# Molecular Mechanisms of Gradient Sensing in Ewing's Sarcoma Cells

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**Citation**

Beletkaia, E., Fenz, S. F., Snaar-Jagalska, E., Hogendoorn, P., & Schmidt, T. (2012). Molecular Mechanisms of Gradient Sensing in Ewing's Sarcoma Cells. *Biophysical Journal*, *102*(3), 513A-513A. Retrieved from https://hdl.handle.net/1887/99987

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**Note:** To cite this publication please use the final published version (if applicable).

small-fluorophore labeled antibodies. We observe confined diffusion, directed diffusion, as well as an immobile fraction of receptors and show how ligand binding affects the short-range and long-range diffusion coefficients.

**2614-Pos Board B384**
**Molecular Mechanisms of Gradient Sensing in Ewing's Sarcoma Cells**
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Tumor growth and metastasis are processes exploiting chemotaxis - directed cell movement in a chemical gradient. Currently, a lot is known about the receptors and chemokine molecules responsible for chemotaxis. One essential pair is the G-protein coupled receptor CXCR4 and its ligand stromal cell-derived factor-1 (SDF-1). This pair was recently shown to play an important role in Ewing's sarcoma metastasis. The pathways following chemokine-receptor binding are known as well, but there is still not enough understanding of the molecular mechanisms of gradient sensing.
Here we investigate a cell line derived from Ewing's sarcoma. Using single molecule imaging and microfluidics techniques, we study the cell's response to stimulation with SDF-1. For this purpose we analyze local receptor stoichiometries, dynamics of multimerization and analyze receptor mobility. In an integral approach those molecular insights are complemented by whole cell behavior characterization, like mobility in controlled chemokine gradients. Results of this work will highlight the molecular machinery of chemokine gradient recognition and its effect on cell motility in the context of cancer spreading.

**2615-Pos Board B385**
**Molecular Simulations Revealing Dynamics of a Chemokine Receptor Homodimer**
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Mutation resistance is currently a problem for anti-HIV therapies that inhibit protease and reverse transcriptase mechanisms. HIV penetration is initiated by viral surface proteins interacting with host cell receptors, such as the G protein-coupled receptor (GPCR) CXC Chemokine Receptor 4 (CXCR4), and targeting this protein represents a viable solution to resistance setbacks. Recently, multiple crystal structures of CXCR4 have been solved, which include notable differences from other GPCR structures available, including a likely biologically relevant homodimer interface. These structures allude to unique dynamics and possible ligand design strategies. Explicit atomic level molecular dynamics simulations of CXCR4 have been carried out, and are discussed in the context of current trends and observations of GPCR dynamics, modeling and functional modulation. Investigation of monomer-monomer interactions were emphasized, and compared to crystal structure observations.

**2616-Pos Board B386**
**Screening of GPCR C-Terminal Tails for Interactions with PSD-95 - A Quantitative Approach to Identify and Characterize GPCR-PDZ Interactions**
Thor C. Møller[1,2], **Volker F. Wirth**[1,2], Nina I. Roberts[1,2],
Birgitte P.S. Jacky[1], Anders Bach[3], Kristian Strømgaard[3],
Thue W. Schwartz[1], Karen L. Martinez[1,2].
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Scaffolding proteins containing PDZ domains are among the most abundant interaction partners of G protein-coupled receptors (GPCRs). Discovery and characterization of GPCR-PDZ interactions are important steps in the understanding of these interactions and how they affect the function of GPCRs. We have used the prototypical PDZ domain scaffold postsynaptic density protein 95 (PSD-95) to develop a generic high-throughput compatible approach to accelerate the description of GPCR-PDZ interactions. By screening of two libraries of GPCR C-terminal tails, we have identified a number of novel GPCR interactions with PSD-95, e.g. four of the somatostatin receptors (SSTRs), the neuropeptide Y receptor Y2 and the chemokine receptor CXCR2. These in vitro findings correlated well with the interactions in HEK293 cells, which shows the potential for discovery of new interactions. We show that a fluorescence polarization-based assay has higher sensitivity than a pull-down assay for primary screening of GPCR-PDZ interactions. Quantitative characterization showed inhibition constants (Ki values) around 100 μM or lower for known GPCR-PSD-95 interactions, and Ki values ranging from below 100 μM to the detection limit of 1000 μM for the identified interactions. Quantitative characterization is useful to evaluate the significance of an interaction and to compare the results with other studies. The results obtained with different lengths of the receptor (full-length GPCR, the full cytosolic C-terminal tail and peptides containing only the PDZ motif) and of the PDZ protein (full-length PSD-95 and isolated PDZ domains) were generally in close agreement.

**2617-Pos Board B387**
**Multiscale Simulations Suggest a Mechanism for Integrin Inside-Out Activation**
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Integrins are large heterodimeric cell surface adhesion receptors which are central components of focal adhesion complexes and are crucial for a variety of signal transduction events. They are 'activated' to a high affinity state by the formation of an intracellular complex between the integrin β-subunit tail, the membrane and talin, a process known as 'inside-out activation'. The head domain of talin plays a key role in the formation of this complex. In this study, activation of the integrin αIIb/β3 dimer by the talin head domain was probed using a multiscale approach that combines coarse-grained and atomistic molecular dynamics (MD) simulations. A number of novel insights emerge from these studies including: i) the important role of residues F992 and F993 of the integrin αIIb subunit in stabilizing the αIIb/β3 dimer 'off' state; ii) the crucial role of negatively charged moieties in talin F2-F3/membrane interactions; iii) how interactions of the talin F2-F3 domain with negatively charged lipid headgroups in the membrane induce a reorientation of the β transmembrane (TM) domain; iv) how an increase in the tilt angle of the β TM domain relative to the bilayer normal helps to destabilize the α/β TM interaction promoting a scissor-like motion of the integrin TM helices. On the basis of these results, a model of integrin inside-out activation by talin is proposed which explains how talin facilitates the rearrangement of the α and β integrin TM subunits, thus switching the integrin conformation towards an active high affinity state.

**2618-Pos Board B388**
**Single Molecule Study of Thrombospondin-1 Receptors in the Endothelial Cell Plasma Membrane**
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Thrombospondin-1 (TSP-1) is a potent anti-angiogenic factor downregulated in many tumors. CD36 and $\beta_1$-integrin are two of its key receptors, mediating its anti-angiogenic activity by initiating signaling cascades that inhibit endothelial cell migration and promote apoptosis. Receptor clustering on the plasma membrane is thought to be important for initiating these signaling cascades. However, little is known about the mechanisms that contribute to CD36 and $\beta_1$-integrin clustering and how they lead to downstream signals. In this study, we used quantitative single-molecule and super-resolution imaging to measure the dynamics and spatial organization of CD36 and $\beta_1$-integrin in human microvascular endothelial cells (HMVECs). We compared receptor dynamics and spatial organization between unstimulated cells and cells exposed to TSP-1 or 3TSR, a small subdomain of TSP1 which primarily binds to CD36 and $\beta_1$-integrin. We found that treatments with either TSP-1 or 3TSR, at doses that lead to HMVEC apoptosis, result in a significant increase in CD36 mobility in a $\beta_1$-integrin-dependent manner. We also found that treatment with TSP-1 increases Src phosphorylation at focal adhesions in a CD36 dependent manner. Based on our data, we propose that CD36 and $\beta_1$-integrin work together to initiate cellular signaling downstream of TSP-1 binding.

**2619-Pos Board B389**
**Large Scale, High Resolution Models of Receptor Tyrosine Kinase Signaling Networks**
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Mathematical models based upon the biochemical reactions that effectively define these networks have much promise as a tool for studying cell-signaling networks. Such models could be studied computationally to generate hypotheses that can be tested experimentally. A major obstacle for developing such models is ''combinatorial complexity'', i.e. the number of potential states for the network becomes combinatorially large when post-translational modifications and protein-protein complexes are considered.