developed a dual-chain biosensor based on intermolecular FRET that enables the spatio-temporal dynamics of Rap1 activity to be measured in individual living cells. The biosensor, termed Rgluc-GFP, showed that the binding interaction between cearulene-tagged Rap1 and an affinity reagent fused to yPet. To facilitate expression of the sensor and to reduce cell-to-cell heterogeneity in the stoichiometry of components we have engineered a tandem viral-2A (tv-2A) mediated expression vector that achieves over 99% separation of proteins when both chains of the biosensor are transcribed as a single gene. In living cells this novel Rap1 biosensor showed a > 10-fold increase in FRET upon activation, and is sufficiently bright to image Rap1 activity at concentrations that do not interfere with normal cell motility. The intermolecular design of this biosensor enables native regulation of Rap1 localization by CAAX-box modification and is well suited to fluorescence lifetime imaging microscopy (FLIM), a technique that greatly enhances quantitative accuracy and multiplexing potential.

822-Pos Board B591
Engineered Manipulation of Signaling Networks: Control of Kinase Activation and Interactions Dissects Parallel Src Pathways
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Activation of a kinase in a specific protein complex or subcellular location determines what signaling pathway will be initiated. Current methods typically cannot activate a specific kinase and simultaneously cause it to interact with one specific downstream target. Here we provide such a tool, and use it to determine the role of individual Src-mediated signaling pathways in stimulation of different cell morphological changes. Focal adhesion kinase (FAK) and p130Cas can bind and activate Src kinase, propagating signals through parallel pathways. Localization of Src in the cytoplasm or at the plasma membrane also determines the function of Src. We therefore focused on activation of Src acting through FAK, p130Cas, or at the cytoskeleton versus the plasma membrane. An engineered, “insertable” FKBP12 protein (fKBP) was introduced into the catalytic domain of Src, while FKBP12-rapamycin binding domain (FRB) was attached to the specific downstream effector. This rendered the kinase inactive until rapamycin induced heterodimerization with FRB. Using this technology we restricted Src activation to the complex it formed with FRB-bearing downstream targets. Activation of Src, without targeting specific downstream molecules, led to cell spreading, reorganization of focal adhesions, and the production of filopodia and lamellipodial protrusions. Using the new approach (RapR-TAP), we showed that activation of Src specifically in complex with FAK led to focal adhesion rearrangement and only slow cell spreading, while activation in complex with p130Cas led to rapid spreading and filopodia formation, but no apparent effects on focal adhesions. Comparing Src activation in the cytosol versus at the plasma membrane showed that membrane localization is necessary to stabilize protrusions, whereas cytoplasmic Src promotes rearrangement of focal adhesions. This demonstrates a broadly generalizable strategy to activate specific kinase-mediated signaling pathways in living cells.

823-Pos Board B592
New Tools for Activation of Src Family Isosforms In Vivo Demonstrate Specific Roles for Each Isoform in Cell Motility
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The Src family kinases (SFK) include nine highly homologous members. To identify unique functions for individual SFKs, we have inserted an engineered FKBP domain into a conserved region of the kinases’ catalytic domain, generating rapamycin-regulated (RapR) analogs1 of Src, Lyn, Yes and Fyn, enabling each isoform to be activated specifically and independently in living cells. The cellular morphodynamics and corresponding subcellular translocation induced by activation of each isoform were characterized using new computational methods, revealing distinct roles for each isoform. While both Src and Fyn activation initially induced spreading, only for Src this was followed by polarized movement. Activation of Src led to kinase redistribution from the perinuclear region to the cell periphery, while Fyn remained uniformly distributed throughout the observation period. Changes in the lipidation or swapping the effector binding domains of each isoform interconverted the cell behaviors and corresponding translocation dynamics. Differences in focal adhesion dynamics driven by Src versus Fyn activation provide a mechanistic model for the distinct morphological changes induced by each kinase.

824-Pos Board B593
Uptake of Ultrasmall Fluorescent Gold Nanoclusters by Live Hela Cells
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The development of advanced fluorescence-based imaging techniques in the life sciences depends significantly on the further development of specific fluorescence markers. Gold nanoclusters (AuNCs) are a novel type of nanomaterials which have attracted great interest in recent years. Composed of only a few atoms, AuNCs exhibit bright tunable fluorescence ranging from the visible to the infrared. Owing to their ultrasmall size, good photophysical properties and low cytotoxicity, fluorescent AuNCs have been recognized as promising probes for live cell labeling and intracellular sensing [1,2]. Still, little is known about the interactions of AuNCs with biological systems. Here we have studied the uptake behavior of water-soluble fluorescent AuNCs by live HeLa cells using confocal fluorescence microscopy. Unlike larger particles, ultrasmall AuNCs accumulate at the plasma membrane before they are internalized and become enwrapped in endosomes and, subsequently, lysosomes. We have also explored the endocytic mechanisms by which AuNCs are internalized by using specific inhibitors.

825-Pos Board B594
Spatial-Temporal Regulation of Spindle Assembly Checkpoint
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During mitosis, microtubule spindle organized by the two spindle poles (SP) needed to properly capture and align chromosomes at the cell equator to ensure faithful chromosome segregation. Improper chromosome-spindle attachment will activate spindle assembly checkpoint proteins (SAC) emanated from this chromosome. Active SAC prevents cyclin B degradation in the cytoplasm and, consequently halts the progression of mitosis. Only after the last chromosome gets properly attached can chromosome segregation begin. Given the tens of chromosomes and everlasting fluctuations in the cell, it remains murky how the silencing of SAC faithfully couples with the last chromosome attachment. We established a theoretical model that describes the mitotic spindle structure (chromosomes, mitotic spindles, and spindle poles) as a coherent transport system. Depending on whether the chromosome is properly attached, SAC and cyclin B circulate within the transport system via dyneins, and exchange with the cytoplasm in accordance to their well-known biochemical regulations. The basis of such coherent transport is the compartmentalization underlined by direct and/or indirect protein binding affinities with these mitotic structures. Our model results show that such transport system recapitulates the observed spatial-temporal pattern of SAC and cyclin B. More importantly, the transport system establishes a robust and sensitive mechanism for silencing SAC activity in accordance with the status of chromosome-spindle attachments.

826-Pos Board B595
Chromatographic Migration of Escherichia Coli to Glucose is due to Additive Contributions from Phosphotranserase System and Trg Receptor Sensing Mechanism
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The chromatographic response of Escherichia coli to glucose is mediated by two distinct intracellular mechanisms, namely, a receptor mediated response through Trg, and a PhosphoTranserase System (PTS) sugar uptake mechanism. The study by Neumann et al. (www.pnas.org/cgi/doi/10.1073/pnas.1205307109) has recently demonstrated that the signals from the PTS and the Trg sensing mechanism is integrated to yield an additive intracellular response in E. coli wherein the relative kinase activity is weaker for the PTS compared to the Trg mediated response. While this additive property, through the two mechanisms, has been shown at the signalling level, its consequence on the chromatographic migration of a population of cells remains to be demonstrated. We performed experiments with a wild type E. coli K12 strain and a mutant lacking Trg to determine the drift velocity at various locations along a microchannel for varying gradients of glucose and, a non-metabolizable analogue of glucose, 2-Deoxy-D-glucose. The experiments demonstrated that the individual and integrated contributions of the two mechanisms at the intracellular level in response to glucose is closely reflected at the phenotypic level. We also measured the run speed, clockwise bias, cell diffusivity, and relate these measurements to the two mechanisms. We compare the chromatographic