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Probing the Properties of Cells and Cell Surfaces with the Atomic Force Microscope

M.W. McElfresh, R.E. Rudd, R. Balhorn, J. Belak

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INTRODUCTION

We have developed new techniques based on atomic force microscopy (AFM) to image, and to quantify the strength of, specific receptor sites on the membrane of a living cell. AFM has developed rapidly during the past decade, providing nanometer scale resolution in the imaging of biological materials ranging in size from single molecules to intact cells. By monitoring the cantilever deflection during approach-retraction cycles (i.e. force-volume curves), the unbinding forces have been determined for various ligand-receptor pairs. It is now possible to use a single receptor molecule bound to the tip of an AFM cantilever to map the locations of ligands bound on solid surfaces, opening the door for new "recognition mapping" methods. The goal of our project was to develop recognition mapping for living cells and cell membranes, a major step forward.

This project contributes to LLNL's national security mission by creating a method to measure the real time response of individual living cells to a range of perturbations. The properties of living cells change in response to wide variety of external stimuli ranging from chemical and thermal changes in their environment to their response to infection or poisoning by bacteria and toxins, like those manifested by bio- and chemical warfare agents. The technique we are developing provides a new, non-destructive means to detect resulting changes in the properties of the cell membrane at the nanoscale. These methods provide a means for evaluating molecules that could be used as the targeting molecules in sensor systems.

The scope of the project was the development of the experimental technique to make recognition measurements on living cells, the development of an associated computational model, and a proof of principle application to a well-known cell system. The computational model of the overall mechanical response of the cell was developed in order to deconvolute the mechanical response of the cell from the local surface interactions of interest. These local interactions include recognition (or binding) events between molecules bound to an AFM tip (e.g. an antibody) and molecules, or receptors on the cell surface (e.g. the respective antigen).

RESULTS

In FY01, the project has produced significant results in three areas: AFM measurement of cellular mechanical properties, analytic model building to describe large deformations of the membrane, and implementation of numerical models of indentation. Stunning topographic measurements of the shape of the revitalized bull sperm cell, including

hitherto unobtainable detail of the equatorial segment, and force-displacement measurements were made for the three cell segments. The latest characterization techniques were used to measure the shape of the AFM tip in order to parameterize the model. A new analytic model for large deformations of cell membranes was developed. This is a major step forward in the modeling of cell deformations, replacing the Hertz model and linear elastic models. The model has been coded in C^1 finite elements and used thus far to study the deformation in high symmetry. We have also developed high resolution 3D finite element models of the cell that will be used to simulate more generic deformations.

In FY01 the team included Professor Eveline Baesu of the Mechanical Engineering Department of the University of Nebraska, Lincoln. Professor Baesu is an expert in the theoretical modeling of membranes and worked closely with R. Rudd of our team to develop a new theoretical modeling strategy applicable to cell membranes. We also collaborated with Dr. Mike Allen of Biometrology. Dr. Allen is an expert in the use of the AFM on biological systems and worked on the AFM of sperm cells during his thesis work with R. Balhorn. Several papers resulted form this work including one in the Proceedings of the National Academy of Sciences.

In FY02, the project produced significant results in several areas. Nanostamped patterns of specific self-assembled-monolayers (SAMs) were fabricated and AFM tips derivatized with Concanavalin-A (Con-A) were used to test the binding of the target molecules of interest between an AFM tip and rigid surface bound molecules. The Con-A derivatized tips were then used to study living cells prior to, and after, the acrosomal reaction. Recognition events were observed for cells for which the acrosomal reaction had been induced. In addition, unprecedented topographic images of the post acrosomal cell showing the dramatic change in shape of the cell were obtained. In FY02, the theoretical modeling effort made significant progress by incorporating the constitutive properties of the membrane into a more realistic model of the cell. The most recent model now makes it possible to move beyond axial symmetry and on to realistic cell shapes.

In addition, a surprising experimental result was observed. Sperm cells were found to stick to the AFM cantilever for periods of time during which it was possible to monitor the force produced by the sperm flagella. Several papers were submitted in FY02 and several invited talks were presented.

In FY03, Dr. Tim Ratto joined the project as a postdoc. Dr. Ratto introduced the group to recent developments in analysis of recognition microscopy data (i.e. force spectral analysis). The interactions between Con-A tethered on the AFM tip and mannose residues tethered to the surface were studied and analyzed in detail. It was shown that by using 20 nm tethers to attach each molecules to their respective surfaces, it was possible to distinguish non-specific binding events from the specific binding events associated with the Con-A to mannose bonding. This allowed resolving the bond energy with much greater precision than in any previous molecular bonding studies. In addition, bonding events requiring double the force to break the bonds were observed. This appears to be

the first clear example of multivalent binding in a biological system studied by recognition microscopy.

PRESENTATIONS AND PUBLICATIONS

Presentations:

Invited Presentations:

R. E. Rudd, M. McElfresh, et al., "Modeling of the Mechanical Deformation of Living Cells in AFM, Chemical Engineering Seminar," UC-Davis, Davis, CA, November 4, 2002.

M. McElfresh, et al., "Probing the Properties of Cells and Cell Surfaces with the Atomic Force Microscope," presented at:

- University of Nebraska, Physics Colloquium, Lincoln, NE, October 10, 2002.
- SDSU, Physics Colloquium, San Diego, CA, October 23, 2002.
- University of South Carolina, Physics Colloquium, January 21, 2003.
- UC Davis, DAS, Davis Campus, CA, April 4, 2003.
- UC Davis, Physics Seminar, Davis Campus, CA, February, 2003.

R. E. Rudd, M. McElfresh, et al., "Modeling of the Deformation of Living Cells Induced during Indentation by an Atomic Force Microscope," Materials Seminar, Oxford University, June 11, 2002.

E. Baesu, M. McElfresh, et al, "Continuum Modeling of Cell Membranes," 14th National Congress of Theoretical and Applied Mechanics, Blacksburg, VA, June 23, 2002.

R. E. Rudd, M. McElfresh, et al., "Multiscale Modeling of Transition Metal Plasticity and Living Cell Mechanics," Biocomplexity V Conference, Notre Dame, IN, August 15, 2003.

Contributed Presentations:

"Modeling of the Deformation of a Cell Membrane Probed by Atomic Force Microscopy," R. E. Rudd, M. McElfresh, et al., Fall Meeting of the Materials Research Society, Boston, December 3, 2002.

"Modeling AFM Induced Mechanical Deformation of Living Cells," R. E. Rudd, M. McElfresh, et al., Nanotech2003, San Francisco, February 26, 2003.

"Mono and Multivalency in Tethered Protein-Carbohydrate Bonds", T. V. Ratto, K. Langry, R. E. Rudd, R. Balhorn, M. McElfresh, Biophysical Society Meeting, February 14-18, 2004.

"Force Spectroscopy of the Double-Tethered Concanavalin-A Mannose Bond", T. V. Ratto, K. Langry, R. E. Rudd, R. Balhorn, M. J. Allen, M. McElfresh, Nano and Bio-Nanoscience Research Meeting, Lawrence Berkeley National Laboratory, June 11, 2003.

"Force Spectroscopy of the Double-Tethered ConcanavalinA Mannose Bond", T. V. Ratto, K. Langry, R. E. Rudd, R. Balhorn, M. J. Allen, M. McElfresh, Seeing at the Nanoscale Conference, University of California, Santa Barbara, August 24-27, 2003

Publications:

Conference Proceedings:

R. E. Rudd, M. McElfresh, R. Balhorn, M. J. Allen, and J. Belak, "Modeling AFM Induced Mechanical Deformation of Living Cells," in Proc. Intl. Conf. Comput. Nanoscience (Nanotech/ICCN'03), San Francisco, CA, February 2003, M. Laudon and B. Romanowicz, eds. (Computational Pub, Boston, 2003), V.1, pp.138-141.

R. E. Rudd, M. McElfresh, E. Baesu, R. Balhorn, M. J. Allen, and J. Belak, "Modeling of the Deformation of Living Cells Induced by Atomic Force Microscopy," in Proc. Intl. Conf. Comput. Nanoscience (ICCN'02), San Juan, Puerto Rico, April 2002, M. Laudon and B. Romanowicz, eds. (Computational Pub, Boston, 2002), pp. 73-6. UCRL-JC-146706

T. V. Ratto, K. Langry, R. E. Rudd, R. Balhorn, M. McElfresh, "Mono and Multivalency in Tethered Protein-Carbohydrate Bonds", ACS Conference Proceeding (in press), Anaheim, CA, March 28-April 1, 2004

Refereed Publications:

McElfresh, M., E. Baesu, R. Balhorn, J. Belak, M. J. Allen, and R.E. Rudd "Combining constitutive materials modeling with atomic force microscopy to understand the mechanical properties of living cells," *Proceedings of the National Academy of Sciences of the United States of America* Vol 99, Pages 6493-6497, 2002. UCRL-JC-145763

D. Steigmann, J. Belak, M. McElfresh and R.E. Rudd, "On the variational theory of cell-membrane equilibria," *Interfaces and Free boundaries, Modeling, Analysis, and Computation*; in press (2002).

E. Baesu, R. E. Rudd, J. Belak and M. McElfresh, "Continuum Modeling of Cell Membranes," Intl. J. Non-linear Mech. 38, 1473-1479 (2003). UCRL-JC-150482

Ratto, T.V., Langry K., Rudd, R.E., Balhorn, R, Allen, M.J., McElfresh, M., "Force Spectroscopy of the Double-Tethered Concanavalin-A Mannose Bond"**,** *Biophysical Journal*, 2004 86 (4) (in press)

M. J. Allen, J. Silveira, R. E. Rudd, R. Balhorn and M. McElfresh, "Direct Measurement of a Discrete Force-Pulse Driven by Axonemal Dynein Motors in Live Sperm," submitted to Proc. Natl. Acad. Sci. (2003).

Ratto, T.V., Langry K., Rudd, R.E., Balhorn, R., McElfresh, M. “Mono and Multivalency in Tethered Protein-Carbohydrate Bonds”, Manuscript in preparation.