

Design and Synthesis of Peptidomimetic Transcription Factors

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Purpose of the project/Problem to be solved:

To synthesize and characterize a protein-functionalized tetrameric molecule capable of binding selectively to specific promoter regions in DNA and which may hold the capacity for future development into a switchable system which can be turned on or off using differential chemical signals/cues.

Background and Introduction:

DNA is the molecule that stores genetic information in almost all forms of life. Following the processes of transcription and translation, this information is expressed in the form of proteins which carry out various cellular functions. However, the ultimate site of regulation for the expression of these proteins exists at the level of transcription.

The essential product of transcription is a second messenger molecule called mRNA. The synthesis of mRNA is catalyzed by an enzyme called RNA polymerase II, which only becomes active upon interaction with certain proteins called transcription factors. Normally, these transcription factors lie dormant in the cell, but become activated upon interaction with other signaling mechanisms (e.g. via the interaction with some ligand). This activation of the transcription factor typically induces a change in the conformation of the protein that then affords an extremely specific interaction with a recognition sequence near the 5' end the DNA sequence. The site specificity of this interaction is remarkable given that an individual transcription factor will recognize some small sequence of base pairs among millions that exist in the genome.

Statement of proposed project:

Our research is focused on the bZIP family of transcription factors, most notably GCN4. The objective is to design and synthesize miniature peptidomimetic molecules that retain the capacity to bind to DNA with great specificity. A few examples have appeared in the recent literature, with most mimicking directly the natural system (shown below in **Figure 1**).

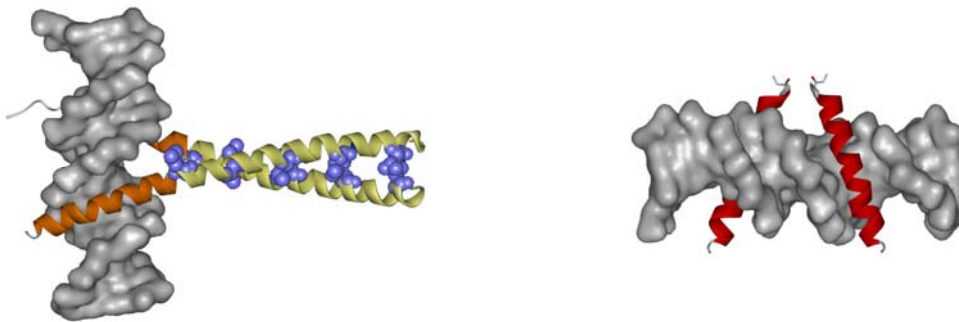


Figure 1. Binding of the bZIP transcription factor, GCN4, to DNA (left) and binding of a modified synthetic min-protein to the same sequence of DNA (right).

Our efforts are directed toward the design of molecular constructs that can be functionalized with up to 4 different synthetic mini-proteins. In addition, our ultimate goal is to design a system that is not only capable of recognizing more than one specific binding site in the genome, but also possesses the capability of being switched on and off (bound vs. unbound) as shown in **Figure 2**.

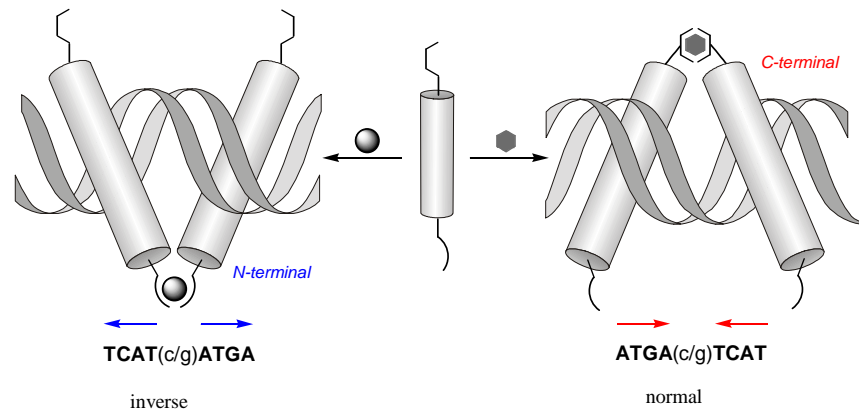


Figure 2. Orthogonally functionalized system that can be selectively tuned to recognize either the normal DNA binding site or its inverse site.

Currently, we are interested in the design of tetrameric molecules in which the bZIP binding motifs appear on the periphery of a core organic structure (see **Figure 3** below). Once realized, we intend to demonstrate the DNA binding capacity of the construct under various conditions (that will promote either on or off modes of binding) by CD and gel mobility shift assays.

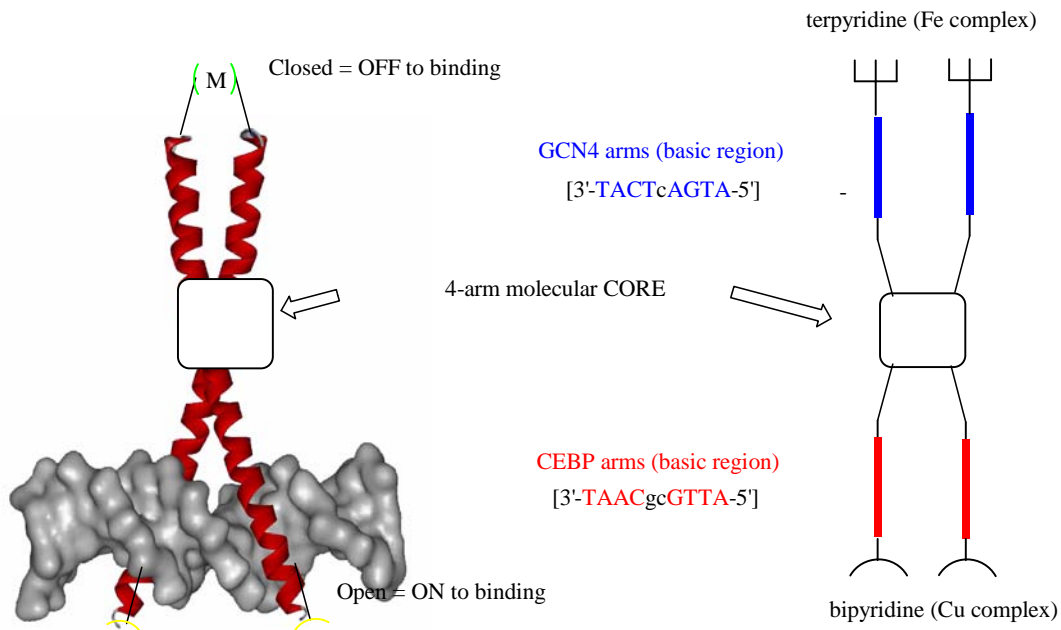


Figure 2. Tetrameric switchable system designed around a central molecular core.