Application of homology modeling to study the function of proteins and their interactions with ligands

Krzysztof Kuchta

Abstract

Homology modeling is considered to be the most accurate of the computational structure prediction methods. In my studies I used homology modeling to investigate the function of proteins and their interactions with ligands. I focused on two types of proteins: eukaryotic translation initiation factors 4E (eIF4Es) and non-canonical poly(A) polymerases (PAPs). Biochemical binding studies and detailed analysis of homology models gave better insight into the molecular characteristic of varying cap-binding abilities of *Arabidopsis* and *Drosophila* eIF4E isoforms. Comparison of the 3D model of fungal CutA with solved crystal structures of poly(U) polymerase (PUP) Cid1 and non-canonical poly(A) polymerase Trf4, together with site-directed mutagenesis, allowed to define an unusual nucleotide-binding site, in which the unique nucleotide recognition motif present in the non-canonical PAPs and PUPs is mainly responsible for the observed preference of CutA toward cytidine. Finally, comprehensive analysis of various biological information available in literature and databases combined with numerous sequence and structure analyses, including a state-of-the-art distant homology detection, fold recognition and 3D modeling, allowed to classify uncharacterized FAM46 family members to cytoplasmic and/or nuclear non-canonical poly(A) polymerases. Presented studies show that homology modeling might be successfully used in explaining biochemical data related to substrate binding and specificity, planning new biochemical experiments to change the enzyme specificity and annotating protein function.