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 2.5 Å
  • 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
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 structure?
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

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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?

- 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?
Electron-crystallographic Refinement of the Structure of Bacteriorhodopsin

N. Grigorieff¹, T. A. Ceska¹, K. H. Downing², J. M. Baldwin¹ and R. Henderson¹
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