7.61 Fall 2006

9.11.06

Comment cards

Pace: - 1
Slow-5
Too slow in some parts
OK -11
A bit rushed – 1

Use chalkboard more
Examples when there are problems in interpreting results.

Lodish edition 4 pages.

Questions:

How do binding parameters provide insights about larger biological system? Varies. For example, Kd values can be compared to ambient concentrations of ligands to determine if there is a match or discordance. This can be used to help assess the specificity and function. Also, it can be used to determine if the receptor (e.g., endocytic rather than signaling) functions at saturation and the principal issue is the levels of expression for controlling physiology. Sometimes discordance has permitted to identification of co-receptors or co-ligands.

Examples of negative and positive cooperativity?

Check Pubmed with search: receptor cooperative binding

EMBO J. 2006 Aug 31; [Epub ahead of print] **Single-molecule analysis of epidermal growth factor binding on the surface of living cells.**

Teramura Y, Ichinose J, Takagi H, Nishida K, Yanagida T, Sako Y.

Global cellular responses induced by epidermal growth factor (EGF) receptor (EGFR) occur immediately with a less than 1% occupancy among tens of thousands of EGFR molecules on single cell surface. Activation of EGFR requires the formation of a signaling dimer of EGFR bound with a single ligand to each molecule. How sufficient numbers of signaling dimers are formed at such low occupancy rate is still not known. Here, we have analyzed the kinetics of EGF binding and the formation of the signaling dimer using single-molecule imaging and mathematical modeling. A small number of EGFR on the cell surface formed dimeric binding sites, which bound EGF two orders of magnitude faster than the monomeric binding sites. There was a positive cooperative binding of EGF to the dimeric binding sites through a newly discovered kinetic intermediate. These two mechanisms facilitate the formation of signaling dimers of EGF/EGFR complexes.

Nat Cell Biol. 2000 Mar;2(3):168-72. **Comment in:** Nat Cell Biol. 2000 Mar;2(3):E37. **Single-molecule imaging of EGFR signalling on the surface of living cells.** **Sako Y, Minoghchi S, Yanagida T.**

The early events in signal transduction from the epidermal growth factor (EGF) receptor (EGFR) are dimerization and autophosphorylation of the receptor, induced by binding of EGF. Here we observe these events in living cells by visualizing single molecules of fluorescent-dye-labelled EGF in the plasma membrane of A431 carcinoma cells. Single-molecule tracking reveals that the predominant mechanism of dimerization involves the formation of a cell-surface complex of one EGF molecule and an EGFR dimer, followed by the direct arrest of a second EGF molecule, indicating that the EGFR dimers were probably preformed before the binding of the second EGF molecule. Single-molecule fluorescence-resonance energy transfer shows that EGF-EGFR complexes indeed form dimers at the molecular level. Use of a monoclonal antibody specific to the phosphorylated (activated) EGFR reveals that the EGFR becomes phosphorylated after dimerization.

Biophys J. 2005 Dec;89(6):3686-700. **Effects of receptor clustering on ligand dissociation kinetics: theory and simulations.**

Gopalakrishnan M, Forsten-Williams K, Nugent MA, Tauber UC.

Department of Biological Physics, Max-Planck-Institut fur Physik Komplexer Systeme, Dresden, Germany.

Receptor-ligand binding is a critical first step in signal transduction and the duration of the interaction can impact signal generation. In mammalian cells, clustering of receptors may be facilitated by heterogeneous zones of lipids, known as lipid rafts. In vitro experiments show that disruption of rafts significantly alters the dissociation of fibroblast growth factor-2 (FGF-2) from heparan sulfate proteoglycans (HSPGs), co-receptors for FGF-2. In this article, we develop a continuum stochastic formalism to address how receptor clustering might influence ligand rebinding. We find that clusters reduce the effective dissociation rate dramatically when the clusters are dense and the overall surface density of receptors is low. The effect is much less pronounced in the case of high receptor

density and shows nonmonotonic behavior with time. These predictions are verified via lattice Monte Carlo simulations. Comparison with FGF-2-HSPG experimental results is made and suggests that the theory could be used to analyze similar biological systems. We further present an analysis of an additional cooperative internal-diffusion model that might be used by other systems to increase ligand retention when simple rebinding is insufficient.

J Mol Neurosci. 2005;26(2-3):155-60. **Oligomers of D2 dopamine receptors: evidence from ligand binding.Strange PG.**
There is increasing evidence that G protein-coupled receptors form oligomers and that this might be important for their function. We have studied this phenomenon for the D2 dopamine receptor and have shown using a variety of biochemical and biophysical techniques that this receptor forms dimers or higher-order oligomers. Using ligand-binding studies, we have also found evidence that this oligomer formation has functional relevance. Thus, for the receptor expressed in either CHO cells or Sf 9 insect cells, the binding properties of several radioligands (in saturation, competition, and dissociation assays) do not conform to those expected for a monomeric receptor with a single binding site. We propose that the receptors exist in oligomers with homotropic and heterotropic negatively cooperative interactions between ligands.

Is there amplification of the signal mediated by nuclear receptors? Yes, multiple rounds of transcription can produce a very large amplification from a single receptor bound to hormone on the promoter of a gene.

Do nuclear receptors return to the cytoplasm? Yes, when the ligands dissociate, the NR can be released and return to the cytoplasm (see progesterone receptor slide in the handout).

Is an inverse agonist samae as a repressor? Not really normally considered in this way, but I am not absolutely sure what you mean when you say 'repressor'.

When iodinating proteins can one get diIodotyrosine as well as MIT? Yes.

Problem with details of the 4 estrogen pathways – see me for more, not sure how to help.

Will lecture notes be available on-line before the lectures? Sometimes. Those for lec 1 and 3 were (if the site was working). Of course, please DON'T look ahead unless you found it hard to follow the lecture (Socratic method…).

Is it necessary to review thermodynamics of binding for this course? If you haven't looked at deltaG and Kd for a while, you should review at least at the very elementary level in the two textbooks.

How much of an excess of cold is used for hot+cold exps? 40-100-fold.