**Notes on simulation units**

ODE-based models were originally designed to simulate binding reactions in a homogenous, well-mixed solution. As such, the units of second order rate constants were usually expressed in units of molar and seconds (or minutes for slow reactions). When models were based on cells, a variety of different units were used to accommodate the heterogenous nature of the system. For example, in my original models, I expressed receptor concentrations in terms of receptors/cell. The concentration of EGF was in terms of molar. This worked fine, yielding an equilibration constant in terms of molar and binding rates in terms of molecules per cell per sec. Things are more complex if you want to generalize a model to accommodate cells of different sizes, shapes and receptor expression and to include reactions on the cell surface, the internal membrane leaflet and various compartments. This requires setting a “volume” of the cells. This is not technically accurate because binding of a soluble ligand to a membrane-anchored receptor is much more efficient because of steric and orientation effects (e.g. the ligand cannot get “behind” the receptor). Membrane localization seems to increase the efficiency of binding ~250 fold (DOI: 10.1007/978-1-4419-5913-3\_73). As long as we are consistent with our units, we should be able to interconvert units between systems.

Forward rate constant: The original units are per cell (M-1 sec-1). Although ligand concentrations are easy to set, we normally use variable numbers of cells expressing variable number of receptors. We can normalize to each cell, but then we would have to scale down the medium volume to per cell.

Let’s say that you have 1x105 receptors per cell. If a cell has a volume of 2 pl (a reasonable estimate), then the molar equivalent would be ((1x105/AN)/2 pl), which is 8.3E-8 M. Thus, if a cell had a single receptor, it would be 8.3E-13 M. Thus, 100,000 receptors per cell would be 1x105 \* 8.3E-13 M, or 8.3E-8 molar (83nM). If we convert everything to molar, then we have to convert all copies-per-cell protein values to concentration. One issue we must consider is ligand depletion at low ligand concentrations because the media needs to be scaled with the cells. Assume 106 cells per 10ml of media, then 10-2L/106 = 10-8 L

As you go from 0->100% receptor occupancy, you are increasing the concentration of PY sites at the plasma membrane. Half-maximum Ras activation is between 300-2000 occupied receptors, which presumably reflects half-maximum binding of an effector because the number of receptors is nowhere near limiting. If we take the 2000 receptor value as a conservative Km value, the effective receptor concentration (one site per receptor) would yield a value of 1.66nM. However, concentrating the receptors at the membrane increases its effective concentration by approximately 250 (Sites reference). So, this level of activated receptors would be seen by free cytoplasmic adaptors as ~0.4µM. How far off are calculated adaptor affinities?

The number of Grb2 in HMEC cells is ~43K. This translates to a concentration of 35nM. In the paper by Morimatsu (DOI: 10.1073/pnas.0701330104), the apparent dissociation equilibrium constant for membrane-anchored EGFR binding to Grb2 was experimentally estimated from the total on- and off- times for each binding site. The average was 97, 340, and 650 nM at 1, 10, and 100 nM Grb2, respectively. At 35nM, this would be ~500nM, or 0.5µM, which is close to observed half-maximum binding of Grb2-SOS to the EGFR.