SMA-based Membrane Active Polymers for Membrane Protein Structural Biology

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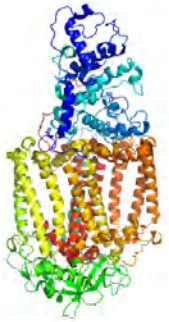
Milestones in the History of Membrane Protein Structural Biology

1975
Bacterial
Rhodopsin



(1985) Photosynthesis Reaction Center

1988

Robert Huber
Hartmut Michel
Johann Deisenhofer

Detergent DDM

(1998) KcsA Potassium Channel

2003

Peter Agre
Roderick MacKinnon

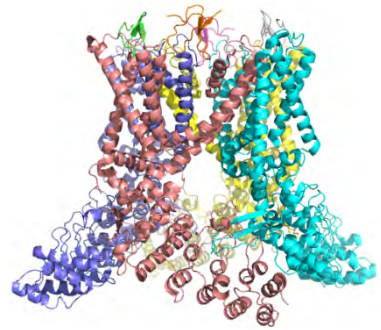
Hetero-overexpression

(2011) β2 Adrenergic Receptor

2012

Robert Lefkowitz
Brian K. Kobilka

Lipid Cubic Phase

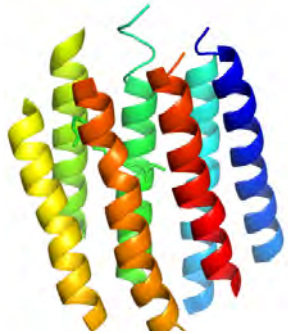


(2013) TRPV1 Channel

2017

Joachim Frank
Jacques Dubochet
Richard Henderson

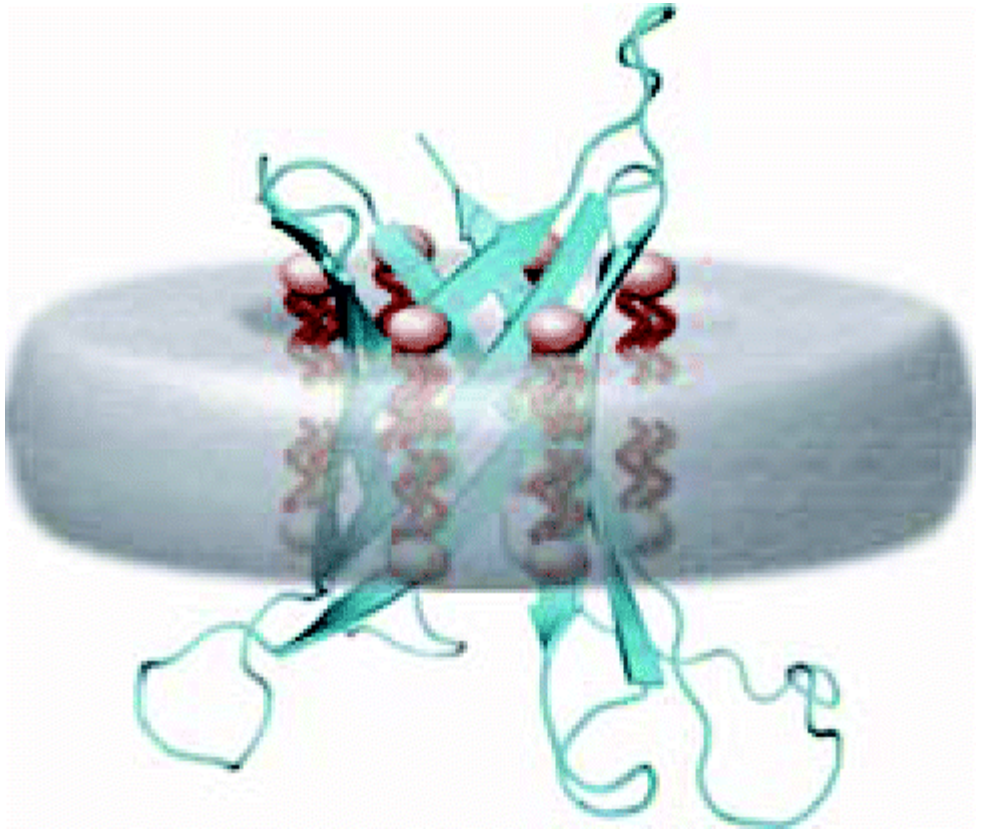
Single particle Cryo-EM



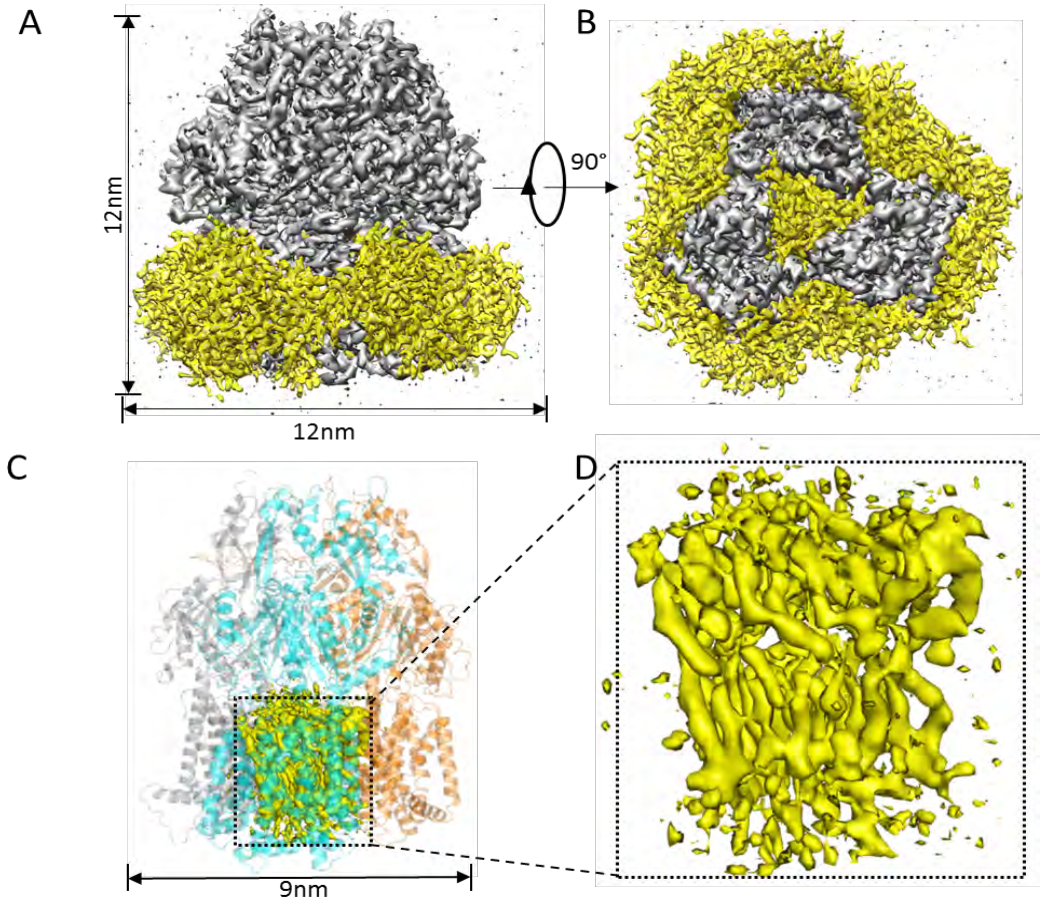
1990
Bacterial
Rhodopsin

Membrane Proteins Solubilized Intact in Lipid Containing Nanoparticles Bounded by Styrene Maleic Acid Copolymer

Timothy J. Knowles, Rachael Finka, Corinne Smith, Yu-Pin Lin,
Tim Dafforn, Michael Overduin (2009) JACS

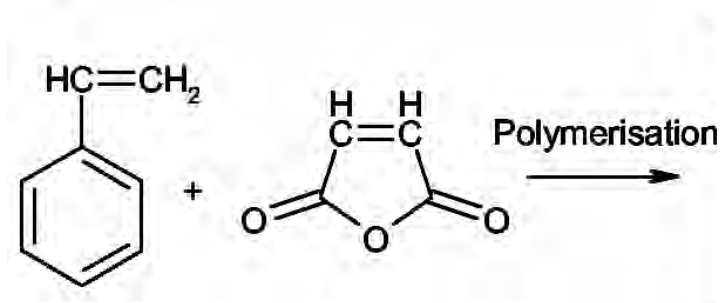


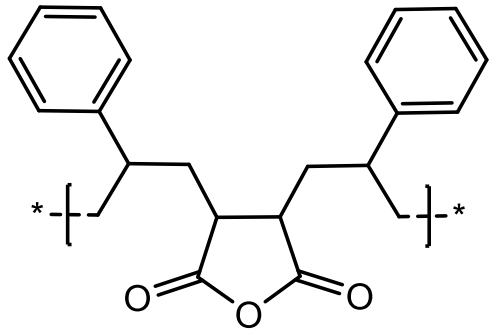
Lipid Bilayer Associated with AcrB



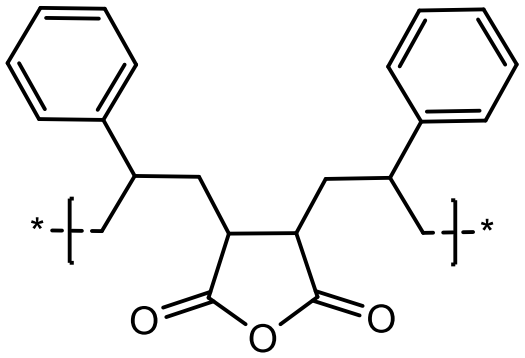
Qiu W., et al., Structure and activity of lipid bilayer within a membrane-protein transporter *Proc Natl Acad Sci U S A.* 2018 **115**, 12985-12990.

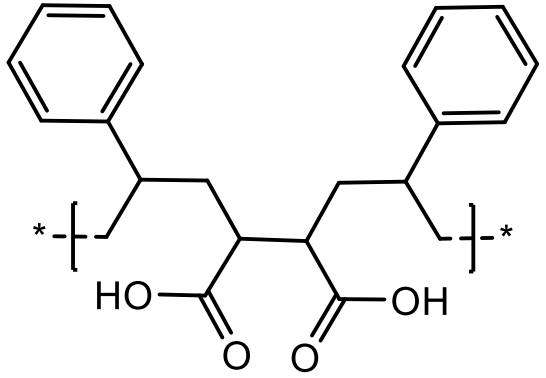
Styrene Maleic Acid (SMA) Co-polymers





Styrene **Maleic Anhydride** **Co-polymer**





1M NaOH, 100 °C

Membrane–inactive form

Membrane–active form

SMA2000, 7.5 kDa
Maximum linear length about 240Å

Styrene Maleic Acid (SMA) Co-polymers

1. SMA 1000
2. SMA 2000*
3. SMA 3000*
4. SMA 4000
5. SMA 1000I
6. SMA 2000I*
7. SMA 3000I*
8. SMA 4000I
9. SMA EF-30*
10. SMA EF-40
11. SMA EF-60
12. SMA EF-80
13. SMA 1440*
14. SMA 2021
15. SMA 2625*
16. SMA 3840
17. SMA 17352*

Membrane Active Polymers
Current Challenges and Opportunities

High-resolution structure determination:

Compatibility to divalent ions:

Compatibility to lower pH conditions:

Compatibility to both lower pH value conditions and divalent ions:

Solubility efficiency:

Nanoparticles sizes:

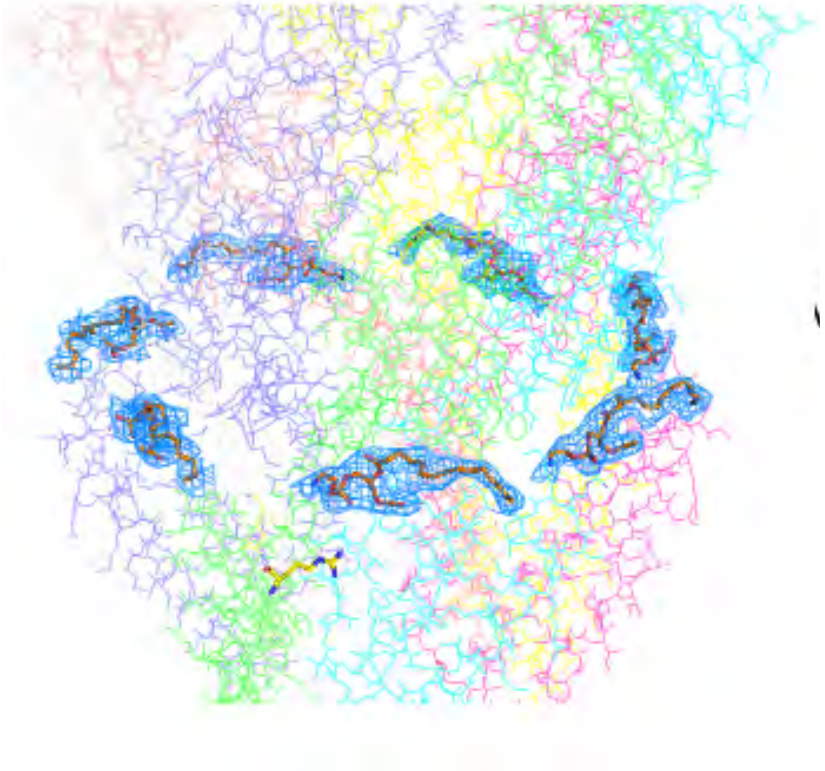
Continued

Enzyme activity: TSPO, Ca^{2+} dependent P-ATPase.

Channels: Mechanosensitive Channels

Transporters: ABC Transporters

Receptors: GPCRs



SMA-based Membrane Active Polymer Library and Native Cell Membrane Nanoparticles System

S^RMAP-1	S^RMAP-8
S^RMAP-2	S^RMAP-9
S^RMAP-3	S^RMAP-10
S^RMAP-4	S^RMAP-11
S^RMAP-5	S^RMAP-12
S^RMAP-6	S^RMAP-13
S^RMAP-7	…

Each of the polymers has to be tested successfully for high-resolution structure determination. High quality polymers.

Neither compatible to low pH conditions nor divalent ions

Low pH conditions only but not to divalent ions.

Low pH conditions and divalent ions.

Membrane protein with small transmembrane domains.

membrane protein with large transmembrane domains.

Bacterial cell membrane
Yeast cell membrane
Plant cell membrane
Insect cell membrane
Human cell membrane

SMA-based Stimuli-Responsive Membrane-active Polymers

- **SMA copolymer**

  SMA copolymer for membrane proteins extraction was first reported in 2009.

- **Stimuli-Responsive Membrane-Active Polymers (S^{R}MA-P1, S^{R}MA-P2, S^{R}MA-P3…)**

S^{R}MA-P1

Precipitation before centrifugation

Precipitation after centrifugation

S^{R}MA-P1 precipitates in the presence of divalent ions (10mM $CaCl_2$).

S^{R}MA-P2

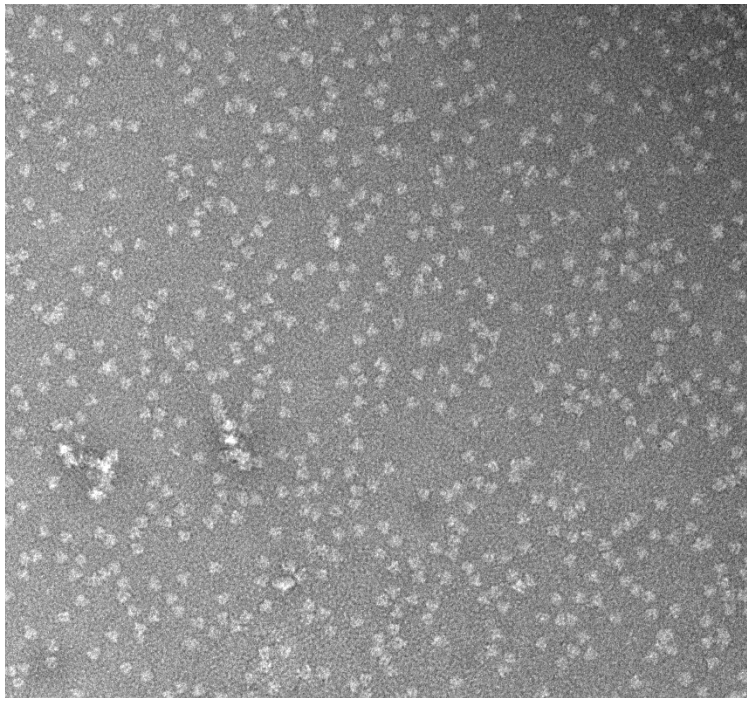
Clear solution before centrifugation

Clear solution after centrifugation

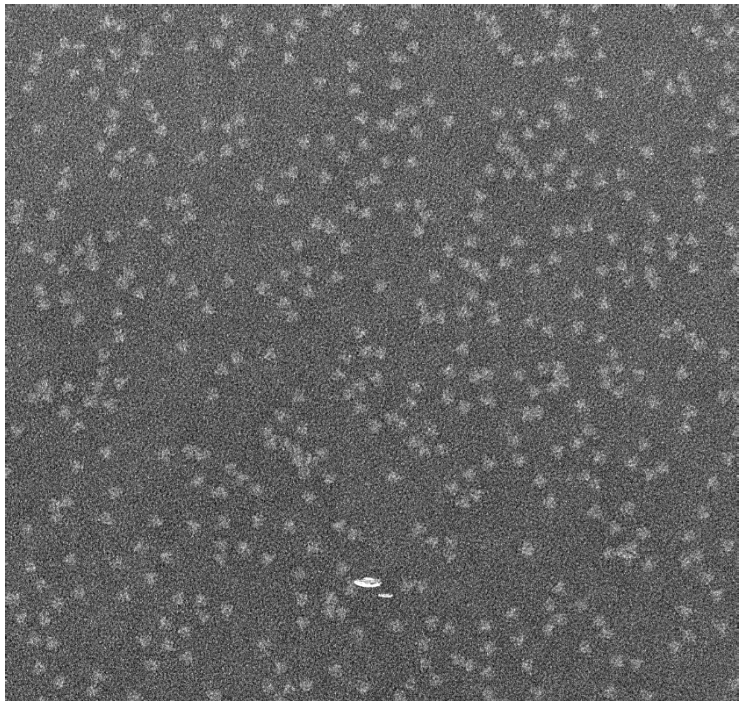
S^{R}MA-P2 does not precipitate in the presence of divalent ions (10mM $CaCl_2$).

Knowles T, et al*, J. Am. Chem. Soc.,* 2009. 131: 7484-7485

Native cell membrane nanoparticles

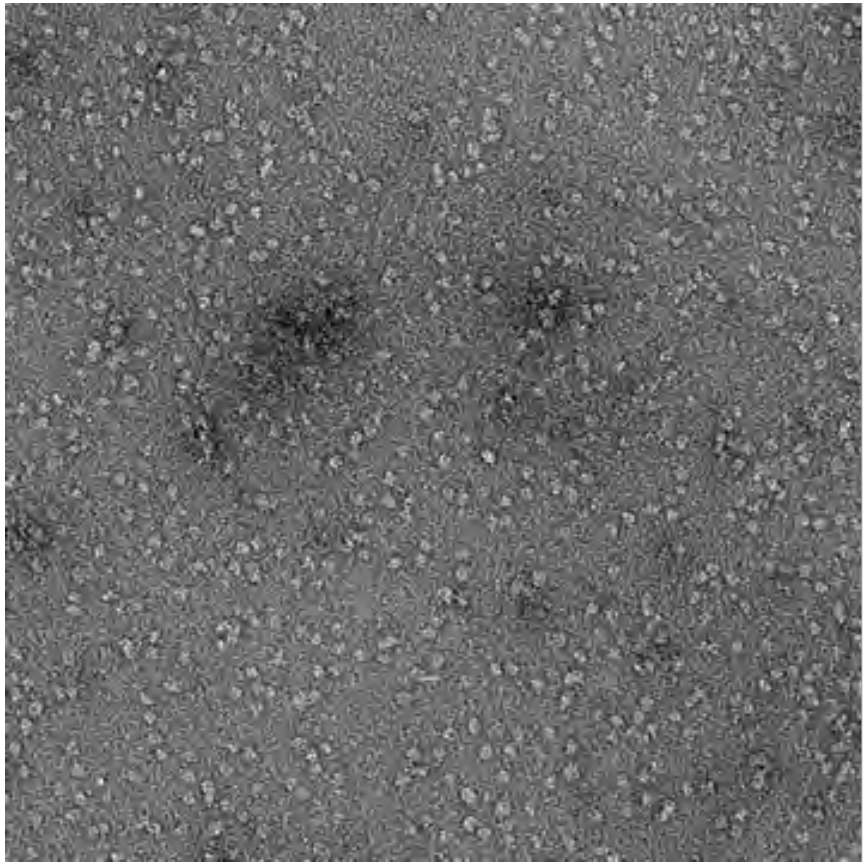


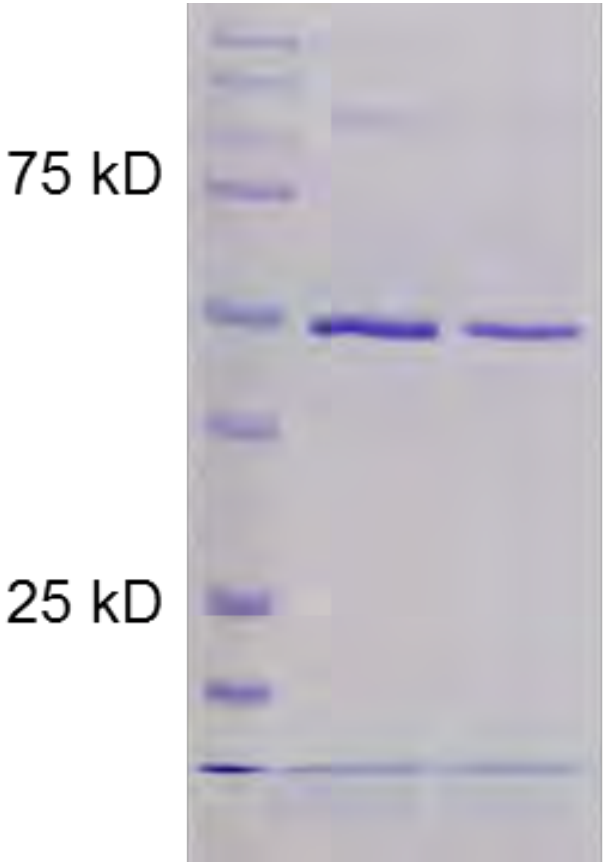
Native cell membrane nanoparticles prepared with S^{R}MA-P1 polymer.

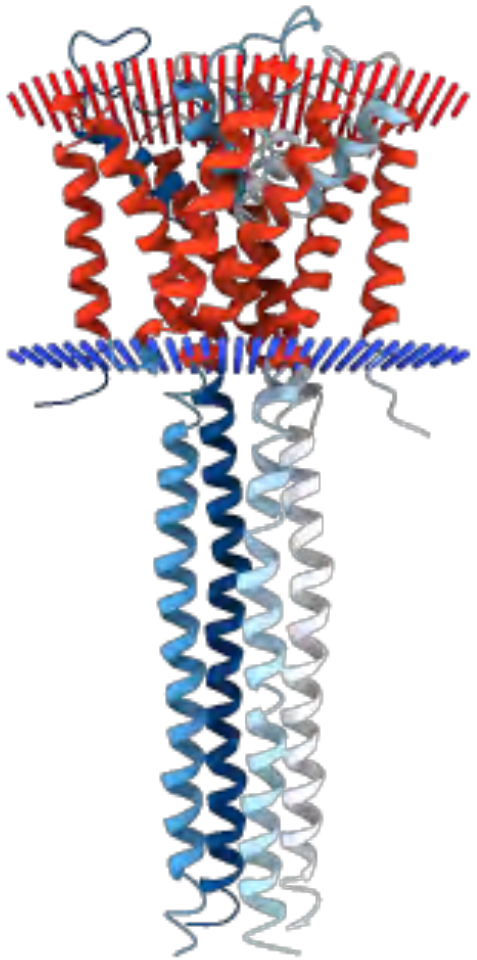


Native cell membrane nanoparticles prepared with S^{R}MA-P2 polymer.

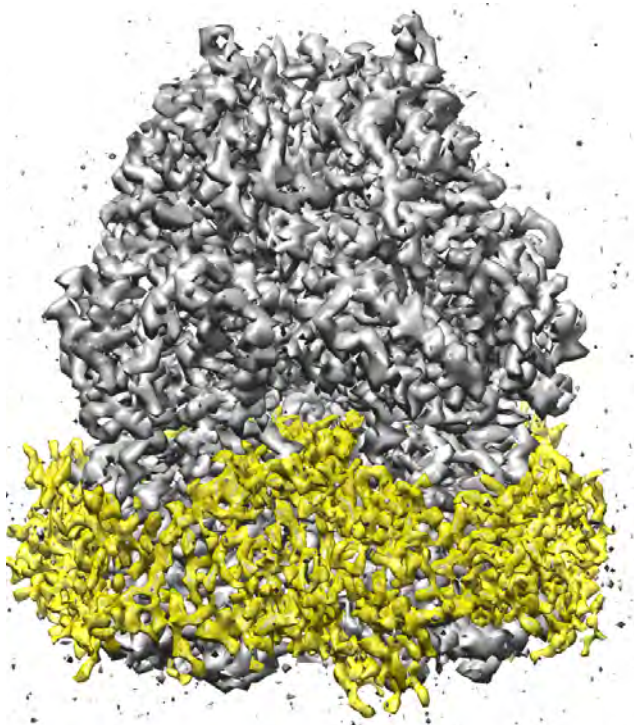
Small Nanoparticles

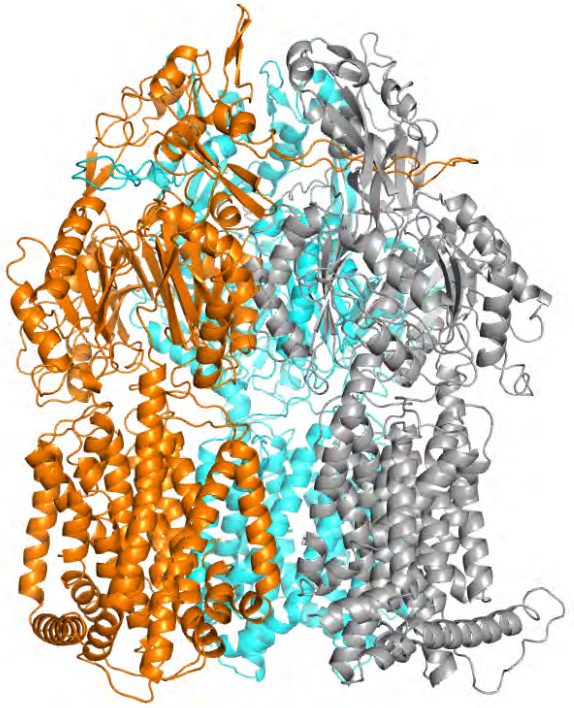






KcsA





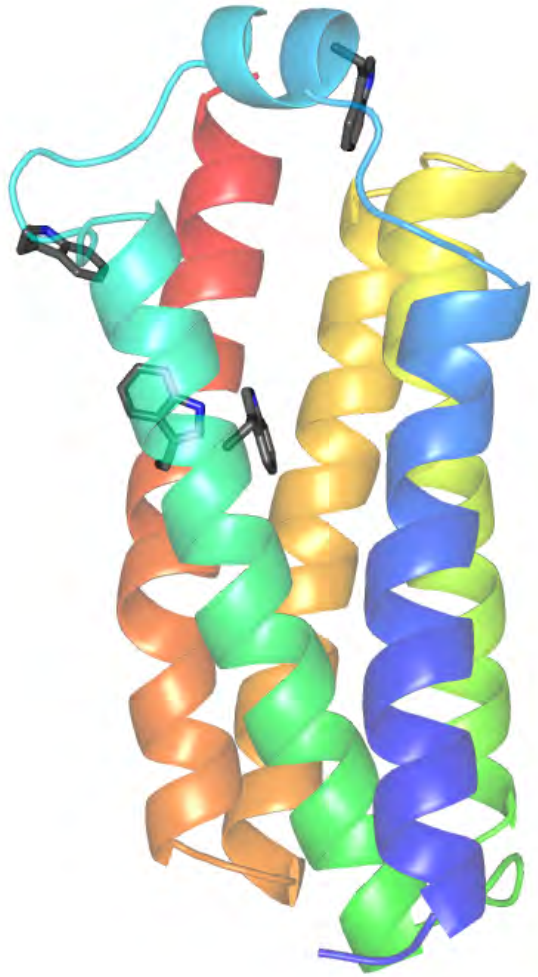
Functional Study of Tryptophan-rich Sensory Protein (TSPO)

- TSPO proteins share very conserved structure and function. In human, it was identified as peripheral benzodiazepine receptor (PBR).

- 5 transmembrane helices.

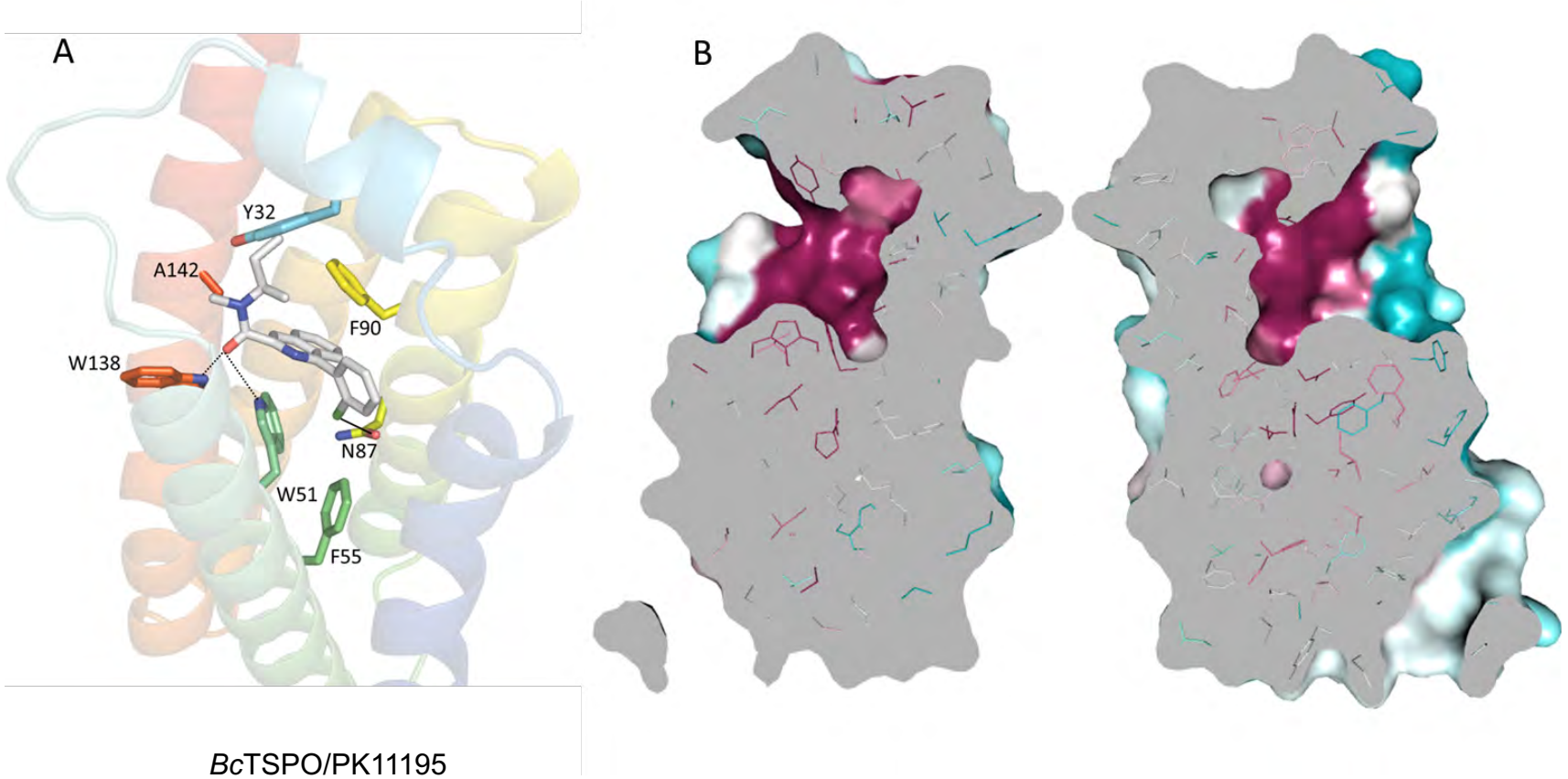
- TSPO is an enzyme.

Crystal structures of *Bacillus cereus* TSPO(*Bc*TSPO)



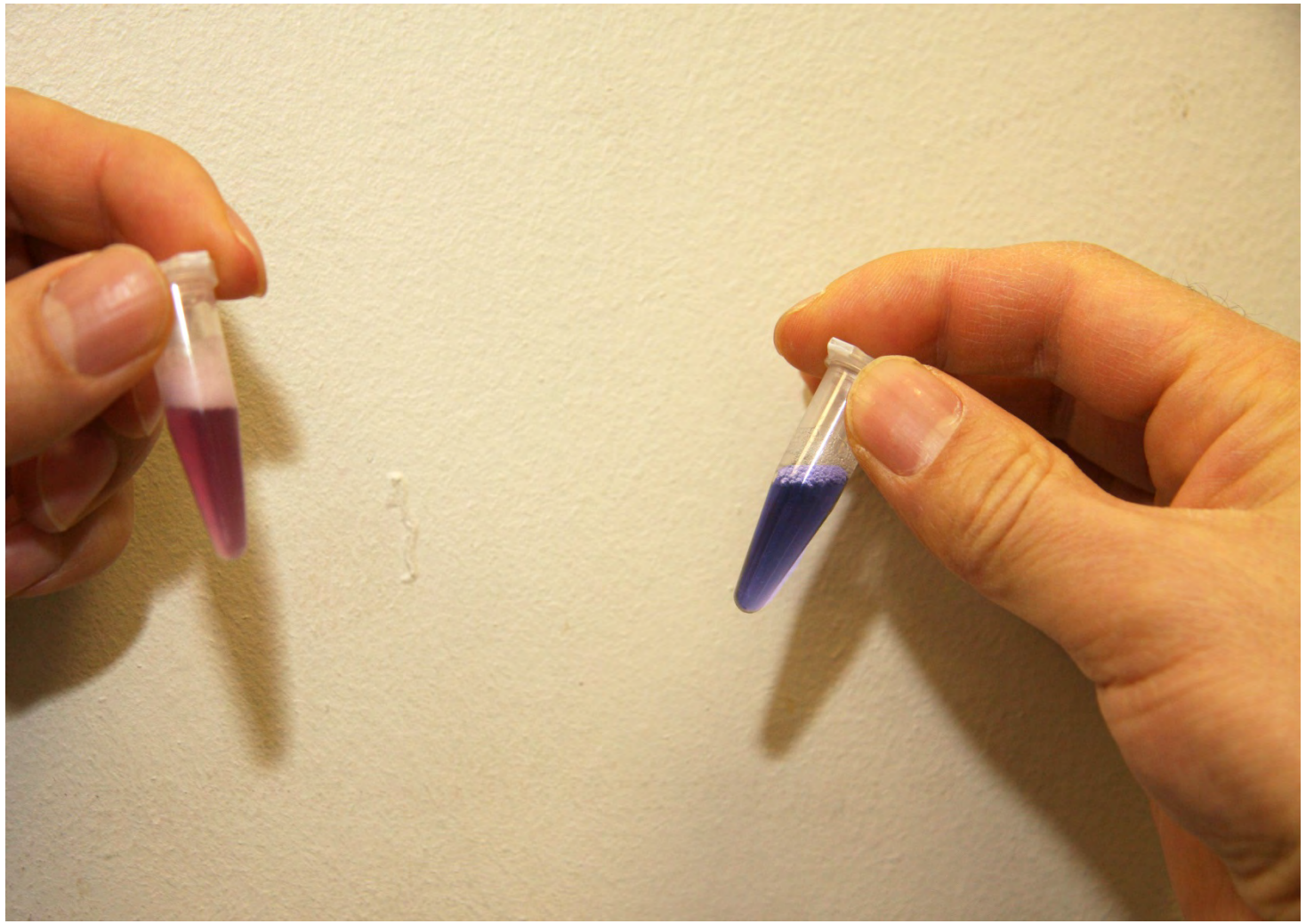
Ginter C., et al. (2013) *Biochemistry*, *52*, 3609–3611.
Guo Y., et al. (2015) *Science*, 2015, 347: 551-555.

Property of the Active Center of *Bc*TSPO

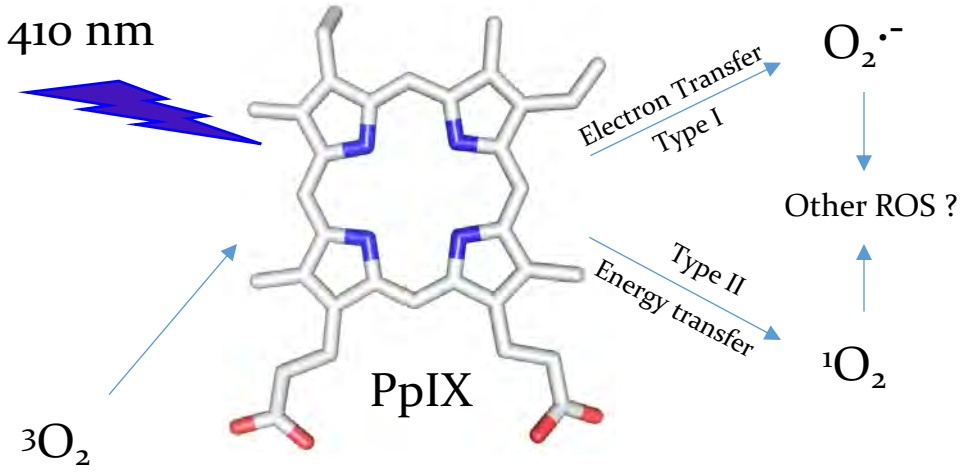


*Bc*TSPO/PK11195

TSPO Catalyzed Color Reaction

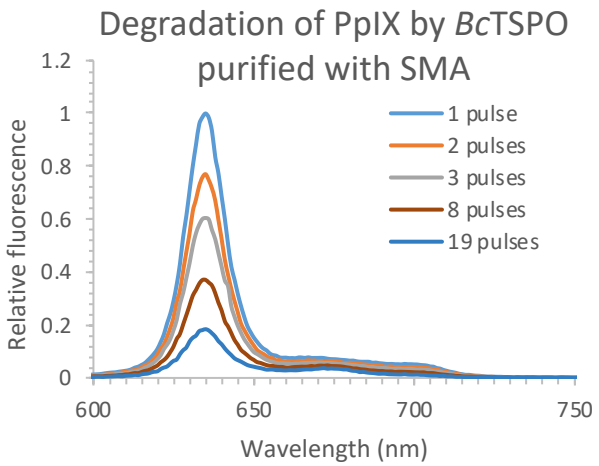


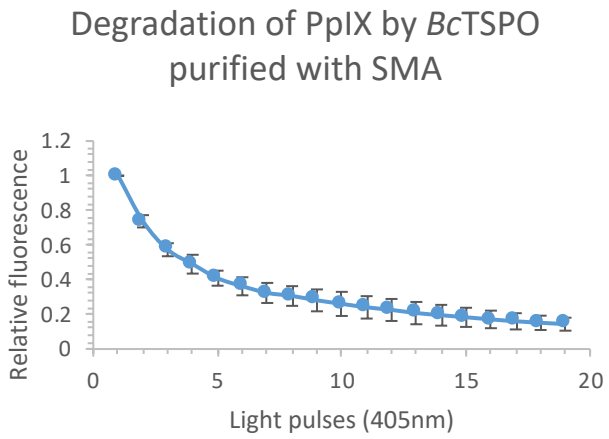
Reaction Between Molecular Oxygen and Photo-excited Protoporphyrin IX (PpIX)

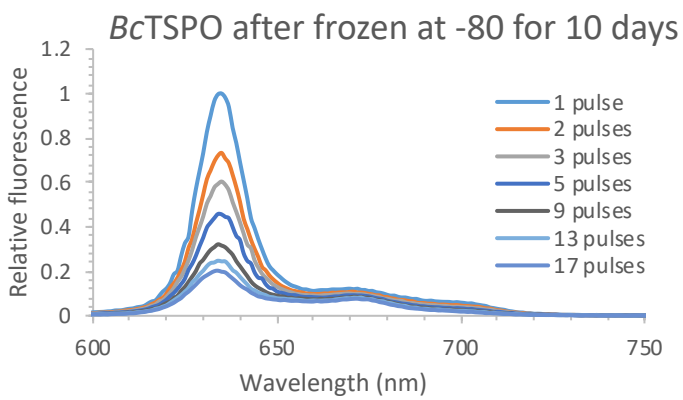


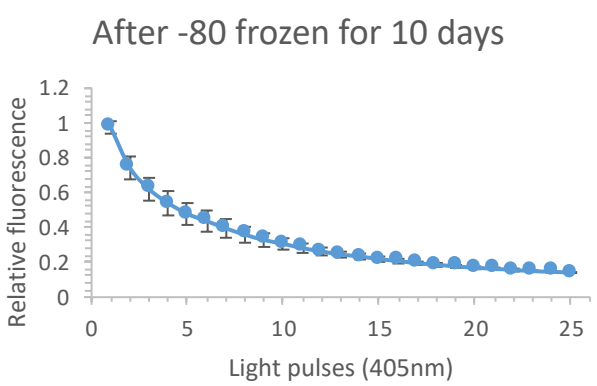
Dalton J. et al., (1972) Nature 235: 388.
Treffry A. and Ainsworth S. (1974) Biochem J. 137: 319-329.
Buettner G. R., and Oberley L.W. (1979) FEBS Letters 98: 18-20.

TSPO Catalyzed Reaction









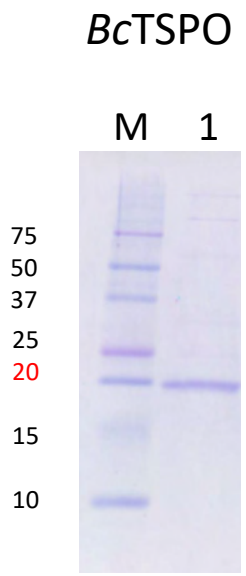
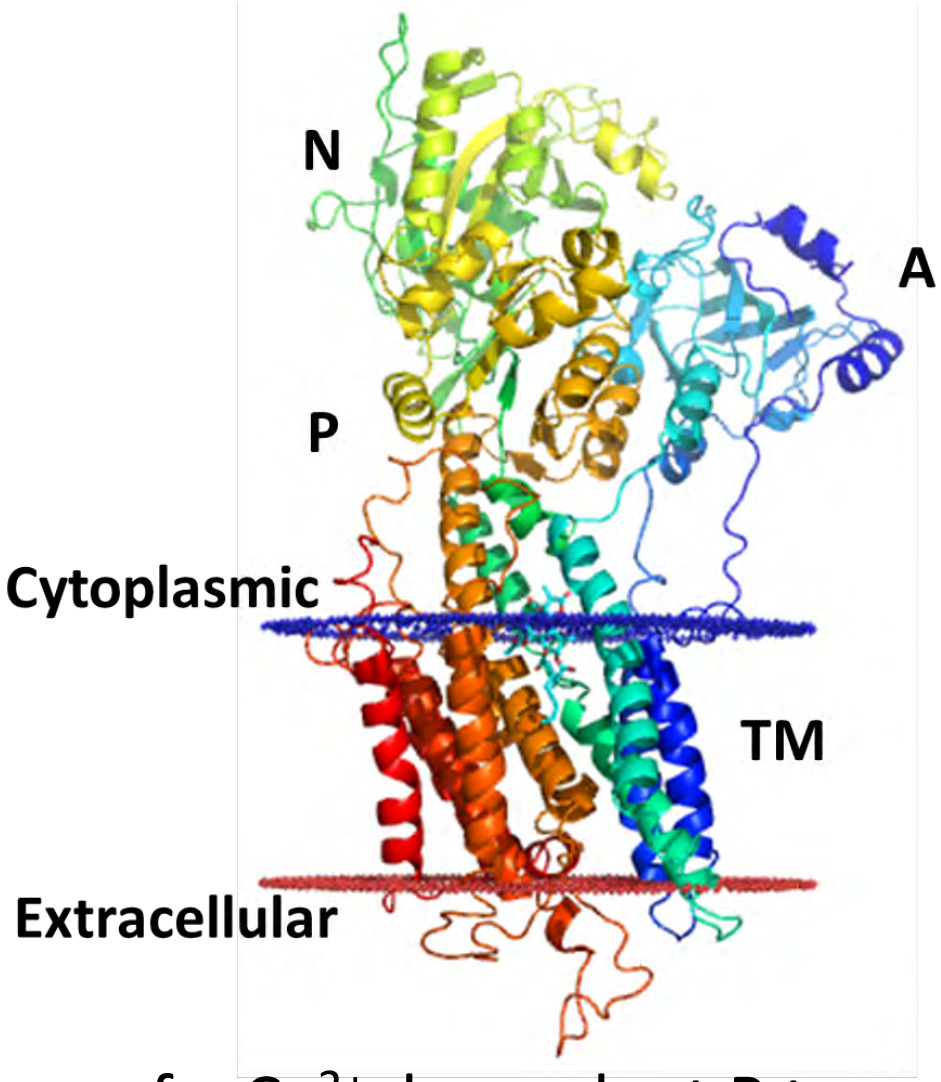


Figure 1

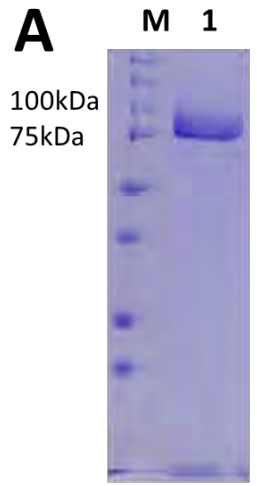
Ca^{2+} Dependent P type ATPase



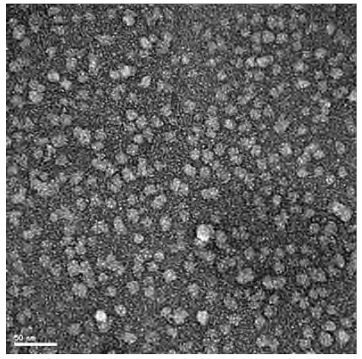
Crystal structure of a Ca^{2+} dependent P type ATPase PDB: 1IWO

Ca^{2+} dependent P type ATPase

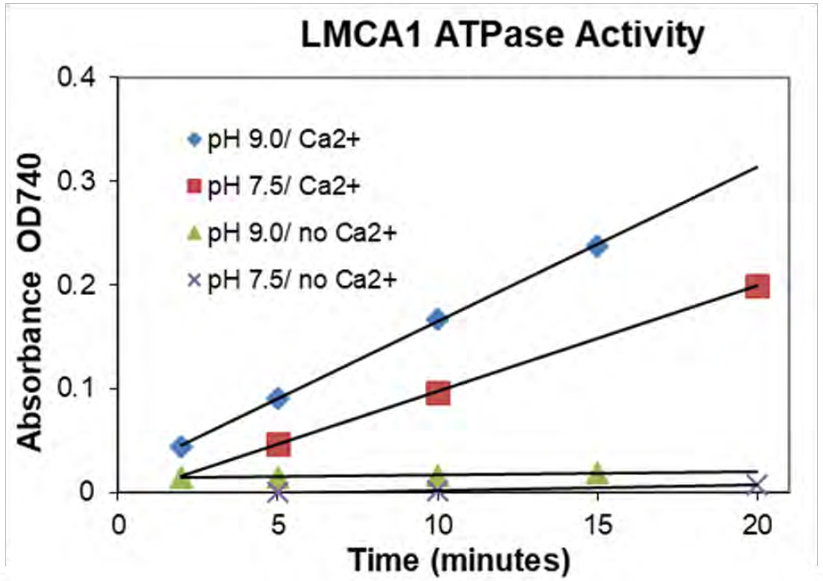
A



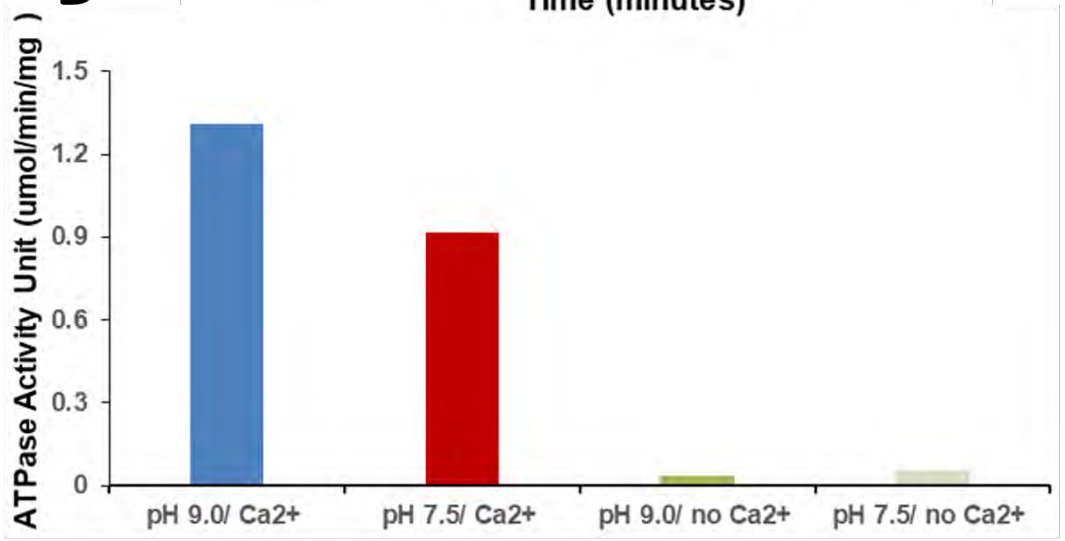
B



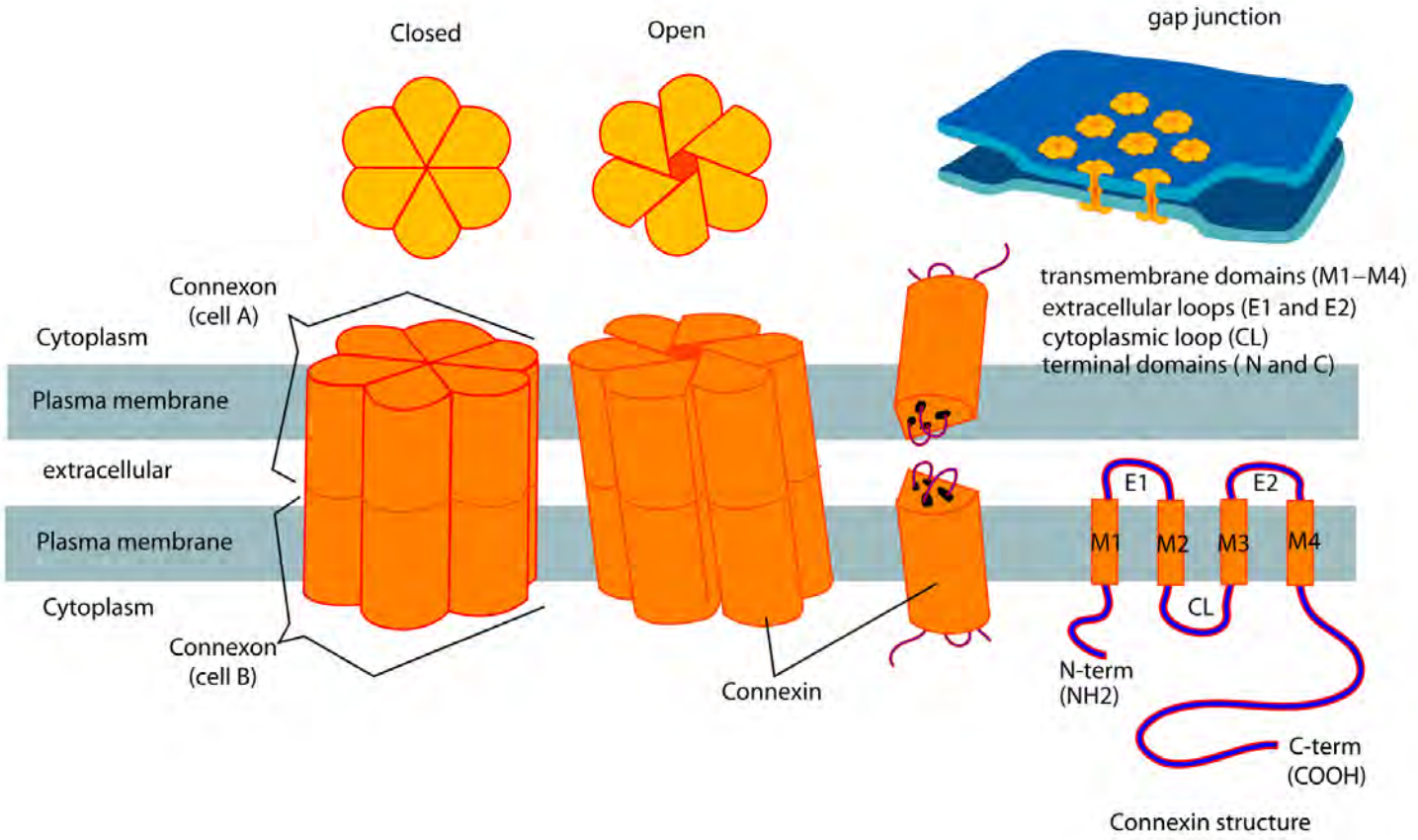
C



D



Calcium Regulated Human Connexin Channels



https://en.wikipedia.org/wiki/Connexin#/media/File:Connexon_and_connexin_structure.svg

Manual 0 | Blob (Active) 1,210,714 | Template 0 | Total Extracted Particles 391,566

247 Queued 2472 2471 2470 2469 2468 2467

2D 3D

Threshold 0.00 0.08 0.37

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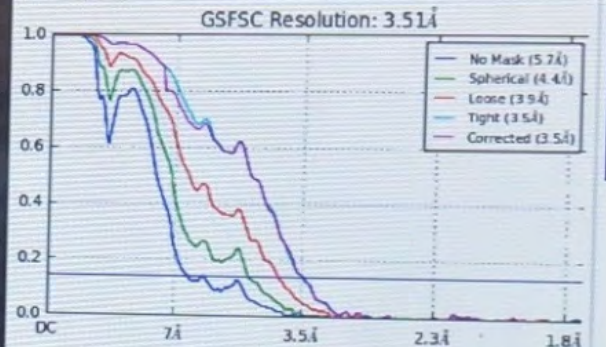
Configuration | Individual | Overview | Browse | Picking | 2DClasses | Abinitio | Refinem

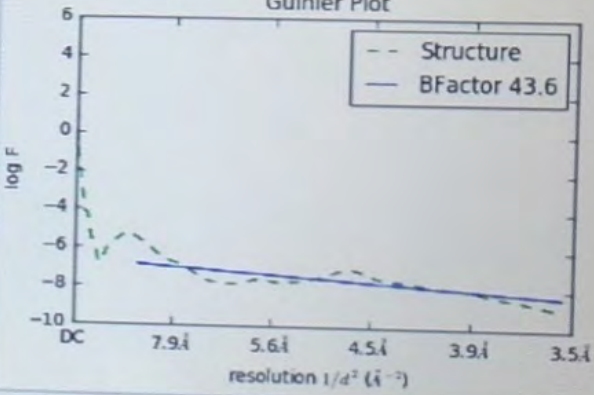
J441 ● Running (110.2 mins. elapsed) 161,766 Particles Done iteration 9 Configure

Fourier Shell Correlation

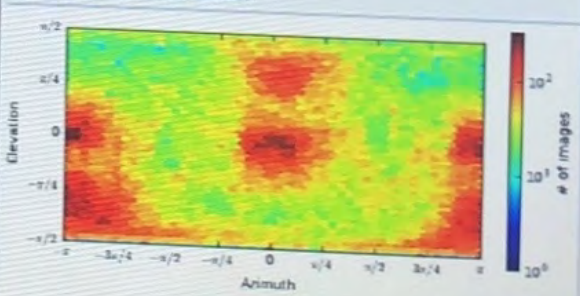


Fourier Space Slices

Guinier Plot



Orientation Distribution



Real Space Slices

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Thursday March 12, 2020 9:22 AM ● 1 Active Session

Logout

Conclusions

- A membrane active polymer library is need for membrane protein structural biology.

- We set a high standard to develop membrane active polymers.

  high-resolution structures of memteins.

- SMA co-polymers are good start materials to develop membrane active polymers, but all potential novel polymers will be considered.

Acknowledgements

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  Weihua Qiu, Claudio Catalano, Kyle Kroeck, Hoang Trinh, Katie Shedden, Jeannie Kilgore
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  - **VCU School of Pharmacy**
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    (Native Cell Membrane Nanoparticles System)