

Biological Opportunities with Solution Scattering

XDL 2011

Brian R. Crane

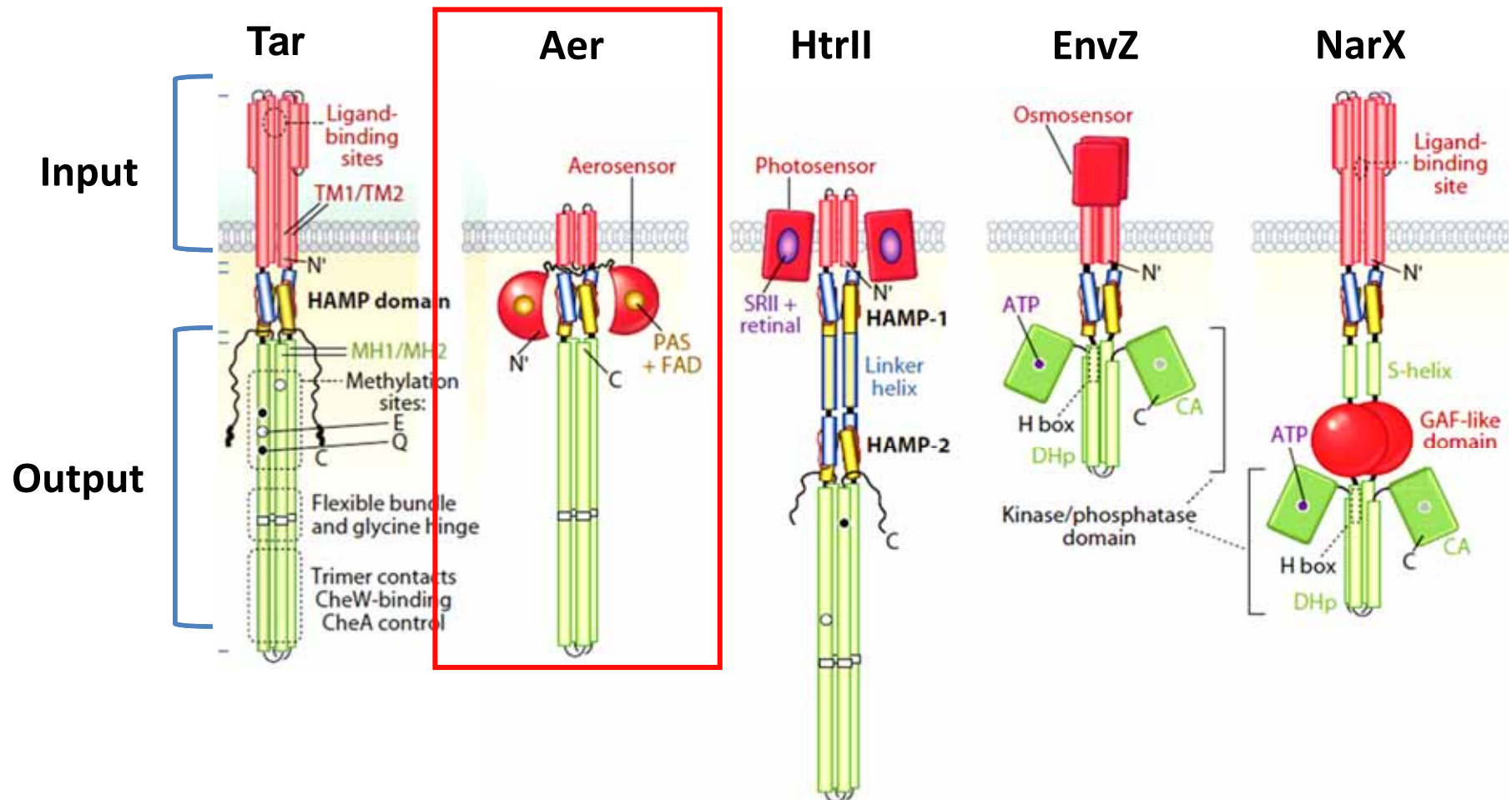
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Cornell University

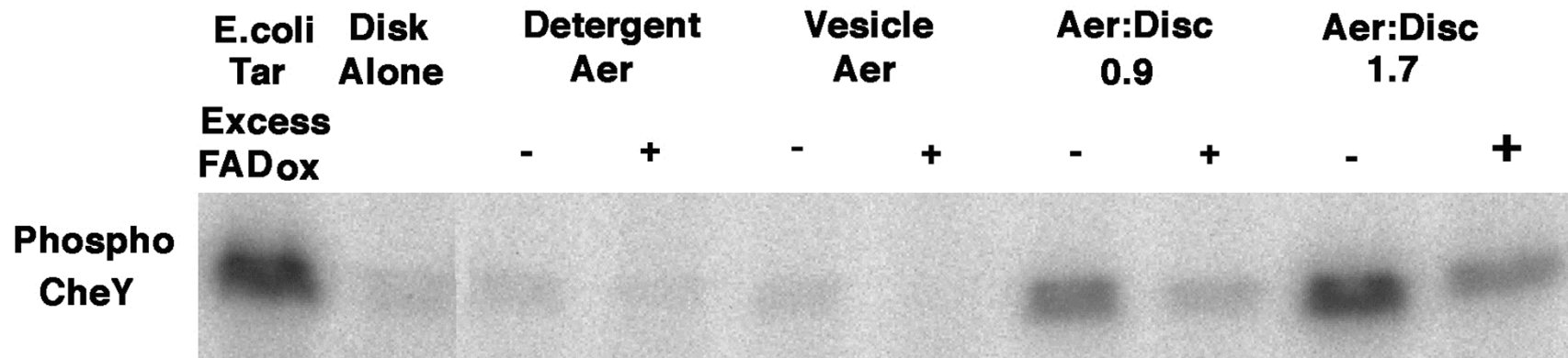
Bacterial Transmembrane Receptors

Histidine kinases, adenylyl cyclases, methyl-accepting chemotaxis proteins, photosensors, energy sensors, phosphatases



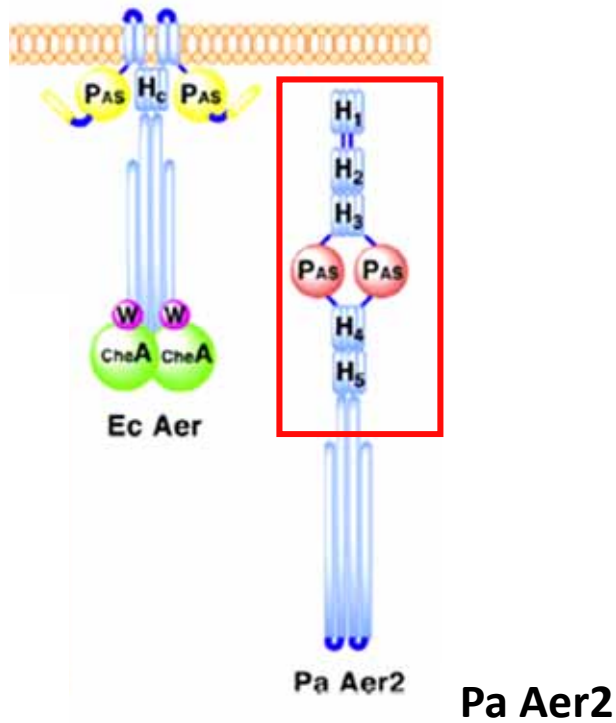
Many proteins don't function properly in the choice environment of analysis

Aer phosphorylation Assays (CheA/CheW/CheY)



Joanne Widom, Mingshan Li and Jerry Hazelbauer

Aerotaxis (energy/redox) receptors



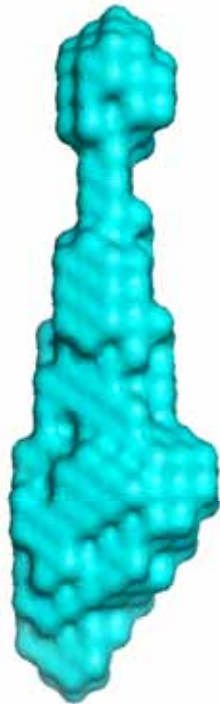
Airola et. al., *Structure*, 2010

- Aer : EcAer homolog, 1 HAMP membrane-bound, aerotaxis
 - Aer2
 - Soluble
 - Contains 5 HAMP domains
 - Role of Aer2 is unclear
 - Mediate response to diatomic gas (O₂, CO, NO) in *E. coli*
- (Watts et. al., *Mol. Micro.* 2011)

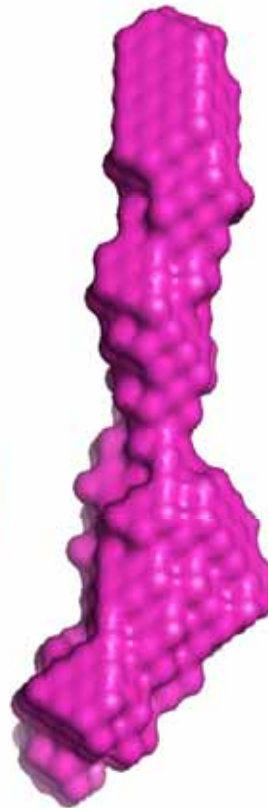
SAXS models indicate a linear domain arrangement



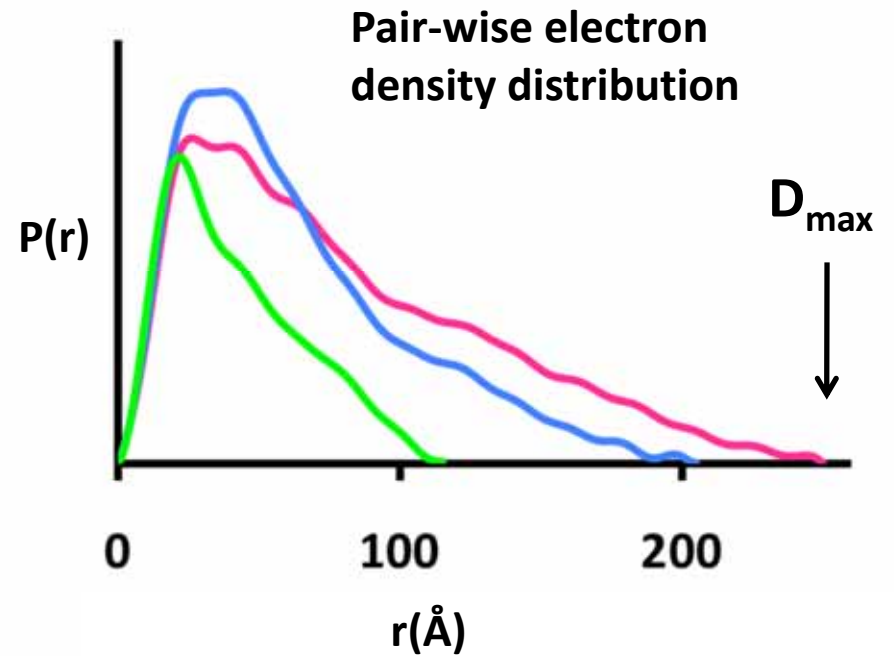
1-
172



1-
317

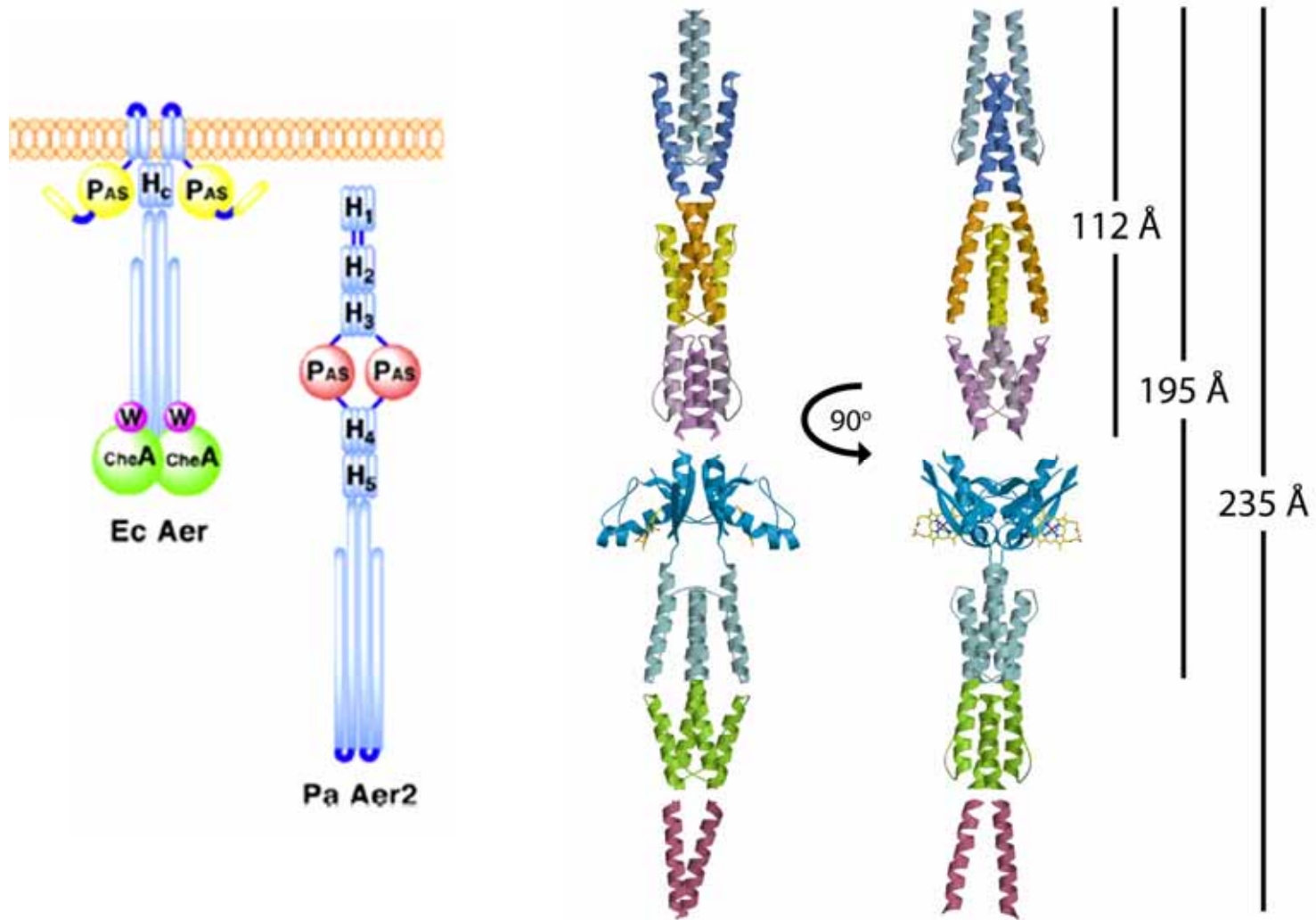


1-
402

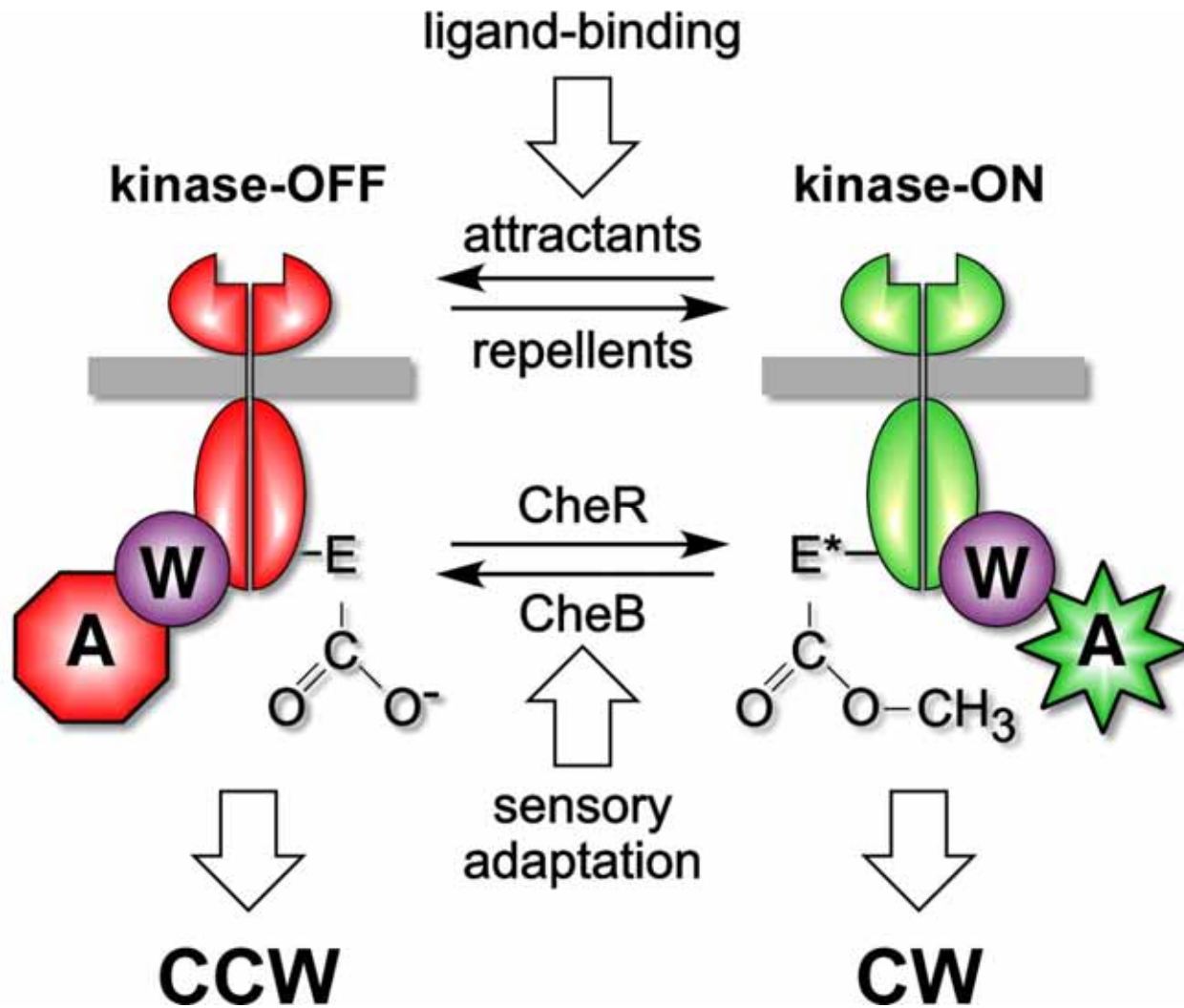


protein	Dmax (Å) : P(r)	Dmax (Å) : model
Aer2 1-172	115	112
Aer2 1-317	205	193
Aer2 1-402	250	238

Domain Orientations in the Sensing Region



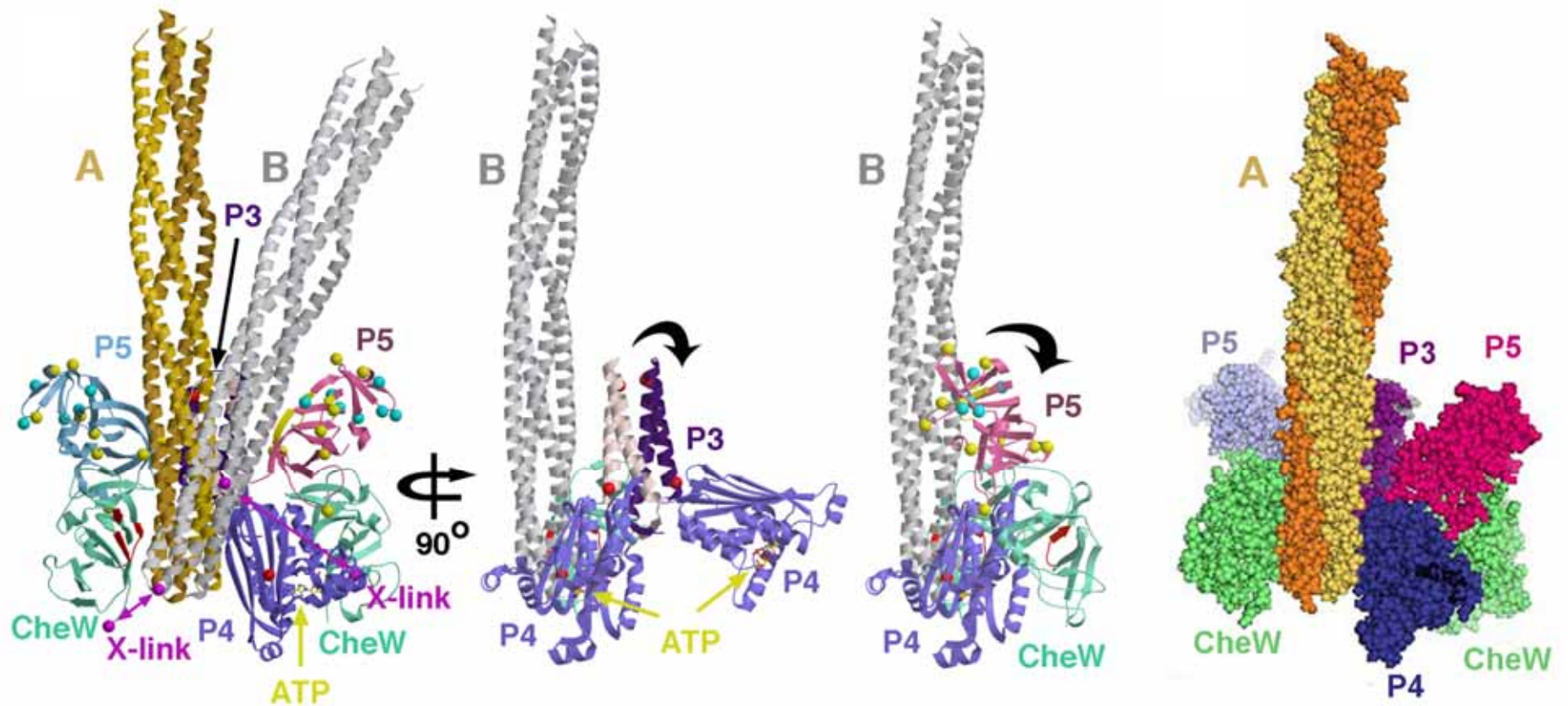
Chemoreceptors MCPs



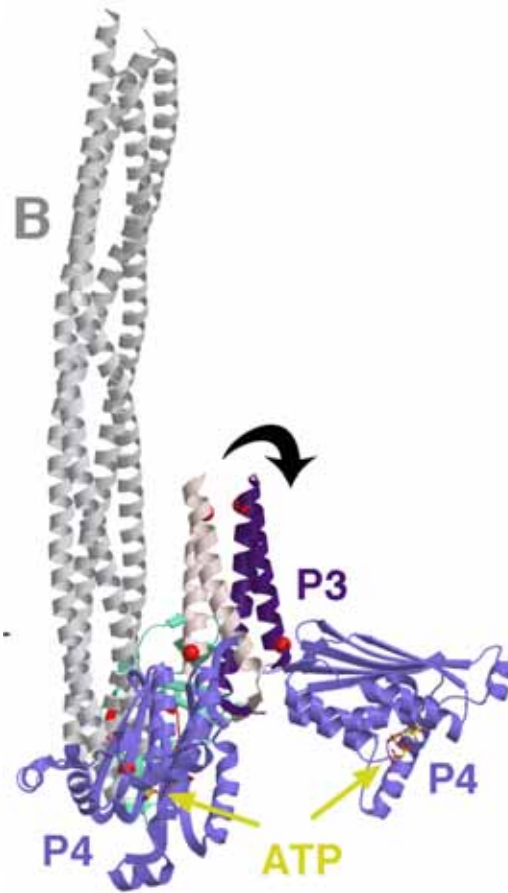
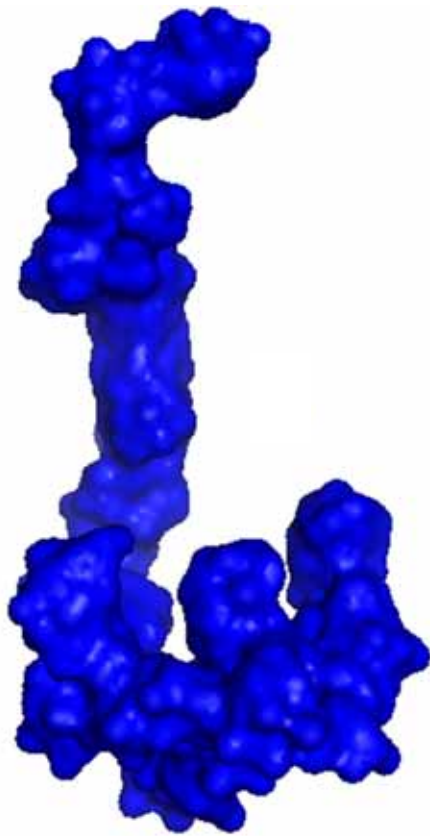
CheA:CheW:MCP Ternary Complex determined by pulsed dipolar ESR

- P5, - W

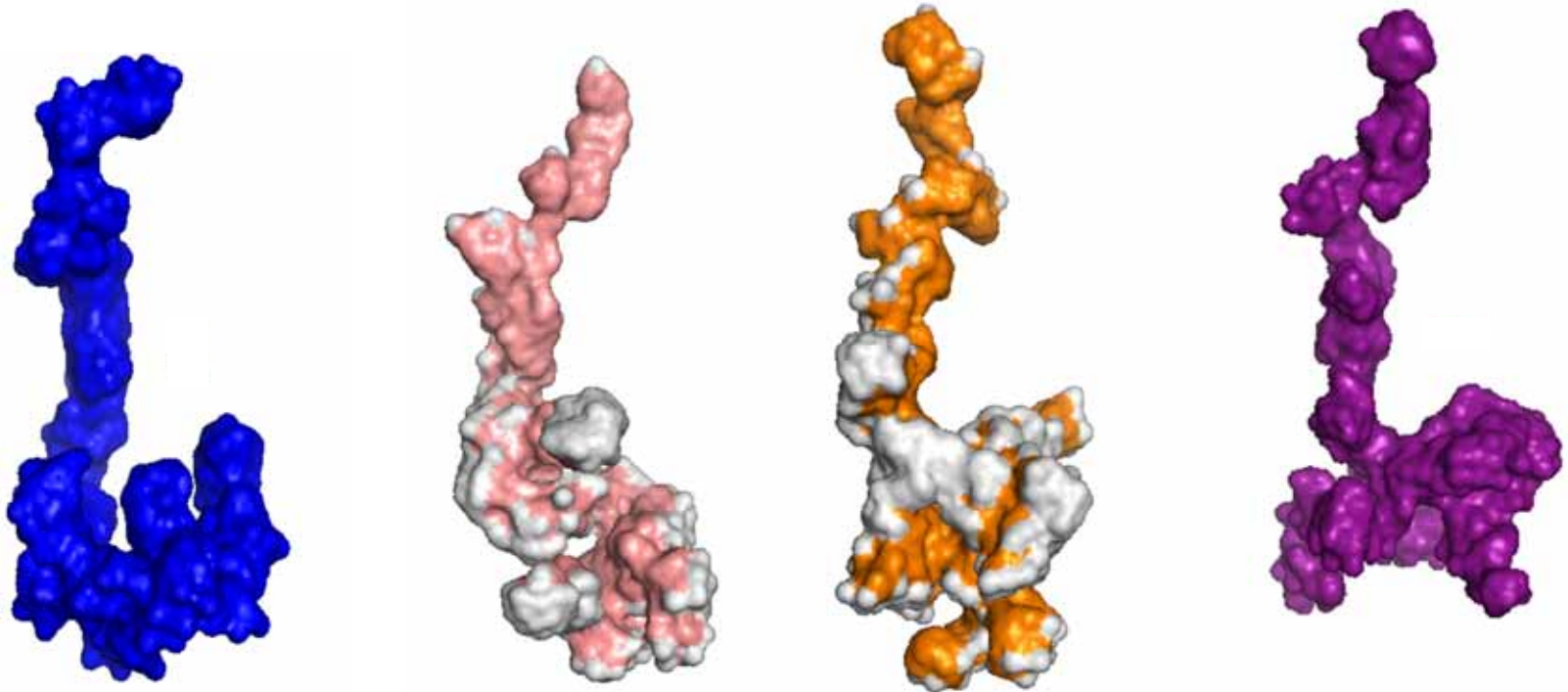
- P3



GASBOR Envelopes generated of the ternary complex



Variability in envelopes, but they all show kinase binding at one end in an asymmetric manner

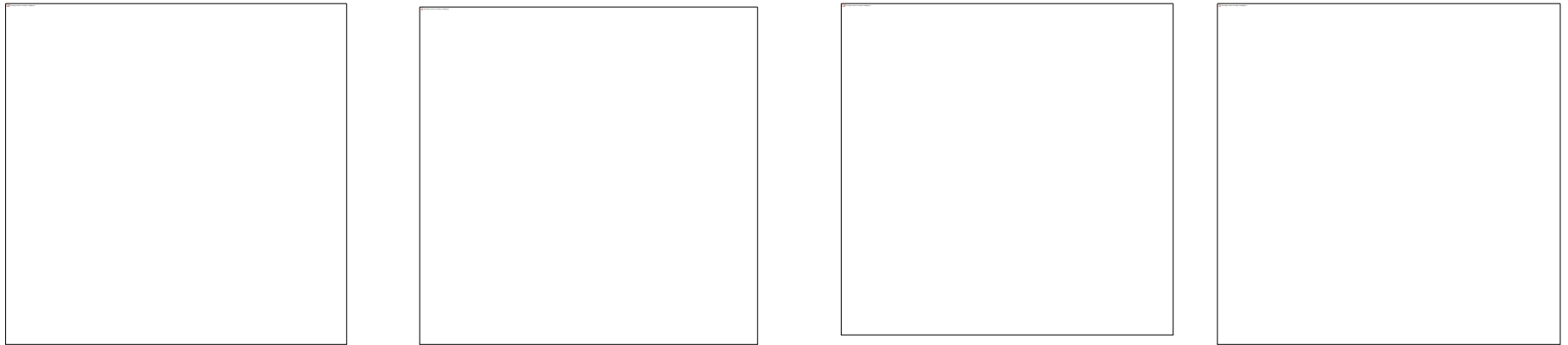


This is great! - what if you had data on oriented samples?

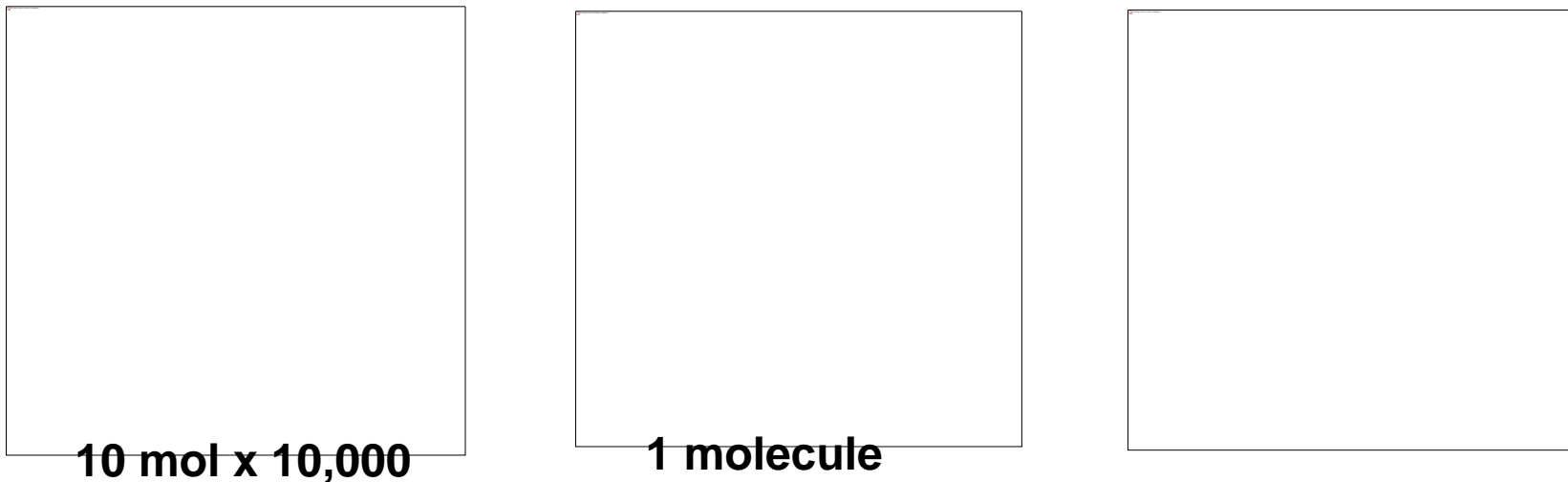
Direct phasing of partially oriented samples - John Spence and Coworkers

Saldin, etl al. Phys Rev B 81 174105 (2010); Saldin et al.
New J Phys. 12 (2010) 035014; Kam (1977, 1980, 1982)

Gold Particles



Projection of a Potassium channel oriented in a membrane



10 mol x 10,000

1 molecule

Alignment methods to augment solution scattering

Restraints from angular correlations of the molecular transform with *ab initio* and structure-informed reconstruction methods.

How far could (modest) alignment of a molecules get you?

Alignment methods

Magnetic Fields - Even with paramagnetic molecule - order parameters of 10^{-3} - No Way

Electric Fields - Static - Protein dipole moment - 200-1000 D - still need 10^9 V/m - ion mobility, electrolysis etc.

Non-resonant Nd:YAG ($I \sim 10^{12}$ W/cm²; $\lambda = 1064$ nm) 3×10^9 V/m

Molecules ionize at $> 3 \times 10^{10}$ V/m

But - anisotropy of the polarization tensor is what matters, not the permanent dipole moment

Small molecules in gas phase - done, in solution, simulated. - big molecules, not known

Would polarization anisotropy be enough? - simulations say ~
100 Å³ (J. Chem Phys (2004) 120 9123) - probably OK

Proteins α anisotropy - 1000-10,000 Å³ (depends on ϵ) (Colloids
and Surfaces B (2007) 56 19)

Problems

Time scale for re-orientation - on the order of rotational diffusion - 10-50 nsec
- maybe too long for the pulse

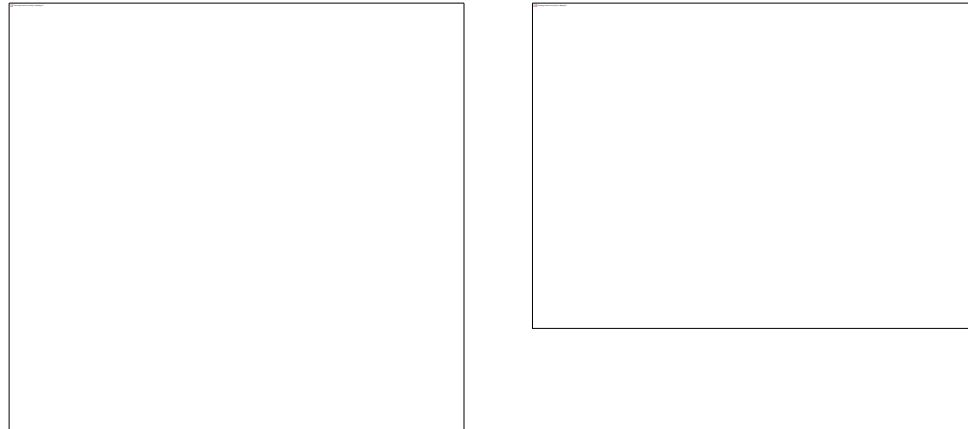
Would the molecule distort instead or re-orient? - probably at least to
some extent.

Aligned, but not directional - up and down - apply a direct field
too?

Effect of permanent and induced fields from neighboring molecules

Physical Alignment - Force and Media

Shear flow - amyloid and protein fibers (Biomacromolecules, 8 77, 2007)



Alignment media - lipids, polymers, phage, nanodiscs, native membranes, SAMS, patterning on surfaces

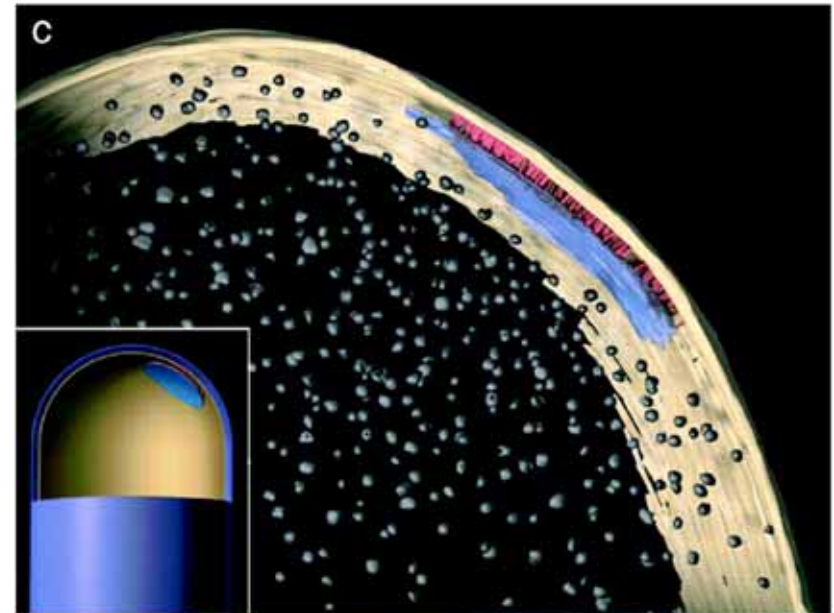
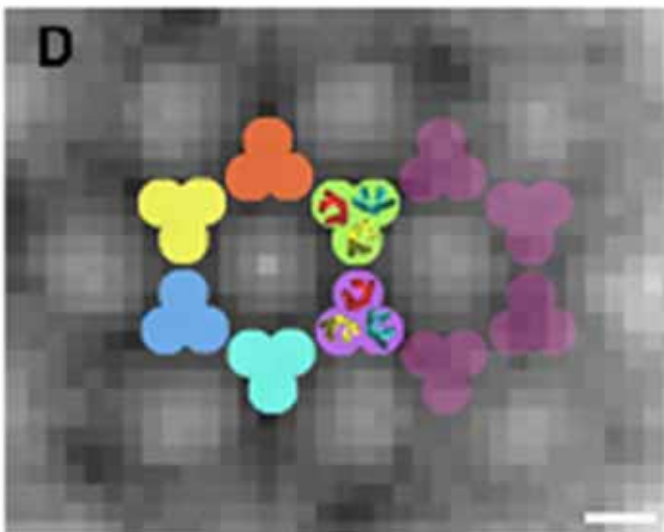
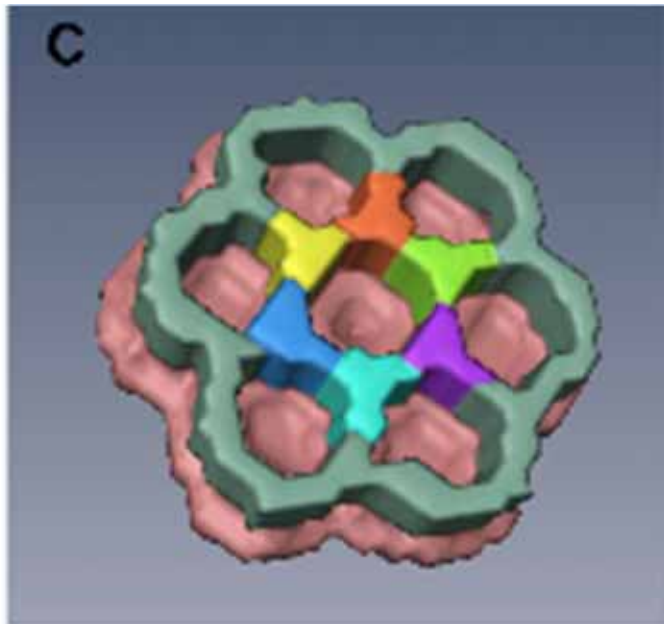
Poor diffracting crystals

Membrane enrichment techniques for mammalian membrane proteins (retroviral protein induced proteoliposomes)

Better contrast - Anomalous signals (very weak)? Heavy atom probes?

“Natural Alignment” -

Chemoreceptors form clusters at the poles of cells



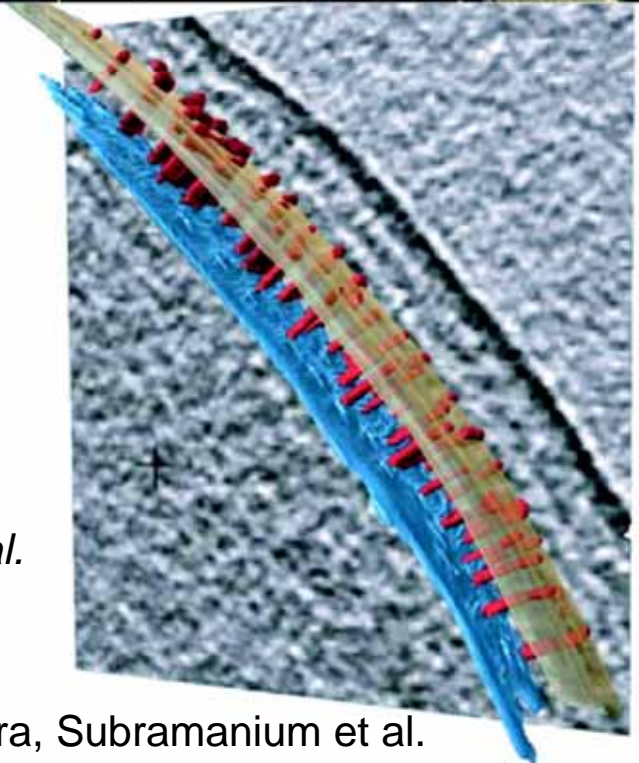
Receptor arrays have the same hexagonal symmetry in many bacteria

Briegel, Jensen PNAS (2009).

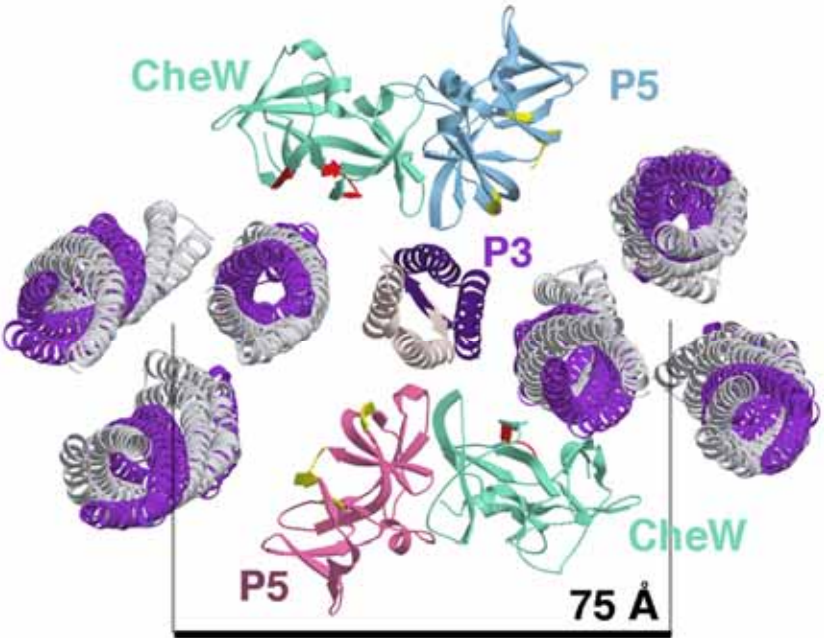


Zhang, Subramaniam *et al.* PNAS (2007) **104** 3777

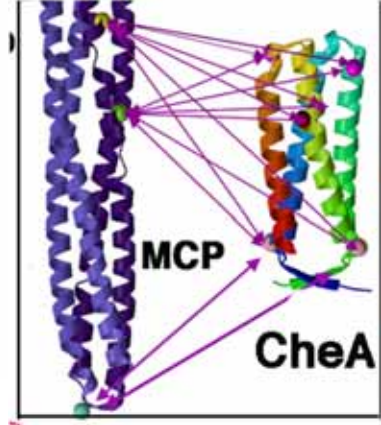
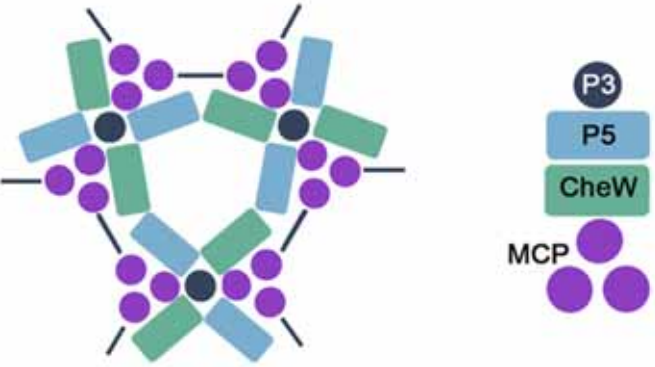
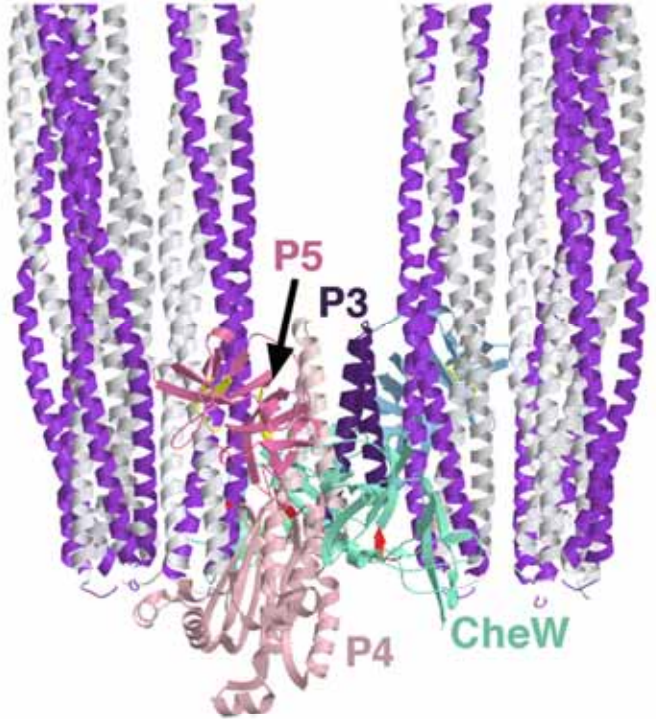
Briegel, Jensen *et al.* Mol Micro. (2008); Kursigara, Subramaniam *et al.* J. Bact. (2008);



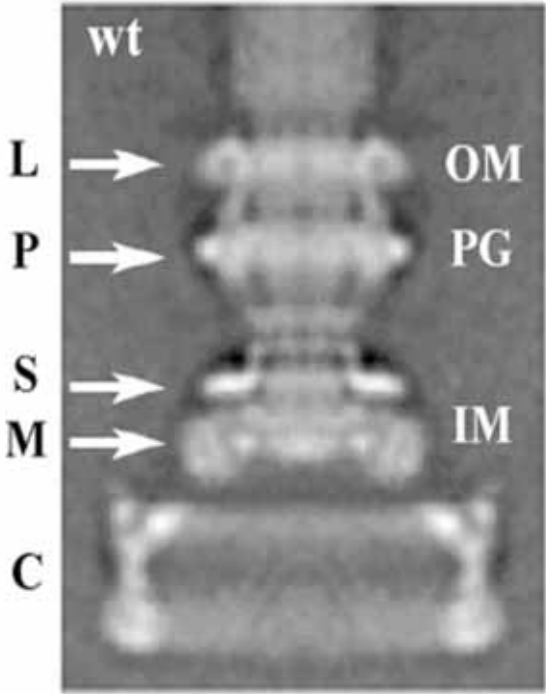
Model for The Membrane Receptor Arrays



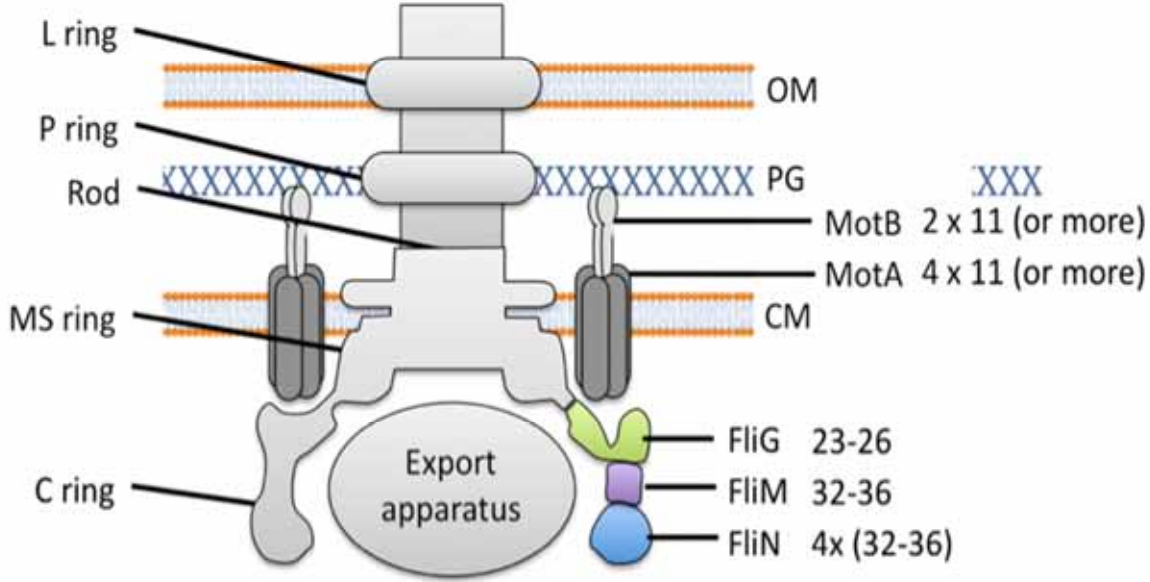
90°



The Flagellar Rotor



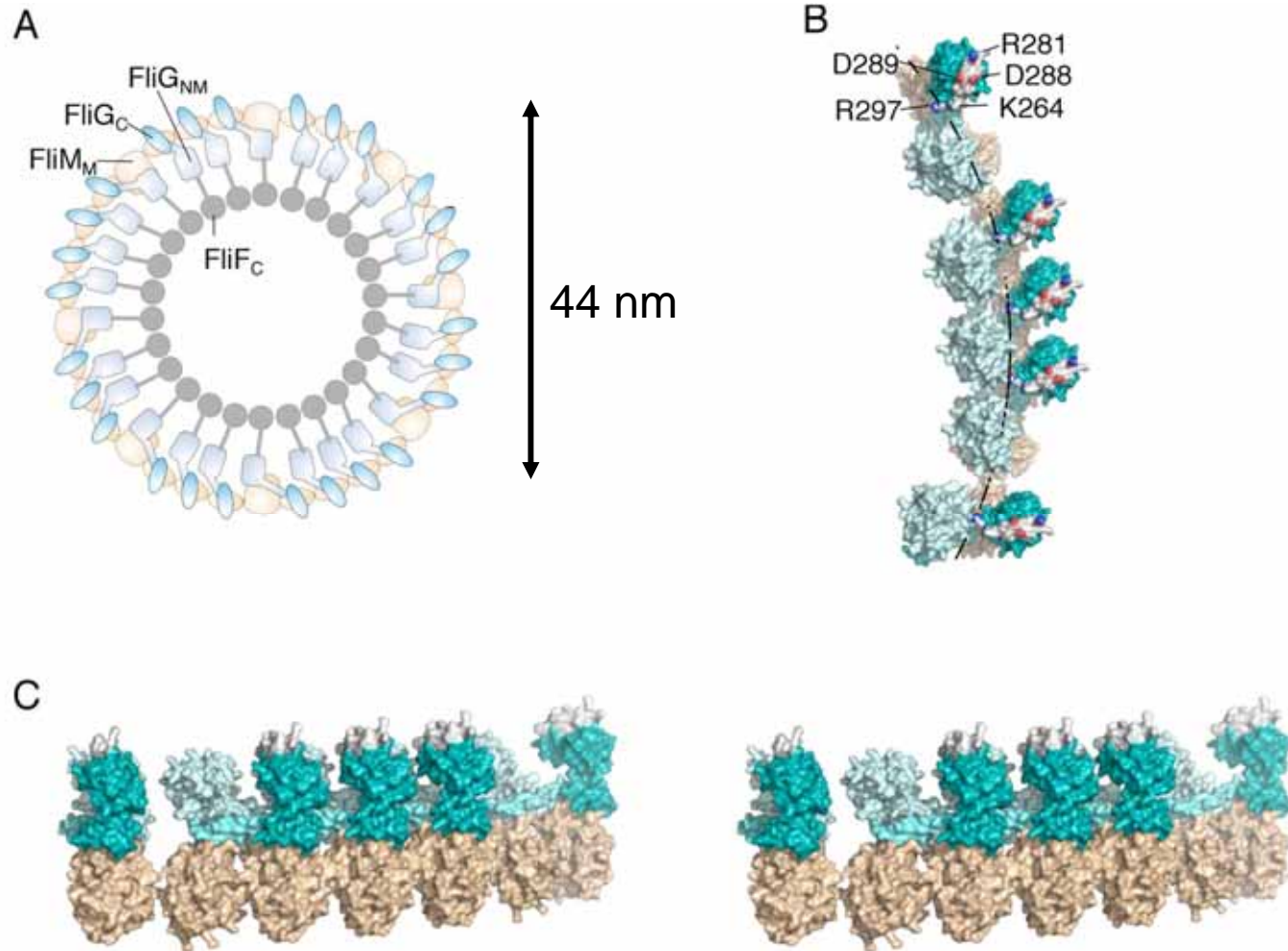
Thomas, DeRosier
Salmonella



Liu, Norris et al (2009) J. Bact. 191:5026

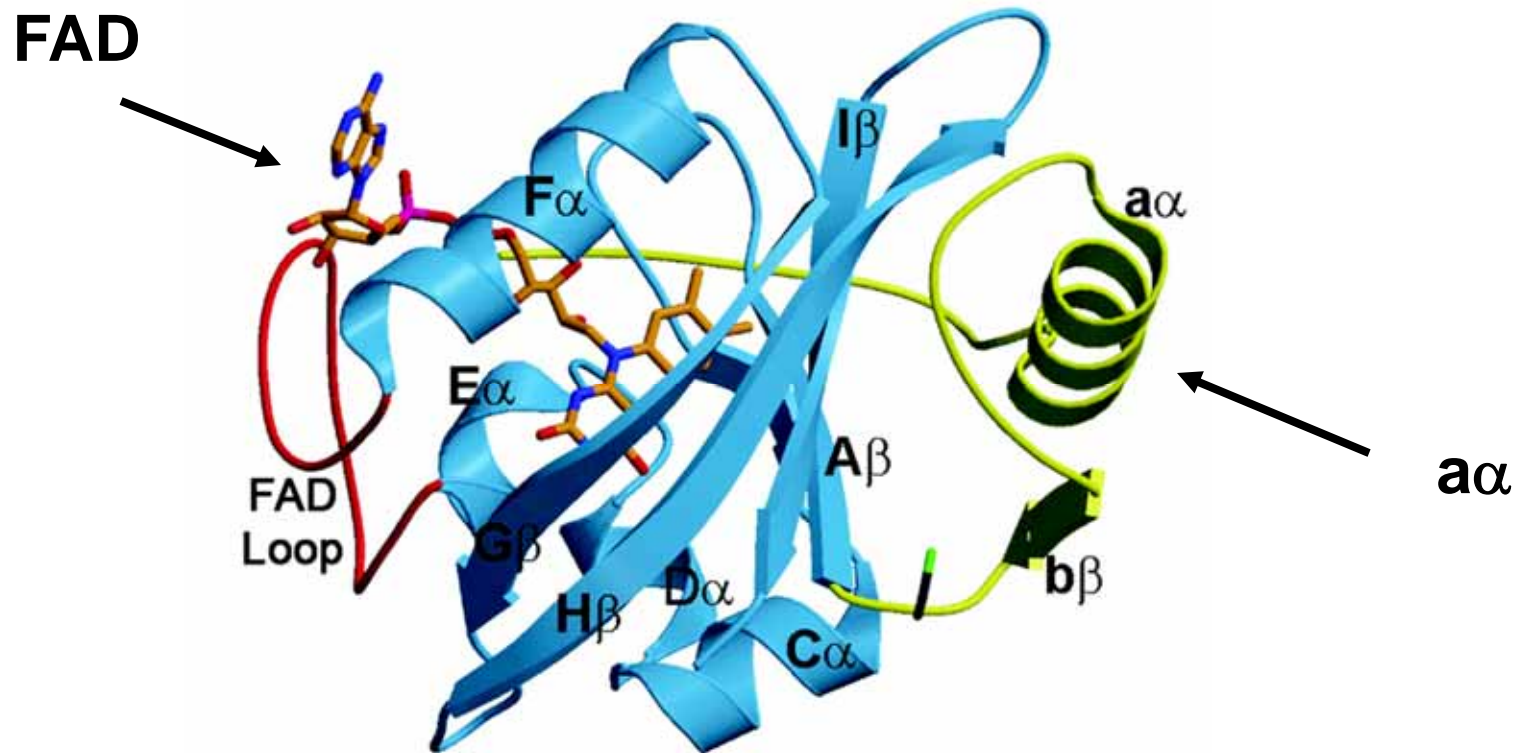
B. burgdorferi

Radially symmetric copies of switch complexes in the flagellar rotor

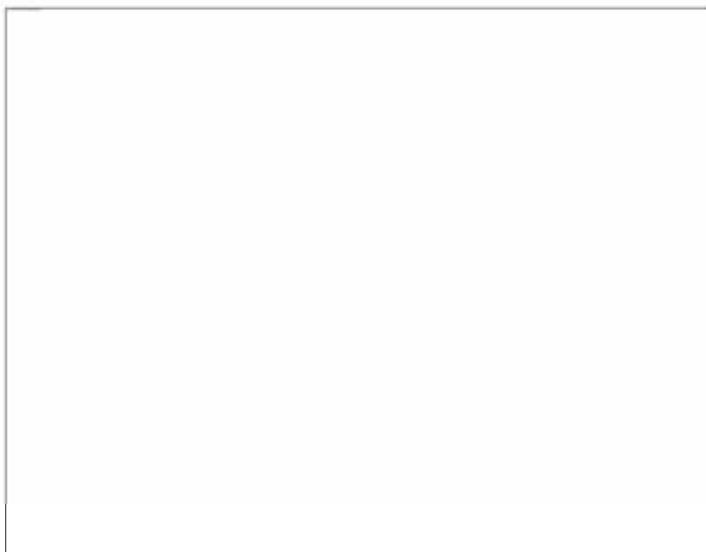


Paul et al (2011) EMBO J. Park et al (2006) *Proc. Natl. Acad. Sci.*

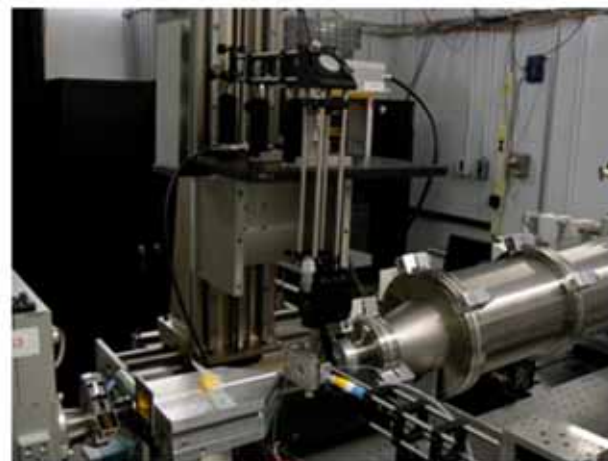
Time resolved SAXS on photoactivatable systems



Time-resolved SAXS to monitor VVD Light Induced Conformational Change

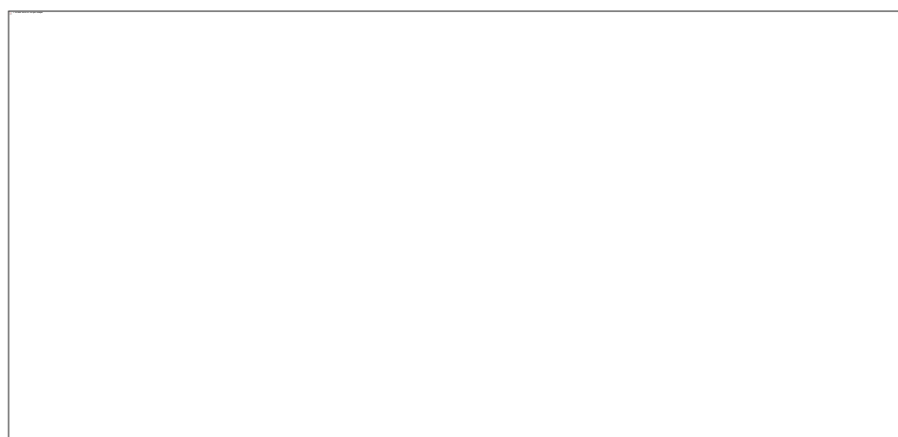


Guinier Plot

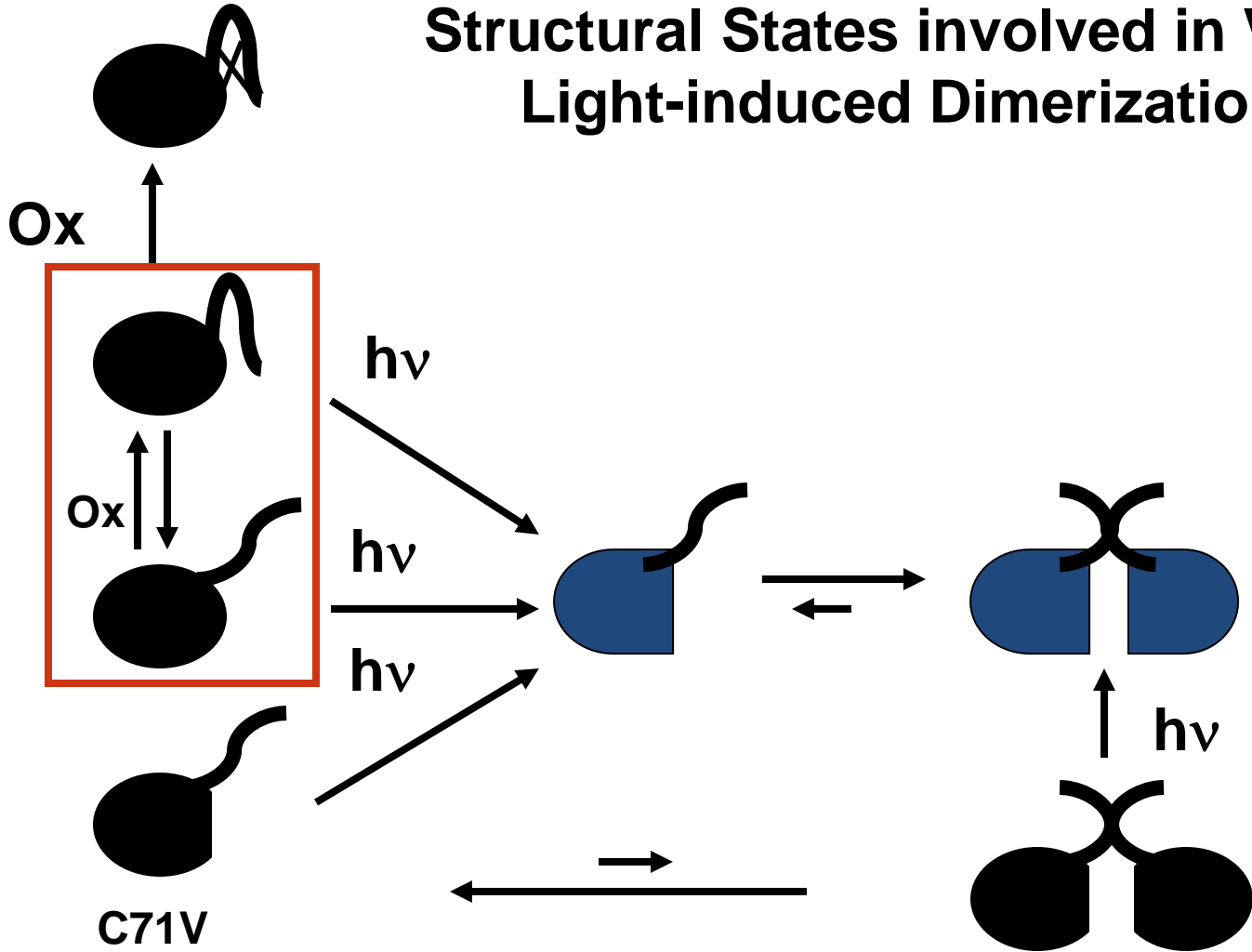


20 msec

Kratky Plot



Structural States involved in VVD Light-induced Dimerization



Conclusions

Much potential for solution scattering in a high flux, small focus regime

It's already an over achieving technique - what will increased orientational restraints bring?

You will be sample limited, but you always are - there will be work arounds

You don't need high resolution and fast times to answer important questions



Cornell University

Chemistry and Chemical Biology

Michael Airola
Joanne Widom
Abiola Pollard

Alexandrine Bilwes-Crane
Loma Linda University

Kylie Watts

CHES, ALS (SIBYLS Beam Line)

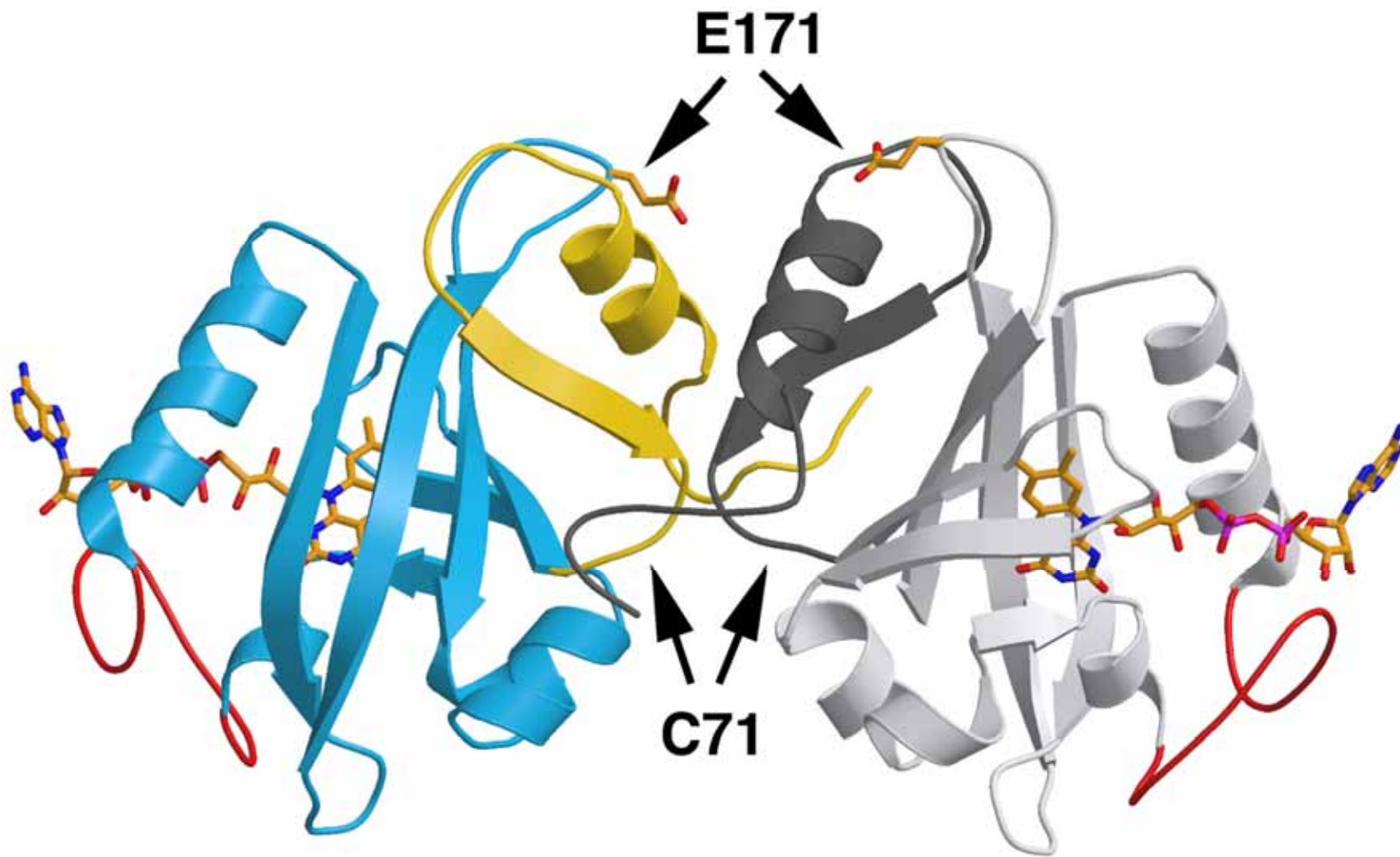
Applied and Engineering Physics

Lois Pollack
Jessica Lamb

Advanced Center for ESR Technologies (ACERT)

Peter Borbat
Jack Freed

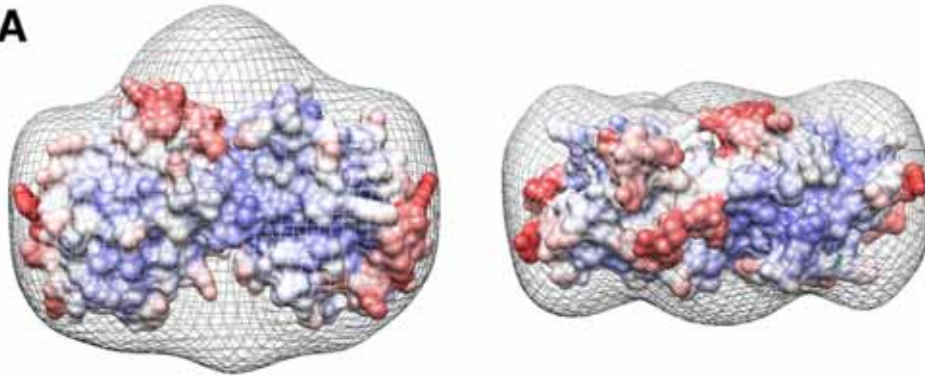
Light-state Dimer Crystal Structure of VVD



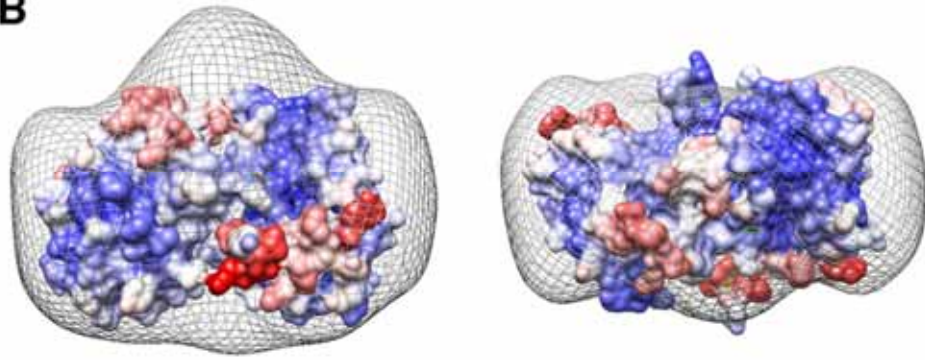
Anand Vaidya

Structure fits the time resolved SAXS envelope quite well

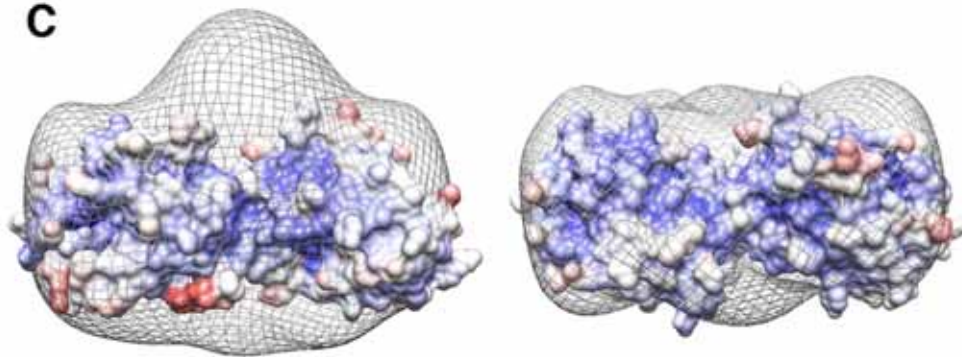
A



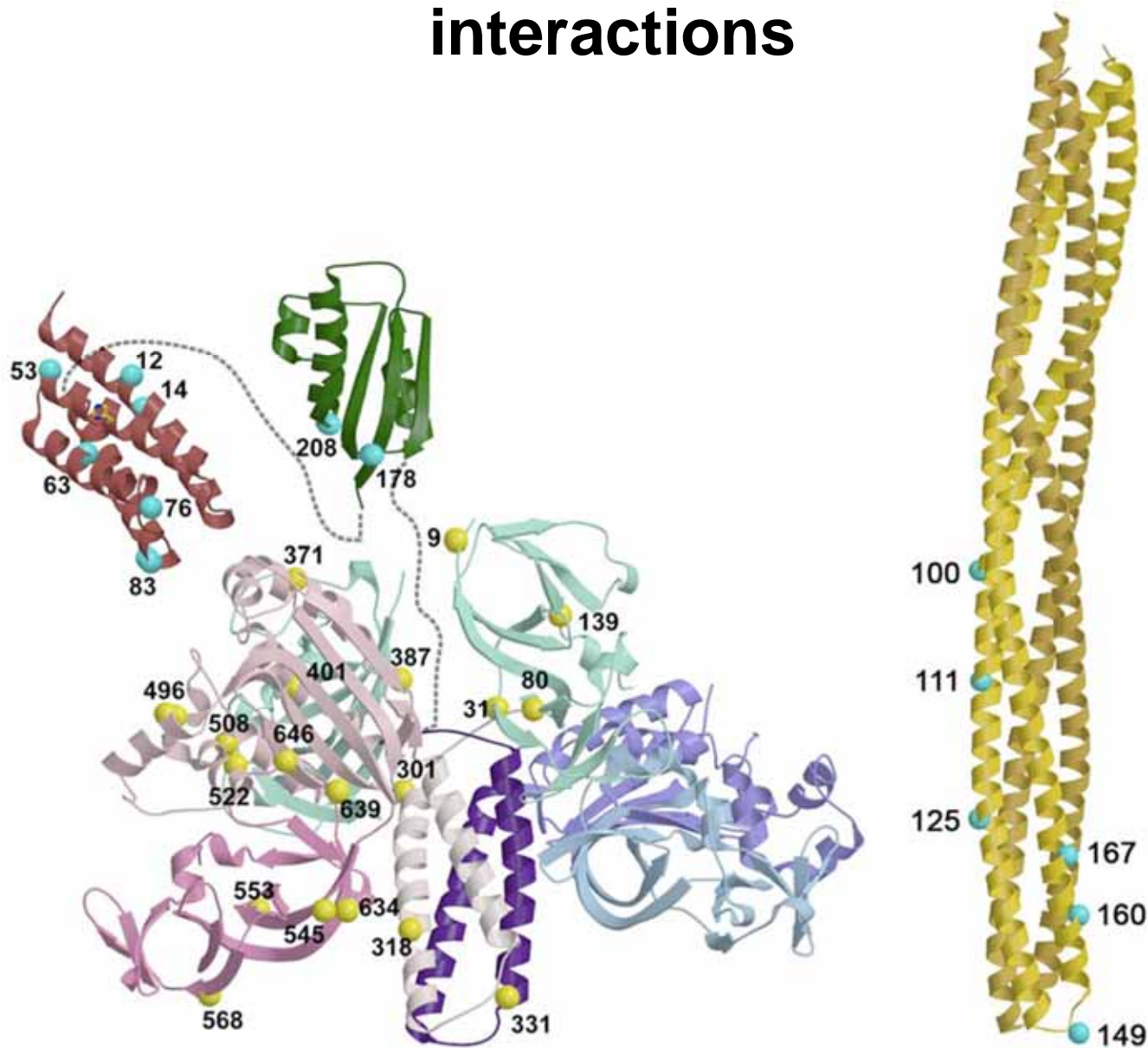
B



C

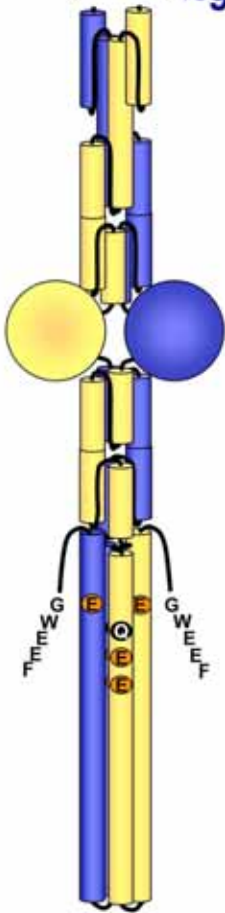


Spin-label sites for probing receptor:CheA:CheW interactions



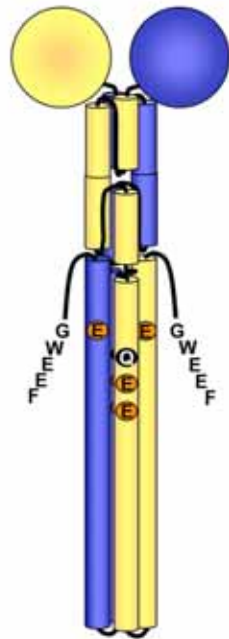
N-Terminal HAMPs are required for function

WT Aer-2
(1-679) ~95% CW

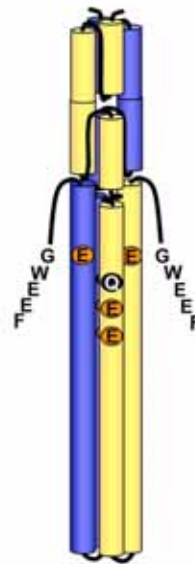


Steady-state motility in air
in BT3388 (mcp-, aer-)

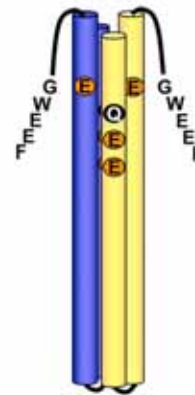
Signal-off
 Δ H1-3 (1-172)



Signal-off
 Δ H1-3-PAS (Δ 1-288) or
 Δ H1-3-PAS-H4 (Δ 1-329)



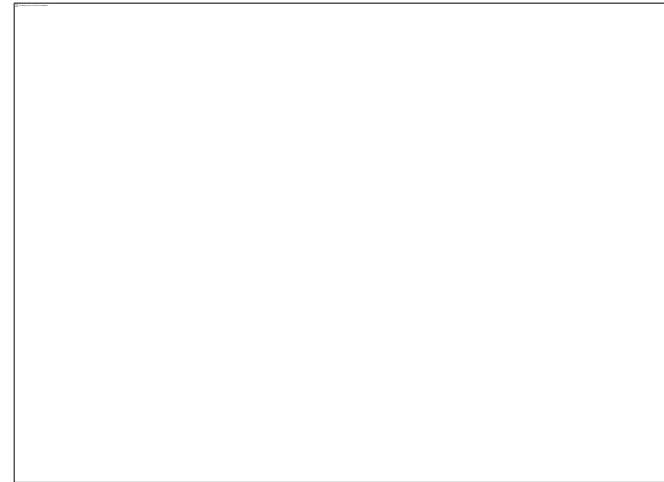
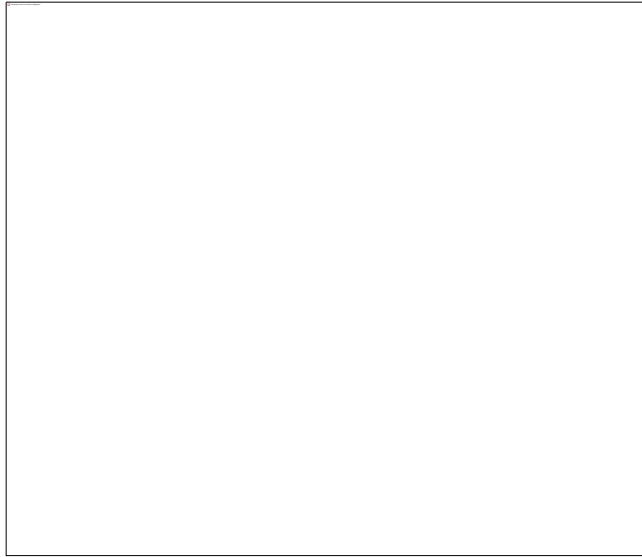
~35% CW
 Δ H1-3-PAS-H4-5 (Δ 1-379)



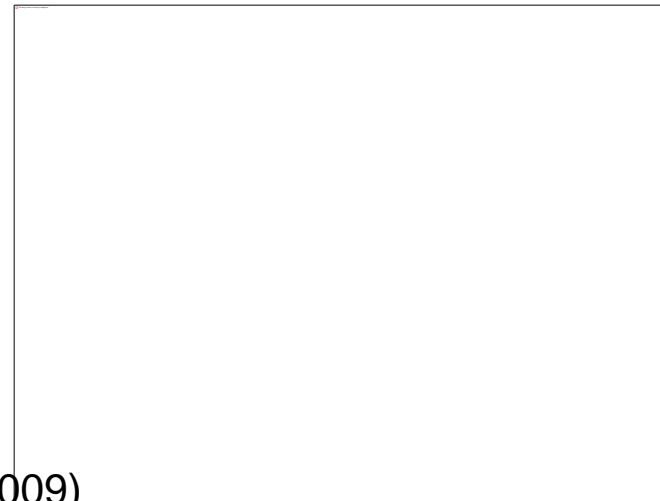
CheA activity
+
-
-
+

Kylie Watts, in preparation

The dark state of Cys71Val forms an extended monomer that is partially dimerized in the absence of light



The extended state can be mimicked by introducing conformational variability at the N-terminus

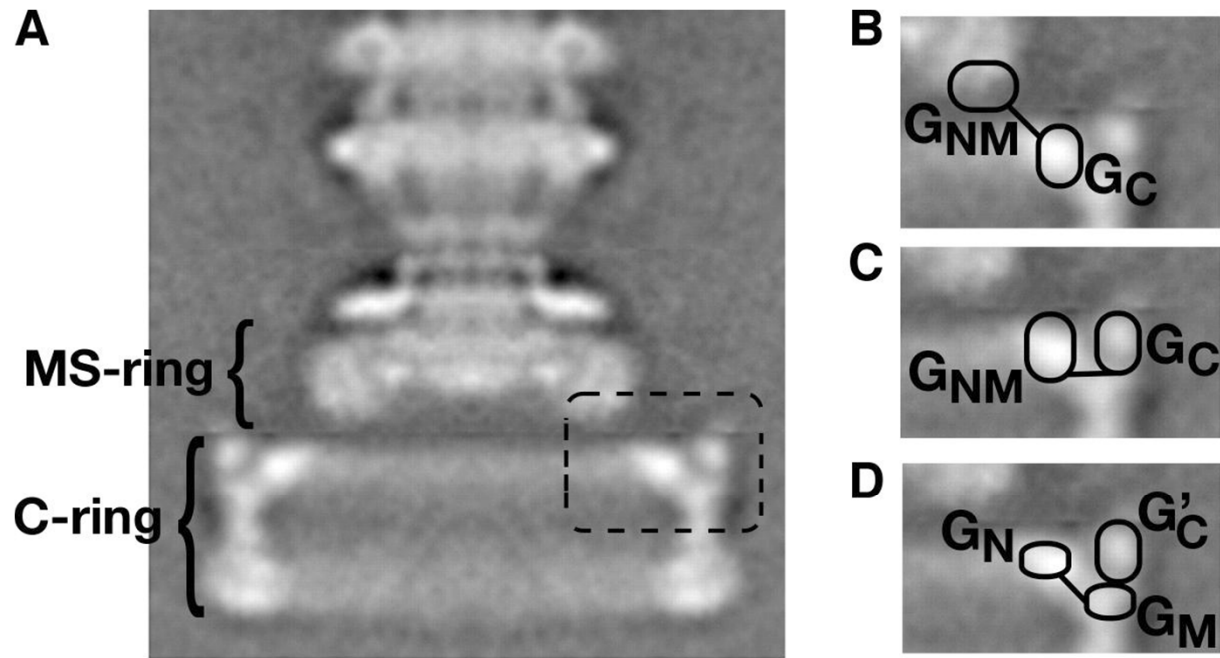


Reconstructed model

Aer2 fragment	Dmax (Å) P(r)	Dmax (Å) <i>Ab initio</i> model
Aer2 1-172	115	112
Aer2 1-317	205	193
Aer2 1-402	250	238

PAS domains in close proximity ... possible role for dimerization?

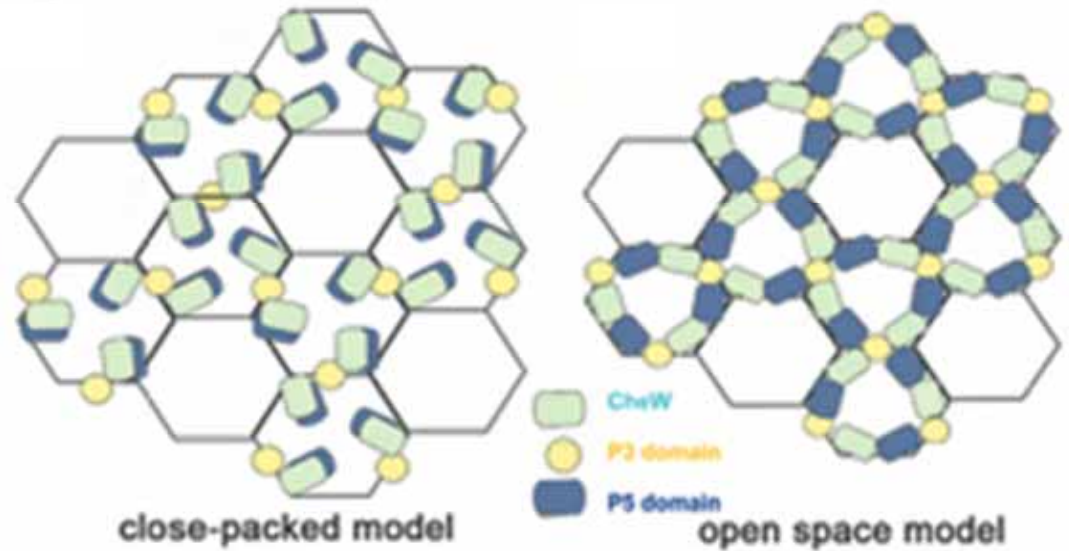
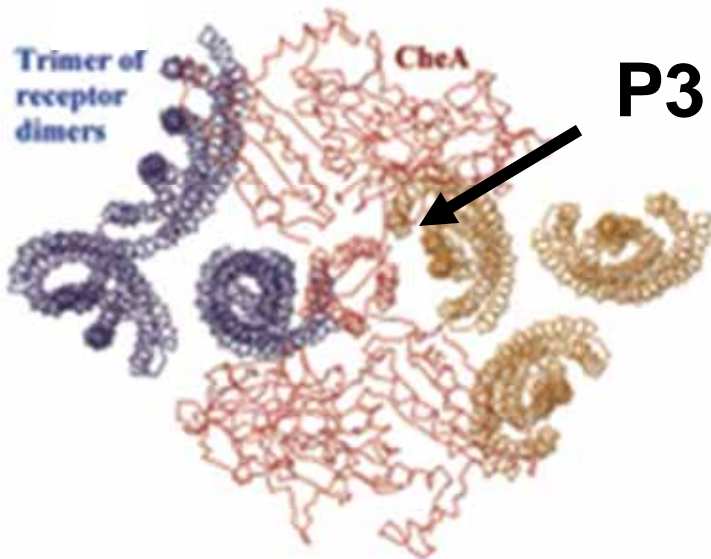
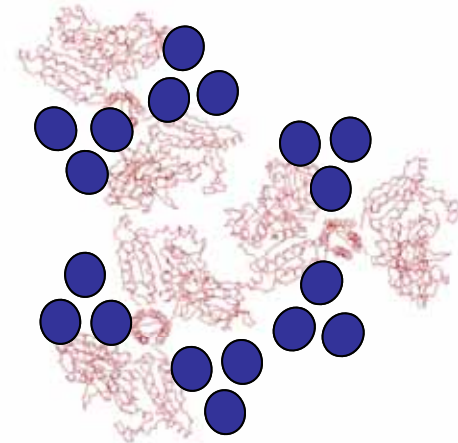
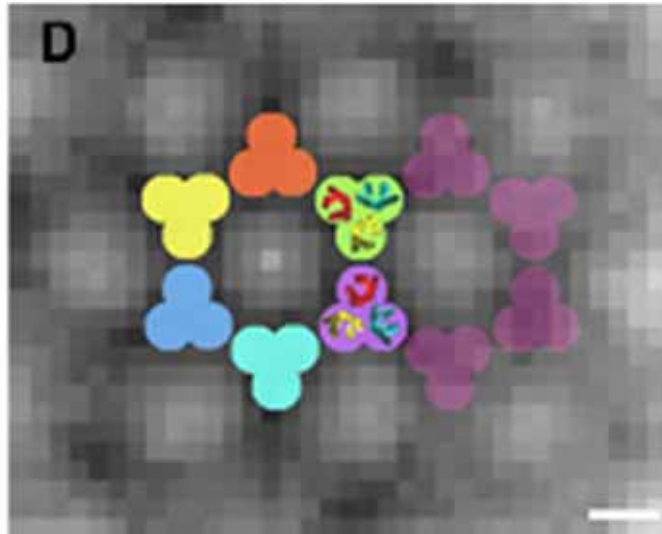




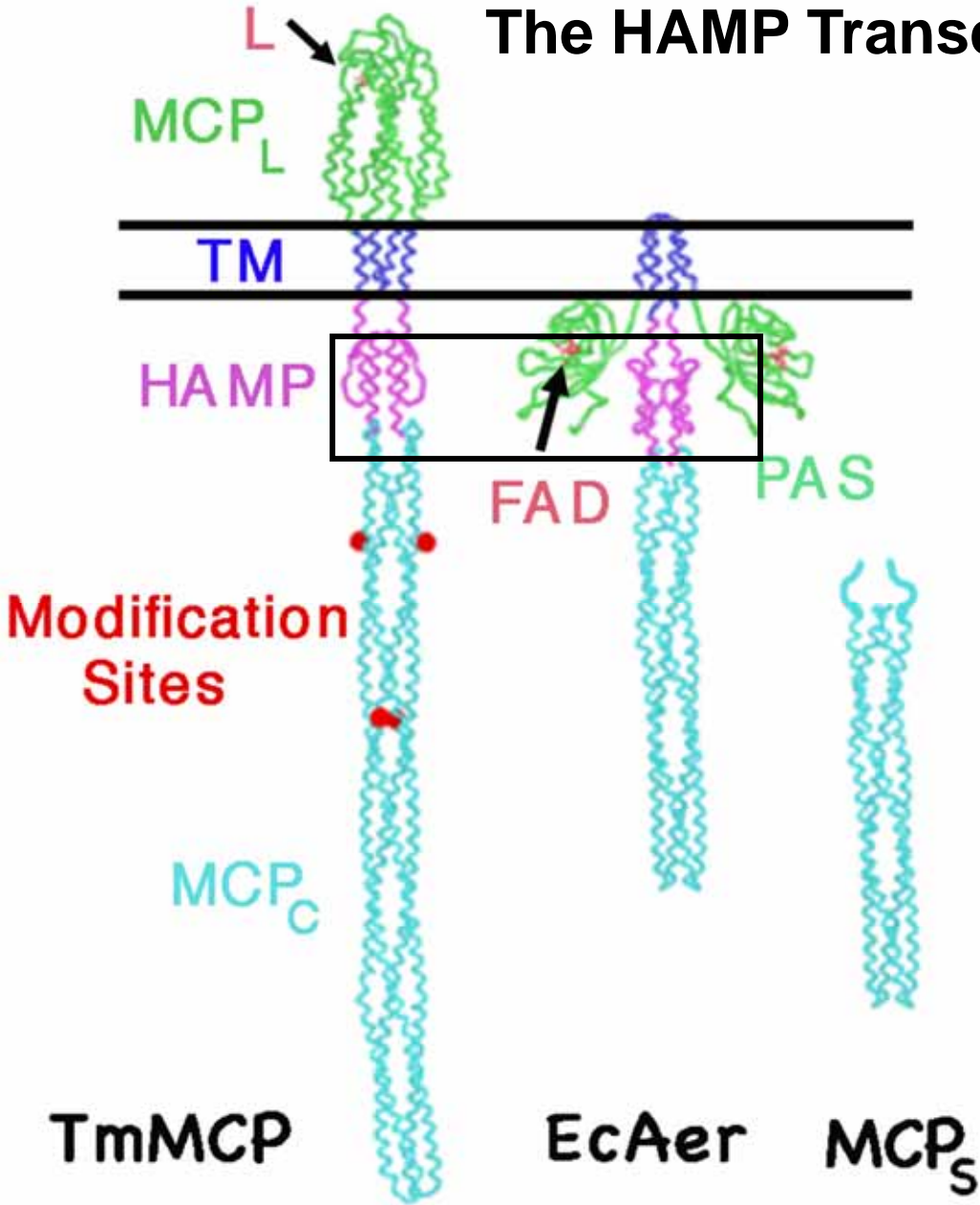
Paul et al., Figure 1

Extension to a lattice model - P3 between trimers?

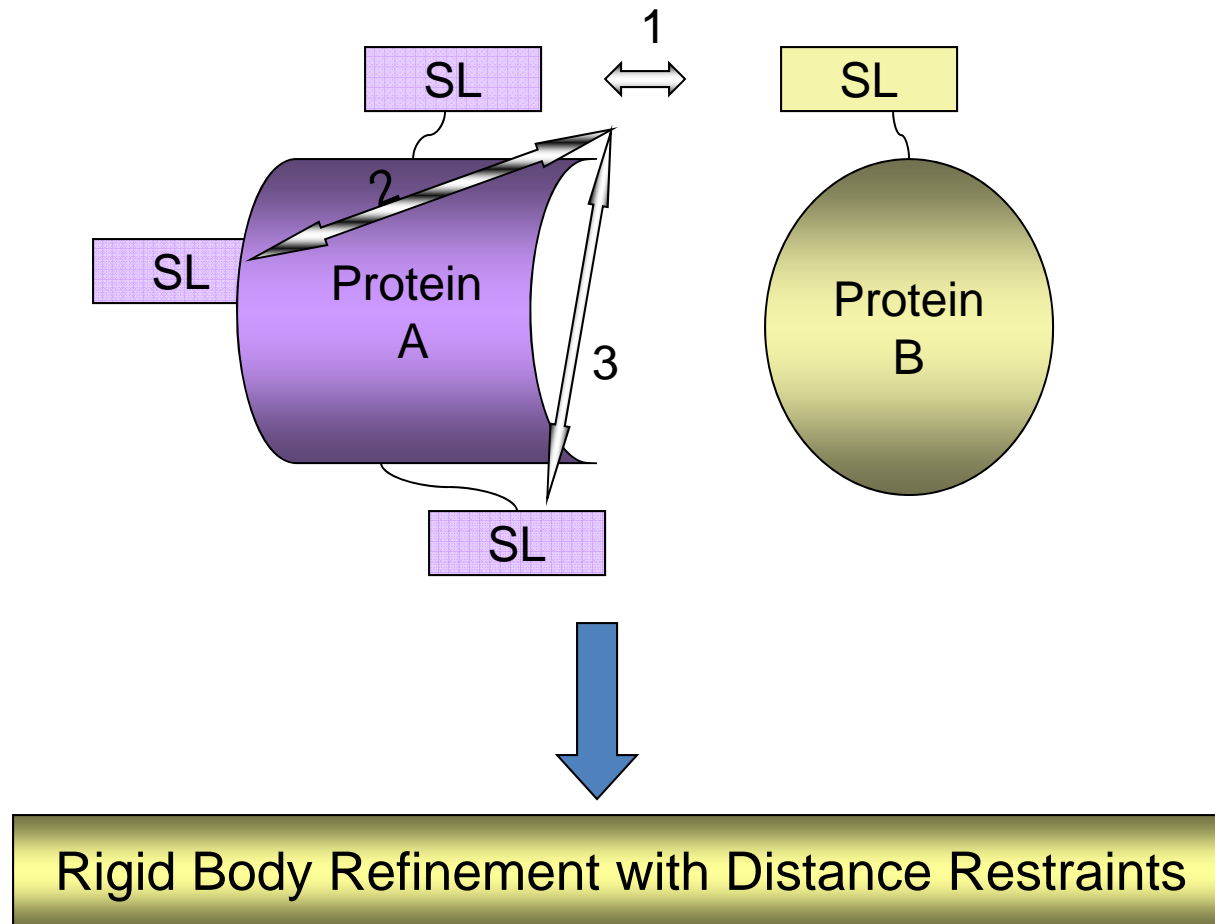
Briegel
et al
(2008,
2009)



The HAMP Transducer module

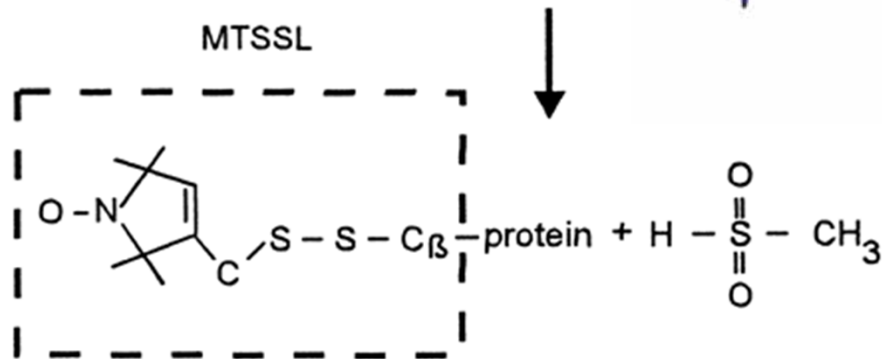
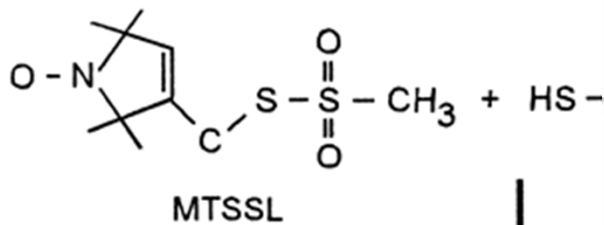
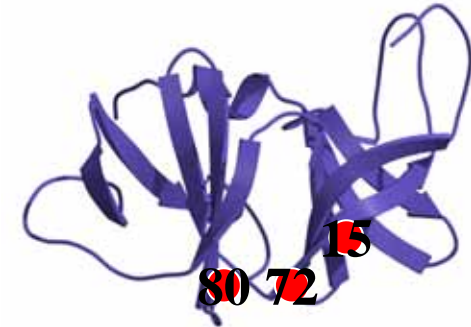
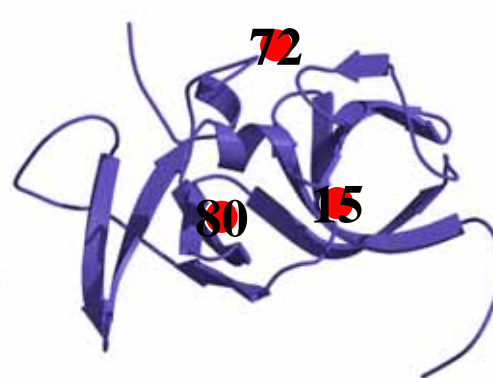
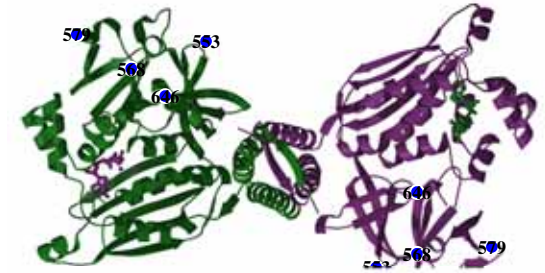
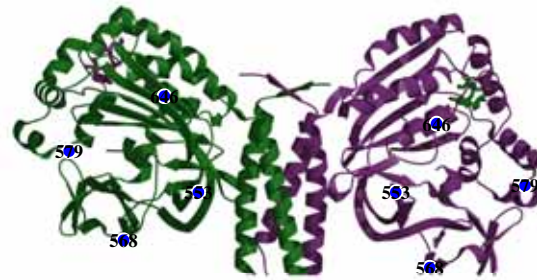


Strategy for structure determination of protein complexes with spin-labeling



Inter-domain distances by spin-labeling

Protein Spin-Labeling



MTSSL

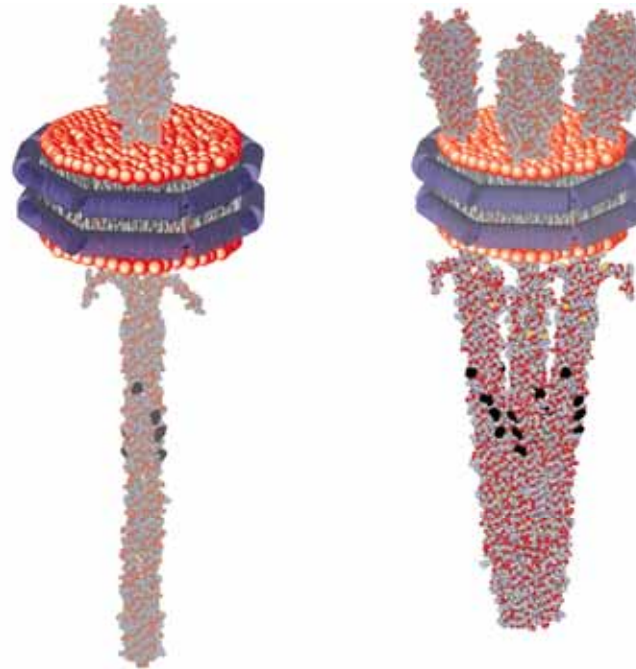
(1-Oxyl-2, 2,5,5-tetramethylpyrroline-3-methyl)methanethiosulfonate)

Nanodisk-embedded chemoreceptors

Nanodisks:

Nath, Atkins and Sligar

Biochemistry (2007) **46** 2059



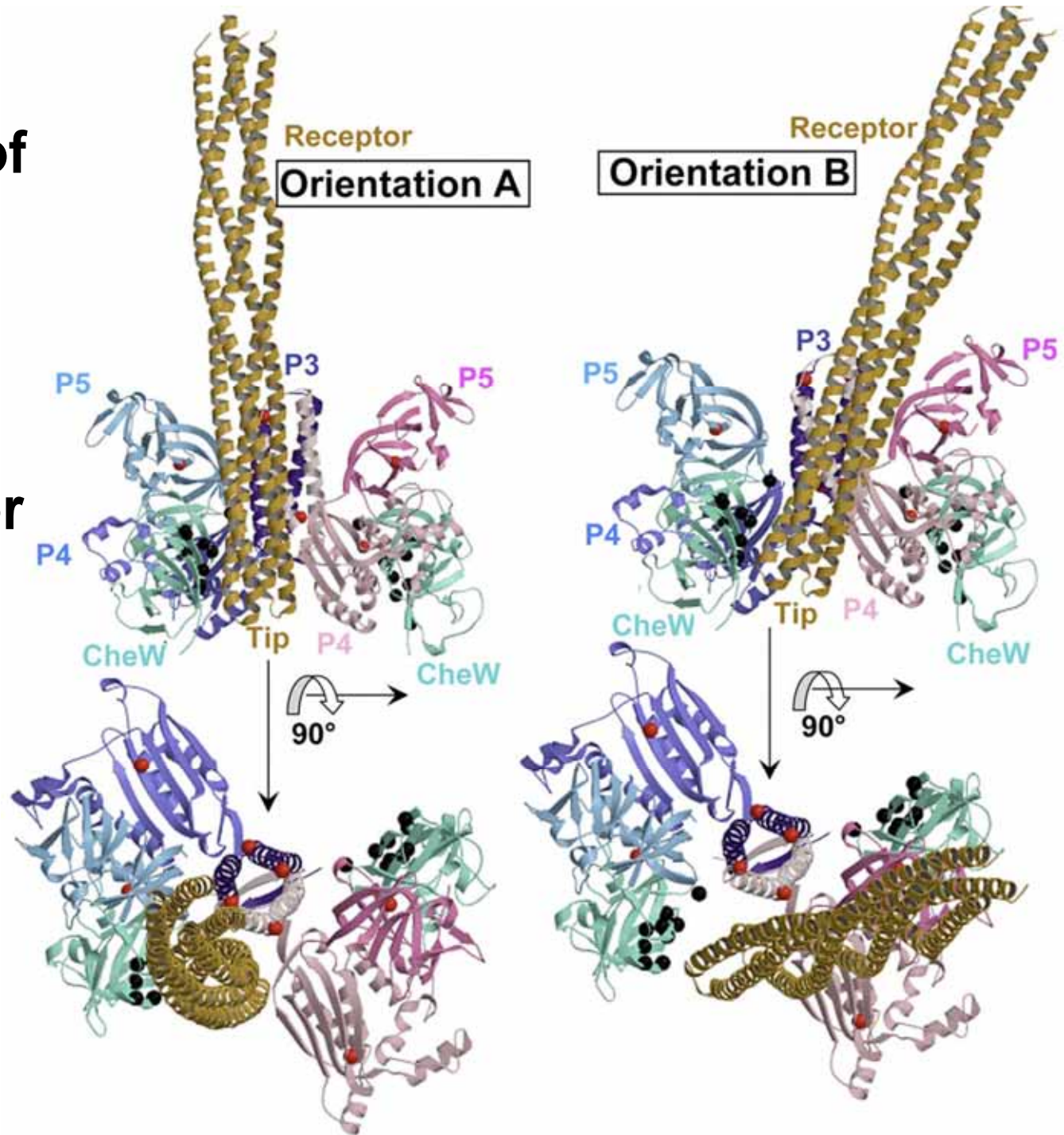
Ligand Binding	yes	yes
Transmembrane Signaling	yes	yes
Adaptational Modification	yes	yes
Kinase Activation/Control	no	yes

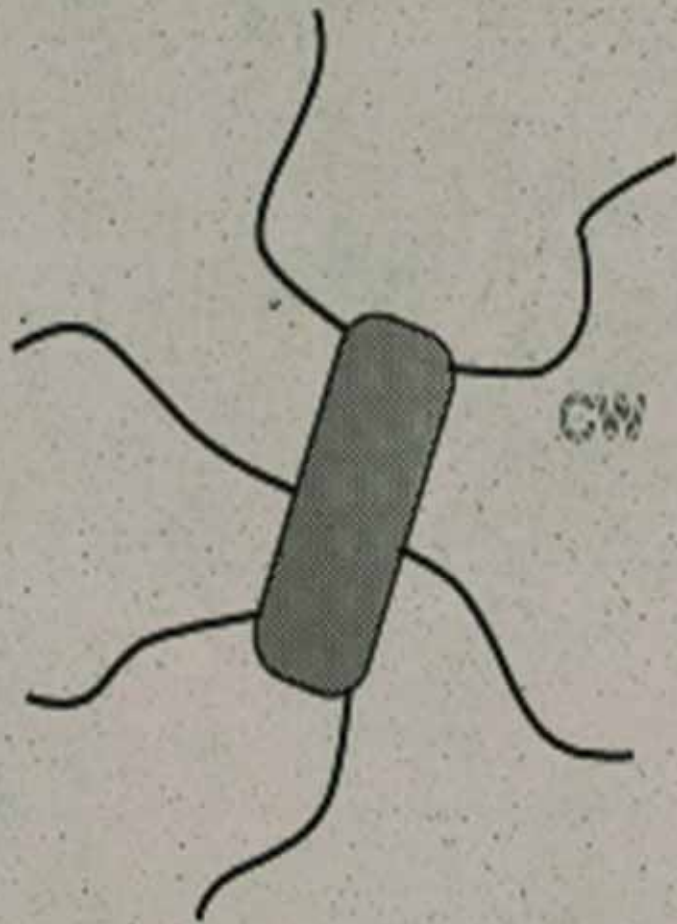
Boldog, Grimme, Li, Sligar and Hazelbauer PNAS (2006) **103** 11509-11514

Boldog, Li and Hazelbauer Meth. Enzymol. (2007) **423** 317-335

Structure of the ternary complex:

Two limits for receptor orientation





Tumble



Smooth