Bacteriorhodopsin

Imaging and force spectroscopy
with FlexAFM and C3000

Bacteriorhodopsin (BR)

- BR is a protein from halobacteria, belonging to the family of Archaea (this is not a bacterium). It acts as proton pump that captures light energy and transfers this to move a proton across the membrane. The thus created proton (or pH) gradient is subsequently used to generate ATP, the chemical energy source of many protein processes inside a cell.

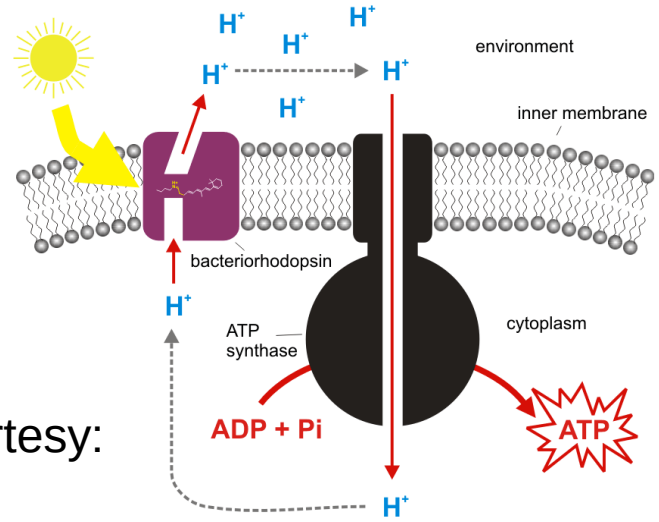
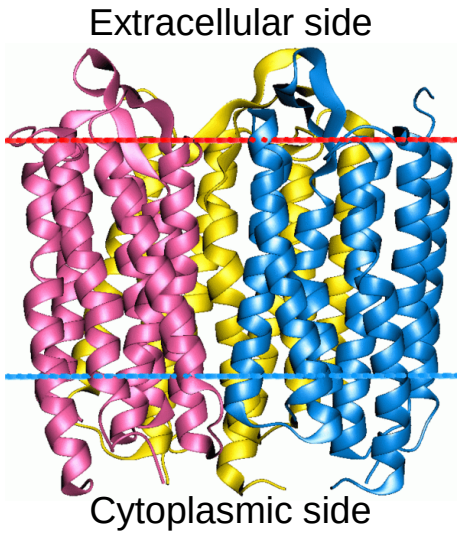


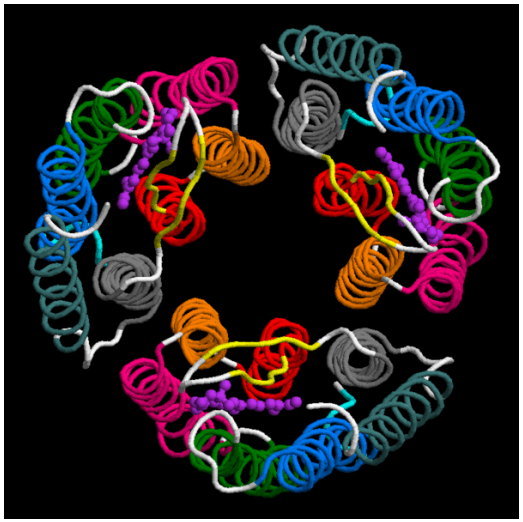
Image courtesy: wikipedia

Great Salt Lake, Utah

Bacteriorhodopsin (BR)

- BR is present at such high density in native membranes that it organizes in a crystalline manner
- The molecules are organized as trimers
- Both sides can be imaged by AFM





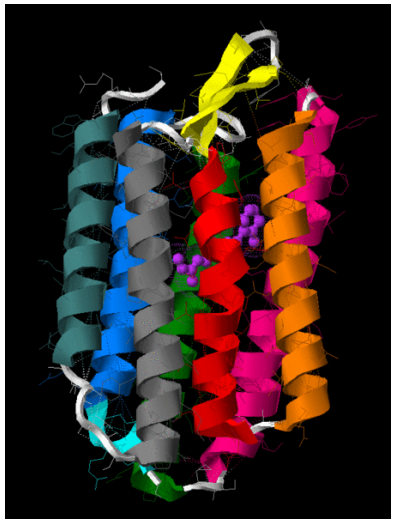
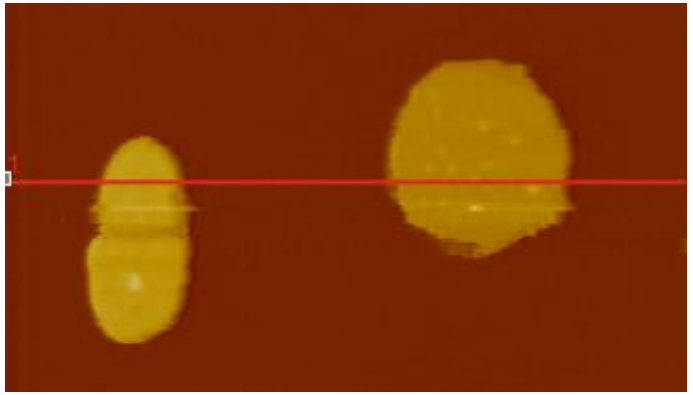
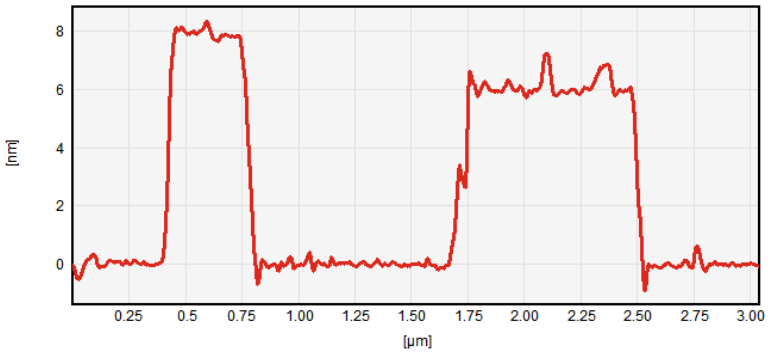


Image courtesy: wikipedia

Imaging Bacteriorhodopsin with FlexAFM

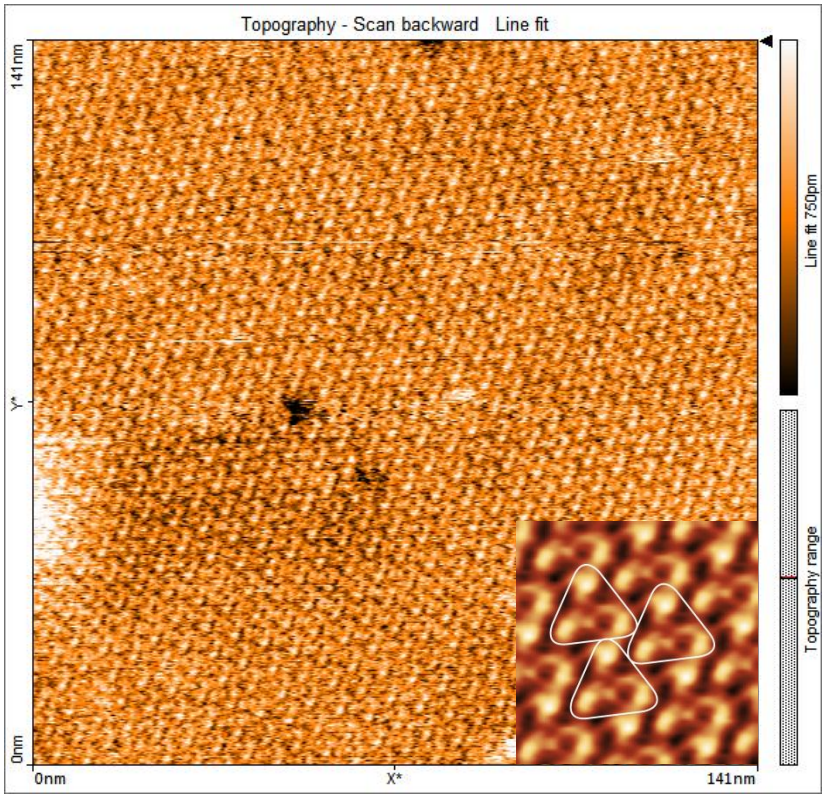
- Bacteriorhodopsin is measured in buffer solution with a FlexAFM and C3000 controller
- Two membrane patches containing bacteriorhodopsin
- Left patch faces extracellular side up
- Right patch faces cytoplasmic side up
- Height difference is attributed to unequal electrostatic interactions between tip and surface
- Data processing: Nanosurf Analysis software





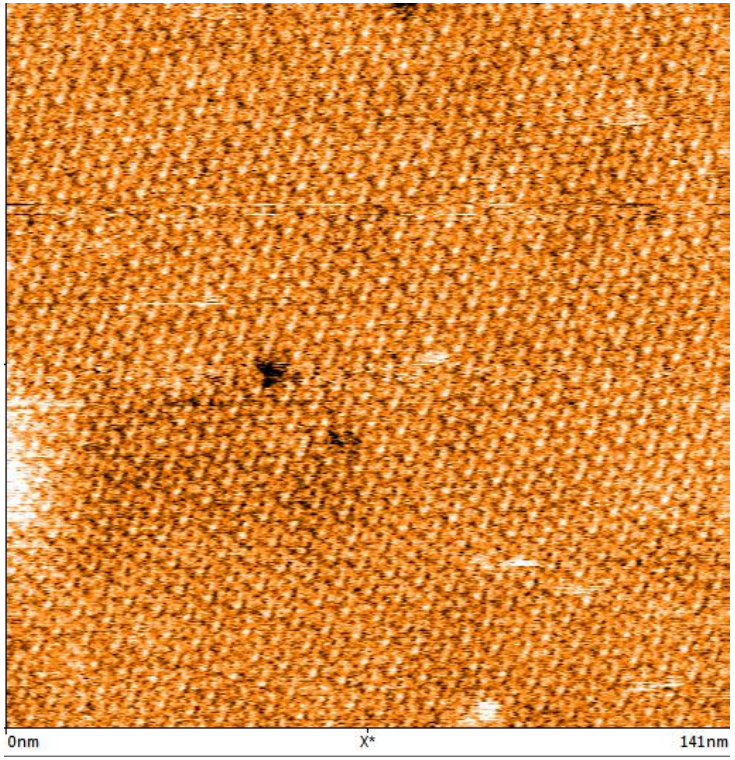
High resolution imaging of the cytoplasmic side of bacteriorhodopsin

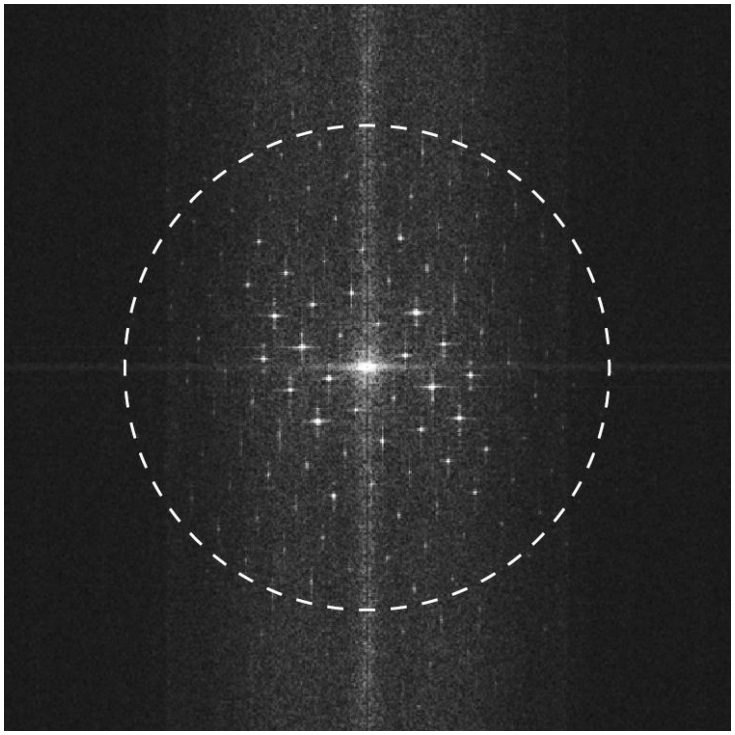
- Image are unfiltered data with linear background correction
- Inset shows correlation average with 3 trimers highlighted by rounded triangles
- Image was measured in static mode
  - Buffer: 150mM NaCl, 25mM $MgCl_2$ & 50mM Tris pH 7.6
  - FlexAFM V3 with 10µm scanner range
  - C3000 controller
  - Cantilever: 0.1N/m
    - Uniqprobe, qp-CONT, Nanosensors
- Calculation cross correlation:
  - IPLT software (free ware)



Estimation of resolution

- For 2-dimensional crystals, the resolution of the recorded data can be estimated from the 2D power spectrum. In the image below, diffraction spots go well beyond 1 nm lateral resolution (circle).

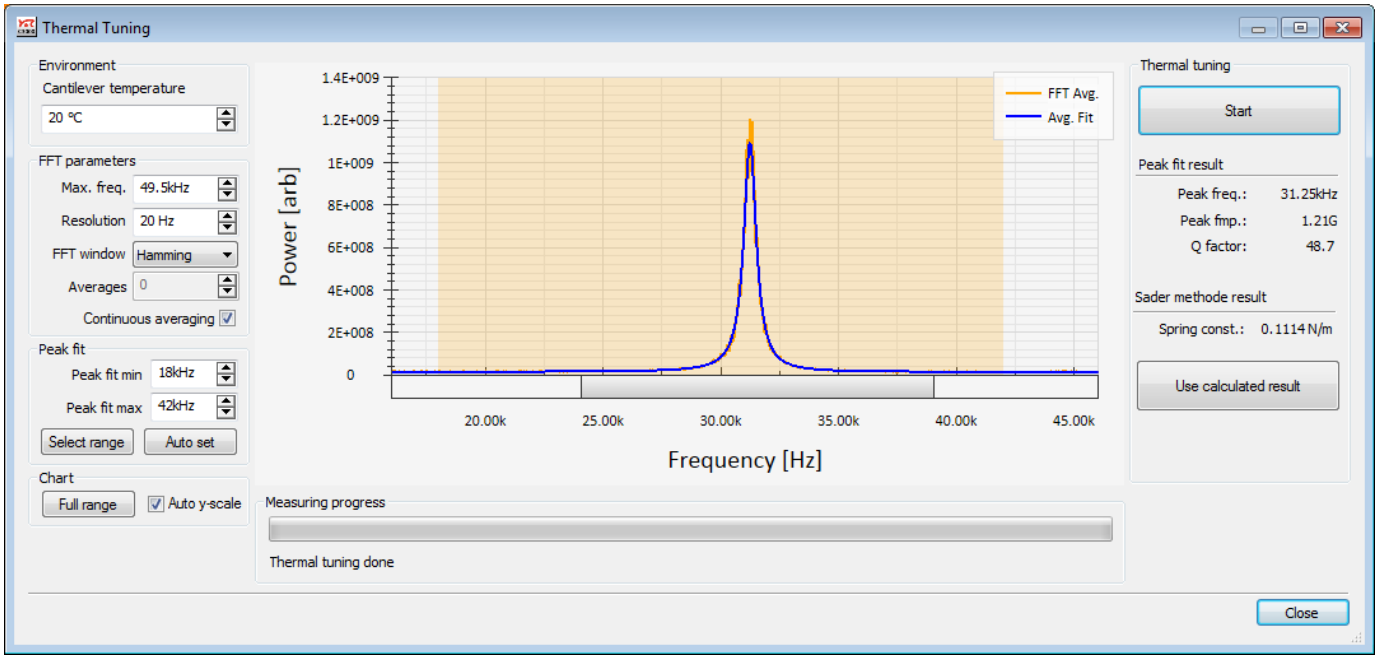




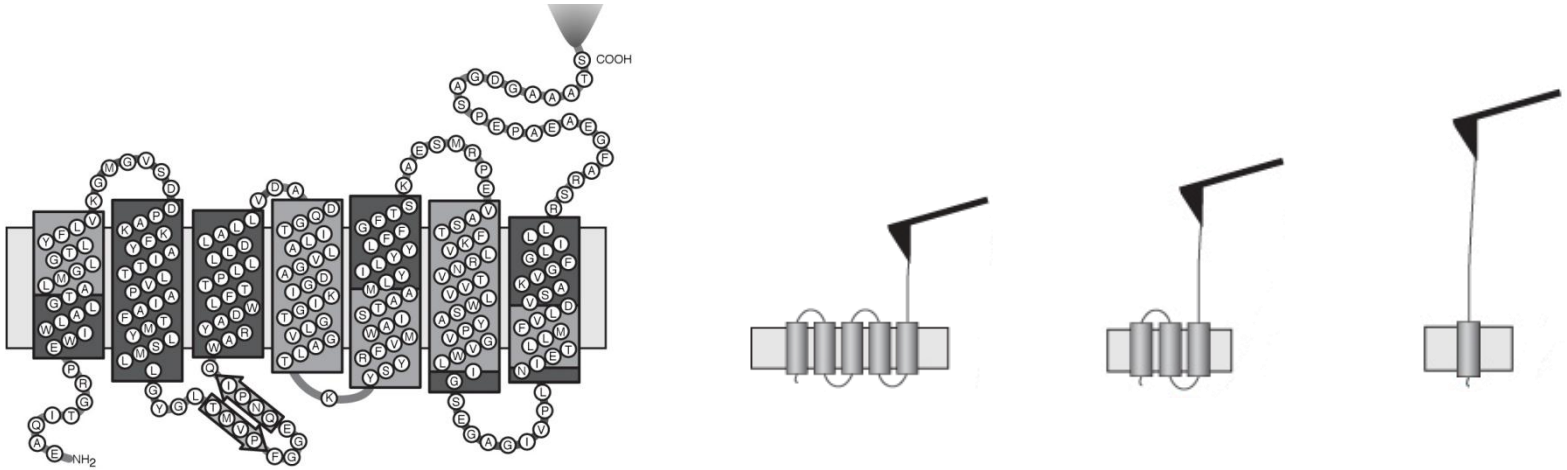
Recorded in buffer with: FlexAFM V3 with 10µm scanner range & C3000 controller

Single molecule force spectroscopy

- Done with soft cantilevers ≤ 0.2N/m
  - E.g. qp-CONT, Nanosensors
- Spring constant must be determined
  - E.g. from thermal noise spectrum using method by John Sader
  - Shown: Thermal noise analysis in C3000 controller



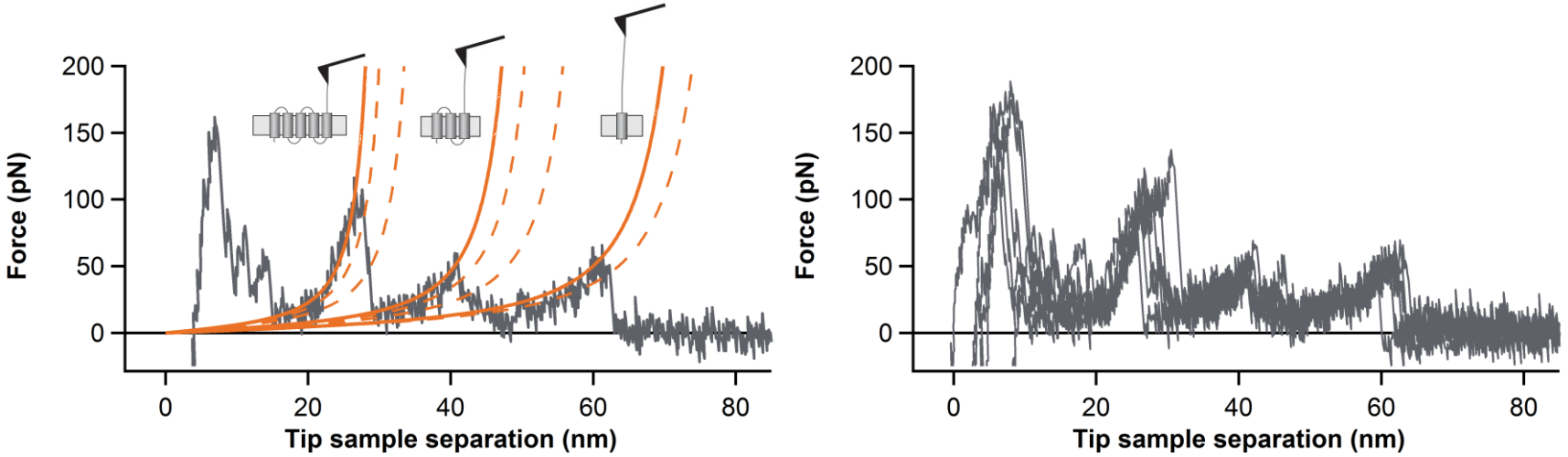
Unfolding bacteriorhodopsin using single molecule force spectroscopy



- Protein can be unfolded step-by-step when pulling from a terminus
- Binding is generally unspecific between tip and protein
- Exact unfolding pathway varies from molecule to molecule, but main barriers exist, e.g. where structure enters the membrane
- Length of the unfolded part can be calculated from worm-like chain (WLC) fits. If a fitted contour length equals 88 amino acids, it means that extra force is needed to pull out the next part starting at aa 89 from the pulling side.

Bippes & Müller 2011, Phys.Rev.Letts

Unfolding bacteriorhodopsin by single molecule force spectroscopy



- Single curve (left) and overlay of multiple curves (right)
- Unfolding pathways vary from molecule to molecule
- Noise level: F_{RMS}=7.98 pN
- WLC contour lengths: 88 amino acids (aa), 148aa, 219aa
- Recorded in buffer solution with:
  - FlexAFM V3 with 10µm scanner range & C3000 controller
  - uniqprobe, qp-CONT, Nanosensors, *k*=0.1N/m