A Possible Mechanism for Phase Separation during Cell Signaling

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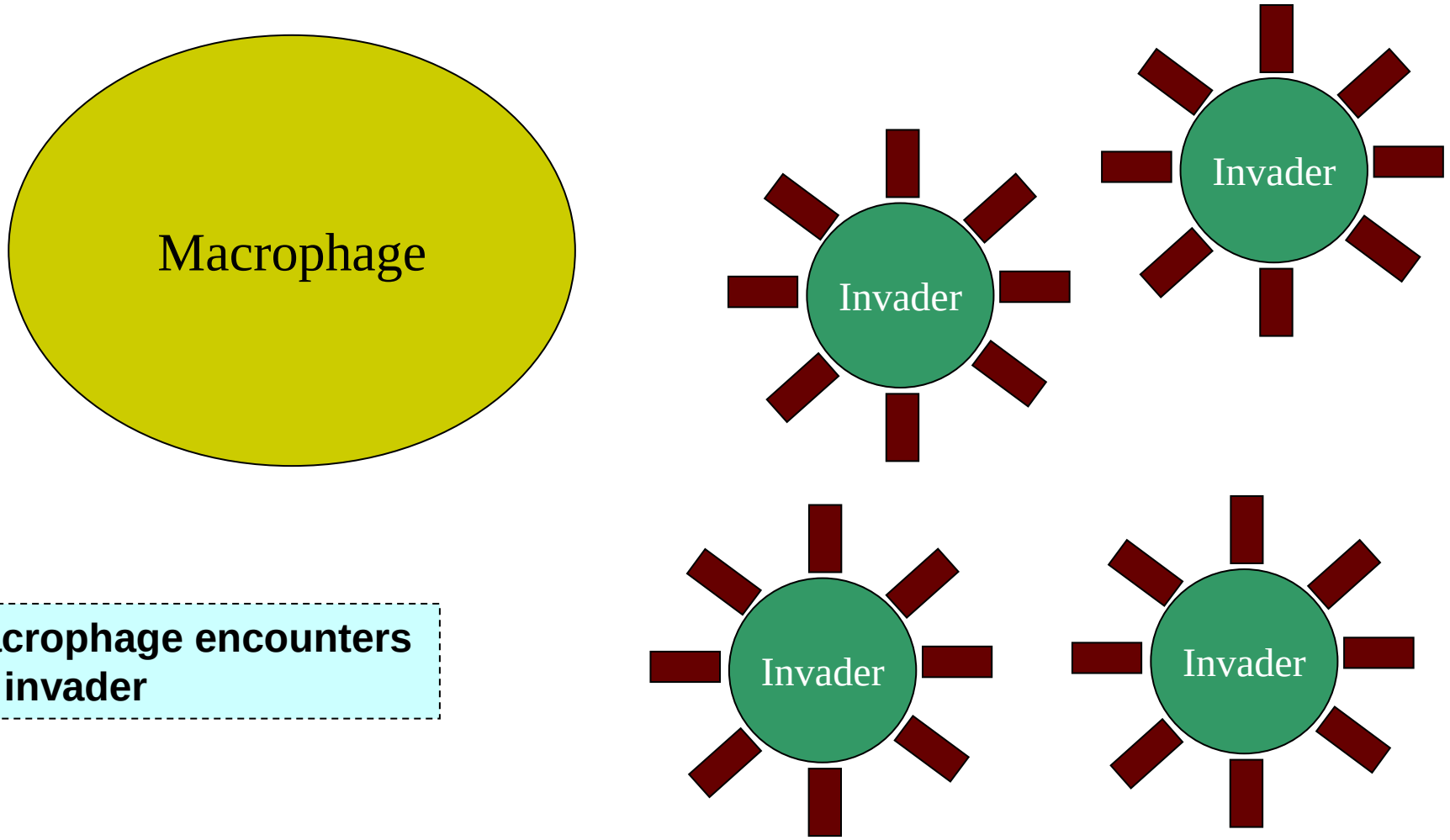
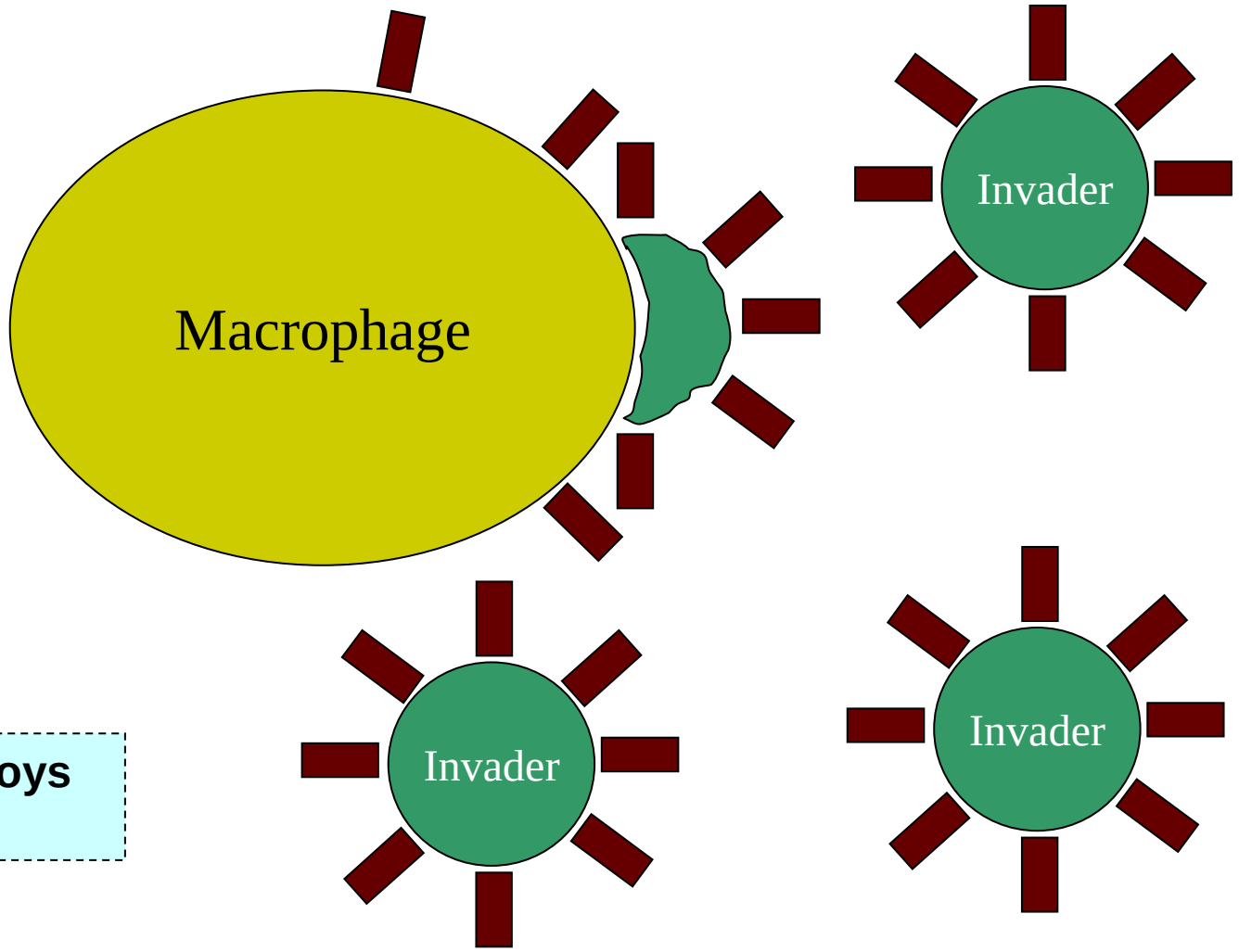
Immune System Signaling (abridged)



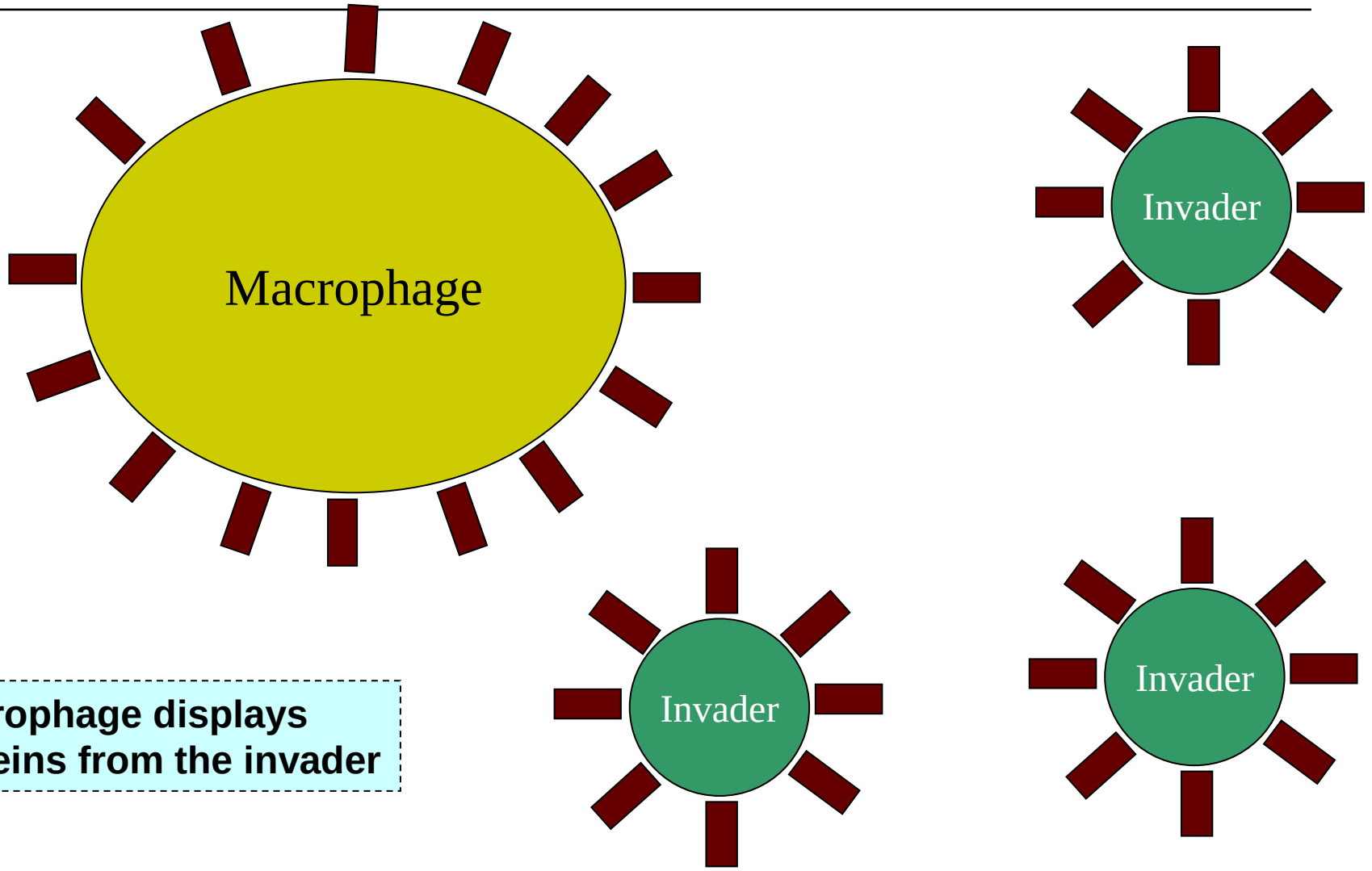
Macrophage encounters an invader

Immune System Signaling (abridged)



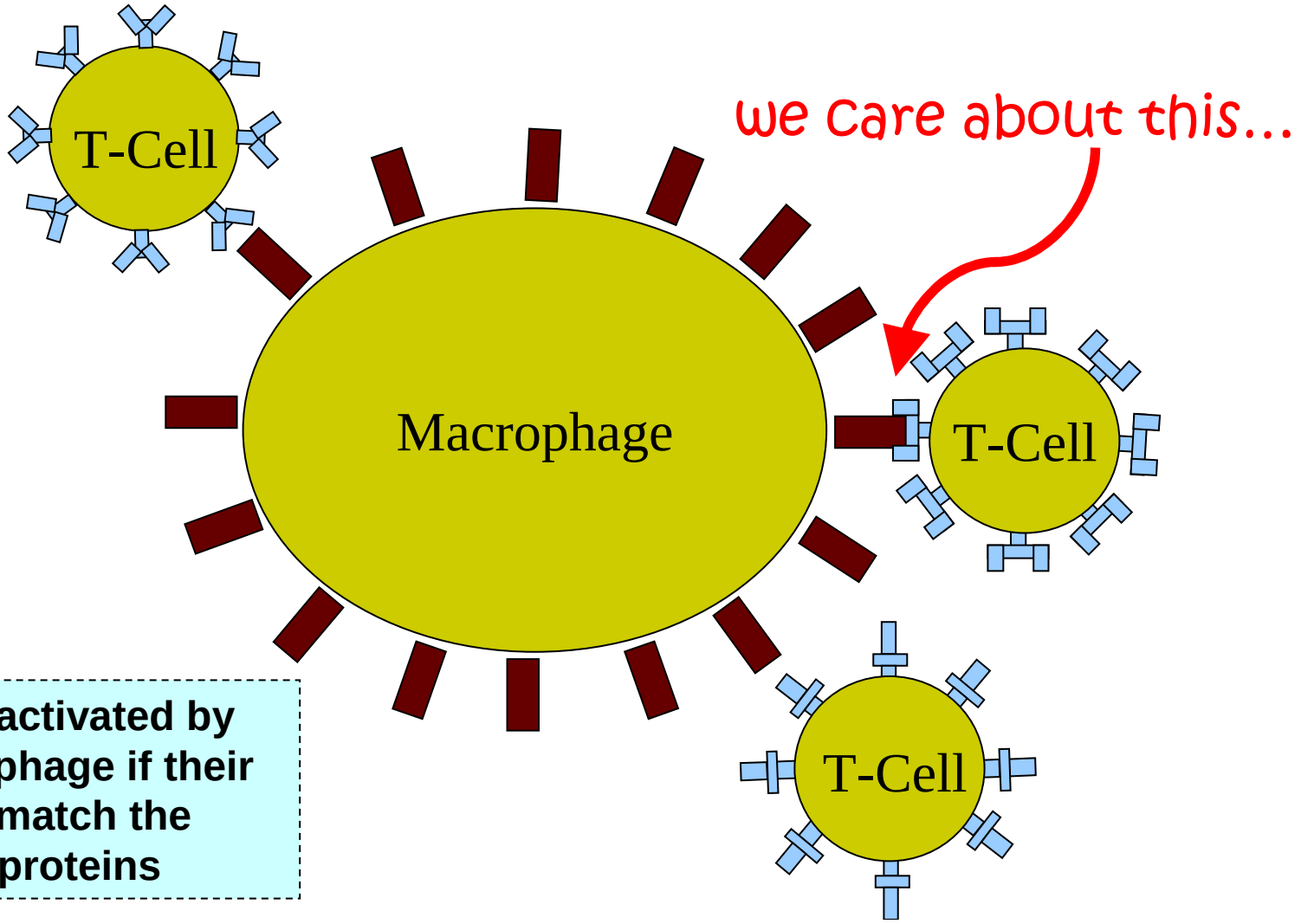
Macrophage destroys the invader

Immune System Signaling (abridged)



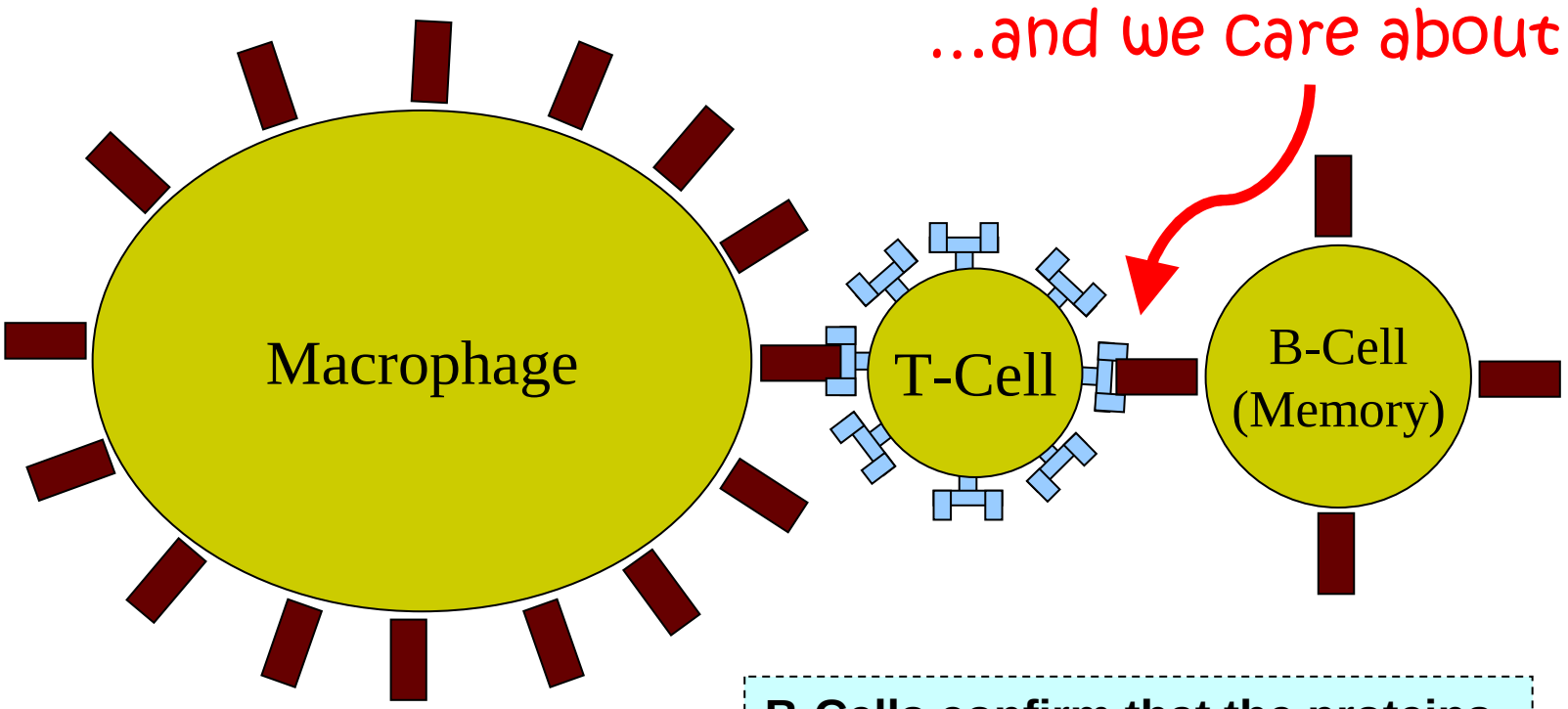
Macrophage displays proteins from the invader

Immune System Signaling (abridged)



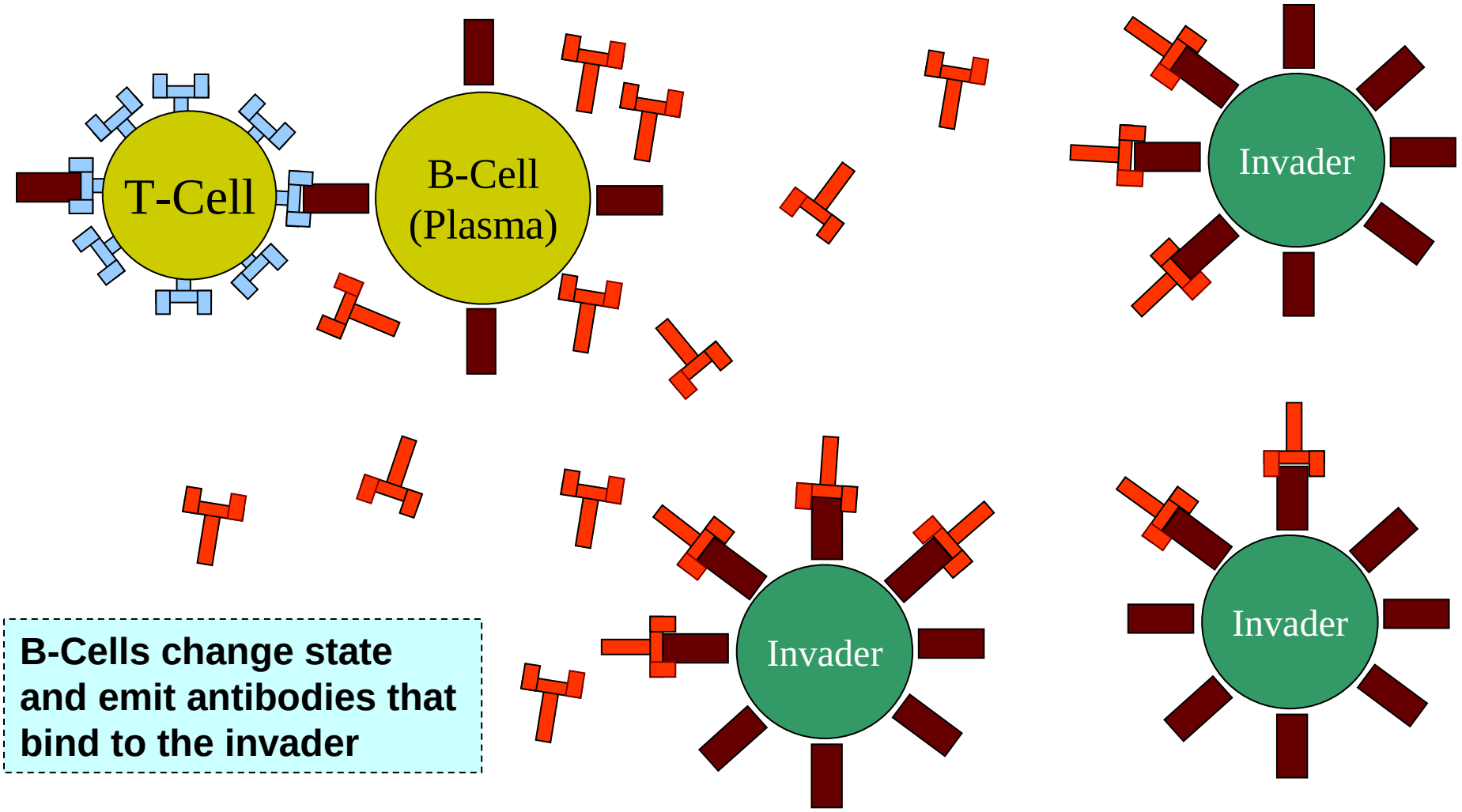
T-Cell are activated by the macrophage if their receptors match the displayed proteins

Immune System Signaling (abridged)



B-Cells confirm that the proteins are from a pathogen encountered previously.

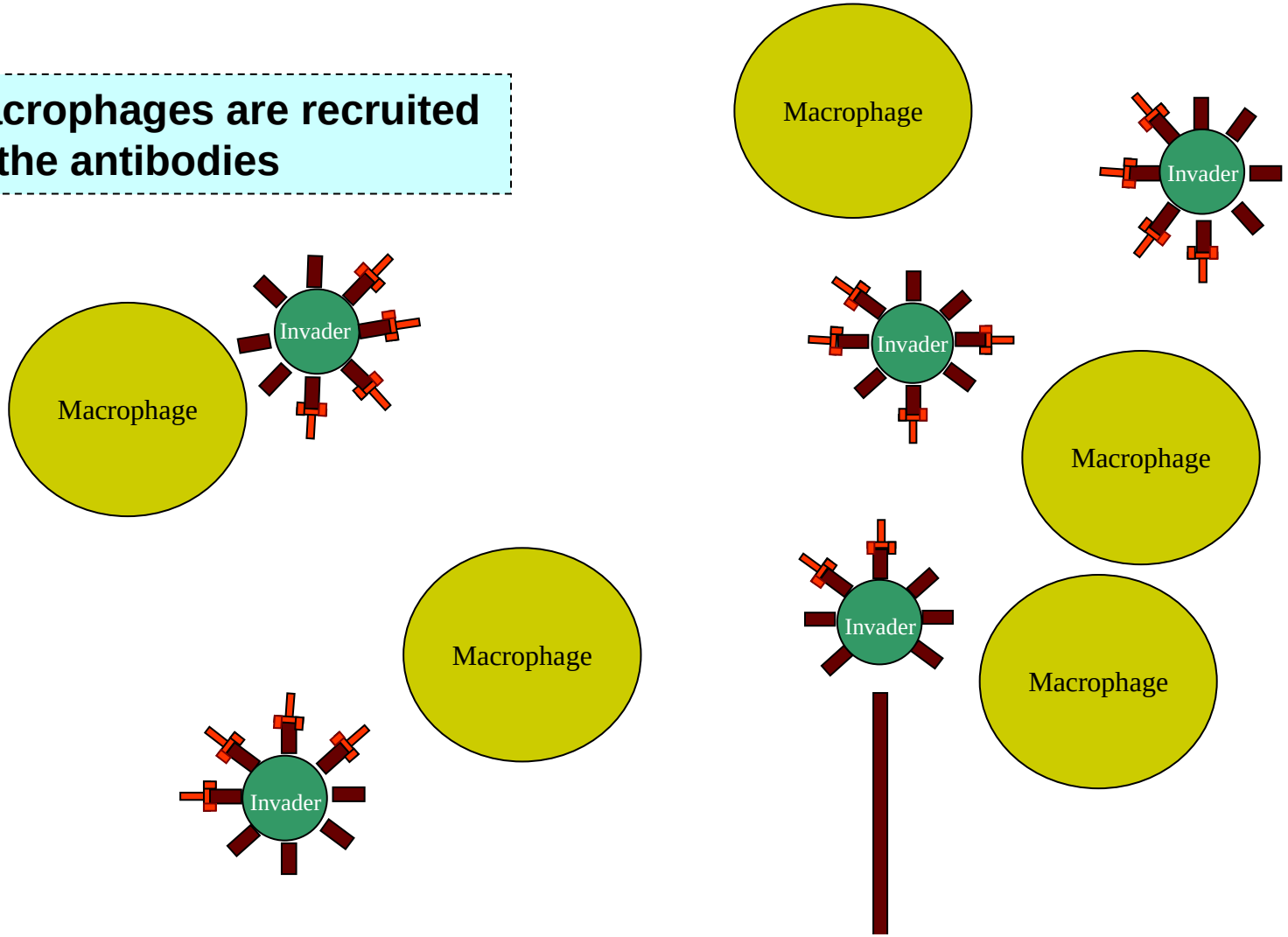
Immune System Signaling (abridged)



B-Cells change state and emit antibodies that bind to the invader

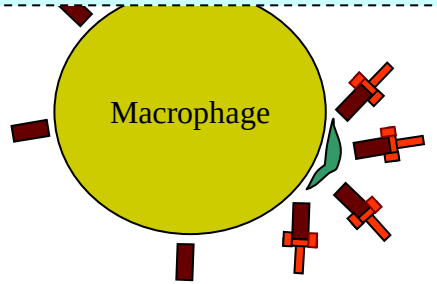
Immune System Signaling (abridged)

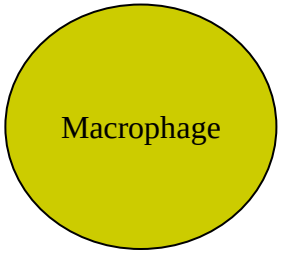
Macrophages are recruited to the antibodies

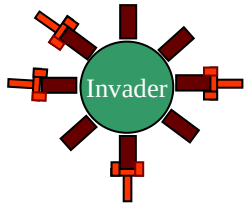


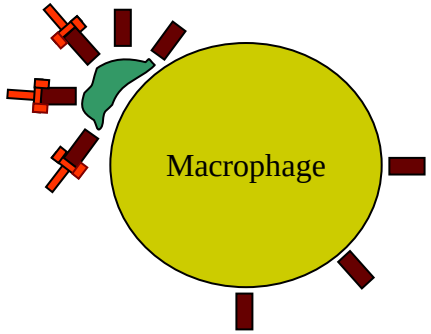
Immune System Signaling (abridged)

Recruited macrophages destroy the invaders and display their proteins.

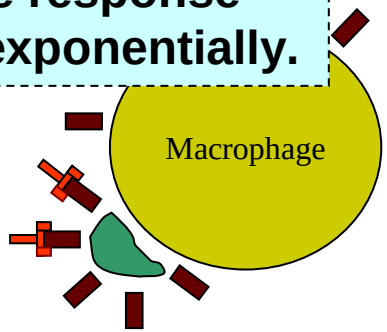


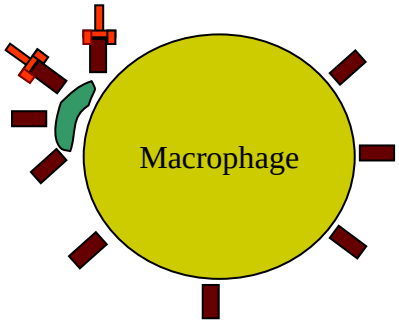




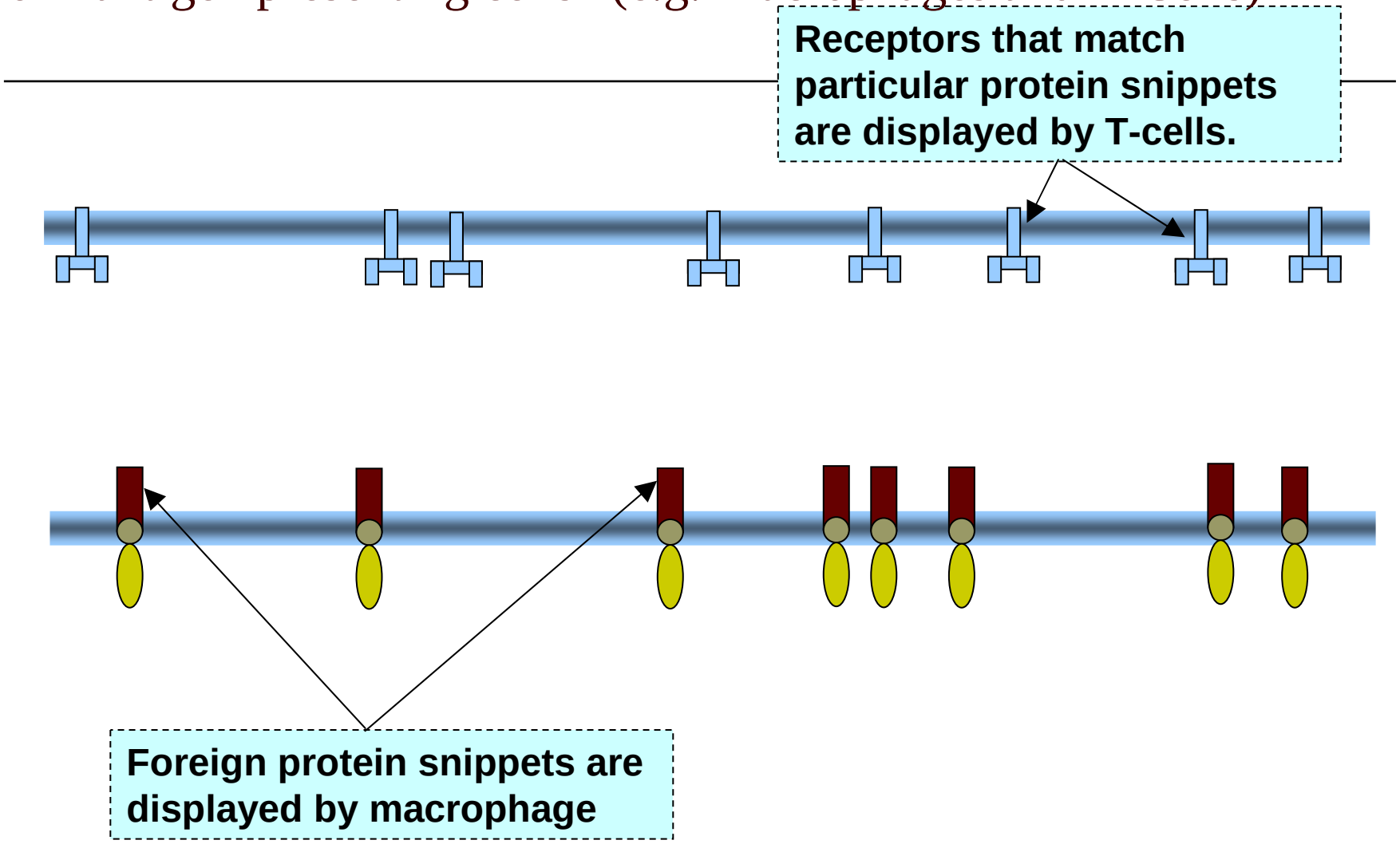


The cycle repeats and the immune response increases exponentially.





In vivo, T cells are stimulated by monovalent binding to ligands on “antigen presenting cells” (e.g. Macrophages and B-Cells)



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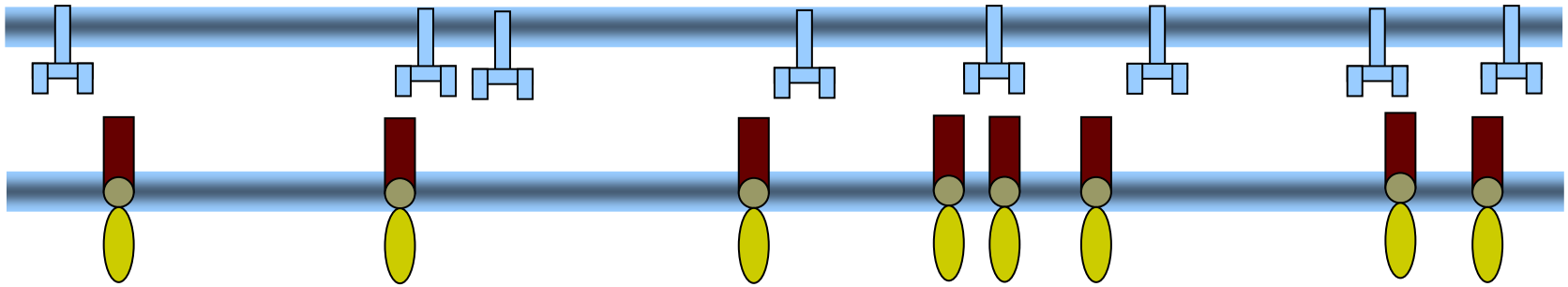

The macrophage comes into contact with various T-cells.

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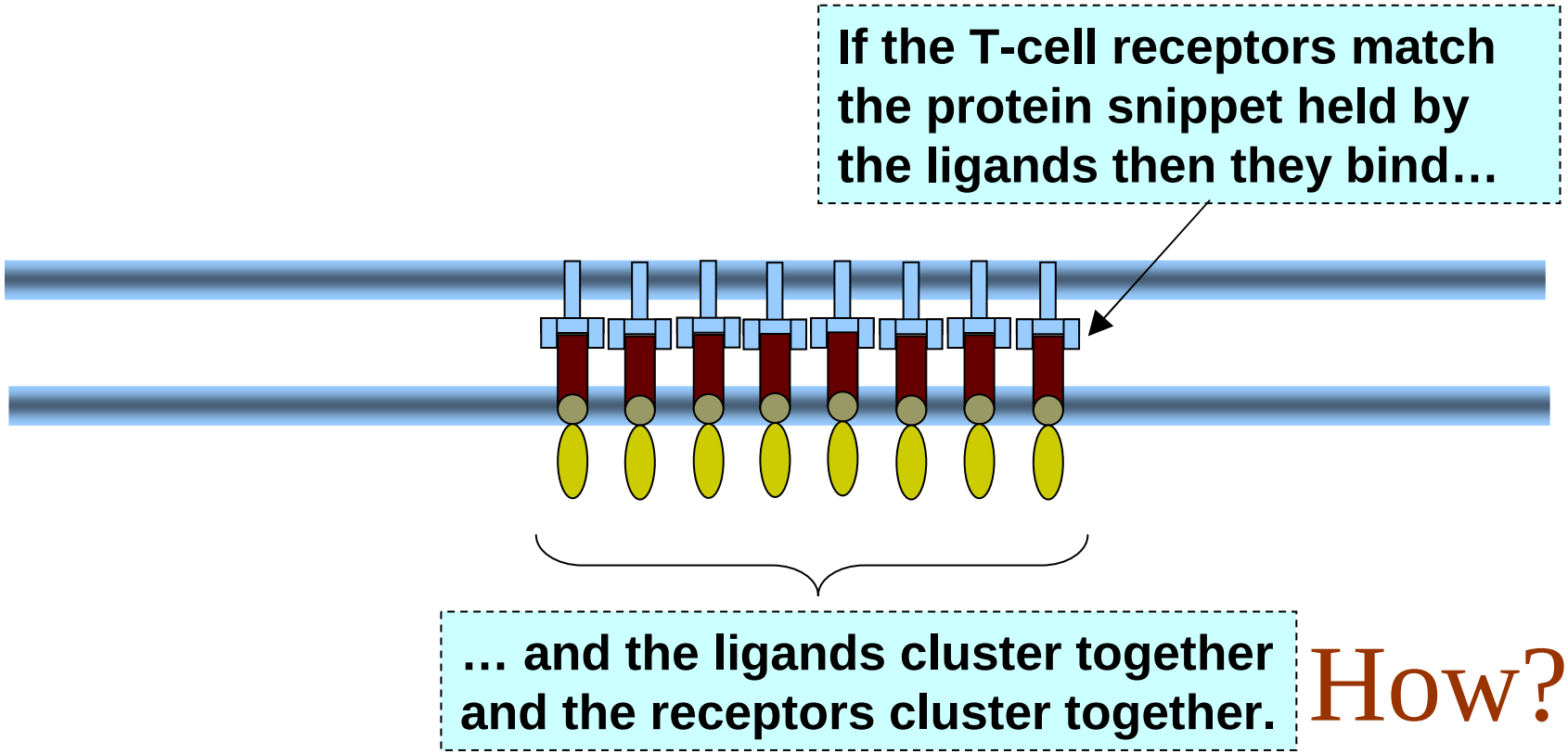
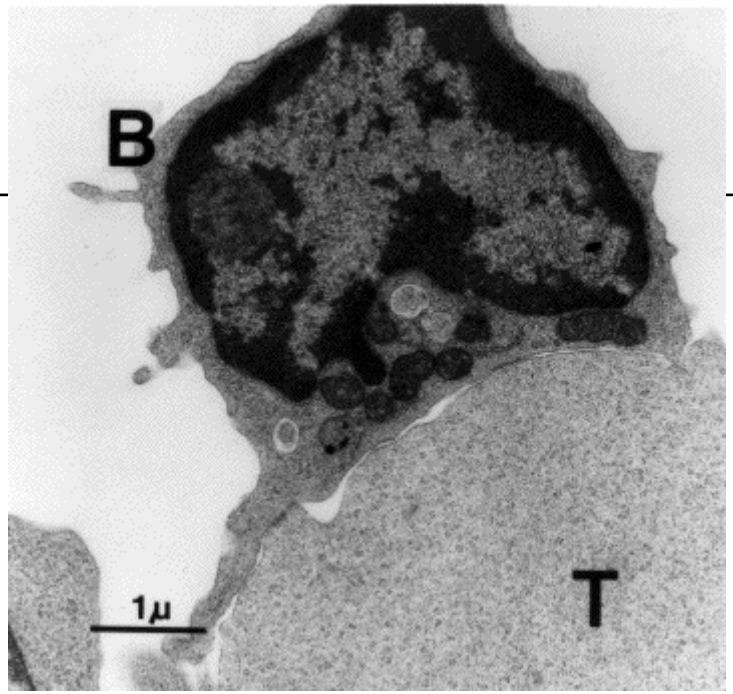
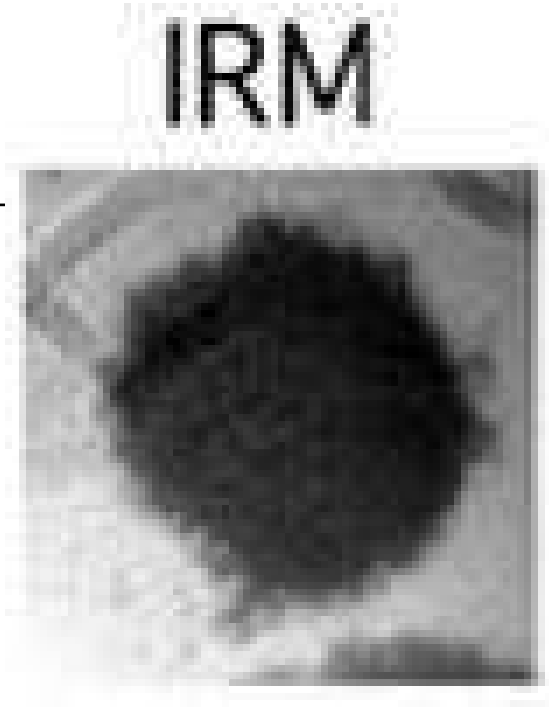


How?





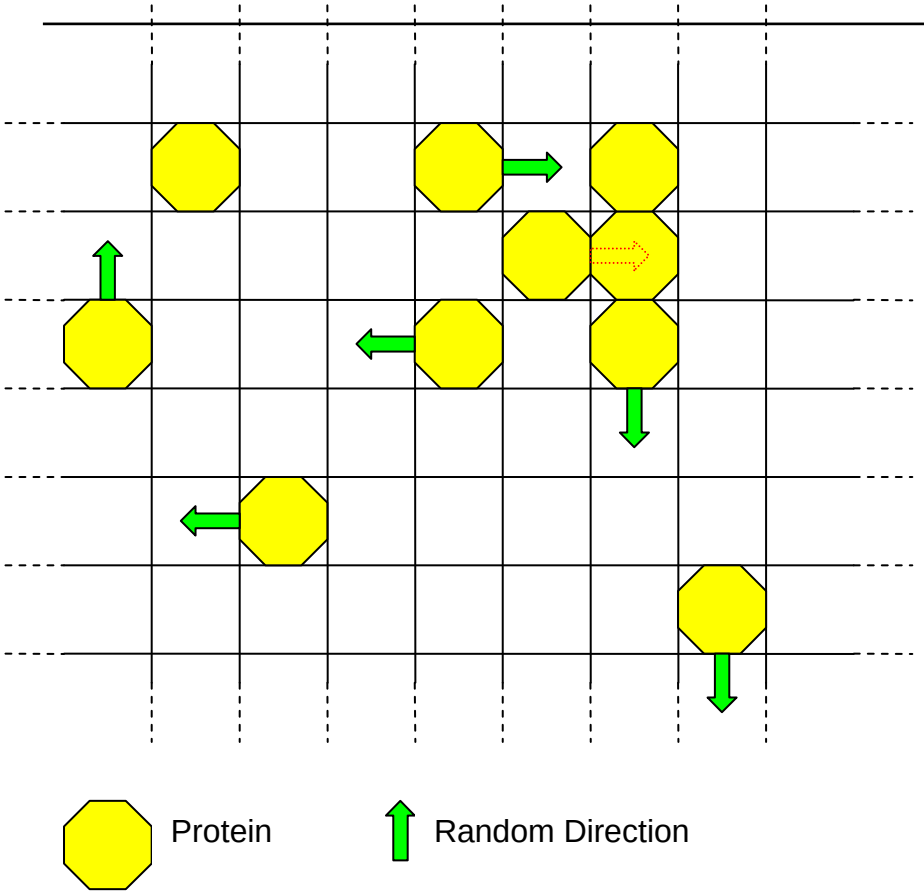
Our hypothesis: Cross Membrane binding between ligand-receptor pairs serves to combine the attractive forces between proteins in their own membranes. This would allow receptor or ligand groups that by themselves are do not cluster to “sum” the attractive forces and cluster.

Sounds simple but we can’t predict how strong the inter-membrane force needs to be in relation to the intra-membrane forces to cause phase separation. So model it!

Our Approach

- Write a model of phase separation on a single membrane
- Confirm that our results match those of previous phase transition models
- Implement two copies of the single membrane model and bring them into contact
- Add a cross-membrane binding force
- Under what circumstances do we get phase separation?

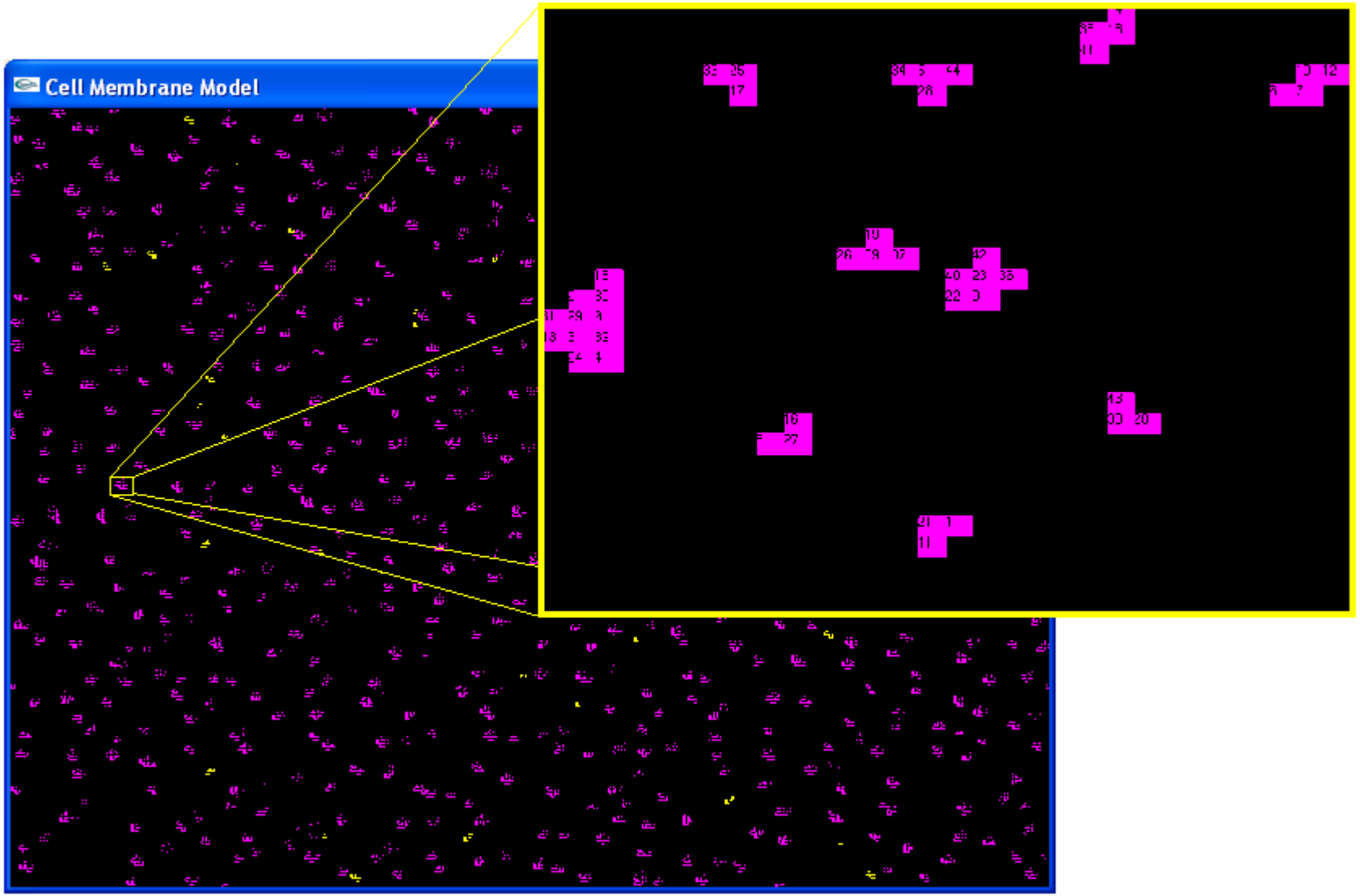
Metropolis Monte Carlo



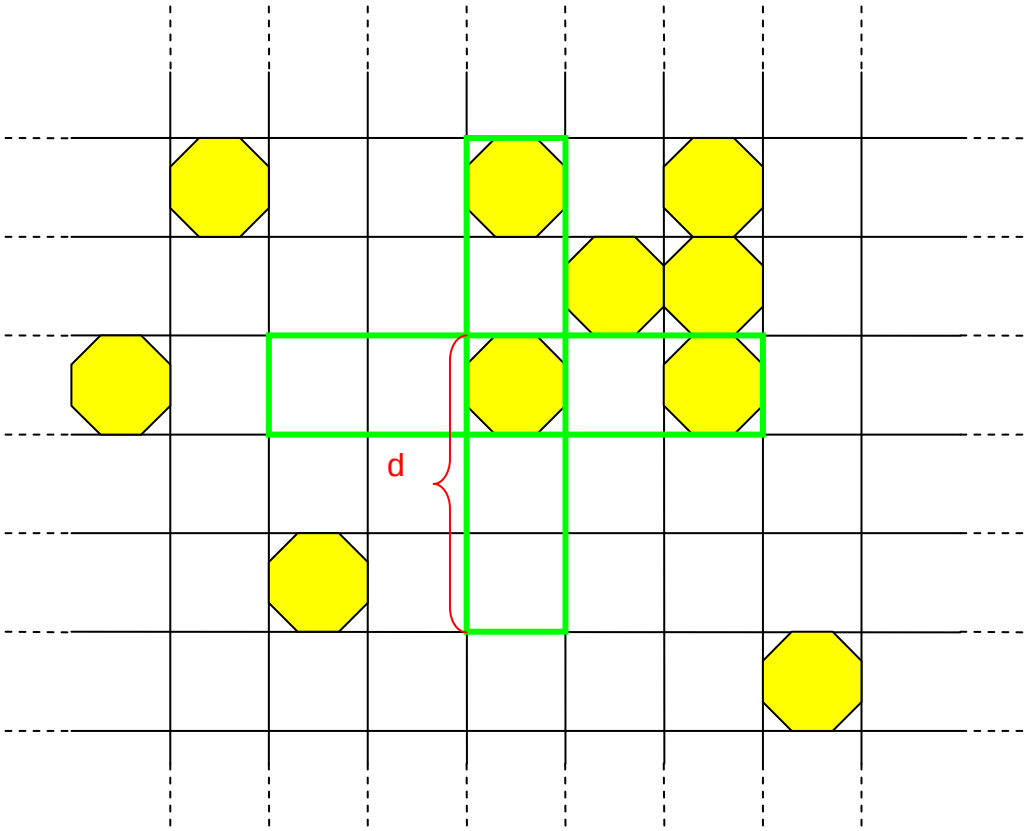
ε = Favorable contact energy (in kT) between neighboring proteins.

- n x n toroidal lattice
- Each site on the lattice can hold a single protein
- At each discrete time-step all proteins choose a random direction to move
- If the energy is reduced the motion is accepted.
- Otherwise the motion is accepted with probability $e^{(-\Delta E/kT)\varepsilon}$.
- Repeat until we are confident that the system is in equilibrium

“Freeze Out:” the problem that isn’t?

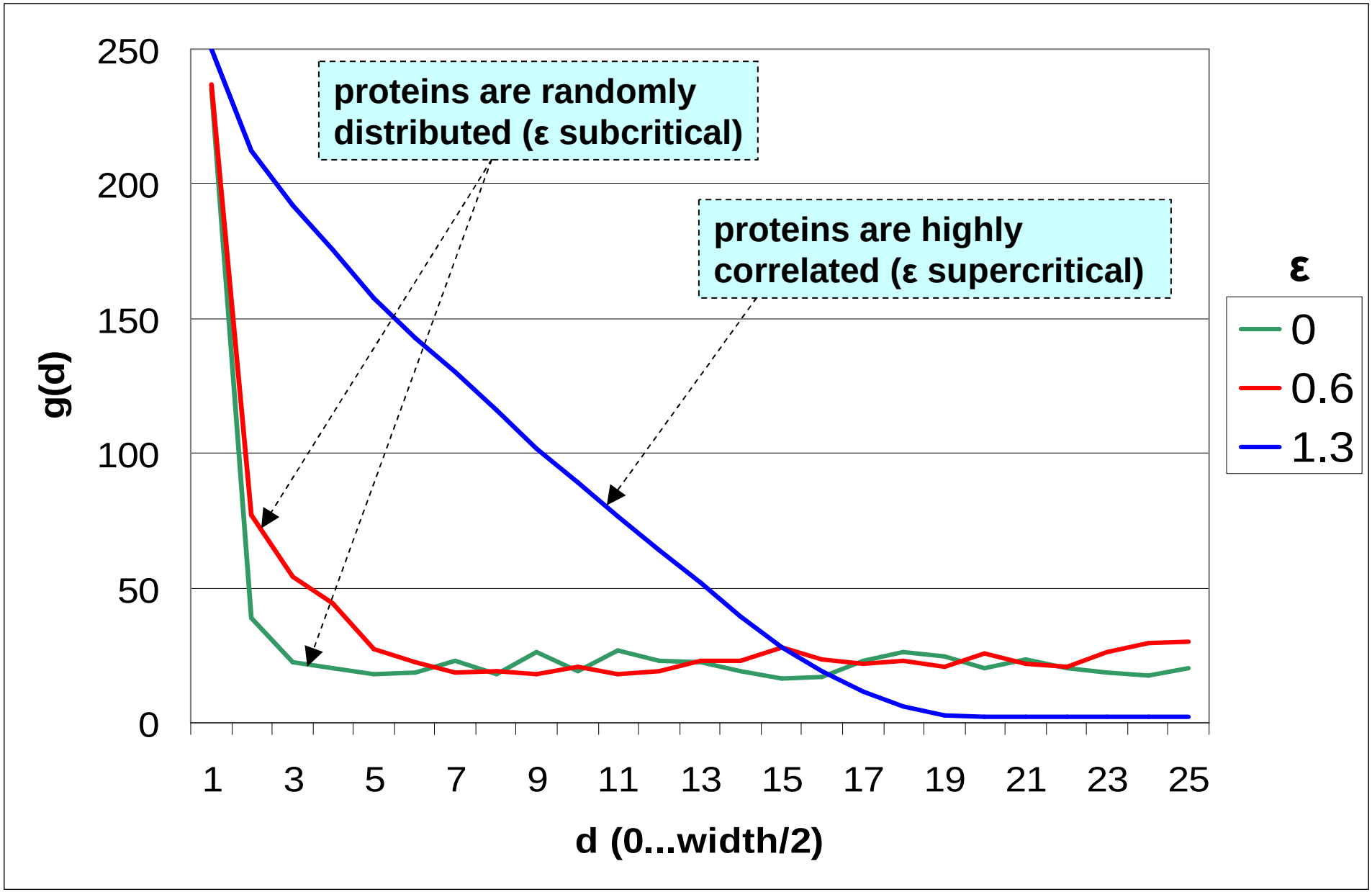


Measuring Phase Separation – Spatial Autocorrelation

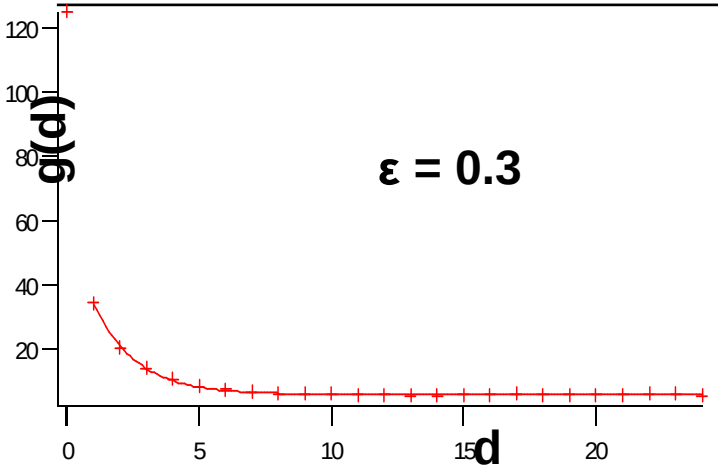


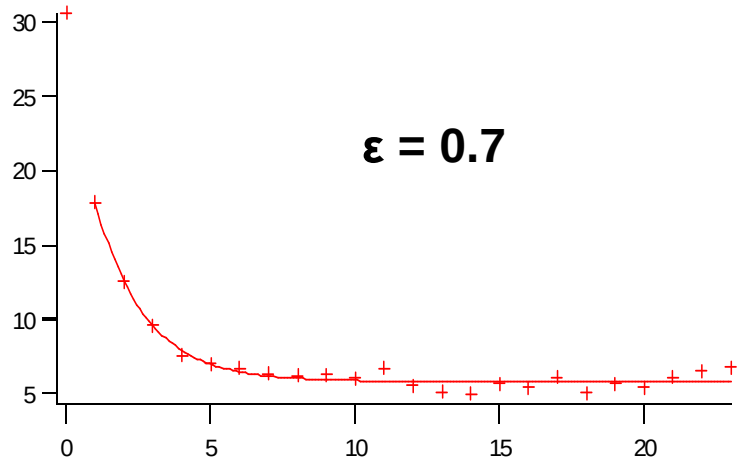
- Autocorrelation Function g(*d*)
- Choose a protein and count the number of proteins at distance *d (then* ÷ *4 .)*

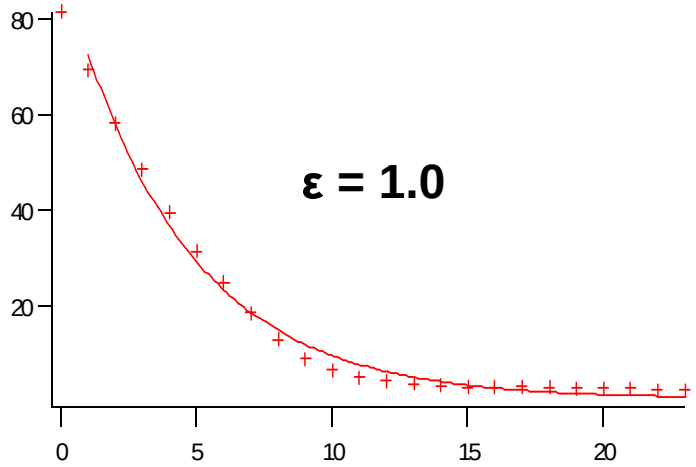
Correlation Functions for Three Values of ε

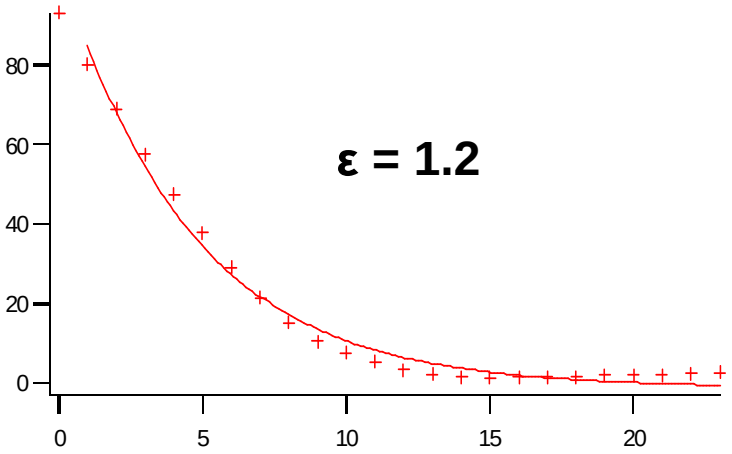


Fit Correlation Functions to an Exponential $y = C_1 e^{C_2 x}$ (fit deteriorates as critical epsilon reached)

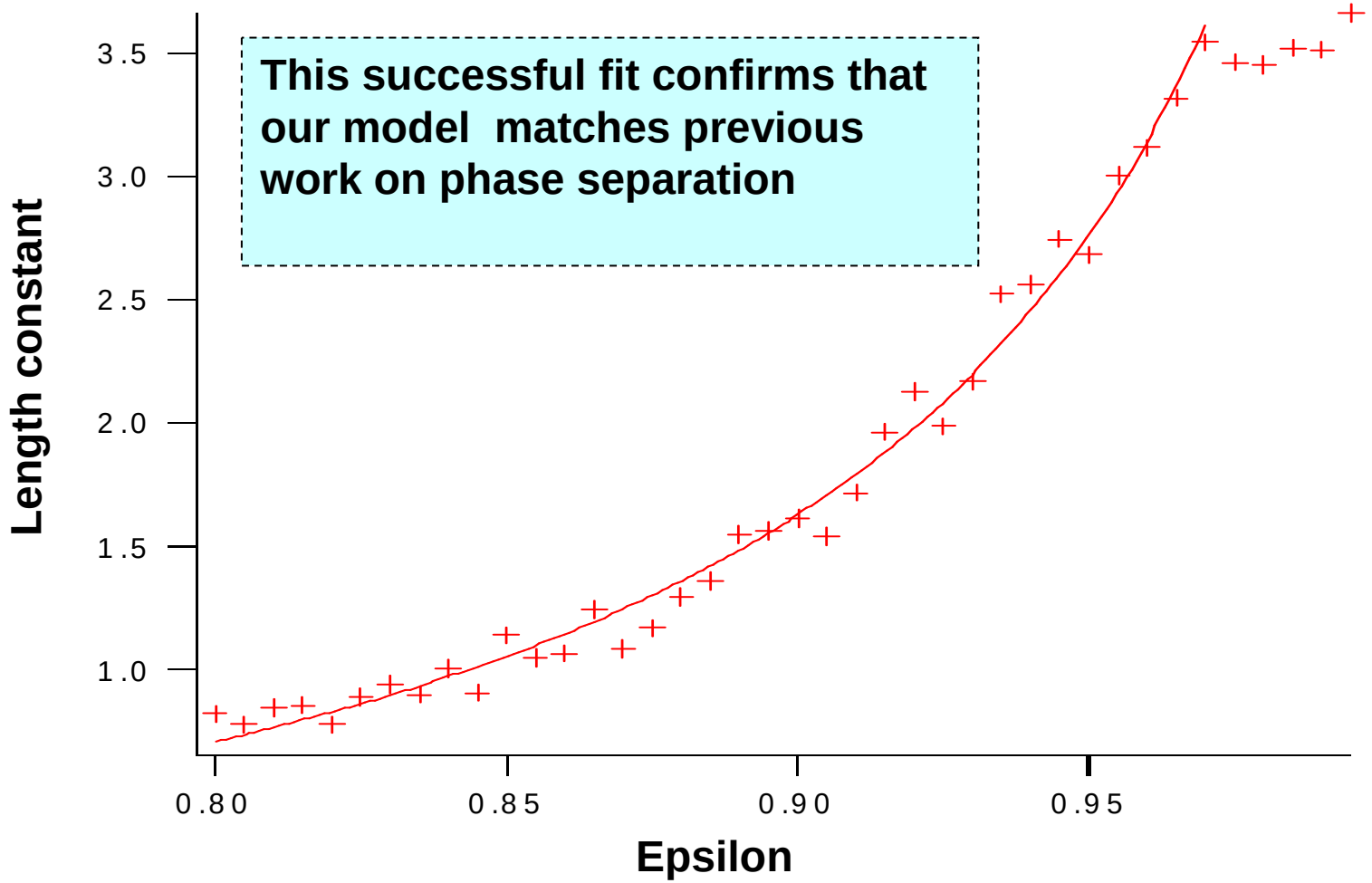








Fit exponential length constants to $\frac{C_1}{\varepsilon - \varepsilon_c} + C_2$ *



*Gould H., and J. Tobochnik An Introduction to Computer Simulation Methods: Applications to Physical Systems, 1996

Finite Size Effects

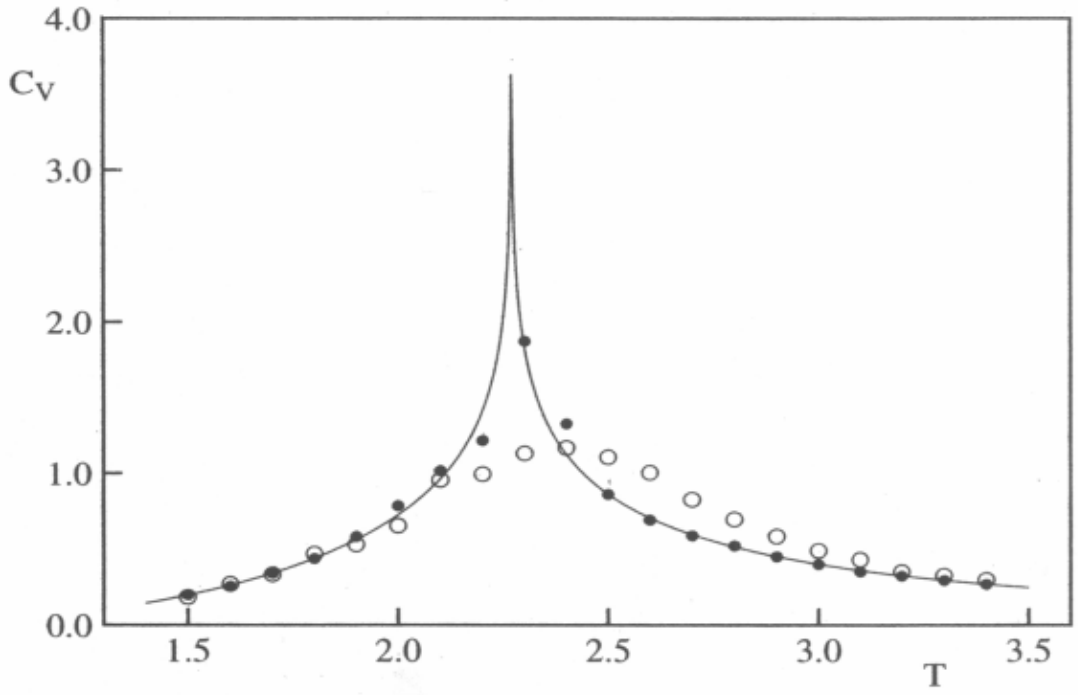
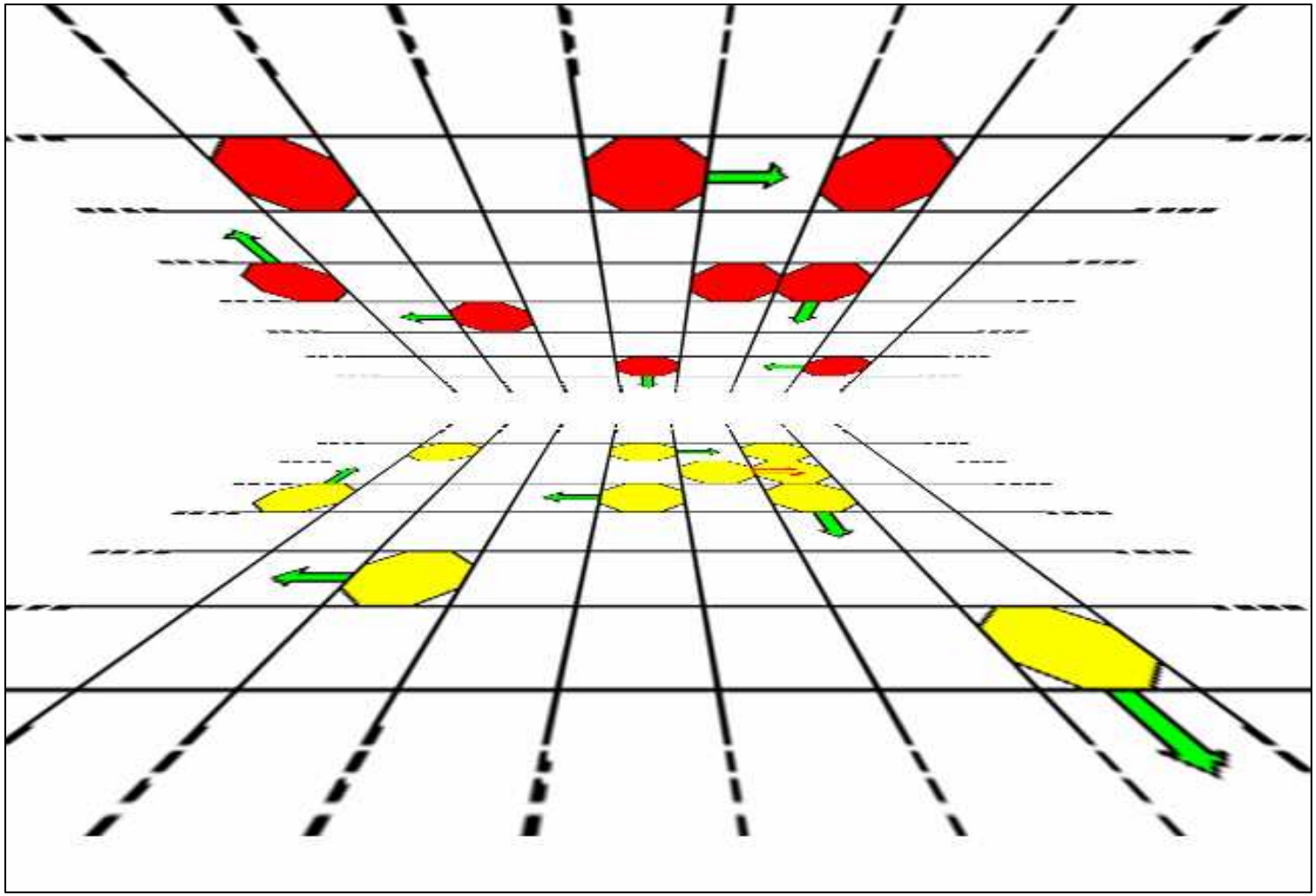


Fig. 17.2 The temperature dependence of the specific heat C (per spin) of the Ising model on a $L = 8$ and $L = 16$ square lattice with periodic boundary conditions. One thousand Monte Carlo steps per spin were used for each value of the temperature. The continuous line represents the temperature dependence of C in the limit of an infinite lattice. (Note that C is infinite at $T = T_c$ for an infinite lattice.)

Two Membrane Model

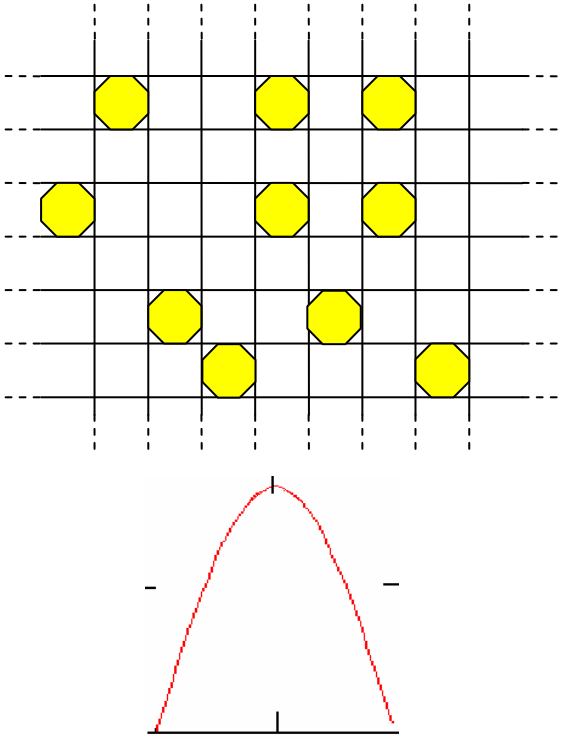


Density variance as a measure of phase separation

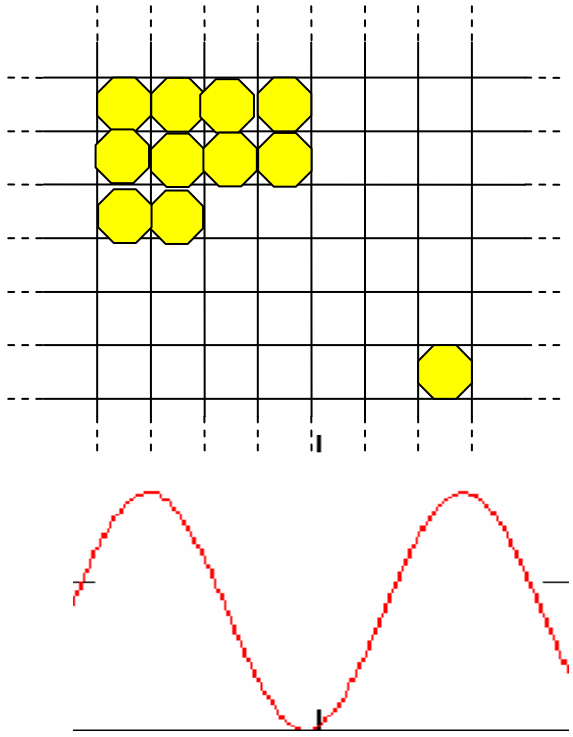
Calculating the autocorrelation, exponents, and critical exponents is too slow

Instead: calculate the protein density for all overlapping 3x3 squares on the lattice

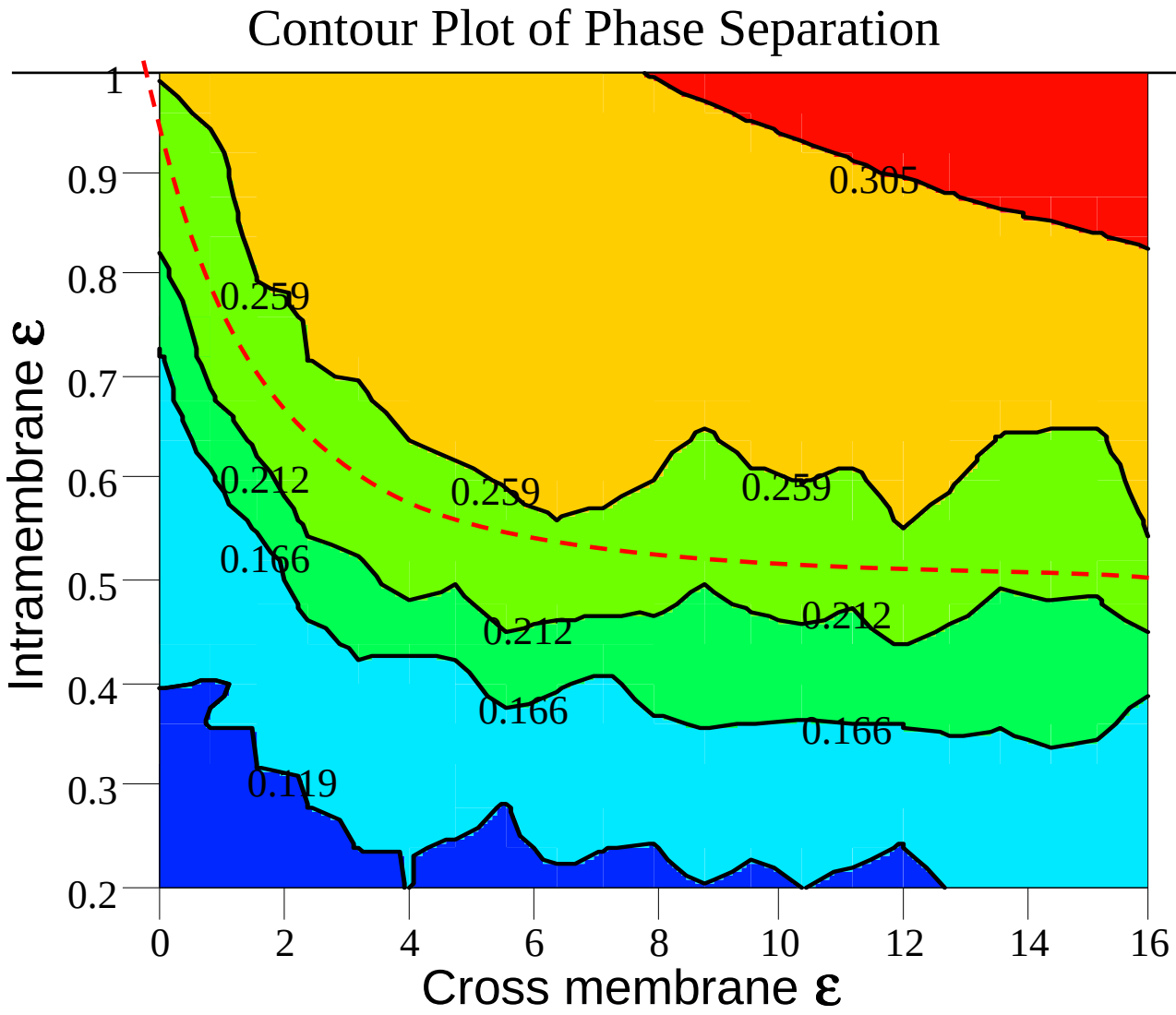
Standard deviation is a measure of phase separation



Low σ, one phase



High σ, two phase



Complete phase separation occurs at 0.27

Random protein distributions have been observed to have z values of between 0.09 and 0.105

Conclusions

The Monte Carlo model confirms our hypothesis that binding between ligand-receptor pairs can cause two groups of proteins to phase separate even when their attractive forces are too low individually.

The model has also provided a picture of how strong the cross membrane binding needs to be in relation to the intra-membrane forces.

We have provided the simplest possible physical explanation for protein phase separation.
(Satisfying from a “Occam’s Razor” point of view).

Further Work

- The model was written in an object oriented style to facilitate prototyping.
- In order to map the 2-d inter intra epsilon space in detail the model needs to be faster
- The predictions about ligand-receptor binding strength need to be grounded against observations of real biological systems.