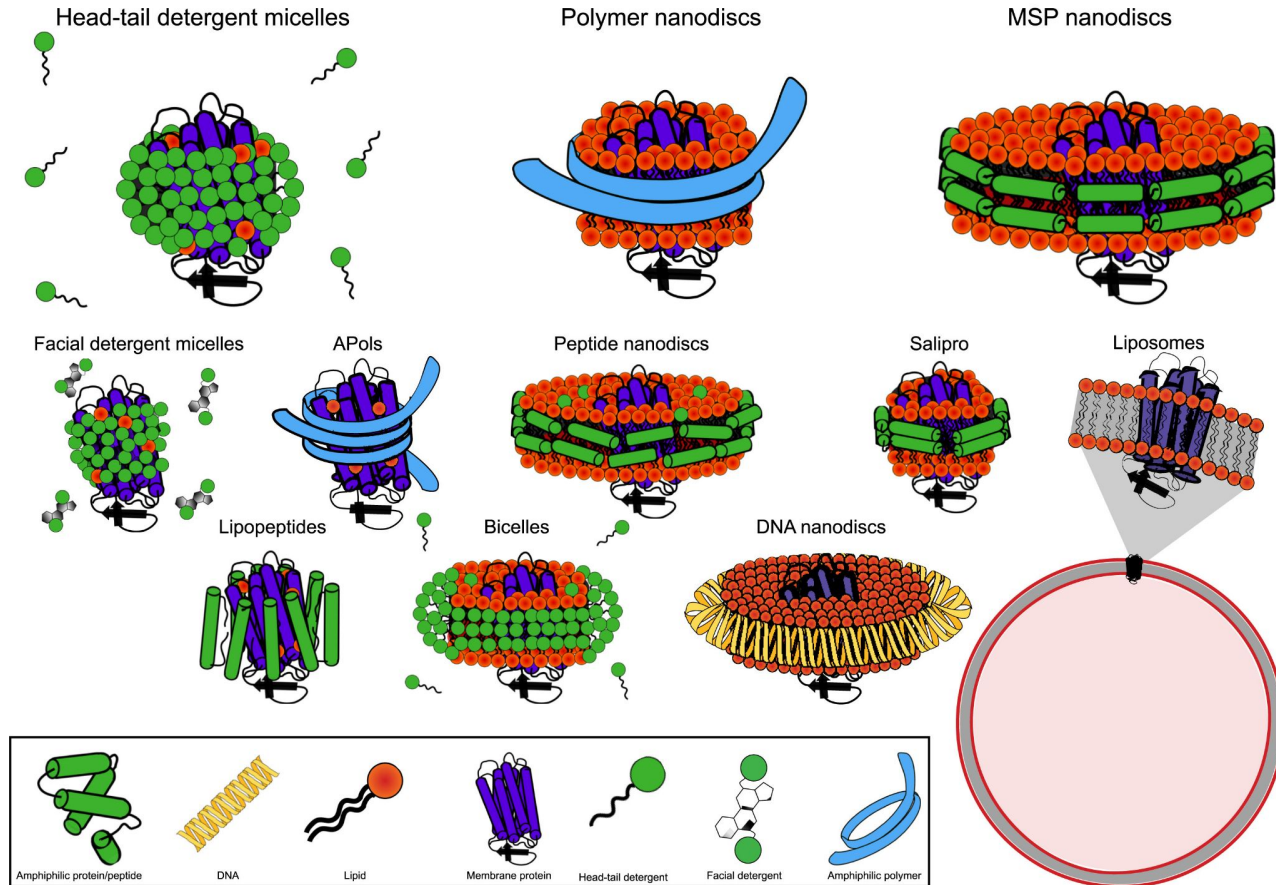
Example of typical membrane protein purification protocol:



- Yield is often lower than for soluble proteins
- Often aggregation prone due to large hydrophobic surfaces
- Integral membrane proteins require membrane mimetics
 - which may need to be handled in later data analysis (i.e. you need to describe the protein-membrane mimetic complex rather than just the protein)
- Conformational changes are often subtle



Several types of membrane mimetics are available



- **Lipid nanodiscs**
- Bicelles
- Amphipols
- Liposomes
- And more

Johansen, N. T., et al. "Travel light: Essential packing for membrane proteins with an active lifestyle." *Biochimie* 205 (2023): 3-26.

Models in analysis for membrane mimetics

Analytical models

- Mathematical expression to describe the system
- Typically describing volumes that have scattering length densities/electron densities

Atomistic models

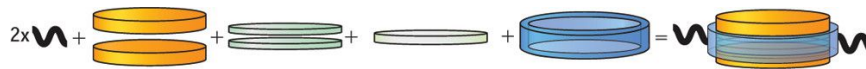
- Can be generated for various membrane mimetics by tools like Charmm-GUI & Shapspyer
- Requires choosing e.g. the number of detergent molecules, a parameter which may need tuning
- Probably need averaging for good fit to SAS data

Ab initio models

- Generates models based on the scattering profile
- Using multiple bead types is beneficial (possible in e.g. MONSA)

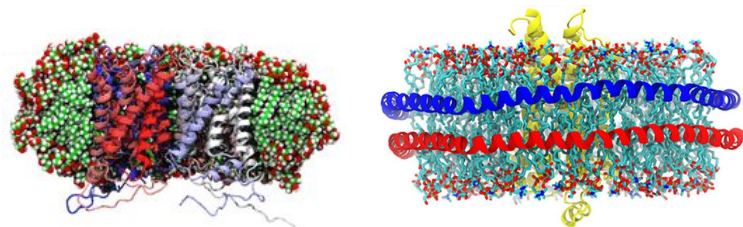
Hybrid models

- Combines different model types
- Often atomistic for the protein and analytical or *ab initio* for the membrane mimetic

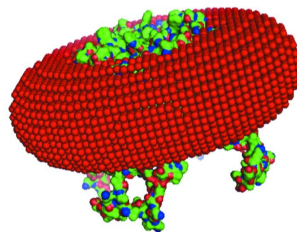


$$A_{tags} + A_{cap} + A_{tails} + A_{meth} + A_{belt} = A_{disc}$$

Skar-Gislinge, N., et al. Journal of the American Chemical Society 132.39 (2010): 13713-13722.

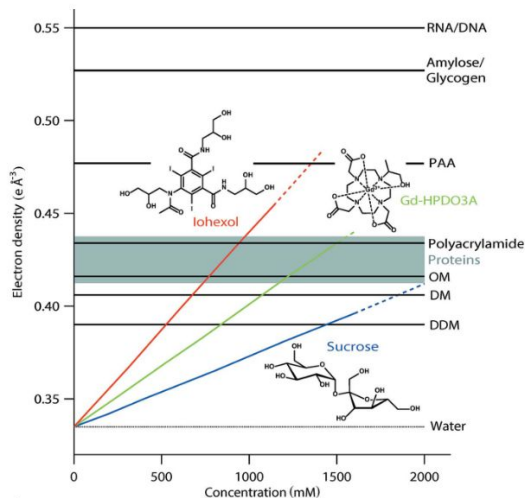


Chen, P., Hub., J.S., The Journal of Physical Chemistry Letters 6.24 (2015): 5116-5121.
Cheng, X., et al. Journal of Chemical Information and Modeling 53.8 (2013): 2171-2180.

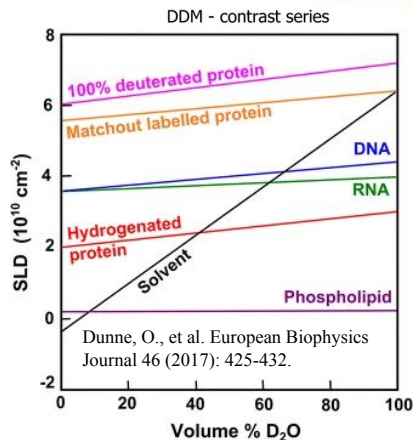


Pérez, J., Koutsioubas, A. "Memprot: a program to model the detergent corona around a membrane protein based on SEC-SAXS data." Acta Crystallographica Section D: Biological Crystallography 71.1 (2015): 86-93.

Avoiding modelling the membrane mimetic: Contrast matching



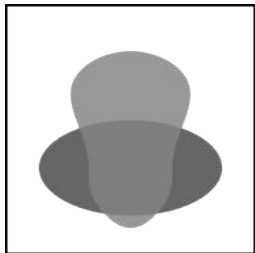
Gabel, F., et al. IUCrJ 6.4 (2019): 521-525.



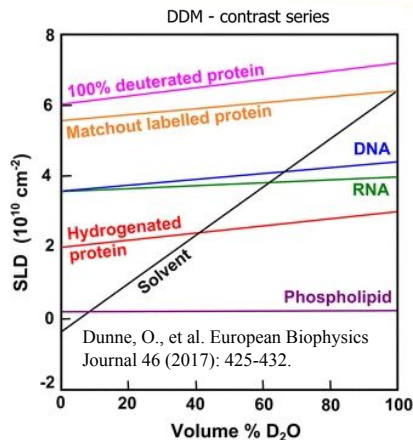
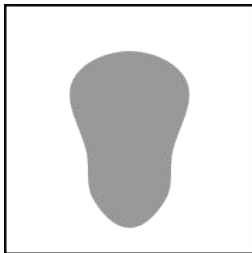
- Match the SLD of the buffer and the membrane mimetic → only protein visible
- Easier to do for neutron scattering than X-ray
 - X-rays: Add e^- rich buffer components, e.g. sugars, glycerol, salts, medical contrast agents
 - Neutrons: Utilize that H and D have different b

Avoiding modelling the membrane mimetic: Contrast matching

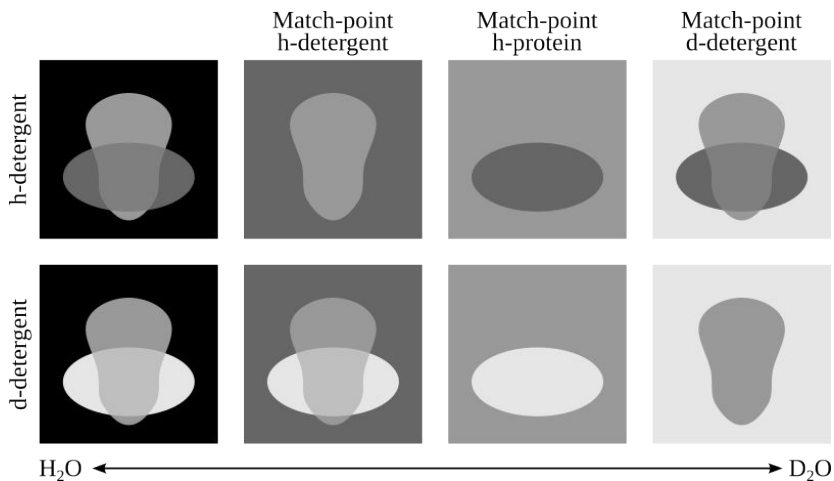
Have:



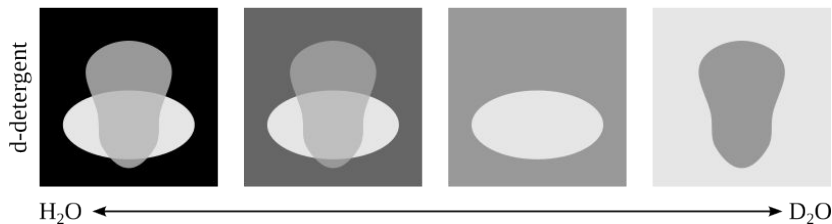
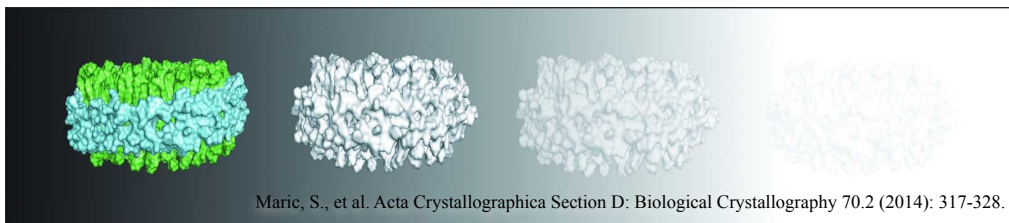
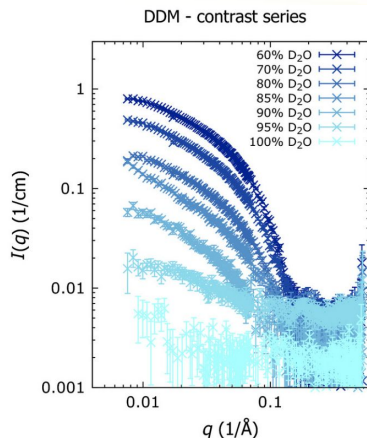
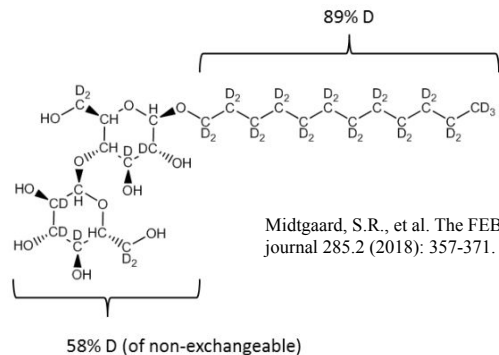
But interested in:



- Match the SLD of the buffer and the membrane mimetic → only protein visible
- Easier to do for neutron scattering than X-ray
 - X-rays: Add e^- rich buffer components, e.g. sugars, glycerol, salts, medical contrast agents
 - Neutrons: Utilize that H and D have different b
- Can match SLD_{buffer} to SLD_{mimetic} by using a suitable amount of D_2O in the buffer
- Can match SLD_{mimetic} to SLD_{buffer} by using deuterated material in the mimetic

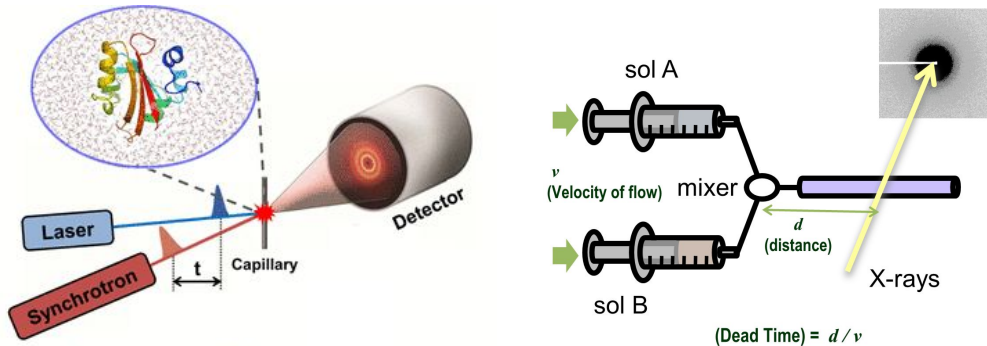
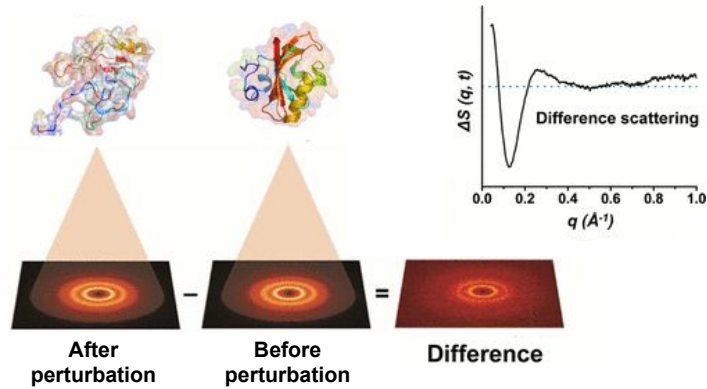


Avoiding modelling the membrane mimetic: Contrast matching



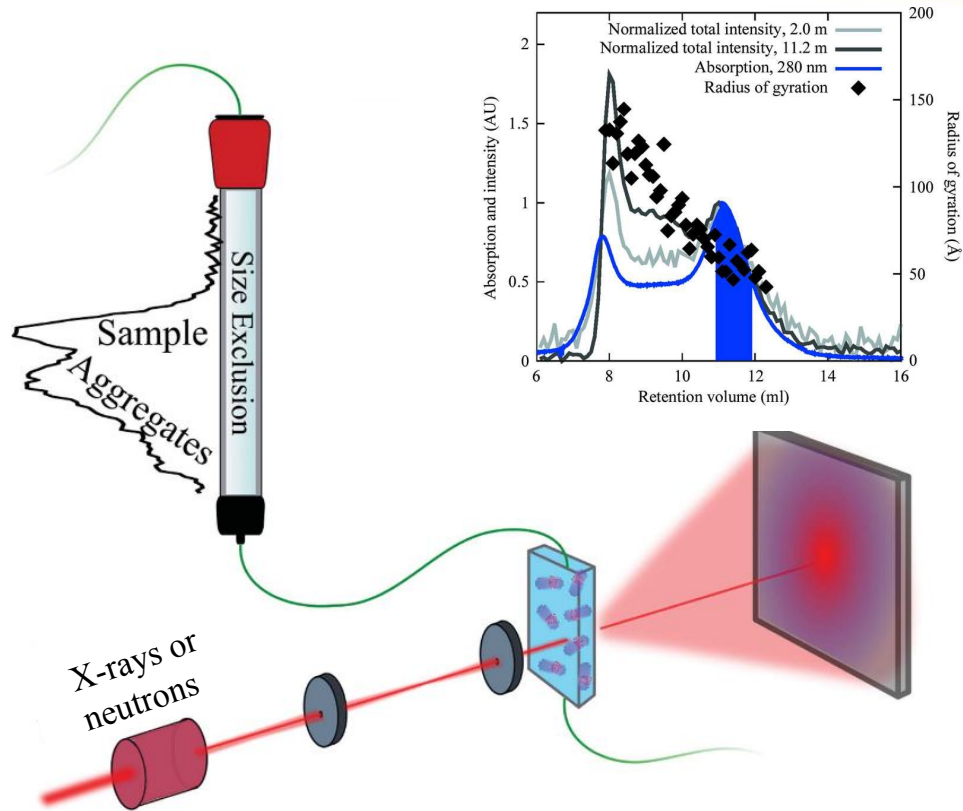
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- Easier to do for neutron scattering than X-ray
 - X-rays: Add e^- rich buffer components, e.g. sugars, glycerol, salts, medical contrast agents
 - Neutrons: Utilize that H and D have different b
- Can match SLD_{buffer} to SLD_{mimetic} by using a suitable amount of D₂O in the buffer
- Can match SLD_{mimetic} to SLD_{buffer} by using deuterated material in the mimetic
 - Deuterated detergents and nanodiscs have been developed
- Usually desirable to match out at high %D₂O
 - Large contrast between D-rich buffer and H-rich protein
 - H has a large incoherent scattering that contribute to the background, so less is good
 - D₂O may exacerbate aggregation issues

Avoiding modelling the membrane mimetic: Difference scattering



- **Difference scattering:** Scattering from a reference condition subtracted from the scattering measured after a perturbation
 - Contains information on how the system has changed
 - If the fluctuations in the perturbation doesn't disturb the membrane mimetic, the data can be fit considering only the membrane protein
 - Modelling generally require a large amount of prior knowledge of the system
- **Time-Resolved X-ray Solution Scattering (TR-XSS)** is the usual measuring technique
 - Laser for light induced perturbations (Pump-probe scheme)
 - Stopped-flow cell for perturbations induced by buffer components

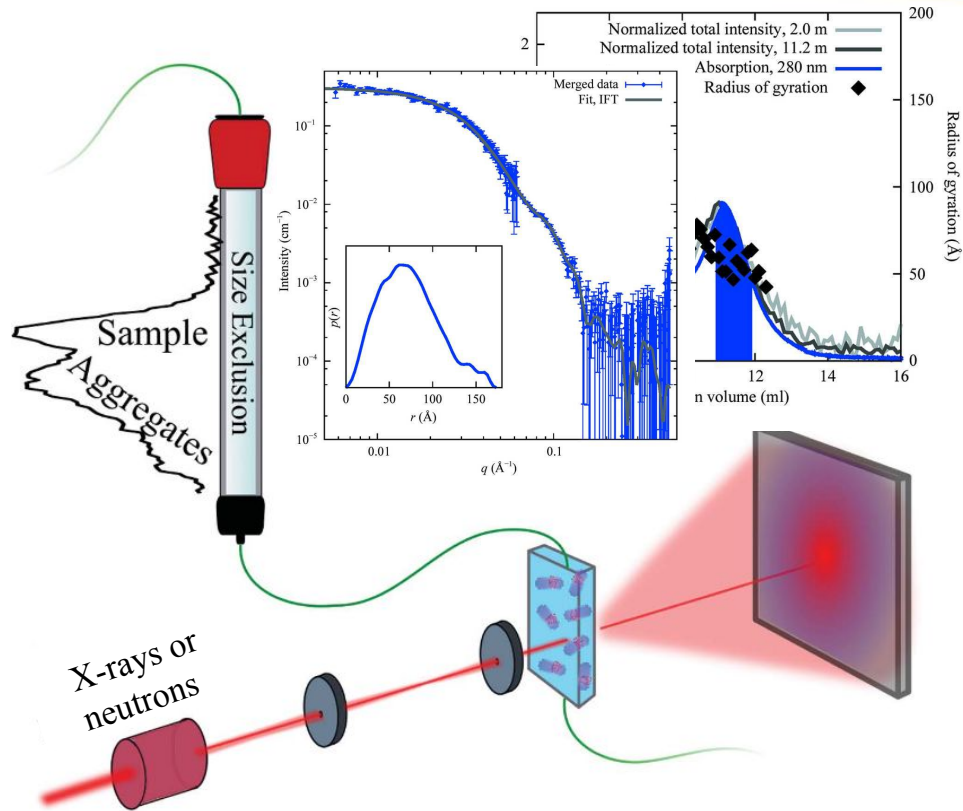
Size Exclusion Chromatography coupled SAS



- Sample passes through a SEC, then goes to an in-line SAS measuring cell
- Separates sample by size
 - Good for separating aggregates from the main protein sample of interest
 - Bring as pure samples as possible and utilize to minimize heterogeneity during the measurement
- Detector images are collected during a short time each, thus recording $I(q)$ as a function of t
 - Scatterogram: $\sum_q I(q,t)$ plotted vs t

Figure adapted from: Johansen, N. T., et al. "Introducing SEC-SANS for studies of complex self-organized biological systems." *Acta Crystallographica Section D: Structural Biology* 74.12 (2018): 1178-1191.

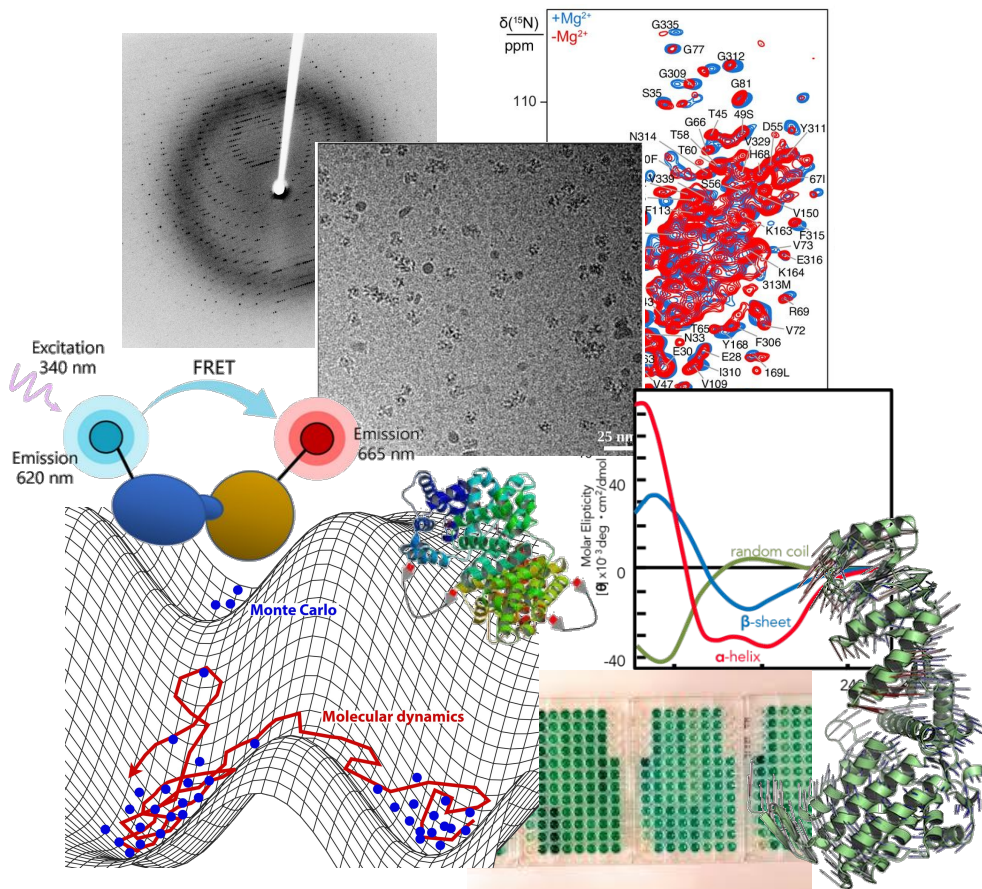
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 - Bring as pure samples as possible and utilize to minimize heterogeneity during the measurement
- Detector images are collected during a short time each, thus recording $I(q)$ as a function of t
 - Scatterogram: $\sum_q I(q,t)$ plotted vs t
 - Scattering profile from averaging select frames chosen based on the chromatogram/scatterogram
- Sample is diluted by the SEC → drawback as high concentration is important for strong signal

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SAS methods are often used in conjunction with other methods



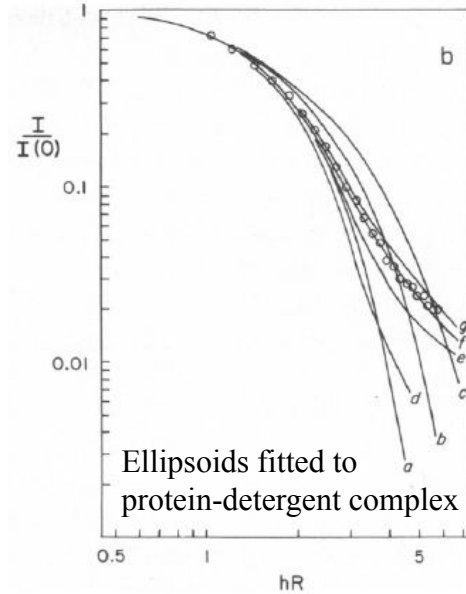
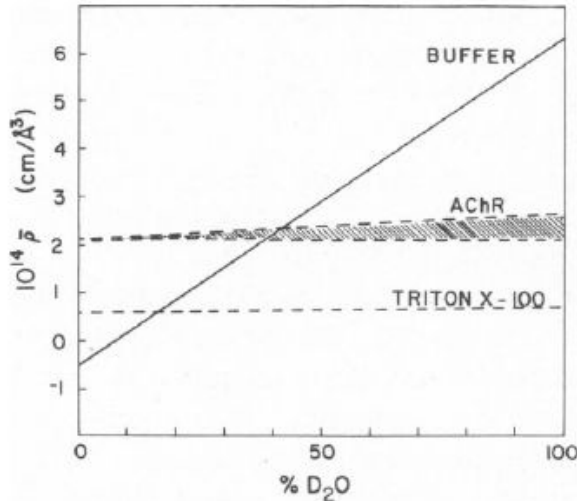
- High resolution structural methods
 - Crystal diffraction
 - Single particle cryo-EM
 - NMR
- Other structural methods
 - DLS, SEC-MALS, FRET, crosslinking, IR, circular dichroism, ect.
- Functional methods
 - E.g. activity assays
- Simulation methods
 - Molecular dynamics simulations
 - Monte Carlo simulations
 - Normal mode analysis

The background features a series of colorful lines in shades of yellow, green, blue, and purple that radiate from the right side towards the left. In the upper left quadrant, there is a faint, light gray grid pattern. The overall aesthetic is clean and modern.

A few examples of SAS on membrane proteins

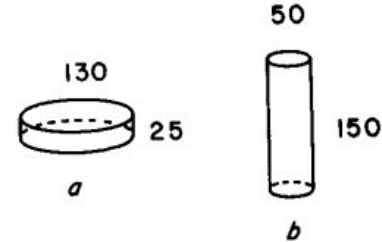
The Nicotinic Acetylcholine Receptors and SANS in 1979

Wise, D. S., Karlin, A., and Schoenborn, B. P. "An analysis by low-angle neutron scattering of the structure of the acetylcholine receptor from *Torpedo californica* in detergent solution." *Biophysical journal* 28.3 (1979): 473-496.



Applying knowledge of the system:

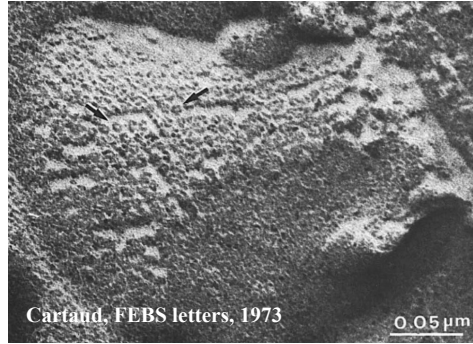
- R_g, V_{molecule}



1:4 ellipsoid
40 x 140 x 140 Å

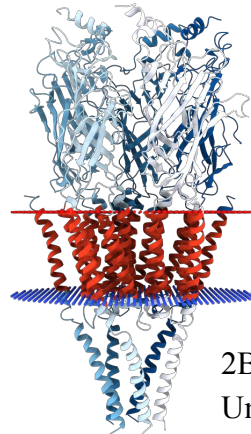
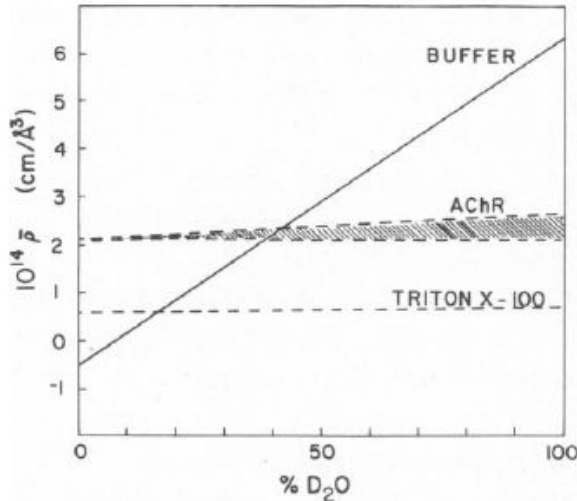
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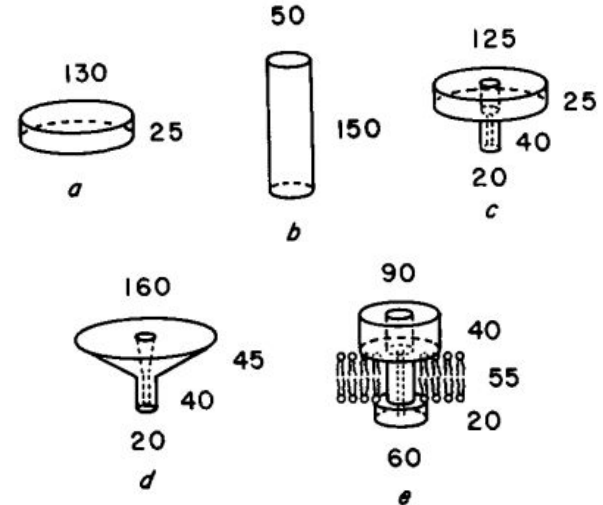


Applying knowledge of the system:

- R_g, V_{molecule}
- Probably have membrane spanning pore
- Estimated dimensions from X-ray diffraction, electron microscopy, ect.

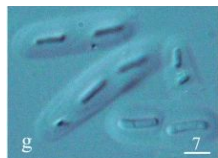


2BG9
Unwin (2005)

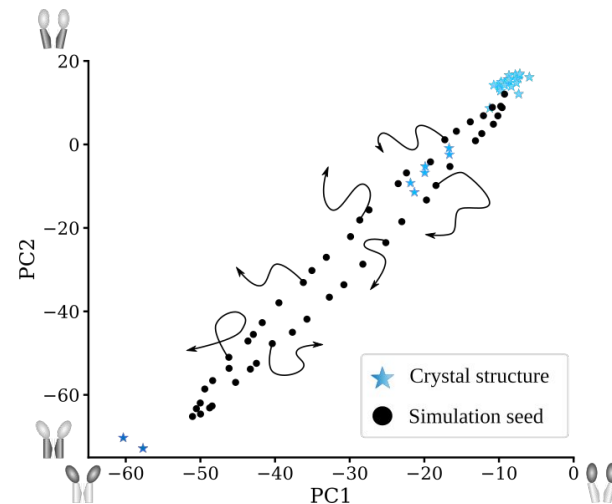
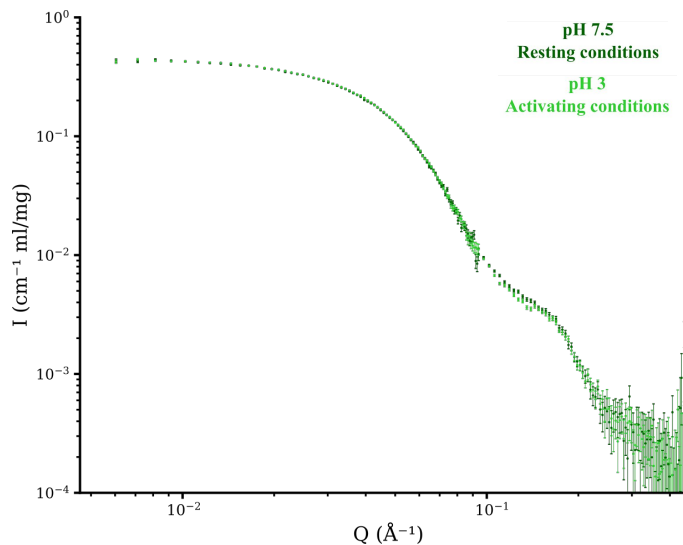
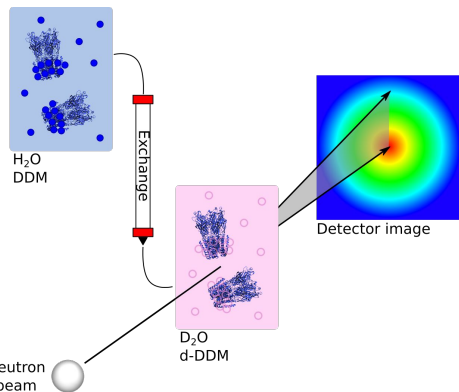
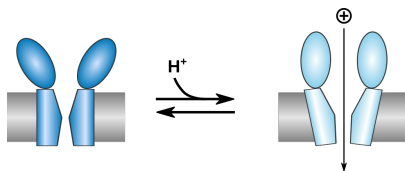


Contemporary SANS on Pentameric Ligand-Gated Ion Channels

Lycksell, M., et al. "Probing solution structure of the pentameric ligand-gated ion channel GLIC by small-angle neutron scattering." *Proceedings of the National Academy of Sciences* 118.37 (2021)

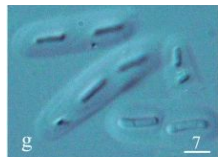


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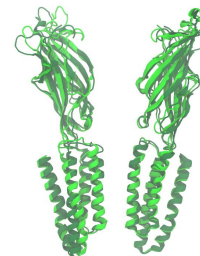
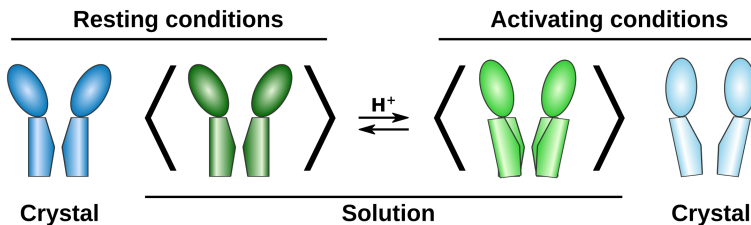
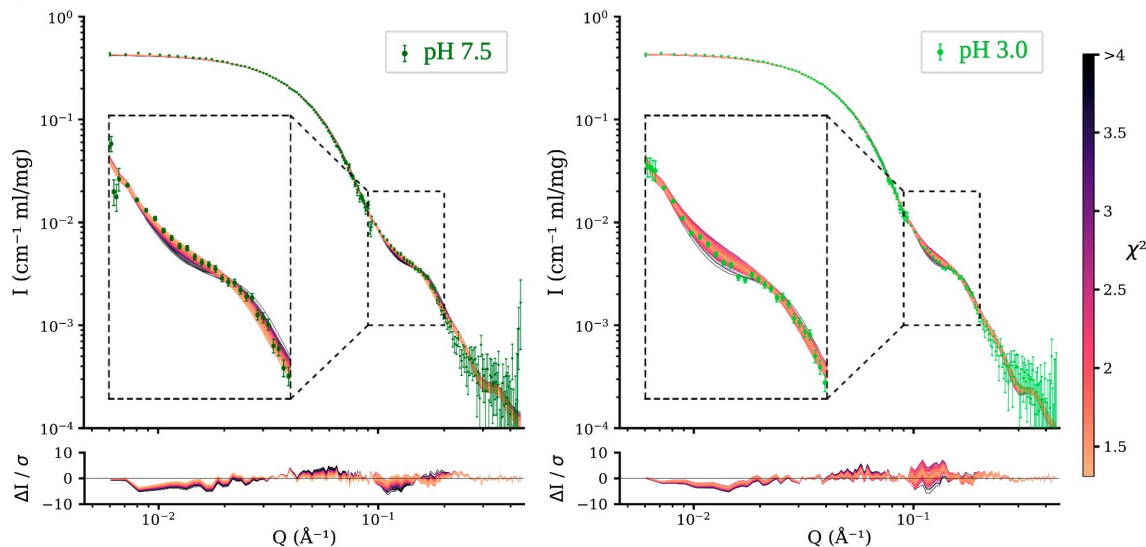
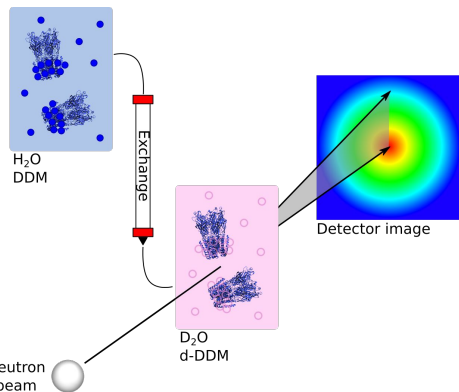
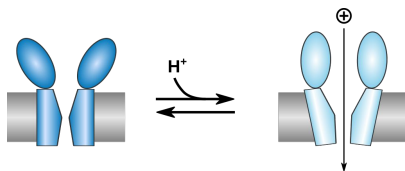


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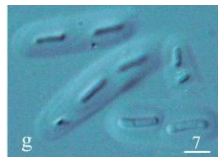


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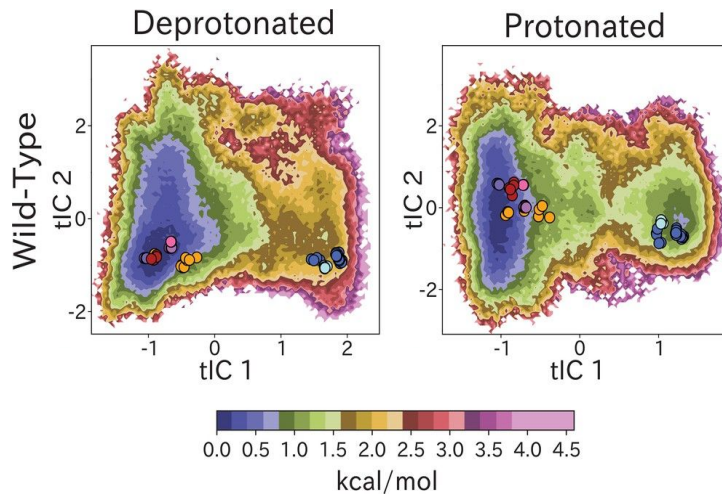


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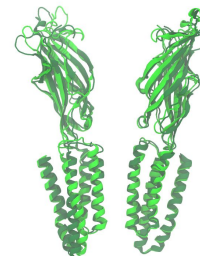
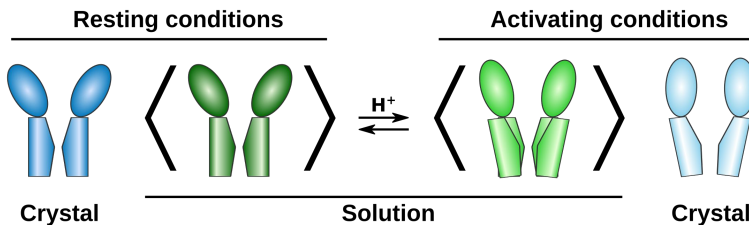
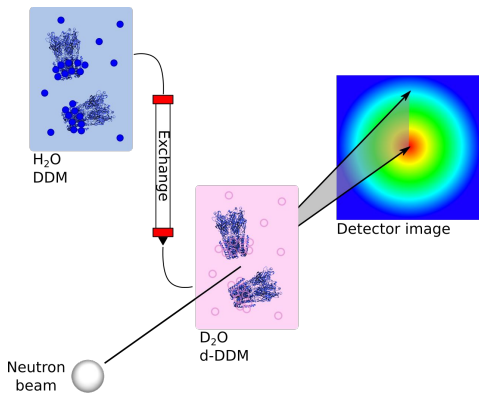
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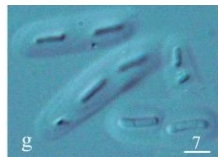


Bergh, C., et al. "Markov state models of proton- and pore-dependent activation in a pentameric ligand-gated ion channel." *Elife* 10 (2021): e68369.

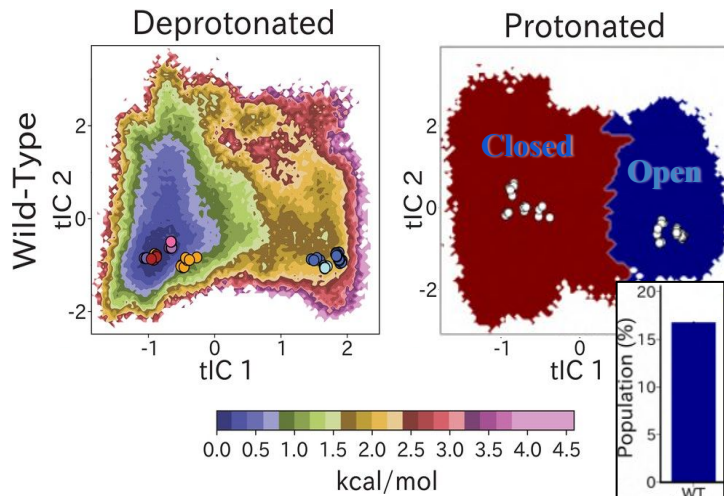


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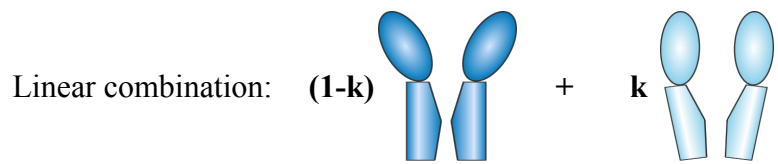
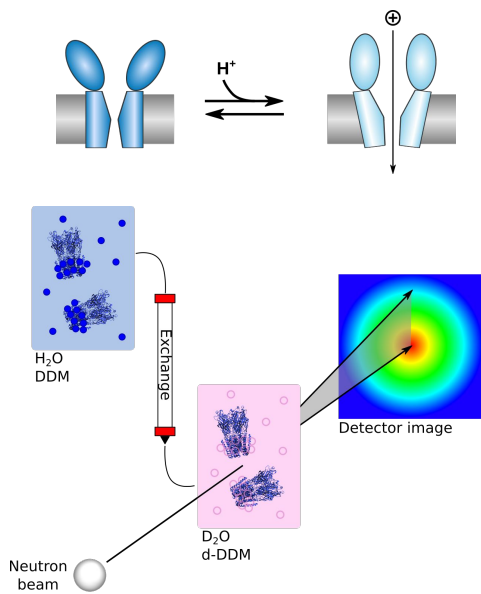
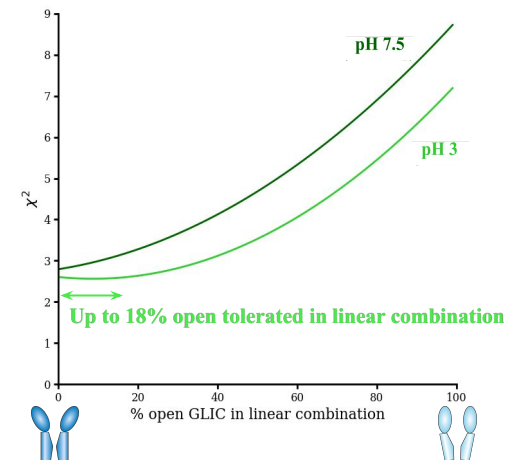
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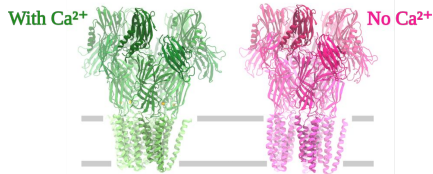


Finding conformations eluding high-res structure determination

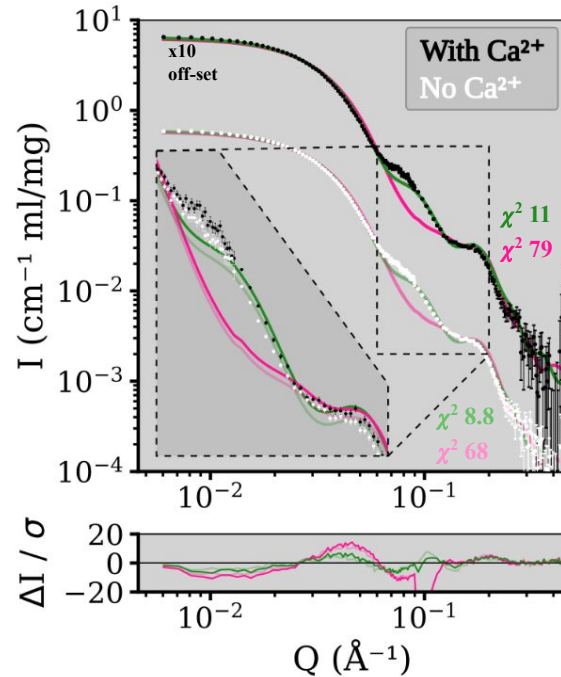
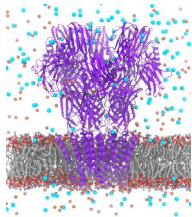
Lycksell, M., Rovšnik, U., et al. "Biophysical characterization of calcium-binding and modulatory-domain dynamics in a pentameric ligand-gated ion channel." Proceedings of the National Academy of Sciences 119.50 (2022)



Wilbanks, E.G., et al. Environmental microbiology 16.11 (2014): 3398-3415.



Hu, H., et al. PNAS 117.24 (2020): 13437-13446.

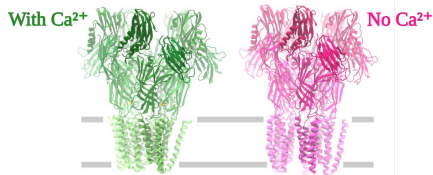


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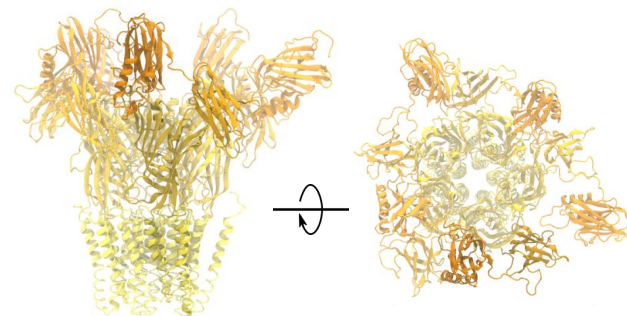
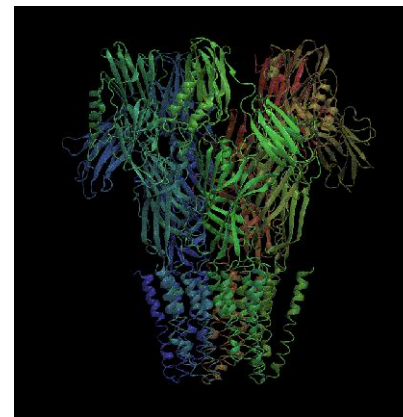
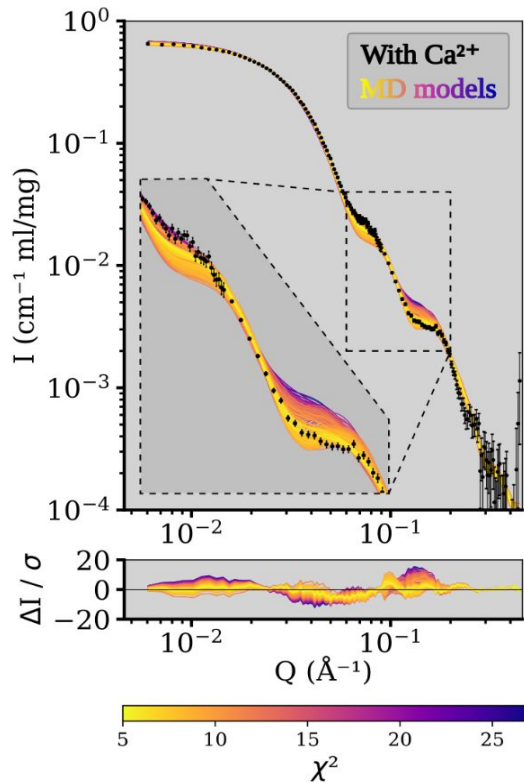
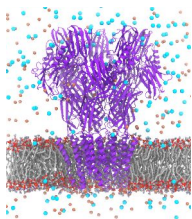
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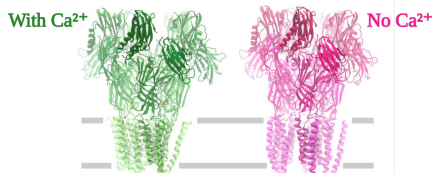


Finding conformations eluding high-res structure determination

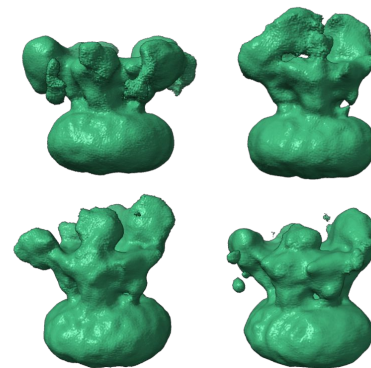
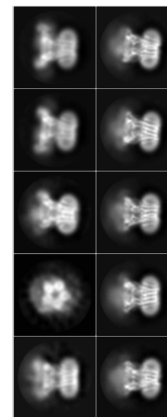
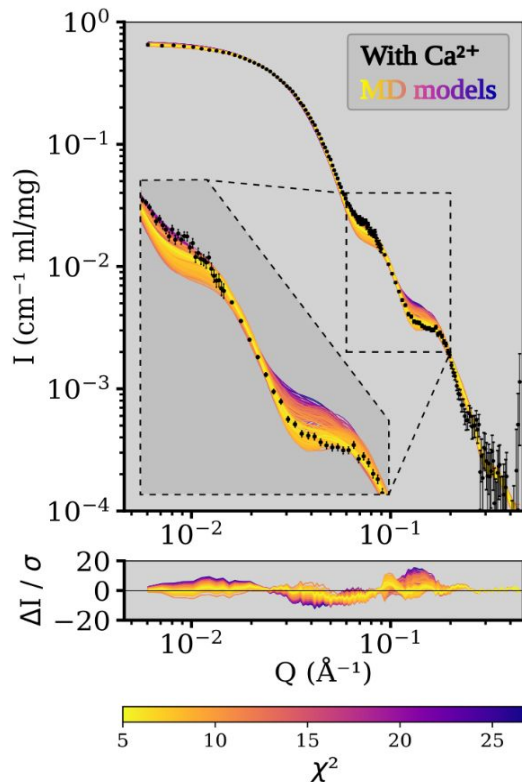
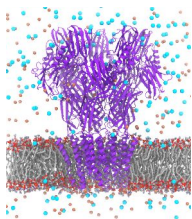
Lycksell, M., Rovšnik, U., et al. "Biophysical characterization of calcium-binding and modulatory-domain dynamics in a pentameric ligand-gated ion channel." *Proceedings of the National Academy of Sciences* 119.50 (2022)



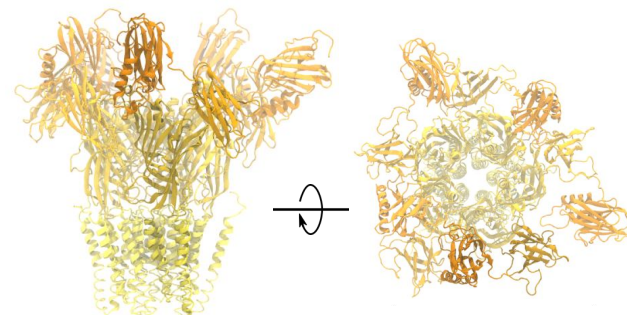
Wilbanks, E.G., et al. *Environmental microbiology* 16.11 (2014): 3398-3415.



Hu, H., et al. *PNAS* 117.24 (2020): 13437-13446.

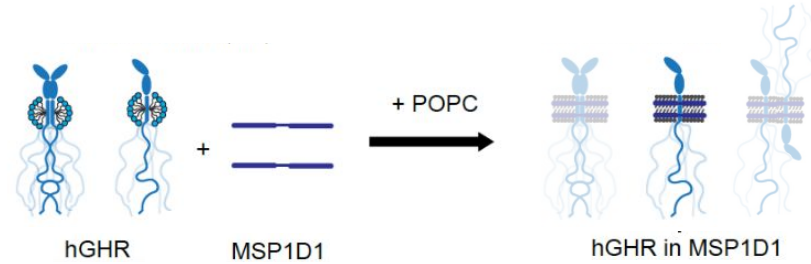
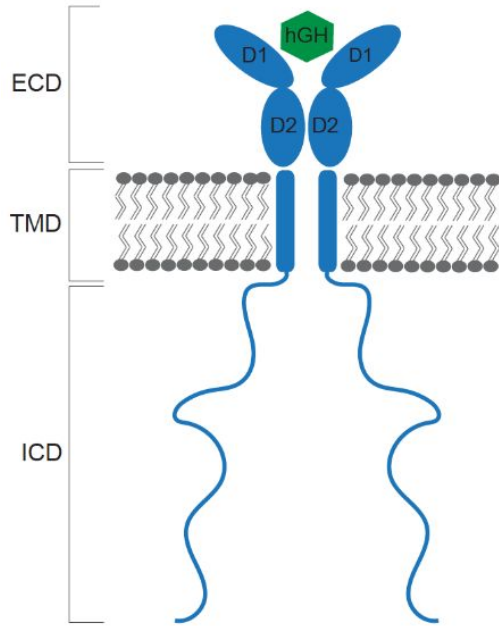


Rovšnik, U., Andén O., Lycksell M., et al., Manuscript

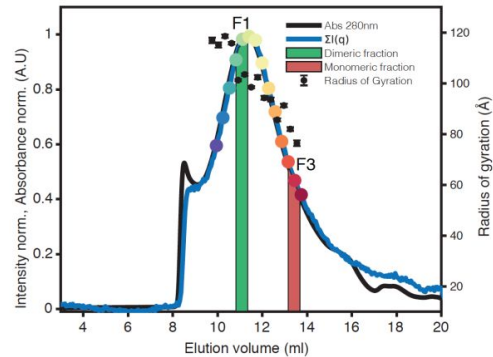


Order and disorder - An integrative structure of the full-length human growth hormone receptor

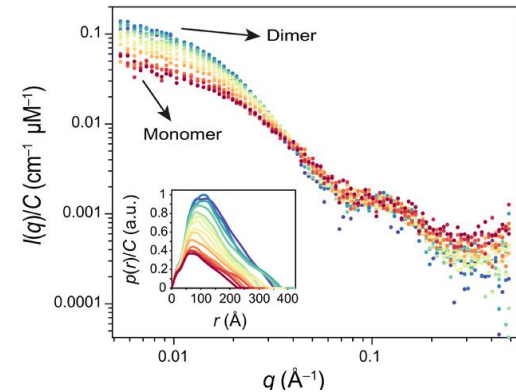
Kassem, N., et al. Science Advances 7.27 (2021): eabh3805.



SEC-SAXS chromatogram & scatterogram

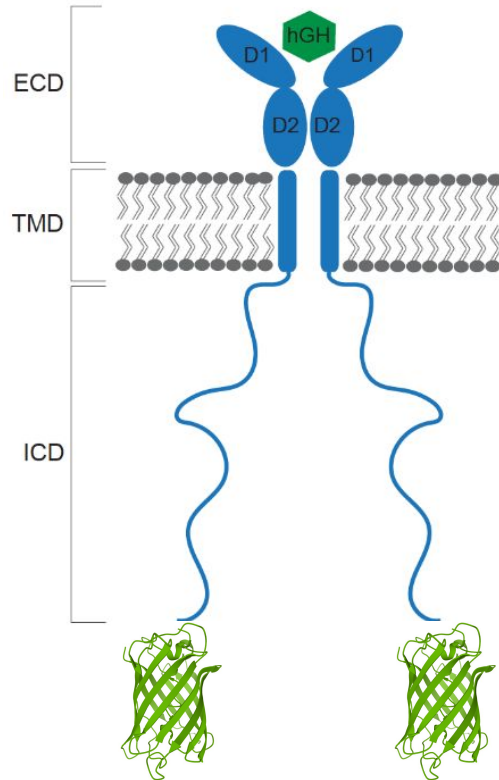


SEC-SAXS scattering profiles

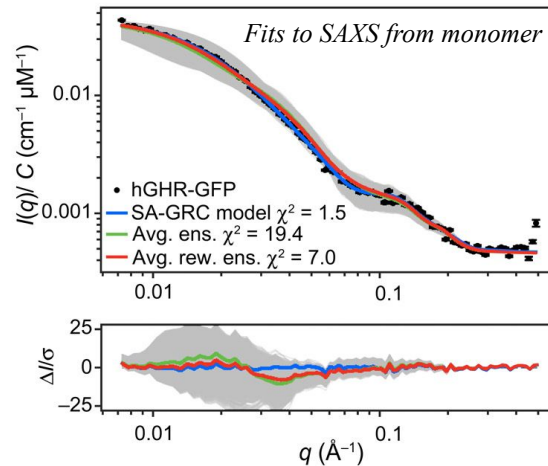
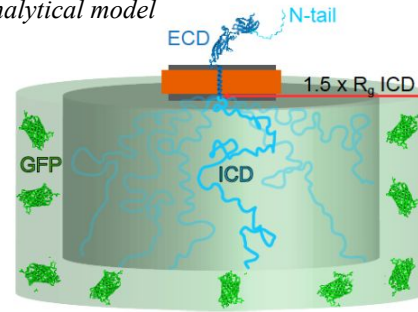


Order and disorder - An integrative structure of the full-length human growth hormone receptor

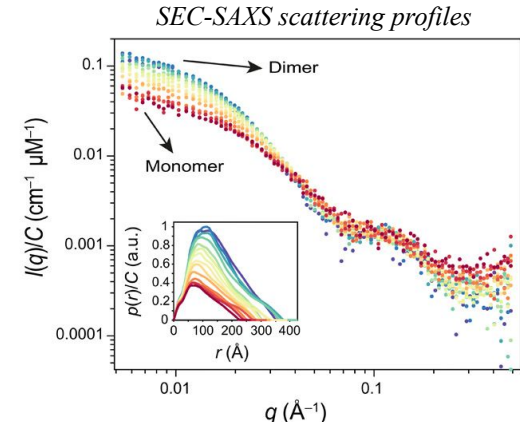
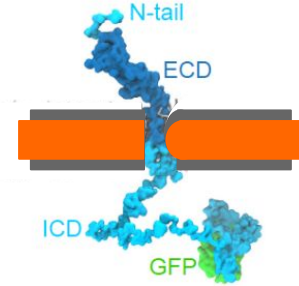
Kassem, N., et al. Science Advances 7.27 (2021): eabh3805.



Semianalytical model

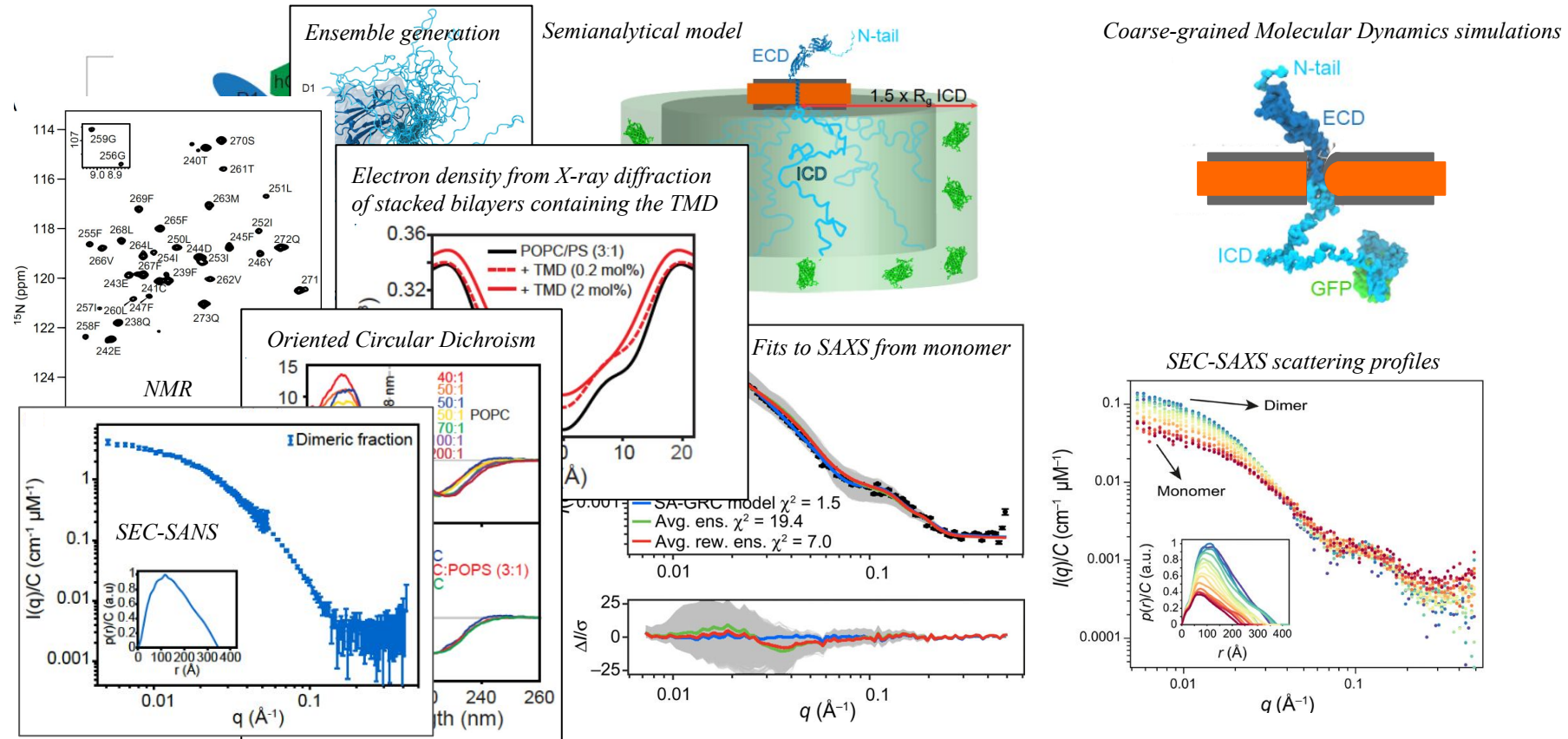


Coarse-grained Molecular Dynamics simulations



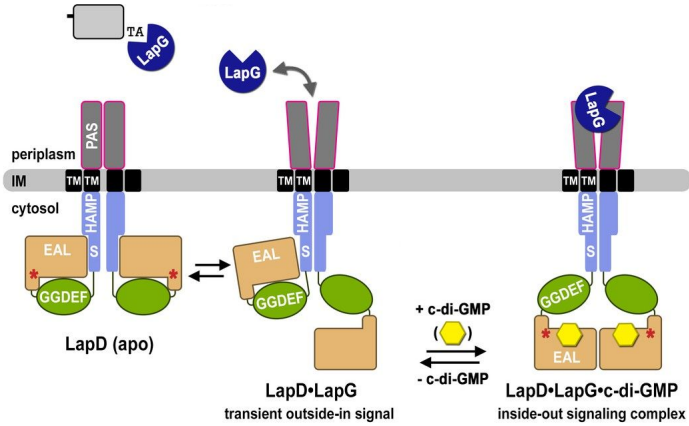
Order and disorder - An integrative structure of the full-length human growth hormone receptor

Kassem, N., et al. *Science Advances* 7.27 (2021): eabh3805.

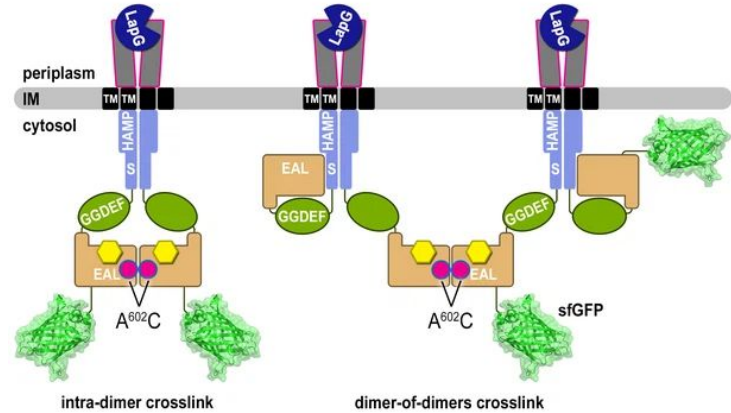


Conformational changes and complex formation observed with SAXS

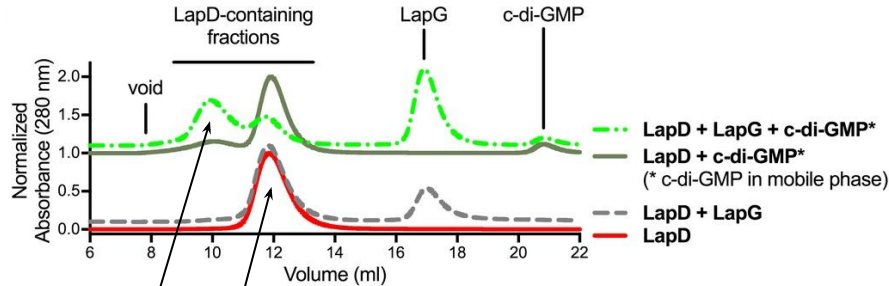
Cooley, R.B., O'Donnell, J.P., Sondermann, H. "Coincidence detection and bi-directional transmembrane signaling control a bacterial second messenger receptor." *Elife* 5 (2016): e21848.



Crosslinking showed where dimers join to form dimer-of-dimers



Chromatograms from SEC-MALS

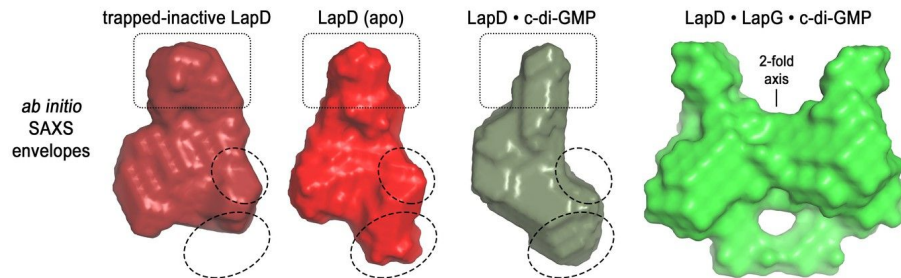
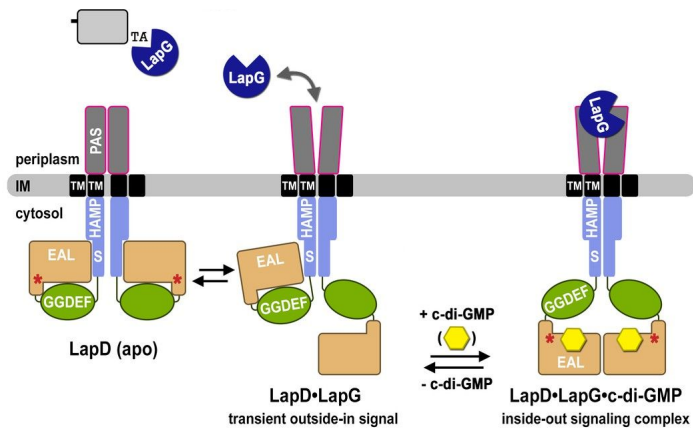


M_w matched LapD dimer

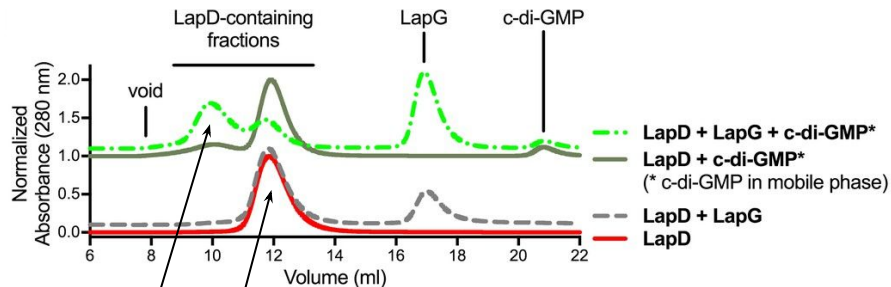
M_w matched LapD dimer-of-dimers

Conformational changes and complex formation observed with SAXS

Cooley, R.B., O'Donnell, J.P., Sondermann, H. "Coincidence detection and bi-directional transmembrane signaling control a bacterial second messenger receptor." *Elife* 5 (2016): e21848.

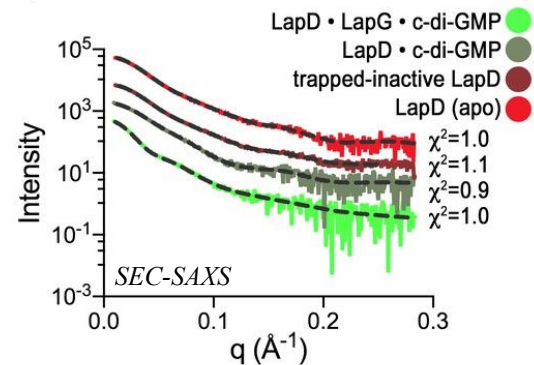


Chromatograms from SEC-MALS



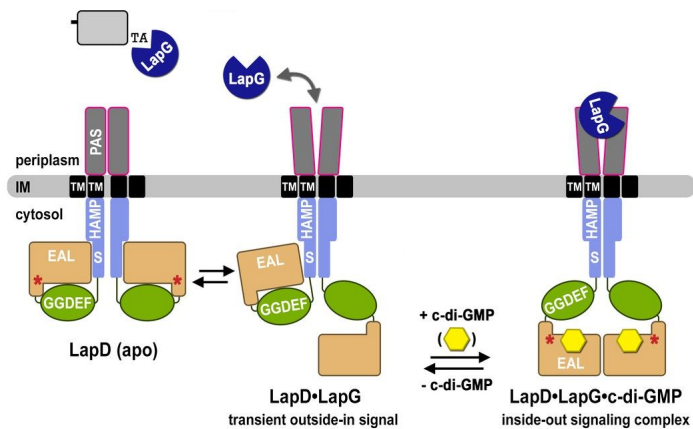
M_w matched LapD dimer

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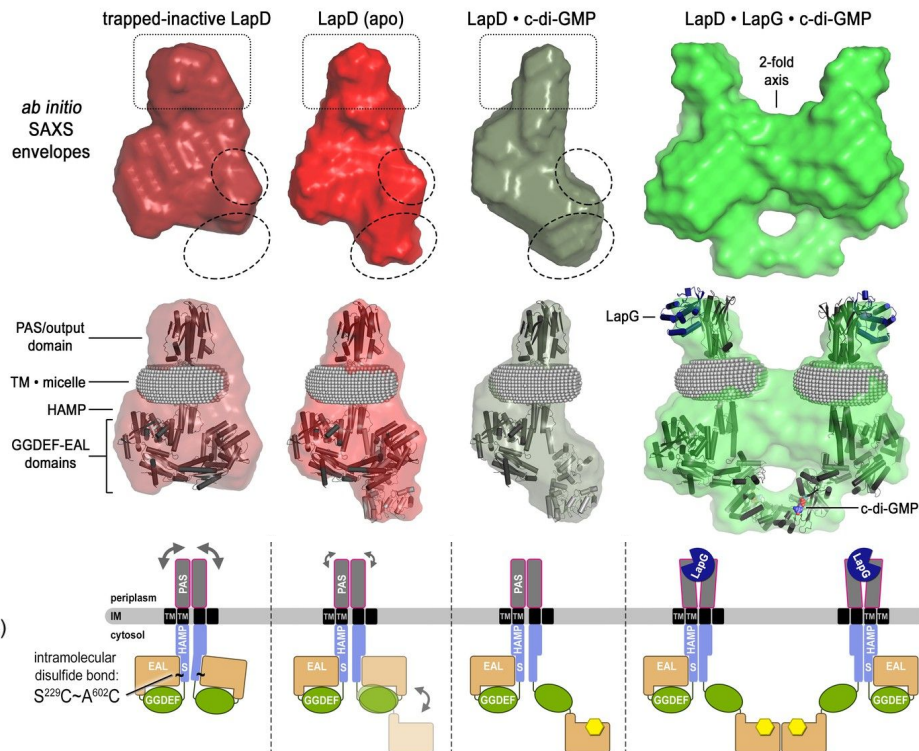
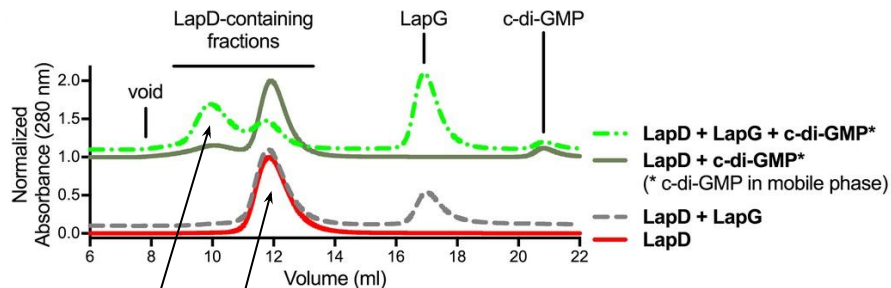


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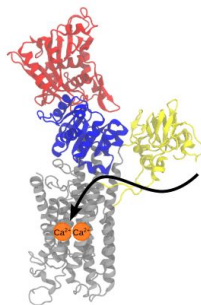
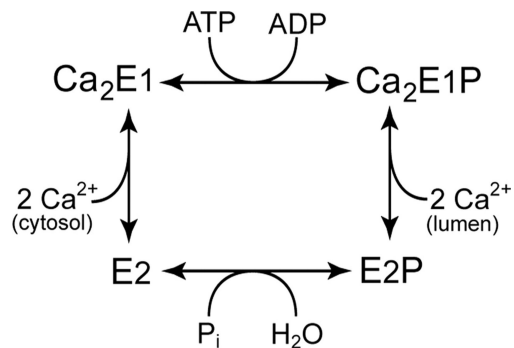
Chromatograms from SEC-MALS



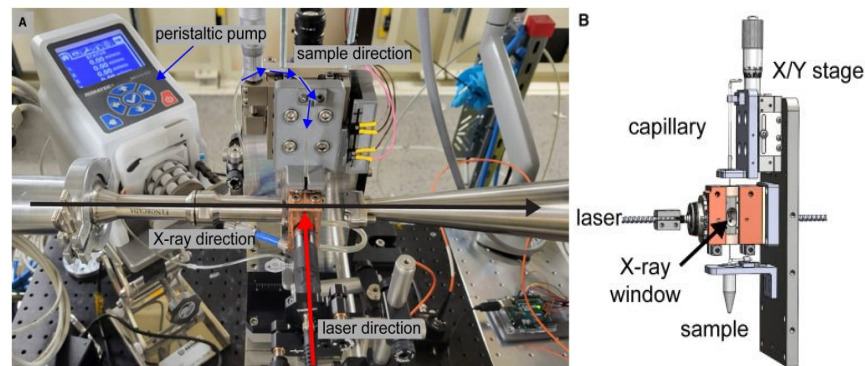
M_w matched LapD dimer-of-dimers

Tracking Ca^{2+} ATPase intermediates in real time by X-ray solution scattering

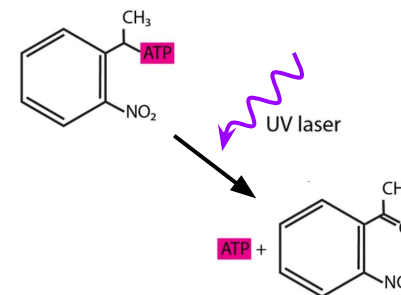
Ravishankar H., et al., *Science Advances* 6 (2020). DOI:10.1126/sciadv.aaz0981



Anthonisen, A.N., et al. *Journal of Biological Chemistry* 281.42 (2006): 31572-31582.



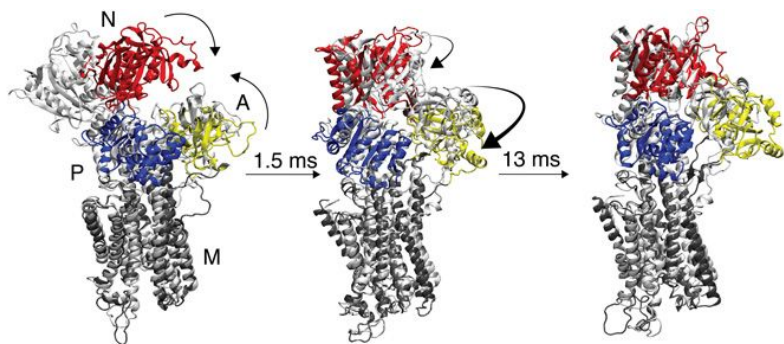
Magkakis, K., Orädd, F., et al. "Real-time structural characterization of protein response to a caged compound by fast detector readout and high-brilliance synchrotron radiation." *Structure* (2024).



Adapted from Orädd, F., Andersson M. *The Journal of Membrane Biology* 254 (2021): 51-64.

Tracking Ca^{2+} ATPase intermediates in real time by X-ray solution scattering

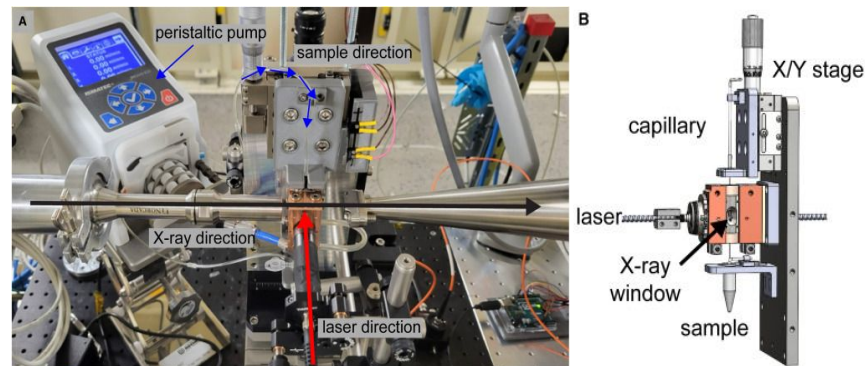
Ravishankar H., et al., *Science Advances* 6 (2020). DOI:10.1126/sciadv.aaz0981



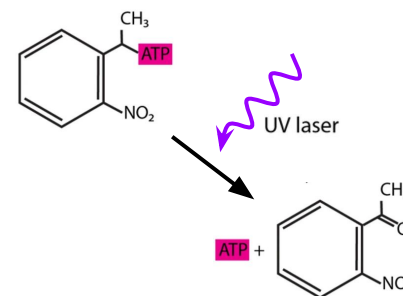
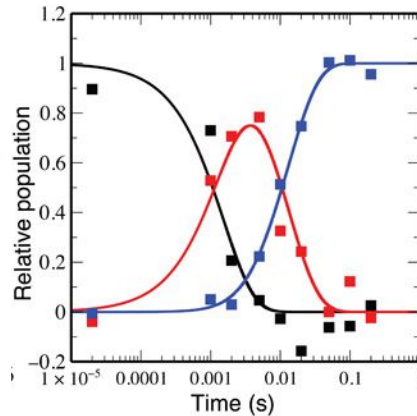
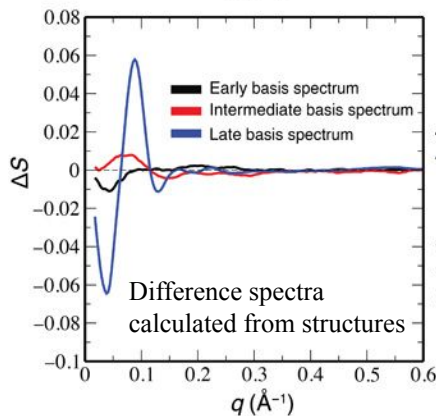
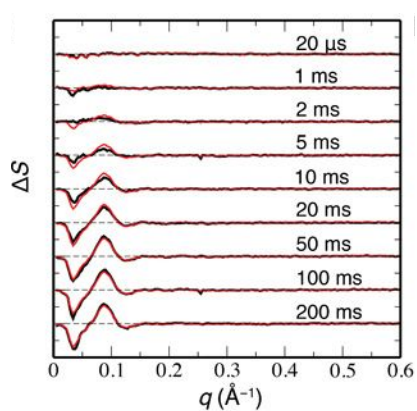
TR-XSS prepulse state versus $[\text{Ca}_2]\text{E1}$ (2C9M)

TR-XSS intermediate state versus $[\text{Ca}_2]\text{E1ATP}$ (3N8G)

TR-XSS late state versus $[\text{Ca}_2]\text{E1P}\cdot\text{ADP}$ (3BA6)



Magkakis, K., Orádd, F., et al. "Real-time structural characterization of protein response to a caged compound by fast detector readout and high-brilliance synchrotron radiation." *Structure* (2024).



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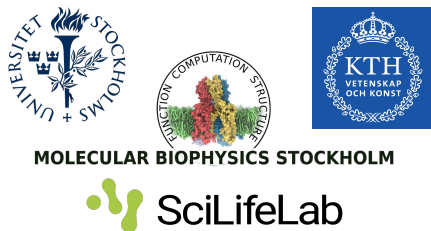
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Rebecca Howard
& group



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Anne Martel
Mark Tully
Petra Pernot



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& group



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ANSTO's National deuteration facility

