Part I: Circular permutation: Database, Prediction and Design

Part II: Structural insights into the catalytic mechanism of Dopamine *N*-Acetyltransferase

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This lecture will consist of two parts, the structural bioinformatics part and structural biology part: **I. Circular permutation: Database, Prediction and Design**

Circular permutation (CP) is a protein structural rearrangement phenomenon, through which nature allows structural homologs to have different locations of termini and thus varied activities, stabilities and functional properties. It can be applied in many fields of protein engineering. The limitation of applying CP lies in its technical complexity, high cost and uncertainty of the viability of the resulting protein variants. Not every position in a protein can be used to create a viable circular permutant, but there is still a lack of practical computational tools for evaluating the positional feasibility of CP before costly experiments are carried out. We have developed an efficient CP search tool, (CPSARST, CP Search Aided by Ramachandran Sequential Transformation), constructed a CP database (CPDB) and designed a comprehensive method for predicting viable CP cleavage sites in proteins. Here we also report the prediction and redesigning of a carbohydrate-binding module family 21 protein (CBM21) to test our tool. CP of the RoCBM21 substantially improved its binding affinity and selectivity towards longer-chain carbohydrates.

II: Structural insights into the catalytic mechanism of Dopamine *N*- Acetyltransferase

The daily cycle of melatonin biosynthesis in mammals is regulated by arylalkylamine *N*-acetyltransferase (EC 2.3.1.87, AANAT), making it an attractive target for therapeutic control of abnormal melatonin production in mood and sleep disorders. *Drosophila melanogaster* dopamine *N*-acetyltransferase (Dat) is an AANAT. Until this report, no insect Dat structure had been solved, and consequently, the structural basis for its acetyl-transfer activity was not well understood. We report herein the high-resolution crystal structure for a ternary complex for *D. melanogaster* Dat/ tryptamine /acetyl coenzyme A obtained using one-edge single-wavelength anomalous diffraction. The binding study by isothermal titration calorimetry suggested that the cofactor bound to Dat first before substrate. Examination of the complex structure indicated that Dat contained a novel AANAT catalytic triad. Site-directed mutagenesis and kinetic study confirmed that Glu47, Ser182, and Ser186 were critical for catalysis. These results suggest that Dat possesses a specialized active site structure dedicated to a catalytic mechanism. According to the ternary complex structure, we also proposed that three aromatic residues (F43, Y64, and F114) in a hydrophobic pocket of Dat may play key roles in substrate specificity. This study provides the structural insights into the enzyme activity and the substrate binding selectivity in Drosophila AANAT.