SMA-based Membrane Active Polymers for Membrane Protein Structural Biology

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Youzhong Guo
Department of Medicinal Chemistry
Institute for Structural Biology, Drug Discovery and Development
School of Pharmacy, Virginia Commonwealth University
Milestones in the History of Membrane Protein Structural Biology

- **1975**: Bacterial Rhodopsin
- **1985**: Photosynthesis Reaction Center
- **1988**: Detergent DDM
- **1988**: Robert Huber
- **1988**: Hartmut Michel
- **1988**: Johann Deisenhofer
- **1990**: Bacterial Rhodopsin
- **1990**: Joachim Frank
- **1998**: KcsA Potassium Channel
- **1998**: Peter Agre
- **1998**: Roderick MacKinnon
- **2003**: Hetero-overexpression

- **2011**: β2 Adrenergic Receptor
- **2011**: Robert Lefkowitz
- **2011**: Brian K. Kobilka
- **2012**: Lipid Cubic Phase

- **2013**: TRPV1 Channel
- **2013**: Joachim Frank
- **2013**: Jacques Dubochet
- **2013**: Richard Henderson
- **2017**: Single particle Cryo-EM
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

Styrene Maleic Acid (SMA) Co-polymer

1M NaOH, 100 °C

Membrane–inactive form

SMA2000, 7.5 kDa
Maximum linear length about 240Å

Membrane–active form
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:
Enzyme activity: TSPO, $\text{Ca}^{2+}$ dependent P-ATPase.

Channels: Mechanosensitive Channels

Transporters: ABC Transporters

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

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

*S^R*MAP-1  *S^R*MAP-8
*S^R*MAP-2  *S^R*MAP-9
*S^R*MAP-3  *S^R*MAP-10
*S^R*MAP-4  *S^R*MAP-11
*S^R*MAP-5  *S^R*MAP-12
*S^R*MAP-6  *S^R*MAP-13
*S^R*MAP-7  ...

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 (SRMA-P1, SRMA-P2, SRMA-P3...)**

  - **SRMA-P1** precipitates in the presence of divalent ions (10mM CaCl₂).
  - **SRMA-P2** does not precipitate in the presence of divalent ions (10mM CaCl₂).

Native cell membrane nanoparticles prepared with $S^RMA$-P1 polymer.

Native cell membrane nanoparticles prepared with $S^RMA$-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 (*BcTSPO*)

Property of the Active Center of BcTSPO

BcTSPO/PK11195
TSPO Catalyzed Color Reaction
Reaction Between Molecular Oxygen and Photo-excited Protoporphyrin IX (PpIX)

TSPO Catalyzed Reaction

Degradation of PpIX by BcTSPO purified with SMA

- 1 pulse
- 2 pulses
- 3 pulses
- 8 pulses
- 19 pulses

Relative fluorescence vs. Wavelength (nm)

Degradation of PpIX by BcTSPO after frozen at -80°C for 10 days

- 1 pulse
- 2 pulses
- 3 pulses
- 5 pulses
- 9 pulses
- 13 pulses
- 17 pulses

Relative fluorescence vs. Wavelength (nm)

BcTSPO

After -80°C frozen for 10 days

Relative fluorescence vs. Light pulses (405nm)

Figure 1
Ca^{2+} Dependent P type ATPase

Crystal structure of a Ca^{2+} dependent P type ATPase PDB: 1IWO
Ca\textsuperscript{2+} dependent P type ATPase
Calcium Regulated Human Connexin Channels
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.
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  (Native Cell Membrane Nanoparticles System)