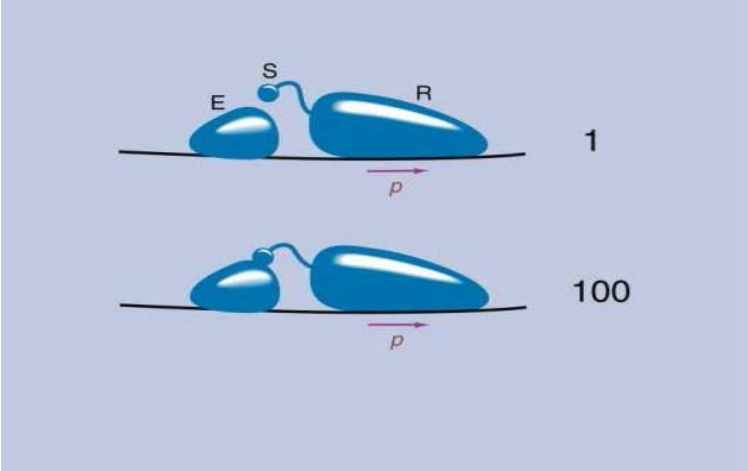
Transcription Factor--RNAP Interactions

Summary: In some cases, the interactions between transcription factors and RNA polymerase can be approximated as being mediated by an interaction domain on a flexible tether. This situation permits a simple estimation of the necessary strength of interaction between the two proteins to generate, for example, a 100-fold stimulation in DNA binding by RNA polymerase.It turns out that very weak interactions can be sufficient, as low as $K_d=10^{-3}$ M.

The Estimation: Consider a promoter with a low affinity for RNA polymerase. If a tight binding site for an activating protein is added nearby, how tightly, for example, must the C-terminal domain of the alpha subunit of polymerase, a-CTD, bind to the protein to increase RNAP binding to the promoter by a factor 100?

When the activating protein is bound nearby and if a-CTD can bind to the activator, at any instant there are two states of polymerase: one in which a-CTD does not contact the activator, and one in which it does.



Because there is no interaction between the activator and polymerase in the noncontacting state, the concentration of polymerase bound to the promoter in this noncontacting state is the same as when activator is absent altogether.The polymerase whose a-CTD is in contact with the activator is thus the extra polymerase that has been recruited to the promoter by the activator. In our case, we want the extra polymerase to be 100 times as much as the original polymerase.

The problem then becomes one of estimating the necessary affinity between a-CTD and the activator, such that when the activator and polymerase are both bound to their DNA sites, there is 100 times as much a-CTD bound to the activator as not bound.

Let us consider the activator to be E and a-CTD to be the substrate S. Then, by the familiar Michaelis-Menton equation or Langmuir binding isotherm,

$$\frac{ES}{E_t} = \frac{S}{K_D + S}$$

where *ES* is activator complexed with a-CTD, *Et* is total activator, K_D is the dissociation constant between the activator and a-CTD, and *S* is the concentration of a-CTD.Normally, *S* represents a solution concentration. In our case, however, and this is crucial, *S* represents the concentration of a-CTD in the vicinity of the activator. (What is relevant to the binding of a-CTD to the activator is the collision frequency between the two.)

The fraction of activator not in complex is then

$$\frac{E}{E_t} = 1 - \frac{S}{K_D + S} = \frac{K_D}{K_D + S}$$

and the ratio of bound to free a-CTD is thus

$$\frac{ES}{E} = \frac{\frac{S}{K_D + S}}{\frac{K_D}{K_D + S}} = \frac{S}{K_D}$$

In our case, we need S/K_D to be 100.

What is the effective local concentration, *S*, of a-CTD, in the vicinity of an activator? This can be estimated by approximating the situation to that of a-CTD being held in the vicinity of the activator by a completely flexible tether of length equal to the linker connecting the two domains of RNA polymerase. In this case, there is a concentration of one a-CTD in a hemisphere of radius equal to the tether length which here will be taken to be about 30 Å.

It is now necessary to convert one molecule per hemisphere to a molar concentration. This concentration is equivalent to that of the molar concentration of tightly packed hemispheres.This concentration *S* is the same as determining how many moles of such hemisphere volumes fit in one liter. This is

$$S = \frac{10^3}{Hemi.Volume} \frac{molecules}{liter} \times \frac{1}{6 \times 10^{23} \frac{molecules}{mole}}$$

The hemisphere volume is approximately 54 x 10^{-21} and thus S~ 1/30 mole/liter.

To obtain a 100 x stimulation, we need S/K_D to be 100, or $K_D = S/100 = 3 \times 10^{-2} \times 10^{-2} = 3 \times 10^{-4}$ M. This is a very weak interaction.

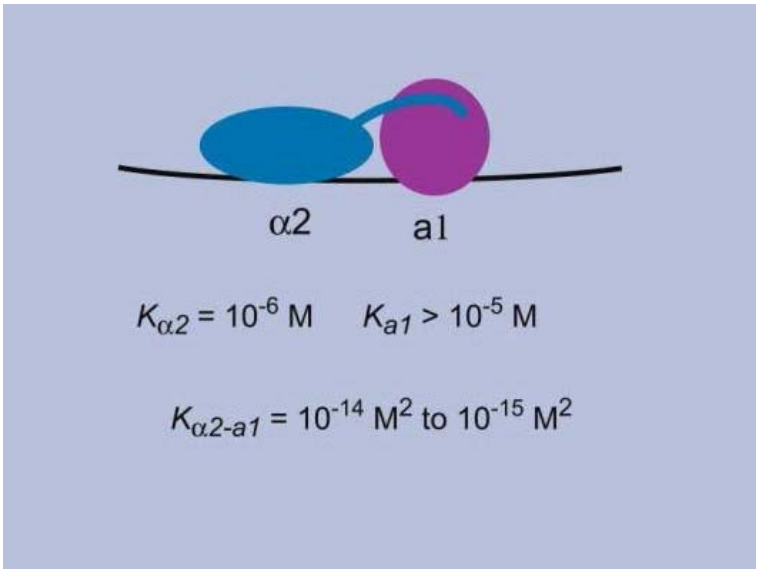
Comments: The fact that transcription activators can function and yet possess weak interactions with RNA polymerase is consistent with the small interaction interface seen between a-CTD and CRP1[1], and the weak interactions seen between MarA[2] and a-CTD and AraC and a-CTD[3].

The same line of reasoning as was applied above leads to the general result that the apparent dissociation constant from DNA of a complex of two proteins that can interact via a flexible tether or arm is

$$K_{AB} = K_A \times K_B \times \frac{K_{arm}}{C_{eff}}$$

where K_A and K_B are the individual dissociation constants, K_{AB} is the dissociation constant for the complex, K_{arm} is the dissociation constant of the interaction between *A* and *B*, and C_{eff} is the effective concentration of the tethered interaction interface[4].

Applying this to the yeast mating type proteins a1 and a2 which interact via an arm of length approximately 10 Å yields a result that closely agrees with experiment.



[1] B. Benoff, H. Yang, C. Lawson, G. Parkinson, J. Liu, E. Blatter, Y. Ebright, H. Berman, R. Ebright Science 297, 1562-1566 (2002).

[2]B. Dangi, A. Gronenborn, J. Rosner, R. Martin, Mol. Micro. 54, 45-59 (2004).

[3]M. Rodgers, R. Schleif, unpublished.

[4] R. Schleif, C. Wolberger, Protein Science 13, 2829-2831 (2004).