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L29

CHARYBDOTOXIN UNBINDING FROM THE *MKV1.3* POTASSIUM CHANNEL: A COMBINED COMPUTATIONAL AND EXPERIMENTAL STUDY

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Charybdotoxin, belonging to the group of so-called scorpion toxins, is a short peptide able to block many voltage-gated potassium channels, such as *mKv1.3,* with high affinity. We use a reliable homology model based on the high-resolution crystal structure of the 94% sequence identical homolog *Kv1.2* for charybdotoxin docking followed by molecular dynamics simulations to investigate the mechanism and energetics of unbinding, tracing the behavior of the channel protein and charybdotoxin during umbrella-sampling simulations as charybdotoxin is moved away from the binding site [1, 2]. The potential of mean force is constructed from the umbrella sampling simulations and combined with Kd and free energy values gained experimentally using the patch-clamp technique to study the free energy of binding at different ion concentrations and the mechanism of the charybdotoxin-*mKv1.3* binding process. A possible charybdotoxin binding mechanism is deduced that includes an initial hydrophobic contact followed by stepwise electrostatic interactions and finally optimization of hydrogen-bonds and salt-bridges.

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[1] Khabiri, M.; Nikouee, A.; Cwiklik, L.; Grissmer, S.; Ettrich, R. *Journal of Physical Chemistry* **B115** (2011) (39): 11490–11500.

[2] Nikouee, A.; Khabiri, M.; Grissmer, S.; Ettrich, R. *Journal of Physical Chemistry* **B116** (2012) (17): 5132-5140.

L30

BINDING-COMPETENT STATES FOR L-ARGININE IN *E. COLI* ARGININE REPRESSOR APOPROTEIN

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Arginine repressor of *E. coli* is a multifunctional hexameric protein that provides feedback regulation of arginine metabolism upon activation by the negatively cooperative binding of L-arginine. Interpretation of this complex system requires an understanding of the protein's conformational landscape. The ~50 kDa hexameric C-terminal domain was studied by 100 ns molecular dynamics simulations in presence and absence of the six L-arg ligands that bind at the trimer-trimer interface. A rotational shift between trimers followed by rotational oscillation occurs in the production phase of the simulations only when L-arg is absent. Analysis of the system reveals that the degree of rotation is correlated with the number of hydrogen bonds across the trimer interface. The trajectory presents frames with one or more apparently open binding sites into which one L-arg could be docked successfully in three different instances, indicating that a binding-competent state of the system is occasionally sampled. Simulations of the resulting singly-liganded systems reveal for the first time that the bind.

L31

DOMAIN INTEGRITY VERIFICATION OF ALTERNATIVE SPLICING - THE DIVAS SERVER

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We present a new method and a server to predict the viability of proteins with globular domains truncated by alternative splicing or chromosomal translocation. The prediction is based on residue-level calculations of exposed hydrophobicity of structural domains with minimum 60% sequence similarity to the query, combined with local disorder values and domain length statistics. The accuracy of the server varies between 75% (for TargetDB) and 88% (Swissprot with protein evidence).

L32

BIOINFORMATIC ANALYSIS OF THE LOCAL DYNAMICS OF PROTEINS AND DNA

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Dynamics of proteins and nucleic acids is as important as their averaged molecular structures. Diffraction images contain information about the local molecular dynamics that is concealed in so-called B-factors (officially temperature displacement factors). To find out how well the crystal-derived B-factors represent the dynamic behavior of atoms and residues of key biomolecules, proteins and DNA, we analyzed distributions of scaled B-factors in a well-curated non-redundant dataset of almost a thousand protein/DNA complexes that has been described in Schneider *et al*. Nucleic Acids Research, ***42*** (2014). The analysis confirmed several expected features of protein and DNA dynamics but it also revealed some surprising facts. Solvent-accessible amino acids have B-factors larger than residues forming the interface with another protein or DNA molecule and the core-forming amino acids have B-factors the lowest so that their movement is very restricted. Really unique feature distinguishing the core-forming amino acids from any other type of amino acid residue is the fact that their side chains are restricted in their movements more than the main chains. The protein core is therefore extremely well packed leaving minimum free space for atomic movements. Surprising are low values of B-factors of the water molecules bridging protein and DNA that are actually significantly lower than the B-factors of DNA phosphates. Solvent-accessible phosphates are extremely flexible; they and DNA bases stiffen significantly upon complexation with proteins but overall are more flexible than amino acids. Unexpected and perhaps to some extent worrisome is also the fact that the features discriminating different types of residues quickly vanish in structures with lower crystallographic resolution. Some of the observed trends are a likely consequence of improper refinement protocols that may need rectifying.

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