

Abstract

A retinal protein complex known as bacteriorhodopsin was investigated by atomic force microscopy to determine whether it possessed qualities that make it favorable for use as a nanoscale actuator or sensor. Bacteriorhodopsin was tested in different forms (layered 2D crystals and disordered clusters), in three different environments (dry air, humidified air, and water), and on a number of surfaces (glass, charged glass, PDAC coated glass, and Gelpak™ adhesive surfaces). Bacteriorhodopsin was not observed to undergo any molecular motion.

Can Molecules Be Used as Machines?

Nature has already solved many technological puzzles. Various organs and macromolecules have developed to convert energy, recognize chemicals, and sense different elements of the environment. For those who wish to solve the same sort of puzzles at the nanoscale level, these aspects of nature may inspire work in specific directions, or they may be altered to apply to the task directly.

We examined whether a specific molecule, bacteriorhodopsin, could be used as a nanoscale actuator or light sensor.

Bacteriorhodopsin

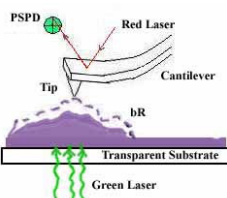
Bacteriorhodopsin (bR) is a protein-retinal complex found in the cell membrane of the archaebacteria *Halobacterium salinarum*. In nature bR operates to provide energy for its organism by creating an ion gradient across the cell membrane. This electric potential is then used to reconstitute ATP from ADP [1].

Bacteriorhodopsin Properties

- Very small structure – $5 \times 5 \times 5$ nanometers
- Responds to light with a change in physical parameters, such as refractive index and shape.
- This photocycle is reversible – at the end of the cycle, the bR is in the same state in which it began.

How to Measure Molecular Motion

An atomic force microscope (AFM) was used to measure the surface of bR as green laser light illuminated the protein in short pulses.

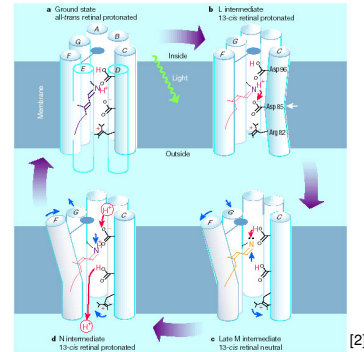


- The cantilever ($\sim 100 \mu\text{m} \times 10 \mu\text{m} \times 2 \mu\text{m}$) hangs over the bR sample, with the tip in contact with its surface.
- The red laser and the position sensitive photo-detector (PSPD) measure the position of the cantilever and, therefore, the position of the surface.
- Short pulses of green laser light provided by the Pulsed Light Delivery System [3] excite the bR photocycle, which should cause conformational changes in the protein.
- Any change in the height of the surface is measured by the AFM.
- An oscilloscope captures the AFM signal and the signal that operates the laser pulse so that surface changes can be compared to the onset of the laser pulse.

The Bacteriorhodopsin Photocycle

Photocycle Properties

- The cycle consists of several intermediate states, each of which is characterized by a label, an absorption maximum, and the position of the hydrogen ion that it is pumping across the membrane.
- The cycle is sensitive to the environment, including the humidity and pH of its surroundings.
- The cycle is initiated by a single photon of green light absorbed by the retinal.
- The cycle takes place over the course of 10 milliseconds (in standard conditions).
- Of principal interest to this investigation is that the protein undergoes conformational changes during the cycle. The largest occurs during the M state (see bottom right picture in the figure below); this conformation is 3.5 \AA in magnitude. It is this conformation that we hoped to measure.

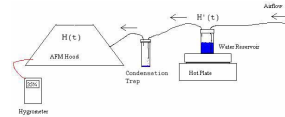


The bacteriorhodopsin photocycle (abbreviated). "Inside" and "outside" refer to the orientation of the bR in the cell membrane.

Humidity

Bacteriorhodopsin requires a humid environment in which to operate. Therefore, a system was developed to humidify the inside of the AFM hood. This system allows us to reach 95% relative humidity.

- Air is bubbled through heated water.
- The humidified air passes through a condensation trap into the AFM hood.
- The sensor of a modified hygrometer is placed inside the hood.
- A model of the humidity as a function of time was developed, assuming that $H(t)$, the humidity of the water reservoir, quickly reaches a humidity of nearly 100%.
- The final humidity expression is given by

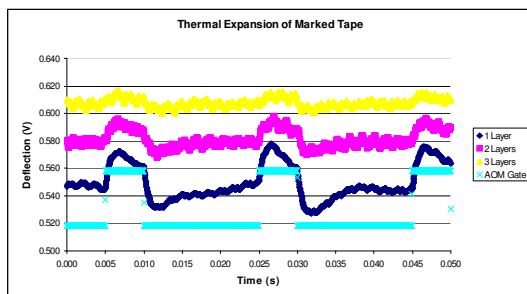


$$H(t) = (H_0 - 1.000) e^{-\frac{\alpha}{125L}t} + 1.000$$

H_0 is the initial humidity inside the hood, α is the rate of airflow, and the volume of the AFM hood is 125L.

Thermal Expansion

This experiment noted no bR motion at any scale in any environment used. Can the AFM detect motions this small? To answer the question, clear Scotch tape was marked with indelible blue ink and placed in the experimental setup. When the laser was pulsed, we could measure the expansion as the laser heated the ink (and probably the tape), which then expanded between 2 \AA and 5 \AA . The bR deformation is 3.5 \AA in the direction of greatest displacement, a value which is clearly within the range measured here.



The AOM Gate voltage simply describes when the laser is on (high voltage) or off (low).

Previous Results

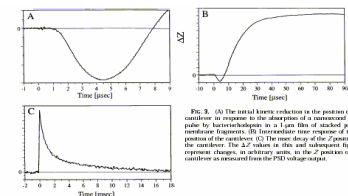


FIG. 3. (A) The initial kinetic reduction in the position of the cantilever in response to the absorption of a resonant laser pulse by bacteriorhodopsin as a 4 μm thin of stacked purple membrane fragments. (B) Intermediate time response of the Z position of the cantilever. (C) The time decay of the Z position of the cantilever. The Z values in this and subsequent figures represent changes in arbitrary units, as the Z position of the cantilever is measured from the PSD voltage output.

Reference [4] used a very similar experimental setup to show bR undergoing motion in highly humidified air ($\sim 85\%$ relative humidity). Note that after an initial compression, bR expanded, then decayed over a time scale of 10ms. These results lack an absolute scale of expansion.

Conclusions

- No bR motion was noted at any scale in dry air, humidified air, or in water. We were unable to reproduce the results of Rouso et al.
- A variety of bR substrates were tested as well: glass, charged glass, polydimethylallylammonium chloride (PDAC), and Gelpak™.
- Another test measured the thermal expansion of marked Scotch tape under illumination by the same laser used on the bR. Expansions of 2 to 5 \AA were measured in this way. These are the same order of magnitude as a single bR conformation change in the direction of its motion.

References

- [1] Vsevolodov, *Biomolecular Electronics: An Introduction Via Photosensitive Proteins* (Birkhäuser, Boston, 1998).
- [2] Kühlbrandt, *Nature* **406**, 569 – 570 (2000).
- [3] Thoreson, Apparatus to Deliver Light to the Tip-Sample Interface of an Atomic Force Microscope. M.S. Thesis at Worcester Polytechnic Institute, Worcester, MA, USA (2002).
- [4] Rouso, Khachatryan, Brodsky, Nachusti, Ottolenghi, Sheves, and Lewis, *Journal of Structural Biology*. **119**, 158 – 164 (1997).
- [5] We are grateful to Professor Robert Birge and Jason Hillebrecht of the University of Connecticut for the bR samples and to Sigma Xi for support.