Structural Analysis of the dsDNA- v. ssDNA-binding Protein Interface

Background
Shape and base readout have been shown to be important determinants of double-stranded DNA (dsDNA) binding and specificity. The goal of this project is to examine the readout mechanisms of single-stranded DNA (ssDNA) binding proteins and how these interactions confer recognition, especially in comparison to dsDNA recognition.

The Question....
Given a protein structure, are there structural features that define the DNA binding properties of a protein... specifically in the recognition of dsDNA v. ssDNA.

Hypothesis
The structural and biochemical properties of a DNA-binding protein allow for specific base and shape readouts that differentiate between ssDNA and dsDNA binding.

Charge and Shape Complementarity
Working hypothesis- The charge and shape properties of dsDNA and ssDNA are complementary to the charge and shape properties of the DNA-binding protein interface.

Like dsDNA-binding proteins, recognition of ssDNA appears to be driven by the interplay of charge and shape complementation (last week’s data). The negative charge and high degree of conformation freedom of ssDNA is reflected in the binding surface of ssDNA-binding proteins. In addition, the size of ssDNA (being ½ the size of dsDNA) is also reflected in the properties of the protein interface.

This week’s focus was to look at the charge characteristics of the ssDNA-binding protein interface. To do this, the scale of the APBS electrostatic potential maxima was changed from ±1 to ±5 (kT/e).

Tra1- 2A0l

RecD2- 3GPL
**Chemistry Complementarity**

**Working Hypothesis** - the conformational freedom of ssDNA allows for more hydrophobic interactions between bases and amino acid residues... therefore the ssDNA-binding protein interface should possess a greater number of solvent accessible hydrophobic side chains that facilitate binding interactions with ssDNA.

PDBe Pisa was used to calculate the change in solvation energy upon ligand binding. The solvation energy gain of the interface is determined by calculating the difference in solvation energies of all residues between dissociated and associated structures. The change in solvation energy is represented by $\Delta G$ where a positive $\Delta G$ value makes a negative contribution to the solvation energy at the interface, therefore representing a hydrophobic effect. T-test shows that the difference is significant ($P < 0.0001$) between residues of the dsDNA- and ssDNA-binding protein interface (once again, not sure if a comparison in this manner is allowed).
Summary/Running Conclusion

ssDNA recognition uses the same base and shape readout mechanism as dsDNA, however the structure and chemical properties of ssDNA lead to different structural and chemical features of the DNA-binding protein interface that confer binding and specificity.

Manuscripts

*Early stages of induction of anterior head ectodermal properties in Xenopus embryos are mediated by transcriptional cofactor ldb1* Carol Zygar Plautz*, Brett E. Zirkle, Malia J. Deshotel, and Robert M. Grainger

Reviews came back this week and for the most part seemed pretty positive. One additional experiment and clarity/shortening of text/figures was suggested.

*Conserved structural and mechanistic requirements of gp41 MPER-directed antibody neutralization of HIV-1* Ofek, G.*, Zirkle, B.*, Mckee, K., Yang, Y., Zhi-Yong, Y., Nabel G.J., Dimitrov, D., Mascola J.R., Kwong, P.K.

Talked to Adi (Ofek) this week and the plans are to submit by the end of the month (fingers crossed). Manuscript is being edited (sent to him on Monday), structure is close to being ready, and we are awaiting last bit of neutralization data.

Weekly Papers and One Sentence Summary  (red = next weeks papers)

“Structure of Human Na+/H+ Exchanger NHE1 Regulatory Region in Complex with Calmodulin and Ca2+” (for class)

Exactly what the title says… purely a structure paper (no functional assays)

“Structural Basis of Silencing: Sir3 BAH Domain in Complex with a Nucleosome at 3.0A Resolution” Armache et al.

Structural basis for silencing and diseases associated with dysfunction

Lab presentation paper for next Wednesday

Questions/Goals for Next Week

Study- test in molecular genetics Thursday

Wednesday presentation- paper + get APBS and Delphi maps

Would like to look at electrostatic potential v. at specific bases

- Plot similar to Fig. 3 in Nature 2009 paper