Functional integrity of membrane protein rhodopsin solubilized by amphipathic polymers

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Rhodopsin is a member of the G-protein-coupled receptor (GPCR) superfamily

- **GPCRs** – covert extracellular signals into intracellular pathways through the activation of G proteins
- **Rhodopsin** – opsin + 11-cis-retinal (11CR)
Using time-resolved absorption spectroscopy to study the photoactivation of rhodopsin

\[ \Delta A(\lambda, t_i) = A_i(\lambda, t_i) - A_o(\lambda, t_o) \]

\[ \lambda_{\text{max}} = 380\text{nm} \]
\[ \lambda_{\text{max}} = 500\text{nm} \]

DOI: 10.1016/S0076-6879(00)15842-2
Rhodopsin is useful as a tool to study the effects of various solubilizing agents.

**Detergents**
- removes native lipids– reduces light scattering
- decreases stability/alters protein dynamics

**Membrane scaffold proteins (MSPs)**
- detergent-solubilized, lipids are added back in desired ratio
- increases protein stability/retains protein dynamics

**Amphipathic polymers (amphipols)**
- detergent-solubilized or native lipids
- may alter protein dynamics when present in excess
Amphipols used to solubilize bovine rhodopsin directly from native ROS disc membranes

From Greek *rhodon* ‘rose’ + *opsis* ‘sight’

DOI: 10.1021/acs.biomac.1c00274
Testing the photoactivation properties of rhodopsin-SMA(3:1)LPs

While the highest SMA/rhodopsin ratios yielded the most solubilized protein, the rhodopsin did not reach the active (Meta-II) state upon photoactivation

- time-dependent absorption changes up to 45-min. after photolysis showed no noticeable shift toward Meta-II

![Absorption spectra](image)

- **ratios 1-10:** 30% Meta-I$_{480}$ and 70% Meta-II
- **ratio 25:** 55% Meta-I$_{480}$ and 45% Meta-II
- **ratios 50-100:** only Meta-I$_{480}$

DOI: 10.1016/j.bpj.2021.05.008
Using high SMA(3:1)/rhodopsin molar ratios yields extremely slow photokinetics

Rhodopsin-SMALPs made at low ratios (≤10-15):
• follow a reaction mechanism that leads to the active state, although at slower rates

Rhodopsin-SMALPs made at high ratios (20+):
• the reaction path becomes disrupted (formation of 460-nm photoproduct) and the active state is not reached
Reaction progress in LPs is slower compared to the native membrane environment.

- Polymers slow down the reaction steps at the late stages where big conformational changes occur.
- Reaction progress is slower with DIBMA, but excess polymer is less disruptive to reaction path.

DOI: 10.1039/d1nr02419a
Why is the reaction progress slower in LPs compared to native ROS membranes?

Does the rigidity of the protein and its surroundings increase?
  - insertion of the hydrophobic moieties between the unsaturated alkyl chains

Are the hydrophobic moieties interacting with cholesterol binding site?
  - [SMA(2:1)LPs vs. SMA(3:1)LPs] vs. DIBMALPs

DOI: 10.1007/978-94-007-7423-0_5

DOI: 10.1007/s12274-014-0560-6
Mutations of the rhodopsin gene cause autosomal dominant retinitis pigmentosa (adRP)

G51V, R135G, and D190N – opsin folds properly and binds 11CR, but instability leads to retinal degeneration

at what point does rhodopsin begin to misfunction?

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