Poster I-3

Designing C2H2 Zinc Finger Proteins to Target Specific Genomic Sites

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Short Abstract: Consisting of modular nucleic acid binding domains, C2H2 zinc finger proteins provide an excellent framework for engineering new sequence-specific DNA binding proteins. Using binding specificities determined by others, we have developed a program, ZiFit, to locate and rank candidate targets for zinc finger binding within a given DNA sequence.

Long Abstract:

Zinc fingers, the most abundant DNA binding motifs in eukaryotes, provide one of the best understood protein-DNA binding mechanisms. They promise to become valuable tools for genome modification and clinical intervention in disease because they can be used to target proteins, including nucleases and transcription factors, to virtually any desired location in any genome. Consisting of multiple modular and interchangeable nucleic acid binding domains, C2H2 zinc finger proteins provide an excellent framework for engineering new sequence-specific DNA binding proteins (see [1]).

The C2H2 zinc finger protein comprises three individual fingers. Each finger consists of two beta strands and an alpha helix coordinated through a zinc ion via two cysteine and two histidine residues. Positioned within the major groove, the helices form both base-specific and backbone contacts with adjacent nucleotide triplets. Amino acids -1, +3, +6 (with respect to the start of the alpha-helix) each make base-specific contacts with a single nucleotide on the 5' to 3' DNA strand. In addition, the amino acid at position +2 can contact a base preceding the triplet on the 3' to 5' strand.

Using C2H2 zinc finger binding specificities determined by others (e.g. [2], [3]), we have developed a program, ZiFit (see [4]) to locate and rank candidate targets for zinc finger binding within a given DNA sequence. In ongoing work, we are designing and experimentally testing additional zinc finger binding modules and combinations by exploiting knowledge-based approaches that incorporate information such as binding affinities, context dependence, and DNA sequence/structural characteristics. In recent work we have begun to explore molecular dynamics simulations to enhance the design of zinc fingers. By making specific substitutions in either the DNA or protein component of existing zinc finger-DNA co-crystal structures, we can explore the space of zinc finger-DNA complexes to computationally estimate the relative binding free energies and specificities of zinc finger proteins.

References

[1] Zinc Finger Consortium. http://www.zincfingers.org

[2] Segal DJ, Dreier B, Beerli RR, Barbas CF 3rd. (1999) Toward controlling gene expression at will: selection and design of zinc finger domains recognizing each of the 5'-GNN-3' DNA target sequences. Proc Natl Acad Sci U S A. 96(6):2758-63.
[3] Dreier B, Beerli RR, Segal DJ, Flippin JD, Barbas CF 3rd. (2001) Development of zinc finger domains for recognition of the 5'-ANN-3' family of DNA sequences and their use in the construction of artificial transcription factors. J Biol Chem. 276(31):29466-78.
[4] ZiFit. http://bindr.gdcb.iastate.edu/ZFPTFWeb