

2nd Annual National Academies Keck *Futures Initiative* Conference

Designing Nanostructures at the Interface between Biomedical and Physical Systems

Arnold & Mabel Beckman Center, Irvine, California

November 18-21, 2004

Sequence a Single Molecule of Protein

Focus Group Description

Background

The study of protein structure and function is central to understanding living systems. However, the diversity and complexity of proteins render even the simplest characterizations challenging. The most basic level, determining the primary structure, involves sequencing the polypeptide chain. Even state-of-the-art commercial sequencing techniques require picomolar samples, equivalent to micrograms of protein or $\sim 10^{13}$ molecules. In contrast to this scale, laboratory experiments at the forefront of the field can access and manipulate single proteins with various physical techniques. These experiments have already shed light on structure and dynamics. Beyond simple sequencing, the higher-order structure of proteins—linked to understanding the folding process—remains elusive in the general case.

The Problem

As typical methods for determining sequence and structure of proteins require large quantities of the molecule, these studies are often delayed until the requisite quantities are synthesized or purified. In the case of high-resolution crystallography, additional effort is required to crystallize sufficient quantities of the protein. Given the appearance of groundbreaking single-protein studies with new tools, will it soon be possible to sequence a single molecule of protein? Consider a combination of existing techniques or newer techniques which need to be developed; for example:

- Modifications of common amino acid sequencing techniques (filtration, cleavage, etc.)
- Mass spectrometry
- Optical tweezers
- Cantilever-based force measurements
- Nanopores / microfluidics
- Scanning probe methods
- Crystallography
- Electron holography

Initial References

- Ezzell, Carol, Proteins Rule. *Scientific American*, April 2002. p. 42-47.
- Bustamante, Carlos; Macosko, Jed C.; Wuite, Gijs J. L., Grabbing the Cat by the Tail: Manipulating Molecules One by One. *Nature Reviews Molecular Cell Biology*, 2000. 1, p. 130-136.
- Engel, Andreas; Müller, Daniel J., Observing Single Biomolecules at Work with the Atomic Force Microscope. *Nature Structural Biology*, 2000. 7, p 715-718.