
**I. DNEs in dimers, oligomers and polymers.**

For the dimer or even higher order oligomers or polymers (n-mers), if there is no preferential degradation of abnormal monomers and oligomers, the proportion of each n-mer, containing 1, 2,..., p abnormal subunits, is given by the binomial expansion formula: $\binom{n}{p}A^{n-p}a^p$, where $A$ and $a$ are the corresponding molar proportions (i.e. $[A]+[a]=1$ and thus, when both monomers have identical concentrations $[A]=[a]=0.5$). As stated in the text, in the case of a dimer, if only AA is active, a diploid heterozygous $A/a$ organism will display only 25% of activity.

**II. Estimating $K_A$ and $K_{Aa}$. The saturation curve for the interaction between the dimers (AA, Aa or aa) and RR.**

The dimer AA interacts with a symmetric partner (i.e. a homodimeric receptor or a bipartite DNA or RNA sequence, RR). The dimer AA recognizes its target with an affinity constant $K_{AA} = [AA-RR]/[AA][RR]$ and a standard free energy: $\Delta G^{o}_{AA} = -RT\ln K_{AA}$. $\Delta G^{o}_{AA}$ comprises the additive energies of the interactions of each monomer with its target site (i.e. $\Delta G^{o}_{A}$). Thus, $\Delta G^{o}_{AA}$ is proportional to $2\Delta G^{o}_{A}$. This expression for $\Delta G^{o}_{AA}$ is a rough approximation that does not take into account the energy spent in conformational changes and entropic factors (i.e. a monomer can interact with two different R subunits/sites), expected to have a lower impact on $K_{AA}$. It follows that $\Delta G^{o}_{A} = -RT\ln K_A$ is proportional to $-1/2RT\ln K_{AA} = -RT\ln \sqrt{K_{AA}}$ and hence, $K_A$ is proportional to $\sqrt{K_{AA}}$ (for simplicity we will equate them: $K_A = \sqrt{K_{AA}}$). In the case of the interaction between $A/a$ and RR, by analogy, if $a$ has kept some residual binding we will use $K_{Aa} \sim K_A K_a$. If $a$ has lost its binding capacity we will use $K_{Aa} \sim K_A = 1$.

We can study the activity of AA by assessing the degree of saturation (Y) of RR. That is, $Y = [AA-RR]/([RR]+[AA-RR]) = K_{AA}[AA]/(1+K_{AA}[AA])$. Based on the simplifications mentioned above, we can also calculate the fractional saturation of the receptor by AA, Aa and aa when they coexist by using $Y = ([AA-RR]+[Aa-RR]+[aa-RR])/([RR]+[AA-RR]+[Aa-RR]+[aa-RR])$ or, more explicitly:
\[ Y = \frac{K_{AA} / RR.AA + K_{Aa} / RR.Aa + K_{aa} / RR.aa}{1 + K_{AA} / RR.AA + K_{Aa} / RR.Aa + K_{aa} / RR.aa} \]

The values of the Ks are estimated as outlined above. The relative concentrations of AA, Aa and aa are calculated using the Newton’s binomial expansion formula given above.

Parameters for Figure 3. Panel A: K_{AA}=2.10^8 \text{ M}, K_{Aa}=2.10^6 \text{ M} and K_{aa}=2.10^4 \text{ M}. In panel B K_{AA}=2.10^8 \text{ M}, K_{Aa}=7.10^7 \text{ M} and K_{aa}=2.5x10^7 \text{ M}. Concentrations of A or A+a (in equimolarity) range between 5.10^{-10}-10^{-7} \text{ M}.

III. DNE in transcription: the meaning of synergy.

In our model, the species that contributes the most to transcription is the promoter occupied by 2 molecules of activator: pAA. The key ingredient to explain this is synergy. Briefly, let us assume that the constant for the association between pA and the polymerase (pol) is K_{polA}. This constant is linked to the free energy of the process by the Arrhenius equation \( \Delta G^\circ_{pA-pol} = -RT\ln K_{polA} \).

Invoking free energy additivity, we assume that the energy of the interactions between pAA and the polymerase is about 2 times greater (see Zlotnick, 1994). Thus, \( \Delta G^\circ_{pAA-pol} \sim 2RT\ln K_{polA} = -RT\ln K_{polA}^2 \). Hence, the constant for the association of pAA and the polymerase is \( \sim K_{polA}^2 \). This estimate of \( \Delta G^\circ_{pAA-pol} \) is, as explained above, an oversimplification. Concerning a, partial transactivation activity is represented by K_{pola} (for p_a + pol) and by K_{pola}^2 (for paa + pol). Under these assumptions an equation for the transcriptional response TR as a function of the concentration of A (and a) can be derived as described by Veitia (2003) and Veitia and Nijhout (2006).

The derivation of the expression for the transcriptional response (Y or TR) for a promoter with 2 sites is based on the model described in Veitia (2003) and Veitia and Nijhout (2006). If we follow this reference step by step, allowing that each monomer interacts with DNA and with the polymerase with different affinities \( (K_A, K_a, K_{polA} \text{ and } K_{pola}) \) we obtain:
\[
Y = \frac{1}{f} \frac{2K_A A (k_{polA}) + 2K_a a (k_{pola}) + kK_A^2 A^2 (k_{polA}) + 2 k' K_A a A a (k_{polA} k_{pola}) + k' K_a^2 a^2 (k_{pola})^2}{1 + 2K_A A + 2K_a a + kK_A^2 A + 2 k' K_A A a + k' K_a^2 a^2}
\]

where \( f \) is a scaling factor (to normalize the curves). Cooperativity is taken into account by including the interaction coefficients \( k \) and \( k' \). We assume that cooperativity \((k')\) is the same to produce \( p_A a \), \( p_A A \) or \( p_a a \) (or it does not exist at all, depending on the particular DN). In figure 6D, for simplicity \( K_A = K_a \). The linear terms in the numerator can be neglected.

Parameters for fig. 6D. \( K_A = K_a = 10^7 \), \( k = 50 \), \( k' = 50 \) (normal cooperativity \( A - A, A - a, a - A \) or \( a - a \)) or 1 (no cooperativity). \( K_{polA} = 10000 \), \( K_{pola} = 1 \) (no transactivation) or \( 1/2 \) \( K_{polA} \) in the example of partial transactivation. Concentrations of \( A \) or \( A + a \) (equimolarity) range between \( 2 \cdot 10^{-10} - 5 \cdot 10^{-8} \) M.

References
