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11 - 15 October 2010

Classical and QM/MM Simulations of G-protein-coupled Receptors

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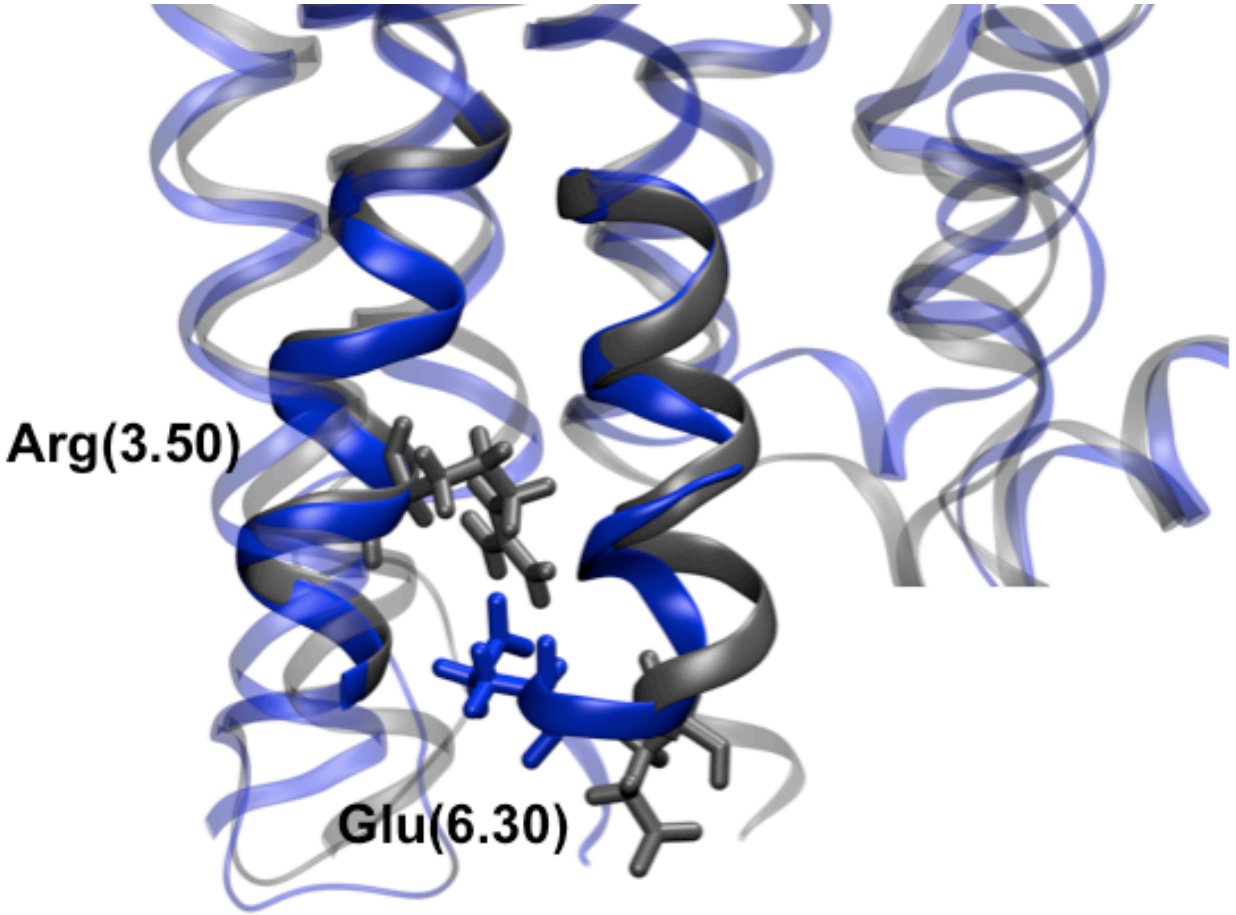
Classical and QM/MM simulations of G-protein coupled receptors

ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE



Stefano Vanni

Trieste, 12/10/2010

Agenda

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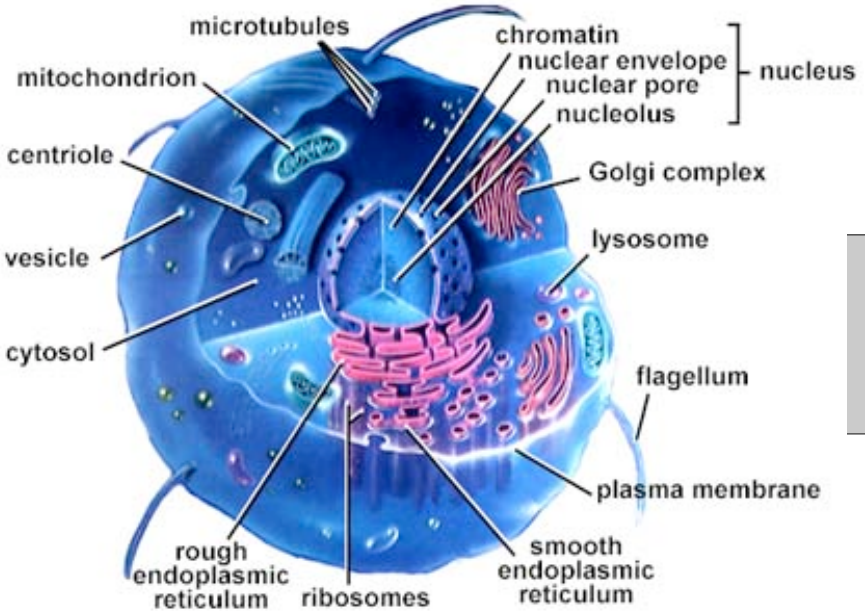
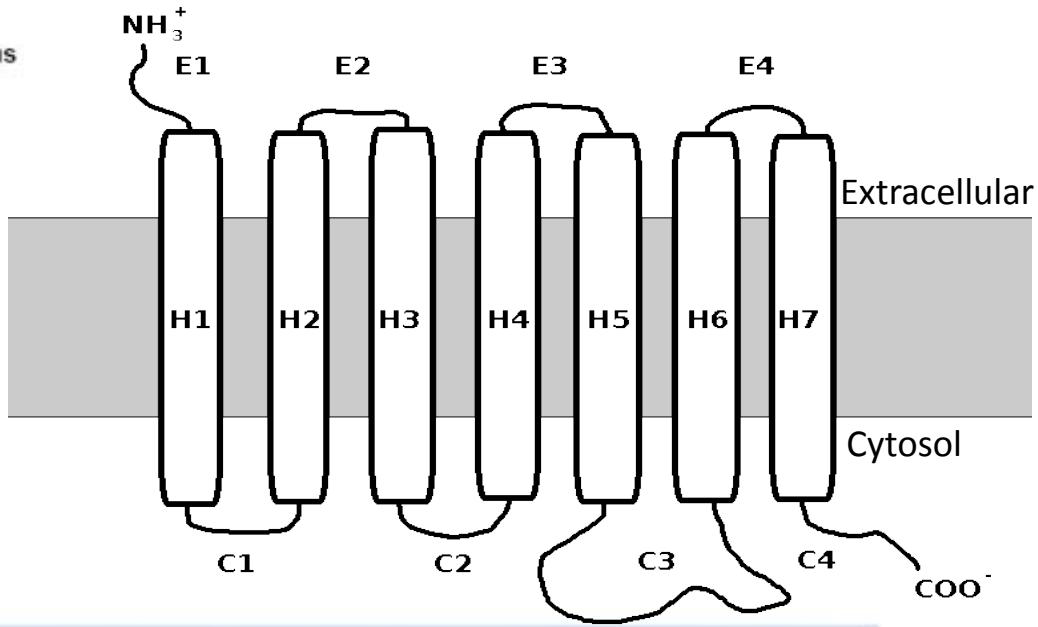
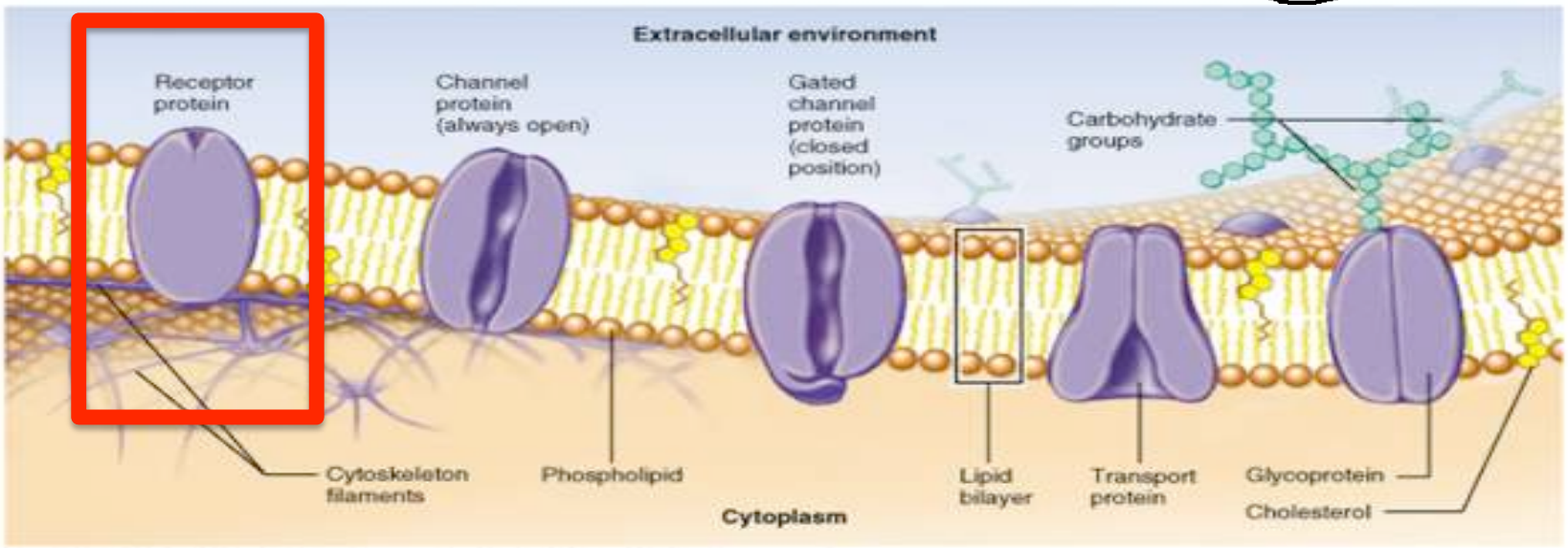
- G-protein coupled receptors (GPCRs) at work: a biological puzzle
- Studying GPCR signal transduction with computer simulations:
  - Adrenergic receptors
  - Rhodopsin
- Conclusions and outlook

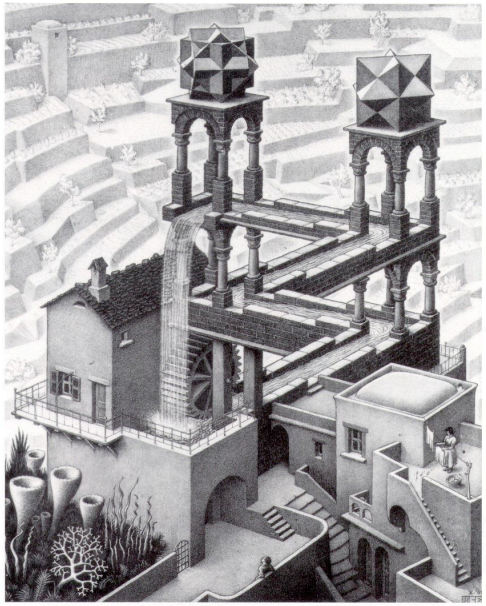
GPCRs: ubiquitous transmembrane proteins (1/2)







GPCRs: ubiquitous transmembrane proteins (2/2)



SMELL

VISION

INFLAMMATORY RESPONSE

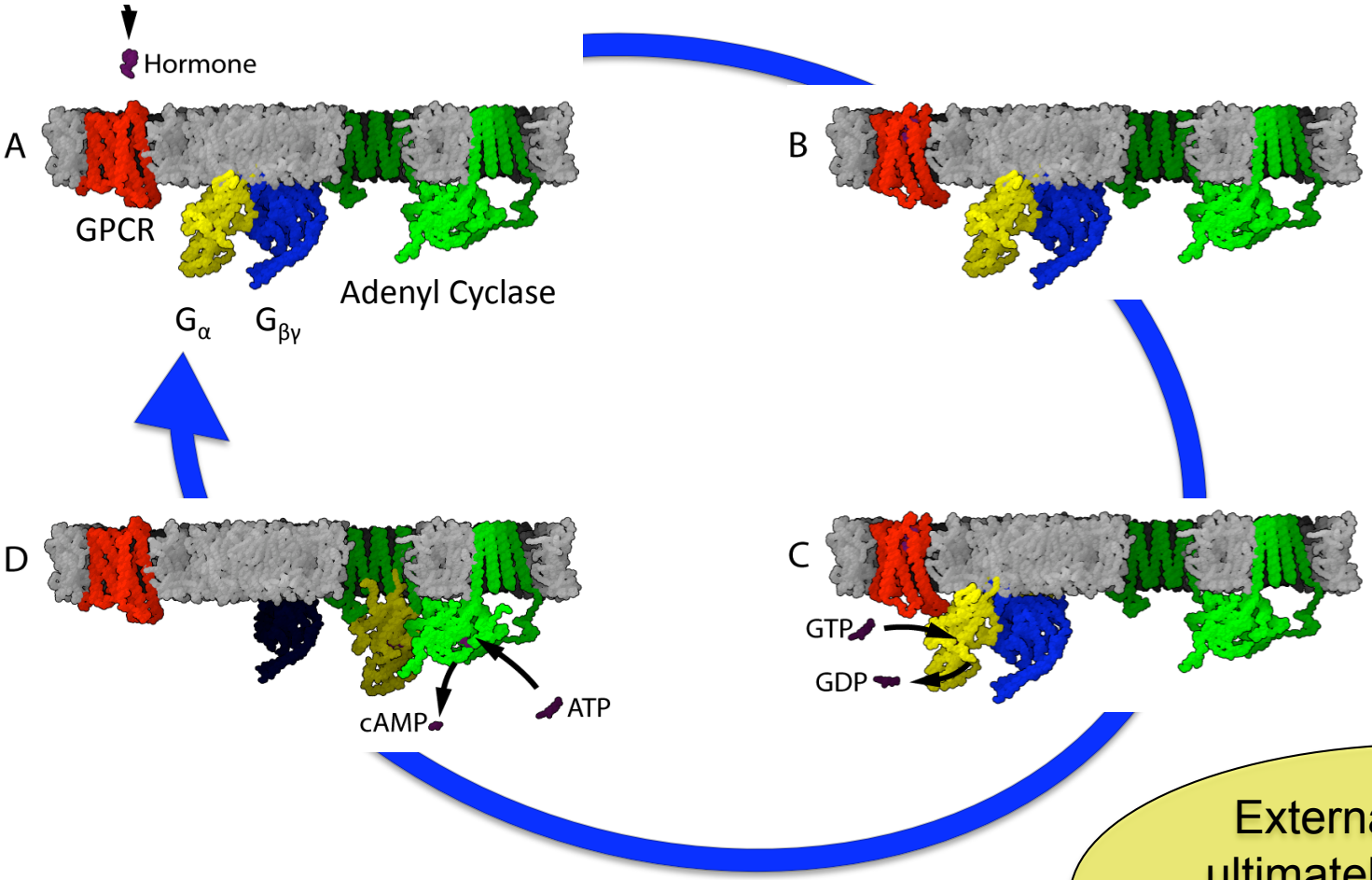
GPCRs are involved in

FIGHT or FLIGHT

MOOD

… and many other signal transduction pathways!

G-protein coupled receptors signalling pathway



External stimulus is ultimately transformed into electrochemical potential

Lefkowitz et al., Ann.Rev.Biochem. **52**, 159 (1983)

GPCRs activation: a simple mechanism?

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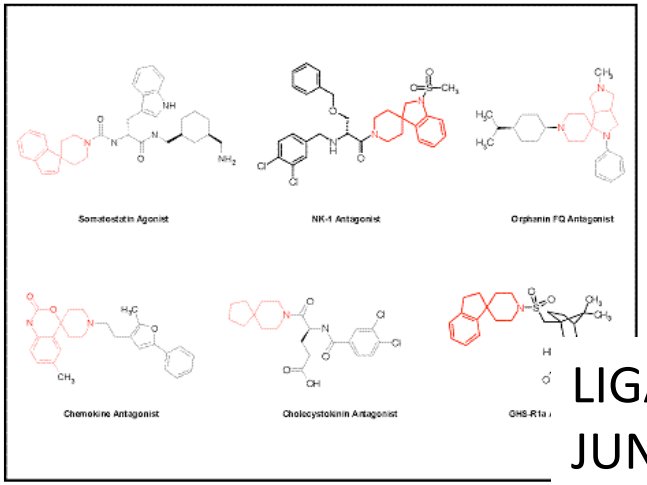
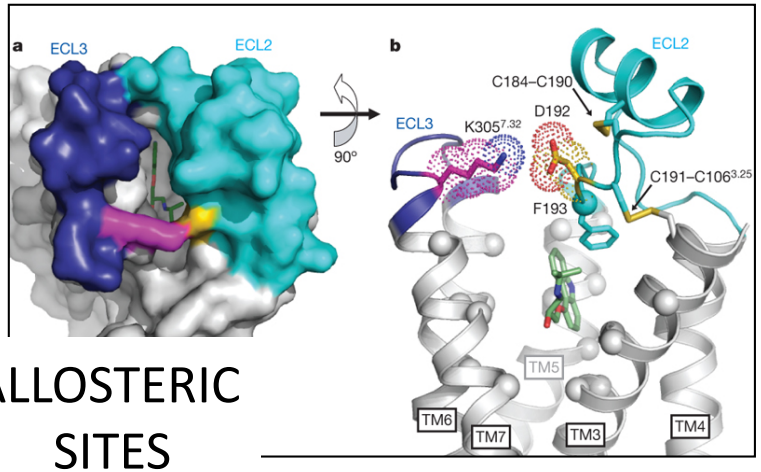


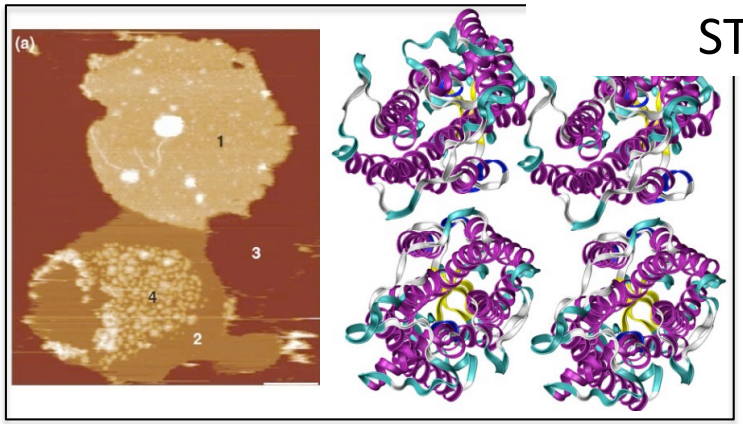
LIGAND JUNGLE



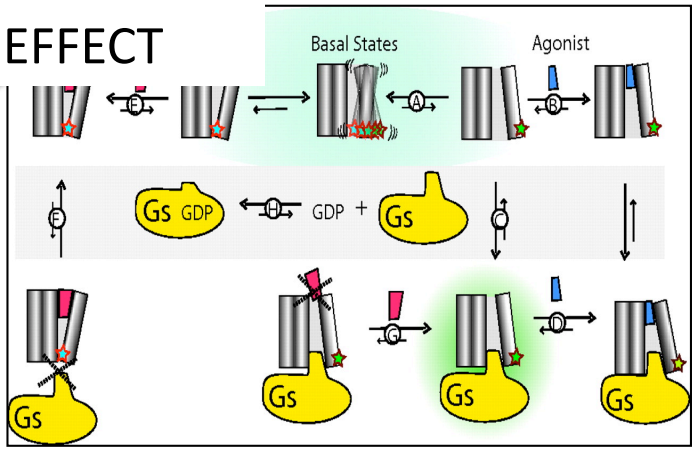
ALLOSTERIC SITES

NOT AT ALL!

OLIGOMERIZATION STATE



G PROTEIN EFFECT



Bokoch et al., Nature, **463**, 108 (2010)
Yao et al., PNAS, **106**, 9501 (2009)

GPCRs signal transduction: theoretical challenges & opportunities

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Time scales	Activation times of milliseconds
Allosteric effects	Signal transduction from the ligand binding site to the G-protein binding site
Cooperativity	Several mutations substantially alter receptor activity.
Multi-scale behaviour	Global changes triggered by local (chemical) events

Classical molecular dynamics

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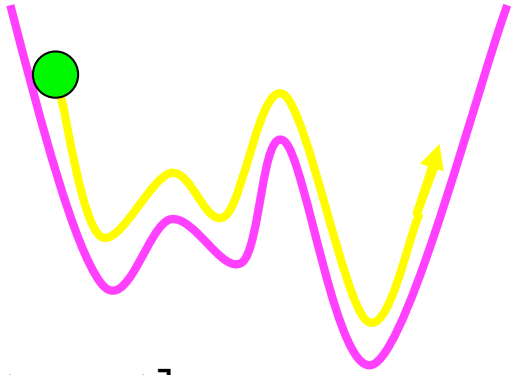
Goal: Follow the time evolution of the system at a finite temperature

Assumption: The motion of atoms can be described with classical mechanics

$$\begin{cases} \sum_i M_i \ddot{\vec{R}}_i = \vec{F}_i \\ (\vec{R}_{io}, \dot{\vec{R}}_{io}) \end{cases}$$

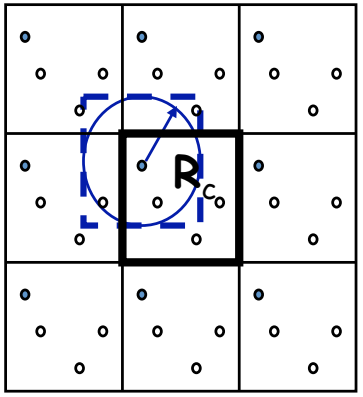
$$\vec{F}_i = -\frac{\delta V(R)}{\delta \vec{R}_i}$$



$$V = \sum_{bond} \frac{1}{2} K_R (R - R_o)^2 + \sum_{angles} \frac{1}{2} K_\theta (\theta - \theta_o)^2 + \sum_{dihedrals} \frac{1}{2} K_\varphi \left[1 + \cos(n\phi + \delta)\right]$$

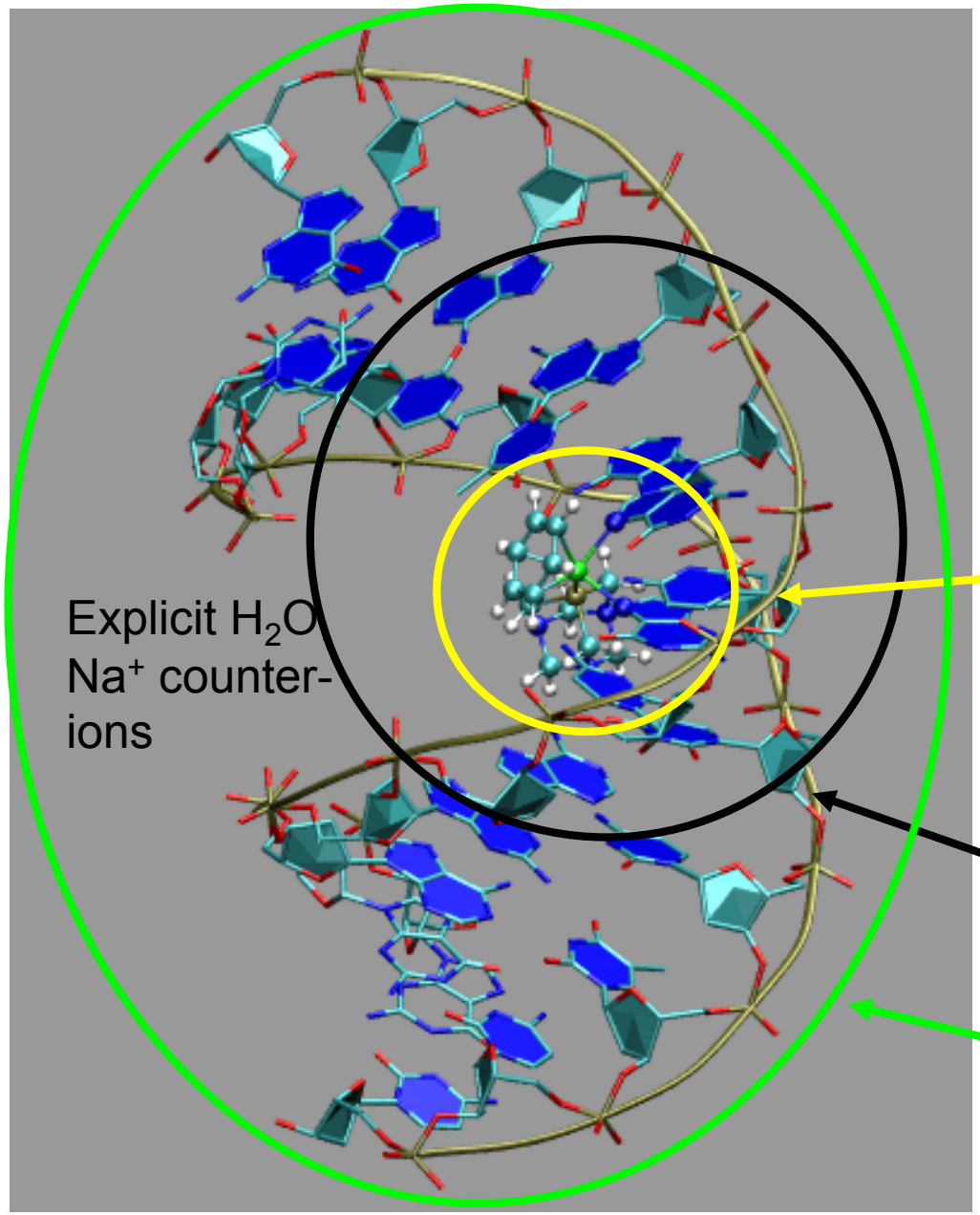
$$+ \sum_{pairs} \left(-\frac{A}{r_{ij}^6} + \frac{B}{r_{ij}^{12}} \right) - \sum_{pairs} \frac{1}{4\pi\varepsilon} \frac{q_i q_j}{r_{ij}}$$

Parametrization with experimental or QM data on small gas phase molecules (plus adaptation to condensed phase environment)



Periodic boundary conditions

Hybrid Quantum Mechanics / Molecular Mechanics scheme



Based on a mixed Hamiltonian:

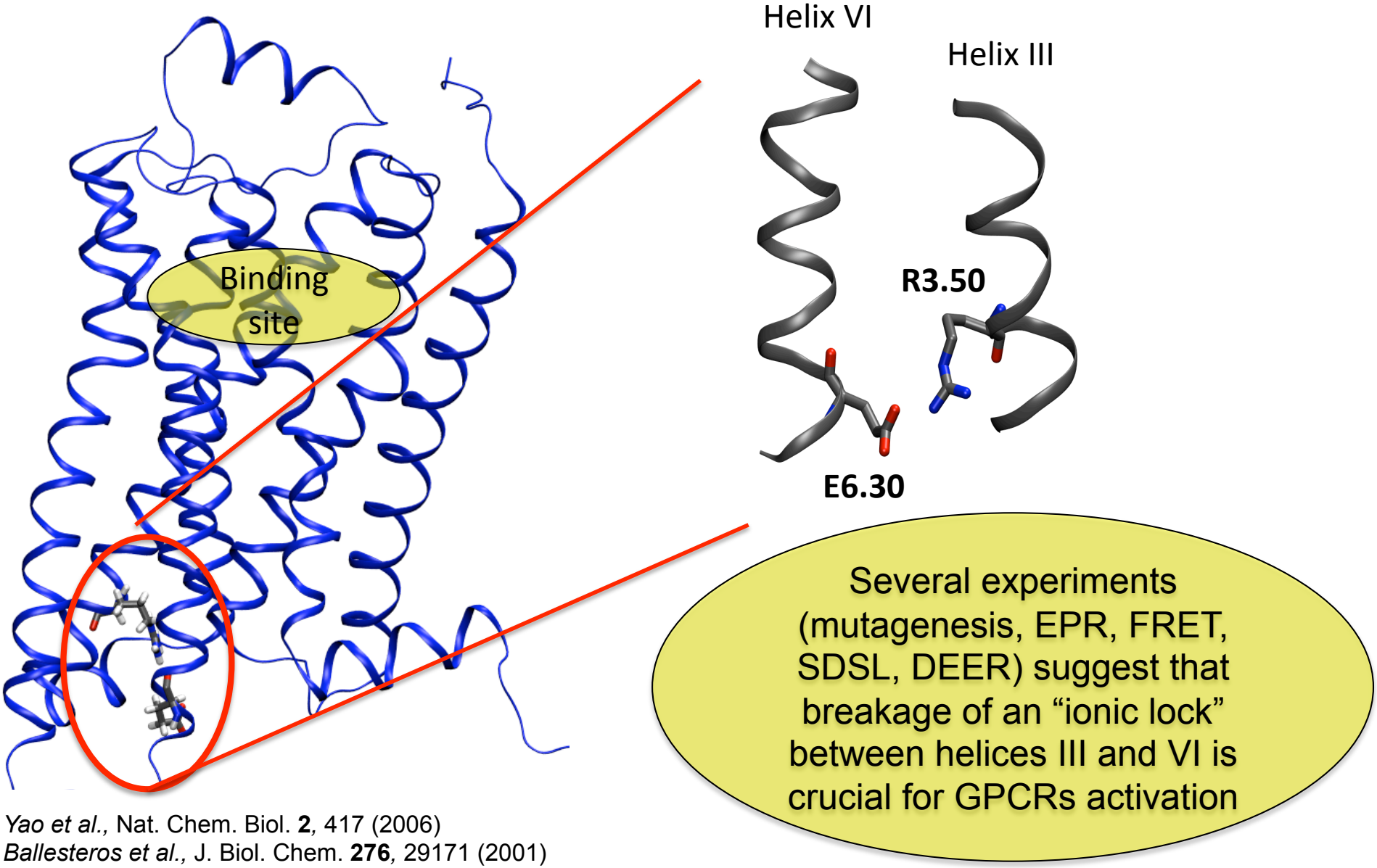
$$H = H_{QM} + H_{MM} + H_{QM\ /MM}$$

QM part: Localized chemically active region: treated with full or semiempirical quantum method

Interface region: Interactions between QM and MM.

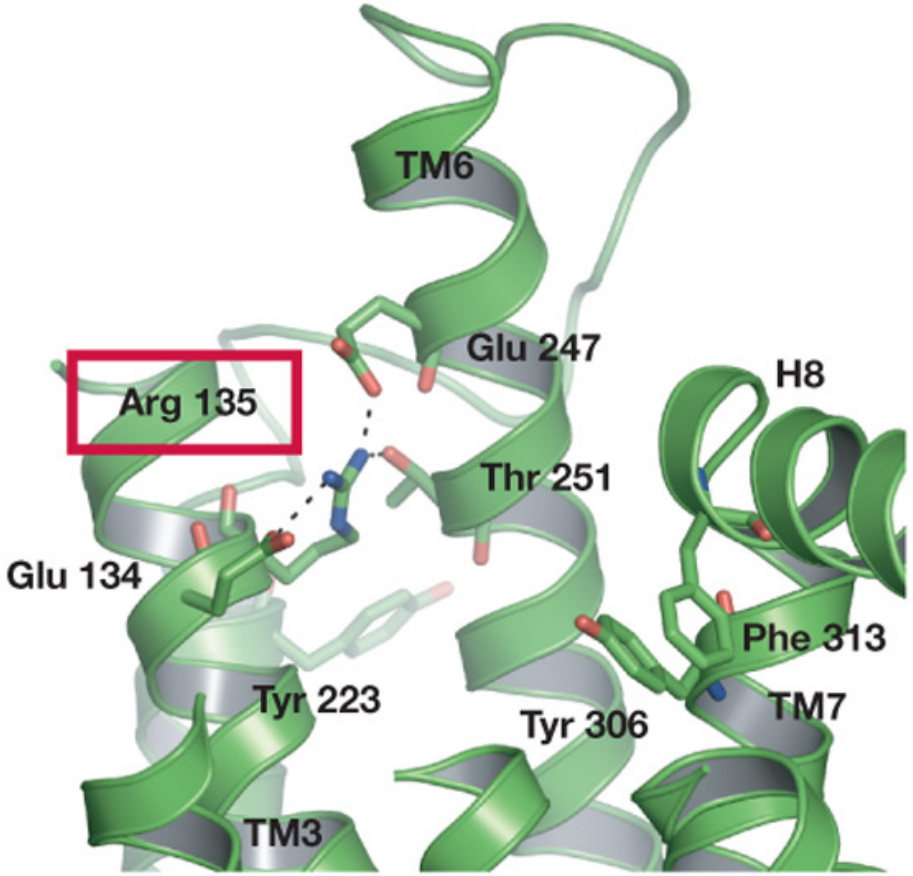
MM part: Environmental effect described with a classical Force Field (Amber, Gromos)

GPCRs activation: a common mechanism?

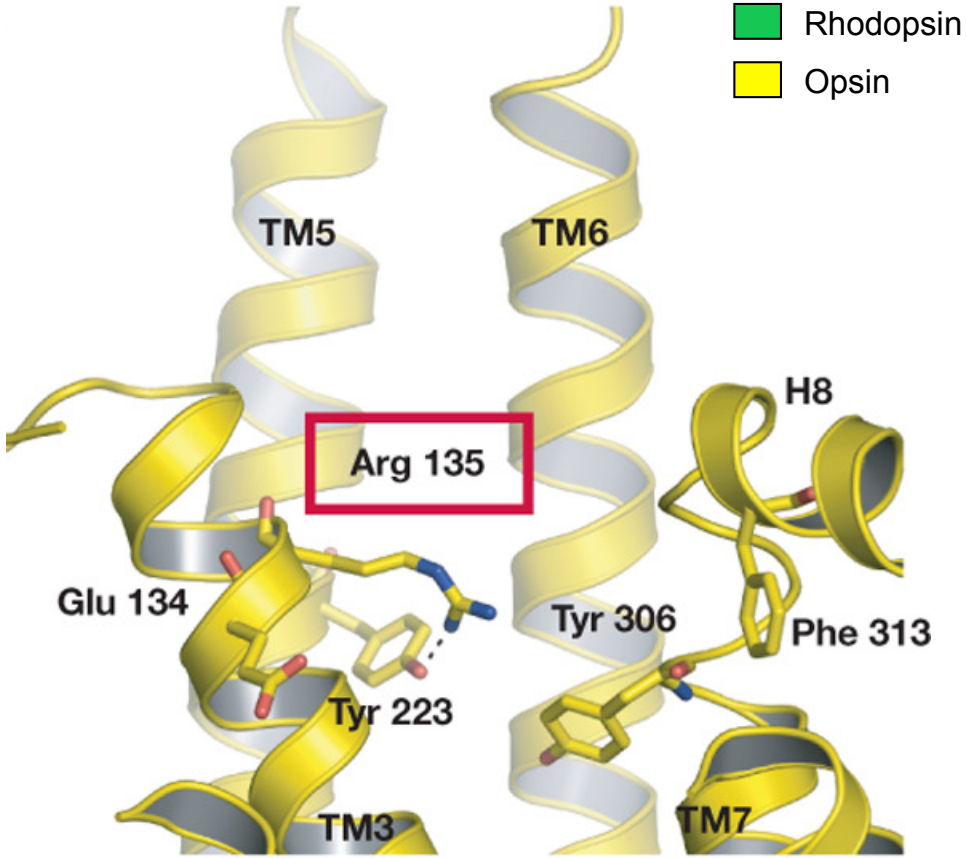


Yao et al., Nat. Chem. Biol. **2**, 417 (2006)
Ballesteros et al., J. Biol. Chem. **276**, 29171 (2001)
Farrens et. *al.*, Science, **274**, 768 (1996)

Crystal structures of dark rhodopsin and active-like opsin



The “ionic lock” is formed in dark rhodopsin and it is absent in “active” opsin



Arg(3.50) directly interacts with a G protein fragment in the crystal structure of opsin

Palczewski et al., Science **289**, 739 (2000)
Scheerer et al., Nature **455**, 497 (2008)

Crystal structures of adrenergic receptors

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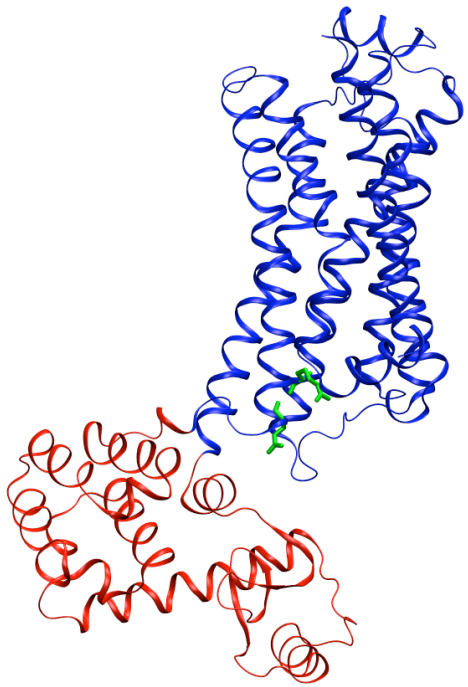
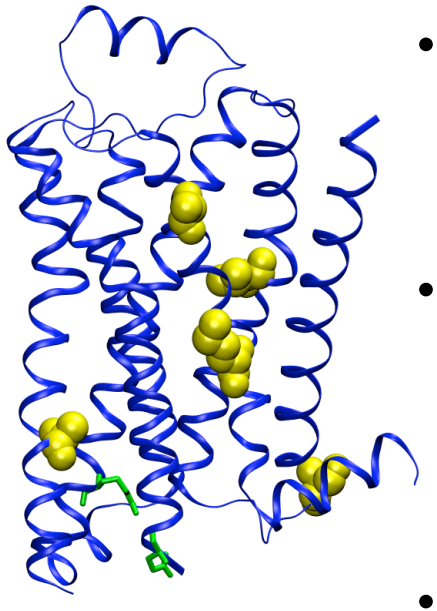
β_2AR-T4L



- Lysozyme engineered
- Bound to the partial inverse agonist carazolol
- Partially active with respect to WT

β_1AR-m23

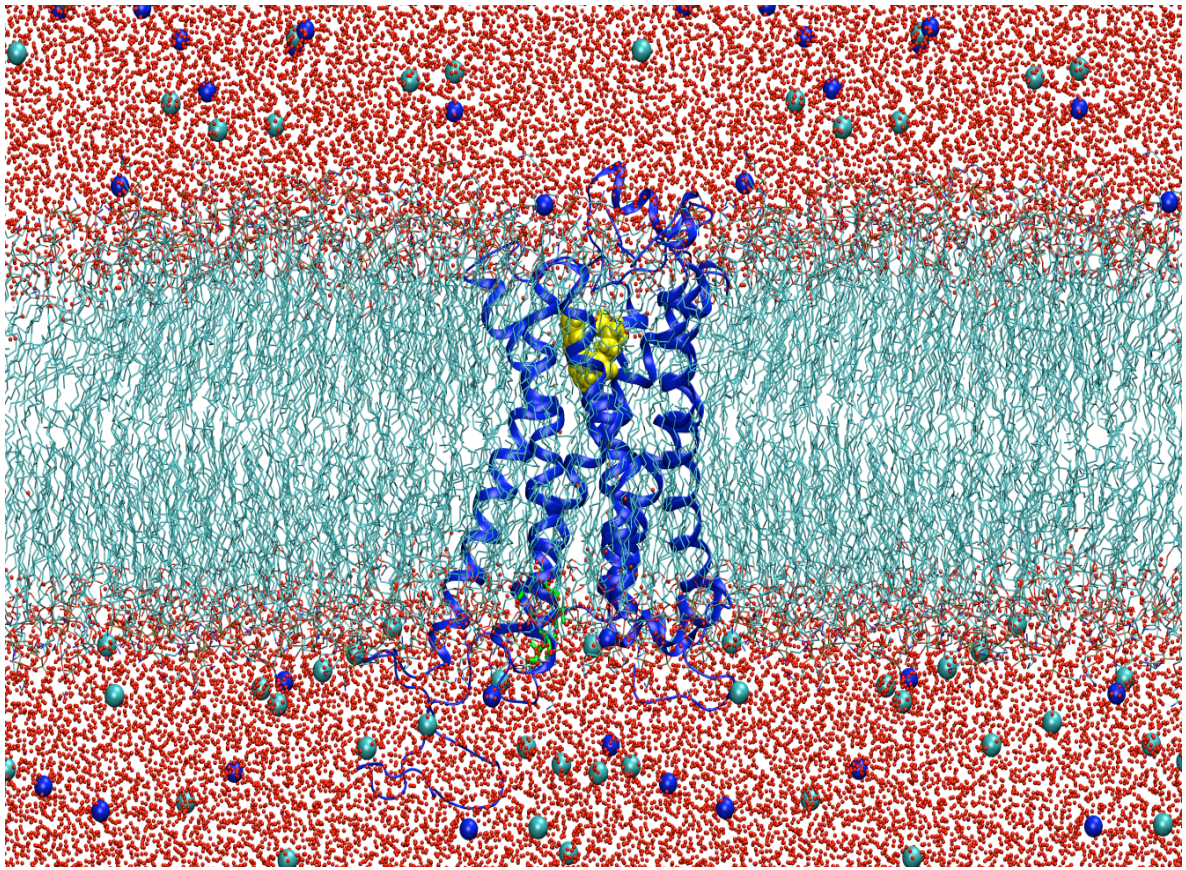


- Thermostabilized via 6 single point mutations
- Bound to the partial inverse agonist cyanopindolol
- Inactive with respect to WT

There is no "ionic lock" between helices III and VI

Cherezov et al., Science **318**, 1258 (2007)
Rosenbaum et al., Science **318**, 1266 (2007)

System set-up



- Reconstructed WT receptor
- Explicit lipid bilayer*
- Physiological ionic strength (0.1M)
- ~100.000 atoms
- 90x90x110 Å box size
- Isothermal-isobaric ensemble
- PBC
- PME electrostatics, 12 Å cutoff
- Amber ff99SB forcefield
- 2 fs integration timestep

* 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine

Root Mean Square Deviation

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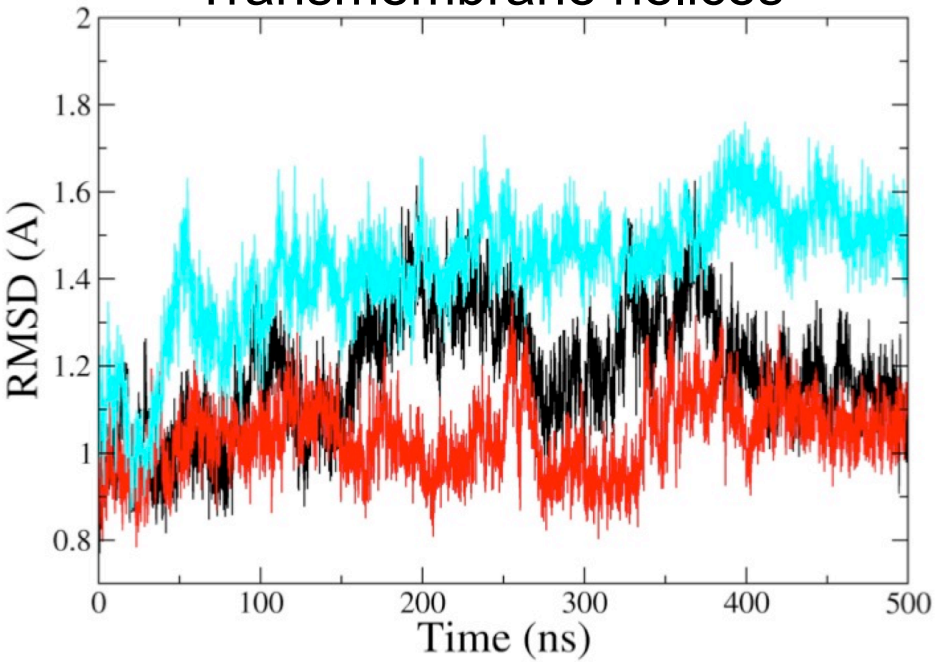
β_2AR wt

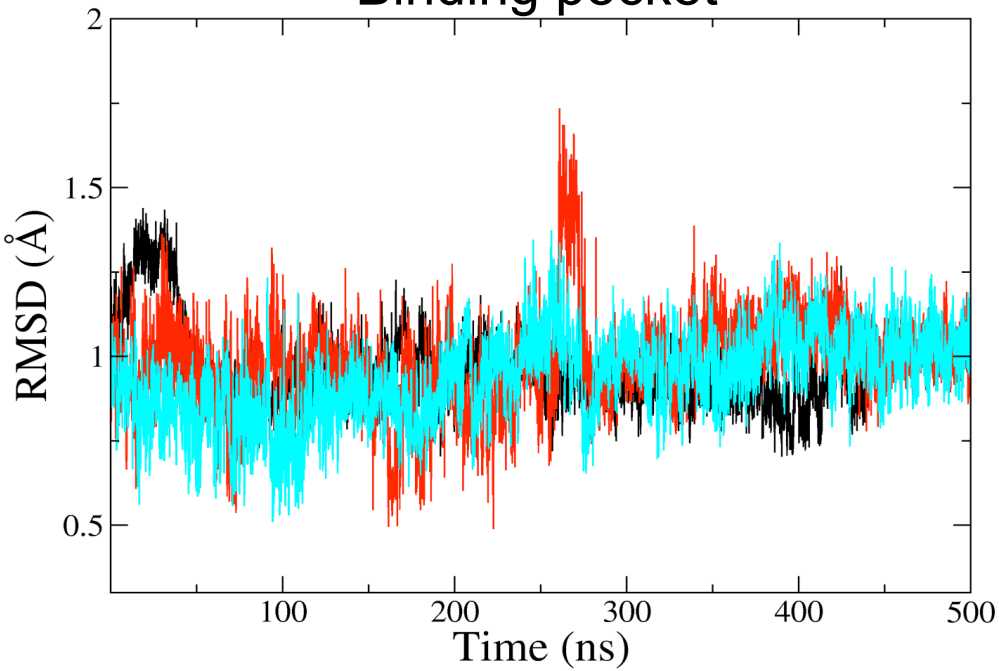
β_1AR wt

β_1AR-m23

Transmembrane helices



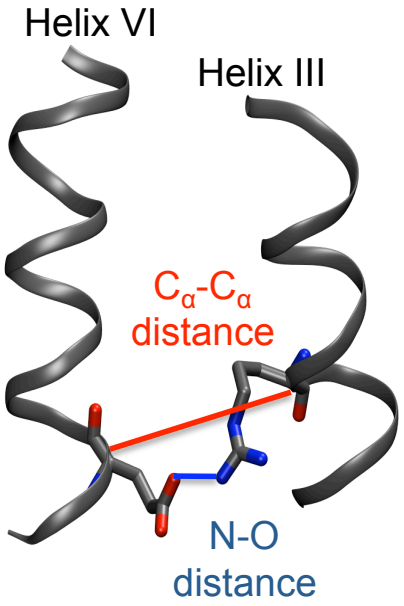
Binding pocket

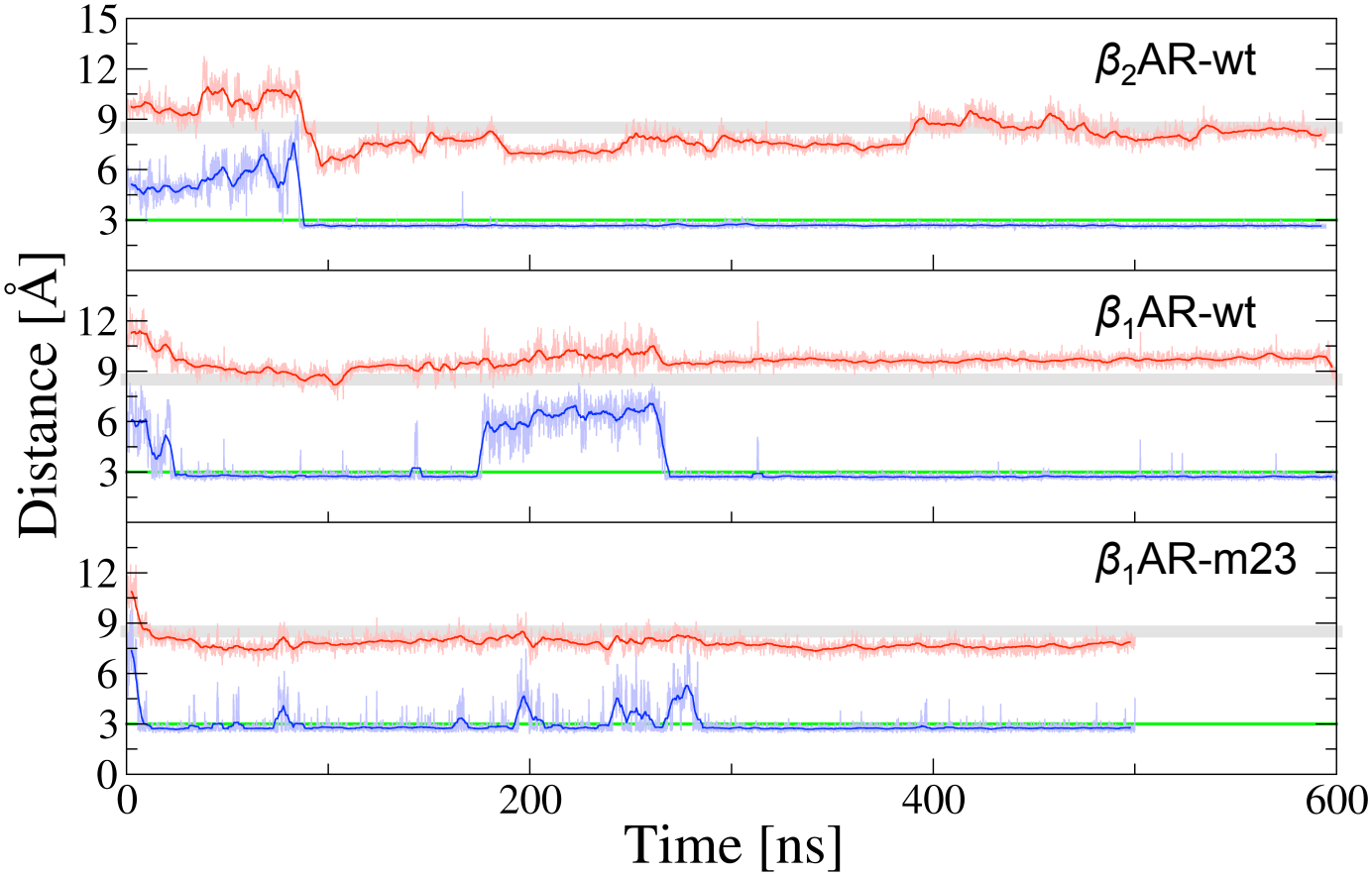


The overall transmembrane structure and the binding pocket remain close to the the X-ray data in the simulations.

SV, M. Neri, I. Tavernelli and U. Rothlisberger, Biochemistry, **48**, 4789 (2009)

“Ionic lock” behaviour in wild type adrenergic receptors





SV, M. Neri, I. Tavernelli and U. Rothlisberger, Biochemistry, **48**, 4789 (2009)
Dror et al. PNAS, **106**, 4689 (2009)

Agonist-receptor interactions

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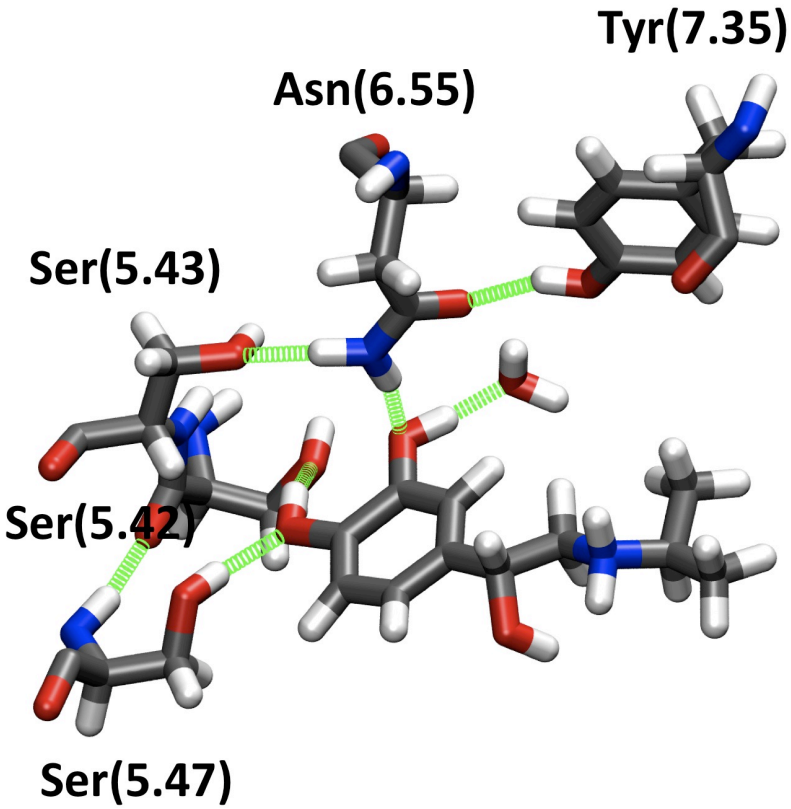
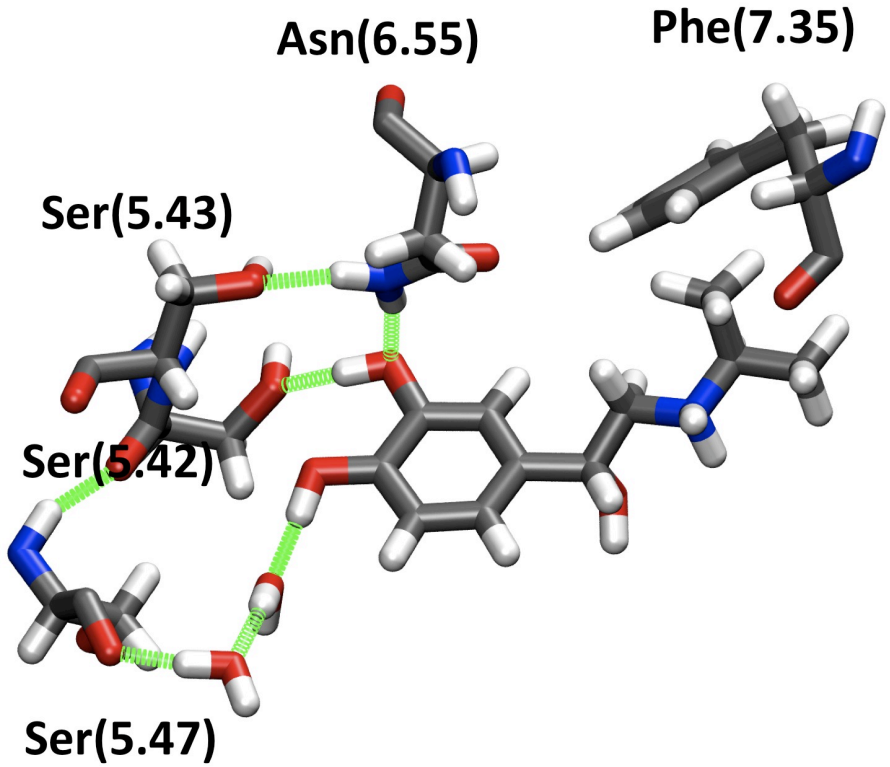
β_2AR



β_1AR



SV, M. Neri, I. Tavernelli and U. Rothlisberger, PLOS Computational Biology, *submitted*

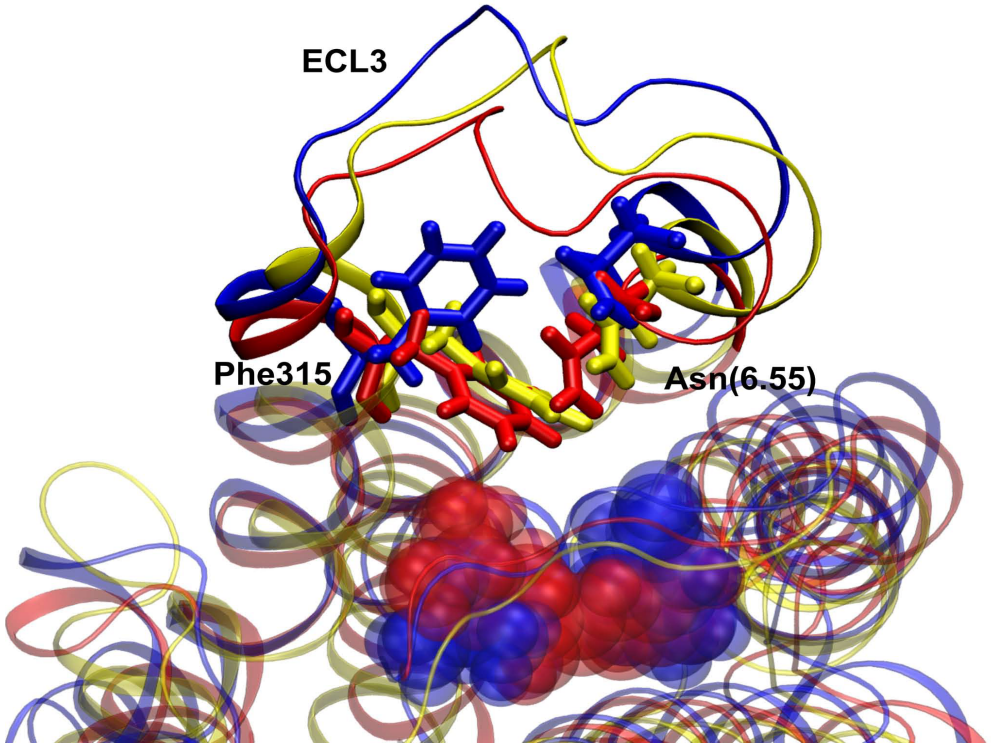
Extracellular loops

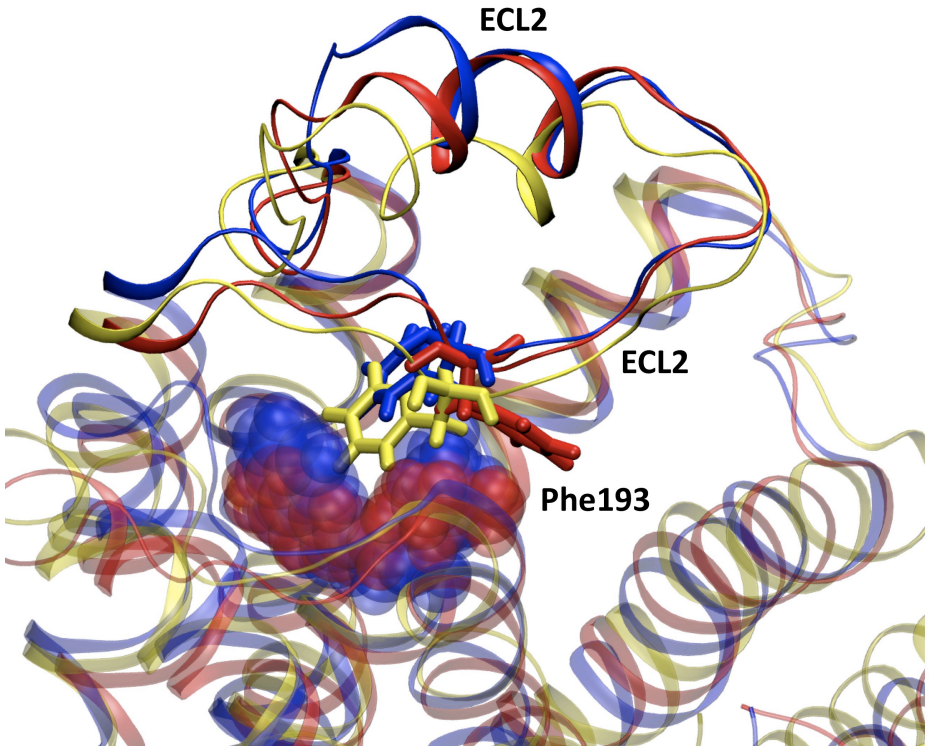
ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

β_2AR - inverse agonist

β_1AR – full agonist

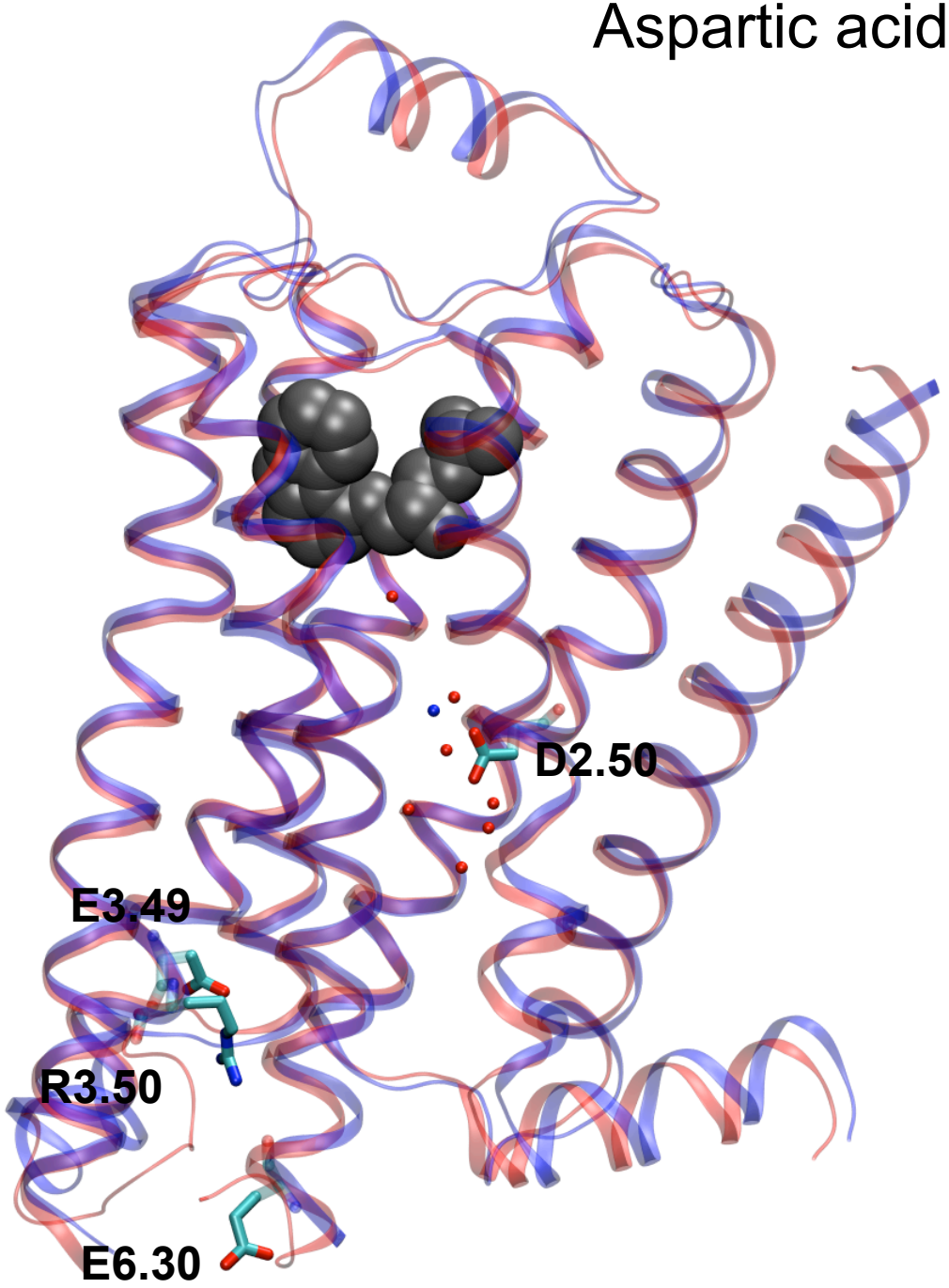
β_1AR - apoform





SV, M. Neri, I. Tavernelli and U. Rothlisberger, PLOS Computational Biology, *submitted*

Aspartic acid Asp(2.50)



- FTIR experiments suggest that Asp(2.50) is protonated in both dark and active state of rhodopsin
- D2.50N reduce coupling to the G-protein in β_1AR and β_2AR, while leads to G-protein uncoupling in other GPCRs
- Binding of Na^+ to Asp(2.50) lowers activity for many GPCRs

SV, M. Neri, I. Tavernelli and U. Rothlisberger, JMB, **397**, 1339, (2010)

Structural and sequence alignment in the transmembrane region of GPCRs

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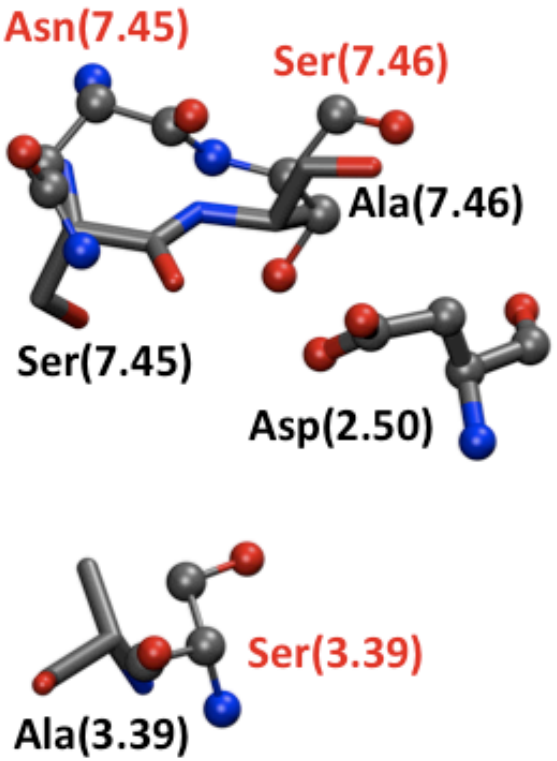


Rhodopsin

β_2AR

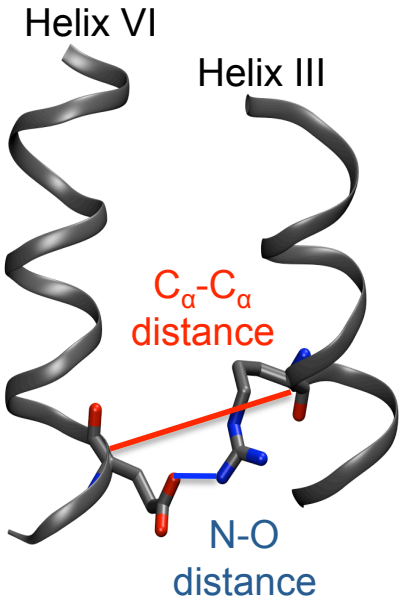
```
                          (2.50)      (3.39)  (7.45-46)
OPSD_BOVIN  ... LAVADLF ... GEIALW ... TSAVYNP ...
OPSU_BRARE  ... ISLAGFI ... GLVTGW ... SSSVYNP ...
OPSG_HUMAN  ... LAVADLA ... GITGLW ... SATIYNP ...
OPSB_HUMAN  ... VSFGGFL ... GLVTGW ... SACIYNP ...
OPSB_ASTFA  ... LAISNLL ... GMVSLW ... ASTVYNP ...
OPSG_ORYLA  ... LAVAGLI ... GQVSLW ... SSALFNP ...

B1AR_HUMAN  ... LASADLV ... VTASIE ... ANSAFNP ...
B2AR_HUMAN  ... LACADLV ... VTASIE ... VNSGFNP ...
B3AR_HUMAN  ... LAAADLV ... VTASIE ... ANSAFNP
ACM1_HUMAN  ... LACADLI ... SNASVM ... VNSTINP ...
ACM2_HUMAN  ... LACADLI ... SNASVM ... INSTINP ...
A1AA_HUMAN  ... LAVADLL ... CTASIM ... LNSCINP ...
A2AA_HUMAN  ... LASADIL ... CTSSIV ... CNSSLNP ...
DADR_HUMAN  ... LAVSDLL ... STASIL ... ANSSLNP ...
HH1R_HUMAN  ... LSVADLI ... STASIF ... INSTLNP ...
5H1B_HUMAN  ... LAVTDLL ... CTASIL ... LNSLINP ...
5H1A_HUMAN  ... LAVTDLM ... CTSSIL ... SNSLLNP
```

Differences in local environment between opsin and non-opsin class A GPCRs regulate the acidity of Asp(2.50)

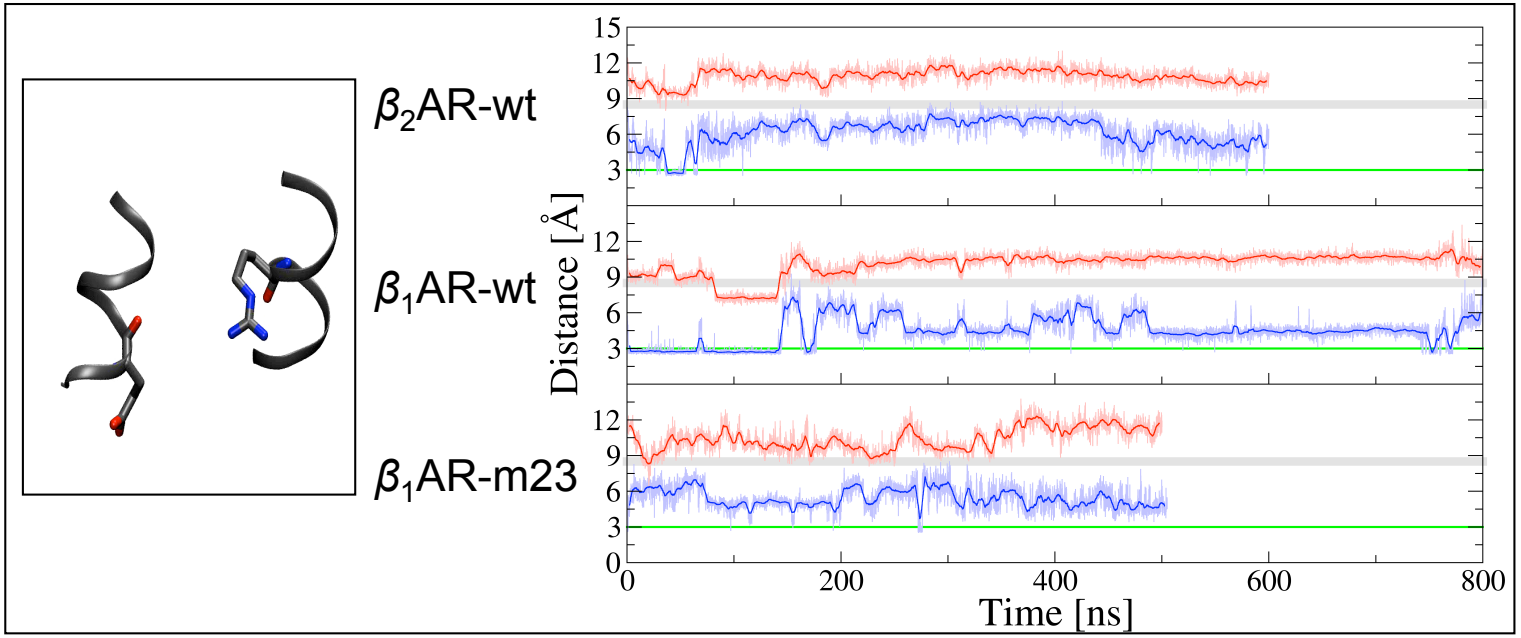
SV, M. Neri, I. Tavernelli and U. Rothlisberger, JMB, **397**, 1339, (2010)

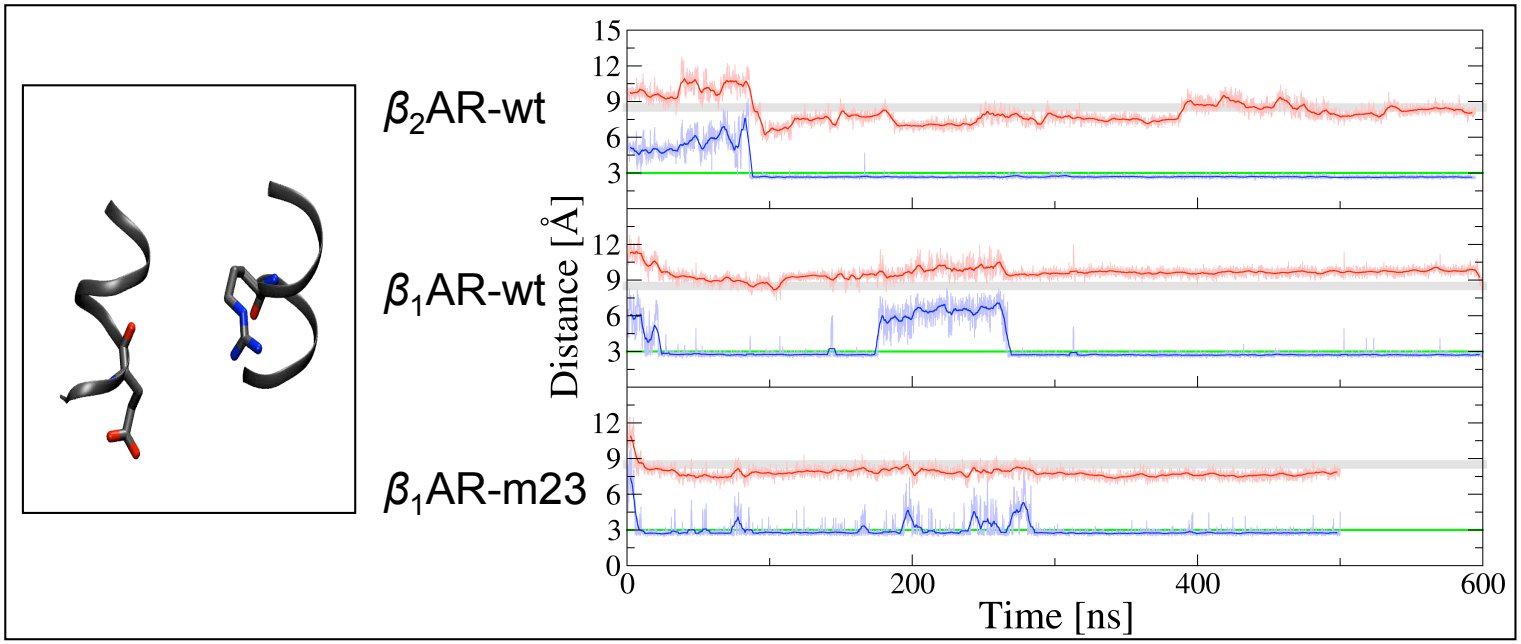
“Ionic lock” behaviour (1/2)

Asp(2.50) deprotonated



Asp(2.50) protonated





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“Ionic lock” behaviour (2/2)

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Asp(2.50) deprotonated

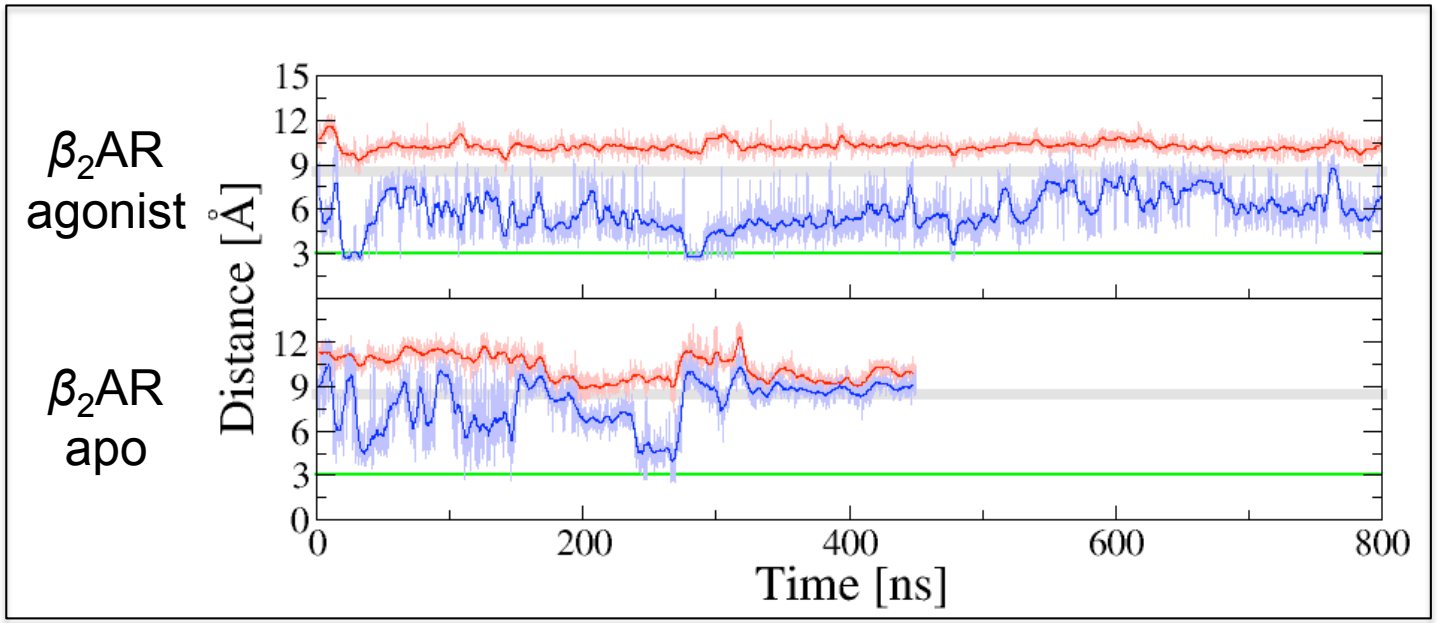
Helix VI

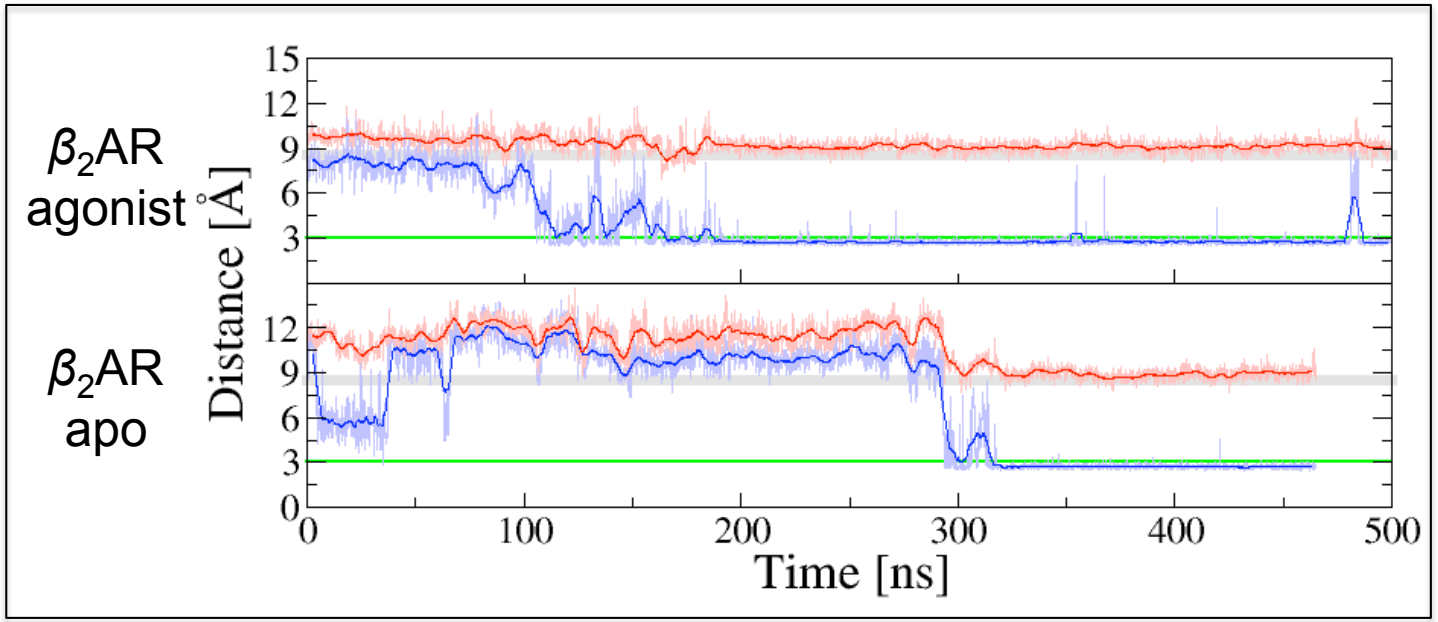
Helix III

C_α-C_α distance

N-O distance

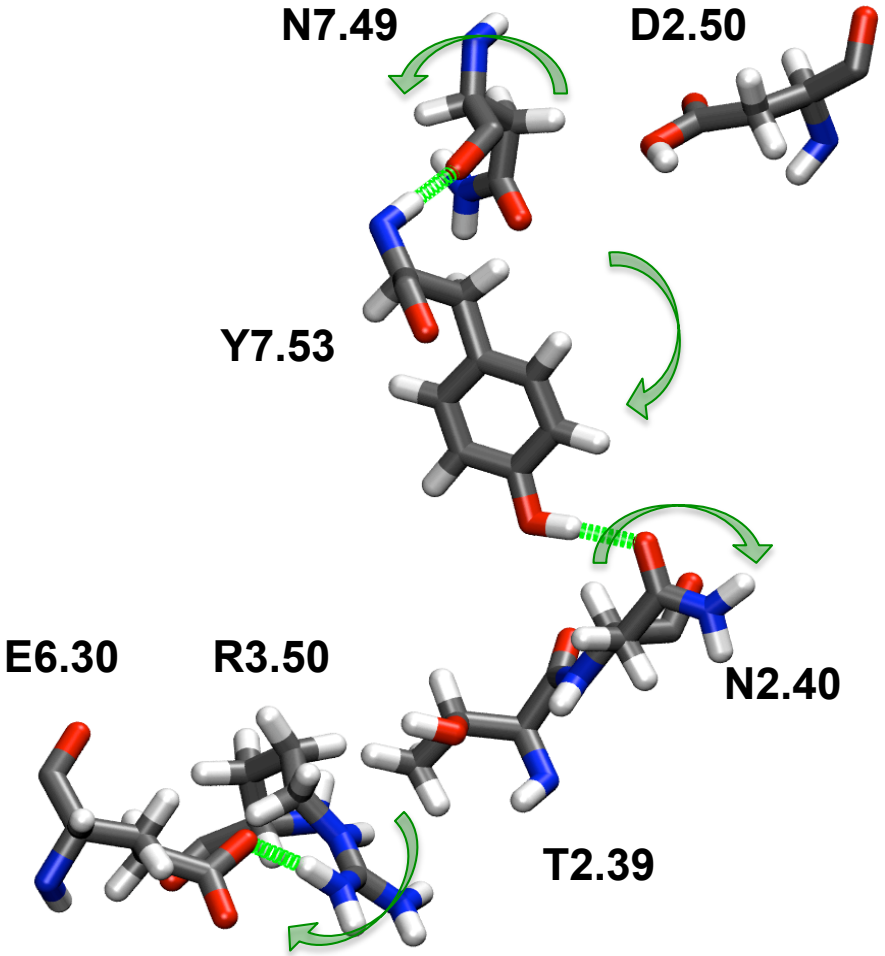
Asp(2.50) protonated

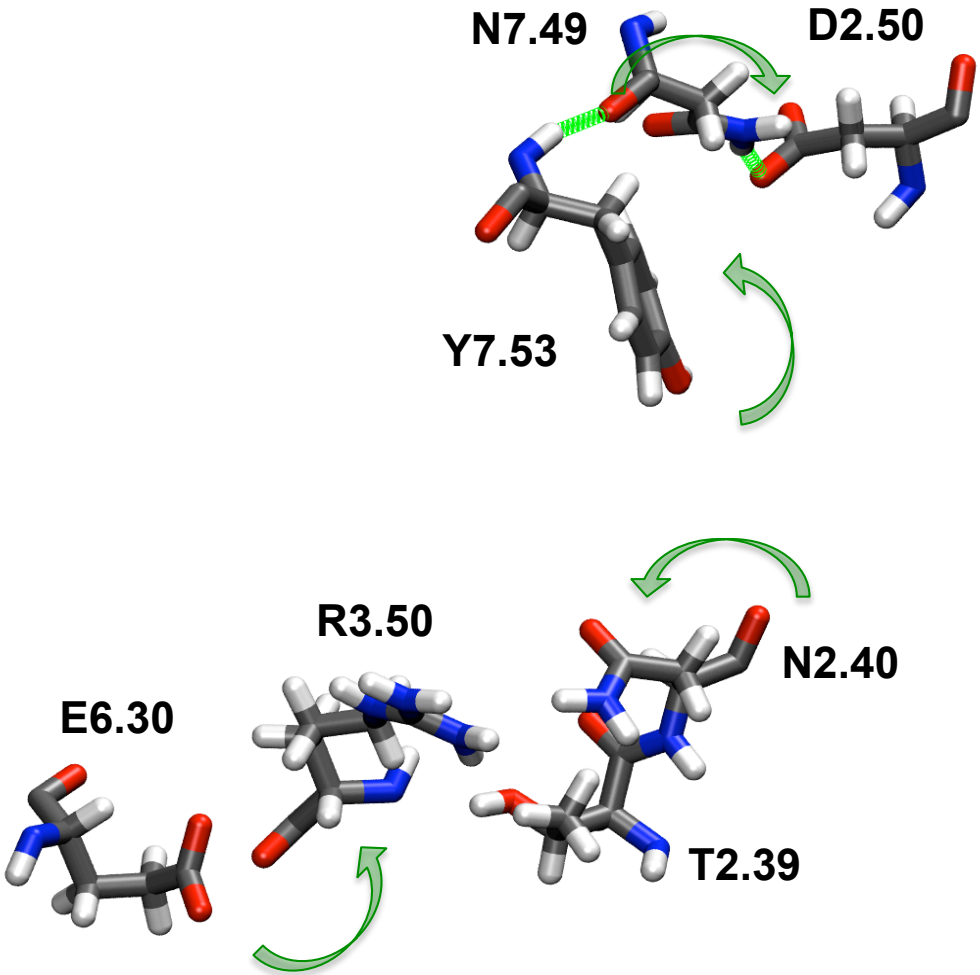




SV, M. Neri, I. Tavernelli and U. Rothlisberger, JMB, **397**, 1339, (2010)

Signal transduction: internal micro-switches





Asp2.50 protonated	β_1AR-wt	β_2AR-wt	Average value
D2.50-N7.49	1%	1%	1%
Y7.53-N2.40	58%	70%	70%
N2.40	98%	97%	97%
"ionic lock"	**99%**	**100%**	**100%**

Asp2.50 deprotonated	β_1AR-wt	β_2AR-wt	Average value
D2.50-N7.49	68%	99%	84%
Y7.53-N2.40	1%	63%	32%
N2.40	37%	0%	19%
"ionic lock"	**6%**	**1%**	**4%**

SV, M. Neri, I. Tavernelli and U. Rothlisberger, JMB, **397**, 1339, (2010)

Cis/Trans Photoisomerization in Rhodopsin: The First Steps in Vision

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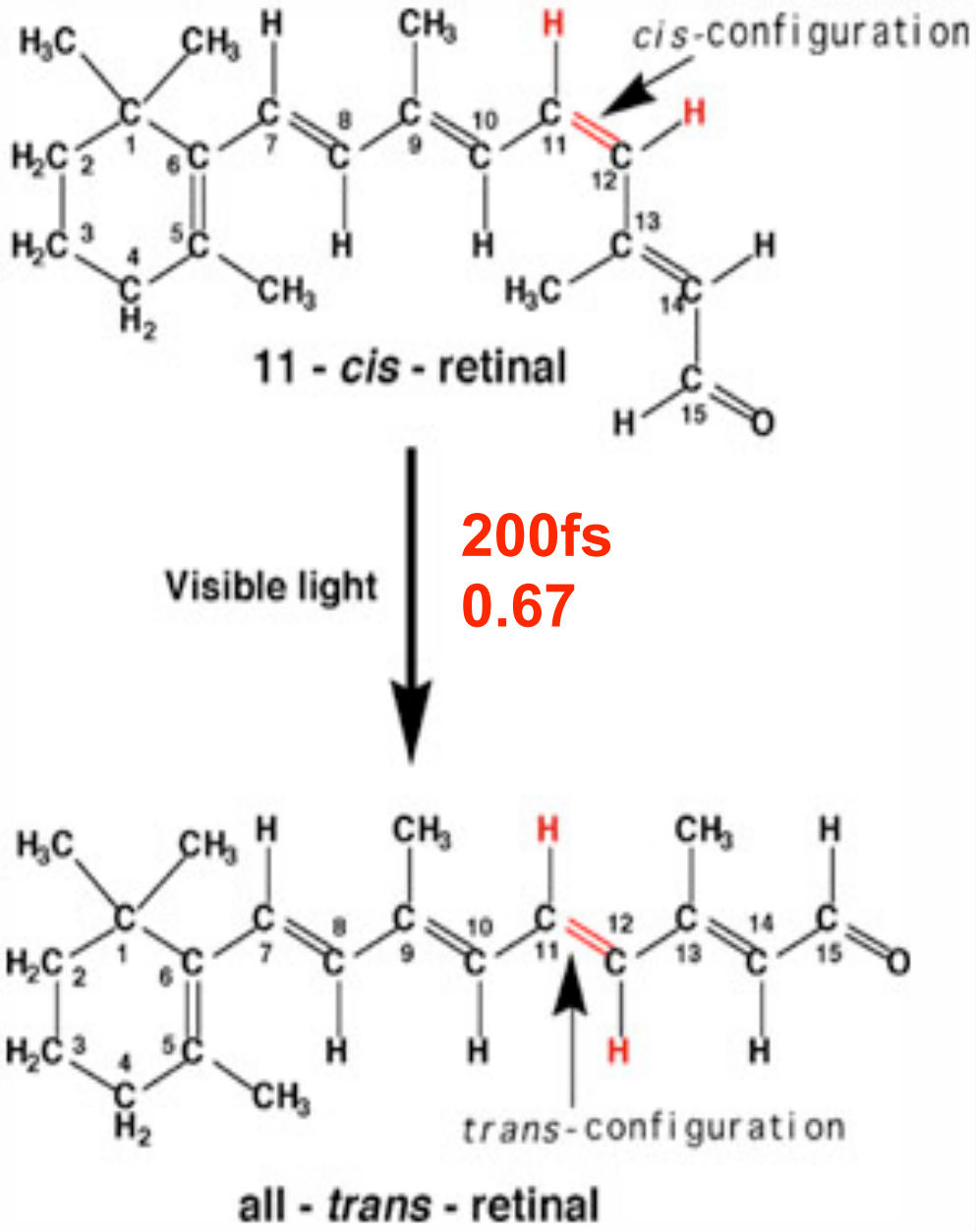
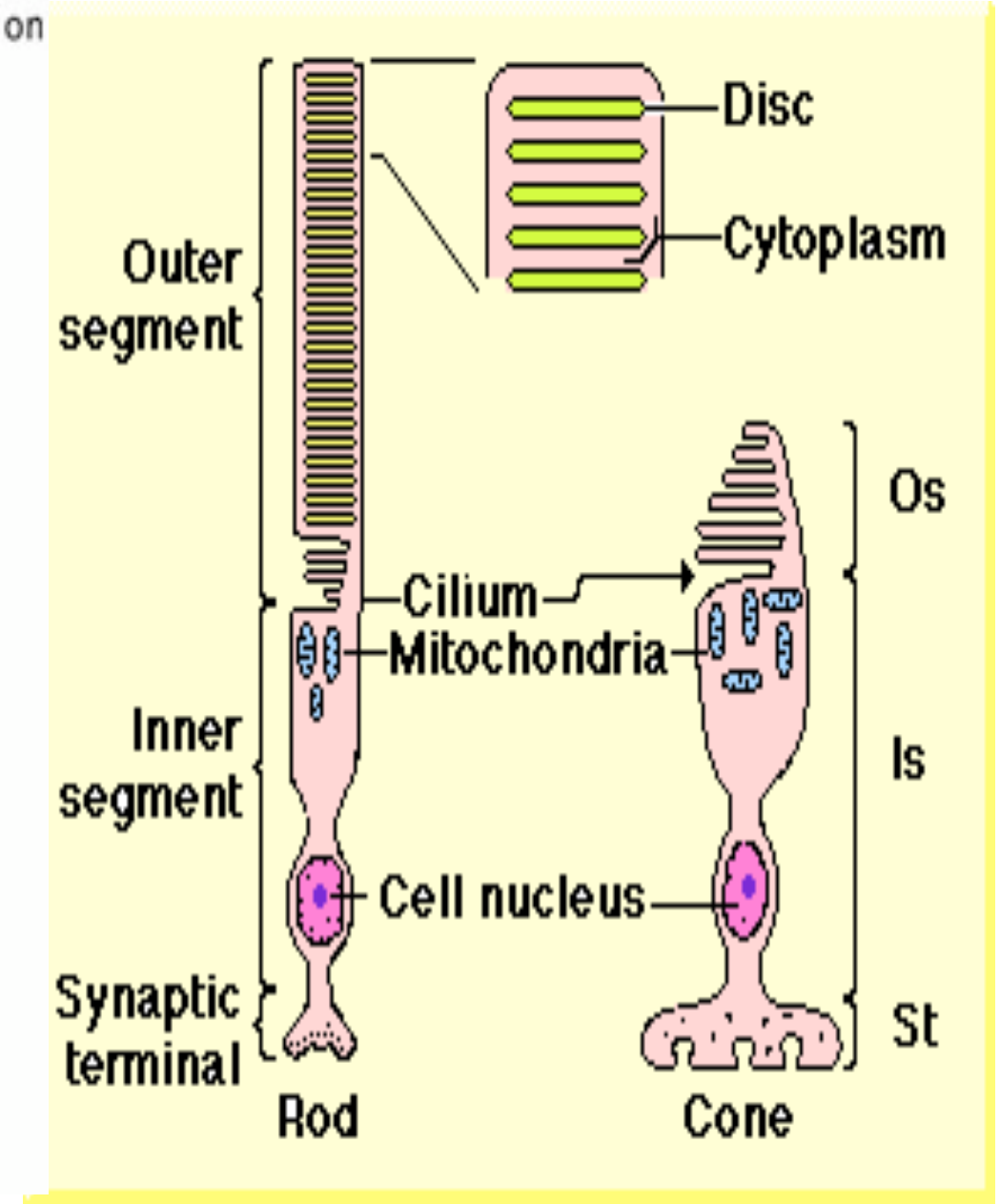
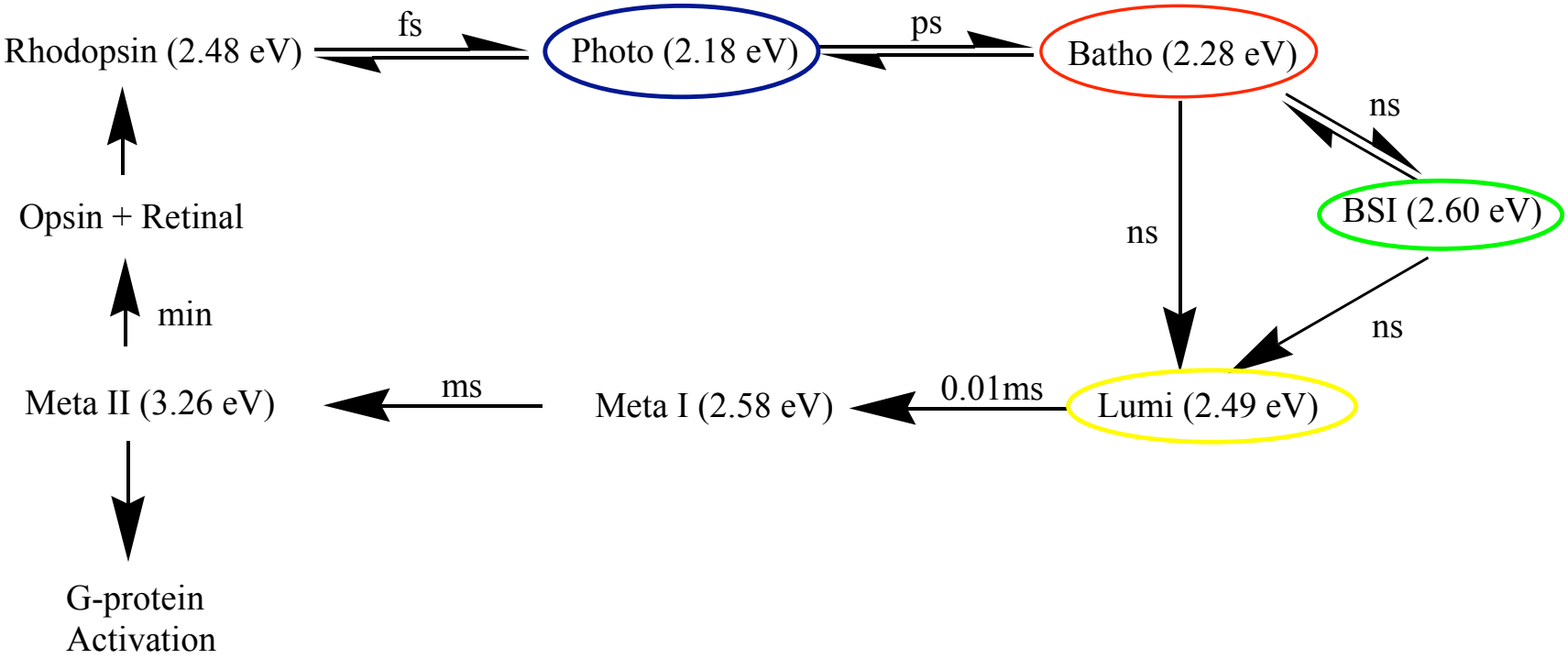
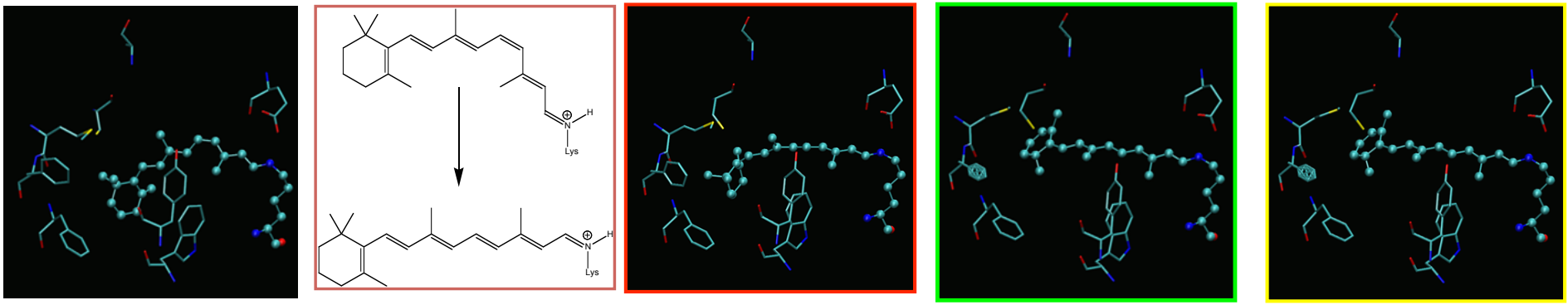




Rhodopsin – the photocycle





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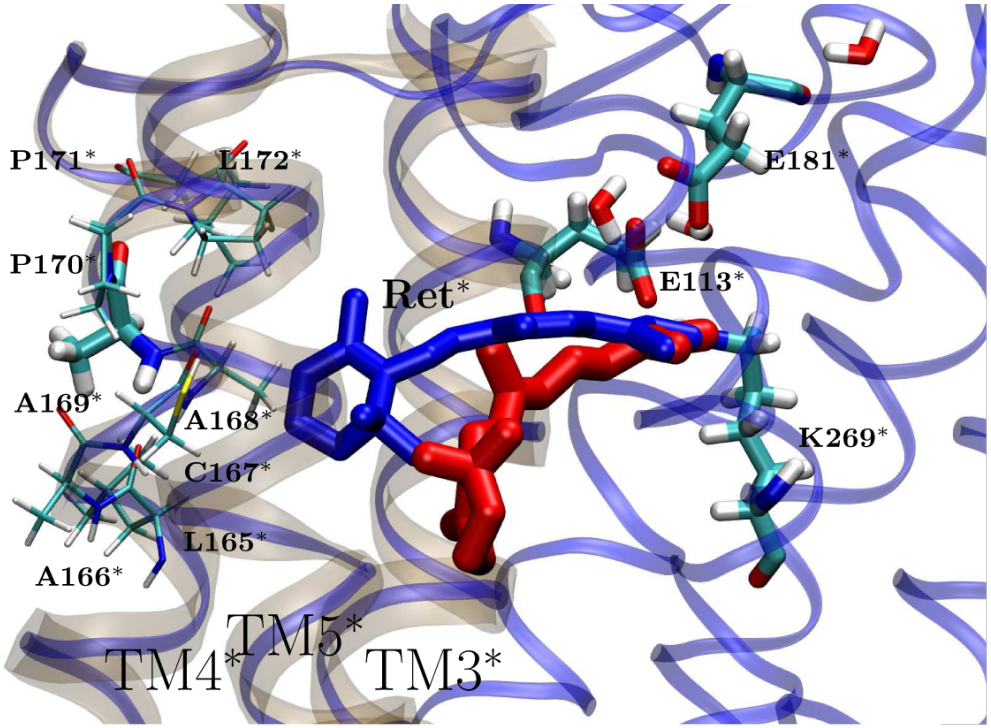
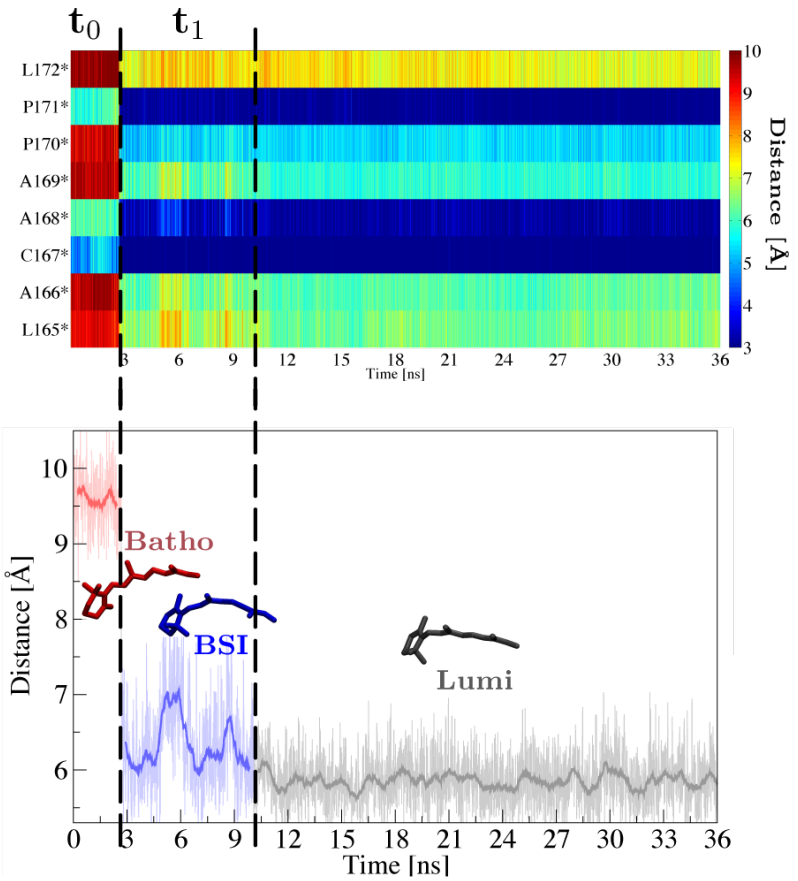
MD simulations of the early steps of signal transduction in rhodopsin





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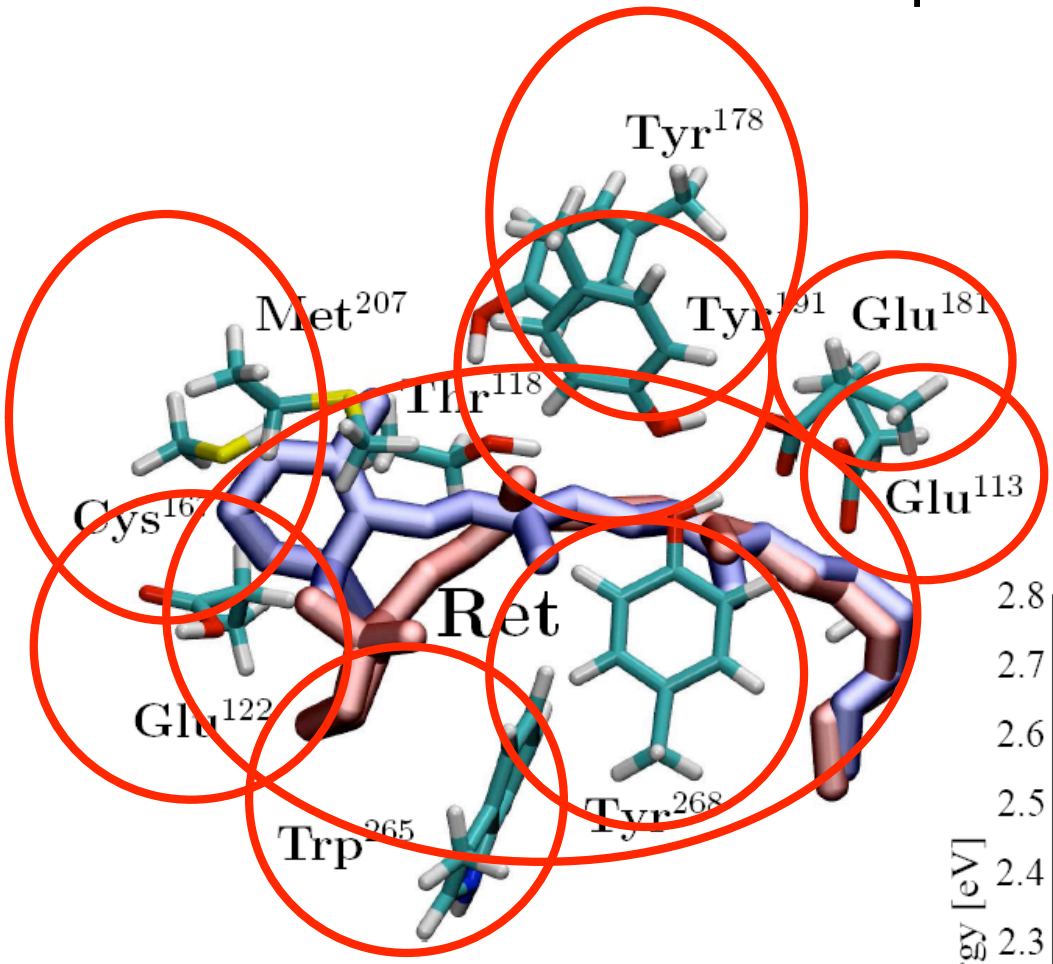
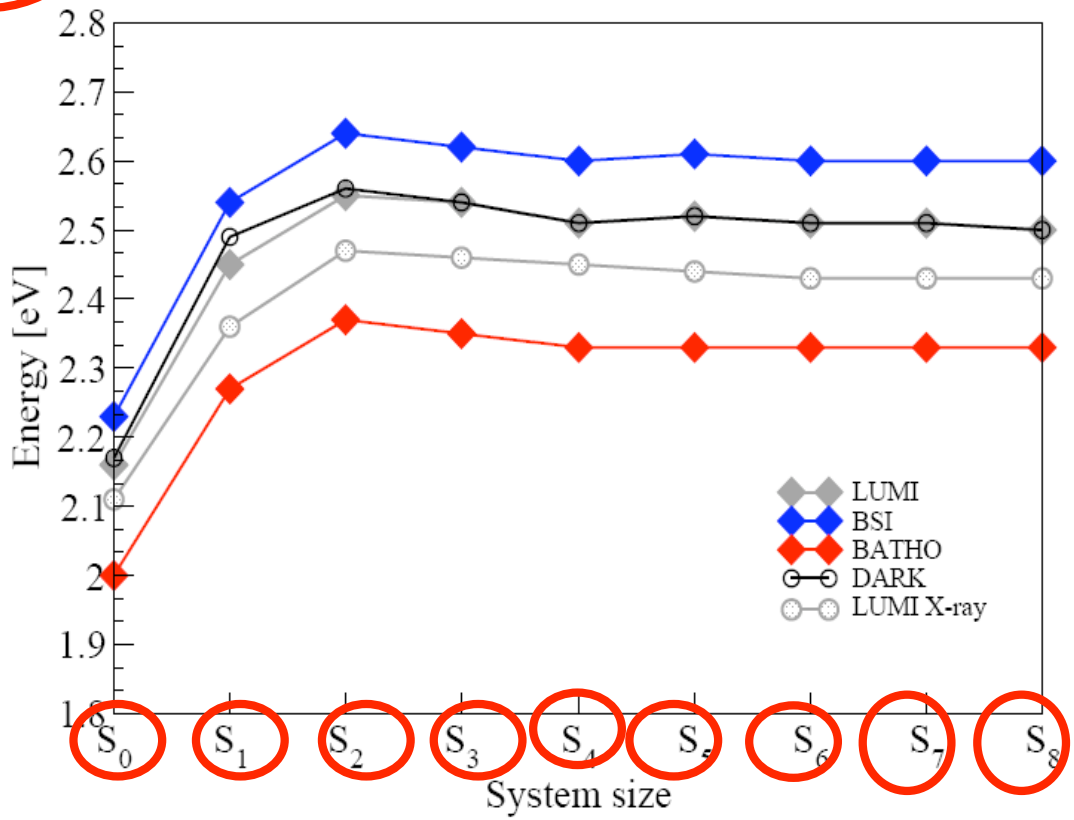
ZINDO Calculations of the Optical Spectra



Thermal sampling of 200 configurations per intermediate at the QM(DFT)/MM level

Size Dependence



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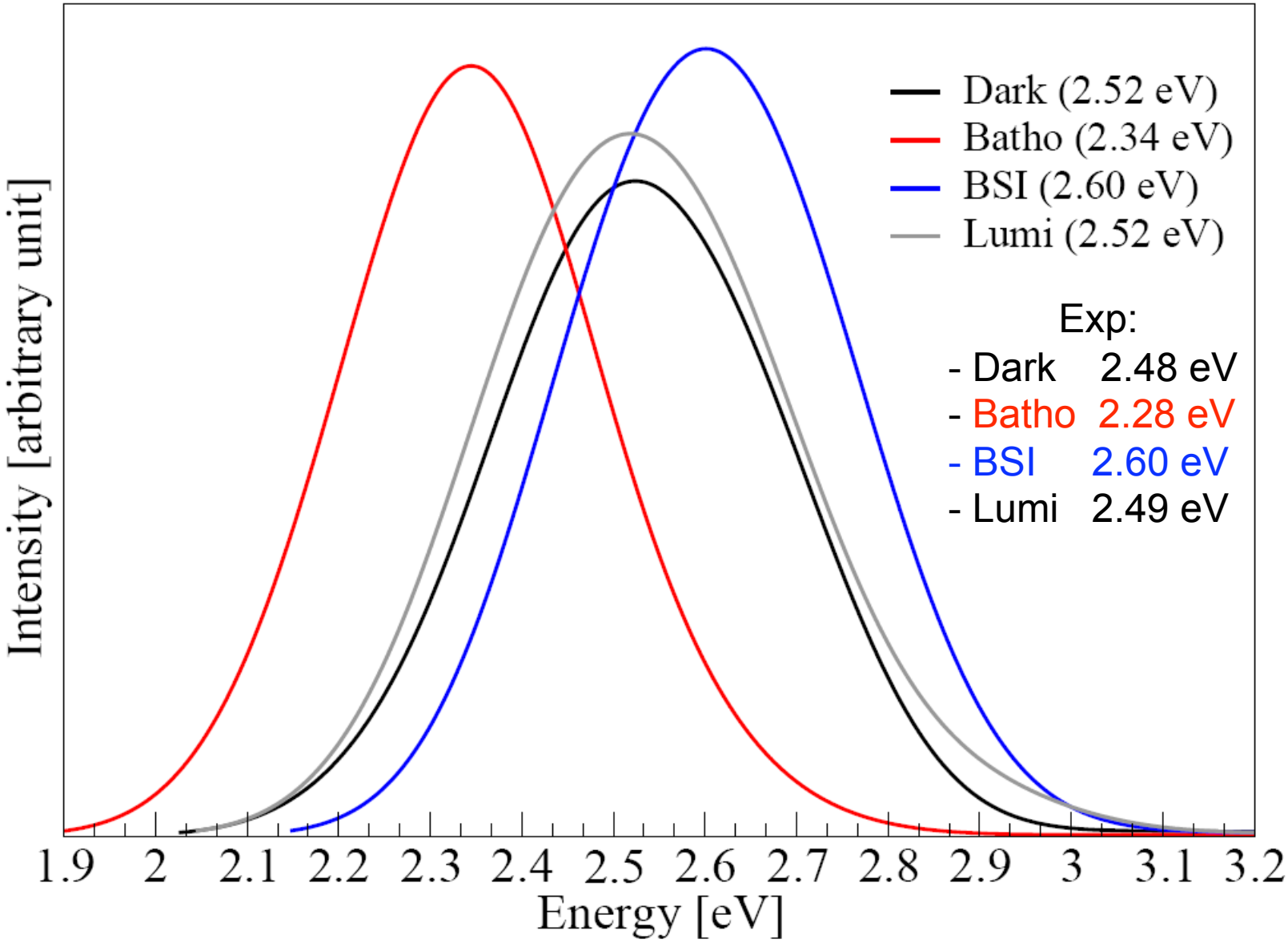
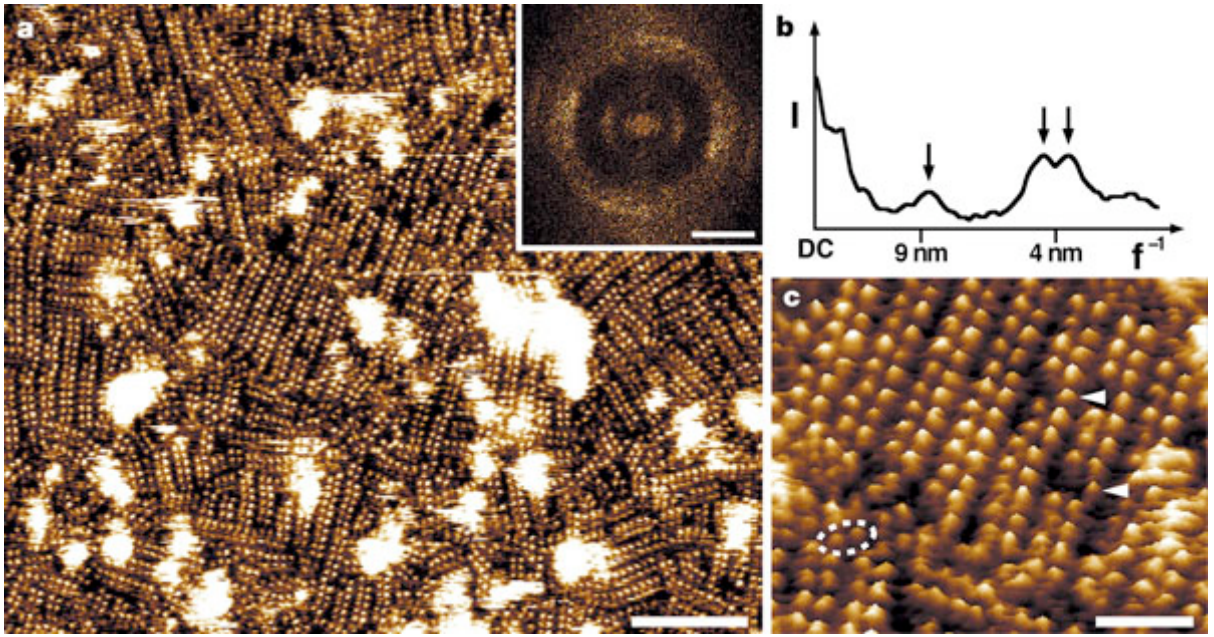
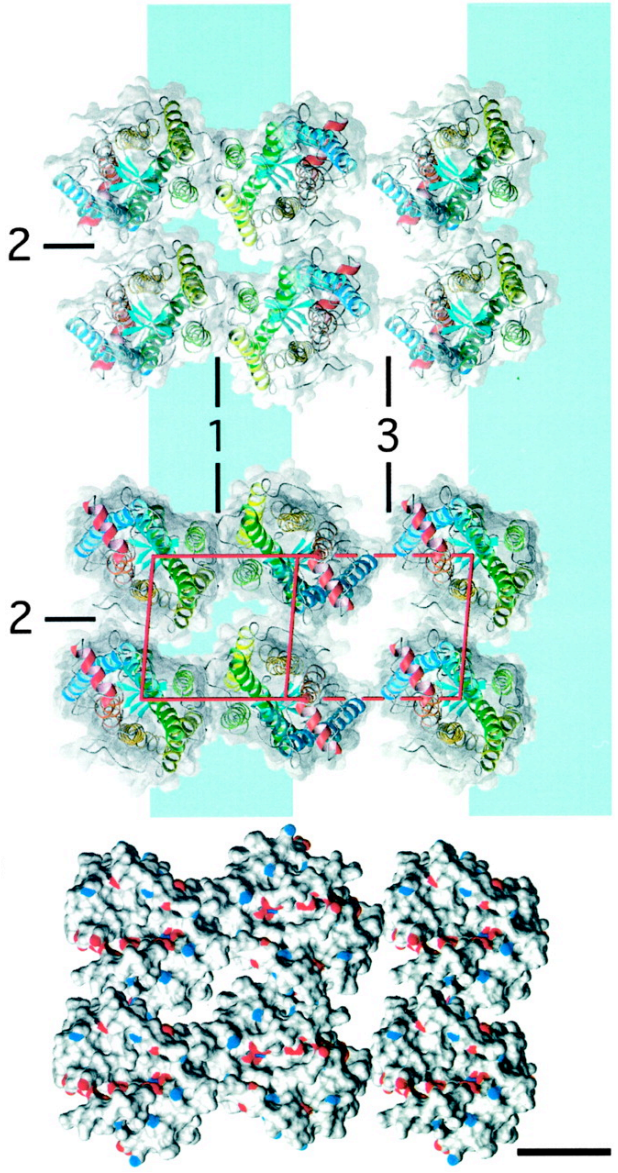


Rhodopsin oligomeric state





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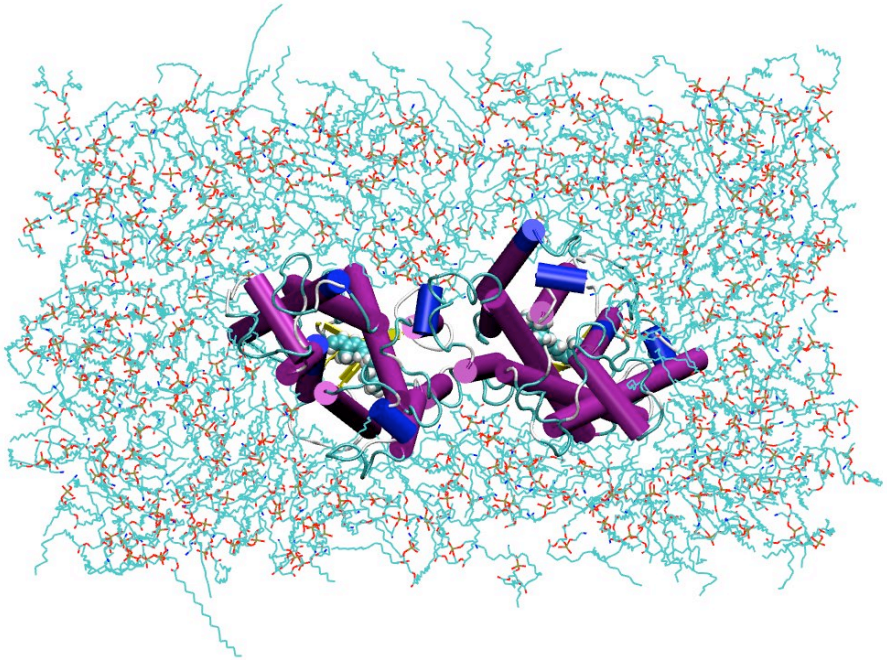
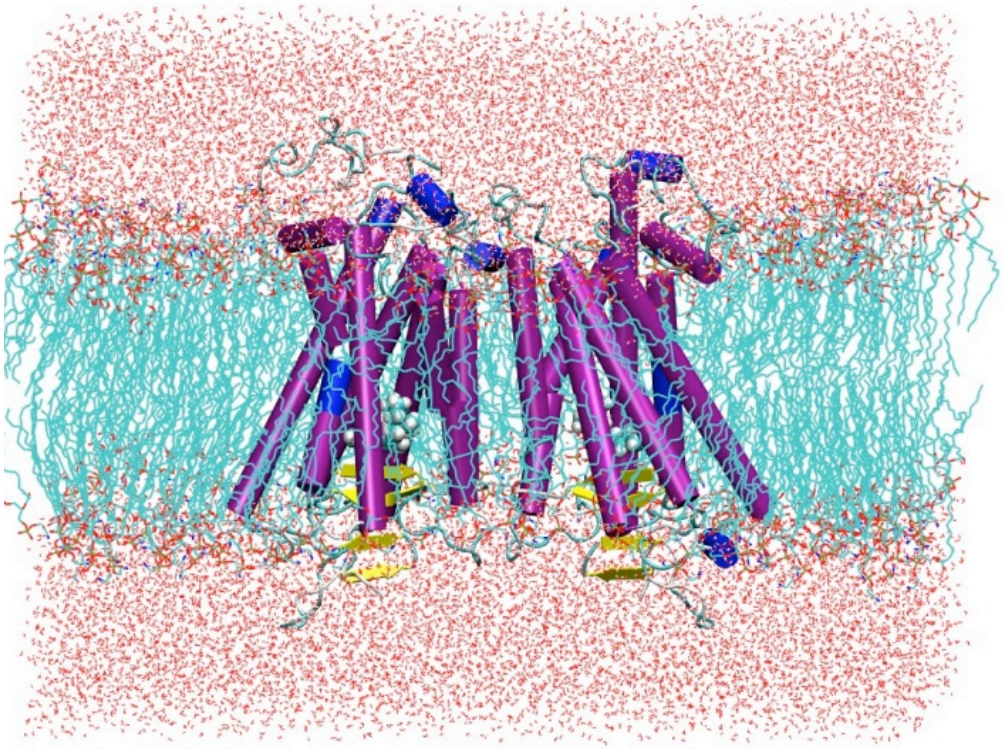
Rhodopsin dimer system





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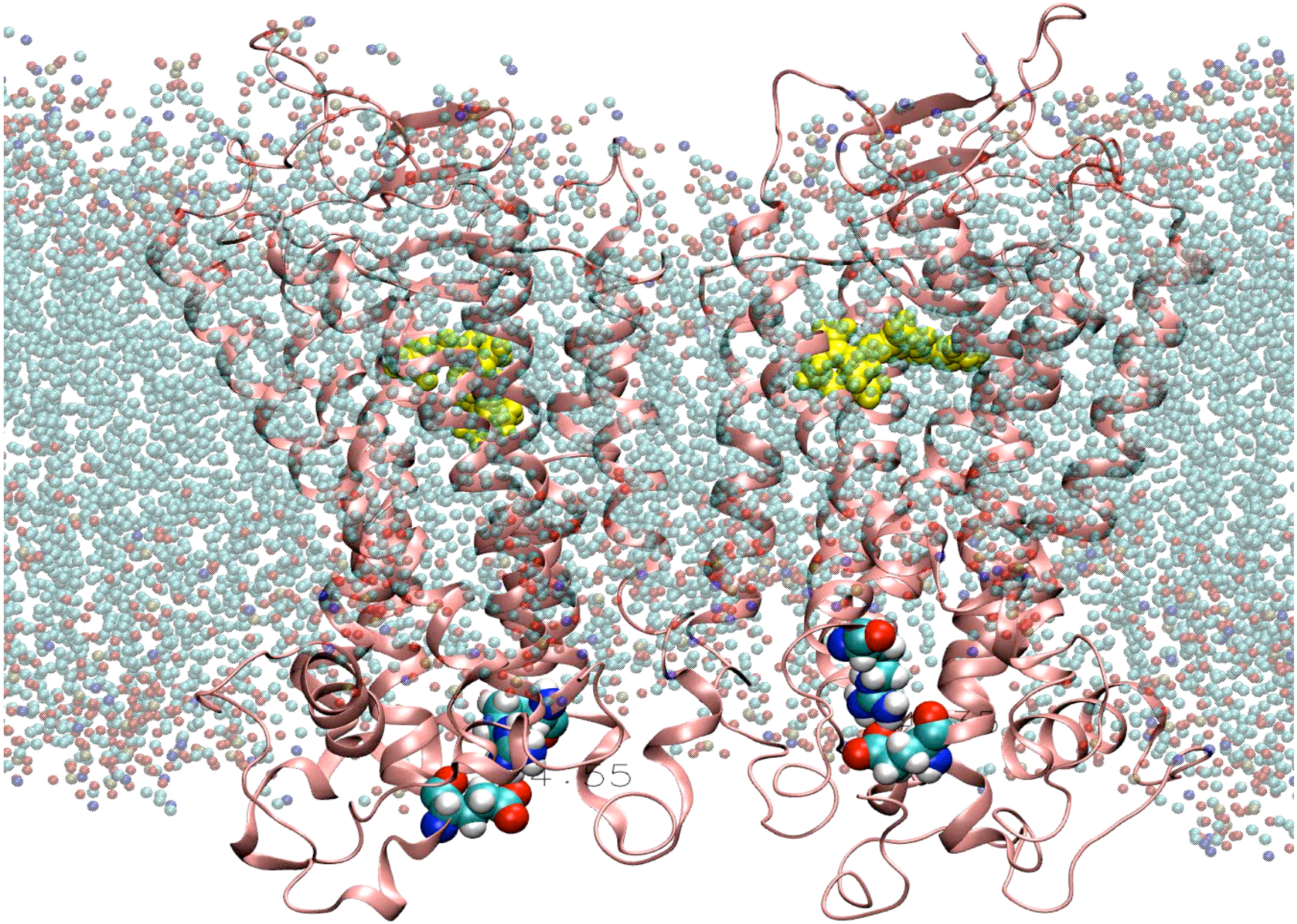
Rhodopsin dimer activation pathway



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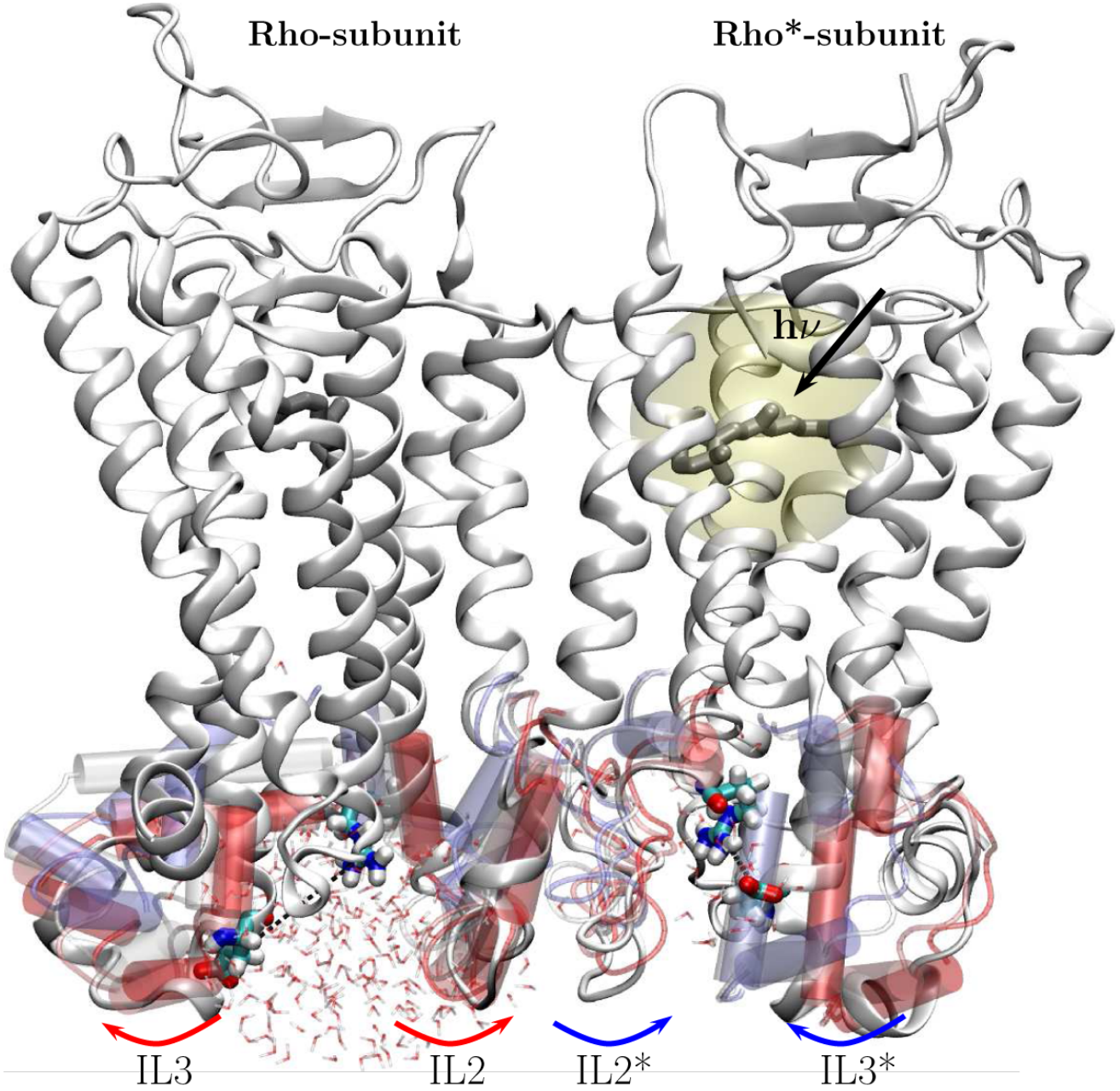
Rhodopsin dimer activation pathway



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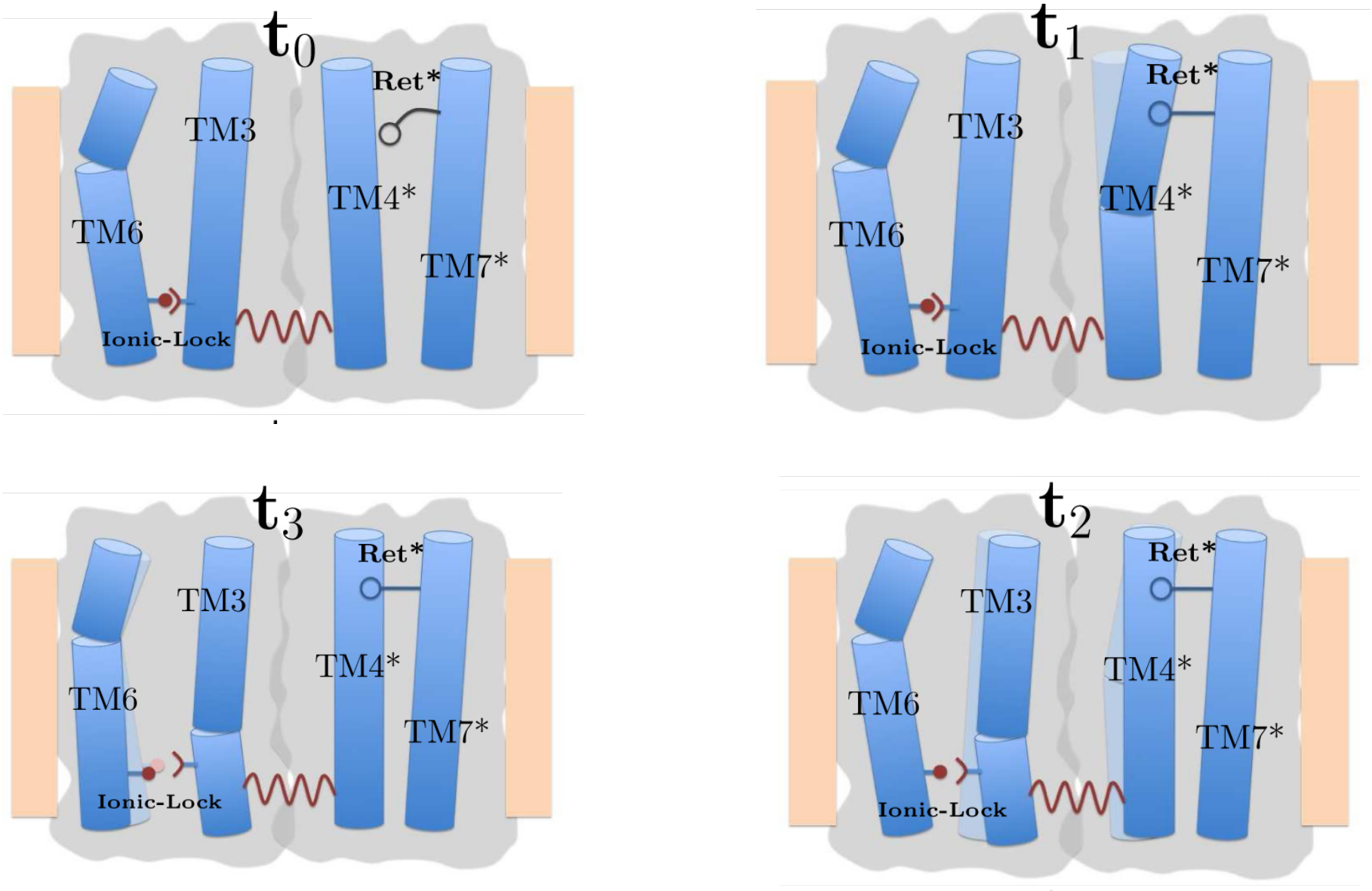
Rhodopsin dimer activation mechanism



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Conclusions

ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

- We can determine the influence of engineering techniques on receptor native conformation
- We can study the effects of mutations or protonation state changes
- We can identify signal transduction pathways that involve spatially distant allosteric sites
- We can reproduce spectroscopic experiments to spectrally characterize the signaling pathway.
- We can study how the environment can affect receptor signaling

Acknowledgments

Marilisa Neri Ivano Tavernelli

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