Design of Protein-DNA Dimerizers

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for my family

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Abstract

Genes are regulated by proteins called transcription factors that bind to DNA in a sequence-specific manner and modulate the rate of transcription. Mutated transcription factors often lead to abnormal gene expression, developmental defects, and disease. This thesis describes the design of chemicals called protein-DNA dimerizers that mimic natural transcription factor protein-DNA complexes. In the long-term, it is hoped that these dimerizers will be able to engage or even replace mutant transcription factors and artificially regulate gene expression in living cells. Specifically, programmable DNA-binding pyrrole-imidazole polyamides conjugated to YPWM peptide motifs incorporating various linker domains facilitate the binding of a natural transcription factor, extradenticle, to DNA. From a design point of view, it has been explored what the minimum size and shape (branched or linear) is that will ultimately be optimal for cell uptake with adequate functional potency in the transcriptional apparatus. Branched dimerizers are shown to function with a minimal WM dipeptide protein-binding domain *in vitro* up to 37 °C, and linear dimerizers are shown to function with WMK tripeptides up to 20 °C. Collectively, branched and linear dimerizers can facilitate protein binding to DNA from 2 base pair overlap sites to ones that reach 6 base pairs apart. Polyamide-WM-fluorescein conjugates are also found to be cell permeable in several cell lines including HeLa, MCF-7, and PC3. These studies provide insight into the importance of linker length and composition, binding-site spacing and orientation, and the protein-binding domain content that are important for the optimization of protein DNA-dimerizers suitable for biological experiments.

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