**Poster Presentation**

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Structure-based engineering of designed armadillo repeat proteins.

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The specific recognition of macromolecules is key for many applications in biochemical research, medical diagnostics and disease treatment. Currently the development of new recognition molecules depends on the immunization of lab animals or combinatorial biochemistry techniques. Since both approaches are elaborate and require the availability of sufficient amounts of stable target molecules we are developing a modular system that allows a rational design of peptide recognition modules. This system is based on the armadillo repeat scaffold, because natural armadillo repeat proteins bind their targets in extended anti-parallel conformations with very regular binding topologies. The development of designed armadillo repeat proteins (dArmRPs) consisting entirely of identical internal repeats (full-consensus design) has been described [1]. Although dArmRP with 2nd generation capping repeats were predominantly monomeric in solution, crystal structures of dArmRP with different numbers of internal repeats revealed domain-swapped N-caps [2]. Redesign of the N-cap significantly improved thermodynamic stability and abrogated swapping of N-caps. These 3rd generation dArmRP recognize full-consensus peptides with nanomolar affinities. The dissociation constants depend on the lengths of the targeted peptides and the number of internal repeats. Three crystal structures of complexes between dArmRPs with five or six internal repeats and the targeted full-consensus (KR)5 peptide (either free or fused to the N- and C-terminii of globular proteins) confirm that the binding mode fulfills the expected regular topology. Further crystal structures of complexes between dArmRPs and the mismatch (RR)5 peptide revealed that the dArmRPs recognize their target peptides in a side-chain specific manner. Several crystal structures confirm that 3rd generation dArmRPs behave as stable and monomeric molecules that allow the selective recognition of the targeted peptide with the expected topology. Therefore, the rational assembly of binding modules from a pool of dipeptide-specific armadillo repeats to recognize peptides with given sequences should indeed be possible.

[1] Parmeggiani, F., Pellarin, R., Larsen, A. P., et al. (2008). Designed armadillo repeat proteins as general peptide-binding scaffolds: consensus design and computational optimization of the hydrophobic core. J. Mol. Biol. 376, 1282-1304., [2] Madhurantakam, C, Varadamsetty, G, Grütter, M. G, et al. (2012). Structure-based optimization of designed Armadillo-repeat proteins. Protein Sci. 21, 1015-1028.
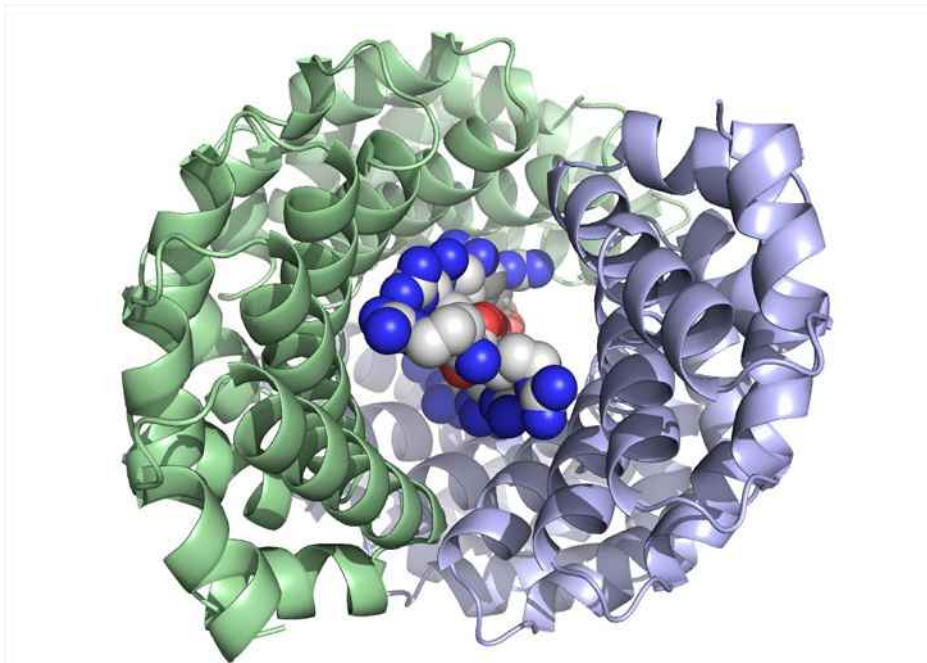
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