Functional integrity of membrane protein rhodopsin solubilized by amphipathic polymers

Stephanie Pitch

Ph.D. candidate - Kliger Lab

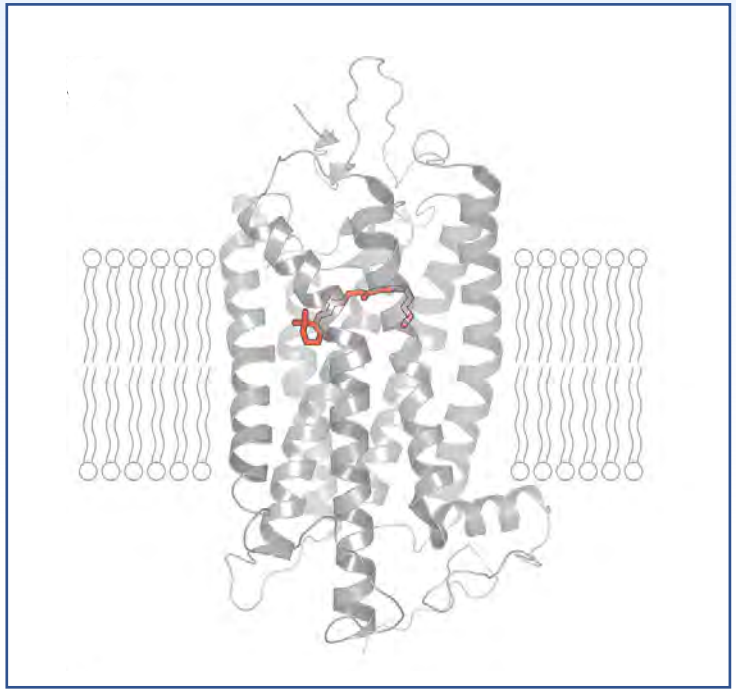
UC Santa Cruz

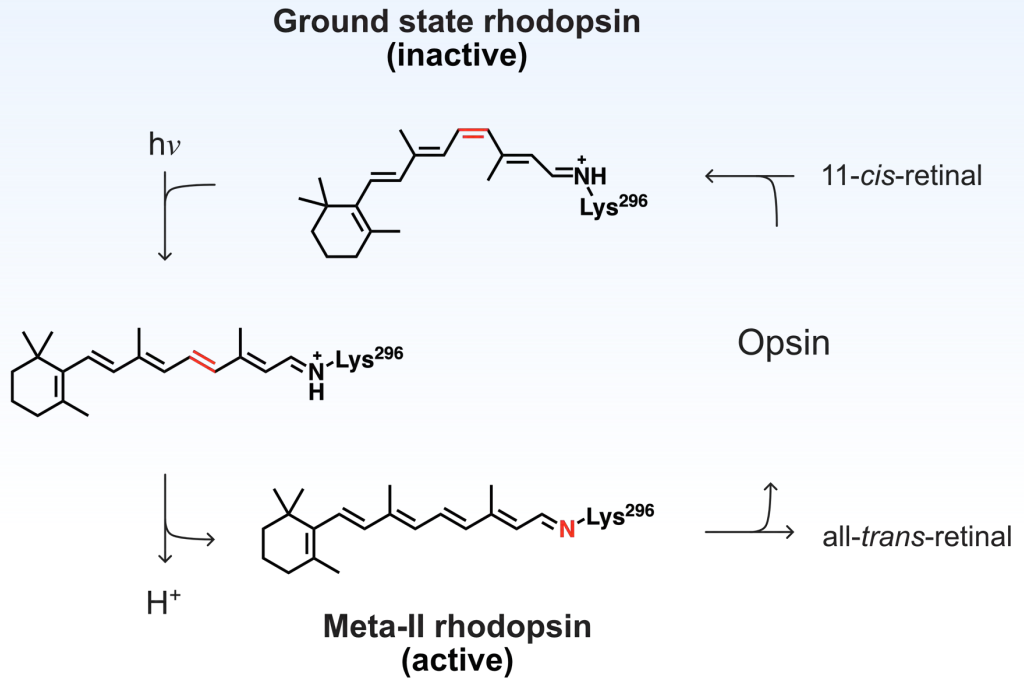
SMALP Conference

1 Oct. 2021

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

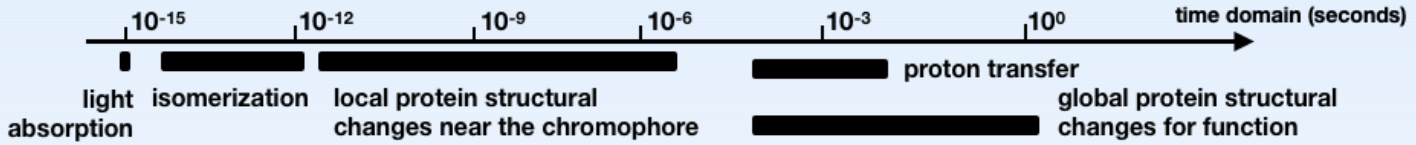
- **GPCRs –** covert extracellular signals into intracellular pathways through the activation of G proteins
- **Rhodopsin –** opsin + 11-*cis*-retinal (11CR)





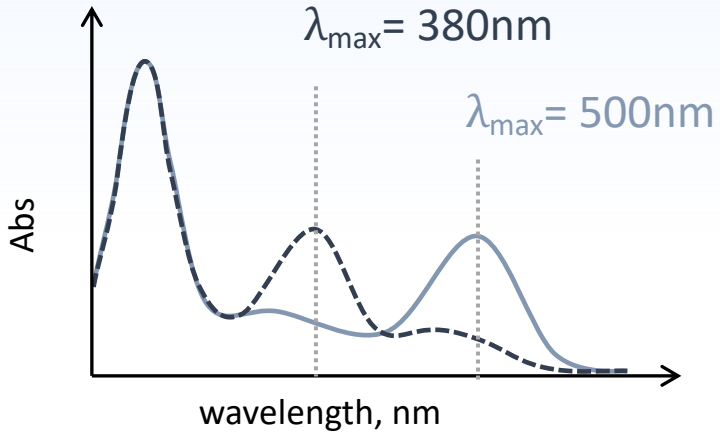
DOI: 10.1021/acs.chemrev.7b00723
DOI: 10.1021/cr400107q

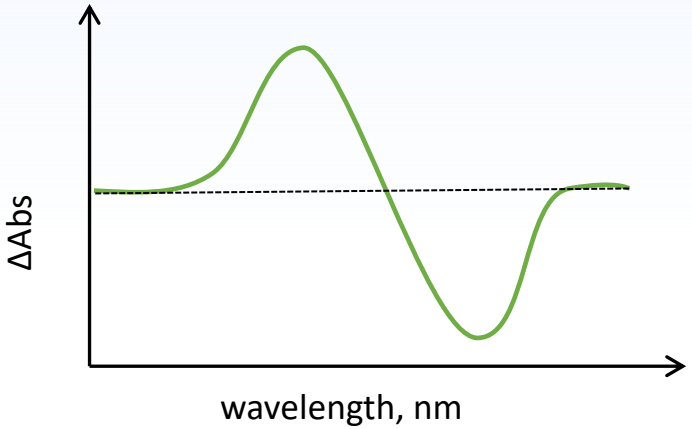
Using time-resolved absorption spectroscopy to study the photoactivation of rhodopsin



Abs_{dark} $\xrightarrow[(t_o)]{}$ **photolysis** $\xrightarrow[(t_i)]{\text{delay time}}$ Abs_{bleach}

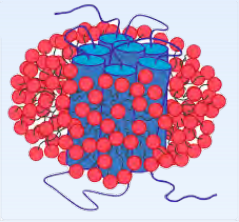
$$\Delta\mathbf{A}(\boldsymbol{\lambda}, \boldsymbol{t_i}) = \mathbf{A_i}(\boldsymbol{\lambda}, \boldsymbol{t_i}) - \mathbf{A_o}(\boldsymbol{\lambda}, \boldsymbol{t_o})$$





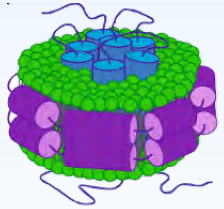
DOI: 10.1016/S0076-6879(00)15842-2

Rhodopsin is useful as a tool to study the effects of various solubilizing agents



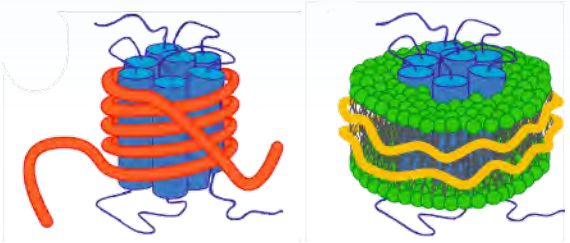
Detergents

- 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

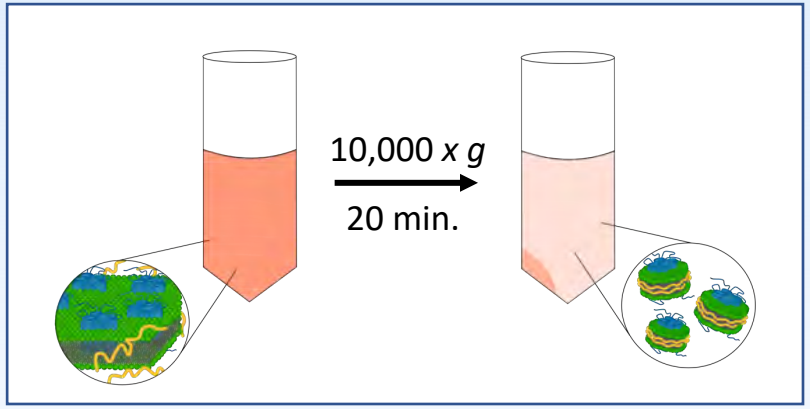


Amphipathic polymers (amphipols)

- detergent-solubilized or native lipids
- may alter protein dynamics when present in excess

DOI: 10.1007/s00249-015-1093-y
DOI: 10.1562/2006-02-01-RA-792
DOI: 10.1038/s41598-018-31925-1
DOI: 10.1021/bi200391a

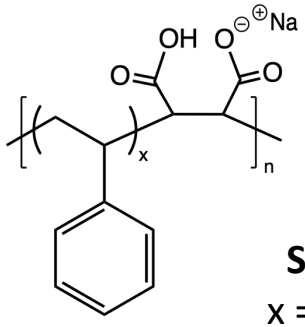
Amphipols used to solubilize bovine rhodopsin directly from native ROS disc membranes



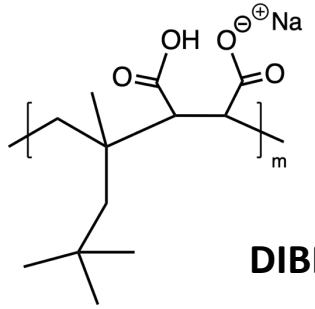
[polymer]: low high

From Greek ***rhodon*** 'rose' + ***opsis*** 'sight'

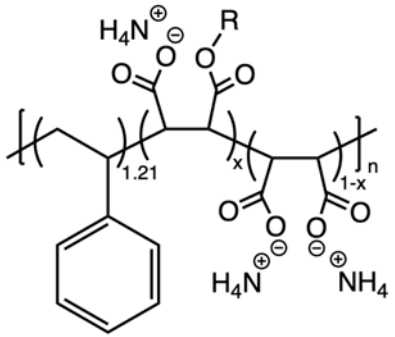
native lipid particles (LPs)

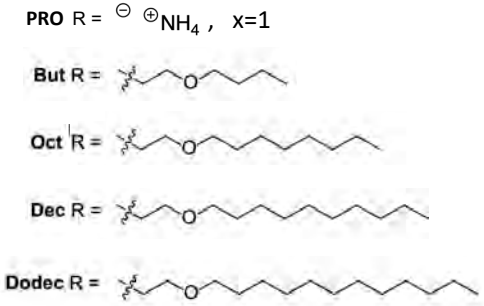


SMA
x = 3, 2



DIBMA





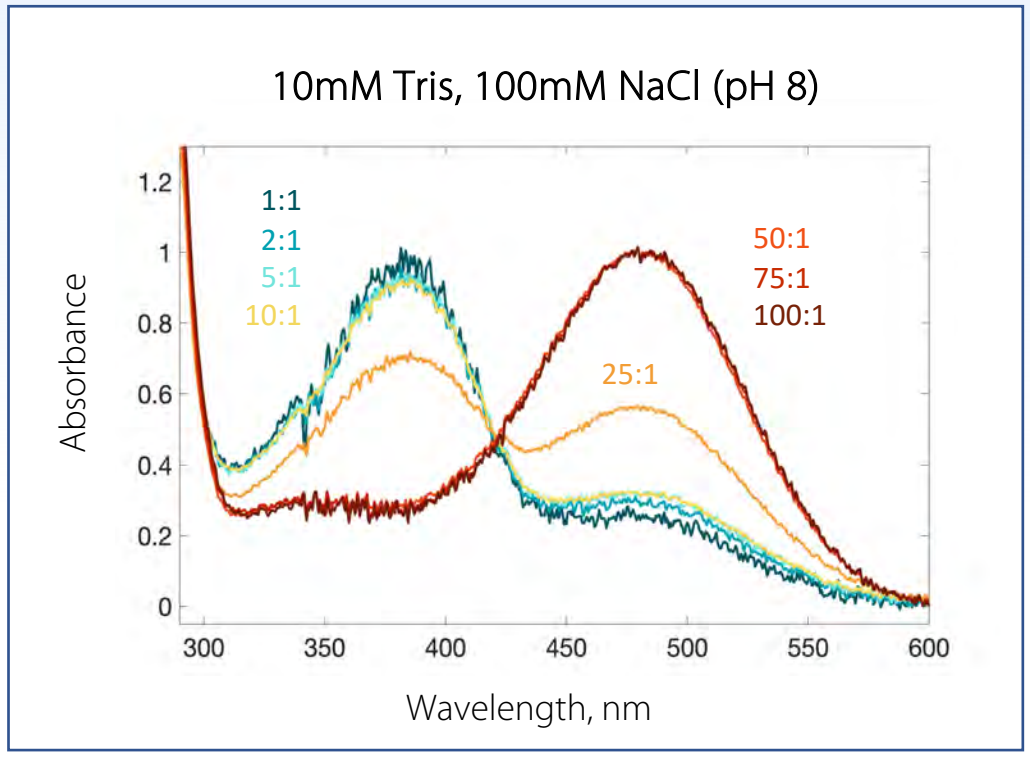
CW02

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



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

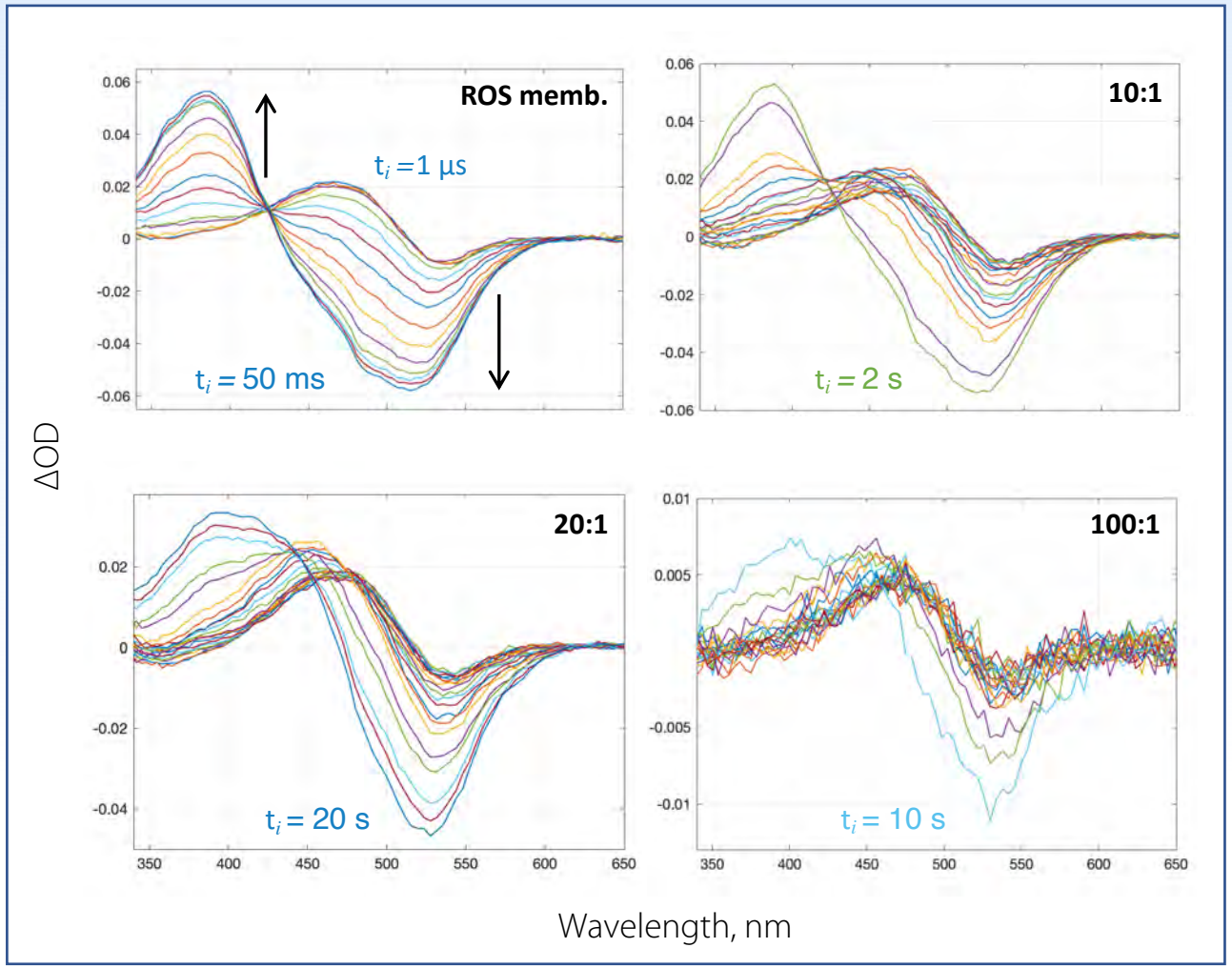
Using high SMA(3:1)/rhodopsin molar ratios yields extremely slow photokinetics

Rhodopsin-SMALPs made at **low** ratios (≤10-15):

- follow a reaction mechanism that leads to the active state, although at slower rates

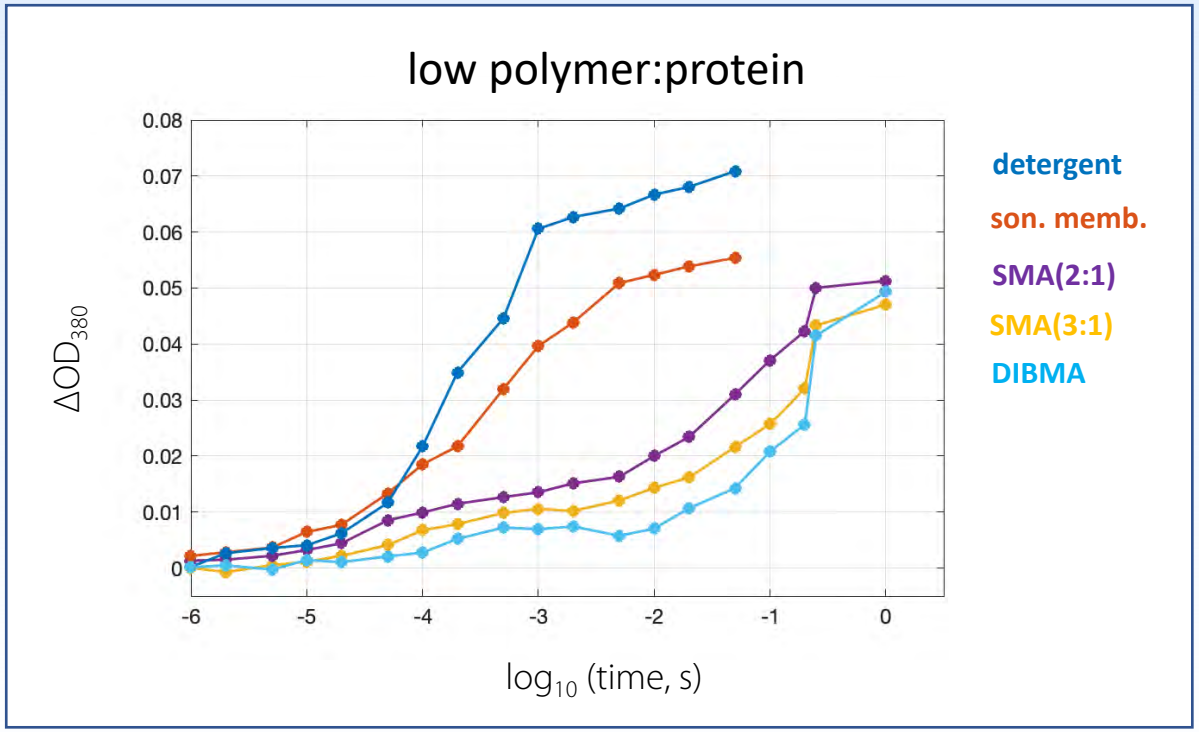
Rhodopsin-SMALPs made at **high** ratios (20+):

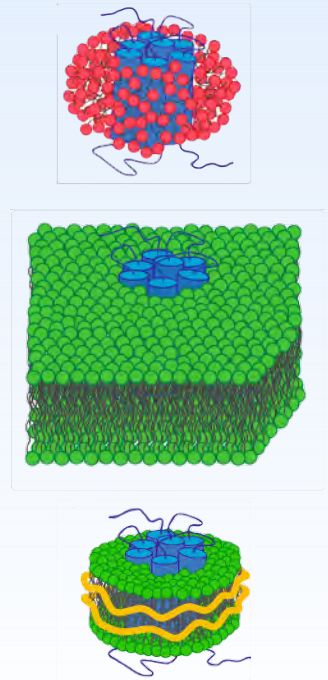
- the reaction path becomes disrupted (formation of 460-nm photoproduct) and the active state is not reached



DOI: 10.1016/j.bpj.2021.09.012

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?

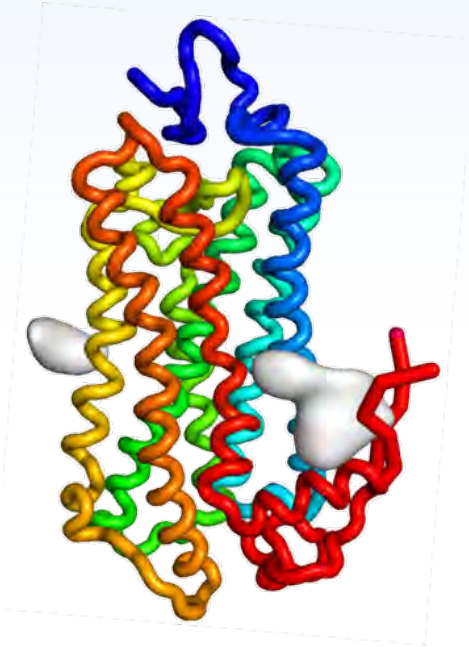
Does the rigidity of the protein and its surroundings increase?

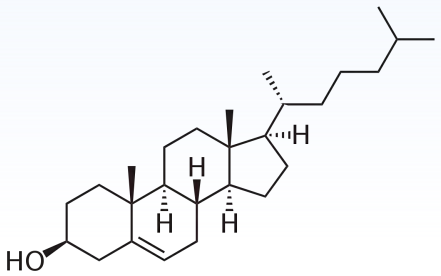
- insertion of the hydrophobic moieties between the unsaturated alkyl chains

Are the hydrophobic moieties interacting with cholesterol binding site?

- [SMA(2:1)LPs vs. SMA(3:1)LPs] vs. DIBMALPs

slower reaction progress

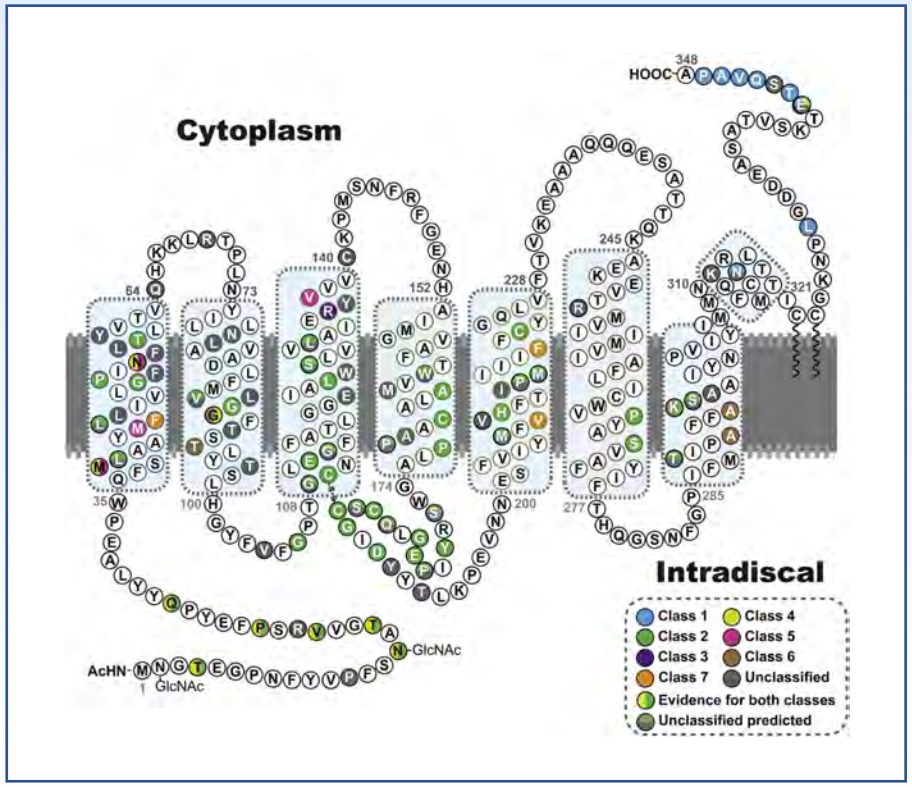




cholesterol

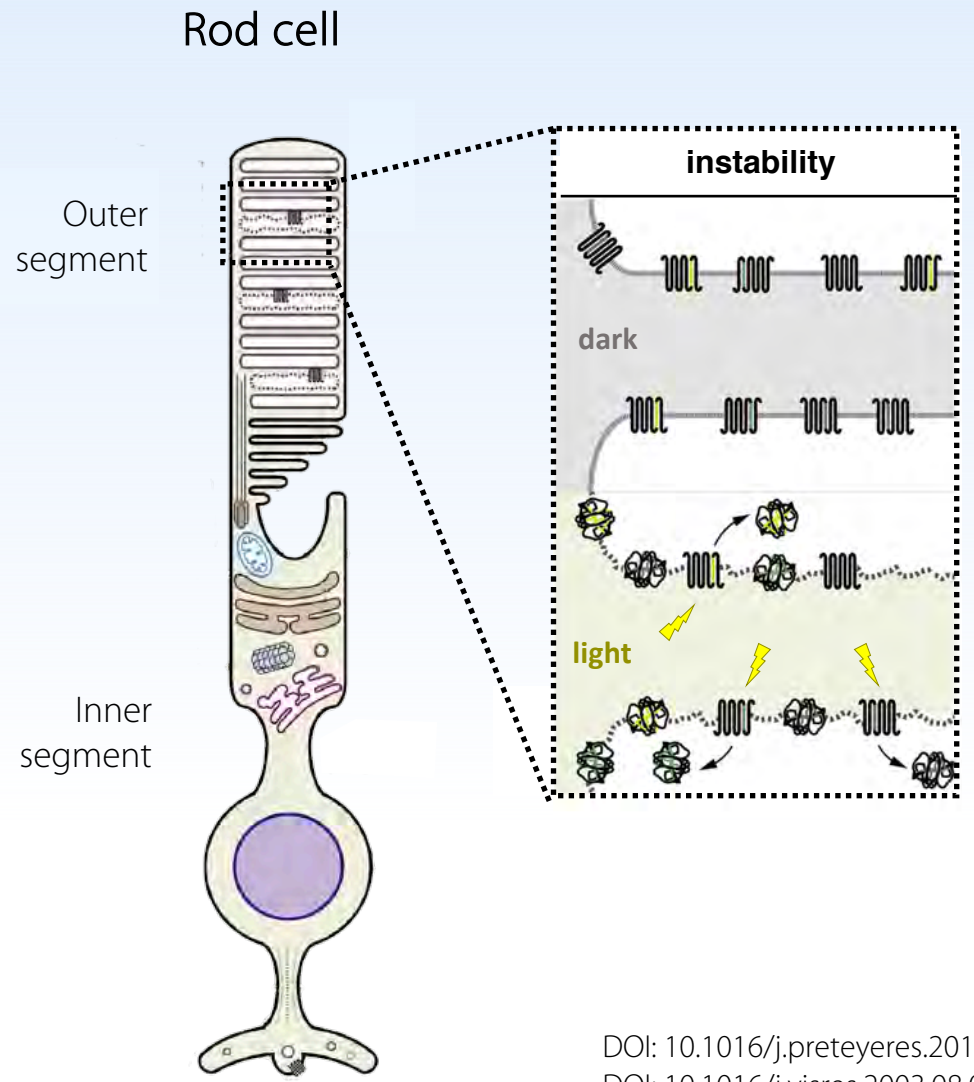
DOI: 10.1007/s12274-014-0560-6
DOI:10.1007/978-94-007-7423-0_5

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?



DOI: 10.1016/j.preteyeres.2017.10.002
DOI: 10.1016/j.visres.2003.08.010
DOI: 10.1111/j.1742-4658.2011.08066.x

Acknowledgments

University of California, Santa Cruz

- David Kliger, PI
- Eefei Chen, Ph.D.
- Jim Lewis, Ph.D.
- Istvan Szundi, Ph.D.
- Chie Funatogawa, Ph.D.
- Pamela Schleissner, grad

Oregon Health & Science University

- David Farrens, Ph.D.
- Jonathon Fay, Ph.D.
- Weekie Yao, grad

University of Tennessee, Knoxville

- Barry Bruce, Ph.D.
- Brian Long, Ph.D.
- Cameron Workman, grad