

## **Lanthanide-binding helix-turn-helix motifs: Structure and function of a designed metallonuclease**

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*De novo* design is a powerful tool to investigate the active site of enzymatic metalloproteins, in a smaller, defined model system. It is also a way to build or combine activity and selectivity in unique ways, not seen biologically. We are utilizing protein design to build artificial endonucleases, and to investigate fundamental questions of metallonuclease structure and function. We have focused on designing constructs comprising geometrically similar turns from unrelated proteins, in particular the Ca-binding EF-hand motif of calmodulin and the helix-turn-helix motif (HTH) of engrailed homeodomain. By substituting the calcium-binding (and thus lanthanide-binding) loop in place of the “turn” of engrailed HTH, a folded, lanthanide and DNA-binding construct was designed. This modular turn substitution approach was employed to design and express a series of four metalloproteins based on the engrailed homeodomain (C1-C4). Circular dichroism studies and molecular modelling were used to compare the overall fold and secondary structure of each to the parental domain, and the NMR solution structure of the best folded La(III)-construct (LaC2) will be discussed. The metallo-homeodomain retains the parental helix-turn-helix structure when bound to lanthanide ions. The DNA-binding and cleavage interactions of these modified proteins, and the recently attained covalent homodimers of C2 will be discussed. (Funding provided by NSF, CHE-0093000)