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Introduction

The Nobel Workshop 'Fundamentals of Biomolecular Function: Nucleic Acids, Proteins, and Membranes', was hosted by the city of Coimbra, Portugal, and the University of Coimbra in May 2005. The workshop focused on three subjects: (i) the functions of biological macromolecules, (ii) the interactions underlying these functions, and (iii) new techniques for studying these interactions. A brief summary of the discussions follows.

Jacob Israelachvili discussed the complexity of molecular interactions among biological macromolecules. Molecules in biological systems often interact far from equilibrium, and the interactions involved are surprisingly poorly understood at the fundamental level. Studies using techniques that Israelachvili has developed for measuring surface forces have revealed unexpected complexities. His studies of near-surface solvent, for example, suggest remarkable differences between short- and long-range interactions. In many biological systems, the concentrations of critical reactants, such as ligands and receptors, are so low that kinetics rather than thermodynamics control interactions.

Perhaps with a hint of irony, Peter Wolynes described protein folding as one of the best understood processes in chemistry. Folding proteins, according to theoretical models, trace pathways across complex potential surfaces with many local minima. Understanding how to structure such surfaces – how to estimate enthalpic and entropic contributions to free energies, how to include solvent, and how to consider multiple processes proceeding simultaneously – remains a central problem in biophysical theory.

By measuring the minute forces required to unfold single RNA molecules, Carlos Bustamante has characterized the non-equilibrium thermodynamics of these processes. He suggested that the behavior of individual molecules typically deviates from equilibrium, and proposed theoretical approaches to analyzing the unfolding process. Bustamante's experiments raise questions about the applicability and universality of the ergotic hypothesis. His studies also suggest that the tertiary structure of RNA – relying mostly on interactions between rigid secondary structure elements – is brittle and inflexible, whereas its secondary structure, which is held together by interactions between stretches of base pairs, is elastic and resilient. Single-molecule studies of this kind help us understand the connections between single-molecule behavior and the behavior of ensembles of molecules.

John Schellman introduced a novel theoretical approach to describe the thermodynamics of the unfolding of proteins. By focusing on solvent-induced melting, he derived an expression that describes how the free energy of unfolding depends on the concentration of co-solvent, or denaturant. The theoretically predicted fraction of denatured protein compares strikingly well with experimental results for ribonuclease T1 and mutants thereof.

Hermann Gaub described a technical *tour-de-force* using atomic force microscopy (AFM) to measure the forces required for pulling a single amino-acid chain of bacteriorhodopsin out of the purple membrane. This new technique is sensitive enough to identify unfolding barriers with the accuracy of a few amino acids (Kessler & Gaub, 2005, *Structure* **14**, 521–527). AFM-based studies of forces in biology have advanced remarkably and are becoming an integral part of single-molecule studies. This technique promises a new window into the interactions that hold proteins together and that allow them to interact with their environment, a lipid bilayer in Gaub's presentation.

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Non-covalent interactions play key roles among biological molecules. Recognition and affinity relies on what George Whitesides termed 'the best sloppy fit'. The free energy of protein–ligand interactions is a complex mixture of terms arising from interactions between the protein and its ligand, the behavior of solvent, interfacial effects (e.g. the hydrophobic effect), and changes in the conformation of protein and ligand. The estimation of these terms, and especially of their entropies, remains a very difficult problem.

Jamie Williamson gave a guided tour of the folding of the 30S subunit of the bacterial ribosome. He described how this process follows a strategy of alternating RNA folding and protein binding events. The hierarchical nature of the folding – with inner parts of the structure nucleating the association of the outer parts – is astonishingly well orchestrated, and a beautiful example of unexpected complexity in nature.

Wayne Hendrickson summarized crystallographic studies of membrane protein structures. He illustrated different mechanisms that have evolved to transduce signals across biological membranes. All involve considerable conformational changes in the molecules involved. For example, the human follicle-stimulating hormone (FSH) and its cognate receptor interact through a 2600 $Å^2$ surface with a dissociation constant in the order of μM to nM, a reaction that exclusively occurs within the membranes. The relationships between the interactions of the molecules and the constraints imposed by the membranes are poorly understood.

Sequence-specific binding of proteins to DNA involves large surface areas and is thus more complicated than simply reading the nucleotide sequence: solvent, various ligands, and intramolecular DNA interactions participate in the process. Richard Lavery described protein–DNA recognition as a process in which many bits of information dictate the resulting forces, interactions and specificity, adhering to the theme of complexity that permeated the proceedings of this workshop.

Recognition through nucleic acid base-pairing, coupled with the formation of catalysts that build sequence specificity into the catalytic site by incorporation of RNA fragments, provide the mechanistic basis of RNA interference (RNAi). Andrew Fire, one of the pioneers behind the discovery of RNAi, reviewed the remarkable history of the discovery of this area of molecular biology, surveyed the many functions now known to rely on the recognition of RNA by RNA, and outlined the method in which small RNA fragments act catalytically in the cell. His essay describes how RNAi serves as part of the immune system, and suggests that RNAi has evolved to provide mechanisms for balancing the control of evolving pathogens and the stability of complex cellular mechanisms.

MAGDALENA ERIKSSON AND GEORGE WHITESIDES
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