## Supplementary Material

### Non-monotonic dose response relationship in steroid hormone receptor-mediated gene expression

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### Table S1. Species, initial values, and ODEs for the SHR-mediated gene expression model.

<table>
<thead>
<tr>
<th>Species</th>
<th>Default initial value</th>
<th>Ordinary differential equations</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>60</td>
<td>( \frac{d[L]}{dt} = 0 )</td>
</tr>
<tr>
<td>X</td>
<td>varied</td>
<td>( \frac{d[X]}{dt} = 0 )</td>
</tr>
<tr>
<td>SHR</td>
<td>120</td>
<td>( \frac{d[SHR]}{dt} = -k_{f01}[SHR] \cdot [L] + k_{b01}[LR] - k_{f02}[SHR] \cdot [X] + k_{b02}[XR] )</td>
</tr>
<tr>
<td>LR</td>
<td>0</td>
<td>( \frac{d[LR]}{dt} = k_{f01}[SHR] \cdot [L] - k_{b01}[LR] - 2k_{f11}[LR]^2 ) [ +2k_{b11}[LRRL]-k_{f13}[XR] \cdot [LR]+k_{b13}[LRRX] ]</td>
</tr>
<tr>
<td>XR</td>
<td>0</td>
<td>( \frac{d[XR]}{dt} = k_{f02}[SHR] \cdot [X] - k_{b02}[XR] - 2k_{f12}[XR]^2 ) [ +2k_{b12}[XRRX]-k_{f13}[XR] \cdot [LR]+k_{b13}[LRRX] ]</td>
</tr>
<tr>
<td>LRRL</td>
<td>0</td>
<td>( \frac{d[LRRL]}{dt} = k_{f11}[LR]^2-k_{b11}[LRRL] ) [ -k_{f21}[LRRL]-[HRE]+k_{b21}[LRRLH] ]</td>
</tr>
<tr>
<td>XRRX</td>
<td>0</td>
<td>( \frac{d[XRRX]}{dt} = k_{f12}[XR]^2-k_{b12}[XRRX] ) [ -k_{f22}[XRRX]-[HRE]+k_{b22}[XRRXH] ]</td>
</tr>
<tr>
<td>LRRX</td>
<td>0</td>
<td>( \frac{d[LRRX]}{dt} = k_{f13}[XR] \cdot [LR]-k_{b13}[LRRX] ) [ -k_{f23}[LRRX]-[HRE]+k_{b23}[LRRXH] ]</td>
</tr>
<tr>
<td>HRE</td>
<td>1</td>
<td>( \frac{d[HRE]}{dt} = -k_{f21}[LRRL]-[HRE]+k_{b21}[LRRLH] ) [ -k_{f22}[XRRX]-[HRE]+k_{b22}[XRRXH] ] [ -k_{f23}[LRRX]-[HRE]+k_{b23}[LRRXH] ]</td>
</tr>
<tr>
<td>LRRLH</td>
<td>0</td>
<td>( \frac{d[LRRLH]}{dt} = k_{f21}[LRRL]-[HRE]-k_{b21}[LRRLH] ) [ -k_{f31}[LRRLH]-[CoA]+k_{b31}[CoALRRLH] ]</td>
</tr>
<tr>
<td>XRRXH</td>
<td>0</td>
<td>( \frac{d[XRRXH]}{dt} = k_{f22}[XRRX]-[HRE]-k_{b22}[XRRXH] ) [ -k_{f32}[XRRXH]-[CoA]+k_{b32}[CoAXRRXH] ] [ -k_{f42}[XRRXH]-[CoR]+k_{b42}[CoXRRXH] ]</td>
</tr>
</tbody>
</table>
LRRXH 0 \[
\frac{d[LRRXH]}{dt} = k_{f131}[LRRX][HRE] - k_{b32}[LRRXH] - k_{f31}[LRRXH][CoA] + k_{b31}[CoALRRXH] - k_{f43}[LRRXH][CoR] + k_{b43}[CoRLRRXH]
\]

CoA 60 \[
\frac{d[CoA]}{dt} = -k_{f31}[LRLHL][CoA] + k_{b31}[CoALRLHL] - k_{f32}[XRRXH][CoA] + k_{b32}[CoAXRRXH] - k_{f33}[LRRXH][CoA] + k_{b33}[CoALRRXH]
\]

CoR 60 \[
\frac{d[CoR]}{dt} = -k_{f42}[XRRXH][CoR] + k_{b42}[CoRXRRXH] - k_{f43}[LRRXH][CoR] + k_{b43}[CoRLRRXH]
\]

CoALRLHL 0 \[
\frac{d[CoALRLHL]}{dt} = k_{f31}[LRLHL][CoA] - k_{b31}[CoALRLHL]
\]

CoAXRRXH 0 \[
\frac{d[CoAXRRXH]}{dt} = k_{f32}[XRRXH][CoA] - k_{b32}[CoAXRRXH]
\]

CoALRRXH 0 \[
\frac{d[CoALRRXH]}{dt} = k_{f33}[LRRXH][CoA] - k_{b33}[CoALRRXH]
\]

CoRXRRXH 0 \[
\frac{d[CoRXRRXH]}{dt} = k_{f42}[XRRXH][CoR] - k_{b42}[CoRXRRXH]
\]

CoRLRRXH 0 \[
\frac{d[CoRLRRXH]}{dt} = k_{f43}[LRRXH][CoR] - k_{b43}[CoRLRRXH]
\]

\[GENE_i\] 1 \[
\frac{d[GENE_i]}{dt} = -(k_{f51}[CoALRRH] + k_{f52}[CoAXRRXH] + k_{f53}[CoALRRXH]) \cdot [GENE_i] + (k_{b51} + k_{b52})[CoAXRRXH] + k_{b53}[CoRLRRXH] \cdot [GENE_a]
\]

\[GENE_a\] 0 \[
\frac{d[GENE_a]}{dt} = (k_{f51}[CoALRRH] + k_{f52}[CoAXRRXH] + k_{f53}[CoALRRXH]) \cdot [GENE_i] - (k_{b51} + k_{b52})[CoRXRRXH] + k_{b53}[CoRLRRXH] \cdot [GENE_a]
\]

PT 0 \[
\frac{d[PT]}{dt} = k_{f6}[GENE_a] - k_{f7}[PT] - k_{f8}[PT]
\]

mRNA 0 \[
\frac{d[mRNA]}{dt} = k_{f8}[PT] - k_{f9}[mRNA]
\]

Protein 0 \[
\frac{d[protein]}{dt} = k_{f10}[mRNA] - k_{f11}[protein]
\]

Note: Initial values represent the copy number of species. With the assumption that the nuclear volume is 100 µm³, the equivalent molar concentrations for L, SHR, CoA, and CoR are 1, 2, 1, and 1 nM, respectively. These values are compatible with the concept that transcription factors and coregulators usually exist in low abundance. The reported concentration of SHRs is generally in the nanomolar range [1, 2].

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<table>
<thead>
<tr>
<th>Parameter</th>
<th>Default value (s⁻¹)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{01}, k_{02}$</td>
<td>$1.66 \times 10^{-5}$</td>
<td>When converted back to molar concentration, the association rate constant $1.66 \times 10^{-5}$/s is equivalent to $1 \times 10^{-3}$/nM/s, which is close to the value of $1.3x$ and $1.61 \times 10^{-3}$/nM/s measured between $17\beta$-estradiol and ER in two separate studies [3, 4]. The dissociation rate constant $1 \times 10^{-3}$/s is close to the value of $1.2x$ and $2 \times 10^{-3}$/s reported in the same two studies. The pair of values used here gives a Kd of 1nM.</td>
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<tr>
<td>$k_{001}, k_{002}$</td>
<td>$1 \times 10^{-3}$</td>
<td></td>
</tr>
<tr>
<td>$k_{11}, k_{12}, k_{13}$</td>
<td>$3.0 \times 10^{-7}$</td>
<td>Reported Kd between SHR monomers such as ER ranges from low nM to around 50 nM [5-7]. The pair of values used here gives a Kd of 12 nM. The dissociation rate constant was set according to the average obtained for ER dimers [6].</td>
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<tr>
<td>$k_{21}, k_{22}, k_{23}$</td>
<td>$1.16 \times 10^{-4}$</td>
<td>These values are derived from binding kinetics measured between estradiol-liganded ER dimer and ERE [8, 9]. This pair of values gives a Kd close to 2nM. AR dimer and ARE were reported to have a Kd of 2 nM [10].</td>
</tr>
<tr>
<td>$k_{31}, k_{32}, k_{33}$</td>
<td>$2.32 \times 10^{-5}$</td>
<td>Reported Kd between ER dimer and various coactivators ranges from low nM to several hundred nM [11-18]. The pair of values used here gives a Kd of 10 nM. The dissociation rate constant was set the same as that for the receptor dimer and HRE complex, which gives an average lifetime of 67s, compatible with the rapid exchange observed between coregulators and transcription factors [19, 20].</td>
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<tr>
<td>$k_{41}, k_{42}, k_{43}$</td>
<td>$2.32 \times 10^{-5}$</td>
<td>This pair of default values was assumed the same as that for coactivators.</td>
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<tr>
<td>$k_{51}$</td>
<td>$5.6 \times 10^{-4}$</td>
<td>Chromatin decondensation and condensation, representing gene activation and deactivation, is a slow process [21]. The value of $k_{51}$ gives an average GENEa lifetime of 30 min. The values of other parameters were assumed.</td>
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<tr>
<td>$k_{52}, k_{53}$</td>
<td>$3.34 \times 10^{-2}$</td>
<td></td>
</tr>
<tr>
<td>$k_{54}, k_{55}$</td>
<td>$1.67 \times 10^{-3}$</td>
<td></td>
</tr>
<tr>
<td>$k_6$</td>
<td>$2.78 \times 10^{-3}$</td>
<td>With this value, about 10 primary transcripts (PTs) are produced per gene template per hour.</td>
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<tr>
<td>$k_7$</td>
<td>$3.34 \times 10^{-3}$</td>
<td>The ratio of $k_7/k_8$ was set to 2 to reflect the fact that only about 1/3 of PTs become mature mRNA and the rest are degraded within the nucleus [22]. Additionally, it takes about 10~20 min for PTs to mature and translocate to the cytoplasm as mRNA [22]. The value for $k_8$ used here gives an average 10 min for this process, and $k_7$ was then set according to the ratio.</td>
</tr>
<tr>
<td>$k_8$</td>
<td>$1.67 \times 10^{-3}$</td>
<td></td>
</tr>
<tr>
<td>$k_9$</td>
<td>$3.2 \times 10^{-5}$</td>
<td>Half-life values for luciferase, a common reporter gene, were used. This value gives an mRNA half-life of 6 h. It was numerically derived based on a study in which luciferase mRNA was delivered to B16-F10 cells [23]. This half-life is also about the same as Promega (Madison, WI) provided for firefly luciferase mRNA.</td>
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<tr>
<td>$k_{10}$</td>
<td>$4.17 \times 10^{-2}$</td>
<td>This value gives a translation rate of 150 protein molecules per mRNA template per hour, an average found in eukaryotic cells [22, 24].</td>
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<tr>
<td>$k_{20}$</td>
<td>$6.4 \times 10^{-6}$</td>
<td>This value gives a protein half-life of 3 h, which is in the range of 50 min to 3.68 h for firefly luciferase in mammalian cells [25-28].</td>
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</tbody>
</table>
References


