NAI Supported Research, a Case Study: The Origin of Protein Functions

Andrew Pohorille
NASA Ames Research Center, Exobiology Branch, MS 239-4, andrew.pohorille@nasa.gov

Introduction: In the 20 years of its existence, the NAI supported a host of studies that fundamentally advanced the field of astrobiology in terms of both understanding the underlying concepts and developing novel research techniques. In this presentation, I will recount one such case in the field of the origin of life, whereby application of new, powerful experimental techniques combined with state-of-the art computer simulations led to a surprising insight into the origin of protein functions.

In Vitro Protein Evolution: In the NAI supported study, Keefe and Szostak (2001) demonstrated their novel technique of in vitro protein evolution in which a functional protein was selected from trillions of random polypeptide sequences. Subsequent studies revealed that the protein had a structure not found in nature, thus providing direct evidence that not all possible protein folds were explored in biology, a result of considerable significance to biotechnology. The importance of protein evolution studies has been underscored by awarding the 2018 Nobel Prize to researchers who pioneered this approach.

A Puzzle of Primordial Proteins: Almost all modern, water-soluble enzymes form a rigid scaffold containing a well-packed hydrophobic core, which provides structural stability, a largely hydrophilic exterior to maintain water solubility, and a catalytic site. A large fraction of an enzyme is composed of conventional secondary structure elements, α-helices and β-sheets, connected by turns and loops. Based on the universality of these features in modern enzymes, it is usually assumed that their primordial ancestors had the same architecture, but were smaller in size. This assumption, however, creates an evolutionary puzzle. Estimates of the optimal size of proteins with a hydrophobic core and a hydrophilic surface vary between 150 and 200 residues. Although some small proteins have a rudimentary hydrophobic core, they do not exhibit enzymatic activity. These structural requirements of modern enzymes are incompatible with size limitations of primordial enzymes.

Proteins Based on Different Principles: An unexpected clue to the puzzle was uncovered from NAI supported experiments of Seelig and Szostak (2007), who evolved in vitro a protein capable of catalyzing phosphate bond formation between two fragments of RNA. This model of ancestral, water-soluble enzymes has architecture quite different from what is found in biological systems. In contrast to contemporary enzymes that form a hydrophobic core, the model protein has a small, rigid, hydrophilic core stabilized by way of metal ions that tether the ends of a highly flexible catalytic loop. This markedly reduces length requirements for enzymatic activity, as it is no longer needed to surround a hydrophobic core with a hydrophilic exterior exposed to water. Common elements of the secondary structure, such as α-helices and β-sheets, are neither required nor found in this novel architecture. This appears to impart considerable robustness to mutations in the core, even when they affect critical interactions with the metal ligands, as the polypeptide chain not engaged in ordered secondary structure readily undergoes rearrangements to accommodate mutations, as revealed in NAI-supported, large-scale, state-of-the-art computer simulations (Pohorille et al 2017). Proteins possessing similar structure did not require extensive evolutionary optimization of their sequence to achieve activity, a desirable property for increasing the repertoire of functional polymers at the origins of life.

Impact and Outlook: A somewhat serendipitous discovery that enzymes can be built according to a different architecture than commonly believed points to a key strength of in vitro evolution methods: the outcomes are not biased by our preconceptions of what feasible and required. Recently, the technique has been combined with computer modeling and extended to carry out in vitro evolution inside vesicles. This, in turn, opens the doors to evolving protein endowed with other essential cellular functions, such as ion channels, active transporters, signaling molecules, and multiprotein systems. By doing so, we gain powerful tools to address such fundamental questions in the origin of life as coevolution of different functions and the role of chance and necessity. Important discoveries might be just around the corner.

References:

Keefe, A. D. and Szostak, J. W. (2001) Nature, 410(6829), 715-718.

Pohorille, A., Wilson, M. A. and Shannon, G. (2017) *Life*, 7, 23.

Seelig, B. and Szostak, J. W. (2007) *Nature*, 448(7155), 828-831.