

# 針對結構生物學家的蛋白質工程學及結構產生應用實例

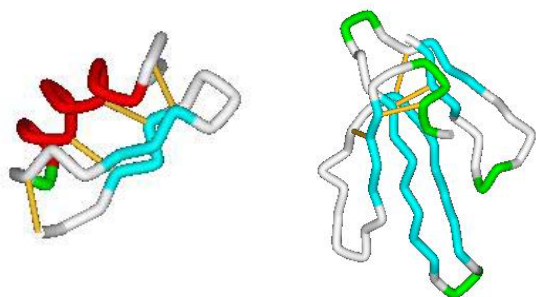
## 毒素分子中的運動與結構變化： 作為蛋白質工程學架構的可能用途

Authors use animal toxins as templates for protein engineering because of high variability in sequence and biologic function. They reported the extensive characterization of the structure and dynamics of two toxin folds, the "three-finger" fold and the short a/b scorpion fold found in snake and scorpion venoms, respectively. These two folds have a very different architecture; the short a/b scorpion fold is highly compact, whereas the "three-finger" fold is a  $\beta$  structure presenting large flexible loops. First, the crystal structure of the snake toxin a was solved at 1.8 Å resolution. The long molecular dynamics simulations (10ns) in water boxes of the snake toxin a and the scorpion charybdotoxin were performed, starting either from the crystal or the solution structure. The trajectories started from the X-ray structure are in agreement with the experimental NMR and X-ray data about the protein dynamics. Both proteins exhibit fast motions with an amplitude correlated to their secondary structure. In contrast, slower motions are essentially only observed in toxin a. The regions submitted to rare motions during the simulations are those that exhibit millisecond time-scale motions. Lastly, the structural variations within each fold family are described.

*Motions and structural variability within toxins: Implication for their use as scaffolds for protein engineering*

Reference: B. GILQUIN, et al, Protein Science (2003) 12, 266;

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## 由分子動力學模擬所帶來的b2-微球蛋白中因 結合銅離子所導致的不穩定性的分子基礎

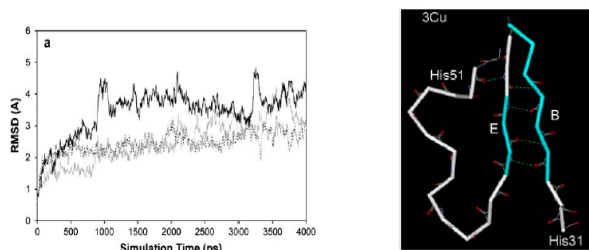
According to experimental data, binding of the  $\text{Cu}^{2+}$  ions destabilized the native state of b2-microglobulin. The partial unfolding of the protein was generally considered an early step toward fibril formation in dialysis-related amyloidosis. To elucidate the effect of ion ligation at atomic detail, a series of molecular dynamics simulations were carried out on apo- b2m systems in explicit aqueous solutions, with varying numbers of bound ions. Simulations at elevated temperature (360K) provide detailed pictures for the process of  $\text{Cu}^{2+}$  binding-induced destabilization of the native structure at the nanosecond timescale, which are in agreement with experiments. Conformational transitions toward partially unfolded states were observed in protein solutions containing bound copper ions at His31 and His51, which is marked by an increase in the protein vibrational entropy, with TS ranging from 30 to 69 kcal/mol. Analysis of the MD trajectories suggests that the changes in the hydrophobic environment near the copper-binding sites lower the barrier of conformational transition and stabilize the more disordered conformation. The results also indicate that the binding of  $\text{Cu}^{2+}$  at His 13 has little effect on the conformational stability, whereas the copper-binding site His13, and to a lesser extent His51, are primarily responsible for the observed changes in the protein conformation and dynamics.

*Molecular basis for the  $\text{Cu}^{2+}$  Binding-Induced Destabilization of b2-Microglobulin Revealed by Molecular Dynamics Simulation*

Reference: Nan-Jie Deng, Lisa Yan et al Biophysical Journal

Volume 90 June 2006 3865;

Used Module: CHARMM



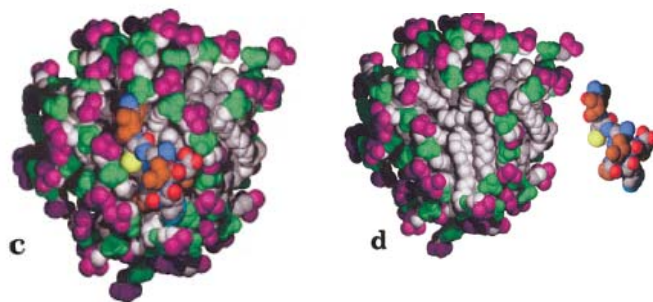
## 髮夾形成蛋白的膠粒結合體的組態： 結合核磁共振與分子動力學之研究

A peptide fragment from a protein hairpin turn region was modified by addition of isoleucine residues to both ends to enhance binding to lipid micelles: the resulting peptide contains the core sequence ICNNPH from an antibody-binding region of hemagglutinin A. NMR diffusion measurements indicated partial binding of the peptide to micelles of n-octylglucoside and significantly stronger binding to dodecylphosphocholine (DPC) micelles. Simulated annealing and conformational analysis using restraints revealed a type I or III hairpin turn between residues N5 and 18 of the DPC-bound peptide. Molecular dynamics simulations of DPC micelles and peptide-micelle complexes suggested that the peptide lies flat on the micelle surface and showed rapid rearrangement of the lipids to accommodate the bound peptide. According to a search performed using the basic local alignment search tool (BLAST), the sequences NPHI and NPHV are present as hairpin turns in eight of the nine proteins whose crystal structures were available. The addition of isoleucine residues and the use of lipid micelles to stabilize hairpin conformations equivalent to those found in proteins generates new possibilities for reproducing biologically important hairpin turns from short, linear peptides.

*Micelle-Bound Conformation of a Hairpin-Forming peptide: Combined NMR and Molecular dynamics Study*

Reference: Ann M. Dixon et al, Biopolymers 65, 284, 2002;

Used Module: CHARMM



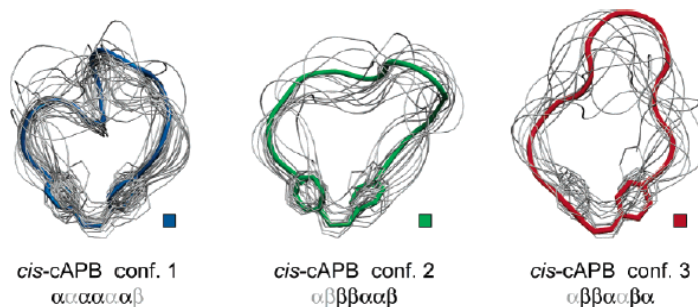
## 以分子動力學模擬所預測的蛋白質多重迴圈構型 與核磁共振的結果一致

The affinity and selectivity of protein-protein interactions can be fine-tuned by varying the size, flexibility, and amino acid composition of involved surface loops. As model for such surface loops, they study the conformational landscape of an octapeptide, whose flexibility is chemically steered by a covalent ring closure integrating an azobenzene dye into and by a disulfide bridge additionally constraining the peptide backbone. Because the covalently integrated azobenzenes dyes can be switched by light between a bent cis state and an elongated trans state, six cyclic peptide models of strongly different flexibilities are obtained. The conformational states of these peptide models are sampled by NMR and by unconstrained molecular dynamics (MD) simulations. Prototypical conformations and the free-energy landscapes in the high-dimensional space spanned by the  $\phi/\psi$  angles at the peptide backbone are obtained by clustering techniques from the MD trajectories. Multiple open-loop conformations are shown to be predicted by MD particularly in the very flexible cases and are shown to comply with the NMR data despite the fact that such open-loop conformations are missing in the refined NMR structures.

*Multiple Loop Conformations of Peptides Predicted by Molecular Dynamics Simulations Are Compatible with Nuclear Magnetic Resonance*

Reference: Heiko Carstens et al Biochemistry 2005, 44, 4829;

Used Module: CHARMM, InsightII



(以上，只是從許多的實例裏精選的一部份。)