Glucocorticoid Receptor-Promoter Interactions: Energetic Dissection Suggests a Framework for Specificity of Steroid Receptor-Mediated Gene Regulation

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Supplementary Information for Figure 6: Simulations of non-competitive and competitive GR and PR-B promoter binding

Figure 6A presents non-competitive simulations of GR and PR-B binding to the GRE_2 promoter, calculating the probability (P) of the fully-ligated promoter:

$$P_{\text{GR}} = \frac{K_{\text{tot-GR}}{}^{2} k_{\text{c,inter-GR}} x_{\text{m-GR}}{}^{4}}{1 + 2K_{\text{tot-GR}} x_{\text{m-GR}}{}^{2} + K_{\text{tot-GR}}{}^{2} k_{\text{c,inter-GR}} x_{\text{m-GR}}{}^{4}} \qquad (1)$$

$$P_{\text{PR-B}} = \frac{K_{\text{tot-PR-B}}{}^{2} k_{\text{c,inter-PR-B}} x_{\text{m-PR-B}}{}^{4}}{1 + 2K_{\text{tot-PR-B}} x_{\text{m-PR-B}}{}^{2} + K_{\text{tot-PR-B}}{}^{2} k_{\text{c,inter-PR-B}} x_{\text{m-PR-B}}{}^{4}} \qquad (2)$$

where x_{m} is the free receptor monomer concentration, K_{tot} is the total binding affinity for saturating a single response element with two receptor monomers, and $k_{\text{c,inter}}$ is the inter-site cooperativity between receptors bound to adjacent palindromic response elements. Note that because these are simulations of non-competitive receptor-promoter binding, the denominator of each equation (Q or the partition function) only describes binding interactions for a single receptor (GR or PR-B).

Figure 6B presents competitive simulations of GR and PR-B pre-formed dimer binding to the GRE_2 promoter using the following equations:

$$P_{\mathrm{GR}} = \frac{k_{\mathrm{c,inter\text{-}GR}} k_{\mathrm{int,d\text{-}GR}}{}^{2} x_{\mathrm{di\text{-}GR}}{}^{2}}{Q} \tag{3}$$

$$P_{\mathrm{PR\text{-}B}} = \frac{k_{\mathrm{c,inter\text{-}PR\text{-}B}} k_{\mathrm{int,d\text{-}PR\text{-}B}}{}^{2} x_{\mathrm{di\text{-}PR\text{-}B}}{}^{2}}{Q} \tag{4}$$

where Q is now defined as:

$$\begin{aligned} Q = {} & 1 + 2k_{\mathrm{int,d\text{-}GR}} x_{\mathrm{di\text{-}GR}} + 2k_{\mathrm{int,d\text{-}PR\text{-}B}} x_{\mathrm{di\text{-}PR\text{-}B}} \\ & + k_{\mathrm{c,inter\text{-}GR}} k_{\mathrm{int,d\text{-}GR}}{}^{2} x_{\mathrm{di\text{-}GR}}{}^{2} + k_{\mathrm{c,inter\text{-}PR\text{-}B}} k_{\mathrm{int,d\text{-}PR\text{-}B}}{}^{2} x_{\mathrm{di\text{-}PR\text{-}B}}{}^{2} \end{aligned} \tag{5}$$

and x_{di} is the concentration of receptor dimer, $k_{\mathrm{c,inter}}$ is the inter-site cooperativity between receptors bound to adjacent palindromic response elements, and $k_{\mathrm{int,d}}$ is the intrinsic affinity of a pre-formed receptor dimer for DNA. Note that the denominator (Q) has been expanded to include all possible binding interactions for both GR and PR-B.

Figure 6C presents a competitive simulation of GR and PR-B monomer binding to the GRE_2 promoter using the following probability of saturation (P) equations:

$$P_{\mathrm{GR}} = \frac{k_{\mathrm{c,inter\text{-}GR}} k_{\mathrm{c,intra\text{-}GR}}{}^{2} k_{\mathrm{int,m\text{-}GR}}{}^{4} x_{\mathrm{m\text{-}GR}}{}^{4}}{Q} \tag{6}$$

$$P_{\text{PR-B}} = \frac{k_{\text{c,inter-PR-B}} k_{\text{c,intra-PR-B}}{}^{2} k_{\text{int,m-PR-B}}{}^{4} x_{\text{m-PR-B}}{}^{4}}{Q} \tag{7}$$

Q is defined as:

$$\begin{aligned} Q = {} & 1 + 4k_{\text{int,m-GR}} x_{\text{m-GR}} + 4k_{\text{int,m-PR-B}} x_{\text{m-PR-B}} + 4k_{\text{int,m-GR}}{}^{2} x_{\text{m-GR}}{}^{2} \\ & + 4k_{\text{int,m-PR-B}}{}^{2} x_{\text{m-PR-B}}{}^{2} + 2k_{\text{c,intrra-GR}} k_{\text{int,m-GR}}{}^{2} x_{\text{m-GR}}{}^{2} \\ & + 2k_{\text{c,intrra-PR-B}} k_{\text{int,m-PR-B}}{}^{2} x_{\text{m-PR-B}}{}^{2} + 4k_{\text{c,intrra-GR}} k_{\text{int,m-GR}}{}^{3} x_{\text{m-GR}}{}^{3} \\ & + 4k_{\text{c,intrra-GR}} k_{\text{int,m-GR}}{}^{3} x_{\text{m-GR}}{}^{3} + k_{\text{c,inter-GR}} k_{\text{c,intra-GR}}{}^{2} k_{\text{int,m-GR}}{}^{4} x_{\text{m-GR}}{}^{4} \\ & + k_{\text{c,inter-PR-B}} k_{\text{c,intra-PR-B}}{}^{2} k_{\text{int,m-PR-B}}{}^{4} x_{\text{m-PR-B}}{}^{4} \end{aligned} \tag{8}$$

where x_{m} is the free receptor monomer concentration, $k_{\text{c,inter}}$ is the inter-site cooperativity between receptors bound to adjacent palindromic response elements, $k_{\text{c,intra}}$ is the intra-site cooperativity between two monomers bound to the same response element, and $k_{\text{int,m}}$ is the intrinsic affinity of a monomeric receptor for DNA.

Figure 6D presents a competitive simulation of GR and PR-B monomer binding to a promoter consisting of a single palindromic response element and a half-site. We assume that receptor monomers can cooperatively interact with receptor dimers bound at another palindromic site with a cooperative term equivalent to that of adjacently bound dimers ($k_{\text{c,inter}}$). The following equations were used:

$$P_{\text{GR}} = \frac{k_{\text{c,inter-GR}} k_{\text{c,intra-GR}} k_{\text{int,m-GR}}{}^{3} x_{\text{m-GR}}{}^{3}}{Q} \tag{9}$$

$$P_{\text{PR-B}} = \frac{k_{\text{c,inter-PR-B}} k_{\text{c,intra-PR-B}} k_{\text{int,m-PR-B}}{}^{3} x_{\text{m-PR-B}}{}^{3}}{Q} \tag{10}$$

Q is now defined as:

$$
\begin{aligned}
Q = {} & 1 + 3k_{\text{int,m-GR}}x_{\text{m-GR}} + 3k_{\text{int,m-PR-B}}x_{\text{m-PR-B}} + 2k_{\text{int,m-GR}}{}^{2}x_{\text{m-GR}}{}^{2} \\
& + 2k_{\text{int,m-PR-B}}{}^{2}x_{\text{m-PR-B}}{}^{2} + k_{\text{c,intra-GR}}k_{\text{int,m-GR}}{}^{2}x_{\text{m-GR}}{}^{2} \\
& + k_{\text{c,intra-PR-B}}k_{\text{int,m-PR-B}}{}^{2}x_{\text{m-PR-B}}{}^{2} + k_{\text{c,inter-GR}}k_{\text{c,intra-GR}}k_{\text{int,m-GR}}{}^{3}x_{\text{m-GR}}{}^{3} \\
& + k_{\text{c,inter-PR-B}}k_{\text{c,intra-PR-B}}k_{\text{int,m-PR-B}}{}^{3}x_{\text{m-PR-B}}{}^{3}
\end{aligned}
\tag{11}
$$

where x_{m} is the free receptor monomer concentration, $k_{\text{c,inter}}$ is the inter-site cooperativity between receptors bound to adjacent palindromic response elements, $k_{\text{c,intra}}$ is the intra-site cooperativity between two monomers bound to the same response element, and $k_{\text{int,m}}$ is the intrinsic affinity of a monomeric receptor for DNA.