Elucidating Structure and Function of Proteins

Gerald Kada, Ph.D.
Agilent Technologies
(Nano Measurements Division)
Austria / USA
The main Species in Biology

Others:
- Lipids
- Carbohydrates
- Small molecules (ions etc.)

From DNA to Proteins

DNA (mostly double stranded),
RNA (mostly single stranded; viral, siRNA, mRNA, tRNA etc)
Structure Analysis: Live Human Rhinovirus protein shell

Watching RNA release from virus by decreasing pH

Structure Analysis: Antibody binding to Protein crystal (Purple membrane) in buffer solution

Kienberger et al, EMBO Reports (2004)
Ultimate Resolution: Higher Harmonics imaging
Bacterial S-Layer protein crystal

- <1 nm resolution on protein crystal in liquid (MAC mode)
- 3 pm amplitude sensitivity in 2nd harm.
- Quantifiable 2nd harmonics amplitude => stiffness data, Young’s modulus map

Preiner et al, PRL 2007
Sleytr et al, PSS 2001

Freeze-etching preparations

Dr. Gerald Kada
NanoMeasurements Div
Agilent Austria
Structure of fragile proteins: Amyloid fibril formation (Alzheimer, Parkinson)

A

Protein

Seed fibril

PEG fibril

Au

B

Elongated fibril

Knowles et al, Science/PNAS 2007

Knowles et al, Science/PNAS 2007
Using Force Spectroscopy to understand Antibody–Antigen Interaction Strength (Function) vs. Intra-molecular Antigen Stability (Structure)

Anti-Sendai Antibody on the Tip, Bacteriorhodopsin on the Surface ("Purple Membrane")

Unravelling single Bacteriorhodopsin

Sequence-dependent pulling pattern – with and without Sendai-loop

Kienberger, Kada et al, JMB 2005
Preiner et al, BJ 2007
Varying the loading rate for $x_\beta$ (binding pocket size), $k_{\text{off}}$ (kinetic off-rate) and $\Delta G$ (Energy)

Kinetics $\Leftrightarrow$ Thermodynamics/affinity

$\Delta G = -RT\ln K_D$

$F^*(r) = \frac{k_B T}{x_\beta} \cdot \ln(r \cdot x_\beta / k_{\text{off}} \cdot k_B T)$

Bell, Evans, Jarzynski, Hummer ‘78,’93,’06

Dr. Gerald Kada
NanoMeasurements Div
Agilent Austria
Close look on modified AFM tip

Fully covered AFM tip

Single Molecule tethering to AFM tip

Topography and Recognition Imaging (TREC)

DNA-protein complexes as stored in the chromosomes (“Chromatin”)

Chromatin plus enzymes

Wang, Lindsay, ASU
Recognition imaging: Identifying protein tags

Protein crystal (S-Layer w *Strep*-tag)

Seeing the location of tagged proteins in a protein crystal using a sensor on the tip

Tang et al, Nano Lett 2008
Atomic force microscopy in bionanotechnology

Atomic force microscopy (AFM) is extensively used for imaging surfaces ranging from micro- to nanometer scales, with the objective of visualizing and characterizing surface textures and shapes. It is the only technique that provides subnanometer resolution under physiological conditions, needed for imaging biological species like proteins and living cells. Measurements of molecular recognition forces provide insights into the function and structure of biomolecular assemblies. Furthermore, in combination with fluorescence microscopy, AFM can identify biomolecules based on fluorescence labeling and high-resolution topography imaging. This review summarizes recently developed techniques for advanced topographical imaging and sensitive force measurements.

Within the field of scanning probe microscopy, atomic force microscopy (AFM) is extensively used in a wide range of disciplines such as life science, solid-state physics and materials science. The AFM has evolved into an imaging method that yields structural details of biological samples such as proteins, nucleic acids, membranes, and cells in their native environment. AFM is a unique technique for providing sub-nanometer resolution at a reasonable signal-to-noise ratio under physiological conditions. As a result of continuous developments in sample preparation, imaging techniques, and manipulation, AFM is now a companion technique of X-ray crystallography and electron microscopy (EM) for the determination of protein structure. For example, it complements EM by allowing visualization of biological samples in buffer that preserve their native structure over extended time periods. AFM does not rely on symmetry-averaging and crystallization, therefore revealing defects and structural anomalies not observable in classical ensemble measurements. Unlike EM, AFM yields three-dimensional maps with an exceptionally good vertical resolution (less than a nanometer).

In addition to high-resolution imaging of proteins, nucleic acids, membranes, and living cells, the measurement of mechanical forces at the molecular level provides detailed insights into the function and structure of biomolecular systems. Inter- and intramolecular interactions can be studied directly at the molecular level, as...
Finally…

Thanks for your attention!

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Contact:
Gerald_Kada@Agilent.com
www.agilent.com/find/nano