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Supplementary Data
Stability versus exchange: A paradox in DNA replication

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MASTER EQUATION SYSTEMS

Our model is formulated in terms of master equations, which have the virtue that the mean life-time of any state can be written as the solution to a simple (albeit elaborate) linear equation. We used Mathematica (Wolfram Research, Inc.; Champaign, Illinois) to solve all equations. See the Methods section of the main text for details of the approach; here we only report the equations that are too lengthy to reproduce in the main text.

Stability under Dilution

The full reaction scheme for the stability under dilution is shown in Fig. S1. The corresponding master equation system, Equation (1), can be written in matrix form as

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{pmatrix}P_\emptyset\\P_{01}\\P_{10}\\P_{11}\end{pmatrix}=\begin{pmatrix}0 & k_\mathrm{off} & k_\mathrm{off} & 0\\0 & -(Nr_{12}+k_\mathrm{off}) & 0 & k_\mathrm{off}\\0 & 0 & -(r_{12}+k_\mathrm{off}) & k_\mathrm{off}\\0 & Nr_{12} & r_{12} & -2k_\mathrm{off}\end{pmatrix}\begin{pmatrix}P_\emptyset\\P_{01}\\P_{10}\\P_{11}\end{pmatrix} \tag{S1}$$

The 4×4 matrix is the K defined by Equation (3) in the main text, and solving Equation (2) in the main text shows that the mean life-time from the 11 state is given by

$$\tau_d=\frac{Nr_{12}^2+2(N+1)r_{12}k_\mathrm{off}+3k_\mathrm{off}^2}{k_\mathrm{off}^2\left((N+1)r_{12}+2k_\mathrm{off}\right)} \tag{S2}$$

as used in the main text, Equation (4).

It may be of some interest to generalize this analysis to the case of completely arbitrary rate constants (Fig. S2) for which the master equation system [Equation (1)] can be written in matrix form as

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{pmatrix}P_\emptyset\\P_{01}\\P_{10}\\P_{11}\end{pmatrix}=\begin{pmatrix}0 & k_{2,\mathrm{off}} & k_{1,\mathrm{off}} & 0\\0 & -(Nr_{21}+k_{2,\mathrm{off}}) & 0 & r_{21,\mathrm{off}}\\0 & 0 & -(r_{12}+k_{1,\mathrm{off}}) & r_{12,\mathrm{off}}\\0 & Nr_{21} & r_{12} & -(r_{21,\mathrm{off}}+r_{12,\mathrm{off}})\end{pmatrix}\begin{pmatrix}P_\emptyset\\P_{01}\\P_{10}\\P_{11}\end{pmatrix} \tag{S3}$$

Again, the 4×4 matrix is the K defined by Equation (3) in the main text, and solving Equation (2) in the main text shows that the mean life-time from the 11 state is given by

$$\tau_d=\frac{(k_{2,\mathrm{off}}+Nr_{21})(r_{12}+r_{12,\mathrm{off}})+(k_{1,\mathrm{off}}+r_{12})(Nr_{21}+r_{21,\mathrm{off}})+k_{1,\mathrm{off}}k_{2,\mathrm{off}}-Nr_{12}r_{21}}{k_{1,\mathrm{off}}r_{12,\mathrm{off}}Nr_{21}+k_{2,\mathrm{off}}r_{12}r_{21,\mathrm{off}}+k_{1,\mathrm{off}}k_{2,\mathrm{off}}(r_{12,\mathrm{off}}+r_{21,\mathrm{off}})}. \tag{S4}$$

For the special case that binding and unbinding to both the primary and secondary sites occur with equal rates ($r_{12}=r_{21}\equiv r_{12}$, $r_{12,\mathrm{off}}=r_{21,\mathrm{off}}\equiv r_{12,\mathrm{off}}$ and $k_{1,\mathrm{off}}=k_{2,\mathrm{off}}=k_\mathrm{off}$) but unbinding from one site while still remaining bound to one is not

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necessarily equivalent to unbinding from a single site ($r_{12,\mathrm{off}}$ not necessarily equal to k_{off}) we find that the mean life-time is given by

$$\tau_d = \frac{k_{\mathrm{off}}^2 + k_{\mathrm{off}}\big((N+1)r_{12} + 2r_{12,\mathrm{off}}\big) + r_{12}\big((N+1)r_{12,\mathrm{off}} + N r_{12}\big)}{k_{\mathrm{off}} r_{12,\mathrm{off}}\big((N+1)r_{12} + 2k_{\mathrm{off}}\big)}. \tag{S5}$$

In the limit that k_{off}/r_{12} and $r_{12,\mathrm{off}}/r_{12}$ are small, we find the expansion

$$\tau_d = \frac{r_{12}}{k_{\mathrm{off}} r_{12,\mathrm{off}}}\left(\frac{N}{N+1} + \cdots\right); \tag{S6}$$

while in the limit r_{12}/k_{off} and $r_{12}/r_{12,\mathrm{off}}$ being small, we find

$$\tau_d = \frac{1}{2}\left(\frac{2}{k_{\mathrm{off}}} + \frac{1}{r_{12,\mathrm{off}}} + \cdots\right). \tag{S7}$$

Stability under Competition for a Single Primary Binding Site ($N=1$)

The full reaction scheme for the stability under competition for a single primary binding site is shown in Fig. S4. The corresponding master equation system [Equation (1)] can be written in matrix form as

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{pmatrix} P_{\emptyset} \\ P_{01} \\ P_{10} \\ P_{11} \\ P_{12} \\ P_{21} \end{pmatrix} = \begin{pmatrix} 0 & k_{\mathrm{off}} & k_{\mathrm{off}} & 0 & k_{\mathrm{off}} & k_{\mathrm{off}} \\ 0 & -(r_{\mathrm{on}}+r_{12}+k_{\mathrm{off}}) & 0 & k_{\mathrm{off}} & 0 & k_{\mathrm{off}} \\ 0 & 0 & -(r_{\mathrm{on}}+r_{12}+k_{\mathrm{off}}) & k_{\mathrm{off}} & k_{\mathrm{off}} & 0 \\ 0 & r_{12} & r_{12} & -2k_{\mathrm{off}} & 0 & 0 \\ 0 & 0 & r_{\mathrm{on}} & 0 & -2k_{\mathrm{off}} & 0 \\ 0 & r_{\mathrm{on}} & 0 & 0 & 0 & -2k_{\mathrm{off}} \end{pmatrix}\begin{pmatrix} P_{\emptyset} \\ P_{01} \\ P_{10} \\ P_{11} \\ P_{12} \\ P_{21} \end{pmatrix} \tag{S8}$$

Again, the 6×6 matrix is the K defined by Equation (3) in the main text, and solving Equation (2) in the main text shows that the mean life-time from the 11 state is given by

$$\tau_c = \frac{3r_{\mathrm{on}} + 2r_{12} + 6k_{\mathrm{off}}}{2k_{\mathrm{off}}\,(r_{\mathrm{on}} + 2k_{\mathrm{off}})} \tag{S9}$$

as used in the main text [Equation (6)].

Stability under Competition for Two Primary Binding Sites ($N=2$)

The full reaction scheme for the stability under competition for two primary binding sites is shown in Fig. S5. The corresponding master equation system [Equation (1)] can be written in matrix form as

$$\frac{\mathrm{d}P}{\mathrm{d}t} = KP \tag{S10}$$

where

$$P = (P_{\emptyset}\ P_{001}\ P_{010}\ P_{011}\ P_{021}\ P_{120}\ P_{012}\ P_{121}\ P_{221}\ P_{122})^T \tag{S11}$$

and

$$K=\begin{pmatrix}
0 & k_{\mathrm{off}} & k_{\mathrm{off}} & 0 & k_{\mathrm{off}} & \ldots \\
\ldots & k_{\mathrm{off}} & k_{\mathrm{off}} & 0 & k_{\mathrm{off}} & k_{\mathrm{off}} \\[1ex]
0 & -(2r_{\mathrm{on}}+2r_{12}+k_{\mathrm{off}}) & 0 & k_{\mathrm{off}} & k_{\mathrm{off}} & \ldots \\
\ldots & 0 & 0 & 0 & 0 & 0 \\[1ex]
0 & 0 & -(2r_{\mathrm{on}}+r_{12}+k_{\mathrm{off}}) & k_{\mathrm{off}} & 0 & \ldots \\
\ldots & k_{\mathrm{off}} & k_{\mathrm{off}} & 0 & 0 & 0 \\[1ex]
0 & 2r_{12} & r_{12} & -(r_{\mathrm{on}}+2k_{\mathrm{off}}) & 0 & \ldots \\
\ldots & 0 & 0 & k_{\mathrm{off}} & 0 & 0 \\[1ex]
0 & 2r_{\mathrm{on}} & 0 & 0 & -(r_{\mathrm{on}}+r_{12}+2k_{\mathrm{off}}) & \ldots \\
\ldots & 0 & 0 & k_{\mathrm{off}} & 2k_{\mathrm{off}} & 0 \\[1ex]
0 & 0 & r_{\mathrm{on}} & 0 & 0 & \ldots \\
\ldots & -(r_{\mathrm{on}}+2r_{12}+2k_{\mathrm{off}}) & 0 & k_{\mathrm{off}} & 0 & k_{\mathrm{off}} \\[1ex]
0 & 0 & r_{\mathrm{on}} & 0 & 0 & \ldots \\
\ldots & 0 & -(r_{\mathrm{on}}+r_{12}+2k_{\mathrm{off}}) & 0 & 0 & k_{\mathrm{off}} \\[1ex]
0 & 0 & 0 & r_{\mathrm{on}} & r_{12} & \ldots \\
\ldots & r_{12} & 0 & -3k_{\mathrm{off}} & 0 & 0 \\[1ex]
0 & 0 & 0 & 0 & r_{\mathrm{on}} & \ldots \\
\ldots & 0 & 0 & 0 & -3k_{\mathrm{off}} & 0 \\[1ex]
0 & 0 & 0 & 0 & 0 & \ldots \\
\ldots & r_{\mathrm{on}}+r_{12} & r_{\mathrm{on}}+r_{12} & 0 & 0 & -3k_{\mathrm{off}}
\end{pmatrix} \tag{S12}$$

Note that this is a 10x10 matrix and the ellipses (…) signify that the row continues on the next line.

Solving Equation (2) in the main text with the K defined by Equation (S12) gives the mean life-time from the 011 state as a rather eloborate expression. It can be written in the form

$$\tau_c k_{\mathrm{off}}=\frac{n}{d} \tag{S13}$$

where the numerator is given by

$$\begin{aligned}
n=&\,648k_{\mathrm{off}}^6+108k_{\mathrm{off}}^5(20r_{12}+19r_{\mathrm{on}})+18k_{\mathrm{off}}^4\left(138r_{12}^2+322r_{12}r_{\mathrm{on}}+149r_{\mathrm{on}}^2\right)+\\
&+2r_{\mathrm{on}}(r_{12}+r_{\mathrm{on}})^2\left(12r_{12}^3+37r_{12}^2r_{\mathrm{on}}+29r_{12}r_{\mathrm{on}}^2+6r_{\mathrm{on}}^3\right)+\\
&+3k_{\mathrm{off}}^3\left(420r_{12}^3+1854r_{12}^2r_{\mathrm{on}}+2044r_{12}r_{\mathrm{on}}^2+617r_{\mathrm{on}}^3\right)+\\
&+k_{\mathrm{off}}^2\left(288r_{12}^4+2307r_{12}^3r_{\mathrm{on}}+4528r_{12}^2r_{\mathrm{on}}^2+3193r_{12}r_{\mathrm{on}}^3+711r_{\mathrm{on}}^4\right)+\\
&+k_{\mathrm{off}}\left(24r_{12}^5+410r_{12}^4r_{\mathrm{on}}+1301r_{12}^3r_{\mathrm{on}}^2+1588r_{12}^2r_{\mathrm{on}}^3+817r_{12}r_{\mathrm{on}}^4+144r_{\mathrm{on}}^5\right)
\end{aligned} \tag{S14}$$

and the denominator by

$$\begin{aligned}
d=&432k_{\mathrm{off}}^{6}+72k_{\mathrm{off}}^{5}(17r_{12}+19r_{\mathrm{on}})+\\
&+2r_{\mathrm{on}}(3r_{12}+r_{\mathrm{on}})\left(r_{12}^{2}+3r_{12}r_{\mathrm{on}}+2r_{\mathrm{on}}^{2}\right)^{2}+\\
&+12k_{\mathrm{off}}^{4}\left(90r_{12}^{2}+275r_{12}r_{\mathrm{on}}+149r_{\mathrm{on}}^{2}\right)+\\
&+2k_{\mathrm{off}}^{3}\left(174r_{12}^{3}+1246r_{12}^{2}r_{\mathrm{on}}+1755r_{12}r_{\mathrm{on}}^{2}+617r_{\mathrm{on}}^{3}\right)+\\
&+k_{\mathrm{off}}r_{\mathrm{on}}\left(105r_{12}^{4}+476r_{12}^{3}r_{\mathrm{on}}+749r_{12}^{2}r_{\mathrm{on}}^{2}+474r_{12}r_{\mathrm{on}}^{3}+96r_{\mathrm{on}}^{4}\right)+\\
&+3k_{\mathrm{off}}^{2}\left(12r_{12}^{4}+256r_{12}^{3}r_{\mathrm{on}}+694r_{12}^{2}r_{\mathrm{on}}^{2}+613r_{12}r_{\mathrm{on}}^{3}+158r_{\mathrm{on}}^{4}\right)
\end{aligned} \tag{S15}$$

as used in the main text (Fig. 4d).

STABILITY UNDER DILUTION IN THE LIMIT OF RAPID RE-BINDING

We may understand the dissociation time under dilution in the limit of rapid re-binding, Equation (5), following an analogous reasoning to the Briggs-Haldane approximation to the Michaelis-Menten mechanism. Thus, in the limit of rapid re-binding, it is reasonable to assume that, after some time, the probability, P_{10}, of having a complex with a molecule bound to only a primary site is roughly constant and likewise for the probability, P_{01}, of having a complex with a molecule bound to the secondary site. Using Equation (S1) this assumption implies that

$$0=\frac{\mathrm{d}P_{01}}{\mathrm{d}t}=-(Nr_{12}+k_{\mathrm{off}})P_{01}+k_{\mathrm{off}}P_{11} \tag{S16}$$

$$0=\frac{\mathrm{d}P_{10}}{\mathrm{d}t}=-(r_{12}+k_{\mathrm{off}})P_{10}+k_{\mathrm{off}}P_{11} \tag{S17}$$

from which we can derive expressions for P_{01} and P_{10} in terms of P_{11}.

The rate of change of the probability, P_{00}, of having a complex with no molecules bound then becomes

$$\frac{\mathrm{d}P_{\emptyset}}{\mathrm{d}t}=k_{\mathrm{off}}(P_{01}+P_{10})=k_{\mathrm{off}}^{2}\left(\frac{1}{Nr_{12}+k_{\mathrm{off}}}+\frac{1}{r_{12}+k_{\mathrm{off}}}\right)P_{11} \tag{S18}$$

where the first equality is just Equation (S1) and the second follows from Equations (S16)-(S17). In Equation (S18) we may identify the factor in front of P_{11} as the total off rate and thus

$$\tau_{d}^{-1}=k_{\mathrm{off}}^{2}\left(\frac{1}{Nr_{12}+k_{\mathrm{off}}}+\frac{1}{r_{12}+k_{\mathrm{off}}}\right) \tag{S19}$$

In the limit of r_{12}/k_{off} large this reduces to Equation (5).

Table S1. Dissociation time in the presence and absence of competing molecules for an example set of kinetic parameters.

				τ_d		
N	k_{off}	r_{12}	c_{bulk}	w/o multiple sites	w/ multiple sites	τ_c
1	$0.1\mathrm{s}^{-1}$	$100\mathrm{s}^{-1}$	$100K_d$	10s	1.39h	1.88 min

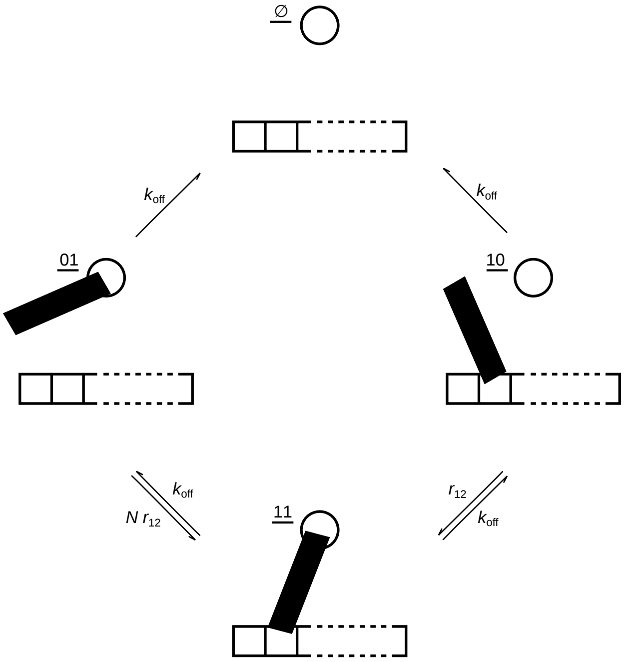

Figure S1. Full reaction scheme for stability under dilution. Reaction rates are defined in Fig. 2. The initially bound molecule is shown in black.

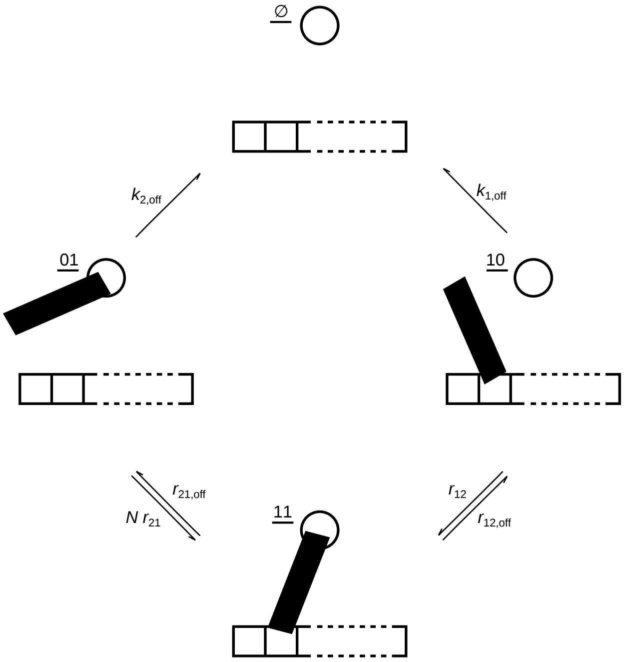

Figure S2. Full reaction scheme for stability under dilution with completely general kinetic rates. The initially bound molecule is shown in black.

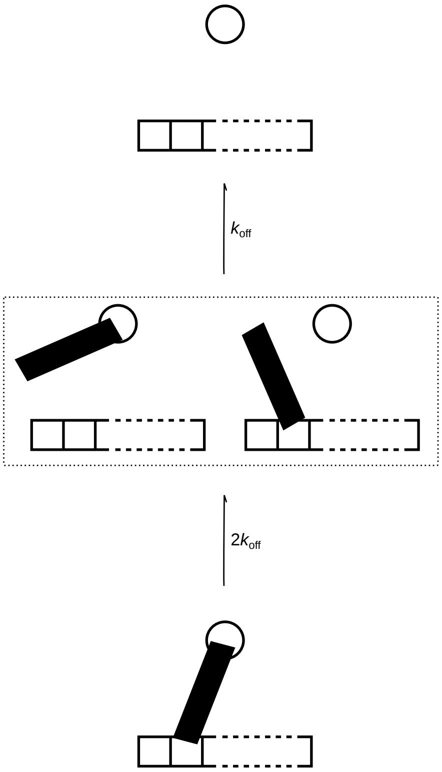

Figure S3. Reaction scheme explaining the results for the mean life-time under dilution in the limit of fast unbinding. Reaction rates are defined in Fig. 2. In the limit of fast unbinding we can neglect the binding processes of the full reaction scheme (*cf.* Fig. S1). Furthermore, the two states where the molecule is bound to only one of the binding site types can be combined (dotted box) giving a factor of 2 in the transition from the initial state. It is then clear that the mean time of the first transition is given by $(2k_{\rm off})^{-1}$, while the mean time of the second is given by $k_{\rm off}^{-1}$. The mean time for the full process of unbinding is given by the sum of these times, because they occur in succession. That is, the mean time for complete unbinding is given by $(2k_{\rm off})^{-1}+k_{\rm off}^{-1}=\frac{3}{2}k_{\rm off}^{-1}$, fully in line with the results presented in the main text for the limit of fast unbinding.

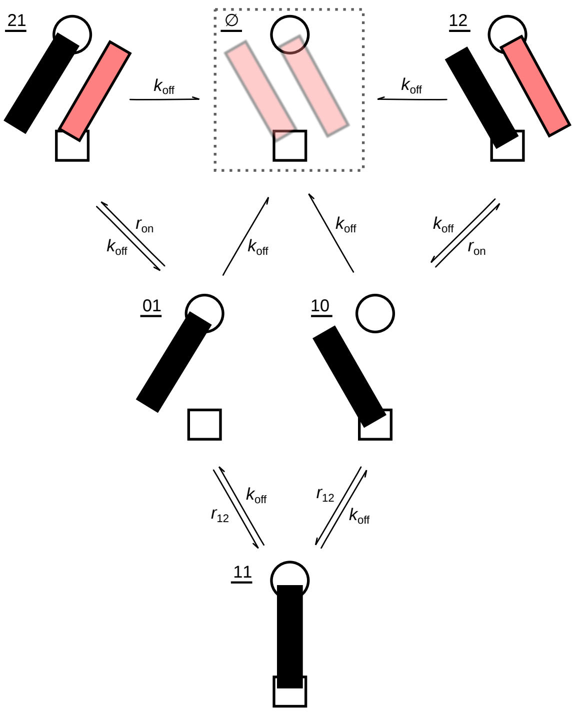

Figure S4. Full reaction scheme for stability under competition from other binding molecules with a single primary binding site ($N=1$). The initially bound molecule is shown in black, while competing molecules are shown in light red. Reaction rates are defined in Fig. 2. (Dotted rectangle) All states without the initially bound molecule (the state with no bound molecule, the state with a competing molecule bound to the upper site and the state with a competing molecule bound to the lower site).

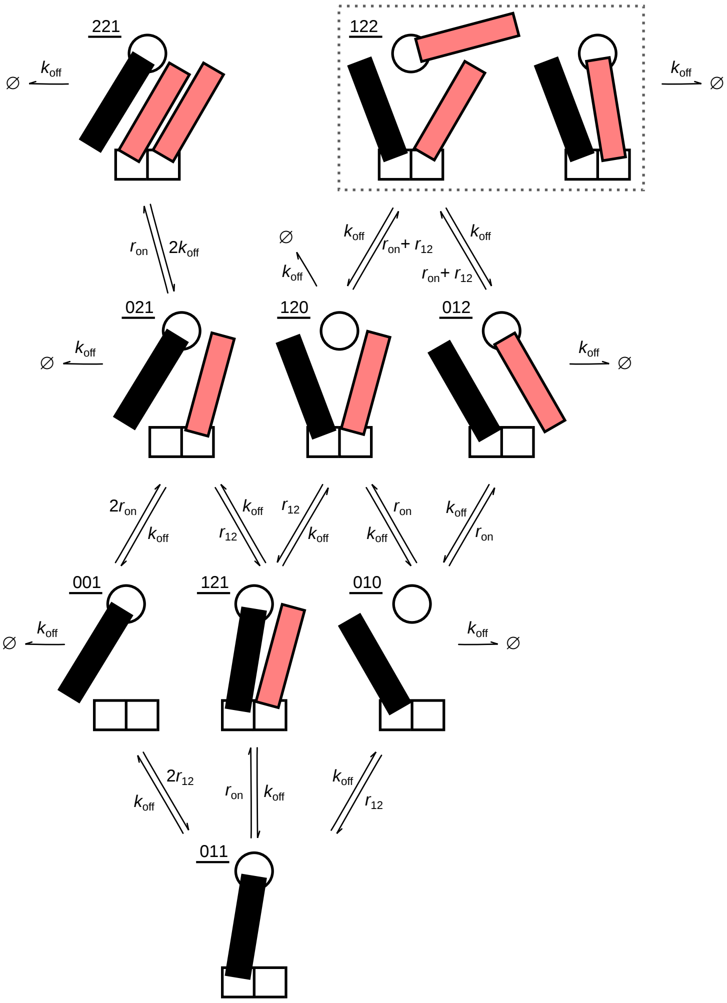

Figure S5. Full reaction scheme for stability under competition from other binding molecules for two primary binding sites ($N = 2$). The initially bound molecule is shown in black, while competing molecules are shown in light red. Reaction rates are defined in Fig. 2. All states without the initially bound molecule are collectively denoted by $\emptyset$ and transitions to this state are drawn separately to avoid reactions crossing over each other. (Dotted rectangle) Two states collected together.