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Multiscale Spatio-Mechanical Regulation of Eph Receptors

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Berkeley
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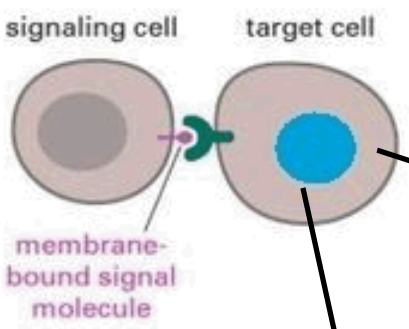
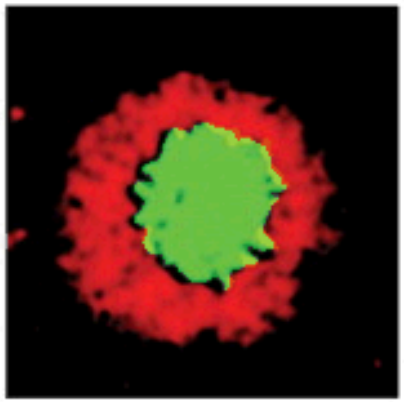
Multiscale Regulation of Cell Signaling

One mechanism by which cells communicate with each other is through direct physical contact

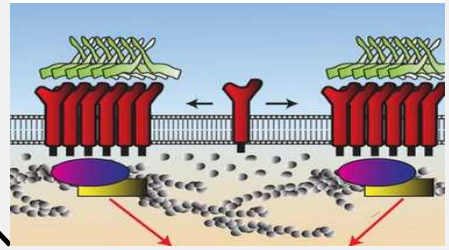
1. Cell-cell signaling



2. Cell-cell Interface



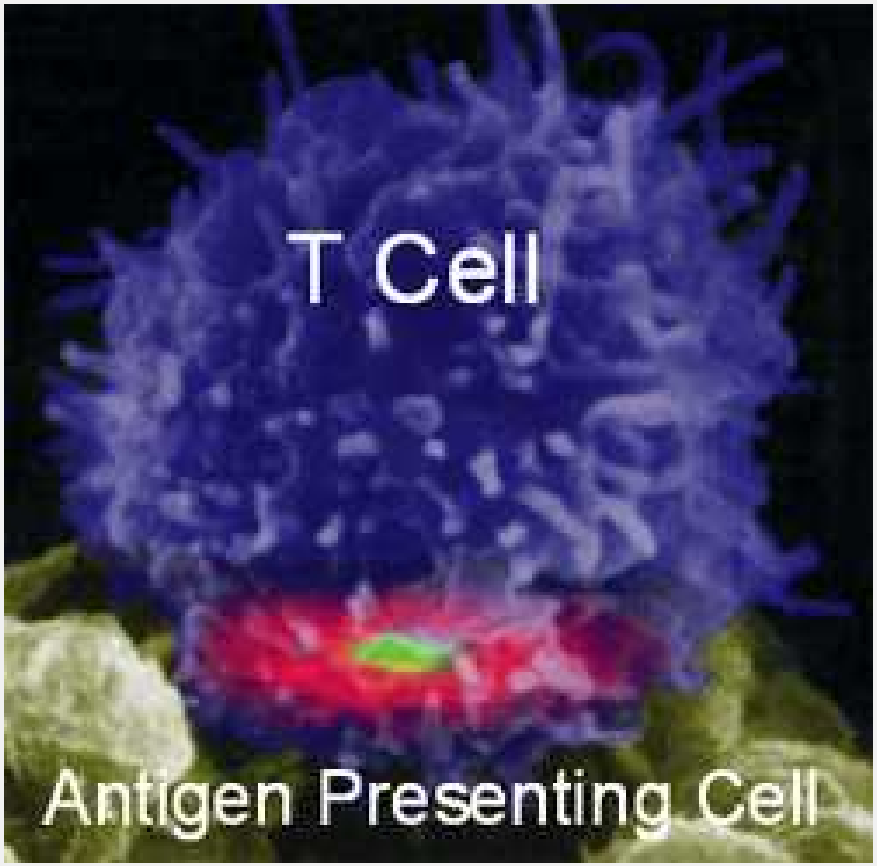
3. Protein Clustering



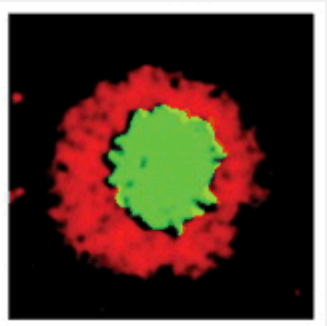
- Cell signaling is “noisy”
  - A single cell input results in a complex cascade of intracellular responses
  - Typically, there are many simultaneous signal inputs, resulting in highly stochastic cell response

Clustering at a Cell-Cell Interface Regulates Signaling

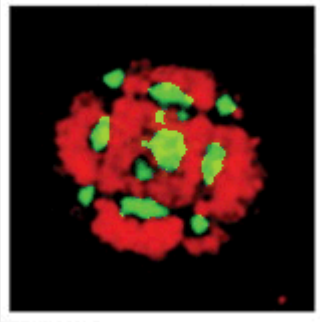
Receptor-ligand organization (clustering) at a membrane is a mechanism for cells to overcome biological noisy signaling environments and impart a downstream signal appropriately



http://www.bme.columbia.edu/~kam/research/research.htm



Correct
Cell Response



Disrupted
Cell Response

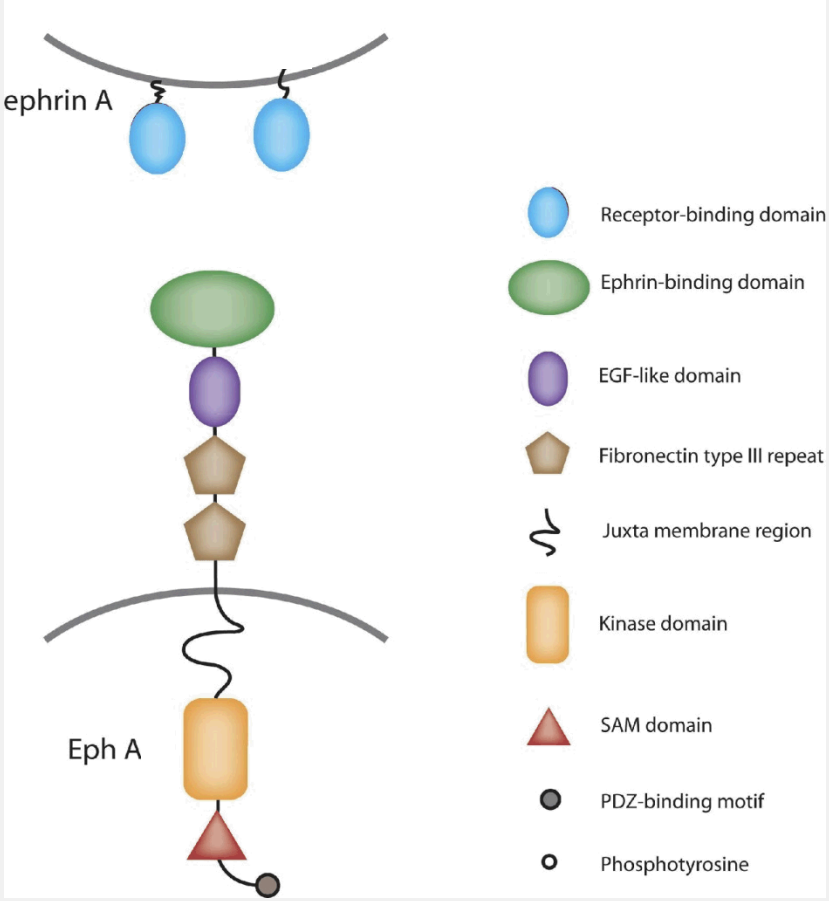
Same # of Signal Inputs!

Mossman, K.D, and Groves, J.T. 2005. *Science*. 18: 1191-3.

Eph Receptor Tyrosine Kinase Signaling

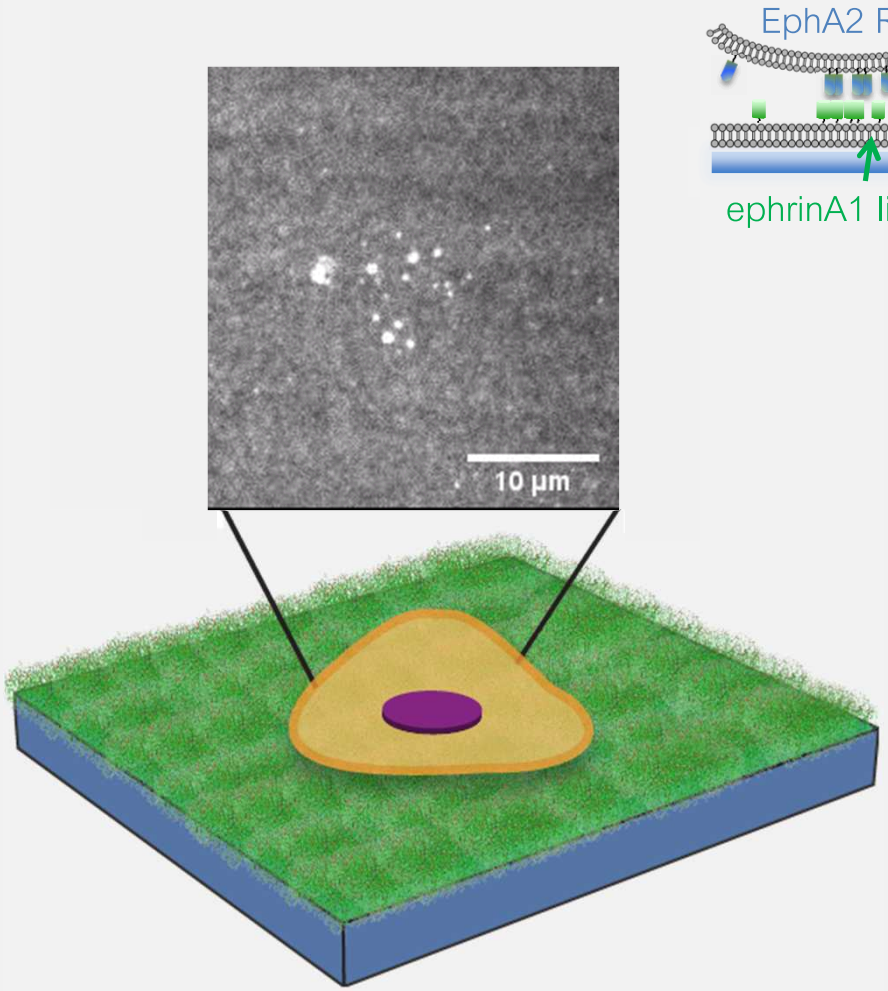
Receptor: EphA2

Ligand: ephrinA1



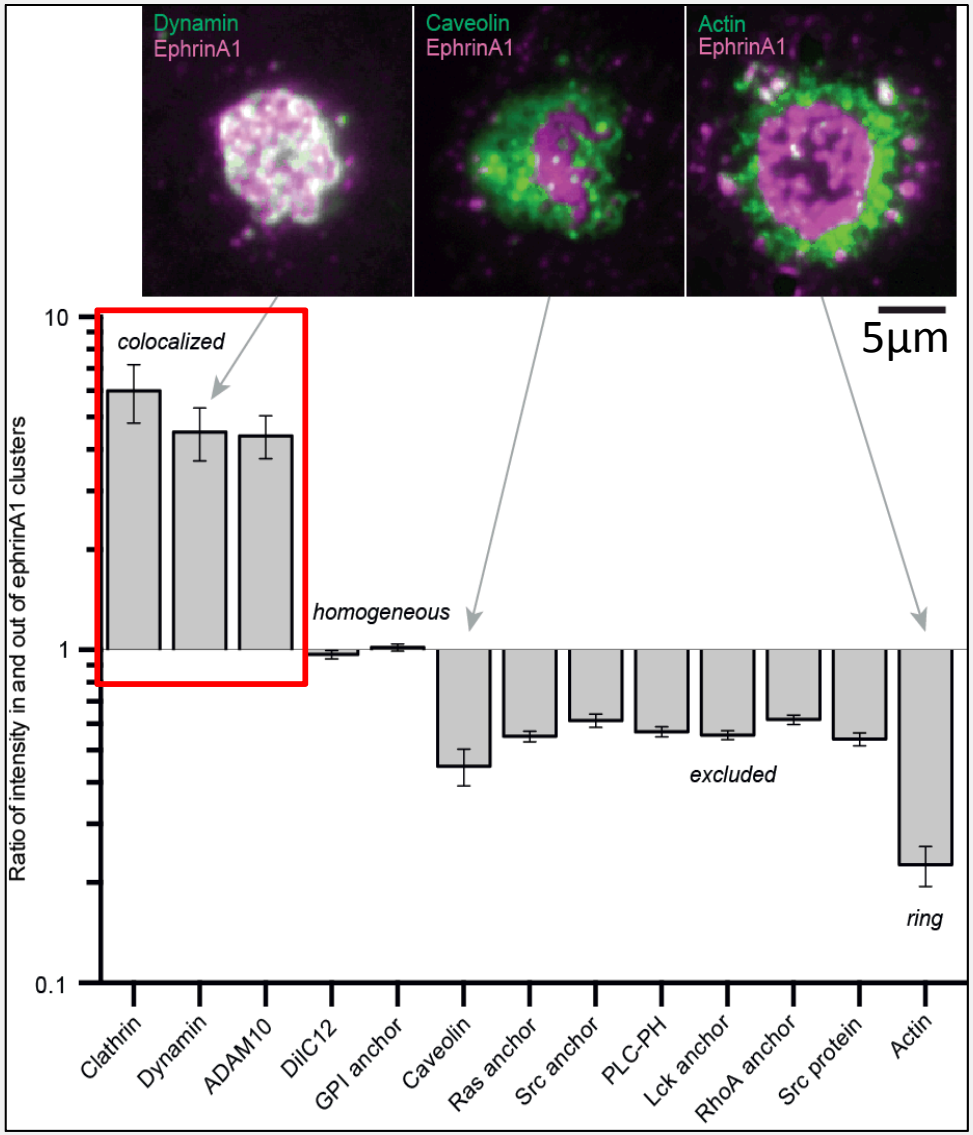
- Bidirectional signaling
  - Bidirectional signaling
- Overexpressed in aggressive cancers
- Highly overexpressed in MDAMB231 cells: invasive and metastatic breast cancer cells
- *Often expressed in triple negative breast cancers (no ER, PR or Her2)*
  - Key therapeutic target

Receptors and Ligands Cluster at the Cell-Membrane Interface

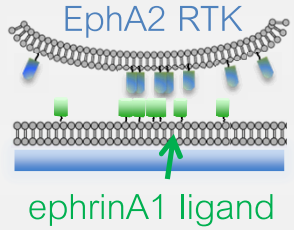


- MDAMB231 cells are seeded onto an ephrinA1 bilayer
- Receptor-ligands undergo higher ordering reorganization at the cell-membrane interface
- High resolution microscopy can be used to probe the importance of spatial organization

EphA2-ephrinA1 Clusters Contain Endocytosis Molecules

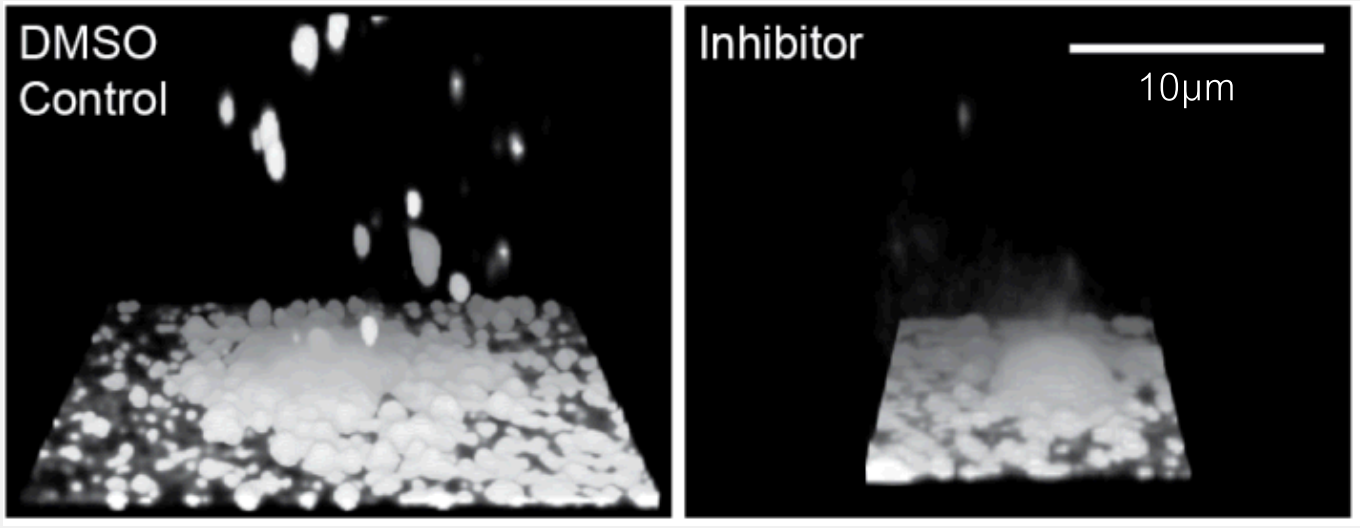


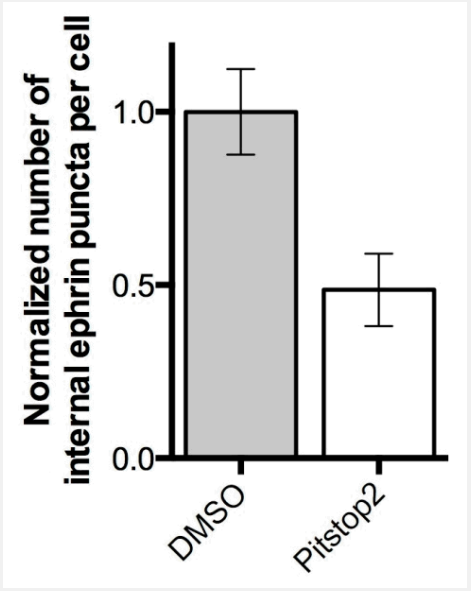
- Localization of signaling molecules to ephrinA1 was measured
- Four types of localization were characterized
  - Colocalization
  - Homogenous distribution
  - Anti-localization
  - Ring formation
- Maybe these clusters are important sites of endocytosis?

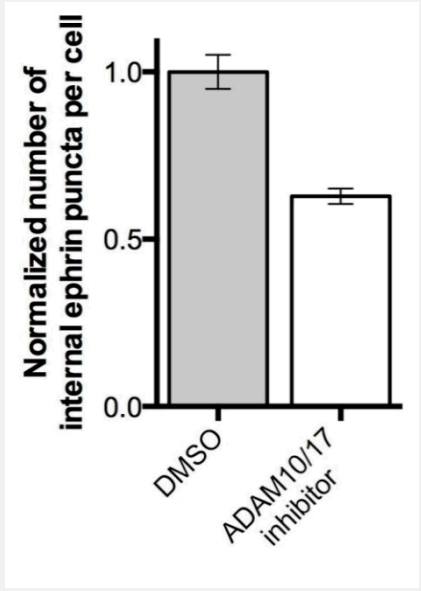


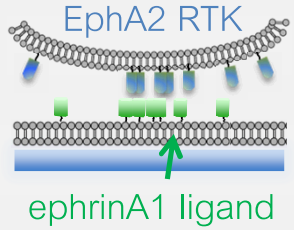
Greene, AC. and Lord, S. *et al.* 2014. *Biophysical Journal* 106: 2196-205.

Trans-Endocytosis Requires Clathrin and ADAM10



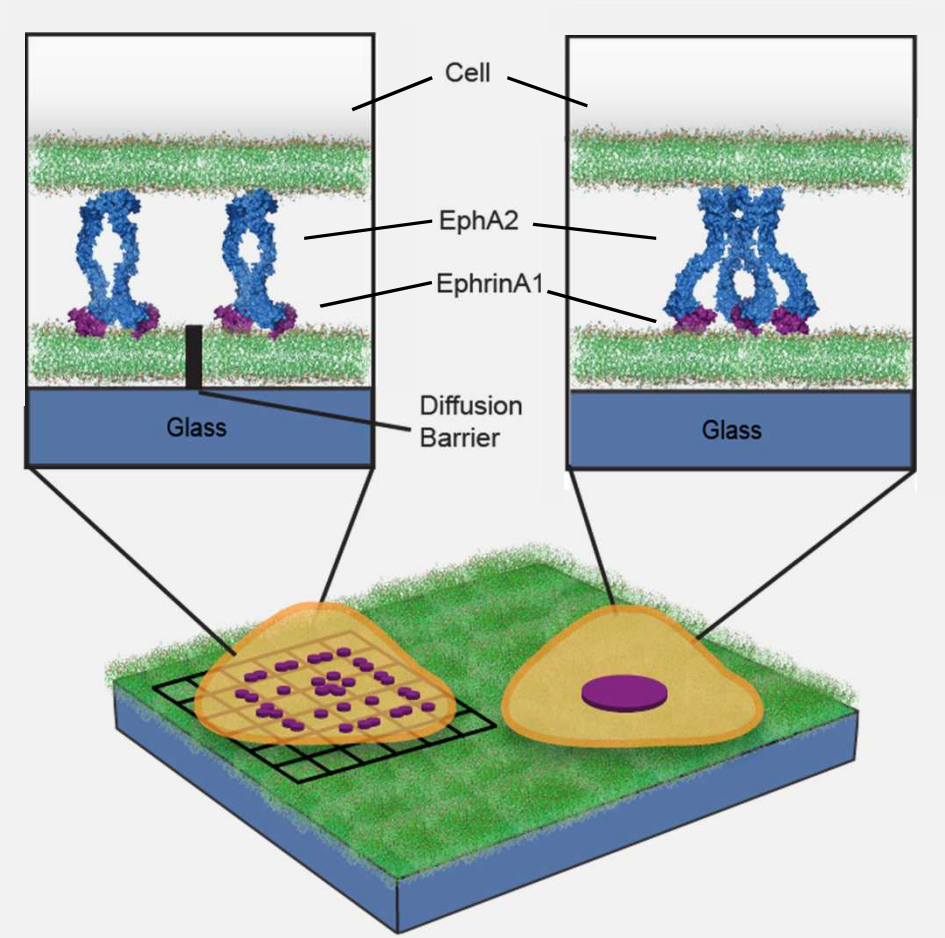




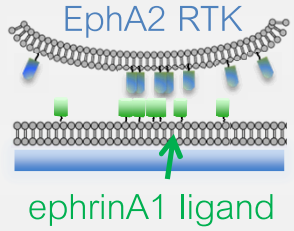


Greene, AC. and Lord, S. *et al.* 2014. *Biophysical Journal* 106: 2196-205.

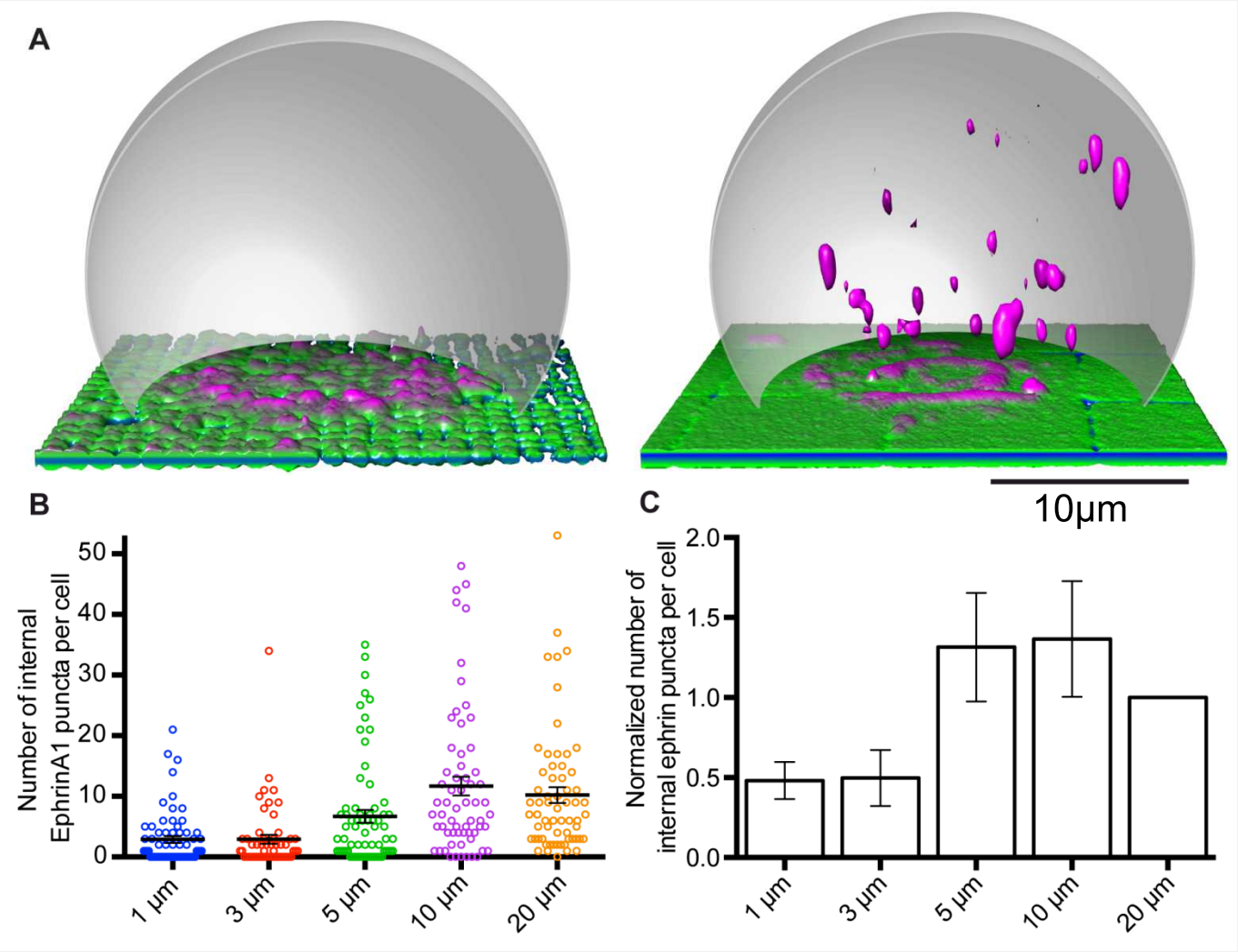
Using Diffusion Barriers to Alter Clustering: Creating a "Spatial Mutation"

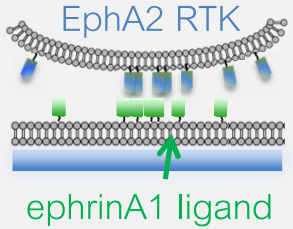


- Using electron beam lithograph, diffusion barriers can be created to restrict receptor-ligand mobility
- This assay allows us to probe the importance of EphA2-ephrinA1 reorganization in the context of downstream signaling



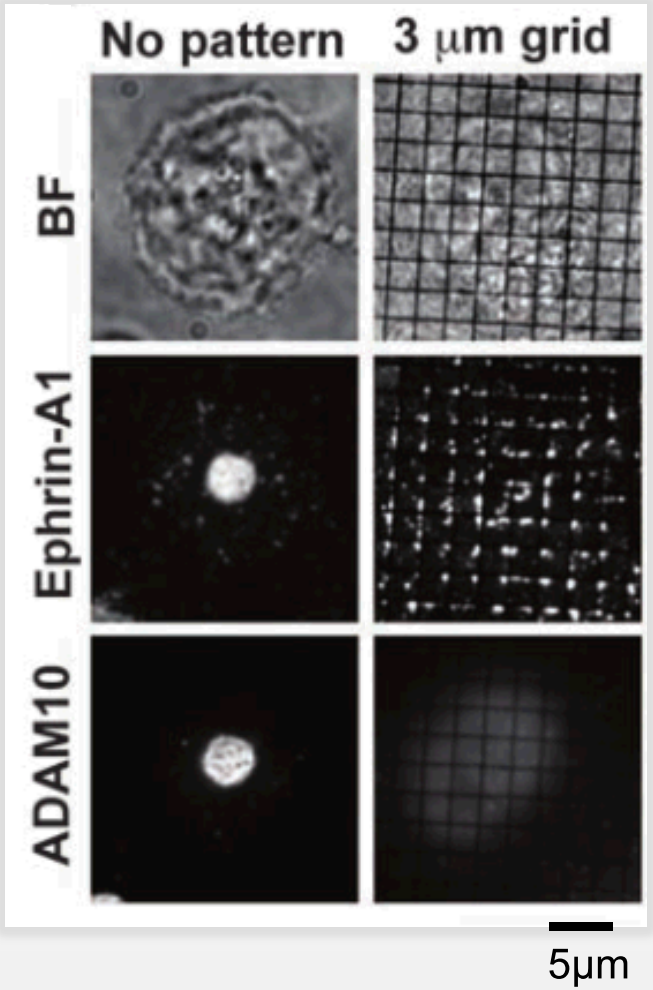
Trans-Endocytosis of EphrinA1 is Altered on Grids

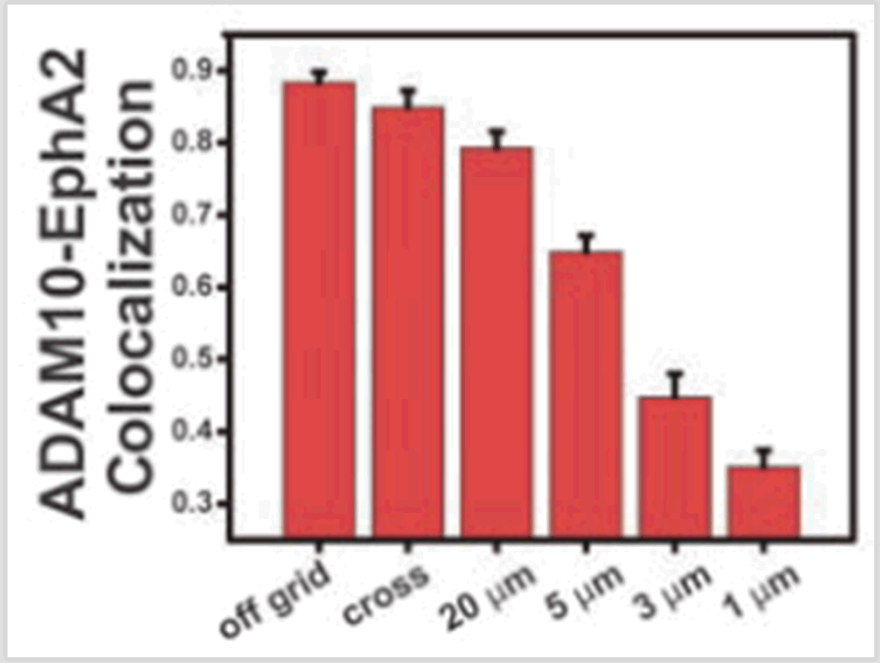




Greene, AC. and Lord, S. *et al.* 2014. *Biophysical Journal* 106: 2196-205.

Recruitment of ADAM10 is Decreased on Grids

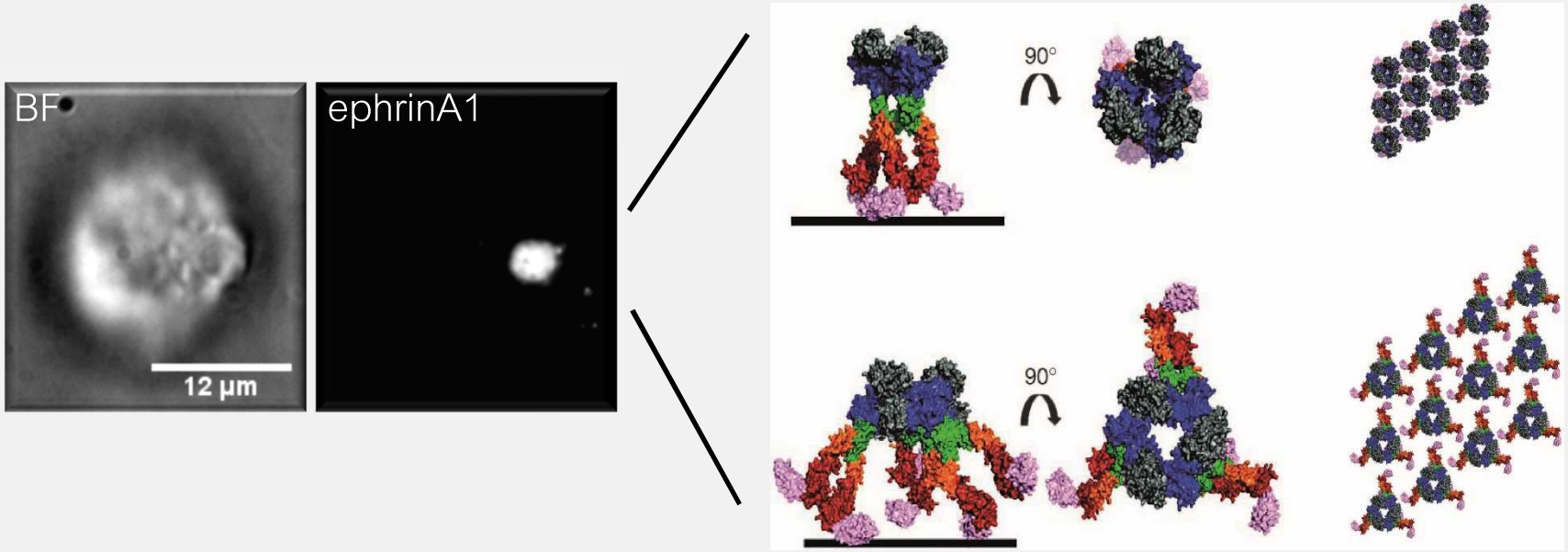




Salaita, K. and Nair P. *et al.* 2010. *Science* 327: 1380-5.

Structure of EphA2 Clustering

How does clustering from the micron down to the nanoscale regulate signaling?

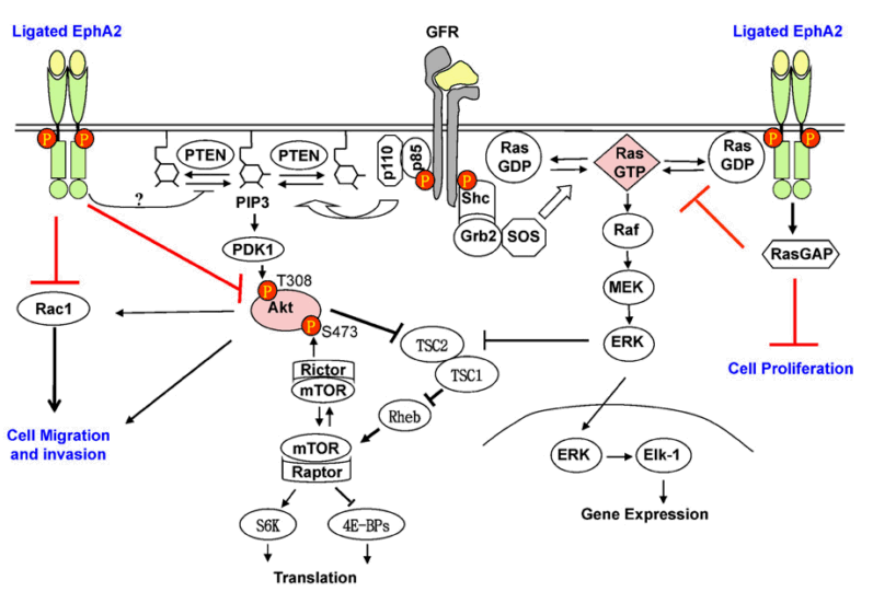


- EphA2 forms large-scale oligomers
  - Both in *cis* with other EphA2 receptors and in *trans* with ephrinA1 ligands

- We need to understand more about Eph molecular structure and clustering and how that alters signaling

Modified from Seiradake, E., *et al*. 2012. *Nat Struct Mol Biol* 20(8): 958-64.

EphA2 Signaling is Complex

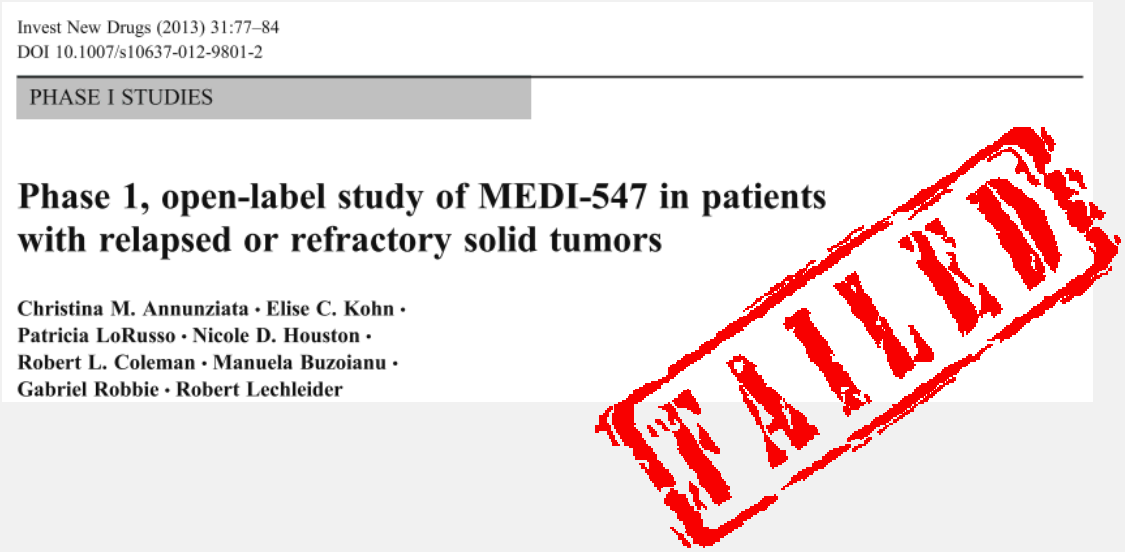


- Requires fine-tuning the balance of signaling based upon ligand-dependent and ligand-independent signaling; complex signaling map!

- How Eph is clustering also changes this signaling map

Miao, H., and Wang, B. 2012. *Semin Cell Dev Biol* 23(1): 16-25.

Why Do We Care About EphA2 Clustering?



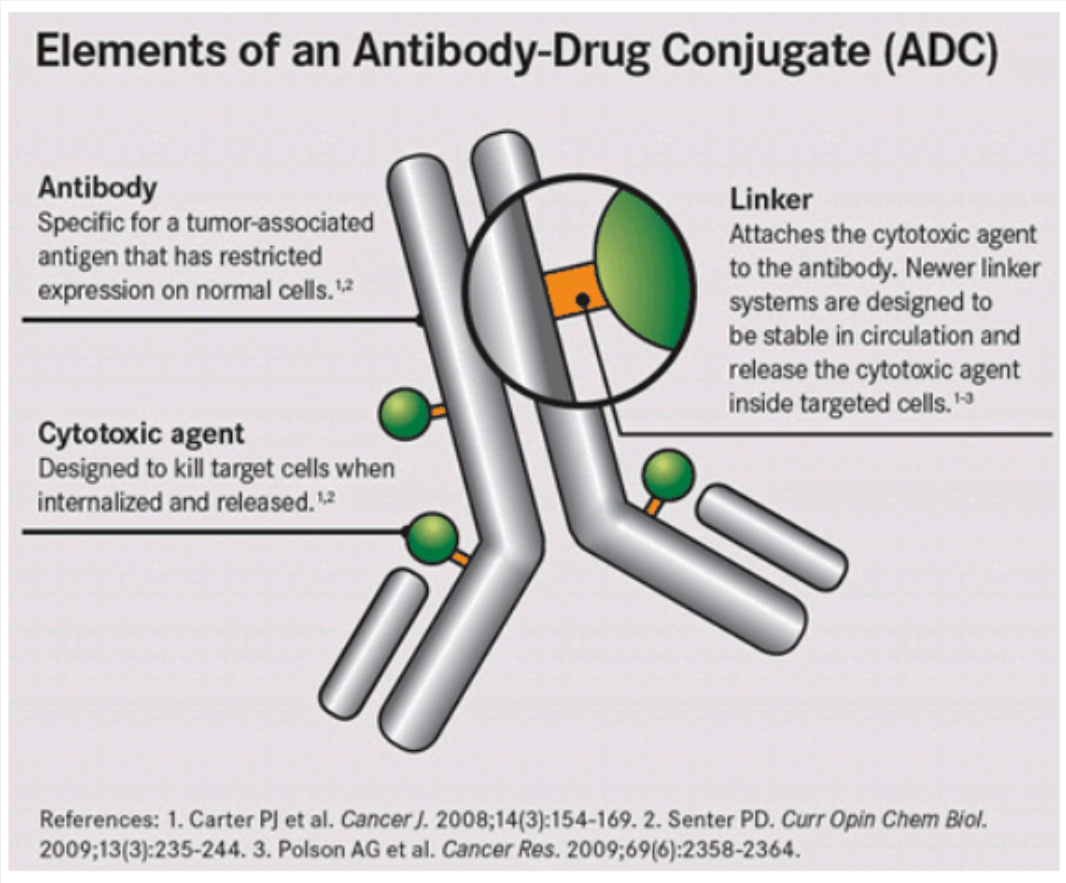
- Drug trial for targeting EphA2 expressing cells (e.g. triple negative breast cancers) failed in the Phase I Trial

- 6 women entered (breast, ovarian, endometrial and colon cancer patients)
  - Trial had disastrous effects; all women withdrew due to adverse affects (hemorrhage, liver disorder, etc.)

Annunziata, CM, *et al*. 2013. *Invest New Drugs* 31(1): 77-84.

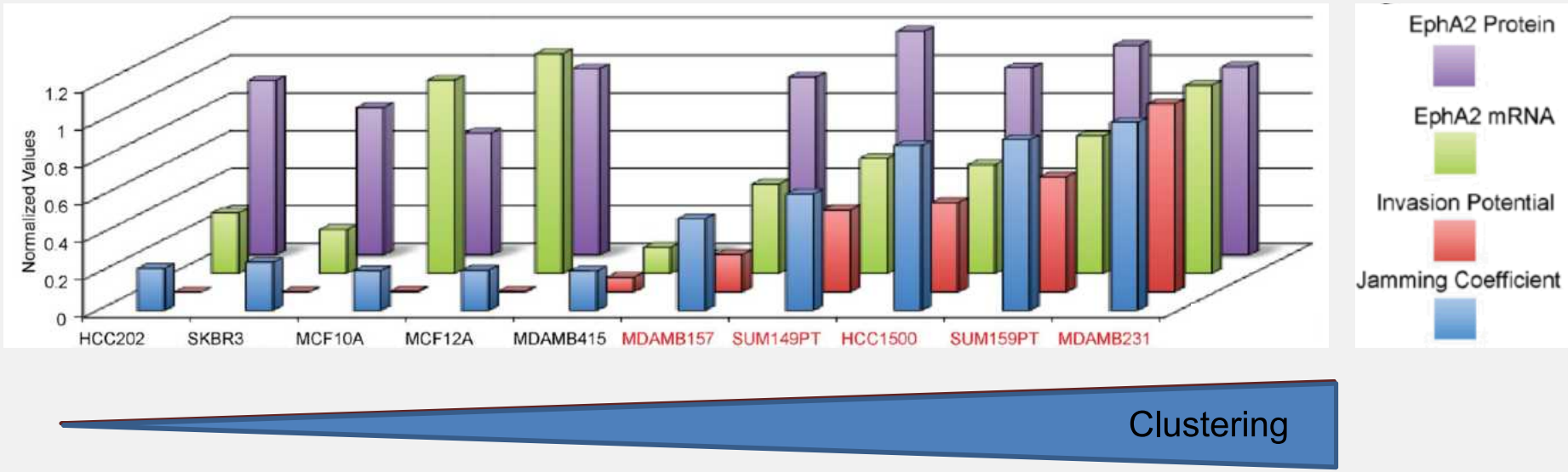
What Went Wrong with the Trial?

- Monoclonal anti-EphA2 antibody covalently attached to auristatin (microtubule inhibitor)
  - Likely NOT due to non-specific toxicity of auristatin
- Likely due to the antibody components of the antibody-drug conjugate
- **The drug induces clustering!**



Clustering Correlates to Disease State

- *The most aggressive cancers have the most tightly clustered EphA2 receptors*

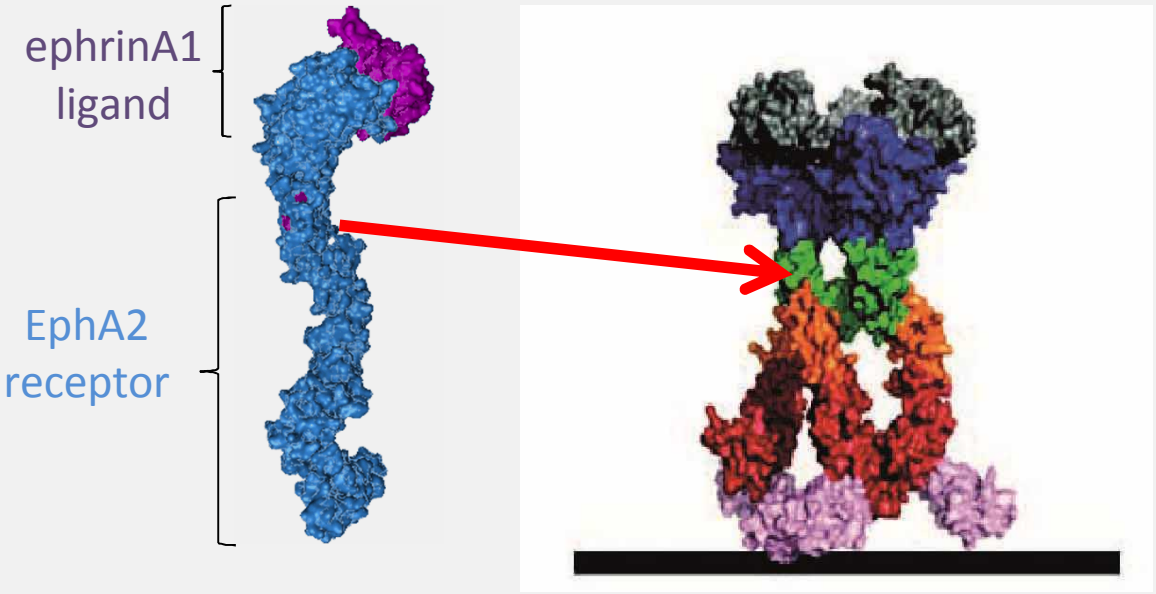


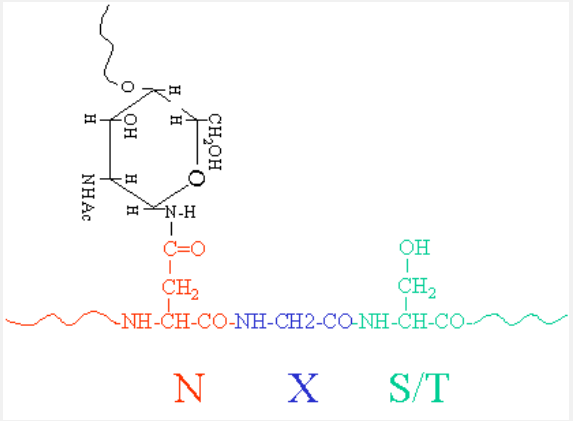
- Invasion potential of these cells only correlates to the EphA2 clustering phenotype

- Tightly clustered EphA2 indicative of more dangerous cancers?
  - Could the antibody-drug target be inducing more EphA2 clustering and causing an increased disease state of the cell?

Lohmuller, T., *et al*. 2013. *Nano Letters,* 13(7): 3059-64.

Genetic Manipulation of EphA2 *cis* Clustering

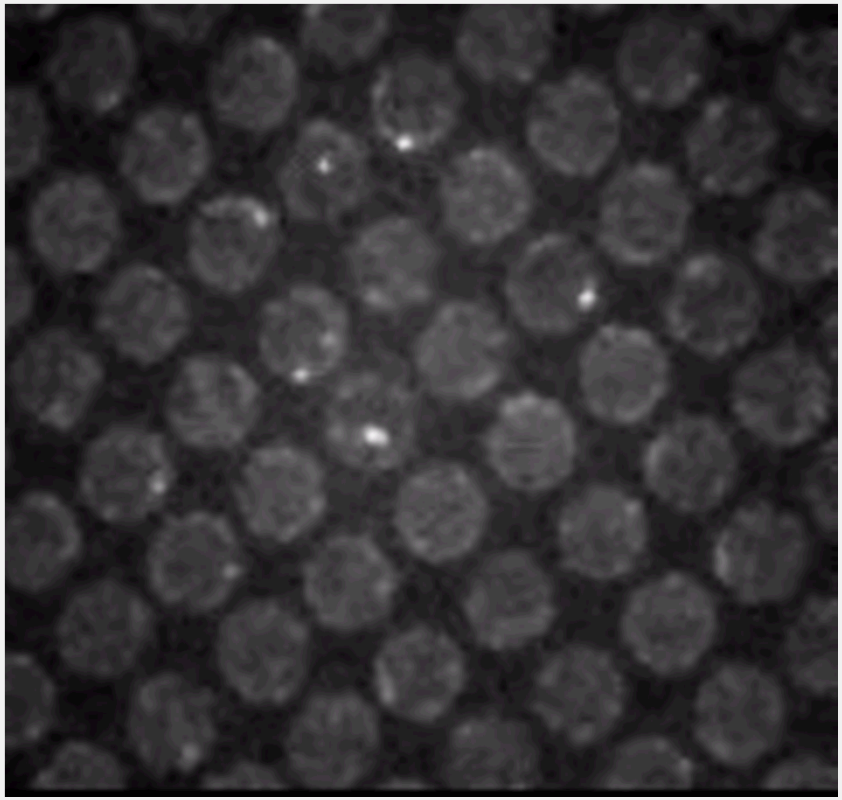




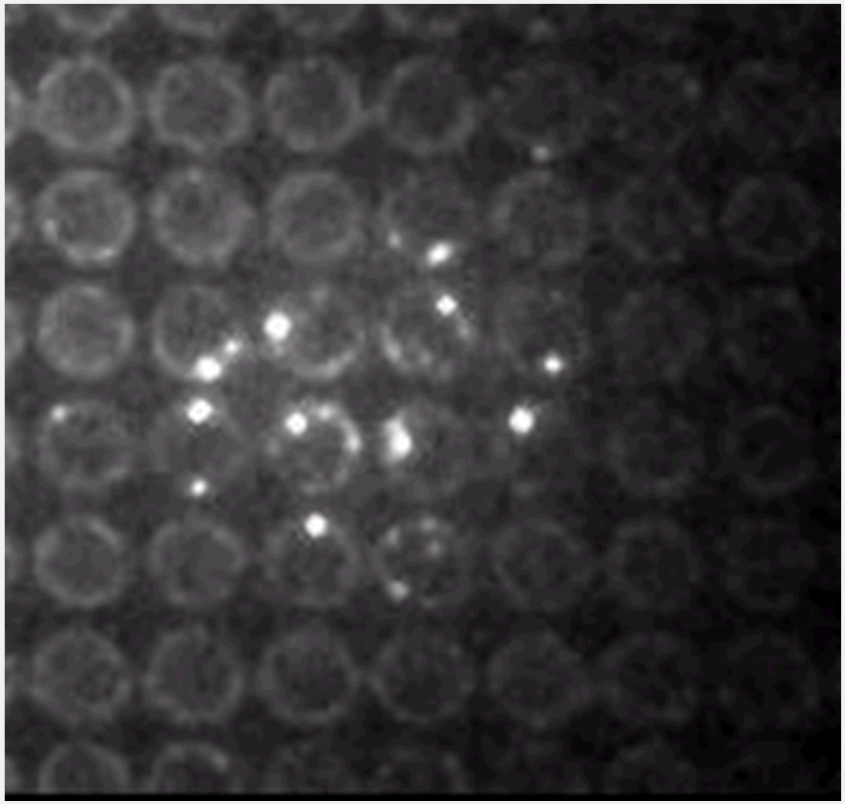
- Disrupting Eph clustering at the nanometer/angstrom scale requires genetic mutations
  - Target the sushi domain of the receptor to disrupt EphA2 *cis* clustering
- To do this in an endogenous context requires genome-editing
- We used CRISPR/Cas9 to permanently introduce two point mutations into the sushi domain resulting in an N-linked glycan in the domain

Mutant Clusters are More Dynamic and Transient

Wildtype



Mutant



5μm

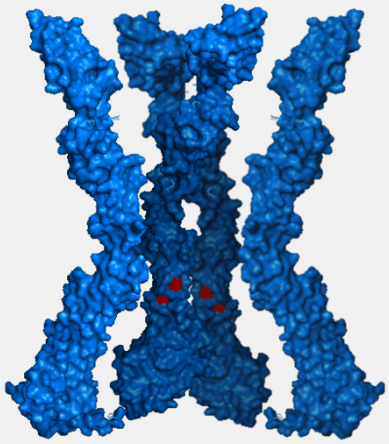
- Ligand density can be confined while adhering the cells using photolithography
- Mutants cluster faster and less definitively

Imaged every 10 seconds for 6 minutes

Movies are 5 frames per second

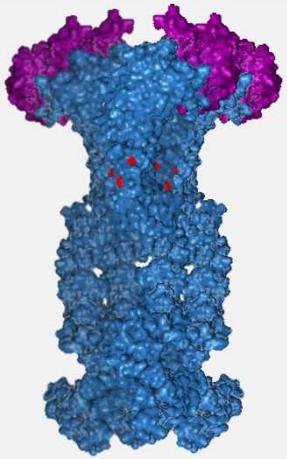
Disrupting EphA2 *cis* Interactions is Necessary for Clustering

Side view of unligated EphA2

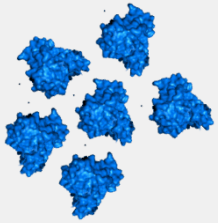


Rearrangement of Eph-Eph interactions

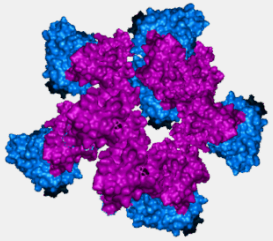
Side view of ligated EphA2



Top-down view of unligated EphA2



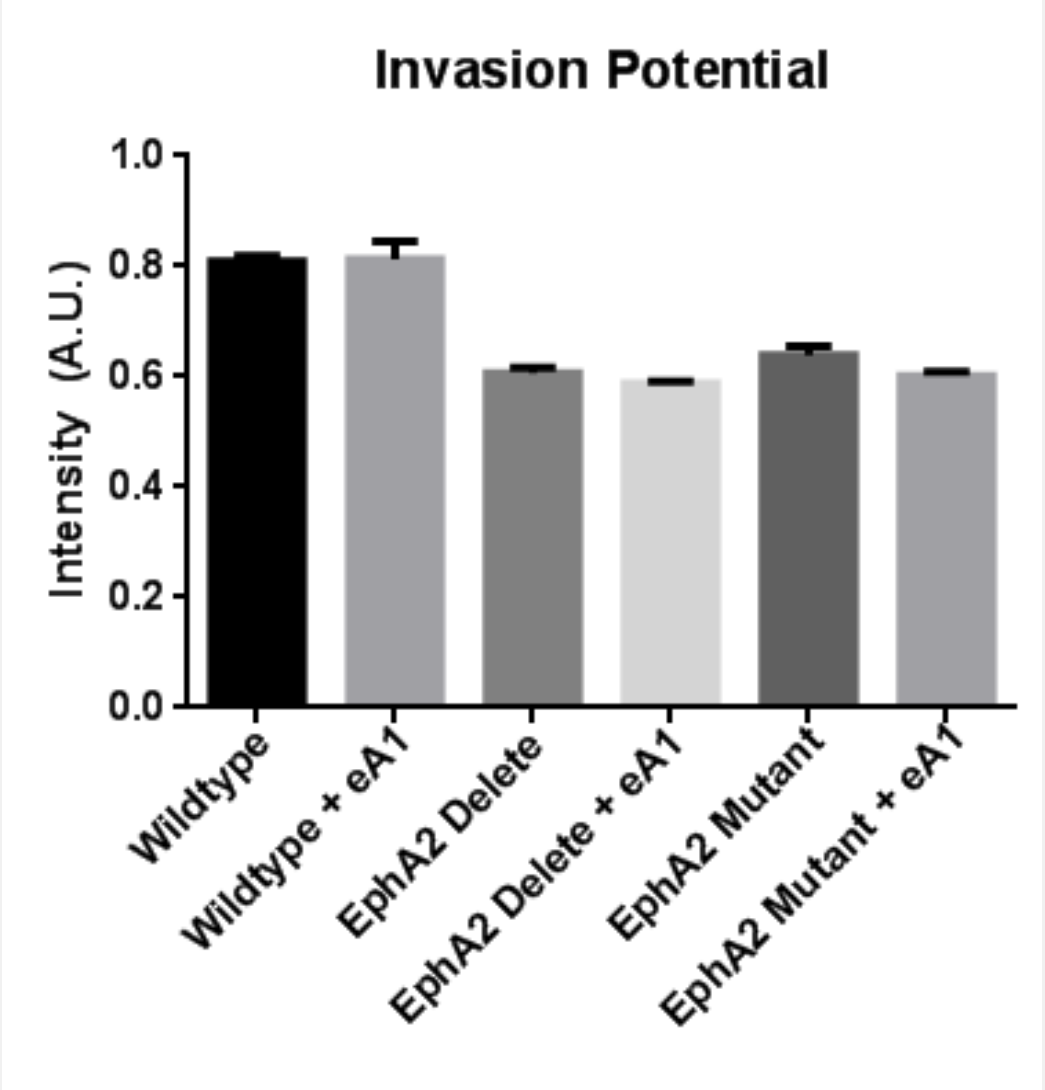
Top-down view of ligated EphA2



- Sushi domain mediates stable Eph-Eph interactions within the cell
- Micron-scale clustering requires binding to ephrinA1 and a disruption of Eph-Eph interactions

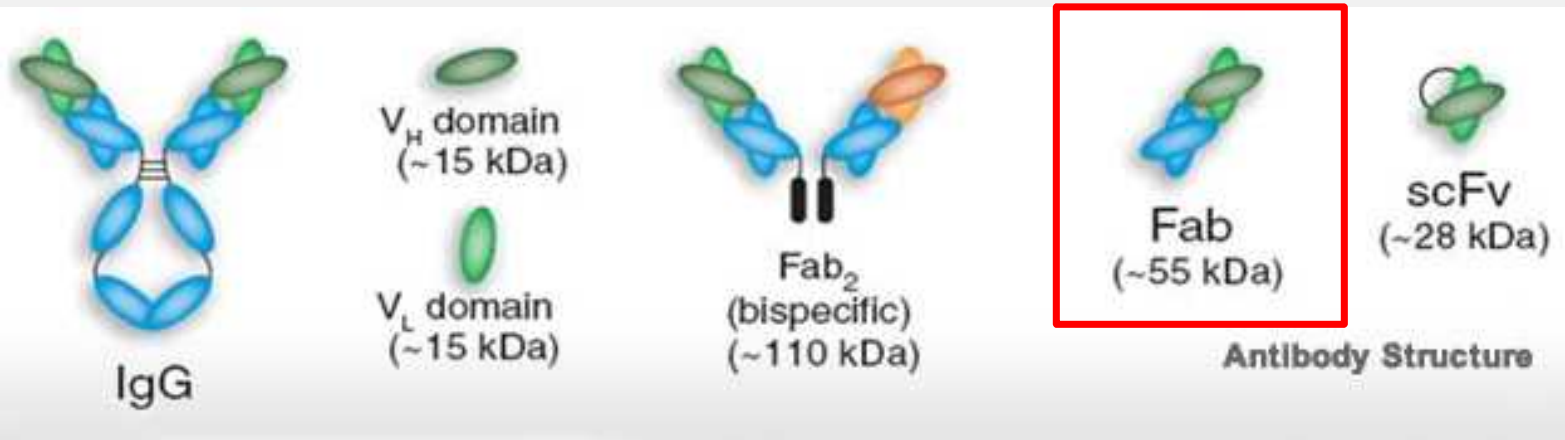
Sushi Domain Mutants are Less Invasive than Wildtype!

- Invasion potential measures the ability of a cell to leave a colony [tumor] and migrate through certain barriers; it is the hallmark of metastasis
- EphA2 with the sushi domain mutation is ~25% less invasive than the wildtype



Conclusions

- Spatio-mechanical regulation of signaling systems is becoming increasingly evident
- EphA2 is the first RTK, to our knowledge, to be regulated in this manner
- Understanding spatio-mechanical regulation of this signaling system will provide insights into the misregulation of EphA2 in disease, particularly cancer
- Alternate strategies for targeting these kinds of receptors can be developed



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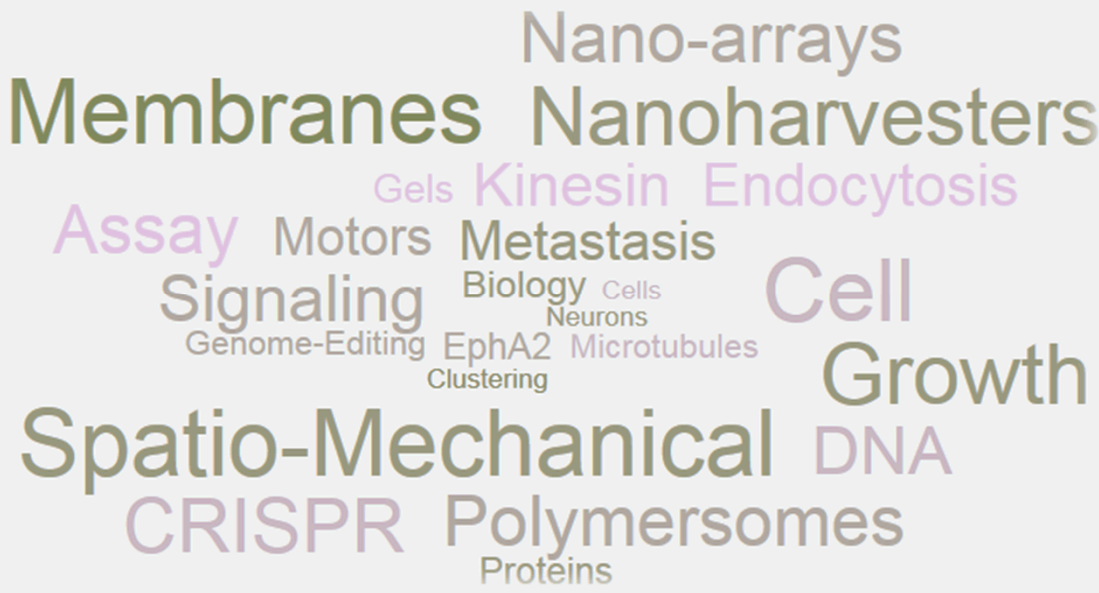

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