Systems I

Ligand-receptor trafficking

Systems Biology

- Nature knows that teamwork is more efficient
- Biochemical reaction networks allow for
  - Filtering out of low-level and noisy signals
  - Amplification of signals when strong responses are necessary
  - Graded or switch-like responses
  - Feedback for regulation
  - Feedforward for multiple time-scale responses
- Molecular processes in cells are essentially all about binding and catalysis (thermo and kinetics)
  - A Mech. Eng. would argue that it's all push and pull
  - A EE might say it's all signal dynamics…

Fundamentals of Kinetic Modeling

- Based on material balances

  {in} – {out} = {accumulation}

- Rate law: describes time course of concentration as function of concentration of all species

$$v = \pm \frac{dC_i}{dt} = f\left(C_A, C_B, C_C, \ldots\right) = kC_A^{\alpha} C_B^{\beta} C_C^{\gamma} \ldots$$

- First-order reaction: probability of a particular molecule undergoing rxn indep. of other molecules

$$\frac{dC}{dt} = -kC$$

$$\frac{dC}{C} = -k\ dt$$

Systems of Reactions

- Parallel reactions

$$A \xrightarrow{k_1} B \qquad -\frac{d[\mathrm{A}]}{dt} = k_1[\mathrm{A}] + k_2[\mathrm{A}] = (k_1 + k_2)[\mathrm{A}]$$

$$A \xrightarrow{k_2} C \qquad \frac{d[\mathrm{B}]}{dt} = k_1[\mathrm{A}] = k_1[\mathrm{A}]_0 e^{-(k_1+k_2)t}$$

- Series reactions

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C \qquad v_1 = -\frac{d[\mathrm{A}]}{dt} = k_1[\mathrm{A}]$$

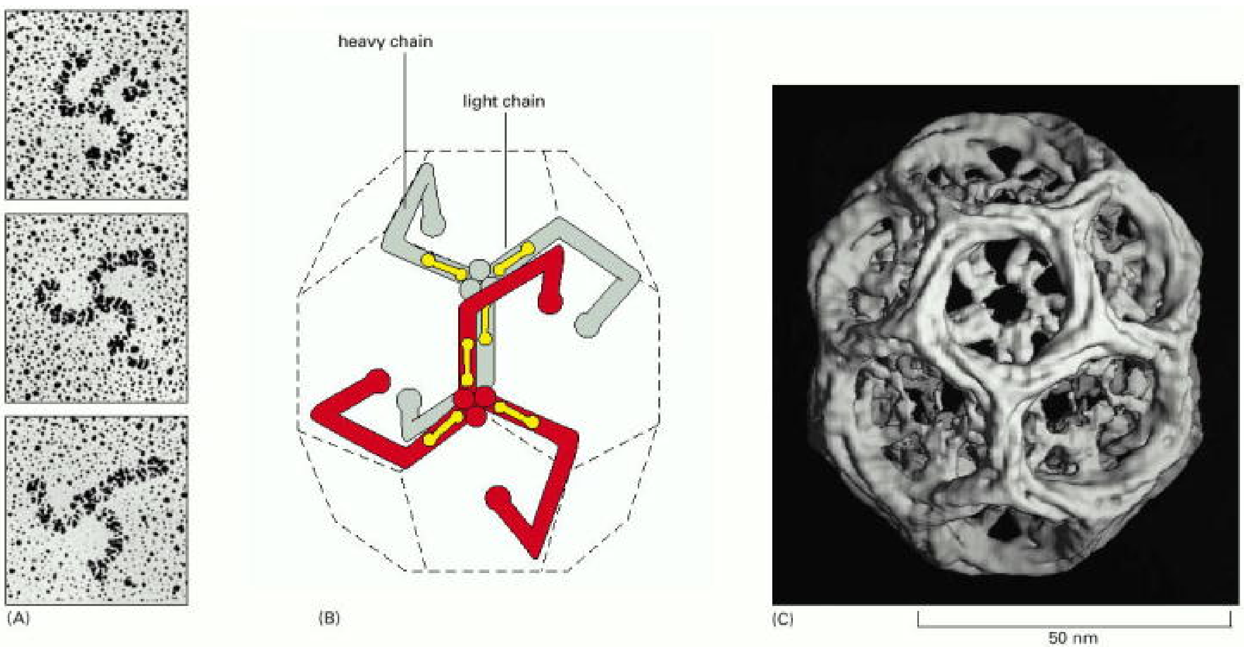
$$\frac{d[\mathrm{B}]}{dt} = k_1[\mathrm{A}] - k_2[\mathrm{B}] = k_1[\mathrm{A}]_0 e^{-k_1 t} - k_2[\mathrm{B}] \Rightarrow [\mathrm{B}] = \frac{k_1}{k_2 - k_1}[\mathrm{A}]_0 \left(e^{-k_1 t} - e^{-k_2 t}\right)$$

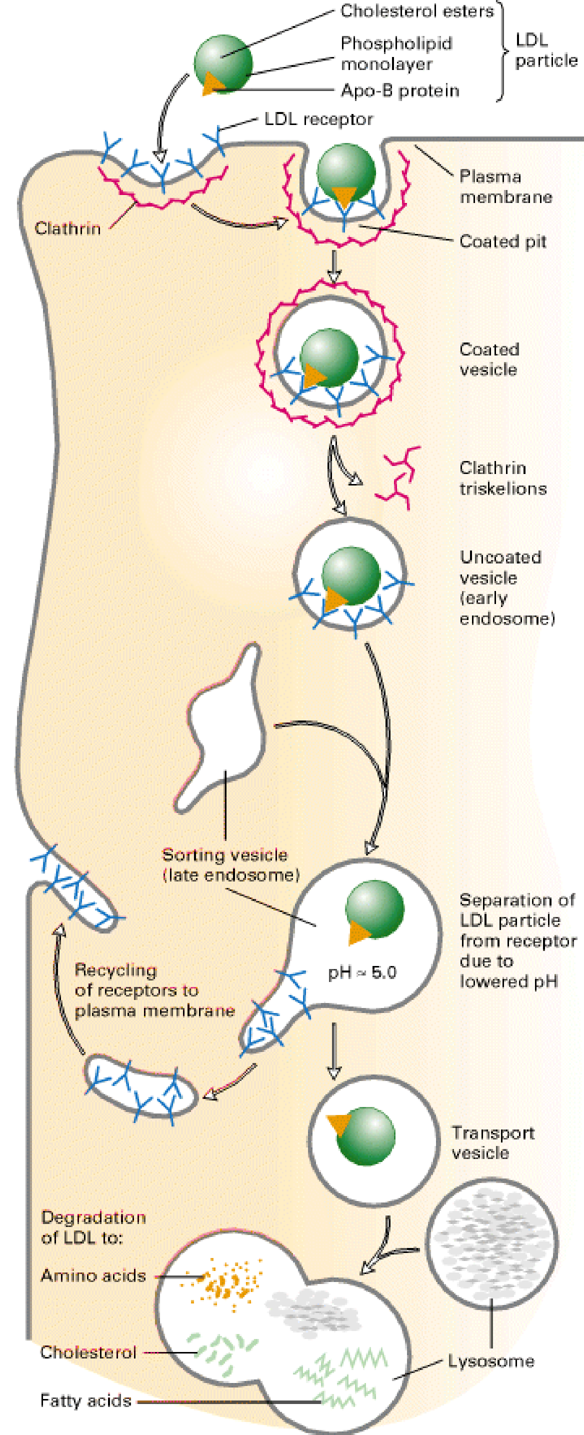
$$[\mathrm{C}] = [\mathrm{A}]_0 \left\{1 - \frac{1}{k_2 - k_1}\left(k_2 e^{-k_1 t} - k_1 e^{-k_2 t}\right)\right\}$$

- Rate-limiting step
  - What happens when $k_1 \gg k_2$?

Receptor-mediated Endocytosis

Clathrin





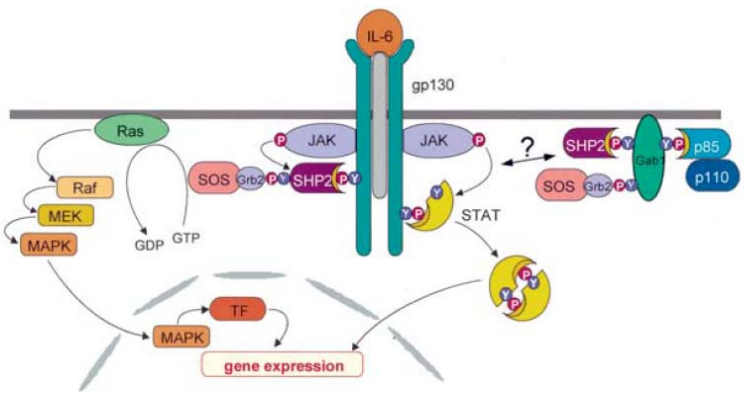
Lodish et al., Molecular Cell Biology

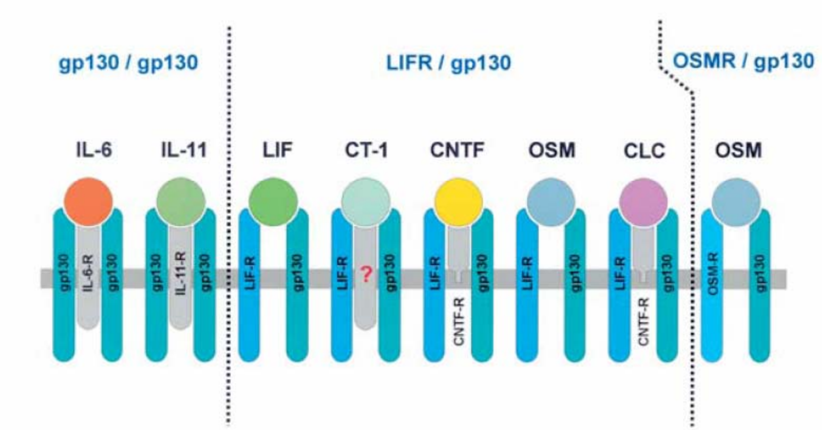
Functions of Ligand-Receptor Systems

- Communicate signals from outside of cell to within
  - Cell-cell communication
  - Systemic communication
- Recognize foreign substances
  - Bacterial pathogens
  - Allergens

Ligand-receptor Binding is Just the First Step…

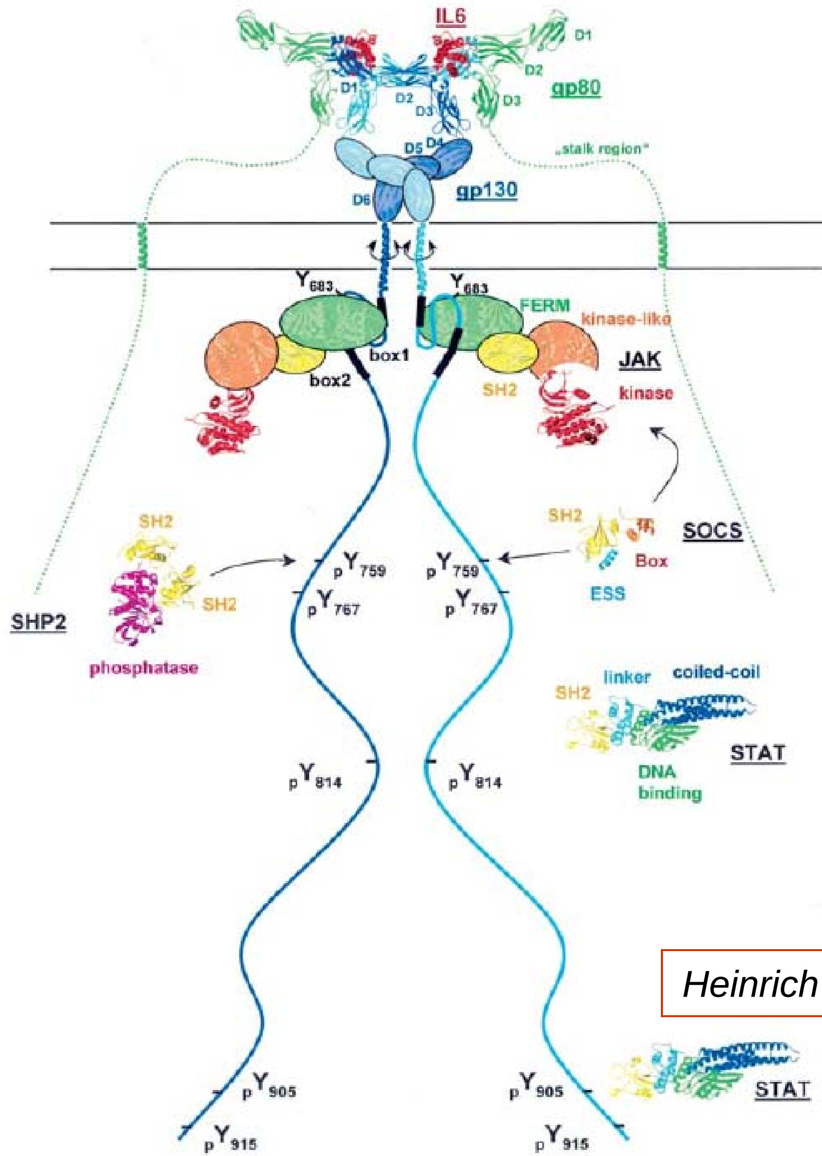
- Example, interleukin-6 is an inflammatory cytokine…
- IL-6 activates Jak/STAT and MAPK pathways
- There is a whole family of cytokines that utilizes the same signaling receptor, gp130





Heinrich et al., *Biochem. J.*, 374:1 (2003)

Insights from Structural Biology



Heinrich et al., Biochem. J., 374:1 (2003)

Putting Ligand-Receptor Binding to Use

- Agonists
  - E.g., cytokines and growth factors
- Antagonists
  - Antibodies and soluble receptors (often blocking cytokines and growth factors)
- Targeting agents
  - Imaging
  - Drug delivery

Agonists

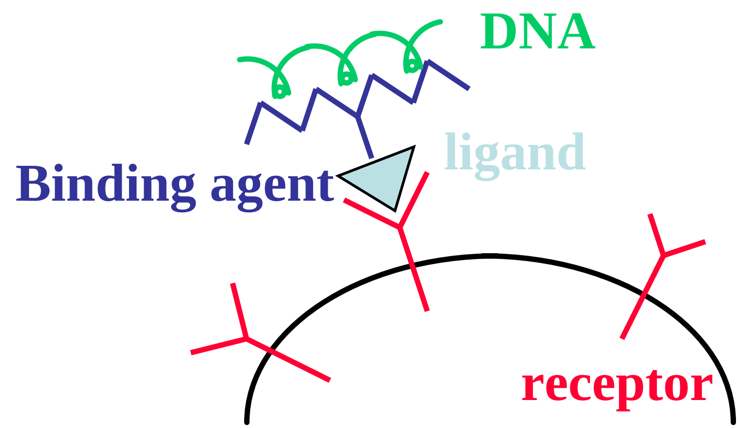
- Goal: to create molecules that potently stimulate downstream responses
  - E.g., GM-CSF, EGF, hyper-IL6
- Considerations
  - Receptor trafficking and sorting
  - Signal transduction
  - Pharmacokinetics

Antagonists

- Antibodies and soluble receptors
  - E.g., anti-EGFR or anti-Her2 therapies for cancer, anti-TNF antibodies for rheumatoid arthritis
- Considerations
  - Kinetics of binding – association rate vs. that of the binding reaction being neutralized (remember affinity usu. governed by dissociation not assoc.
  - Localization of process being targeted
    - E.g., autocrine or juxtacrine signaling

Ligand Targeting

- The distribution of drugs (including genes or oligonucleotides) can be influenced by the attachment of ligands that are recognized by receptors unique to the target cell type

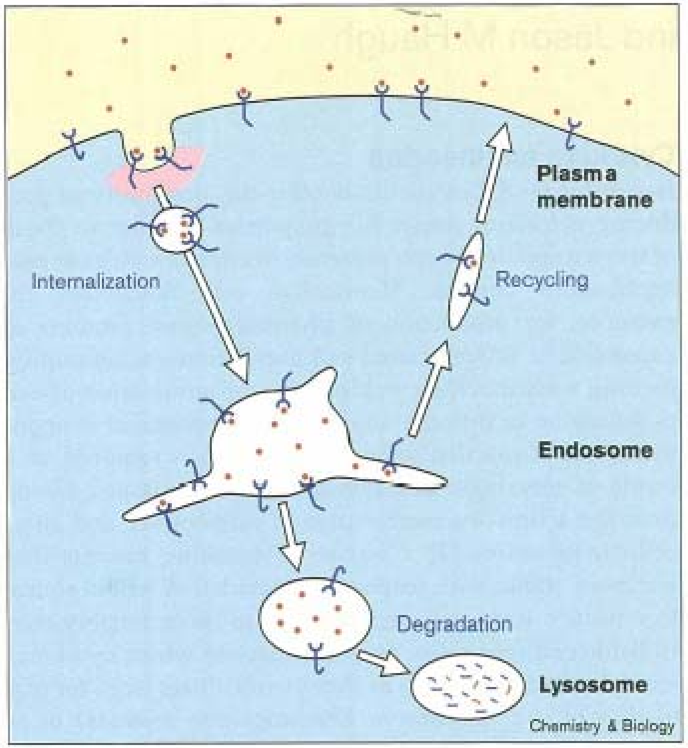


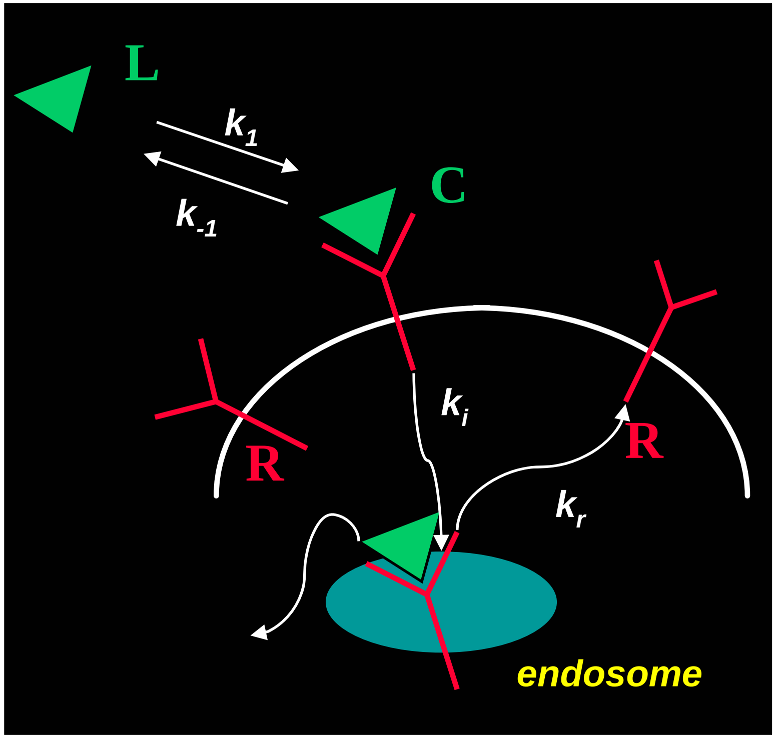
- Limited by receptor number and trafficking routes and rates

- Examples:

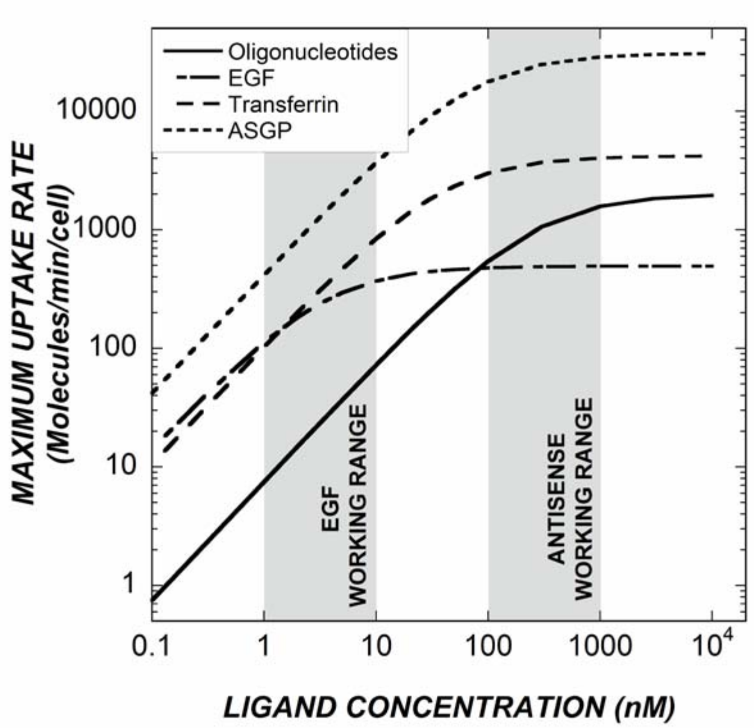
Ligand	Receptor	Cell type targeted
ASGP/galactose	ASGPr	Hepatocytes
EGF/TGF	EGFR	Multiple carcinoma
Folate	Folate R.	Ovarian carcinoma
MAb	E-selectin	Endothelial (inflammation)
HPV capsid	HPVR	Cervical epithelial carcinoma

Quantitative Analysis of Trafficking





Parameter Values



Parameter	EGF	ASGP	Transferrin	Oligonucleotide
R_T (#/cell)	2.5×10^4	2.0×10^5	5.1×10^4	1.0×10^5
k_1 (μM^{-1} min^{-1})	72	2.2	3.0	0.15
k_{-1} (min^{-1})	0.34	0.022	0.09	0.03
k_i (min^{-1})	0.03	0.47	0.20	0.03
k_r (min^{-1})	0.058	0.23	0.14	0.06

Further Reading

- Lodish et al., Molecular Cell Biology, W.H. Freeman, New York
- Lauffenburger and Linderman, Receptors, Oxford University Press, 1993.