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Title (English)

Evolving optimisation of DNA binding in a novel Cro derived protein

Title (Swedish)

Abstract

Interactions between proteins and DNA are essential for biological life processes. However, the understanding of the sequence-specific and high-affinity interaction is incomplete.
Here, *in vitro* evolution was used to study the interactions between helix-turn-helix (HTH) proteins and DNA. A selection system based on Cro, a small HTH protein, was used to select for new DNA binding properties. A phage library was constructed where three of the known five DNA interacting residues were randomly mutated and two residues were subjected to site-directed mutagenisis. Selection was performed at two temperatures (4°C and room temperature) and with a new DNA ligand that is not a natural ligand for wt Cro. After four selection rounds some dominating clones were found. The specificity of these mutants was verified and two clones, DRCLY and WQCSY, were found to be specific for the new ligand. The DRCLY mutant was expressed and purified.

Keywords

Phage display, phage library, single-chain Cro (scCro), DNA ligands and selection.

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