Investigating the spatial organization of VEGF receptors on the cell membrane

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Cell signaling, receptors

- In a multicellular organism,
  - Every cell carries the same ‘genetic information’
  - Cells exhibit many different phenotypes and are organized into higher structures
- Cells select the appropriate behavior (phenotype) based on external inputs
  - Temperature, pressure, pH,..
  - More importantly: communication from the rest of the organism, in the form of chemical signals
  - Important during embryonic development and beyond

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Cell signaling, receptors

- Chemical signals that influence cell behavior are often “growth factors”
  - EGF (endothelial g.f.), VEGF (vascular e.g.f.)
- The molecule typically binds to a receptor
  - VEGFR is the receptor for VEGF, and VEGF is the ligand for VEGFR
- Ligand binding activates the receptor
  - Change in the level of a chemical activity

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Cell signaling, receptors

- Ligand binding is the first step in a chain of transformations
  - Formation of molecular aggregates
  - Activation (phosphorylation) cascades
  - Transport
- The sequence typically results in a change in the pattern of gene expression and specific behaviors of the target cells

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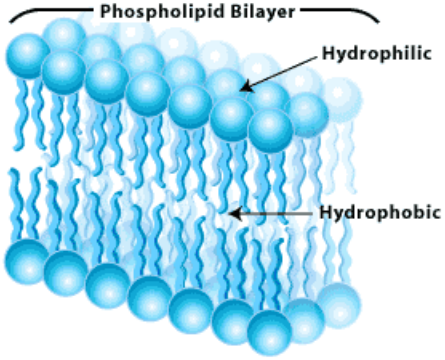
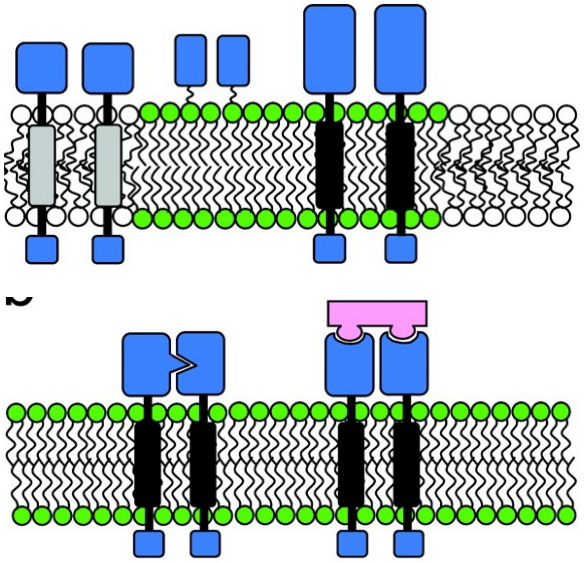
Cell signaling, receptors

- Many important receptor types are located in the cell membrane
  - Confined to the cell membrane, but move more or less freely parallel to it
  - Extracellular and intracellular domains
- Signaling from receptor tyrosine kinases (RTK) requires dimerized, ligand-bound receptors
  - Receptors can bind ligand and dimerize, not necessarily in this order
  - Ligand binding facilitates dimerization
  - Dimerization + ligand binding induces a conformational change in the intracellular domains





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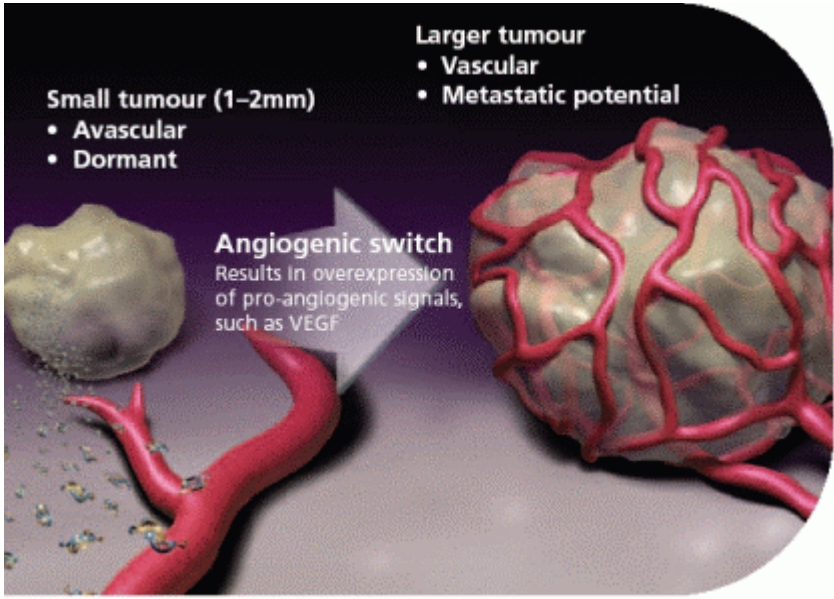
Vascular Endothelial Growth Factor

- Involved in the development of new blood vessels (angiogenesis and vasculogenesis)
- Role in tumor vascularization - *angiogenic switch*
  - Tumors are initially a localized proliferation of anomalous cells, limited by the lack of a dedicated blood supply
  - VEGF is secreted by hypoxic cells
  - Vascular endothelial cells proliferate and migrate toward the VEGF gradient
  - New (albeit irregular) blood vessels are formed
  - Tumor now has its own blood vessels and can grow further
- New drugs target VEGF (avastin)



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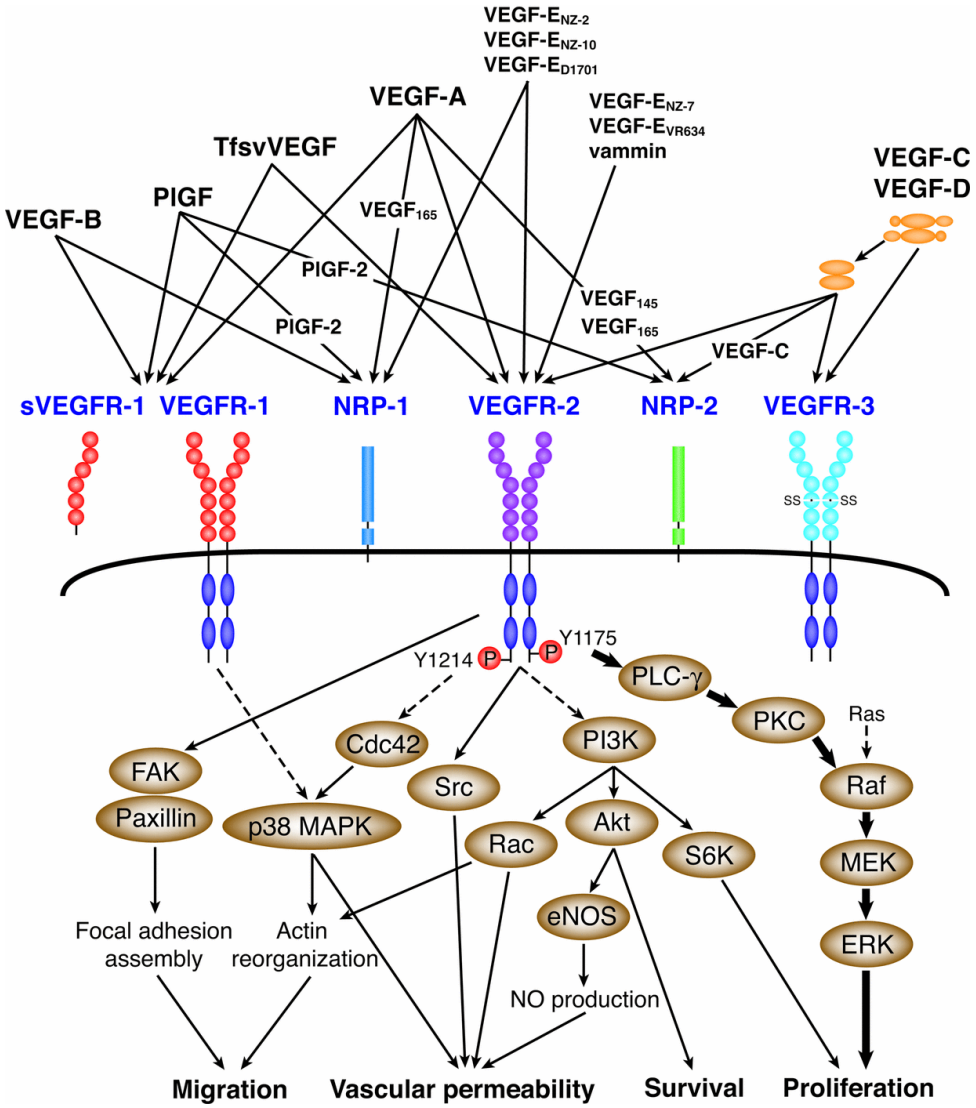
VEGF signaling



- Several types of VEGF (A-E, PlGF)
  - VEGF-A is the best studied
  - splice variants
- Several RTK receptors
  - Flk-1 (VEGFR-1), KDR (VEGFR-2)
  - Typically present together
  - VEGFR1 has higher affinity
  - VEGFR2 more active in signaling
  - VEGFR1 has a soluble splice variant

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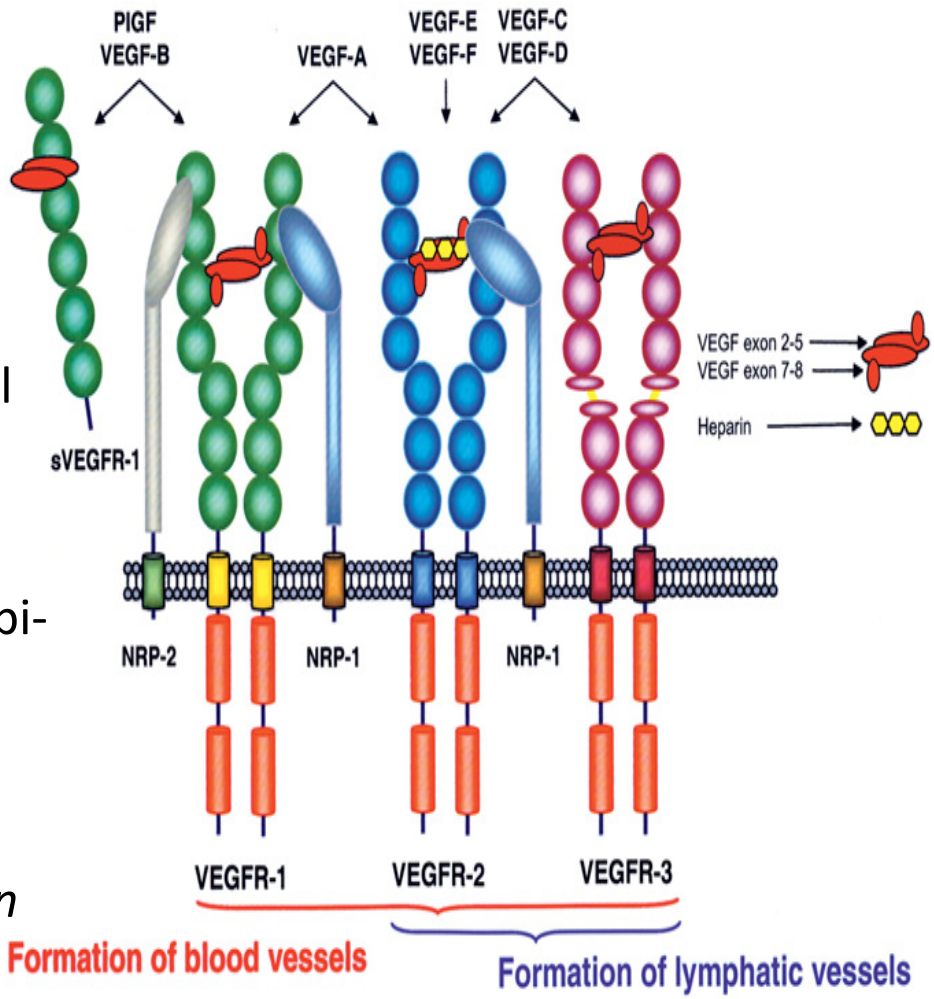
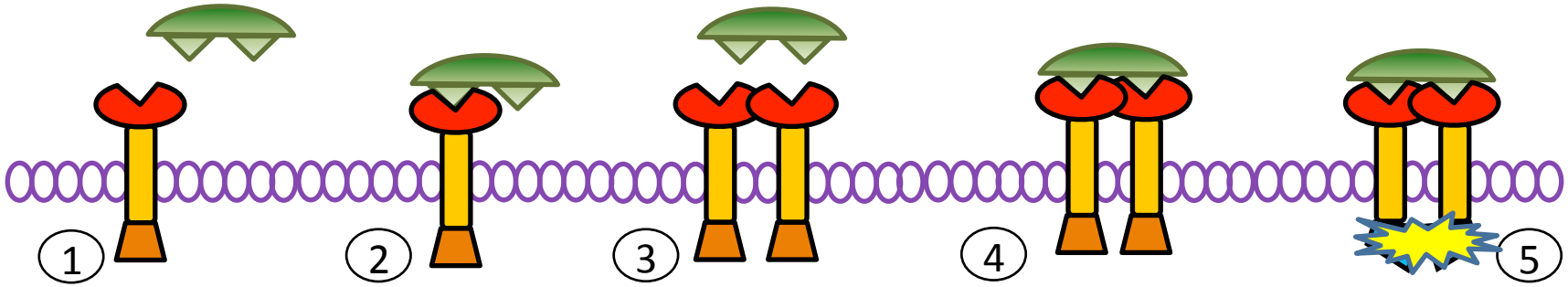
VEGF binding

- Features of VEGF receptors
  - They bind to other membrane proteins (e.g. neuropilin)
  - They form oligomers
  - Activation of VEGFR-2 leads to changes in the cytoskeleton and cell membrane tension
- VEGF ligand is bivalent
  - VEGF is normally a dimer, acts as a bi-valent ligand
  - Two bound VEGF receptors can not form a dimer
  - Phenomenon of *high dose inhibition*



VEGF binding

- Bivalent ligand and monovalent receptor



- A kinetic model is available for one type of ligand (VEGF-A) and two types of receptor (VEGFR-1 & VEGFR-2)

  [MacGabhann and Popel, 2007]

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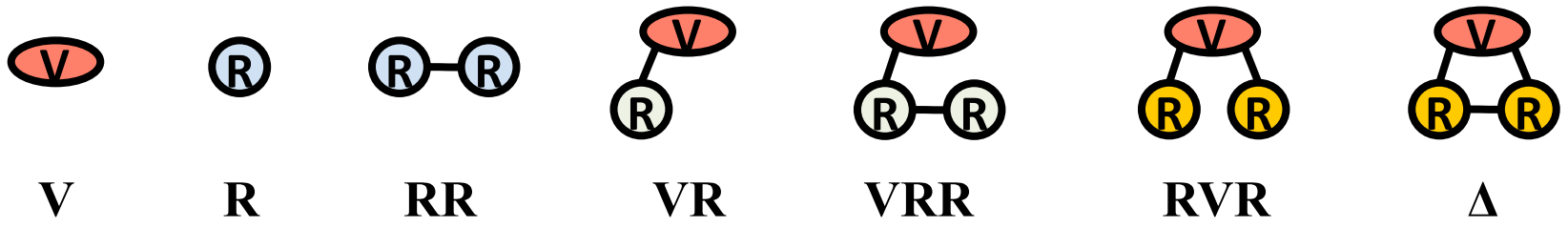
VEGF binding reactions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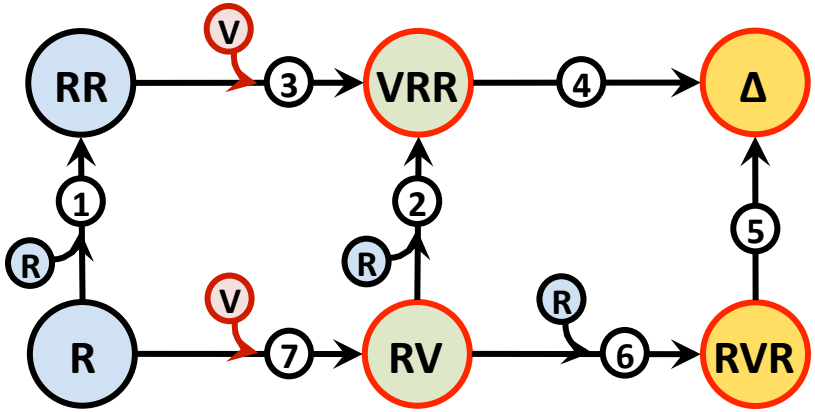
VEGF binding reactions

- One-receptor model:
  - 6 species, one of them NOT on the cell surface (V)
  - 7 reactions, 2 types of bonds (R-R,V-R)
- Formation of a V-R bond may happen in two distinct ways
  - Between a surface species (R or RR) and a volume species (V)
  - Between two surface species (R + RV)
- Formation of activated complex requires one cross-linking step
  - Typically, this is RV + R → RVR
  - Depends on the distribution and mobility of receptors on the surface

$$\Phi_1 = k_{c,RR}[R]^2 - k_{d,RR}[RR]$$
$$\Phi_3 = k_{on,VR}[V] \cdot [RR] - k_{off,VR}[VRR]$$
$$\Phi_5 = \mathrm{k}_{\Delta,\mathrm{RR}}[RVR] - k_{d,RR}[\Delta]$$
$$\Phi_7 = k_{on,VR}\, V \cdot [R] - k_{off,VR}[VR]$$
$$\Phi_2 = k_{c,RR}[R] \cdot [VR] - k_{d,RR}[VRR]$$
$$\Phi_4 = k_{\Delta,\mathrm{VR}}[VRR] - k_{off,VR}[\Delta]$$
$$\Phi_6 = k_{c,VR}[R] \cdot [VR] - k_{off,VR}[RVR]$$



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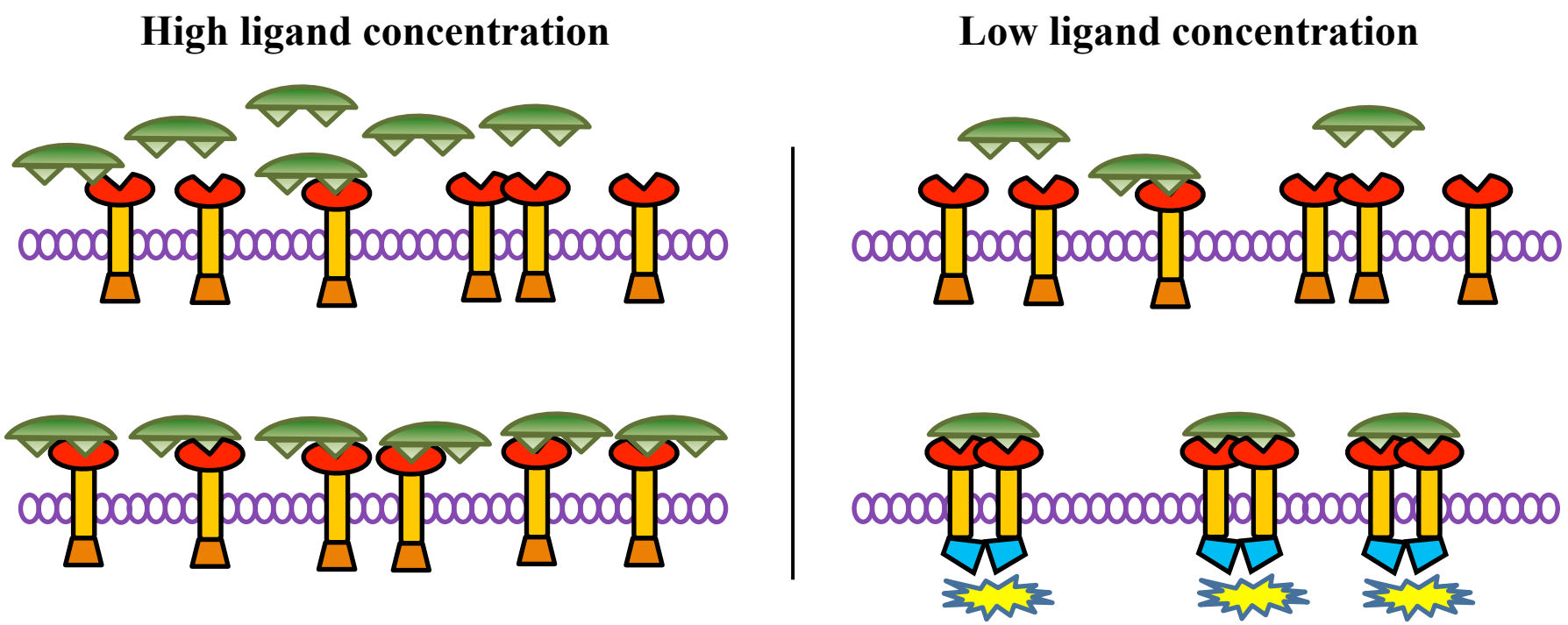
High dose inhibition



- The formation of a second V-R bond requires cross-linking between a ligand-bound receptor (VR) and a free receptor
- If the ligand concentration is high enough, there will be very few free receptors, resulting in few activated complexes

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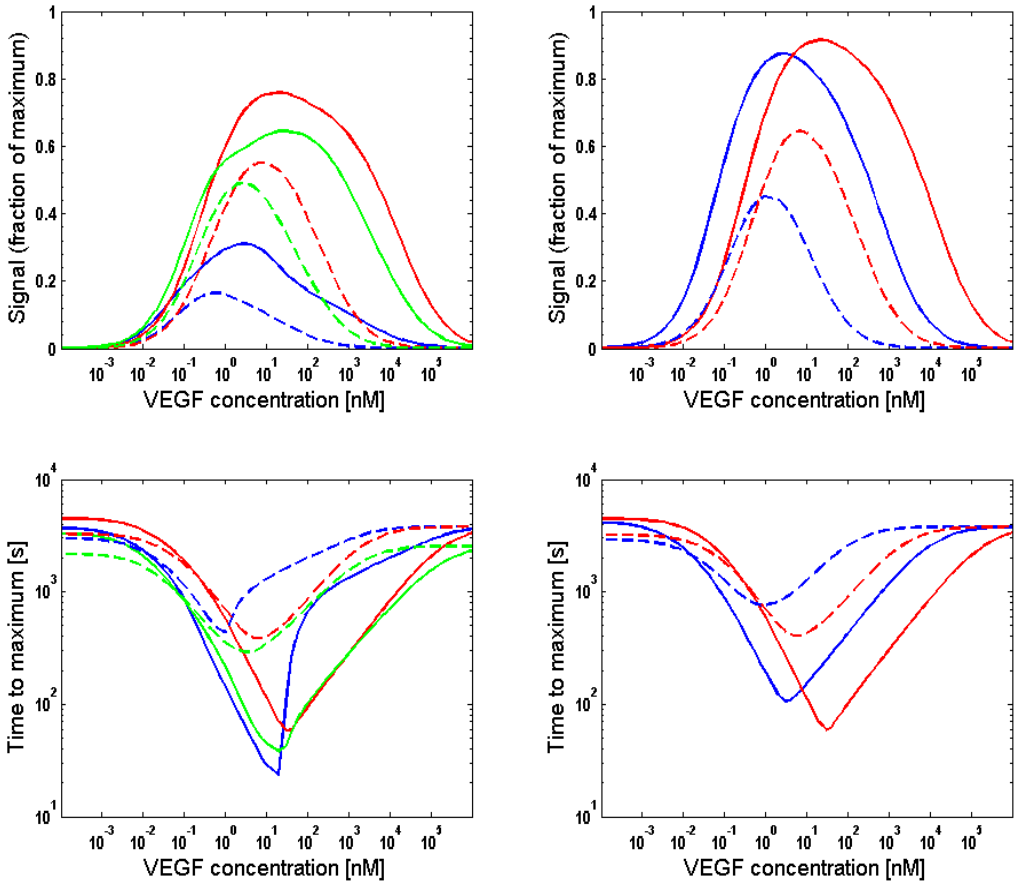
High dose inhibition

- Competition between free and surface-bound ligands (for free receptors)
  - Cross-linking vs. capture rates
- Results in peaked dose response curves
  - Partially overlapping curves for each type of dimer (11,22,12)
- Width of curves increases with the cross-linking rate, i.e.:
  - Higher receptor mobility
  - Receptor concentration
- Figure: activated complexes
  - Peak value (top) time to peak (bottom)
  - Colors: 11,22,12 dimers
  - Dashed line: reduced cross-linking



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High dose inhibition

- HDI is not easily observed with VEGF
- Increased cross-linking rates reduce high dose inhibition
- The cross-linking rate is proportional to the collision rate between receptors
  - Concentration of receptors in a fraction of the cell surface increases the cross-linking rate
  - Increased diffusion rate also increases cross-linking
- Spatial organization has a direct impact on the amplitude and time of the signal response

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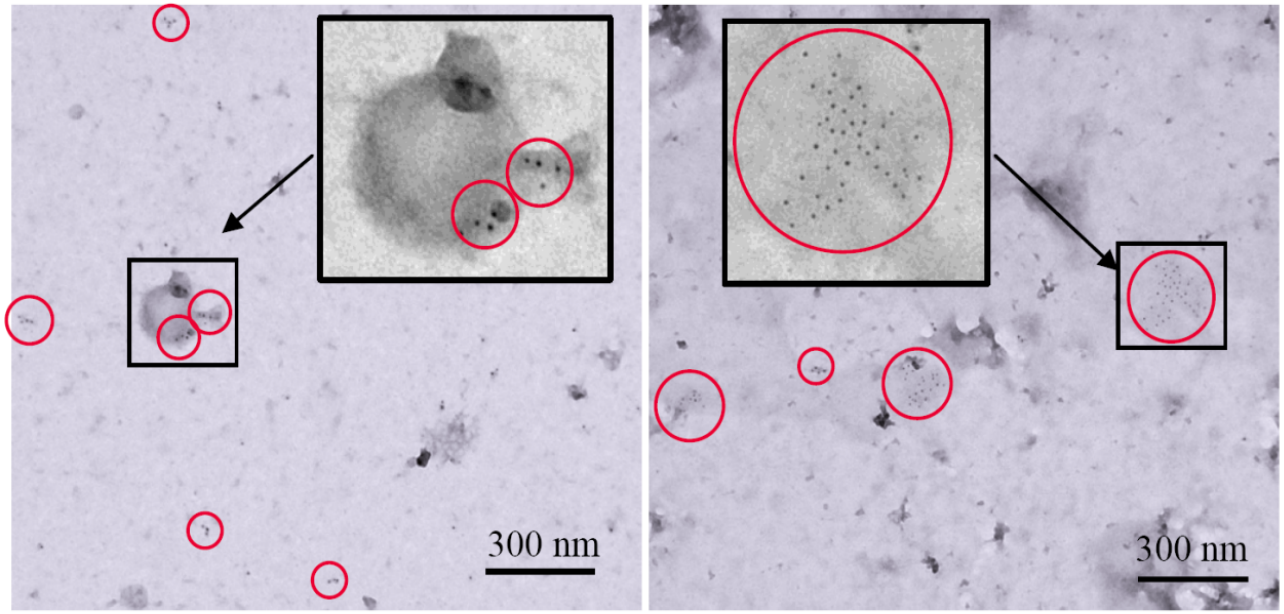
Clustering of receptors

- We need to understand the mechanism of receptor clustering



- Possible sources
  - Collective binding: similar to the 3-2 system, where individual molecules bind to each other in a way that does not saturate
  - Alternative explanation: domain structure

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Domains on the membrane

- Barriers to movement due to elements of the cytoskeleton
  - ‘free’ diffusion of membrane proteins (receptors and others) is inhibited
- Induced microdomain structure
  - Most of the time, receptors are confined to individual microdomains
  - Barrier crossings are possible, but rare
  - Larger molecules / aggregates have reduced mobility, thus are less likely to cross the domain boundaries
- Some microdomains may be ‘sticky’
  - Higher affinity for certain (or possibly, all) types of receptor

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Hypothesis

- Clusters may be induced by the interplay of the domain structure and reduced diffusion of dimers and larger molecular aggregates
- A positive feedback loop:
  - Higher receptor density in a domain increases dimerization and the formation of larger complexes
  - Dimers have reduced mobility, therefore molecules will exit the domain at a lower rate compared to its neighbors
  - Imbalance in exit rates leads to further increase in receptor density
- Can this mechanism generate clustering of a given type of receptor (for instance, VEGFR)?

Simulation methods

- Well-mixed SSA (aka Gillespie / CTMC)

  States = molecule numbers for each species

  Transitions = reaction events

  Propensities = reaction rates

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Simulation methods

- Spatial or kinetic Monte-Carlo

  Lattice of locations (discretization of the space)

  States = location and chemical species of each particle

  Transitions = reaction events or hopping (diffusion)

  Propensities = reaction rates and hopping rates

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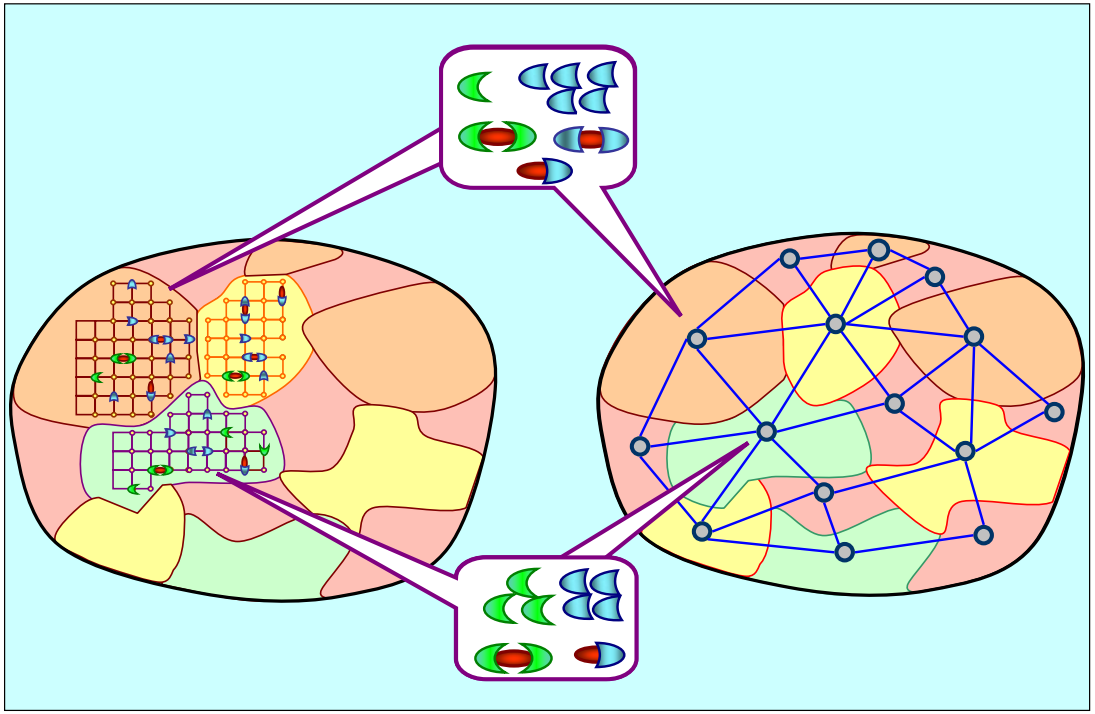
The system of interest

- A patch of the cell membrane (2 x 2 um)
- Domain structure:
  - Lattice (of possible 1-particle locations)
  - Barriers to movement between certain sets of sites
- Previous work:
  - Evidence of emergence of clustering
  - System of many domains is too large for a single simulation
  - Need to simulate several domains to account for the ‘extra’ receptors

Coarse-grained model

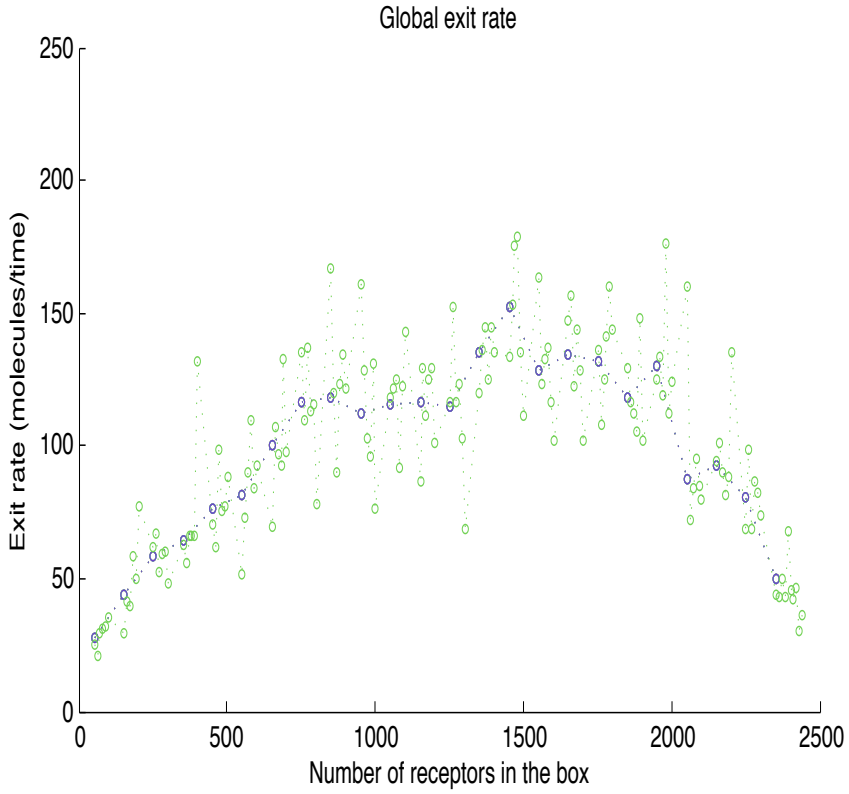
- Spatial coarse-graining
  - Merge the sites of each domain into a single, well-mixed* box



- Gillespie within each box
- Boxes can exchange particles
  - Exit rates obtained from full simulations

Coarse-grained model

- Current work: monomers and dimers
  - Dimers not allowed to exit
- For linear exit rates (for monomers), we can obtain analytical distributions
- Strong evidence of crowding – decreasing global exit rates (above 60% occupancy)
- Two possible interpretations of the coarse-grained model:
  - Microdomains
  - Coarse-graining of diffusion (i.e., would become exact as the box size approaches zero)



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Summary

- Many signaling pathways rely on ligand-induced receptor dimerization
- Spatial distribution and mobility of receptors may control the efficiency of signaling (especially when HDI is possible)
- Receptor clustering and domain stucture are likely connected
- Main theoretical approach is through simulations
- Simulations need to capture molecular detail and cover a large fraction of the membrane

  → a coarse grained approach

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Thank you

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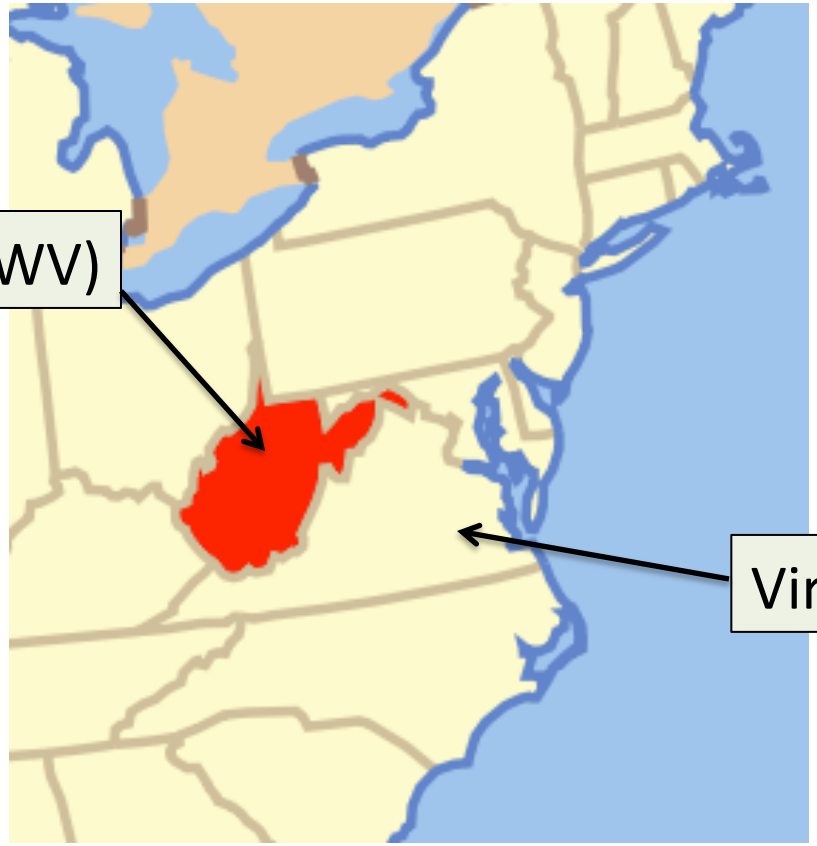
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