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 differences between the dsDNA and ssDNA binding protein interface can be observe qualitatively through the structural and electrostatic potential maps. The goal of the previous week(s) was to write a script to calculate charge at the phosphate\((P_{x,y,z})\), 4’oxygen (\(O4'_{x,y,z}\)), and the 5 carbon (\(C5_{x,y,z}\)) coordinates on a naked protein and measure the distance from the protein in hope of quantifying what is observed in the structures and to provide a foundation for looking at relationships.

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.

Quantifying the Electrostatic of the Binding Interface
Working hypothesis- The charge and shape properties of dsDNA v. ssDNA are complementary to the charge and shape properties of the protein binding interface.

The electrostatic potential as a function of distance was plotted at each \(P_{x,y,z}\), \(O4'_{x,y,z}\), and \(C5_{x,y,z}\) (red, blue, and orange, respectively).
All the data points for pulled for ssDNA- and dsDNA-BPs (whether this is statistically legal or not I don’t know... but just to get an idea).
Looking at the distances alone reveals some interesting properties of ssDNA- versus dsDNA-binding protein interfaces. The distance from C5\textsubscript{x,y,z} in ssDNA is much closer to the protein surface than observed for the dsDNA C5\textsubscript{x,y,z}. This property also holds true for O4'\textsubscript{x,y,z} as well but not P\textsubscript{x,y,z}. This is perhaps a direct reflection of the shape and biochemical properties of DNA within the protein interface. In other words, the conformational freedom of ssDNA gives rise to different shape and chemical properties that result in readout mechanisms different from those resulting from the structure of dsDNA. Therefore, the different readouts may be reflected on the protein surface. The biggest question going forward is how to measure and present such differences in a manner that clearly illustrates biological point.

Weekly Papers + Summary and Applications (purp = previous week’s papers, red = next week’s papers)

Cohen, Fauci, Varmus, and Nabel "Epstein-Barr Virus: An Importance Vaccine Target for Cancer Prevention" Science Translational Medicine 2011

Summation of a NIH meeting on the need for an EBV vaccine.
Chen Lab “Structure of the Epstein-Barr virus major envelope glycoprotein” Nature Structural and Molecular Biology 2006

 Revealed structure of EBV gp350 envelope protein and defined the CR2 binding site through mutagenesis, biochemical assays, and flow cytometry.

 A hypothesis...

 Antibody-mediated neutralization of EBV requires precise targeting of the CR2 binding site on gp350

 In other words...

 Where the antibody binds is important and binding to the CR2 binding site would prevent viral entry. This characteristic has been observed for HIV antibodies where the most potent neutralizers of HIV have been shown to precisely target the CD4 binding site on gp120 (Wu et al. Science 2011). Such a study would require the following, very simplified, experimental flow:

 Isolate patient serum, preferably selecting patients with different abilities to control viral infection

 Probe serum for antibodies capable of recognizing a soluble gp350 resurfaced core protein (ERSC) and but do not bind a mutant ERC (ΔERSC) that is negative for CR2 binding

 Select CR2 binding site specific B cells (ERSC+/ΔERSC) by flow cytometry

 IgG RNA
 cDNA

 Heavy Chain Light Chain

 PCR amplify and express IgG from neutralizing antibodies

 Determine structural basis for antibody-epitope interaction
Infected individuals with influenza and mapped T-cell responses. Preexisting CD4⁺ T-cells (not CD8⁺) correlated with increased viral shedding and less severe symptoms. There was also evidence that showed influenza positive CD4⁺ T-cells exhibit cytolytic activity as well. Overall, it was an interesting paper that reminds you of how the immune system is not the same for every individual and that population dynamics (i.e. diversity, mating behavior, ect) play a key role in determining the immune response.