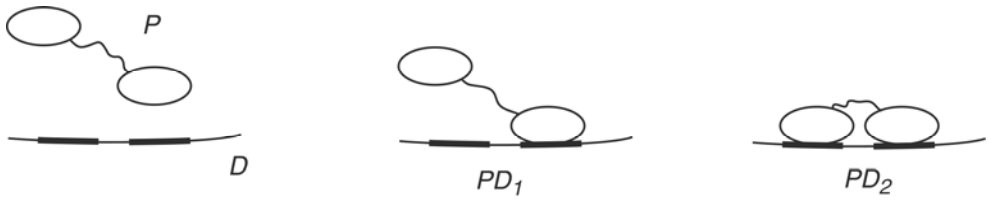
Dimer Binding Affinity in Terms of Monomer Binding Affinity

Suppose two DNA binding domains are connected by a completely flexible linker, what is the binding affinity of the double domain protein in terms of the binding affinity of a single domain?



Consider the three states, protein free in solution, the protein with one monomer bound, and the protein with both monomers bound. The amound of protein bound to DNA by a single DNA binding domain, PD_1, is given from the definition of the monomer dissociation constant K_{M1},

$$K_{M1} = \frac{P \times D}{PD_1}, \text{ or } \quad PD_1 = \frac{P \times D}{K_{M1}}$$

where *P* is the concentration of free protein in solution, *D* is the concentration of DNA, and and K_{M1} is the dissociation constant for a monomer from a DNA site. For the binding of the second monomer to the second DNA site, the same dissociation constant applies, but now the concentration of the DNA reactant is PD_1 and the relevant protein concentration is the concentration of the monomer not bound to DNA in the vicinity of the unoccupied DNA binding site. Define this to be C_{eff}. Thus,

$$K_{M1} = \frac{C_{eff} \times PD_1}{PD_2}$$

and solving this for PD_2 and substituting the value from above for PD_1 gives

$$PD_2 = \frac{C_{eff}}{K_{M1}} \times \frac{P \times D}{K_{M1}}$$

The definition of the overall dissociation constant *K'* between the protein and DNA considers the singly bound and doubly bound species of protein,

$$K' \equiv \frac{P \times D}{PD_1 + PD_2}$$

$$= \frac{P \times D}{\frac{P \times D}{K_{M1}} + \frac{C_{eff} \times P \times D}{K_{M1}^2}}$$

$$= \frac{K_{M1}^2}{K_{M1} + C_{eff}}$$

This result shows that when the linker between the two monomers is long and the effective concentration is very low, the protein binds as a monomer with a dissociation constant of K_{M1} and when

the linker is short and C_{eff} is large with respect to K_{M1}, the effective dissociation constant of the complex is K_{M1}^2 / C_{eff}. For most oligomeric DNA binding proteins utilizing more than one DNA binding site, C_{eff} is high and the binding of the oligomer is much tighter than the binding of the monomer.