

Biological Specificity and Cooperativity (From chance to necessity)

1. Adsorption of ligands on most biomaterials surfaces as a random process. Non-interacting sites.

- (a) *Stochastic independence*. Outcome of n th trial does not depend on outcome of $(n-1)$ st trial.

If Prob. of event $A = P(A)$ and Prob. of event $B = P(B)$, then Prob. of event “first A and then B ” is $P(AB)$.

$P(AB) = P(A) \times P(B)$ if trials are stochastically independent.

- (b) *Independent trials*. With two outcomes only: Bernoulli trials. $p + q = 1$. If probabilities remain constant throughout trials, then for a given sequence of heads (H) and tails (T):

$$P(HHTHT \dots TTH) = ppqpq \dots qq p.$$

Stochastic independence of successive trials. Casino example: Does Nature have memory?

- (c) Random walks of different kinds: The 0.5-power rule.

Kinetic theory of gases: $(\overline{v^2})^{\frac{1}{2}} = (kT/m)^{\frac{1}{2}}$; $v \propto T^{\frac{1}{2}}$.

Diffusion: $(\overline{x^2})^{\frac{1}{2}} = (2Dt)^{\frac{1}{2}}$; $x \propto T^{\frac{1}{2}}$.

Unstretched macromolecule: $(\overline{r^2})^{\frac{1}{2}} = (Nl^2)^{\frac{1}{2}}$; $r \propto N^{\frac{1}{2}}$.

Ligand adsorption onto n identical noninteracting sites of protein molecule:

$$\bar{v} = \text{moles bound ligand} \div \text{moles total protein} = \frac{nk[A]}{1+k[A]}.$$

2. Adsorption on interacting sites.

- (a) Loss of randomness during various physiochemical or biological processes.
Compress a gas.
Impose a concentration gradient.
Stretch a macromolecule.
Adsorb ligand onto protein. Interacting sites.

$$\bar{v} = \text{moles bound ligand} \div \text{moles total protein} = \frac{nk[A]^n}{1+k[A]^n}.$$

- (b) Sigma-shaped curves. Oxygenation of hemoglobin.
- (c) Micelle formation. Cell membrane formation.
- (d) How large need n be to achieve cooperativity? Myoglobin vs. hemoglobin.

3. Cooperative processes.

- (a) Outcome of n th trial depends on outcome of $(n-1)$ st trial.
- (b) How to choose between a car and a goat.
- (c) Enzyme-substrate interaction.
- (d) ECM protein –cell receptor interaction.
- (e) Reversible melting of quaternary structure of collagen.
- (f) Simple one-dimensional statistical model of a critical transition as a cooperative process (See below #5). Nearest-neighbor interactions are sufficient for an “all or none” transition provided that the energy cost of the hybrid state is high enough and the sequence is long enough. The classical “lock-and-key” fit is not necessary.
- (g) A thermodynamic representation of a helix-coil transition: Gibbs free energy and the first-order transition.

4. The unit cell process: Inside and outside the control volume dV .

- (a) Regulators diffuse into and out of control volume dV .
- (b) Cell-matrix interaction inside dV is a cooperative process. Another way of putting it: the cell-matrix interaction is biologically specific.

5. A model of a critical transition from noncooperative to cooperative cell behavior.

What is cooperativity in cell biology? Cells cooperate, i.e., communicate with each other and acquire a common phenotype, following formation of condensed states of biological matter. States of this type comprise cells, usually embedded in matrix, that are arranged in close proximity with each other and frequently function as a single unit. Examples of such organized cell structures are contractile cell capsules surrounding tissues in wounded organs, columns of Schwann cells, and cords formed by epithelial cells during skin synthesis. An example of a cooperative functioning unit is the contractile cell capsule that surrounds skin wounds and nerve wounds during spontaneous healing. Cooperative activity can be disrupted by scaffolds with specific biological activity, occasionally resulting in tissue and organ regeneration.

How do cells cooperate? Cells cooperate by sending to and receiving signals from other cells that lead to a change in phenotype that eventually leads to similarity in behavior. Signals are typically macromolecules, such as growth factors and cytokines of various types. Cell A sends a signal to a cell B in its vicinity by secreting several macromolecules M that travel by diffusion away from cell A, eventually binding on receptors on cell B. After binding on receptors on cell A these macromolecular signals may modify the phenotype of cell B, which now behaves like cell A.

The intensity of communication can be described by I , the rate of binding of macromolecular signals on receptors of cell B. The quantity I is the flux rate of signals transferred to B, i.e., the flux rate of signals that were actually received by B. It may not be possible to detect the cell that has sent out the cells but it is always possible, in principle, to measure the signals that have been received by a cell. The flux rate of signals received can be measured as the number of macromolecules that have become bound per unit surface area of cell B following a defined exposure to cells that emit such macromolecules. Although intensity of communication is required, it does not suffice for cooperative behavior since cells must also modify their phenotype, acquiring a common phenotype that characterizes the entire assembly of cells.

Cells that do not cooperate at all are referred to as noncooperative (“isolated”). Noncooperative cells show an intrinsic behavior that is not affected by the presence of other cells in culture or in a tissue site. An operational definition of an isolated cell is the indifference of its behavior to changing levels of cell density in the system. These cells have maintained their original phenotype either because the intensity of communication with neighboring cells was insufficient for conversion to the cooperative phenotype or because conversion was blocked by a sufficiently high conversion energy.

Critical transition. The behavior of cooperating cells differs markedly from that of isolated cells. When the cell density, or other parameters that are currently under study, a key experimental parameter, is increased above a threshold, cell behavior undergoes a sharp change. An example is the mechanical force exerted by cells on matrix. Below the density threshold cells apply forces that are independent of cell density and are only characteristic of cell type (and other parameters, such as medium composition). Such behavior is characteristic of point sources of mechanical force that act as if they were

stochastically independent. In contrast, above the threshold, the cells apply their individual forces in unison, developing macroscopic tractions that are significantly higher than they would be if the cells acted independently. In addition, cells above the threshold apply their forces with a timing that appears to be correlated, i.e., highly dependent on the activity of other cells in the system. The change in behavior above the critical density level is considered to be an example of an “all or none” transition, somewhat similar to the melting of ice to liquid water.

In biological terms the transition is described as a change in cell phenotype. In a given cell, A, such a change may be brought about by receipt of appropriate signals from other cells nearby. Signals issued from neighbors become bound on specific receptors on A; the bound signal is then transferred into the cytoplasm where it is directed toward the nucleus of A along a specific intracellular pathway. Interaction with the genome brings about transcription of genes that modify the behavior of A along a specific direction. If the phenotype of a large number of cells of the same type as A becomes modified along the same direction, the behavior may become cooperative, i.e., the affected cells all show the same new behavior spatially and temporally.

Change in cell phenotype can be described as an activated process, i.e., one requiring expenditure of a finite amount of energy. Using the terminology of activated rate processes, one can represent the transition in terms of an energy change from a state of noncooperating cells, E_n , to a state comprising cooperating cells, characterized by E_c . The transition to cooperative behavior occurs when noncooperative cells cross over an activation “hump”, E_{nc} . It follows simply from transition state theory that such a transition is likely to take place frequently when E_{nc} is low but it becomes nearly impossible to observe when E_{nc} is forbiddingly high relative to the two lower states on either side of the reaction coordinate.

Derivation of model of a critical transition in cooperative cell behavior. Assume cells exist either in “n” state (“noncooperative”), in which they display the “n” phenotype, or they exist in state “c” (“cooperative”) in which they exhibit the “c” phenotype. Briefly, each phenotype represents a well-defined behavioral state of that cell type. The “n” phenotype can be described as being characteristic of cells that are isolated from each other and may send out but do not receive signals from other cells that might induce a phenotype change from “n” to “c”. With isolated cells either there is little or no communication or else there is intensive communication but the signaling is ineffective in bringing about a phenotype change (the model does not inform about either one of these cases). In contrast, cells displaying the “c” phenotype are close together and receive large numbers of signals from other cells that are effective in changing their phenotype. An example of noncooperating cells are those described by Freyman et al. (Biomaterials 2001. 22:2883-2891): these cells apply mechanical forces to the extracellular matrix independently of each other and the force applied by a system of cells increases in direct proportion to the cell density. An example of cooperating cells is the assembly of myofibroblasts at the edge of a full-thickness skin wound; these cells generate forces that induce the onset of wound contraction and eventually manage to close the wound (Yannas, *Tissue and Organ Regeneration in Adults*, Ch. 9, Springer, 2001).

We will apply the methodology of statistical mechanics. The goal is to determine certain requirement(s) for observing a critical transition from a noncooperative to a cooperative phenotype.

Assume a configuration A of 7 cells as a mini culture (or mini collection) of cooperating (c) and noncooperating cells (n): nccncnn . Assume that these cells occupy a unit volume dV . Another mini culture B of 7 cells, occupies another unit volume dV , and can be described as: nncncnn ; a third is described as cncncnn , and so on for mini cultures C, D etc. and for a very large number of minicultures.

The energy of configuration A will now be represented as the sum of pairwise interactions between nearest-neighbor cells. No other interactions will be assumed to be present. For example, the model excludes consideration of interactions among three or more cells. Cooperative behavior implies collective involvement of a large number of cells. The model will answer the question: If only nearest-neighbor interactions (and no other more complex interactions) are present, can a critical transition still take place?

Interactions between cells in configuration A, nccncnn , that may lead to phenotype change, will be described by interactions between pairs of cells, so that the total energy of interaction for configuration A is:

$$E^A = E_{nc} + E_{cc} + E_{cn} + E_{nc} + E_{cn} + E_{nn}$$

Each of the several configurations A, B, C, etc. has a certain statistical weight and is more or less probable to occur. The statistical weight of each pair-wise interaction is represented by the appropriate Boltzmann factor of statistical mechanics, i.e., as an exponential of the energy of interaction between nearest neighbors. For example, $(-E_{nc} / RT)$ is the energy of the first interaction shown above. The statistical weight of the entire configuration A is:

$$\Omega^A = \exp(-E^A / RT) = \exp \left[-(E_{nc} + E_{cc} + E_{cn} + E_{nc} + E_{cn} + E_{nn}) / RT \right] = \\ \exp(E_{nc} / RT) \cdot \exp(E_{cc} / RT) \cdot \exp(E_{cn} / RT) \dots = g_{nc} \cdot g_{cc} \cdot g_{cn} \cdot g_{nc} \cdot g_{cn} \cdot g_{nn}.$$

The statistical mechanical partition function (“sum over all states”) is the sum of statistical factors for all configurations A, B, C etc.:

$$Z = \Omega_{confA} + \Omega_{confB} + \Omega_{confC} + \dots$$

$$Z = \sum_{All \Omega_j} \prod_{i=1}^{N-1} g_{kl;i}$$

where kl denote the choice of nn , cn , nc , cc ; i identifies the position (first, second, etc.) of a cell in the configuration; j is the number of distinguishable (different) configurations; and N is the number of cells in a configuration.

Use matrix algebra representation to decompose this expression.

Define a 2 x 2 matrix:

$$M \equiv \begin{bmatrix} g_{nn} & g_{cn} \\ g_c & g_{nn} \end{bmatrix}$$

solve using matrix method (see Appendix A)

$$Z = c_1 \lambda_1^{N-1} + c_2 \lambda_2^{N-1}$$

λ_1, λ_2 are roots of the matrix while c_1, c_2 are constants.

Two special cases when:

$$1. \quad g_{nc} \ll (g_{cc} g_{nn})^{\frac{1}{2}}$$

$$Z \approx c_1 g_{cc}^{N-1} + c_2 g_{nn}^{N-1}$$

If N is large (many in a configuration), one term dominates Z even if g_{cc} is only slightly different than (or even equal to) g_{nn} . This occurs when the transition state energy E_{nc} is sufficiently low compared to the energies of the noncooperative or the cooperative states. An all or none transition is predicted from noncooperative to cooperative cell behavior.

$$2. \quad g_{nc} \approx (g_{cc} g_{nn})^{\frac{1}{2}}$$

$$Z \approx c_1 (g_{cc} + g_{nn})^{N-1}$$

Here, the transition state energy E_{nc} is of the same order of magnitude as the energies of the noncooperative and cooperative states. No singularity occurs no matter what the relation of g_{cc} to g_{nn} and no matter what the magnitude of N. Return to randomness.

This model shows that nearest-neighbor interactions are sufficient for an “all or none” cooperative transition provided that the energy cost of the hybrid state is much higher than E_{nn} and E_{cc} and also provided the sequence is long enough (large N).

This model describes certain requirements for an all-or-none cooperative transition but does not predict the conditions under which the transition takes place. Such a condition would be, e.g., a critical threshold of the cell density.

Note. A mathematically identical model can be used to describe a helix-coil transition, i.e., a thermodynamic melting transformation. Since the system is now described in thermodynamic terms it is justified to use statistical thermodynamics arguments (not presented here) in order to relate Z to Gibbs' free energy via the relation (see, e.g., T.L.Hill, *Introduction to Statistical Thermodynamics*):

$$G = -RT \ln Z + PV .$$

As explained above, the two cases describe the outcomes. When Case 1 holds the model predicts that a critical transition will take place at the helix-coil transition temperature (the magnitude of which, however, is not predicted by the model). The important prediction from the model is that only nearest-neighbor interactions suffice for the critical transition. When Case 2 holds, the model predicts that a critical transition will not take place. (See Ref. 1 for literature on the helix-coil transition.)

Appendix A **Use of Matrix Algebra to Decompose Z (Ref. 2)**

The roots (eigenvalues) of M are solutions of the secular (polynomial) equation $|M - \lambda I| = 0$, a polynomial of second degree. M has two roots, λ_1 and λ_2 , since it is a 2x2 matrix. (For details of individual steps in the derivation see an introductory text on Matrix Algebra.)

M is related to the diagonal matrix D by a similarity transformation. Diagonalize M to help in raising M to the $N-1$ power. Since M and D are similar, so are their $N-1$ powers.

$$\text{Diagonalize } M: Q^{-1}MQ = D \Rightarrow M = QDQ^{-1}$$

Q chosen so that:

$$Q^{-1}Q = \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix} \text{ (the identity matrix)}$$

$$Q = \begin{bmatrix} q_{11} & q_{12} \\ q_{21} & q_{22} \end{bmatrix} \text{ and } Q^{-1} = \begin{bmatrix} p_{11} & p_{12} \\ p_{21} & p_{22} \end{bmatrix}$$

$$Z = [1,1]M^{N-1} \begin{bmatrix} 1 \\ 1 \end{bmatrix} = [1,1]QD^{N-1}Q^{-1} \begin{bmatrix} 1 \\ 1 \end{bmatrix} =$$

$$= \begin{bmatrix} 1 & 1 \end{bmatrix} \begin{bmatrix} q_{11} & q_{12} \\ q_{21} & q_{22} \end{bmatrix} \begin{bmatrix} \lambda_1^{N-1} & 0 \\ 0 & \lambda_2^{N-1} \end{bmatrix} \begin{bmatrix} p_{11} & p_{12} \\ p_{21} & p_{22} \end{bmatrix} \begin{bmatrix} 1 \\ 1 \end{bmatrix} =$$

= (drop matrix notation after multiplication) =

$$= (q_{11} + q_{21})(p_{11} + p_{12})\lambda_1^{N-1} + (q_{12} + q_{22})(p_{21} + p_{22})\lambda_2^{N-1}$$

$$= c_1\lambda_1^{N-1} + c_2\lambda_2^{N-1}$$

$$\text{Here, } D \equiv \begin{bmatrix} \lambda_1 & 0 \\ 0 & \lambda_2 \end{bmatrix} \text{ and } D^{N-1} \equiv \begin{bmatrix} \lambda_1^{N-1} & 0 \\ 0 & \lambda_2^{N-1} \end{bmatrix}$$

$$\text{To find roots where } M = \begin{bmatrix} g_{hh} & g_{hr} \\ g_{rh} & g_{rr} \end{bmatrix}$$

$$\begin{vmatrix} g_{hh-\lambda} & g_{hr} \\ g_{rh} & g_{rr} - \lambda \end{vmatrix} = 0 \text{ (quadratic equation)}$$

$$\lambda_{1,2} = \frac{g_{hh} + g_{rr}}{2} \pm \sqrt{\frac{(g_{hh} - g_{rr})^2}{4} + g_{hr}g_{rh}}$$

Two special cases:

Case 1.

$$g_{hr} \ll \sqrt{g_{hh}g_{rr}}$$

$$\lambda_{1,2} = \frac{g_{hh} + g_{rr}}{2} \pm \sqrt{\frac{g_{hh}^2 + g_{rr}^2 - 2g_{hh}g_{rr} + 4g_{hr}g_{rh}}{4}}$$

$$= \frac{g_{hh} + g_{rr}}{2} \pm \sqrt{\frac{(g_{hh} - g_{rr})^2}{4}}$$

$$= \frac{g_{hh} + g_{rr}}{2} \pm \frac{g_{hh} + g_{rr}}{2} \begin{cases} g_{hh} \\ g_{rr} \end{cases}$$

$$Z = c_1g_{hh}^{N-1} + c_2g_{rr}^{N-1}$$

Case 2.

$$\begin{aligned}
 g_{hr} &\cong \sqrt{g_{hh}g_{rr}}; g_{hr}^2 \cong g_{hh}g_{rr} \\
 \lambda_{1,2} &= \frac{g_{hh} + g_{rr}}{2} \pm \sqrt{\frac{g_{hh}^2 + g_{rr}^2 - 2g_{hh}g_{rr} + 4g_{hr}g_{rh}}{4}} \\
 &= \frac{g_{hh} + g_{rr}}{2} \pm \sqrt{\frac{(g_{hh} + g_{rr})^2}{4}} \\
 &= \frac{g_{hh} + g_{rr}}{2} \pm \frac{g_{hh} + g_{rr}}{2} \begin{pmatrix} g_{hh} + g_{rr} \\ 0 \end{pmatrix} \\
 Z &= c_1 (g_{hh} + g_{rr})^{N-1}
 \end{aligned}$$

References.

1. The original theoretical model of the helix-coil transition model was described by B.H. Zimm and J. K. Bragg, Theory of the Phase Transition between Helix and Random Coil in Polypeptide Chains, J Chem Phys 31: 526-535, 1959. See also A.V. Tobolsky, Cell Model for Diffuse Phase transition, J Chem Phys 41:1160-1162, 1964.
2. H.A. Kramers and G.H. Wannier, Statistics of the Two-Dimensional Ferromagnet. Part I, Phys. Rev. 60:252-262, 1941, originally showed that the task of solving for the partition function can be reduced to finding the largest eigenvalue of some matrix.

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