Synthetic Biology for Nano- and Atomic-Scale Macromolecular Structure Determination

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Synthetic biology is an emerging area that involves the design and construction of new or existing biological parts. Gene synthesis and assembly have been coupled to X-ray crystallographic techniques as a strategic synthetic biology approach for the structural determination of macromolecules. A PCR-based gene synthesis method will be described on how coding region assembly of proteins potential for drug targets can be easily performed. The gene synthesis procedure is based on sequential assembly such that homogeneous DNA products can be obtained after each synthesis step without extensive manipulation or purification requirements. Coupling the gene synthesis procedure to *in vivo* homologous recombination techniques allows efficient subcloning and site-directed mutagenesis for error correction. Recombinant proteins important for pharmaceutical leads have been assembled or modified using synthetic biology techniques for recombinant expression and crystallization for structure determination by X-ray and Neutron crystallography. In particular, the soluble inorganic pyrophosphatase (IPPase) has been one of the proteins of focus. IPPase in an enzyme that catalyzes the hydrolysis of inorganic pyrophosphate (PPi) to form orthophosphate (Pi). The action of this enzyme shifts the overall equilibrium in favor of synthesis during a number of ATP-dependent cellular processes such as in the polymerization of nucleic acids, production of coenzymes and proteins and sulfate assimilation pathways. The structures determined include the recombinant IPPase bound to Mg^{+2}, Ca^{+2}, Br^{-}, SO_2^{-2} or PO_4^{-2} involving those with non-hydrolyzed and hydrolyzed pyrophosphate complexes. All the crystallographic structures provide snapshots of the active site corresponding to different stages of the hydrolysis of inorganic pyrophosphate. As a result, a structure-based model of IPPase catalysis is devised showing the enzyme's low-energy conformations, hydration states, movements and nucleophile generation within the active site.