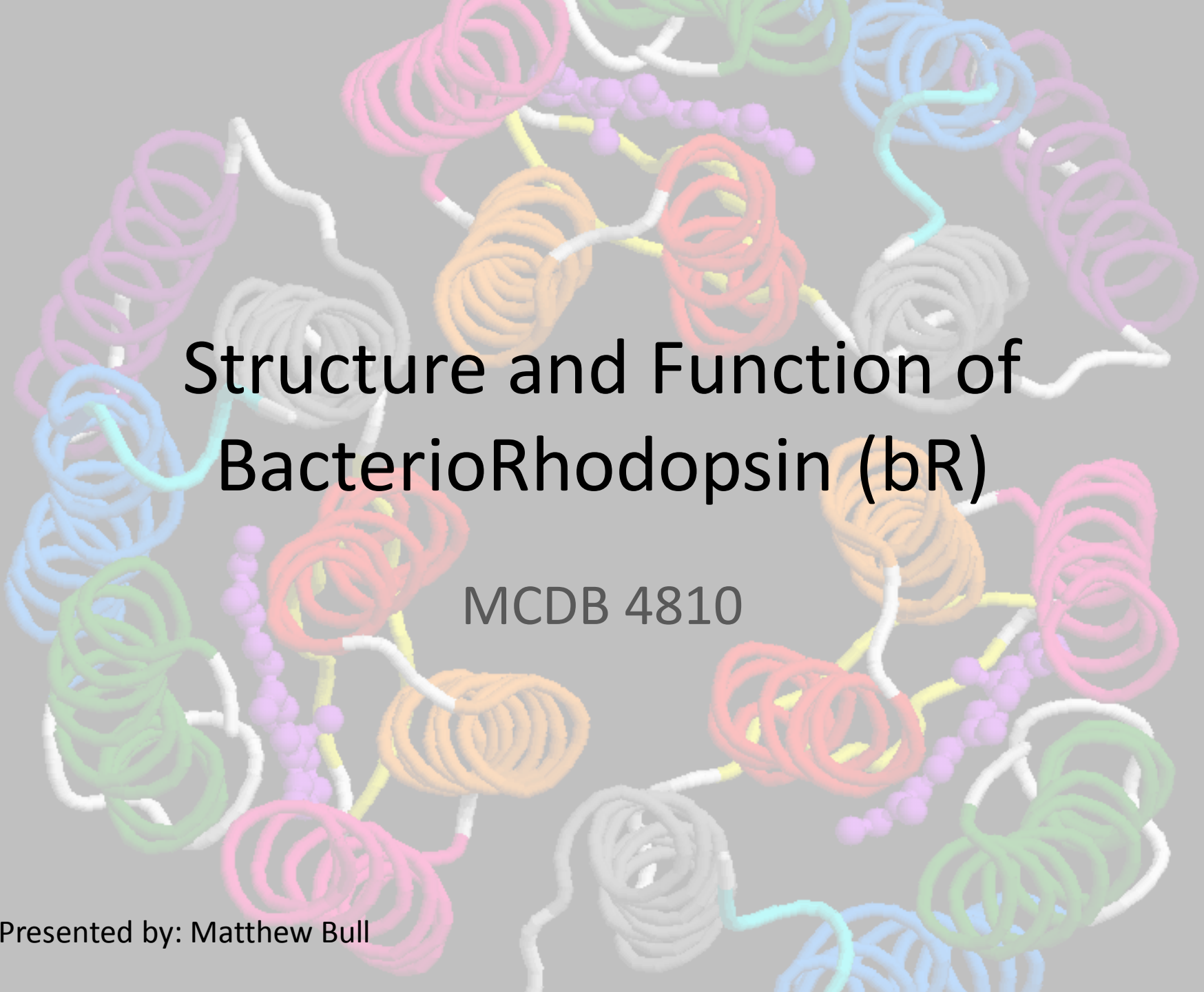


Structure and Function of BacterioRhodopsin (bR)

MCDB 4810

Presented by: Matthew Bull

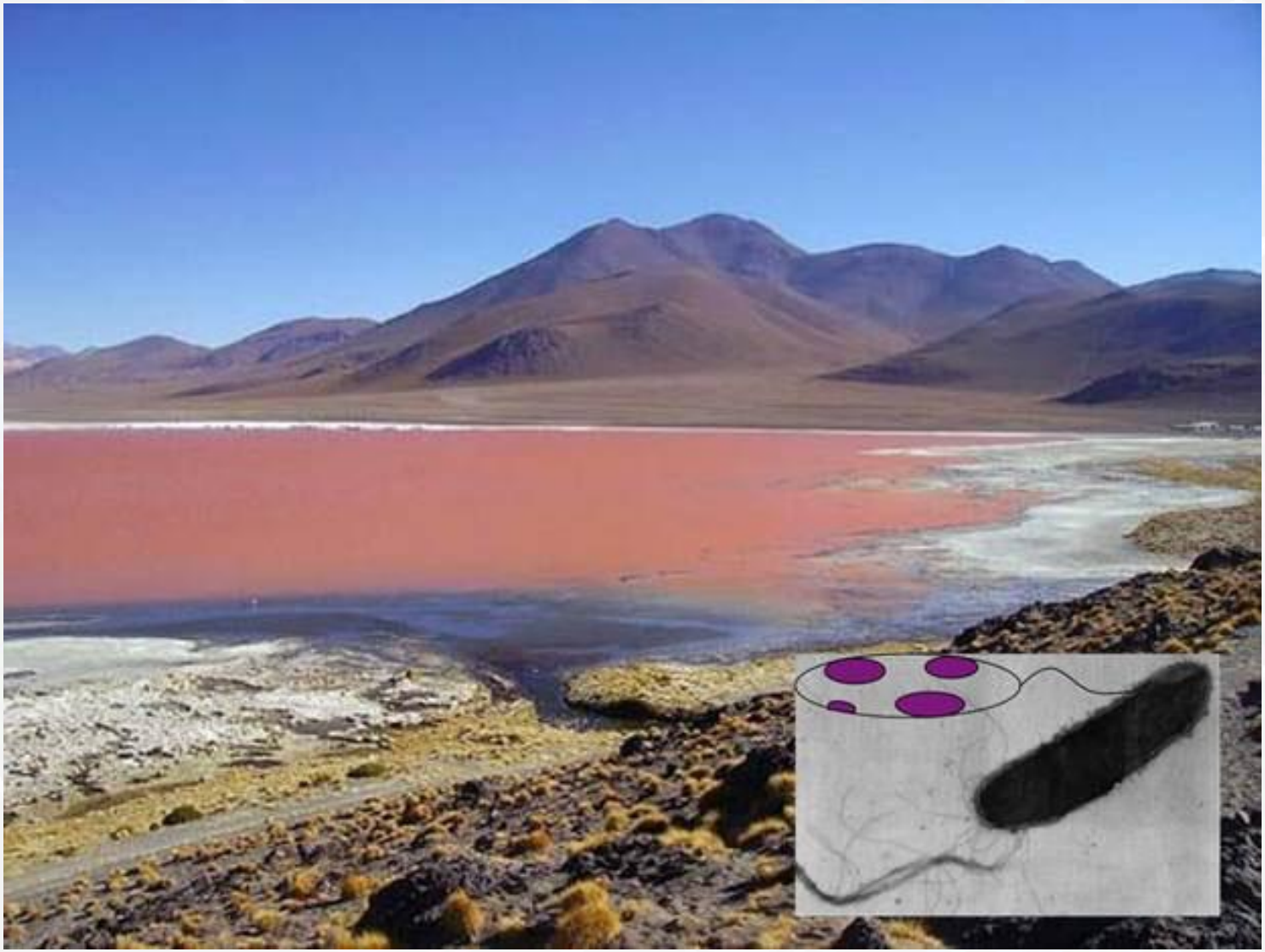
Objectives

- Practice assessing the quality of x-ray and electron-beam crystallographic structure determinations
- Investigate how structure informs function
- Discuss the structure determination and refinement of a seminal membrane protein: Bacteriorhodopsin (bR)

Overview

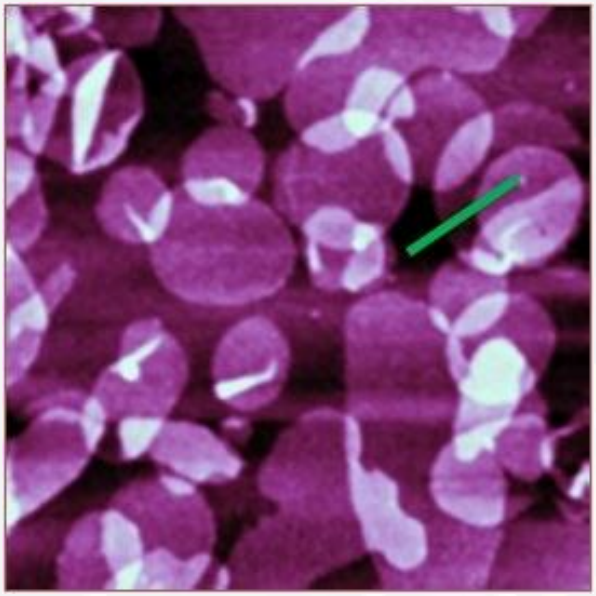
- Context: the accepted bR structural model
- Quick discussion of methods for investigating folding and stability of bR
- Improvement of bR structure by x-ray crystallography to 2.5Å
- Refinement of bR by electron-crystallographic means

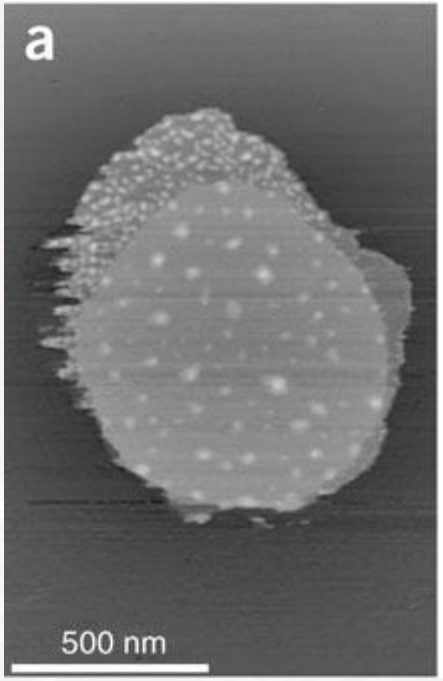
Where is bR found?

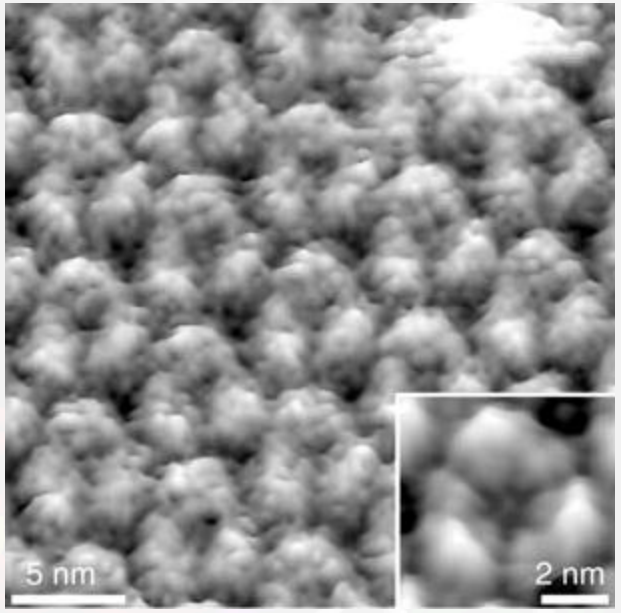


(www.bioch.ox.ac.uk/...)

bR is natively in dense, periodic trimers known as purple membrane

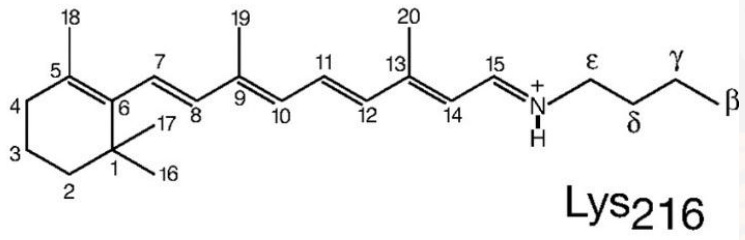




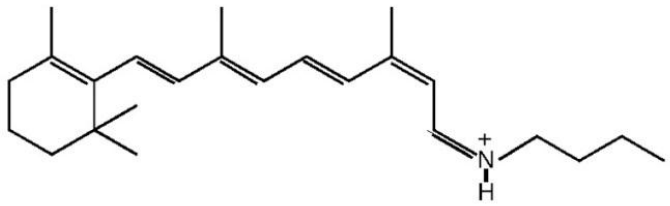


bR undergoes structural confirmation changes in light

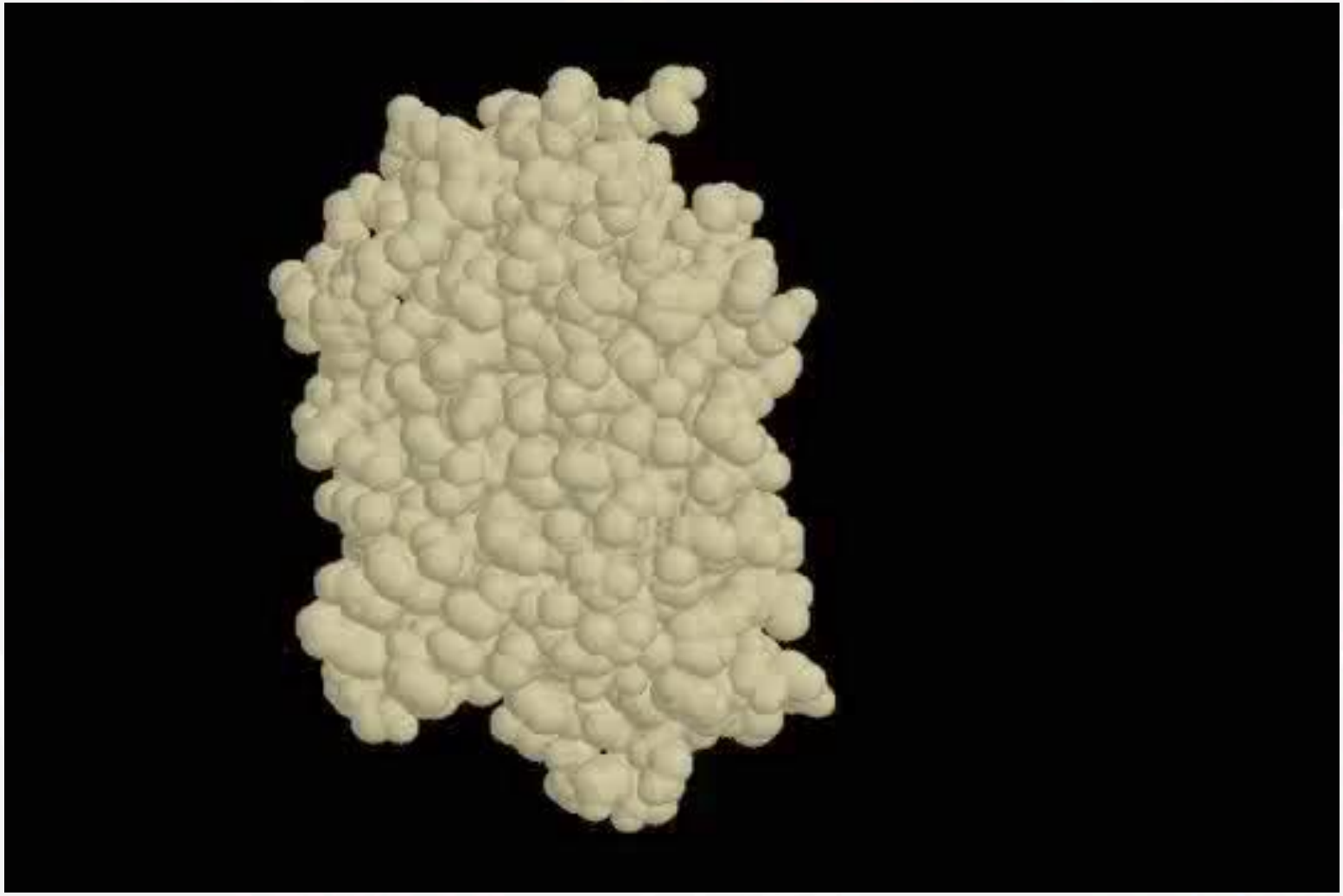
13-*trans*, 15-*anti* (bR_{568})



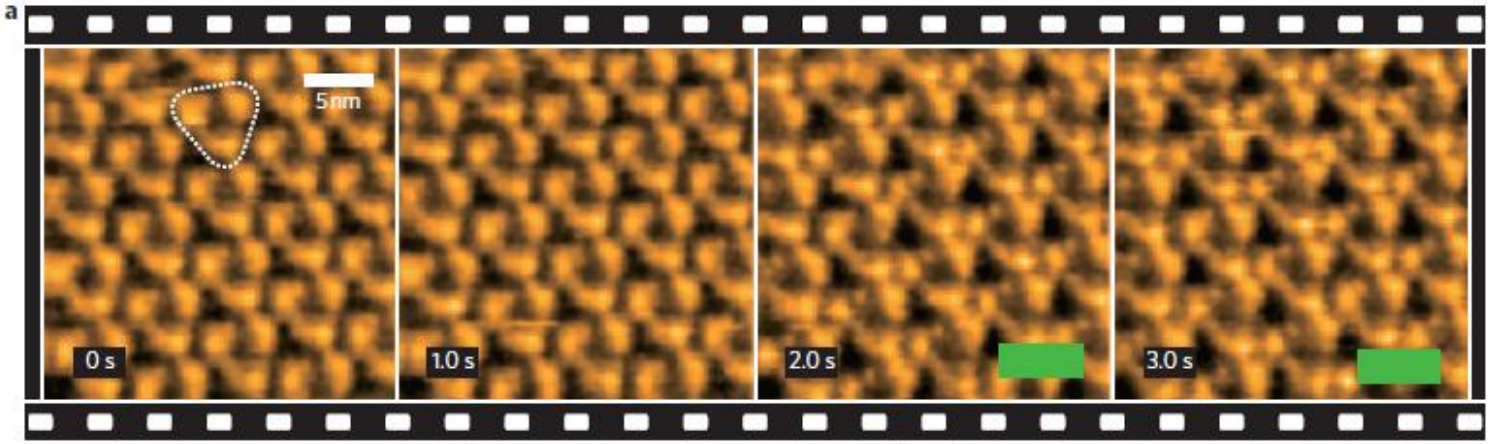
13-*cis*, 15-*syn* (bR_{555})

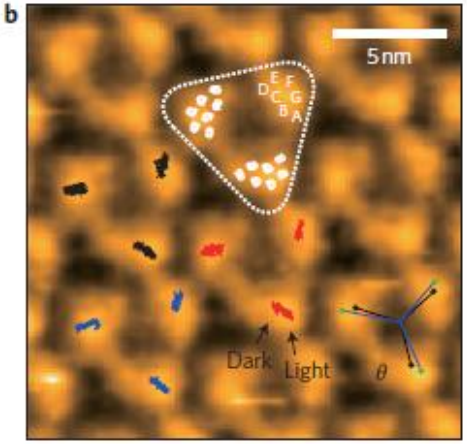


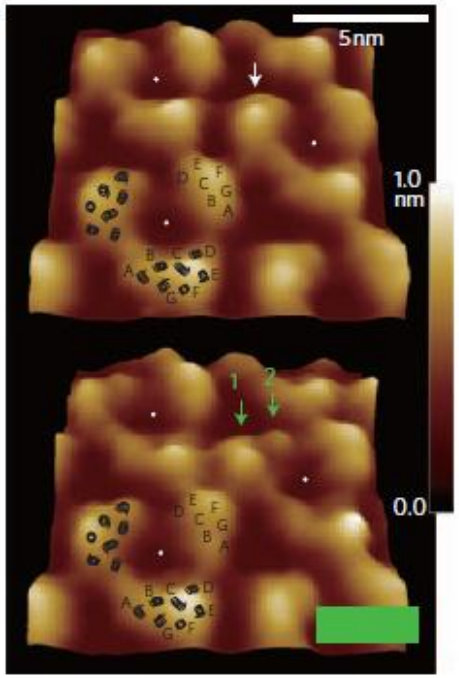
What is bR's physiological function?

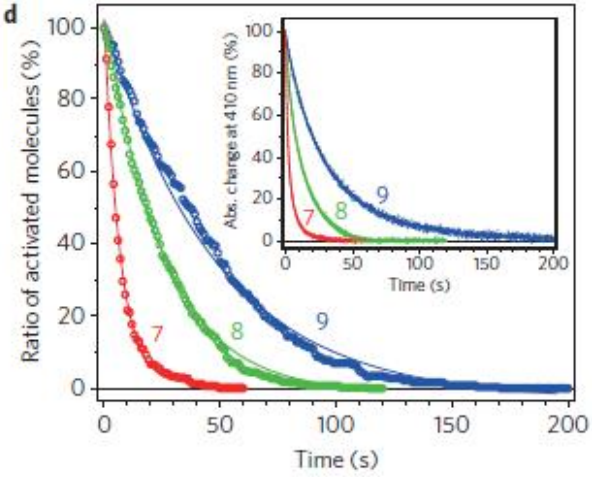


Why is bR exciting for biophysicists?





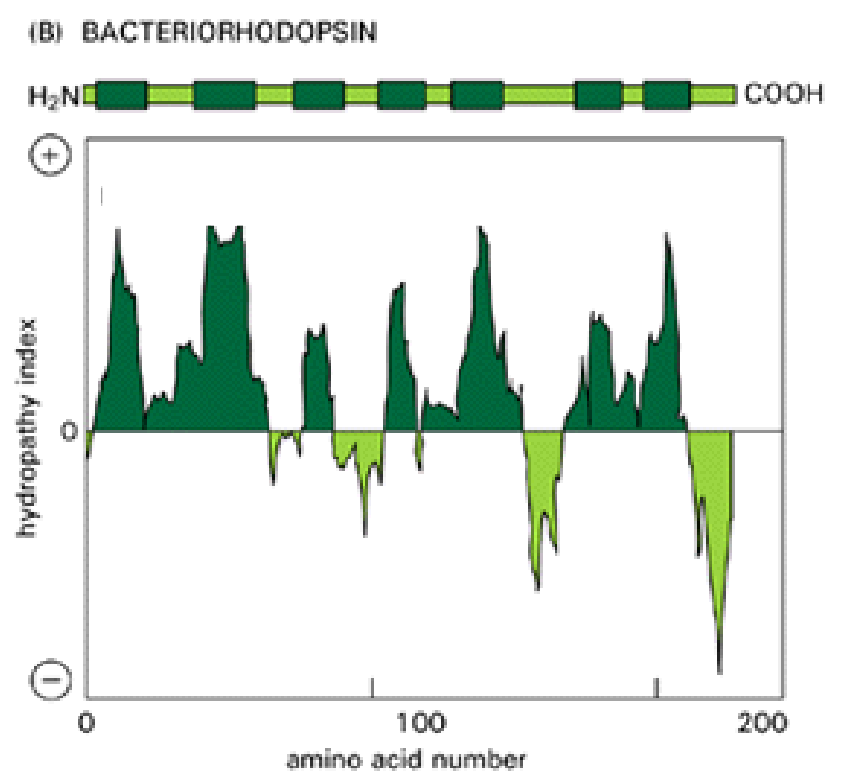


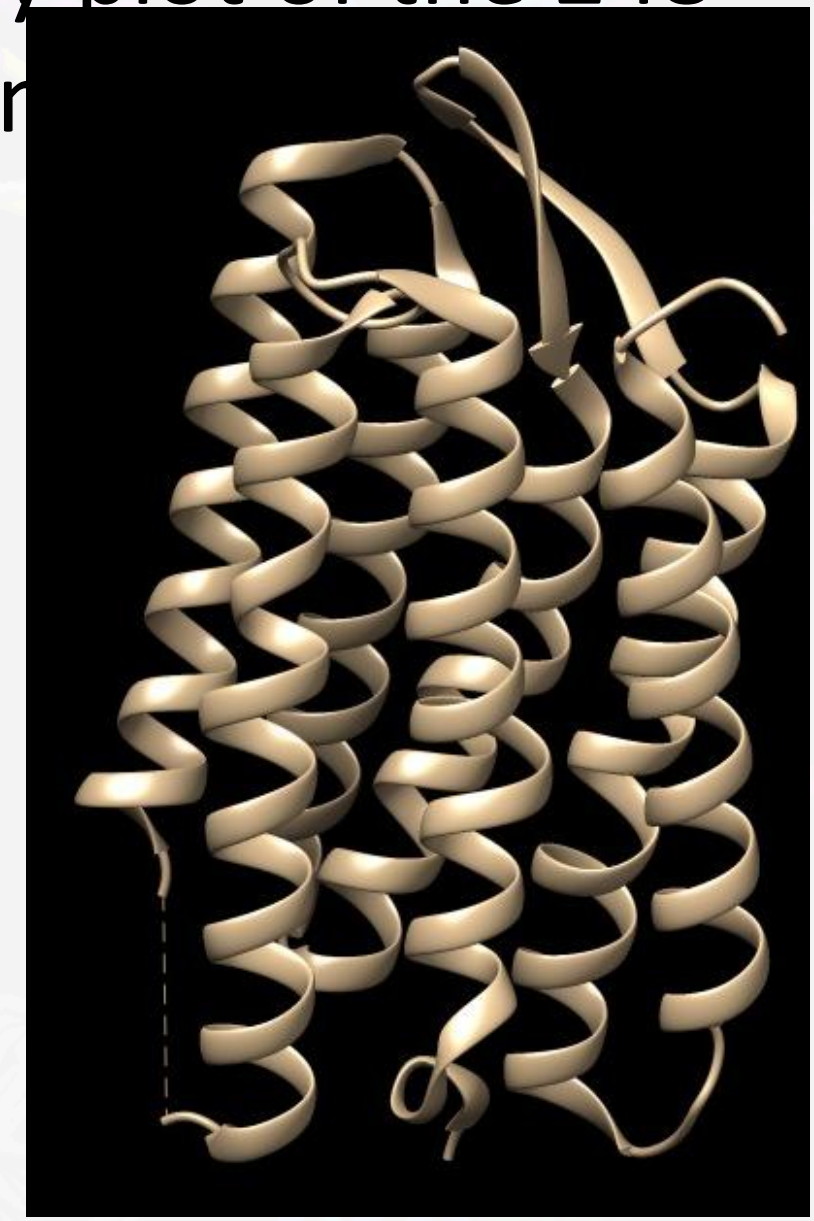


Questions to consider

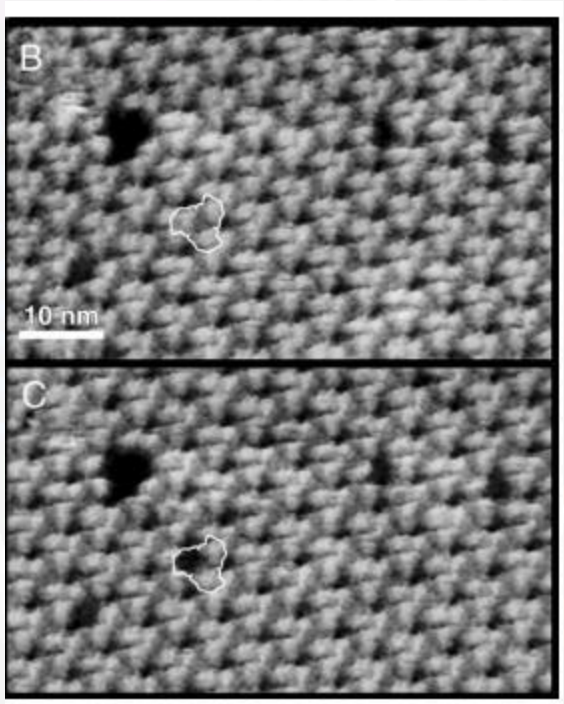
- How can structural determination inform the role of water molecules in the proton transfer?
- Why might proton pumping out of the cell be a valuable asset?
- Why does it matter how the bR orients itself in the membrane? How is this controlled?

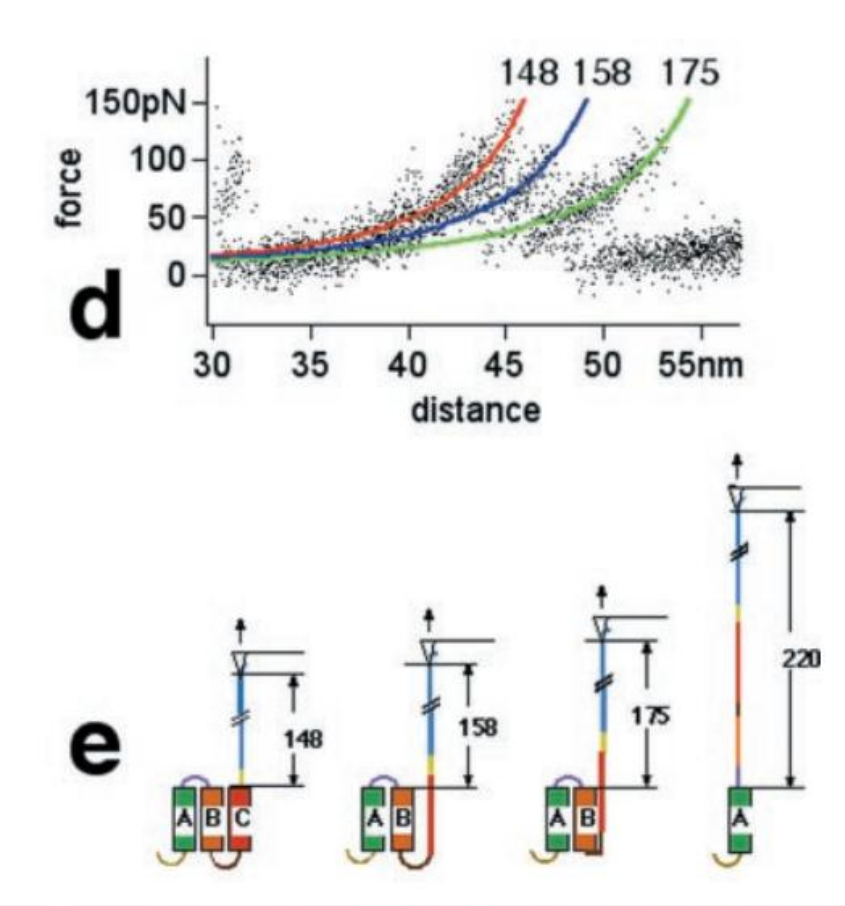
Does a hydropathy plot of the 248 residues inform





AFM can probe the single-molecule stability of bR embedded in purple membrane





Questions to consider

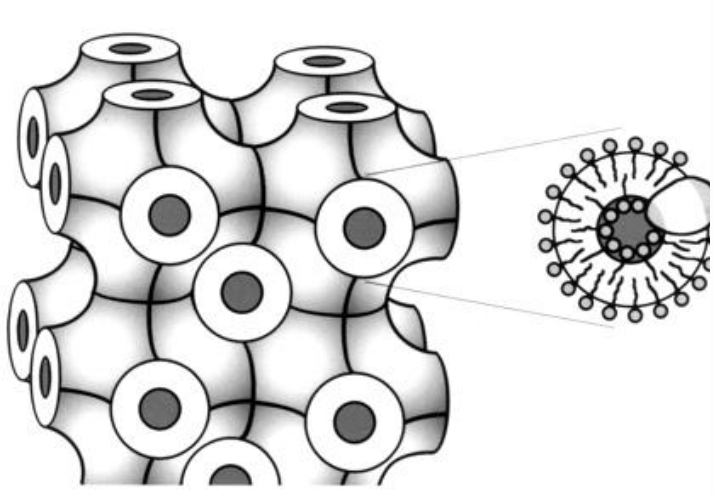
- Why might trimer formation assist molecular stability?

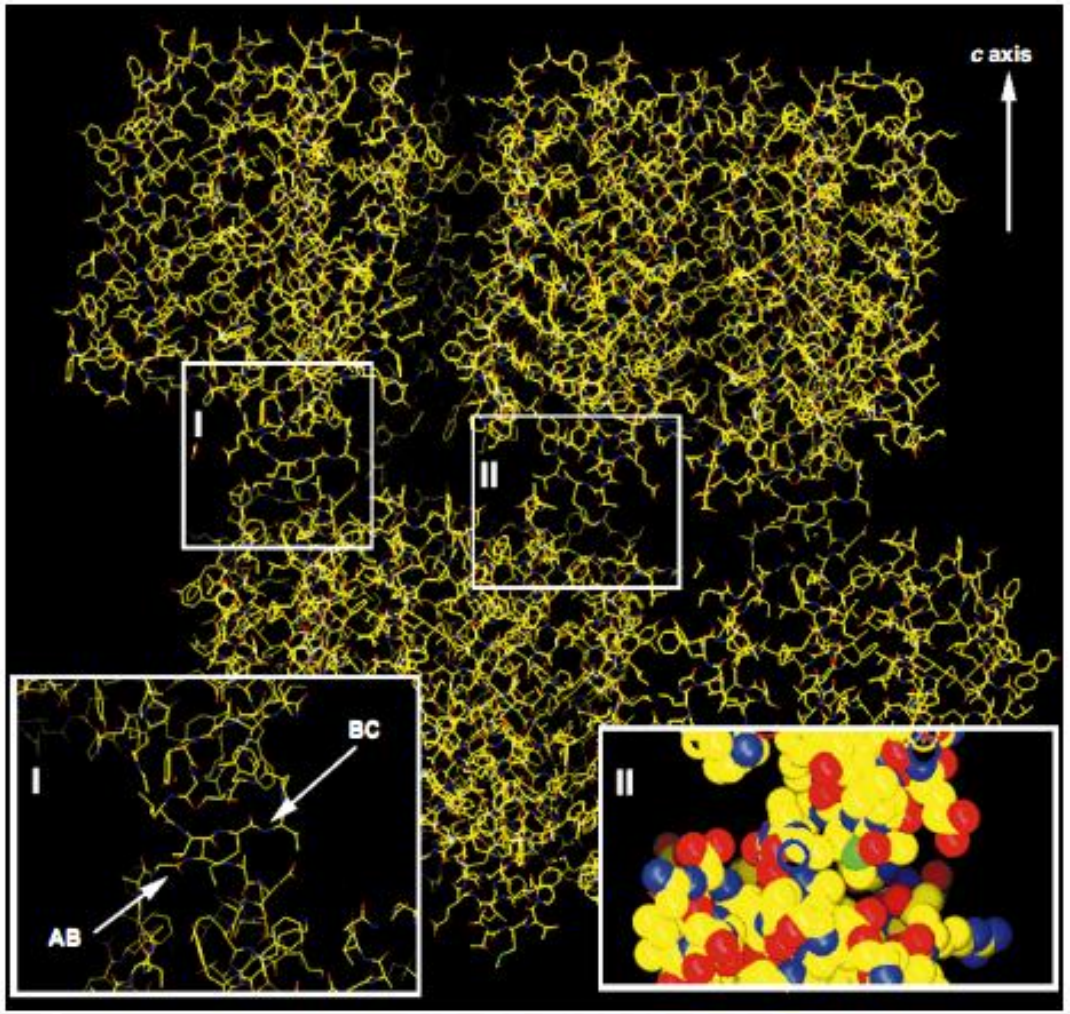
X-ray Structure of Bacteriorhodopsin at 2.5 Angstroms from Microcrystals Grown in Lipidic Cubic Phases

Eva Pebay-Peyroula, Gabriele Rummel, Jurg P. Rosenbusch, Ehud M. Landau

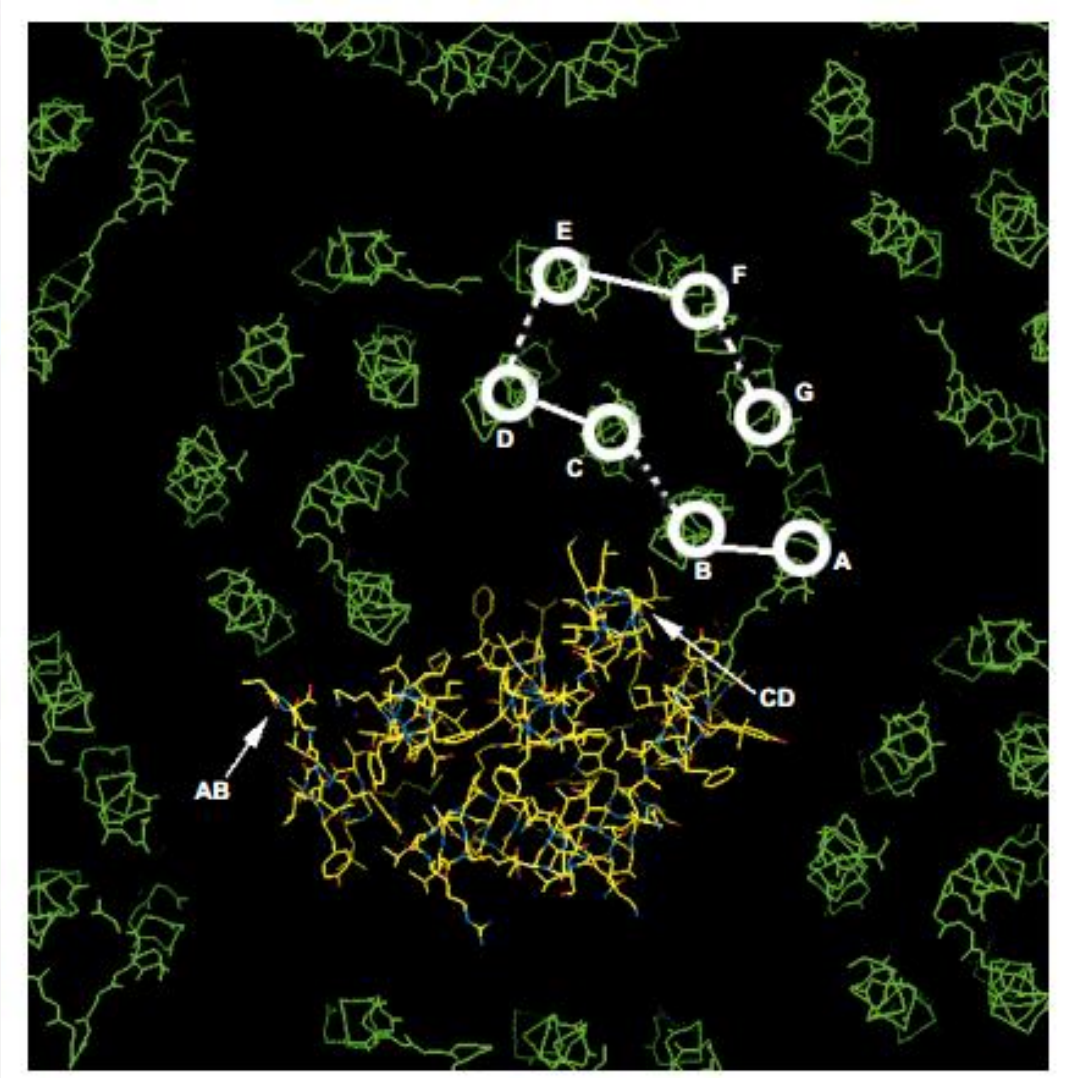
SCIENCE • VOL. 277 • 12 SEPTEMBER 1997 • www.sciencemag.org

Lipidic cubic phase with monoolien, water and membrane protein enables stacked crystal formation

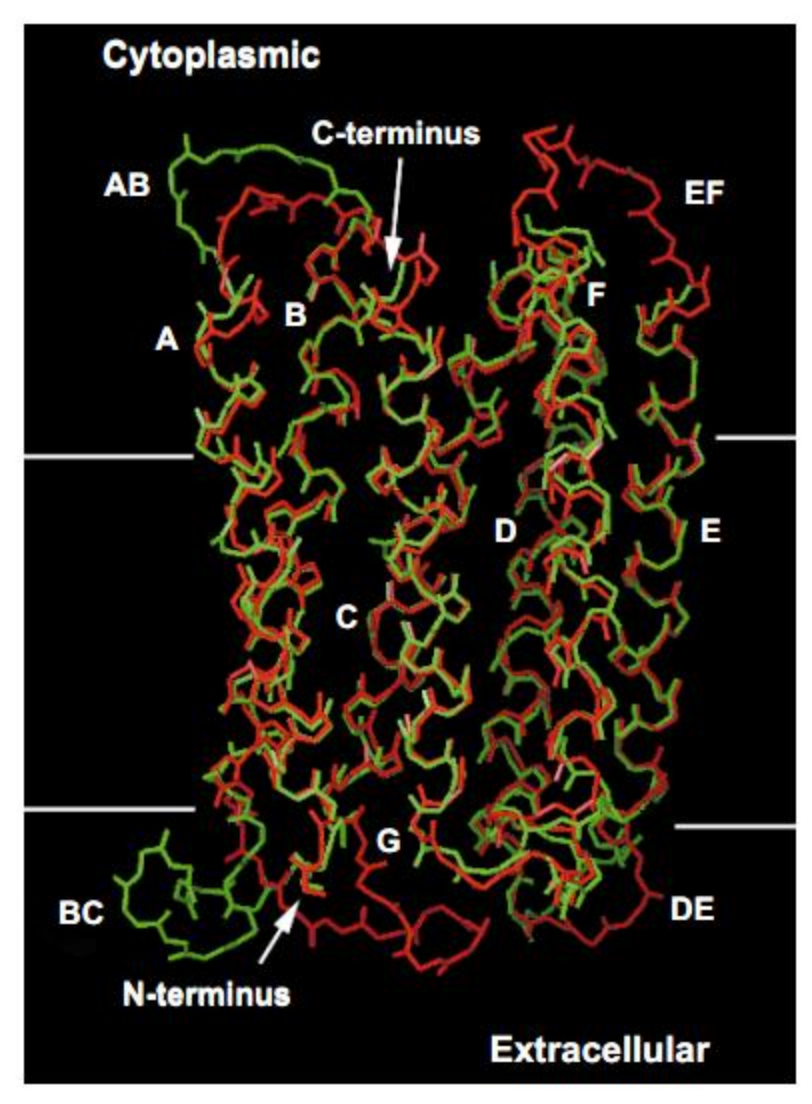




The model along the c-axis showed trimer lattices



Refined model from electron beam analysis overlaid with current x-ray structure

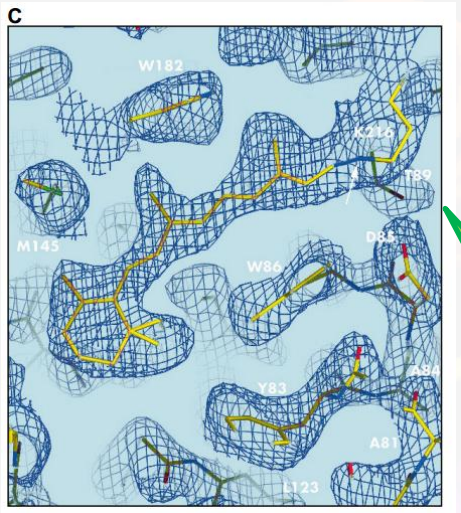


Red: EM-Structure
Green: Current Structure

Given the differences, should we trust this analysis? Does it meet our criteria?

Resolution (Å)	10 to 2.4
$R_{sym}(I)$* (%)	10
Completeness (%)	91
Multiplicity	2
Refinement (5 to 2.5 Å)	
No. of unique reflections ($F > 3\sigma$)	5893
R_{free}† (%)	32.7
R factor‡ (%)	22.1
Non-hydrogen atoms	1761
Protein	1597
Cofactor	20
Water§	144
rms deviation on bond length (Å)	0.016
rms deviation on bond angles (°)	2.29
Average *B* factors (Å^2)	
Protein	54.1
Cofactor	54.6
Water§	57.0

Refined by molecular replacement



Chain continuous
Sequence fits Density

On average “poorly defined”

Questions to consider

- Review: How would the sampling of the diffraction pattern change if the bR unit cell spacing was increased?
- What is the difference between the AB loop and the BC loop indicate about bR? Anything significant beyond the temperature factor?
- Does this model seem reasonable? What factors draw your attention as questionable?

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JMB

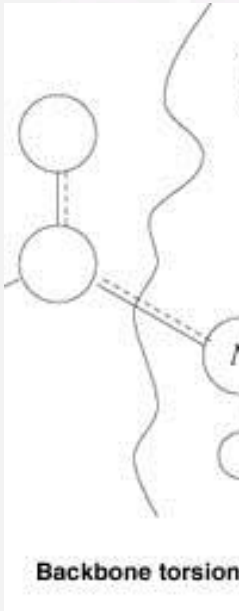
Electron-crystallographic Refinement of the Structure of Bacteriorhodopsin

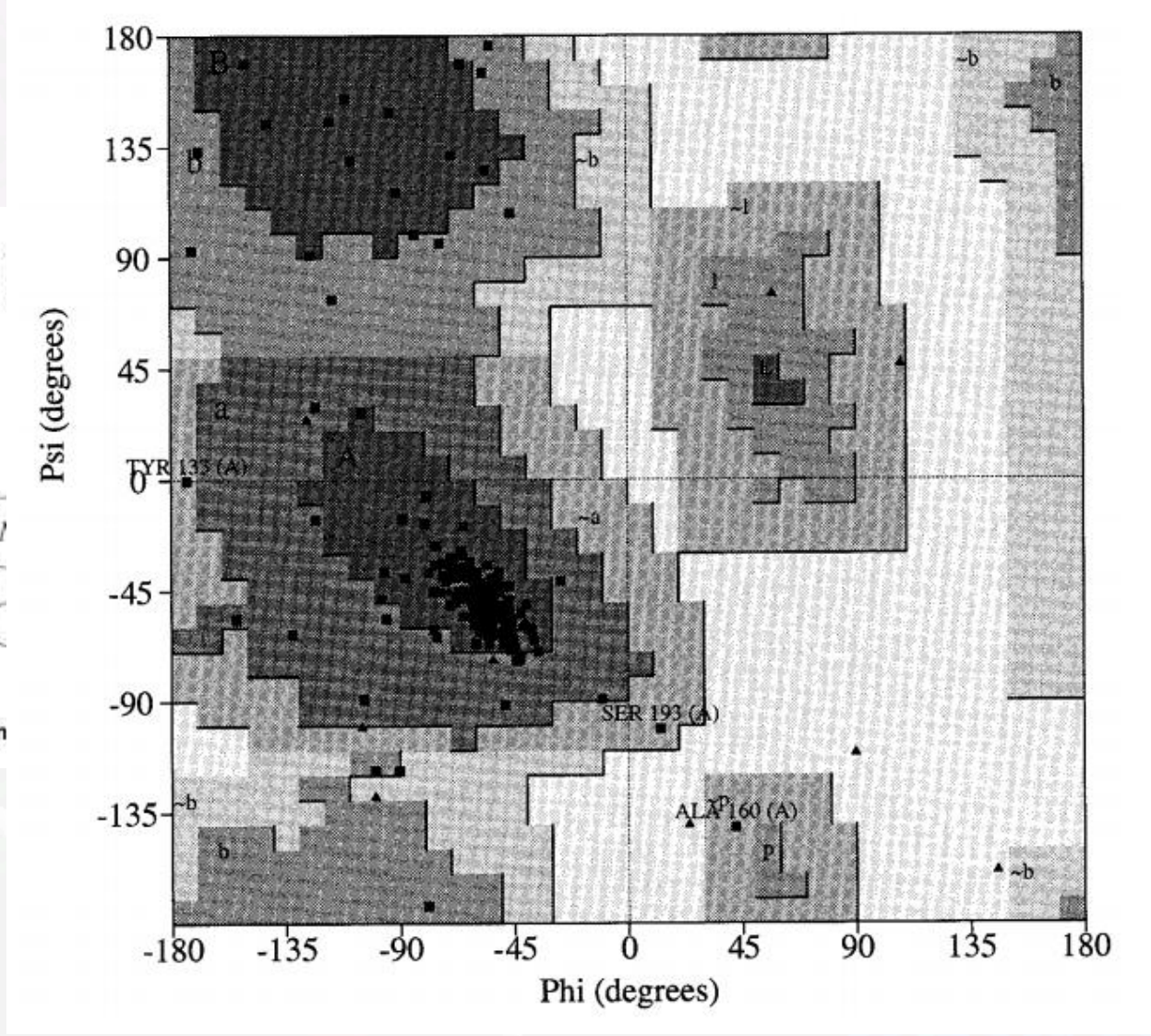
N. Grigorieff[1], T. A. Ceska[1], K. H. Downing[2], J. M. Baldwin[1] and R. Henderson[1]

A useful discussion of unique features of e-beam refinement

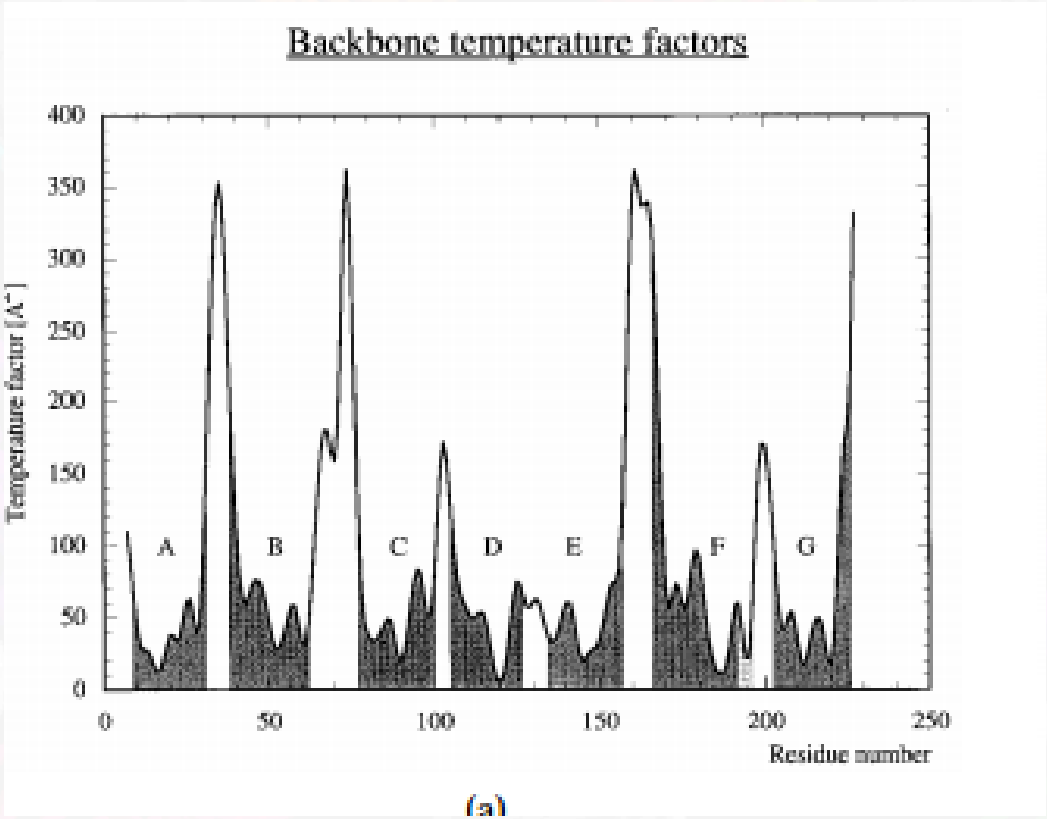
- High Quantum efficiency scattering
- Higher elastic/inelastic scattering ratio
- Potential for multiple scattering
- The presence of diffuse scattering backgrounds

Discussion of a Ramachandran plot for service in refinement





The temperature factor as a function of amino acid sequence



Questions to consider

- How does the R_free factor improve objectivity in modeling?
- What difficulties might need to be addressed in NMR corroborating structures?
- Is bR well enough characterized that it can be used as a standard system of study to assess the accuracy of future techniques?

Thanks for your time

- I hope you felt this discussion was useful. If you have suggestions for improving for Thursday, I would be happy to do my best to implement them. Thanks.
  - Matt