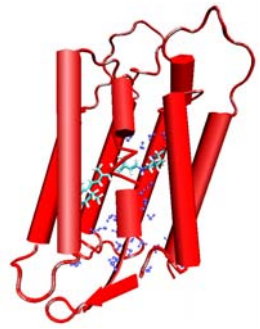
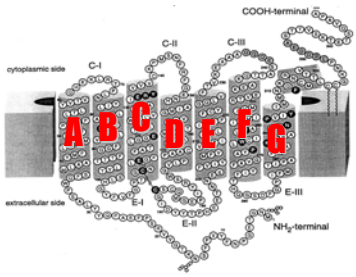
Structure-Function Relationship of Retinal Proteins



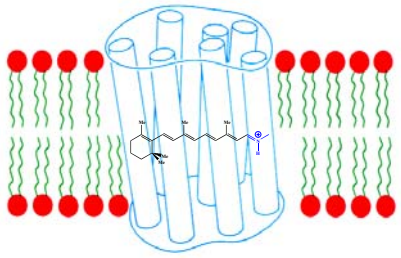
Structure of Retinal Proteins

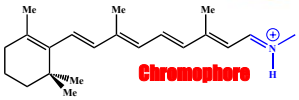


GPCRs

Retinal proteins or rhodopsins belong to the superfamily of seven-transmembrane helical (7TM) proteins. Seven helices, with N-terminus on the extracellular side and C-terminus on the cytoplasmic side of the membrane (not necessarily G-protein coupled)

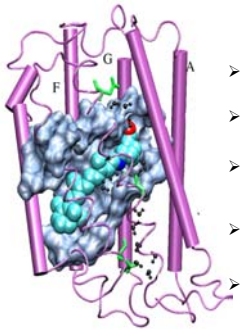
Retinal Proteins -- Rhodopsins





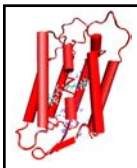
- Covalently linked to a lysine
- Usually protonated Schiff base
- all-*trans* and 11-*cis* isomers

Bacteriorhodopsin -- bR

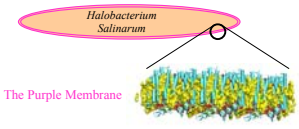


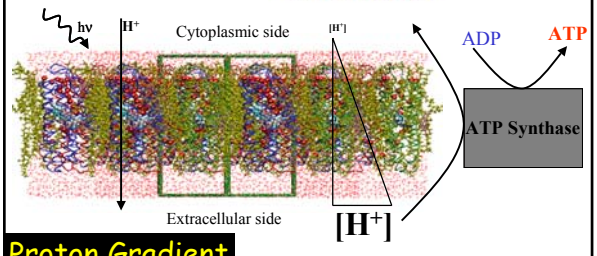
- The simplest ion pump in biology
- The simplest photosynthetic center
- The best characterized membrane protein
- Technological applications in molecular electronics
- The first membrane protein with a known atomic-detail 3D structures

bR role in Bioenergetics



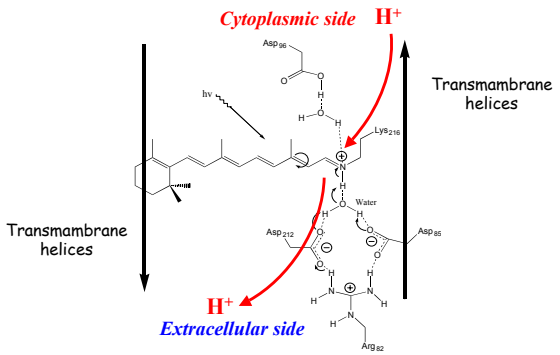
Light



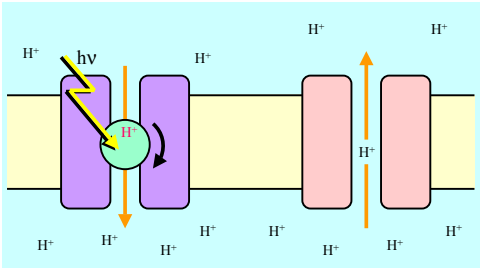


Proton Gradient

Schematic proton path in bacteriorhodopsin

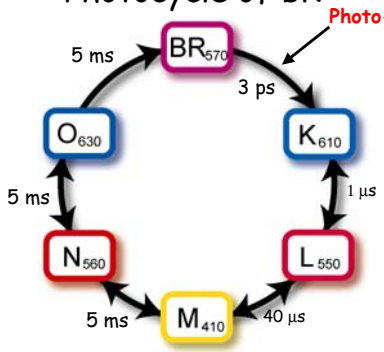


Active Channels Need a 'Switch' Mechanism



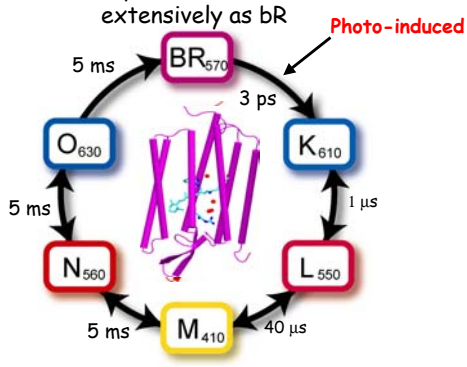
What is the switch in bR? How does it work?

Photocycle of bR



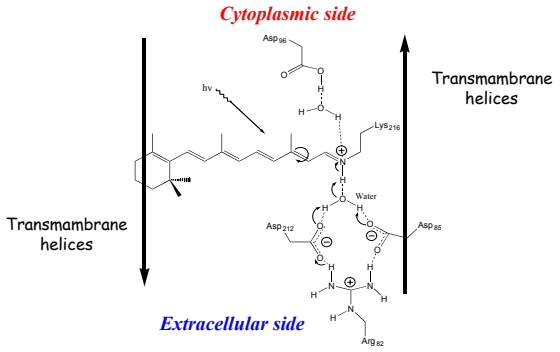
All intermediates are trapped in low temperature and have been characterized by vibrational and absorption spectroscopy.

No membrane protein has been studied as extensively as bR

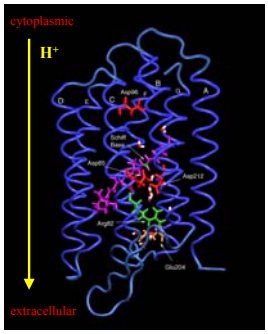


All intermediates have also been characterized by X-ray crystallography!

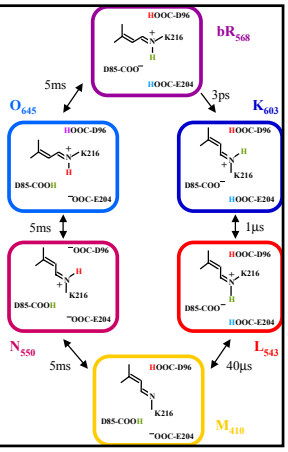
Schematic proton path in bacteriorhodopsin



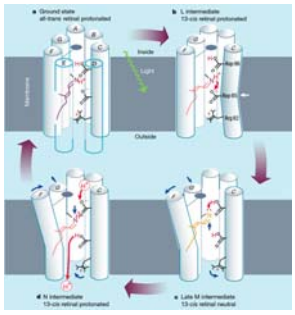
BR's Photocycle



light driven proton pump

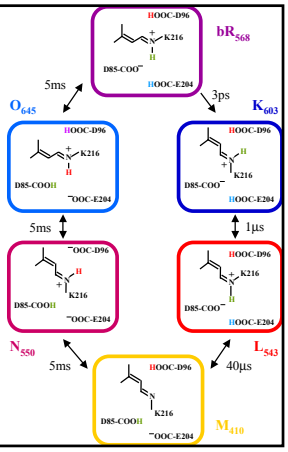


BR's Photocycle

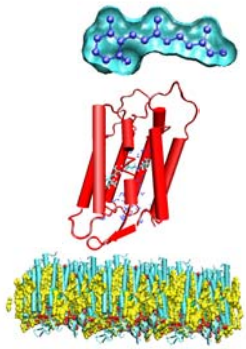


Conformational Change of Helices

Kuhlbarandt, Nature, 406,569 (2000)



Study of bR at three levels

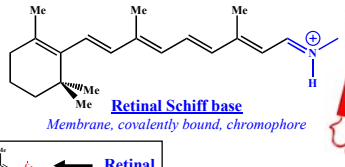


Chromophore
- Analysis of the structure
- Calculation of excited state dynamics

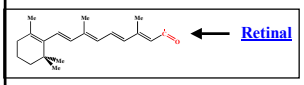
Protein
- Chromophore-protein interaction
- QM-MM calculations
- MD simulation of the photocycle

bR in the purple membrane
Modeling of the protein in *lipid bilayers*

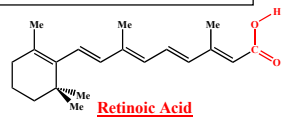
Retinoids



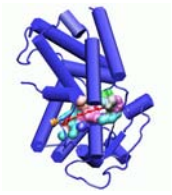
Retinal Schiff base
Membrane, covalently bound, chromophore



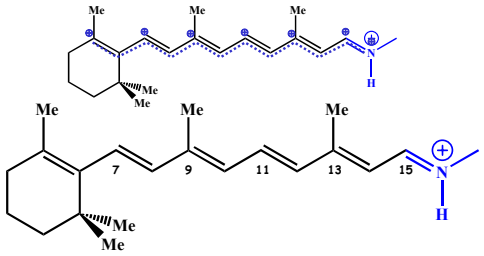
Retinal



Retinoic Acid
Nucleus, receptor site, ligand (no photoactivity)



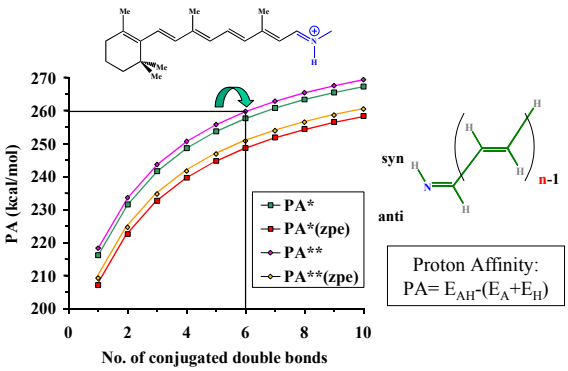
Unconventioanl chemistry



The necessity of quantum mechanical treatment of the chromophore:
Conjugated π-electronic system, delocalization
The effect of protein matrix on the ligand

QM is expensive – Most of the time, one needs to use models

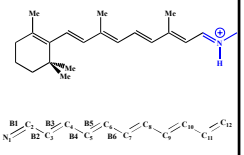
Effect of Conjugation on pK_a (Gas Phase Proton Affinity)



Proton Affinity:
$PA = E_{AH} - (E_A + E_H)$

Effect of the methyl groups on pK_a

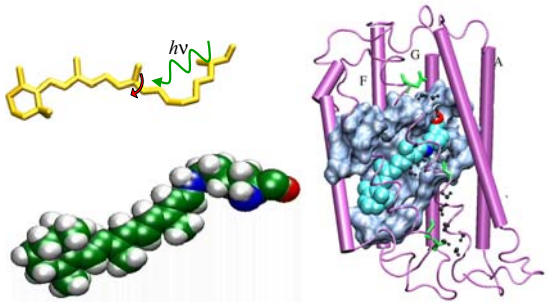
Species	6-31G*	6-31G* (zpe)	6-31G**	6-31G** (zpe)
PSB6	**260.61**	**251.64**	**262.66**	**253.69**
4-met	261.44	252.60	263.54	254.70
5-met	261.03	252.11	263.10	254.18
7-met	261.32	252.40	263.39	254.47
8-met	261.39	252.51	263.49	254.61
12cis-met	262.91	254.04	265.00	256.14
12trans-met	263.41	254.52	265.51	256.63
N-met	260.46	251.03	262.52	253.10
4,8-dimet	262.13	253.38	264.29	255.53
12,12-dimet	264.92	256.08	267.09	258.24
N,4,8-trimet	261.76	252.50	263.92	254.66
4,8,12,12-tetramet	266.08	257.46	268.33	259.71
N,4,8,12,12-pentamet	265.31	256.14	267.54	258.38
N-met-retinal Schiff base	**266.45**	**257.26**	**268.72**	**259.52**



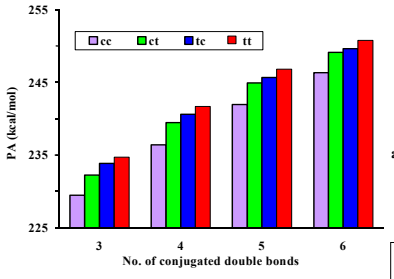
Proton Affinity:
$PA = E_{AH} - (E_A + E_H)$

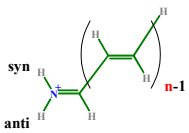
No more room for additional methyl groups on the backbone

What is the effect of isomerization?



Isomerization State and Proton Affinity



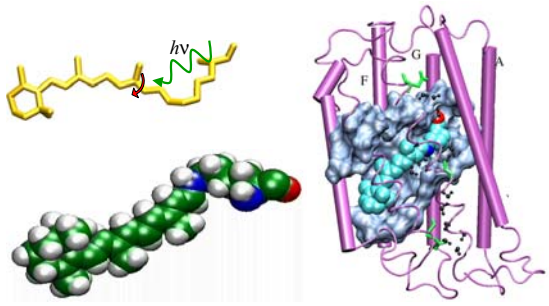


Proton Affinity:
$PA = E_{AH} - (E_A + E_H)$

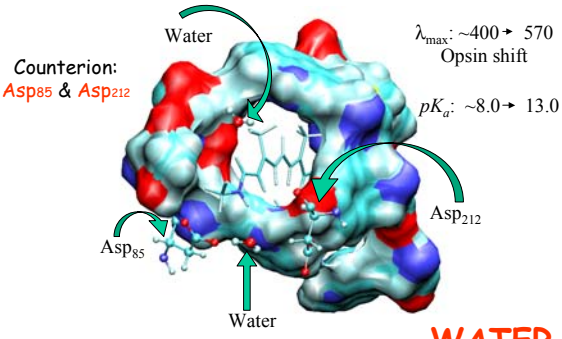
cc: B2,B3-di-*cis* isomer
tc: B2-*strans*, B3-*cis* isomer
ct: B2-s-*cis*, B3-*trans* isomer
tt: all-*trans* isomer.

Isomerization does not have a strong impact on PA!

What is the effect of isomerization?



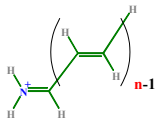
Retinal binding pocket in bR



WATER

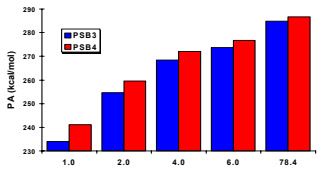
Effect of the environment on PA

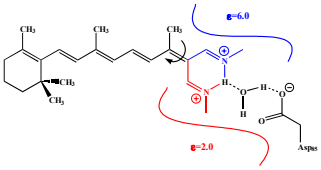
ε	1.0	2.0	4.0	6.0	78.4
acetate	**350.81**		**309.05**		**288.27**
SB1	208.76		256.04		278.02
SB2	223.85		263.36		281.86
SB3	234.00	254.64	268.44	273.78	284.78
SB4	241.15	259.63	271.98	276.77	286.68
SB5	246.39		274.54		288.01
SB6	250.58		276.43		288.93
PMET	258.57		281.23		292.07
RSB	**260.12**		**282.07**		**292.56**



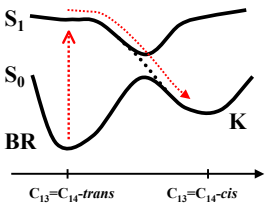
Proton Affinity:
$PA = E_{AH} - (E_A + E_H)$

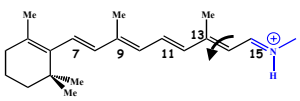
In situ isomerization and pK_a



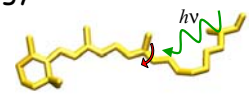


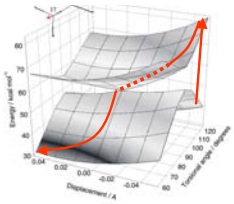
Coupling of electronic excitation and conformational change in bR





Ground and Excited State Potential Energy Surfaces of Retinal

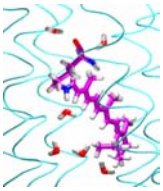


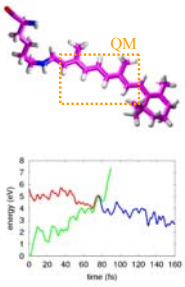


trans

cis

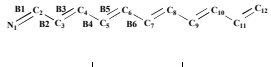
Ab Initio QM/MM Excited State MD Simulation

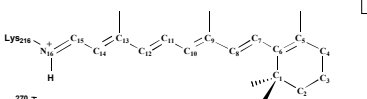




Quantum mechanical (QM) treatment of the chromophore, and force field (MM) treatment of the embedding protein

Isomerization Barriers in retinal





Proton Affinity:
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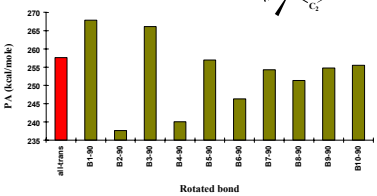
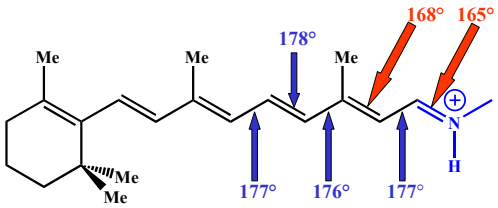


Ground state isomerization

Low barriers against double bond isomerization

A twisted chromophore in bR?



- A twisted chromophore is also experimentally reported.
- X-ray structures of bR report the twisted form of chromophore
- The twist is found around the terminal double bonds
- It may influence pK_a of the chromophore