Supporting Material

Pleomorphic ensembles: formation of large clusters composed of weakly interacting multivalent molecules

Authors: C. V. Falkenberg[1]*, M. L. Blinov[1], L. M. Loew[1]*

Affiliations:

[1] Richard D. Berlin Center for Cell Analysis and Modeling, University of Connecticut Health Center, Farmington, Connecticut, United States of America.

The classical F-S theory:

The theory is described in greater detail in (1-4). Below we list the equations relevant to this manuscript.
Mole fraction, M_{fx}, of x-mers formed by x monomers "A" according to Flory-Stockmayer theory:

$$M_{f\,x} = {}^{N_x}\!\Big/\!\left(N_0\left(1 - p_A\ m/2\right)\right)$$

Where

$$N_x = N_0 \frac{m\,(m\,x - x)!}{(m\,x - 2\,x + 2)!\,x!}\ p_A^{x-1}\left(1 - p_A\right)^{m\,x - 2\,x + 2}$$

Where N_0 is the total number of monomeric units of "A" in the system. Each monomeric unit has valency m. The same expressions are valid for 'symmetric systems' with identical number of molecules A and B, each with valency m. In this case, each site in any molecule A can only bind to sites in molecules type B. The equation for the identification of critical degree of reaction, or percolation threshold, to reach the sol-gel transition in a symmetric equal reactivity system is:

$$p_{A,c} = (m-1)^{-1}$$

The equation for the identification of the sol-gel transition in asymmetric equal reactivity systems (2), with valencies m and n for A and B, respectively:

$$\left(p_A\ p_B\right)_{gel} = (m-1)^{-1}(n-1)^{-1}$$

The critical degree of reaction is valid for both kinetic or equilibrium systems. The critical concentration for reaching the sol-gel transition corresponds to $p_A(t{=}\infty) = p_{A,c}$ for the symmetric system. The critical pair of concentrations is identified by the product $p_A(t{=}\infty)\ p_B(t{=}\infty) = (p_A\ p_B)_{\mathrm{gel}}$ for the asymmetric system.

Given the concentrations c_A and c_B of molecules **A** and **B** respectively, the corresponding valencies m and n, and the binding and unbinding rate constants k_+ and k_-, the critical concentrations are identified by applying the equilibrium condition to the system of equations below. We first define the probabilities of bond for each site type:

$$p_A(t) = \frac{s_{A,b}(t)}{s_{A,b}(t) + s_{A,f}(t)}$$

$$p_B(t) = \frac{s_{B,b}(t)}{s_{B,b}(t) + s_{B,f}(t)}$$

Where the time dependent variables $s_{A,f}$, $s_{A,b}$, $s_{B,f}$, $s_{B,b}$ represent the concentrations of sites type A that are free and bound, and concentration of sites type B that are free and bound, respectively.

All variables are identified by conservation of mass:

$$s_{A,b}(t) = s_{B,b}(t)$$
$$s_{A,b}(t) + s_{A,f}(t) = m\, c_A$$
$$s_{B,b}(t) + s_{B,f}(t) = n\, c_B$$
$$s_{A,f}(t) = m\, c_A - s_{A,b}(t)$$
$$s_{B,f}(t) = n\, c_B - s_{B,b}(t)$$
$$\frac{\partial s_{A,f}(t)}{\partial t} = -\frac{\partial s_{A,b}(t)}{\partial t} = \frac{\partial s_{B,f}(t)}{\partial t} = -\frac{\partial s_{B,b}(t)}{\partial t}$$

$$\frac{\partial s_{A,b}(t)}{\partial t} = k_+\, s_{A,f}(t)\, s_{B,f}(t) - k_-\, s_{A,b}(t)$$

For the convenience of scientific community, we have implemented the classic F-S equations for prediction of equilibrium sol-gel transition diagrams in an openly accessible web based tool at http://vcell.org/SimGel/. But it should be noted that this tool does not implement the new algorithm which is the primary subject of this paper.

Clustering Algorithm

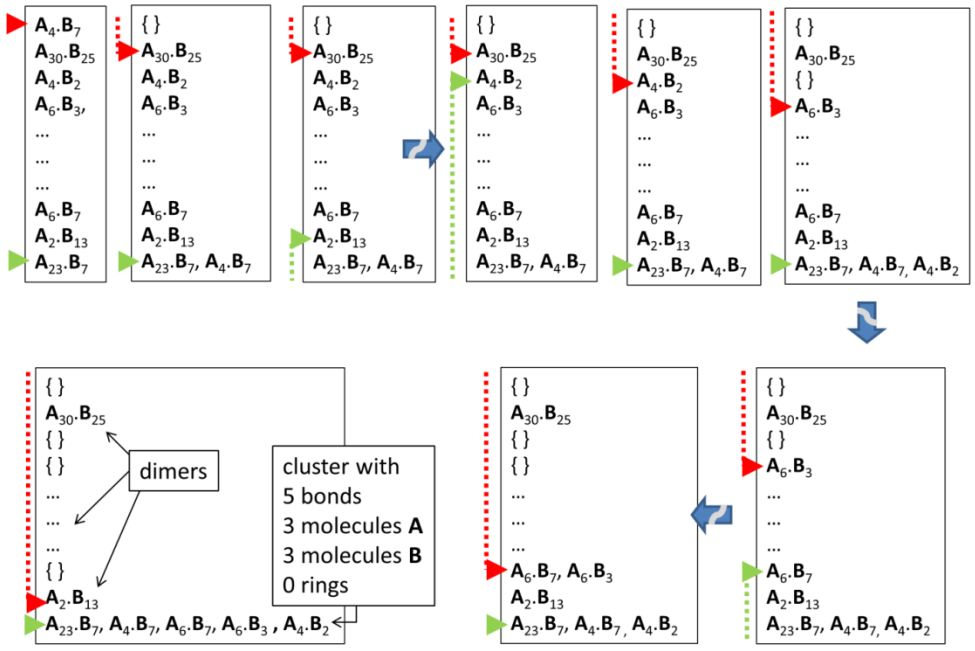

FIGURE S1 Clustering algorithm. Each box in this illustrative example represents the vector of bonds at consecutive iterative steps (clockwise). At each step, the elements of the vector identified by the arrowheads are compared. The bottom arrowhead (green) moves upwards until there is a common molecule in both elements, or it reaches the position of the upper arrowhead (red). If the first test is true, bonds from the element marked by the upper arrowhead are added to the element marked by the lower arrowhead (first step). Large broken blue arrows indicate that intermediate steps are omitted.

The ODE's for TLBR system:

$$\frac{\partial L_1}{\partial t} = 3\ k_{1+}\ L_0\ sR - k_-\ L_1 - 2\ k_{2+}\ L_1\ sR + 2\ k_-\ L_2$$

$$\frac{\partial L_2}{\partial t} = 2\ k_{2+}\ L_1\ sR - 2\ k_-\ L_2 - k_{2+}\ L_2\ sR + 3\ k_-\ L_3$$

$$\frac{\partial L_3}{\partial t} = k_{2+}\ L_2\ sR - 3\ k_-\ L_3$$

Where L_l represents the concentration of ligands with l bonds, and conservation of mass dictates that the total concentration of ligands $c_L = L_0 + L_1 + L_2 + L_3$. The fractions $\rho_{L,l}$ are the ratios between the concentrations of ligands with l bonds and the total ligand concentration:

$$\rho_{L,l} = {L_l}/{c_L} \quad , \qquad l = 0,...,3$$

The total concentration of receptors is $c_{\mathbf{R}}$ and the concentration of free receptor sites sR is obtained from the conservation of mass:

$$sR = 2\ c_R - c_L \sum_{l=1}^{3} (l\ \rho_{L,l})$$

Comparisson of the model with F-S theory

Using our method (Fig.S2), we compared our simulation results with both the analytical solutions and numerical solutions from (4). All three solutions agree up to $p_{A,c}$, which is shown with a vertical line (solid arrowhead). This is consistent with the Falk and Thomas derivations that in a system with a large number of molecules the probability of occurrence of rings is negligible when $p_{A<}\ p_{A,c}$.

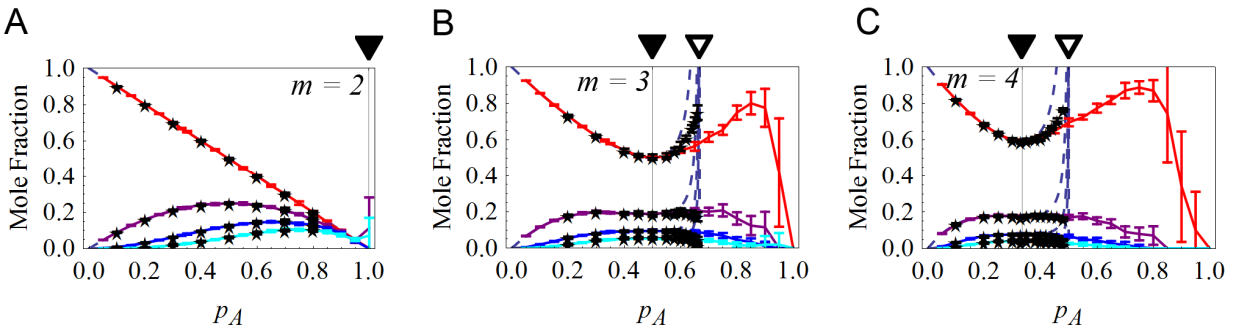

FIGURE S2 Mole fraction for x-mers (curves from top to bottom, x=1 to x=4) for systems with two to four binding sites (m=2 to m=4). Dashed line: analytical solution for mole fraction (3); stars: numerical solution for rings forbidden model (4); solid lines: Hybrid method solution. Each point corresponds to the mean of ten simulations of a symmetric system with 10,000 molecules total. Arrowheads indicate $p_{A,c}$ (solid) and $p_{A,max}$ (open).

Simulation size vs. theory

We compare the simulations and theory over the full range of numerically occurring clusters sizes. In Fig. S3 A-B the results of several simulations are superposed. The lower bound for each mole fraction as a function of simulation size (and m and p_A) is explicit: simulations with larger number of molecules are able to populate a wider range of cluster sizes, which occur at lower frequency. The averaged results over the several simulations are showed in Fig. S3 C-D. Our simulations report slightly higher mean frequency of large clusters than the theory. Similar observations were reported by Sciorino and colleagues when using a Monte Carlo method for 3D systems with 1000 molecules (5). In that study, Flory's theory was compared to systems with combinations of molecules of valencies 2 and 3, and deviations were attributed to a higher mean

valency of the system (larger fraction of trivalent molecules). Here we show that for our model, the agreement between the theory and the numerical solution can be maintained over a wider range of cluster sizes by increasing the size of the system (fivefold increase in size in blue vs. green curves in Fig.S3C-D) or the number of independent simulations used for the averaging (fivefold increase in blue vs. red). The results are consistent for both valencies 2 and 3.

Note that in computational models (or systems with limited number of molecules) the lower bound for the value of the mole fraction in each time point is the inverse of the system size. Consequently, the cluster size distribution is sensitive to the total number of molecules in the system, with greater impact on clusters of lower frequency. It is important to keep in mind that the simulation results are sensitive to the system size as p_A approaches $p_{A,c}$. Meaning, that if the biological system of interest lies on this region, and is composed by an infinitely large number of molecules, the absolute value of the largest cluster in the simulation cannot be trusted. However, for biological systems with a limited number of molecules, the stochastic numerical results may represent a better solution than the analytical theory.

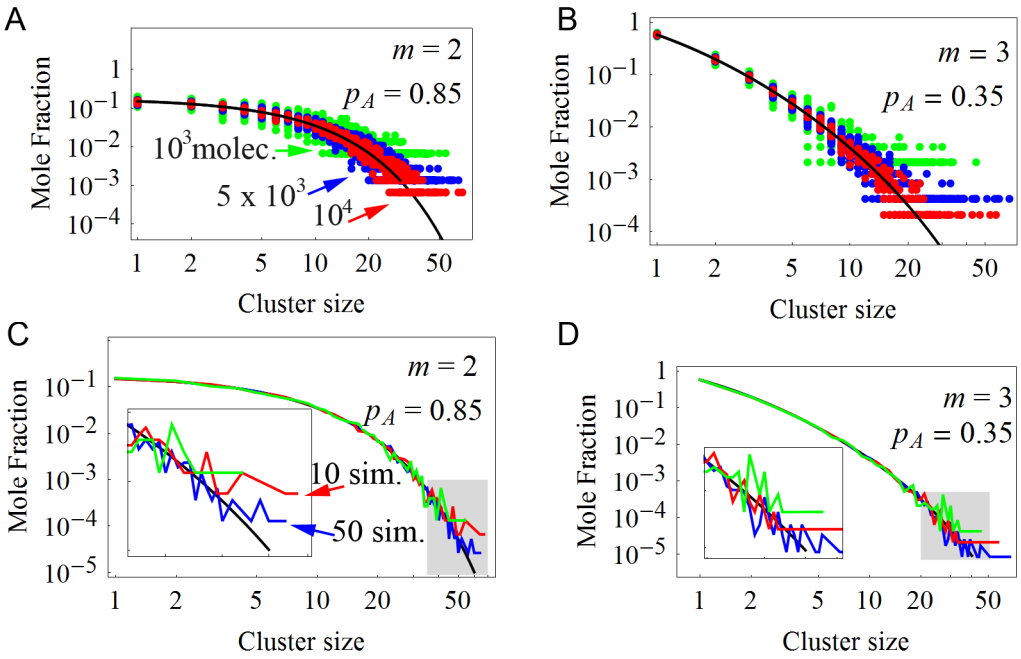

FIGURE S3 Impact of simulation size and number of simulations in the cluster size distribution when $p_A < p_{A,c}$. A-B. Superposition of results for all 10, 50 and 50 simulations with 10000 molecules (red), 5000 (blue) and 1000 (green) molecules, respectively. C-D. Corresponding mean values. Solid black line: analytical solution. Insets correspond to regions in the gray box. A and C, valency m=2. B and D, valency m=3.

Efficiency of the clustering algorithm

The Step 3 of our method was developed so that it is most efficient when the probability of bonds exceeds the percolation threshold. This step is the heaviest part of the algorithm. The

molecules present in each bond will be sequentially compared to the other objects in the list of bonds up to the point when both objects share a common molecule. In a system of monovalent molecules, the largest possible cluster is a dimer and the computational cost scales with the square of the total number of bonds. On the other extreme, systems with high valency and with $p_A > p_{A,c}$, every time a new object is picked from the list of bonds, there is a significant chance that the molecules belonging to this object will also be present in a previously identified cluster. If that is the case, a smaller number of comparisons are necessary.

We use the system with two m-valent molecule types, **A** and **B**, discussed in the manuscript's section "The occurrence of rings in the largest cluster robustly identifies the sol-gel transition" to illustrate the efficiency of the solver. In Fig.S4 we show that once the percolation threshold is met, the computational cost decreases exponentially with the valency of the system. This reduction is the explicit numerical advantage of the assumption that ring formation in the gel phase obeys the exact same rules in both sol and gel phases, therefore no reactions are rejected.

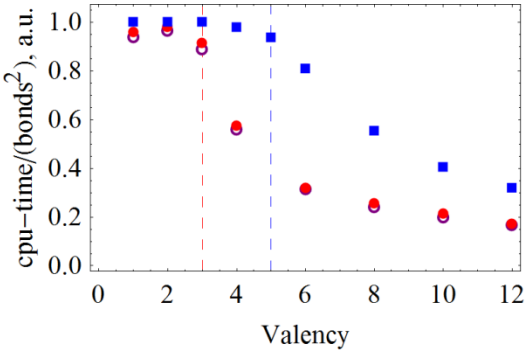

FIGURE S4 Computational cost as a function of number of bonds and valency. The simulation cost scales with the square of the total number of bonds. The ratio cpu-time/(bonds^2) is equivalent for two systems with p_A=0.5 and concentrations 12000 molecules/valency (open circle) or 6000 molecules/valency (solid circles). p_A=0.5 corresponds to the percolation threshold for valency m=3. The solid squares represent the computational cost for simulations with 12000 molecules/valency and p_A=0.25, which matches the percolation threshold for m=5.

Sol-Gel transition for $SH3_3$ and PRM_5 system and the properties of the largest cluster

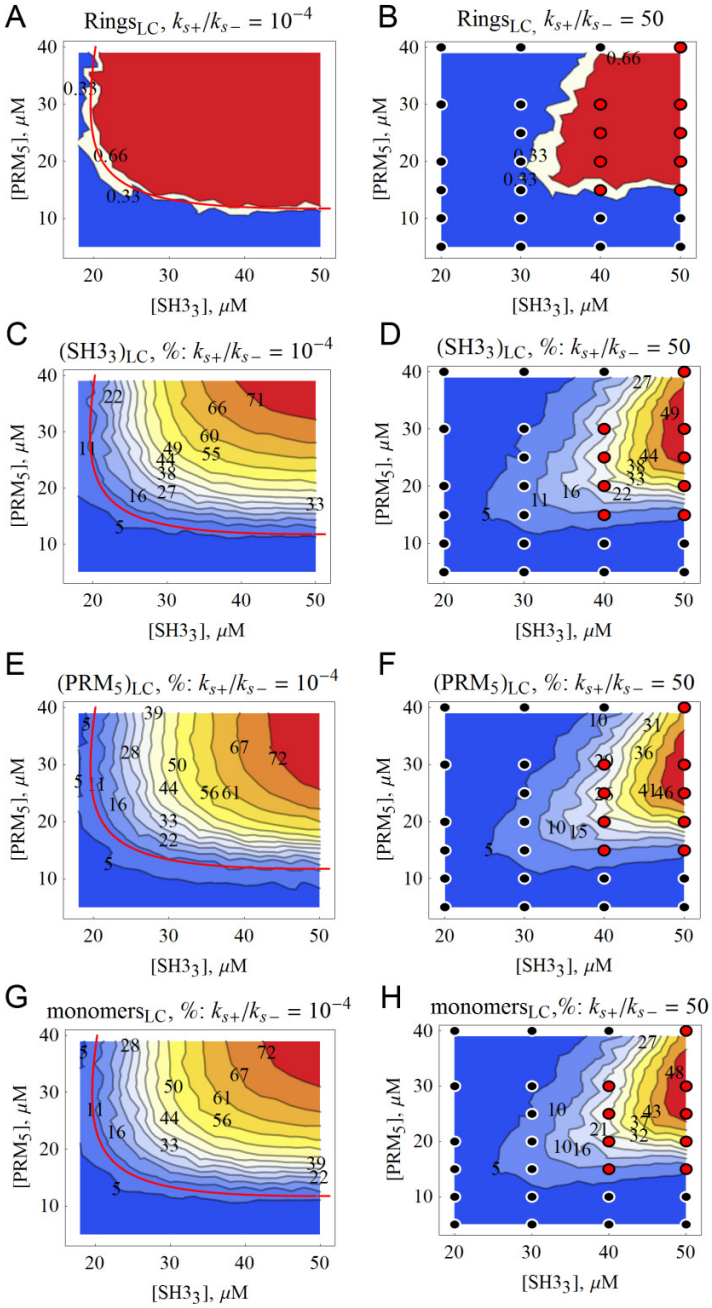

FIGURE S5 Properties of the largest cluster for the trivalent $SH3_3$ and pentavalent PRM_5 system. The average number of rings in the largest cluster (A) is the most robust predictor of the theoretical sol-gel transition interface (red line). The fraction of $SH3_3$ molecules (C), PRM_5 molecules (D) and of all molecules (G) that belong to the largest cluster ranges from 5-11% at the interception with the theoretical curve. B, D, F and H correspond to the same outputs for high synergistic interaction (k_{s+}/k_{s-} =50), superposed with the experimental results from (6).

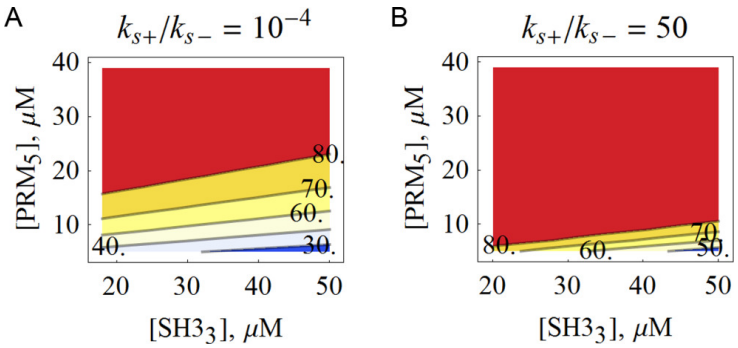

FIGURE S6 Percentage of $SH3_3$ that is bound. Includes small and large clusters, for a system with $SH3_3$ and PRM_5 only. A strong synergistic interaction (B) dramatically reduces the fraction of free monomeric $SH3_3$ in comparison to a system subject to 'equal reactivity' (A).

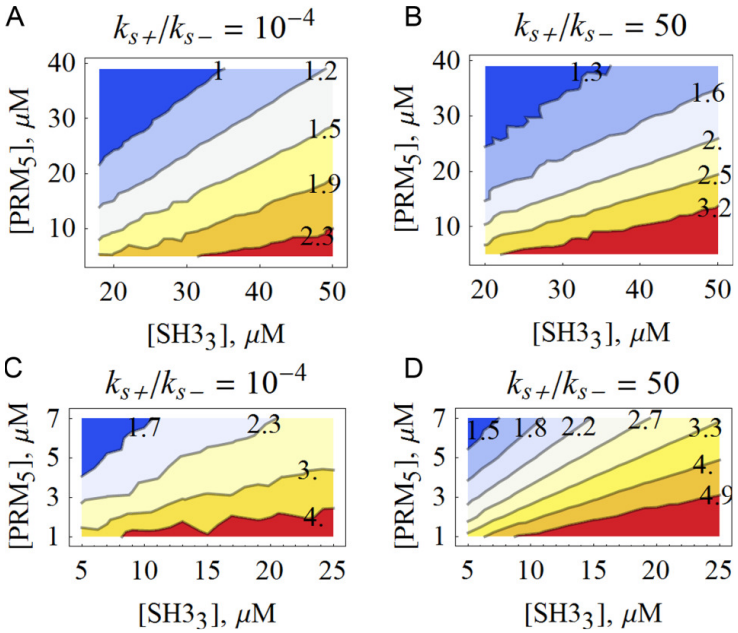

FIGURE S7 Stoichiometry of the largest cluster. Ratio between number of molecules $SH3_3/PRM_5$ in the largest cluster for a system with $SH3_3$ and PRM_5 only (A-B) and with addition of 3 μM of pY_3 (C-D).

The contour plots in Figs.4 and Figs.S5-S7 were generated using 1st order interpolation of the means of 20 simulations per concentration pair. Concentration intervals consist of 2 μM for systems with $SH3_3$ and PRM_5 only (Fig. 4B-C, Fig.S5-S6 and Fig.S7A-B. Concentration intervals consist of 1 μM for PRM_5 concentrations and 2 μM for $SH3_3$ concentrations for Fig.4D-G and Fig.S7C-D.

Supporting References

1. Stockmayer, W. H. 1943. Theory of molecular size distribution and gel formation in branched-chain polymers. The journal of chemical physics. 45-55.
2. Stockmayer, W. H. 1952. Molecular distribution in condensation polymers. Journal of Polymer Science 9:69-71.
3. Flory, P. J. 1953. Principles of polymer chemistry. Cornell University Press, Ithaca.
4. Falk, M., and R. E. Thomas. 1974. Molecular Size Distribution in Random Polyfunctional Condensation with or without Ring Formation: Computer Simulation. Canadian Journal of Chemistry 52:3285-3295.
5. Russo, J., P. Tartaglia, and F. Sciortino. 2009. Reversible gels of patchy particles: Role of the valence. Journal of Chemical Physics 131.
6. Li, P., S. Banjade, H. C. Cheng, S. Kim, B. Chen, L. Guo, M. Llaguno, J. V. Hollingsworth, D. S. King, S. F. Banani, P. S. Russo, Q. X. Jiang, B. T. Nixon, and M. K. Rosen. 2012. Phase transitions in the assembly of multivalent signalling proteins. Nature 483:336-340.

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########################################
## code used to run TLBR using kappa/kasim
########################################


#### Signatures

%agent: A(x1,x2,x3) # Declaration of ligand
%agent: B(x1,x2) # Declaration of receptor

#### Rules - first binding

'a.b11' A(x1,x2,x3),B(x1) -> A(x1!1,x2,x3),B(x1!1) @ 'on_rate' #A binds B
'a..b11' A(x1!1,x2,x3),B(x1!1) -> A(x1,x2,x3),B(x1) @ 'off_rate' #AB dissociation

'a.b12' A(x1,x2,x3),B(x2) -> A(x1!1,x2,x3),B(x2!1) @ 'on_rate' #A binds B
'a..b12' A(x1!1,x2,x3),B(x2!1) -> A(x1,x2,x3),B(x2) @ 'off_rate' #AB dissociation

'a.b21' A(x2,x1,x3),B(x1) -> A(x2!1,x1,x3),B(x1!1) @ 'on_rate' #A binds B
'a..b21' A(x2!1,x1,x3),B(x1!1) -> A(x2,x1,x3),B(x1) @ 'off_rate' #AB dissociation

'a.b22' A(x2,x1,x3),B(x2) -> A(x2!1,x1,x3),B(x2!1) @ 'on_rate' #A binds B
'a..b22' A(x2!1,x1,x3),B(x2!1) -> A(x2,x1,x3),B(x2) @ 'off_rate' #AB dissociation

'a.b31' A(x3,x1,x2),B(x1) -> A(x3!1,x1,x2),B(x1!1) @ 'on_rate' #A binds B
'a..b31' A(x3!1,x1,x2),B(x1!1) -> A(x3,x1,x2),B(x1) @ 'off_rate' #AB dissociation

'a.b32' A(x3,x1,x2),B(x2) -> A(x3!1,x1,x2),B(x2!1) @ 'on_rate' #A binds B
'a..b32' A(x3!1,x1,x2),B(x2!1) -> A(x3,x1,x2),B(x2) @ 'off_rate' #AB dissociation


#### Rules - NEXT binding

'a.b11n1' A(x1,x2!_,x3),B(x1) -> A(x1!1,x2!_,x3),B(x1!1) @ 'on_rate2' #A binds B
'a..b11n1' A(x1!1,x2!_,x3),B(x1!1) -> A(x1,x2!_,x3),B(x1) @ 'off_rate2' #AB dissociation

'a.b12n1' A(x1,x2!_,x3),B(x2) -> A(x1!1,x2!_,x3),B(x2!1) @ 'on_rate2' #A binds B
'a..b12n1' A(x1!1,x2!_,x3),B(x2!1) -> A(x1,x2!_,x3),B(x2) @ 'off_rate2' #AB dissociation

'a.b11n1d' A(x1,x2!_,x3!_),B(x1) -> A(x1!1,x2!_,x3!_),B(x1!1) @ 'on_rate2' #A binds B
'a..b11n1d' A(x1!1,x2!_,x3!_),B(x1!1) -> A(x1,x2!_,x3!_),B(x1) @ 'off_rate2' #AB dissociation

'a.b12n1d' A(x1,x2!_,x3!_),B(x2) -> A(x1!1,x2!_,x3!_),B(x2!1) @ 'on_rate2' #A binds B
'a..b12n1d' A(x1!1,x2!_,x3!_),B(x2!1) -> A(x1,x2!_,x3!_),B(x2) @ 'off_rate2' #AB dissociation


'a.b11n2' A(x1,x3!_,x2),B(x1) -> A(x1!1,x3!_,x2),B(x1!1) @ 'on_rate2' #A binds B
'a..b11n2' A(x1!1,x3!_,x2),B(x1!1) -> A(x1,x3!_,x2),B(x1) @ 'off_rate2' #AB dissociation

'a.b12n2' A(x1,x3!_,x2),B(x2) -> A(x1!1,x3!_,x2),B(x2!1) @ 'on_rate2' #A binds B
'a..b12n2' A(x1!1,x3!_,x2),B(x2!1) -> A(x1,x3!_,x2),B(x2) @ 'off_rate2' #AB dissociation

#------

'a.b21n1' A(x2,x1!_,x3),B(x1) -> A(x2!1,x1!_,x3),B(x1!1) @ 'on_rate2' #A binds B
'a..b21n1' A(x2!1,x1!_,x3),B(x1!1) -> A(x2,x1!_,x3),B(x1) @ 'off_rate2' #AB dissociation

'a.b22n1' A(x2,x1!_,x3),B(x2) -> A(x2!1,x1!_,x3),B(x2!1) @ 'on_rate2' #A binds B
'a..b22n1' A(x2!1,x1!_,x3),B(x2!1) -> A(x2,x1!_,x3),B(x2) @ 'off_rate2' #AB dissociation

'a.b21n2' A(x2,x3!_,x1),B(x1) -> A(x2!1,x3!_,x1),B(x1!1) @ 'on_rate2' #A binds B
'a..b21n2' A(x2!1,x3!_,x1),B(x1!1) -> A(x2,x3!_,x1),B(x1) @ 'off_rate2' #AB dissociation

'a.b22n2' A(x2,x3!_,x1),B(x2) -> A(x2!1,x3!_,x1),B(x2!1) @ 'on_rate2' #A binds B
'a..b22n2' A(x2!1,x3!_,x1),B(x2!1) -> A(x2,x3!_,x1),B(x2) @ 'off_rate2' #AB dissociation

'a.b21n1d' A(x2,x1!_,x3!_),B(x1) -> A(x2!1,x1!_,x3!_),B(x1!1) @ 'on_rate2' #A binds B
'a..b21n1d' A(x2!1,x1!_,x3!_),B(x1!1) -> A(x2,x1!_,x3!_),B(x1) @ 'off_rate2' #AB dissociation

'a.b22n1d' A(x2,x1!_,x3!_),B(x2) -> A(x2!1,x1!_,x3!_),B(x2!1) @ 'on_rate2' #A binds B
'a..b22n1d' A(x2!1,x1!_,x3!_),B(x2!1) -> A(x2,x1!_,x3!_),B(x2) @ 'off_rate2' #AB dissociation

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'a.b31n1' A(x3,x1!_,x2),B(x1) -> A(x3!1,x1!_,x2),B(x1!1) @ 'on_rate2' #A binds B
'a..b31n1' A(x3!1,x1!_,x2),B(x1!1) -> A(x3,x1!_,x2),B(x1) @ 'off_rate2' #AB dissociation

'a.b32n1' A(x3,x1!_,x2),B(x2) -> A(x3!1,x1!_,x2),B(x2!1) @ 'on_rate2' #A binds B
'a..b32n1' A(x3!1,x1!_,x2),B(x2!1) -> A(x3,x1!_,x2),B(x2) @ 'off_rate2' #AB dissociation

'a.b31n2' A(x3,x2!_,x1),B(x1) -> A(x3!1,x2!_,x1),B(x1!1) @ 'on_rate2' #A binds B
'a..b31n2' A(x3!1,x2!_,x1),B(x1!1) -> A(x3,x2!_,x1),B(x1) @ 'off_rate2' #AB dissociation

'a.b32n2' A(x3,x2!_,x1),B(x2) -> A(x3!1,x2!_,x1),B(x2!1) @ 'on_rate2' #A binds B
'a..b32n2' A(x3!1,x2!_,x1),B(x2!1) -> A(x3,x2!_,x1),B(x2) @ 'off_rate2' #AB dissociation

'a.b31n1d' A(x3,x1!_,x2!_),B(x1) -> A(x3!1,x1!_,x2!_),B(x1!1) @ 'on_rate2' #A binds B
'a..b31n1d' A(x3!1,x1!_,x2!_),B(x1!1) -> A(x3,x1!_,x2!_),B(x1) @ 'off_rate2' #AB dissociation

'a.b32n1d' A(x3,x1!_,x2!_),B(x2) -> A(x3!1,x1!_,x2!_),B(x2!1) @ 'on_rate2' #A binds B
'a..b32n1d' A(x3!1,x1!_,x2!_),B(x2!1) -> A(x3,x1!_,x2!_),B(x2) @ 'off_rate2' #AB dissociation


#### Variables

%var: 'on_rate' 1.8E-6 # per molecule per second
%var: 'off_rate' 0.01 # per second

%var: 'on_rate2' 5.6E-4 # per molecule per second
%var: 'off_rate2' 0.01 # per second


%obs: 'Ax1_B' A(x1!_,x2,x3)
%obs: 'Ax2_B' A(x2!_,x1,x3)
%obs: 'Ax3_B' A(x3!_,x2,x1)

%obs: 'Ax3_B2' A(x1!_,x2!_,x3)
%obs: 'Ax2_B2' A(x1!_,x2,x3!_)
%obs: 'Ax1_B2' A(x1,x2!_,x3!_)

%obs: 'Ax_B3' A(x1!_,x2!_,x3!_)



#### Initial conditions

%init: 5000 A()
%init: 300 B()


### %mod: repeat ([T] [mod] 10)=0 do $SNAPSHOT "prefix" until [false]
### %mod: [T]>99 do $SNAPSHOT
%mod: [T]>10 do $SNAPSHOT "sim1c_obs/snap10"
%mod: [T]>20 do $SNAPSHOT "sim1c_obs/snap20"
%mod: [T]>30 do $SNAPSHOT "sim1c_obs/snap30"
%mod: [T]>40 do $SNAPSHOT "sim1c_obs/snap40"
%mod: [T]>45 do $SNAPSHOT "sim1c_obs/snap45"
%mod: [T]>50 do $SNAPSHOT "sim1c_obs/snap50"
%mod: [T]>60 do $SNAPSHOT "sim1c_obs/snap60"
%mod: [T]>75 do $SNAPSHOT "sim1c_obs/snap75"
%mod: [T]>100 do $SNAPSHOT "sim1c_obs/snap100"
%mod: [T]>125 do $SNAPSHOT "sim1c_obs/snap125"
%mod: [T]>150 do $SNAPSHOT "sim1c_obs/snap150"
%mod: [T]>300 do $SNAPSHOT "sim1c_obs/snap300"
%mod: [T]>1000 do $SNAPSHOT "sim1c_obs/snap1000"
```

```
# file modified from RuleMonkey library:
# http://public.tgen.org/rulemonkey/download/models/tlbr.bngl
# Trivalent ligand - bivalent receptor
# Ring closure reaction is included. All possible cyclic
# combinations may form.

begin parameters
t_end      1000
n_steps     100

Lig_tot          5000
Rec_tot          300

kp1        1.8e-06
km1        0.01
kp2        5.6e-04
km2        0.01
kp3        5.6e-04
km3        0.01
end parameters

begin molecule types
L(r,r,r)
R(l,l)
end molecule types

begin species
L(r,r,r)   Lig_tot
R(l,l)           Rec_tot
end species

begin reaction rules

#intermolecular interactions only
L(r,r,r) + R(l) <-> L(r!1,r,r).R(l!1) kp1,km1
L(r,r,r!+) + R(l) <-> L(r!1,r,r!+).R(l!1) kp2,km2
L(r,r!+,r!+) + R(l) <-> L(r!1,r!+,r!+).R(l!1) kp3,km3

# allow intramolecular interactions:
L(r,r!+,r).R(l) <-> L(r!1,r!+,r).R(l!1) kp2,km2
L(r,r!+,r!+).R(l) <-> L(r!1,r!+,r!+).R(l!1) kp3,km3

end reaction rules

begin observables
Species Ligfree   L(r,r,r)
Species Ligbnd1   L(r!+,r,r)
Species Ligbnd2   L(r!+,r!+,r)
Species Ligbnd3   L(r!+,r!+,r!+)

Species Size   R()=1
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end observables

simulate_rm({t_end=>t_end,n_steps=>n_steps});
```