

FRET Biosensors and Live Cell Imaging

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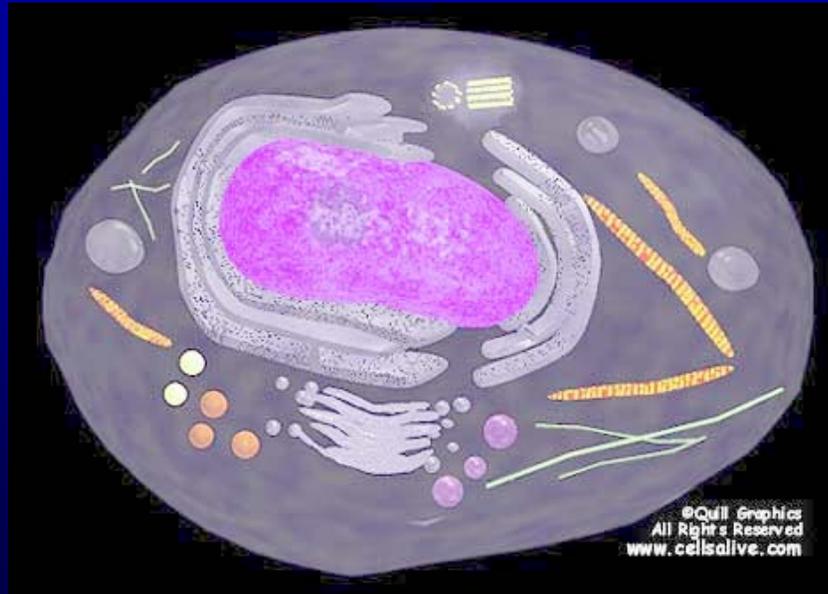
Outline

- 1. Introduction: fluorescent proteins (FPs) and fluorescence resonance energy transfer (FRET)**
- 2. The engineering of FRET biosensors**
- 3. The application of FRET biosensors**

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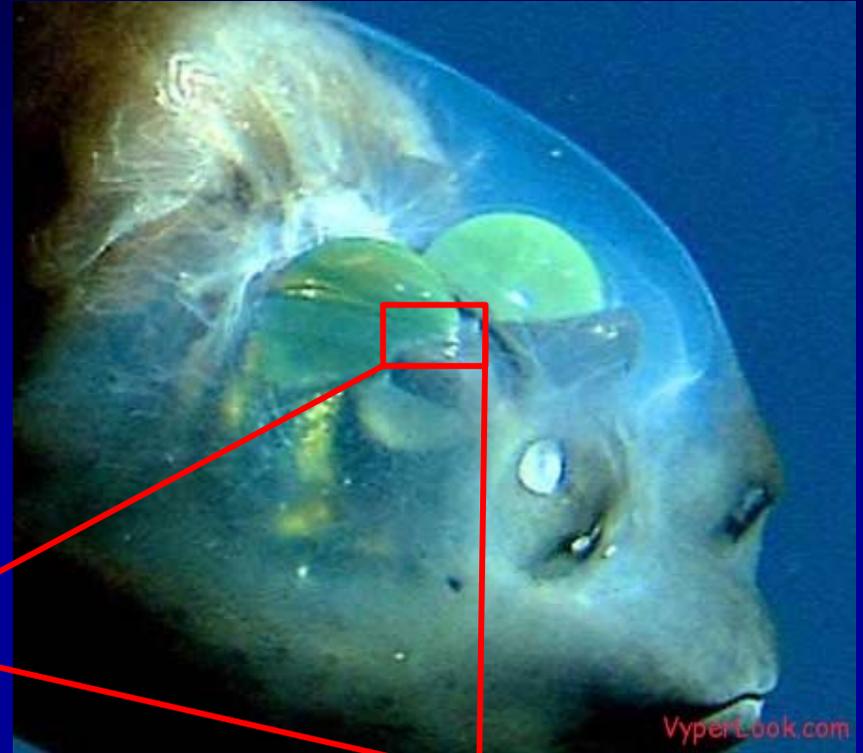
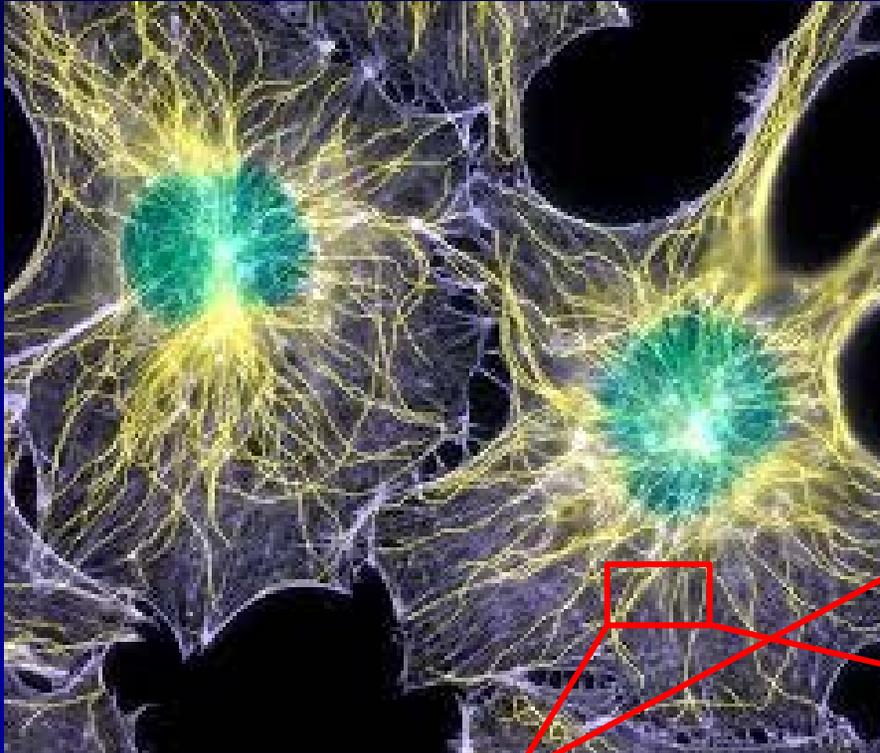
A Cell and A City



Lessons from google Earth/Map, street view



Can we do the same for cells and animals?



Multiple Color FPs

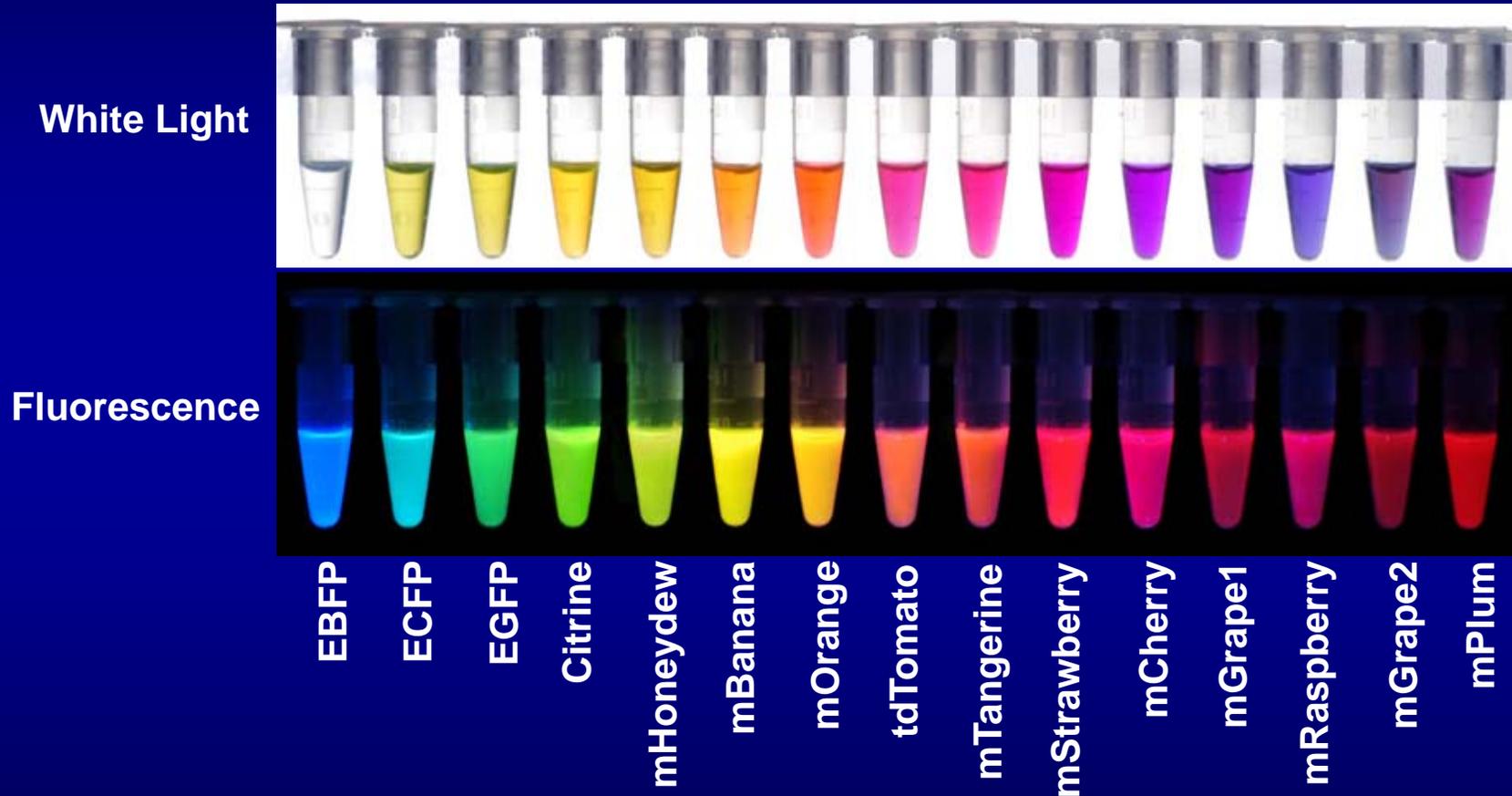
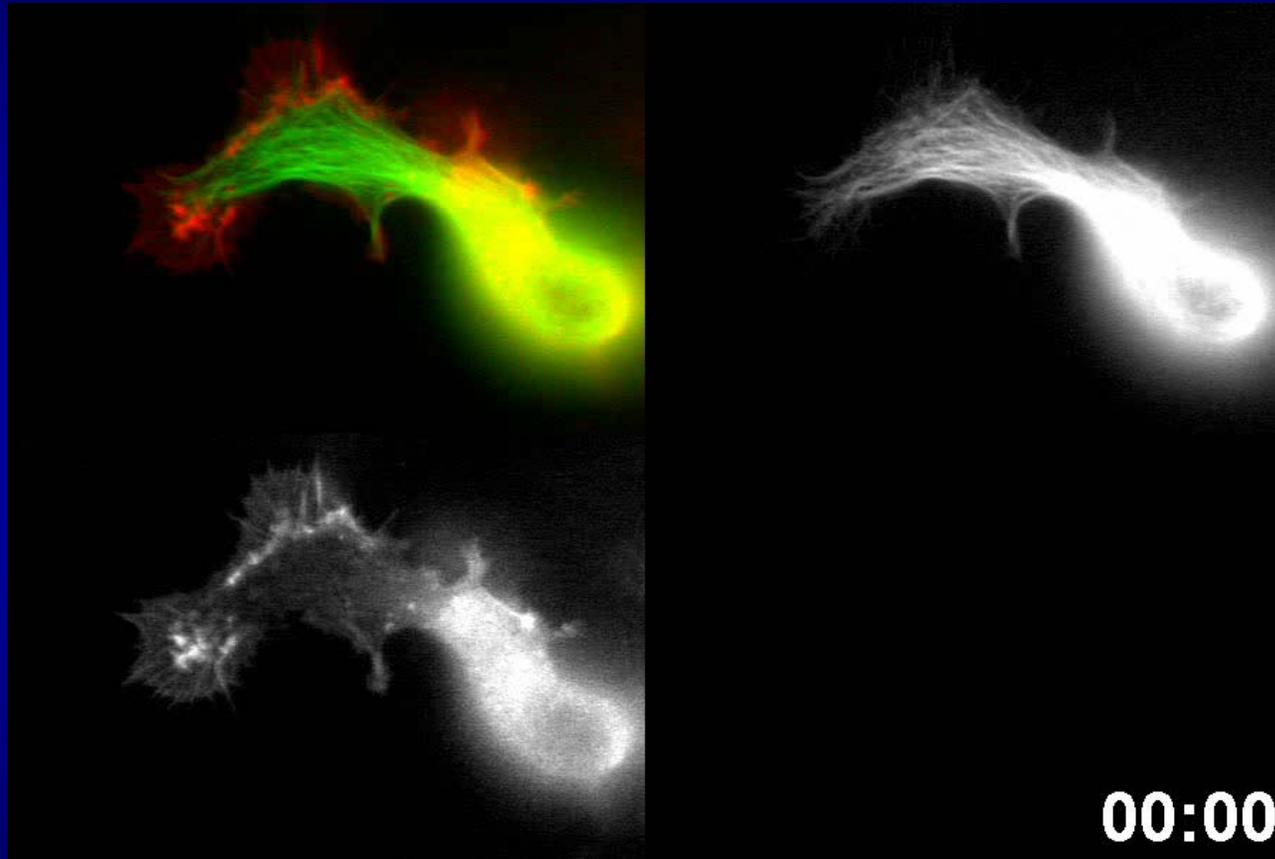


Image courtesy from Roger Y. Tsien

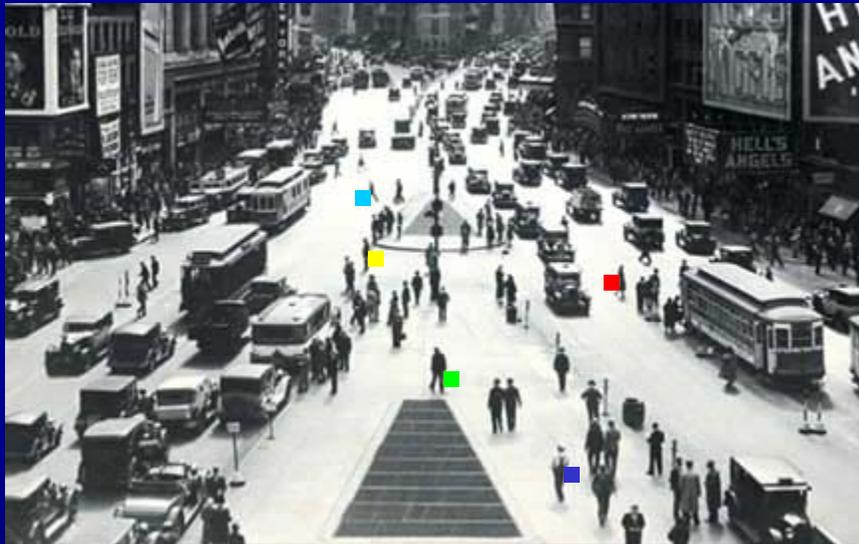
Multiple color visualization



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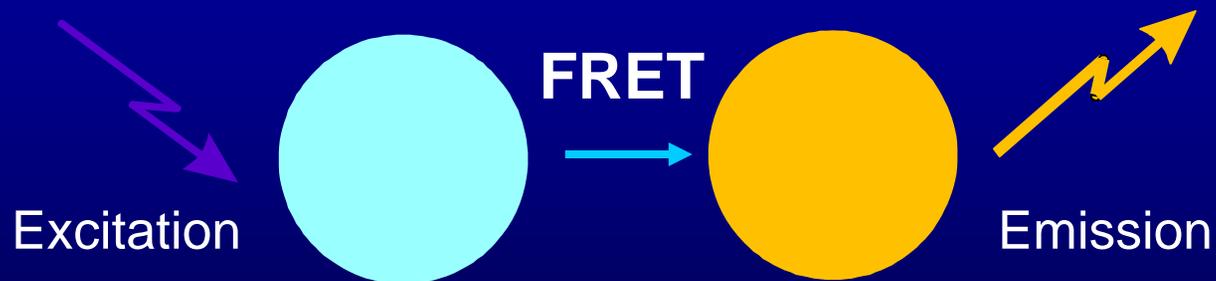
Spy on their Actions!



The Principle of Fluorescence Resonance Energy Transfer (FRET)

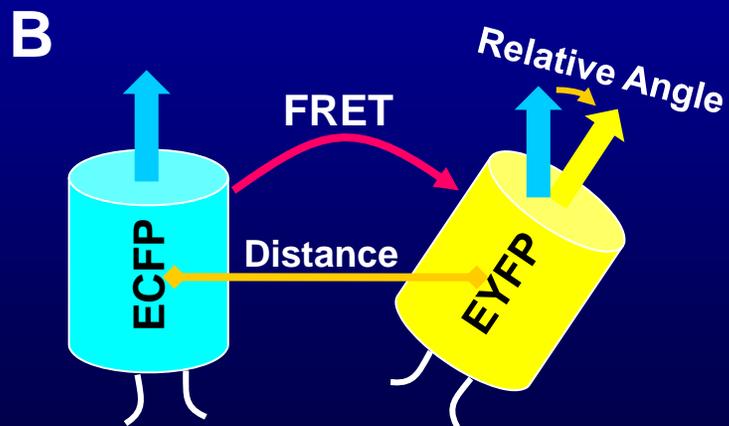
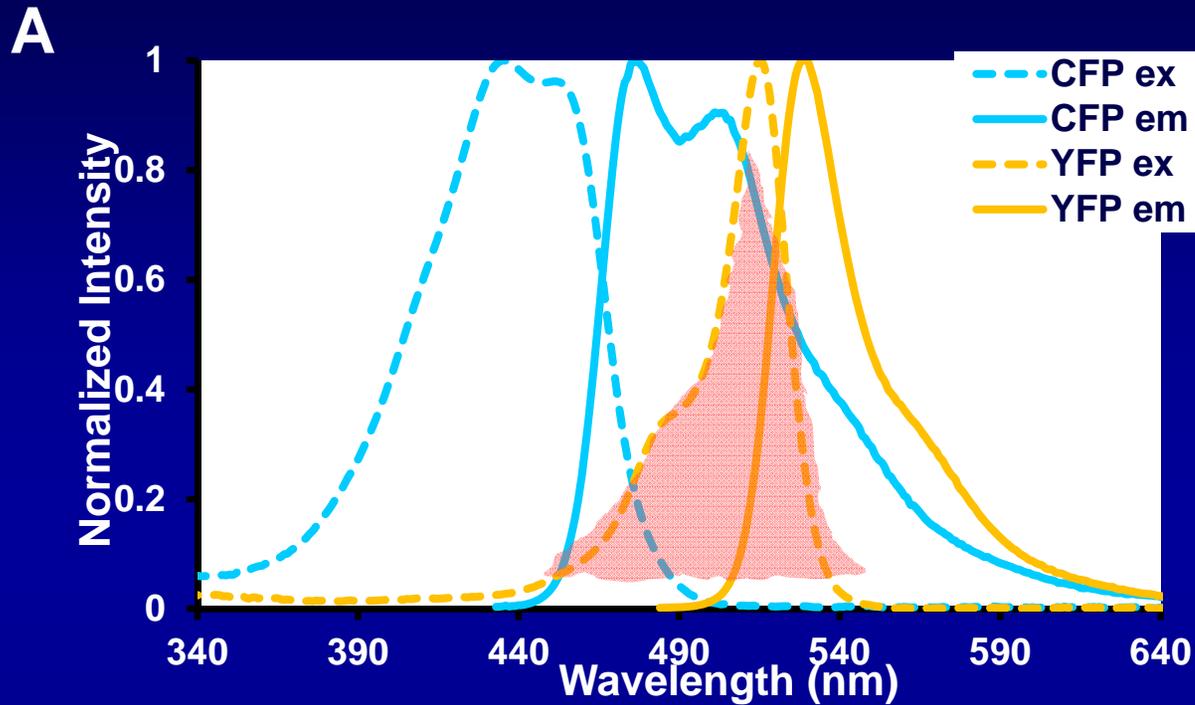


When the fluorophores are far apart: No FRET



When fluorophores are close: FRET occurs

The Principle of Fluorescence Resonance Energy Transfer (FRET)



FRET calculation and measurement

$$E = \frac{1}{1 + (r/R_0)^6}$$

with R_0 being the Förster distance of this pair of donor and acceptor, i.e. the distance at which the energy transfer efficiency is 50%.

$$R_0 = [2.8 \times 10^{17} \cdot \kappa^2 \cdot Q_D \cdot \varepsilon_A \cdot J(\lambda)]^{1/6} \text{ nm} \quad (\text{Equation II})$$

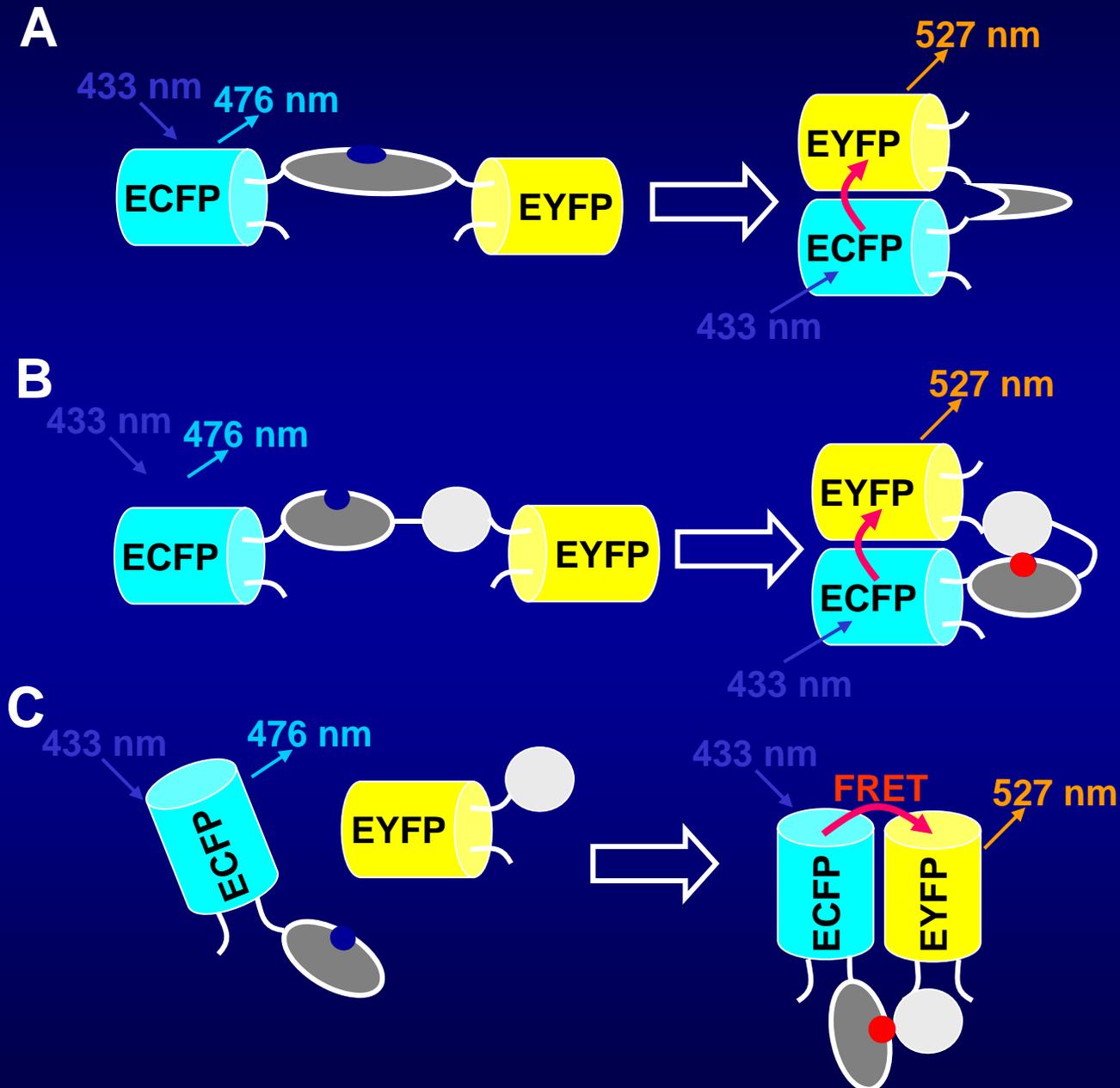
where κ^2 represents the angle between the two fluorophore dipoles, Q_D is the donor quantum yield, ε_A is the maximal acceptor extinction coefficient ($\text{Mol}^{-1} \text{ cm}^{-1}$), and $J(\lambda)$ is the spectral overlap integral between the normalized donor fluorescence, $F_D(\lambda)$, and the acceptor excitation spectra, $E_A(\lambda)$:

$$E = 1 - \tau'_D / \tau_D$$

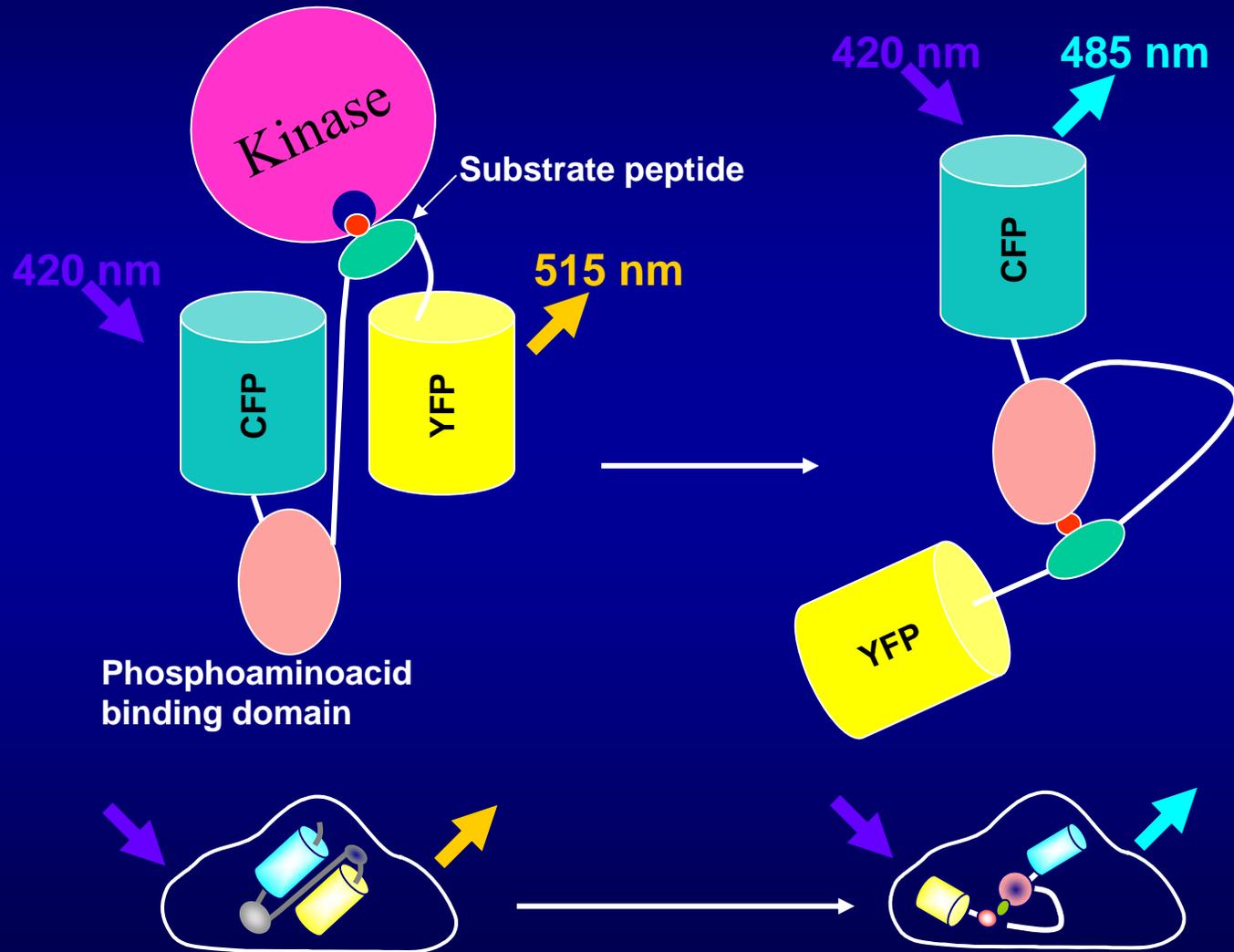
where τ'_D and τ_D are the donor fluorescence lifetimes in the presence and absence of an acceptor

$$E = 1 - F'_D / F_D \quad F'_D \text{ and } F_D \text{ are the donor fluorescence intensities with and without an acceptor.}$$

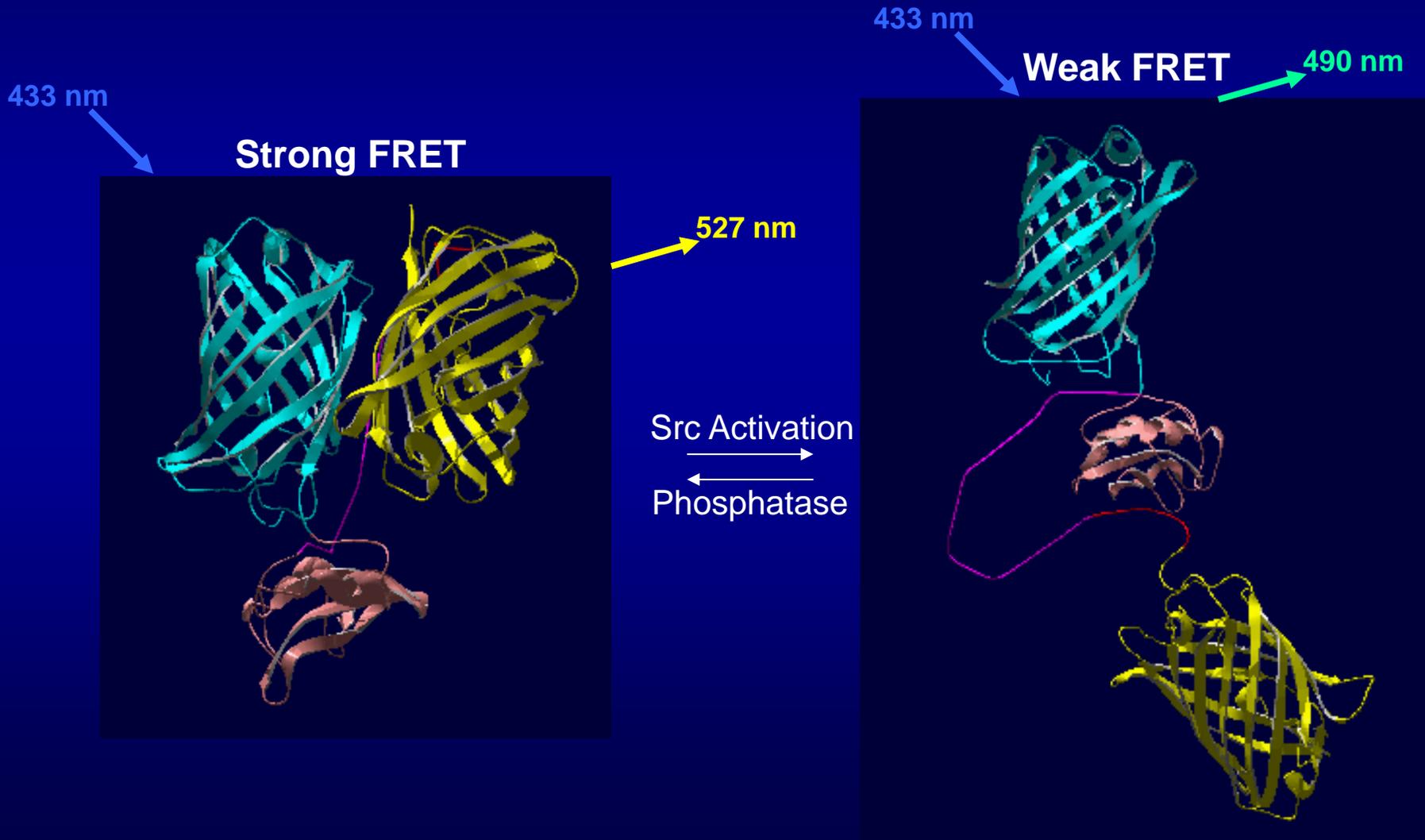
The General Design of FRET-based Fluorescent Probes



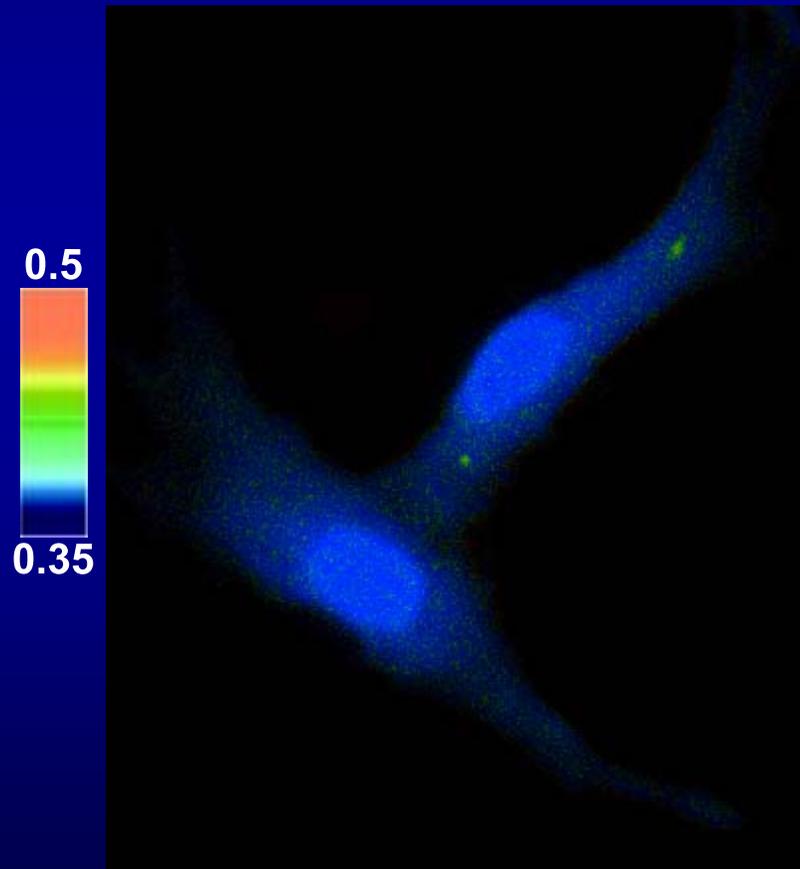
A General Design for Imaging Kinase Activities



General Design Strategy for kinase biosensors

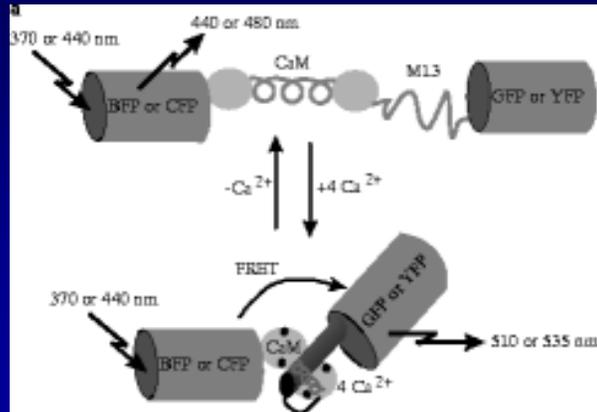


The Src reporter with CFP and YFP monomers



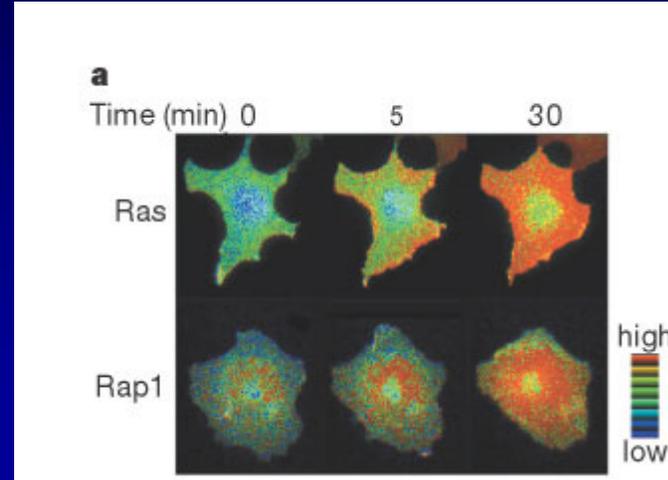
FRET-Based Biosensors

Calcium



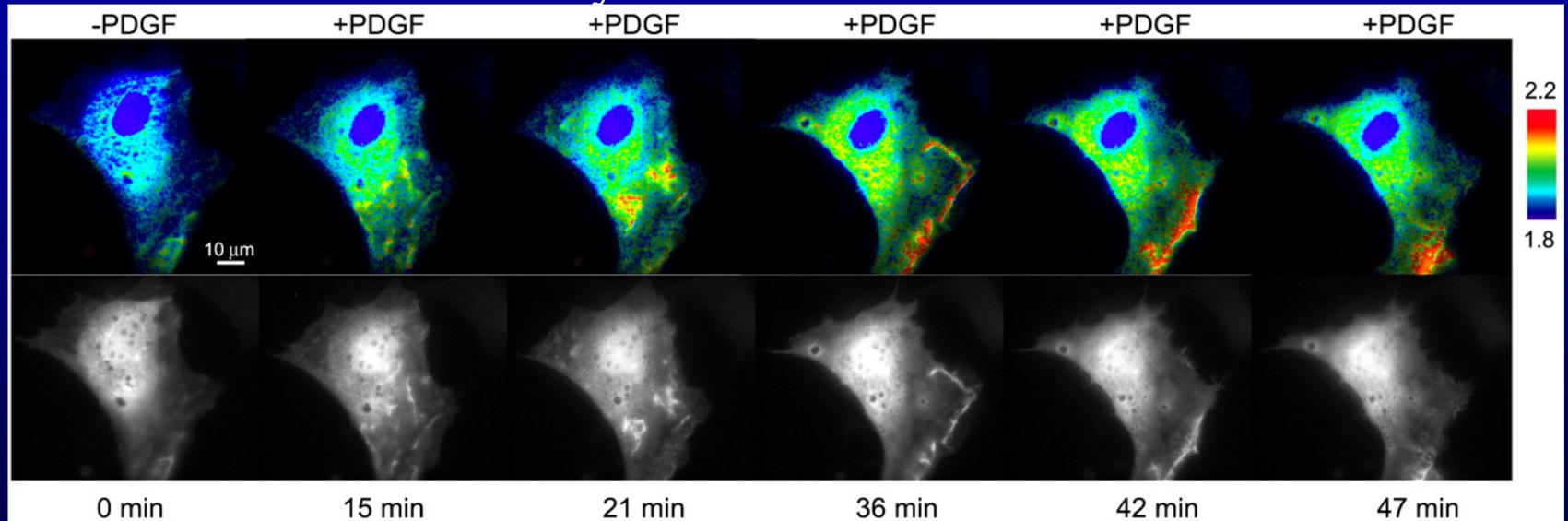
Miyawaki, et al 1997, Nature

Small GTPases Ras and Rap1



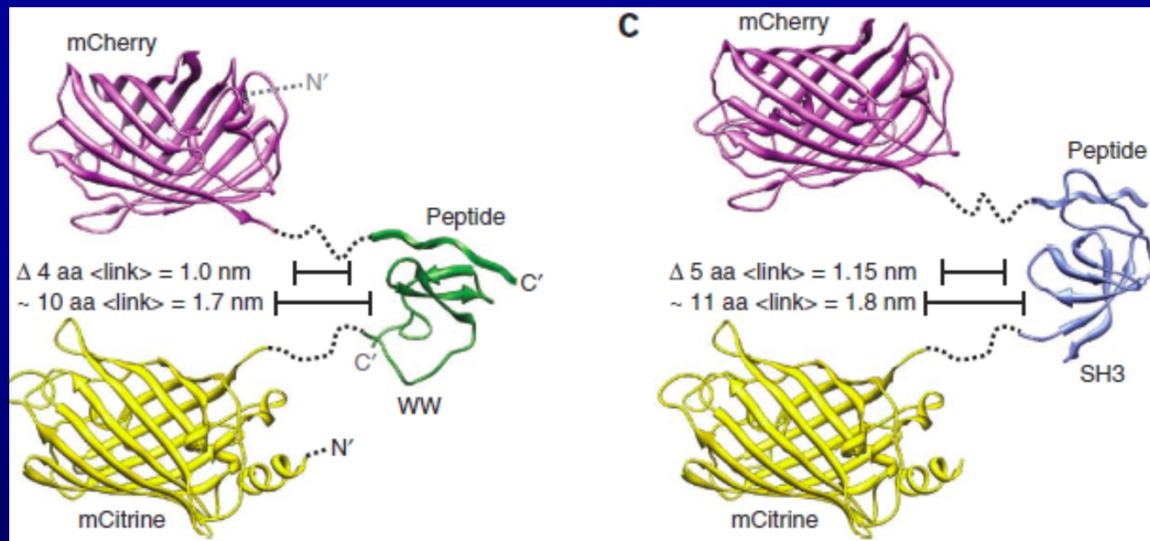
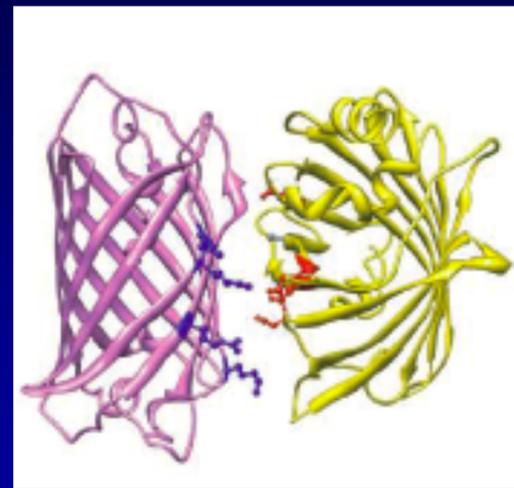
Mochizuki, et al 2001, Nature

Tyrosine Kinase Abl



Ting, et al 2001, PNAS

Enhancing sensitivity of FRET Biosensors



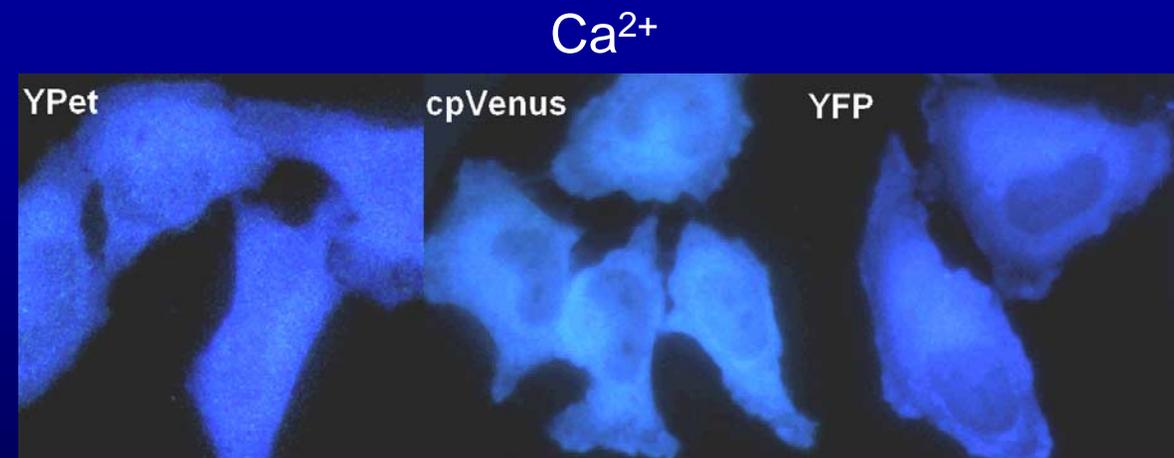
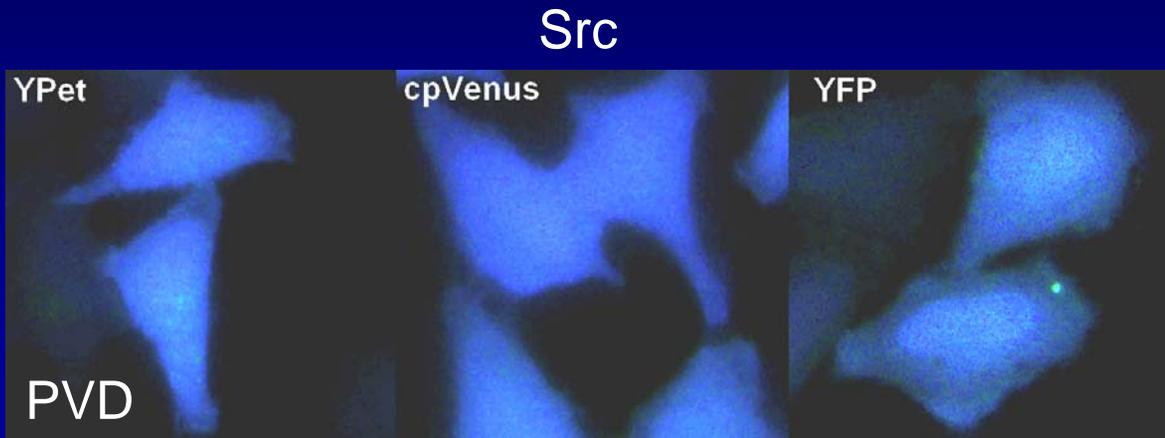
Determination of hierarchical relationship of Src and Rac at subcellular locations with FRET biosensors.

Ouyang M, Sun J, Chien S, Wang Y. Proc Natl Acad Sci U S A. 2008 Sep 23;105(38):14353-8

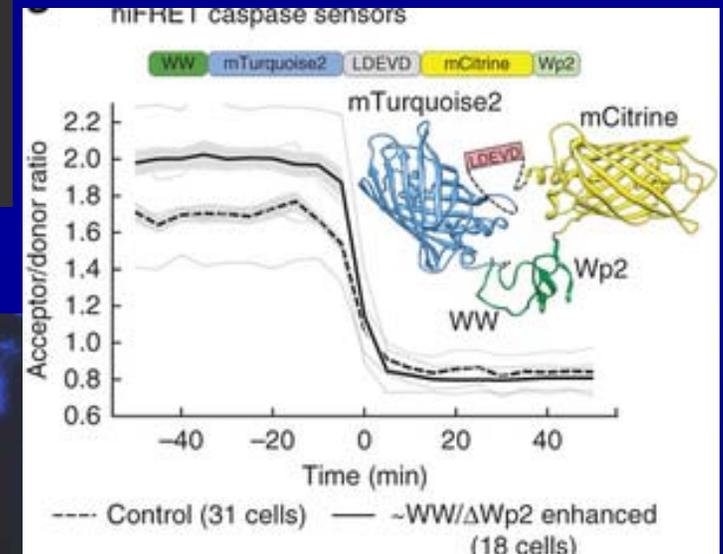
Engineering of weak helper interactions for high-efficiency FRET probes. Grünberg R, Burnier JV, Ferrar T, Beltran-Sastre V, Stricher F, van der Sloot AM, Garcia-Olivas R, Mallabiabarrena A, Sanjuan X, Zimmermann T, Serrano L. Nat Methods. 2013 Oct;10(10):1021-7

The YPet-based FRET Biosensors in Cells

ECFP-Ypet pair



Ouyang, et al. PNAS, 2008



Beltran-Sastre V, et al, NM, 2013

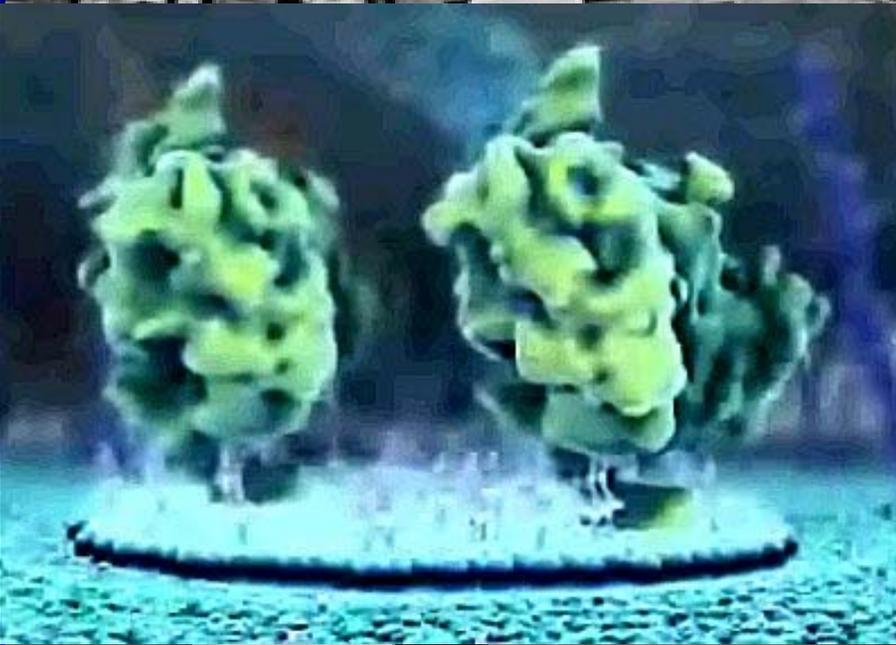
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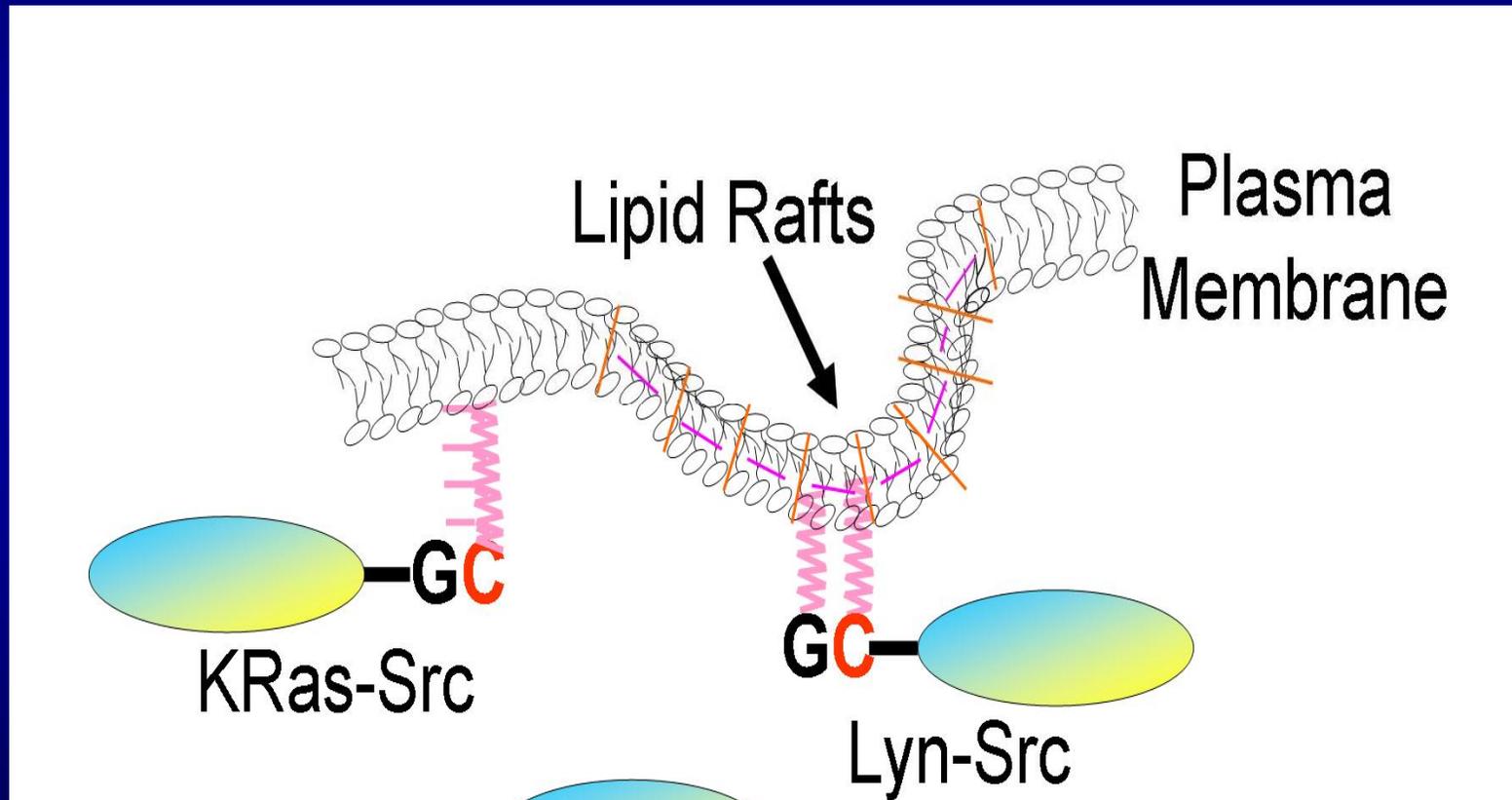
Spy on the Molecular Actions at different subcellular compartments



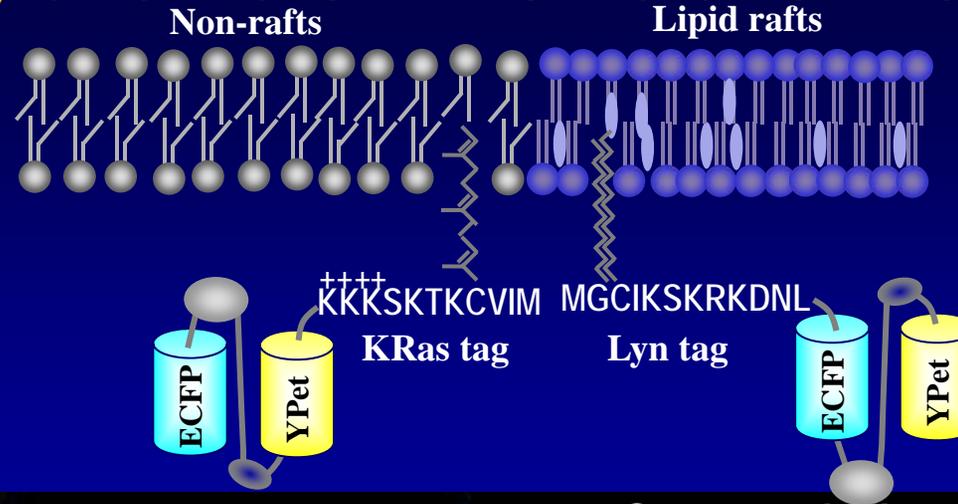
FRET



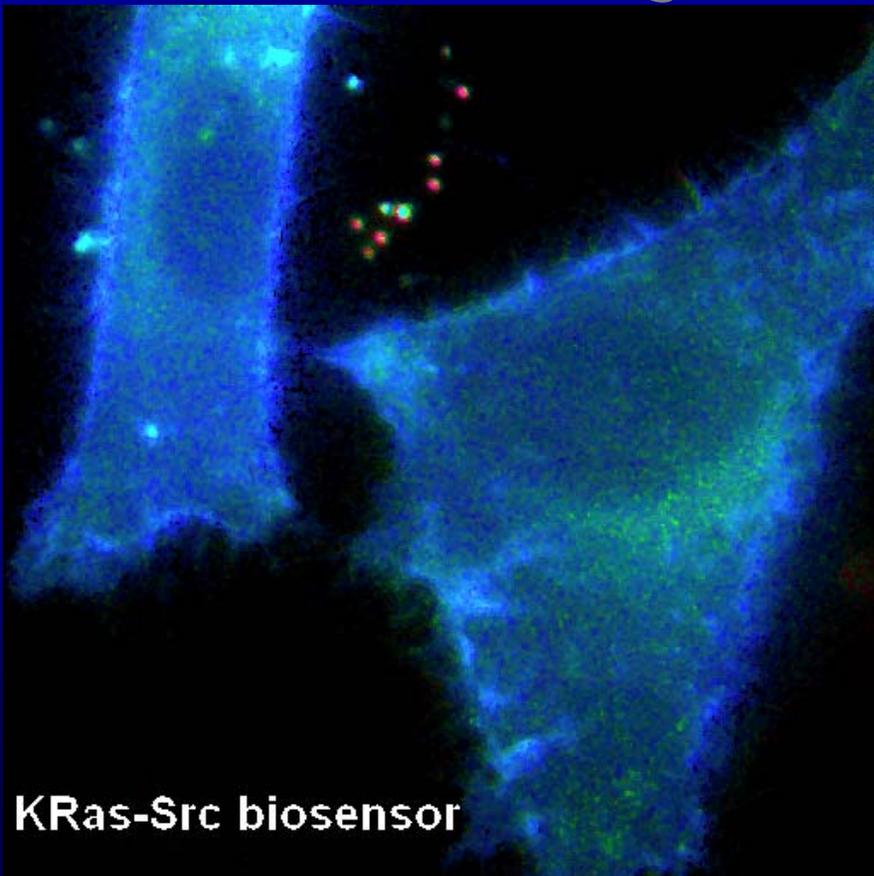
Src Biosensors Targeted to Different microdomains on the Plasma Membrane



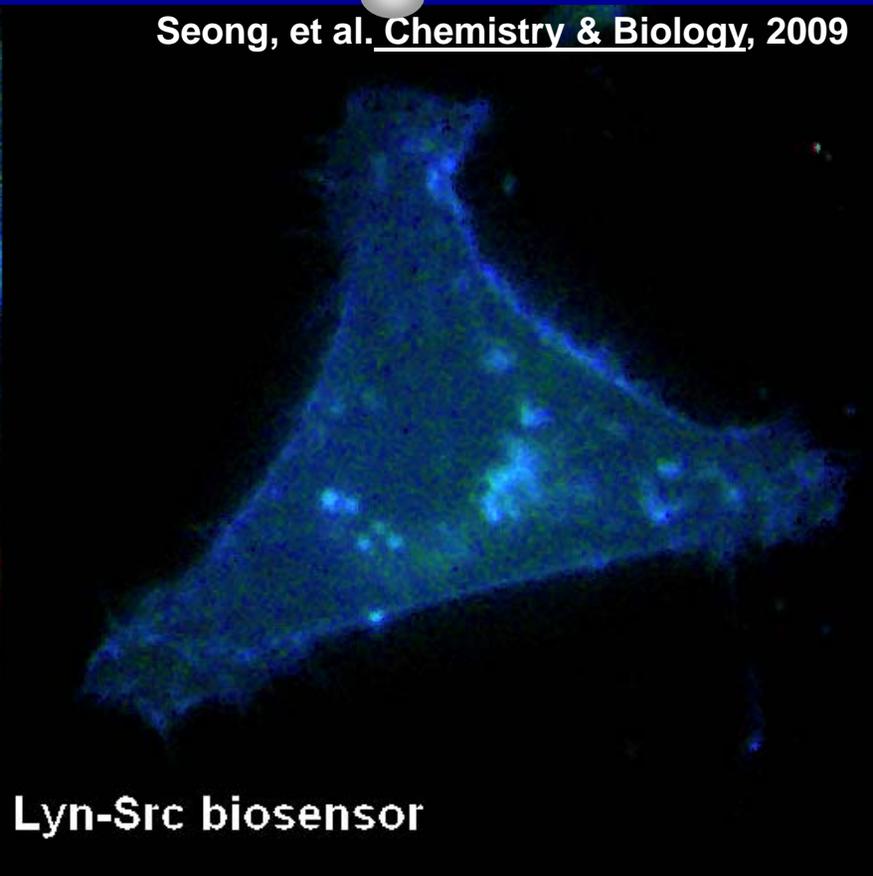
Src activity at different membrane microdomains



Seong, et al. Chemistry & Biology, 2009

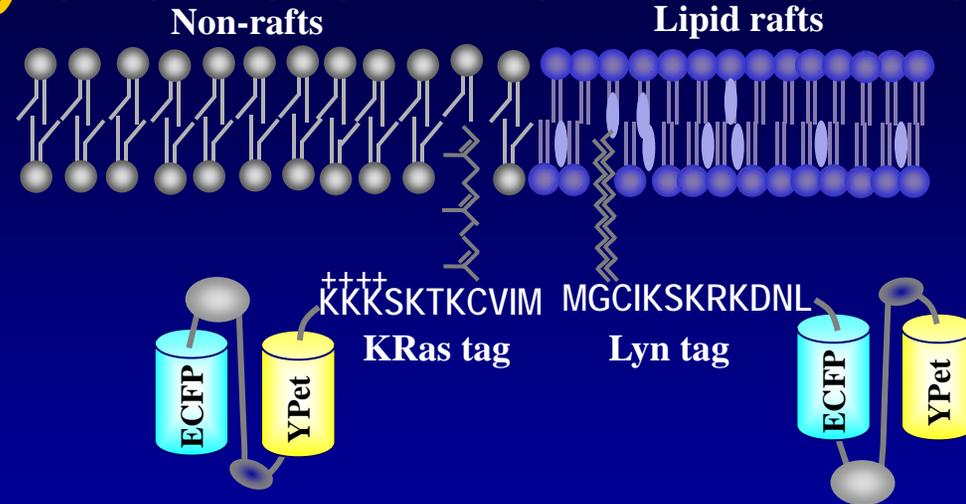


KRas-Src biosensor

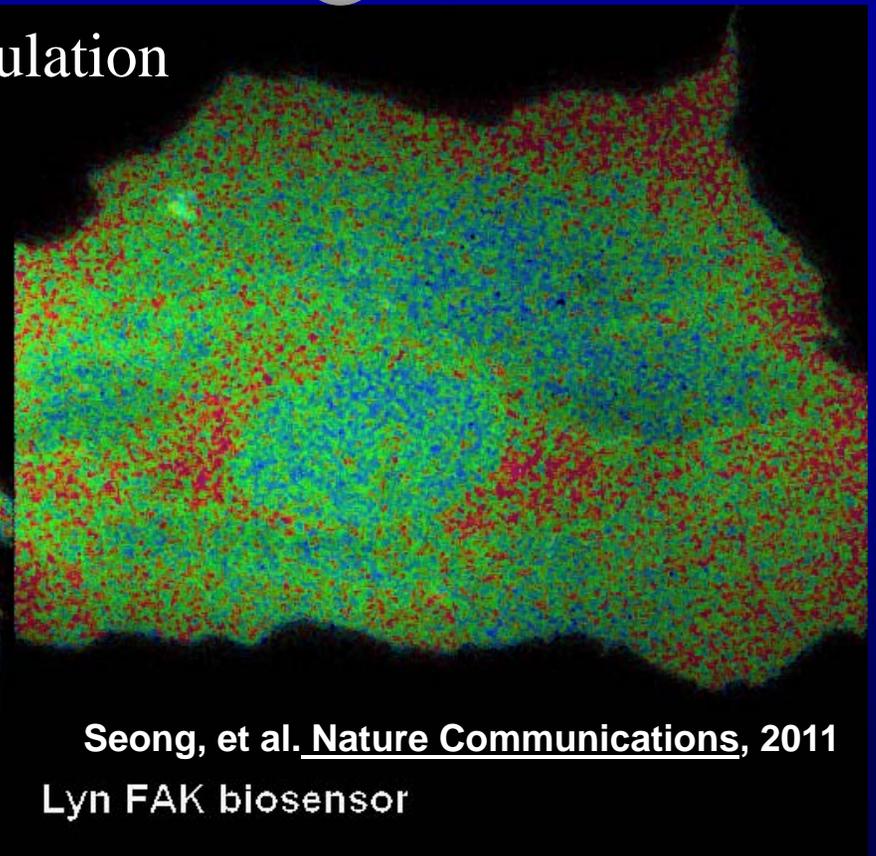
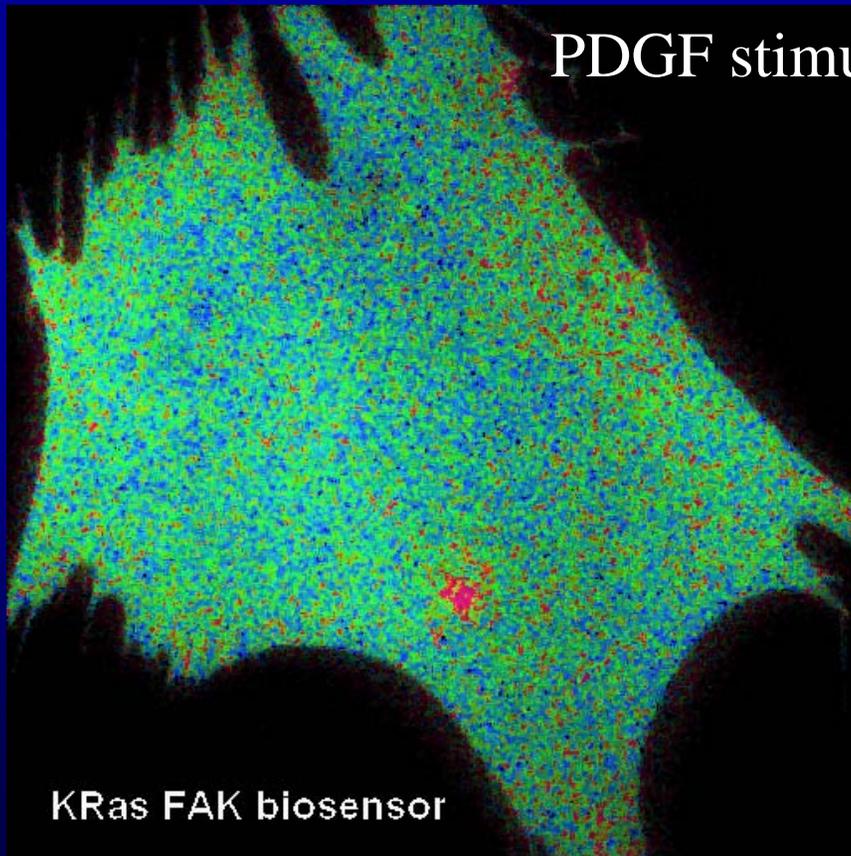
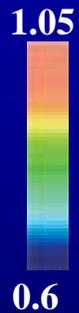


Lyn-Src biosensor

FAK activity at different membrane microdomains



PDGF stimulation

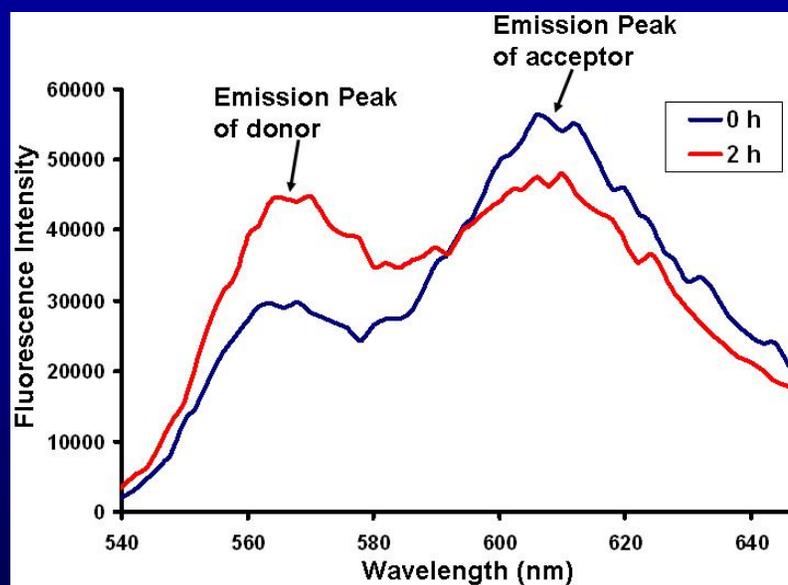
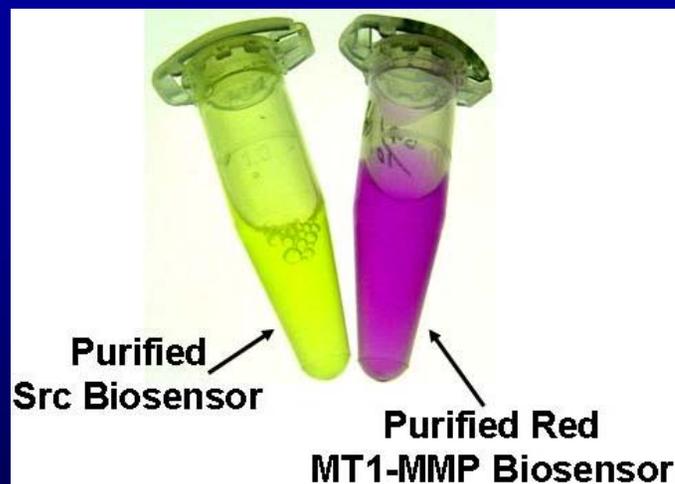
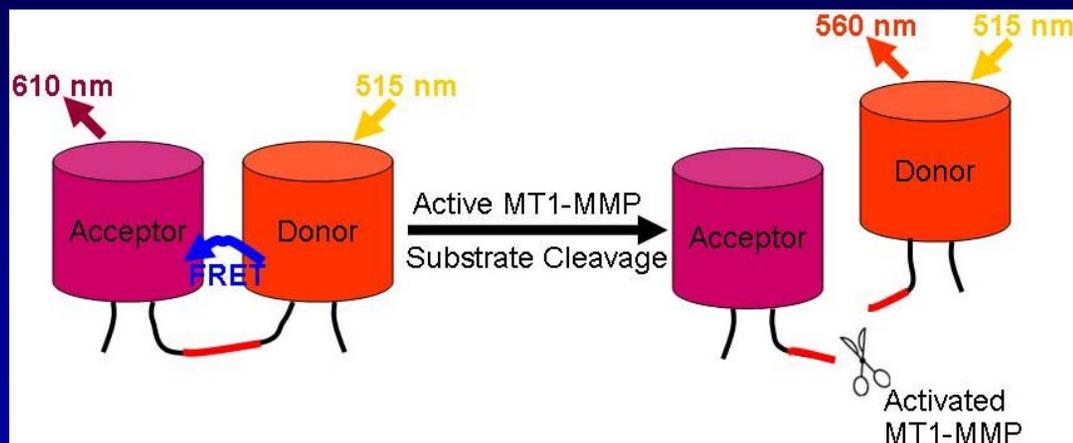


Seong, et al. Nature Communications, 2011

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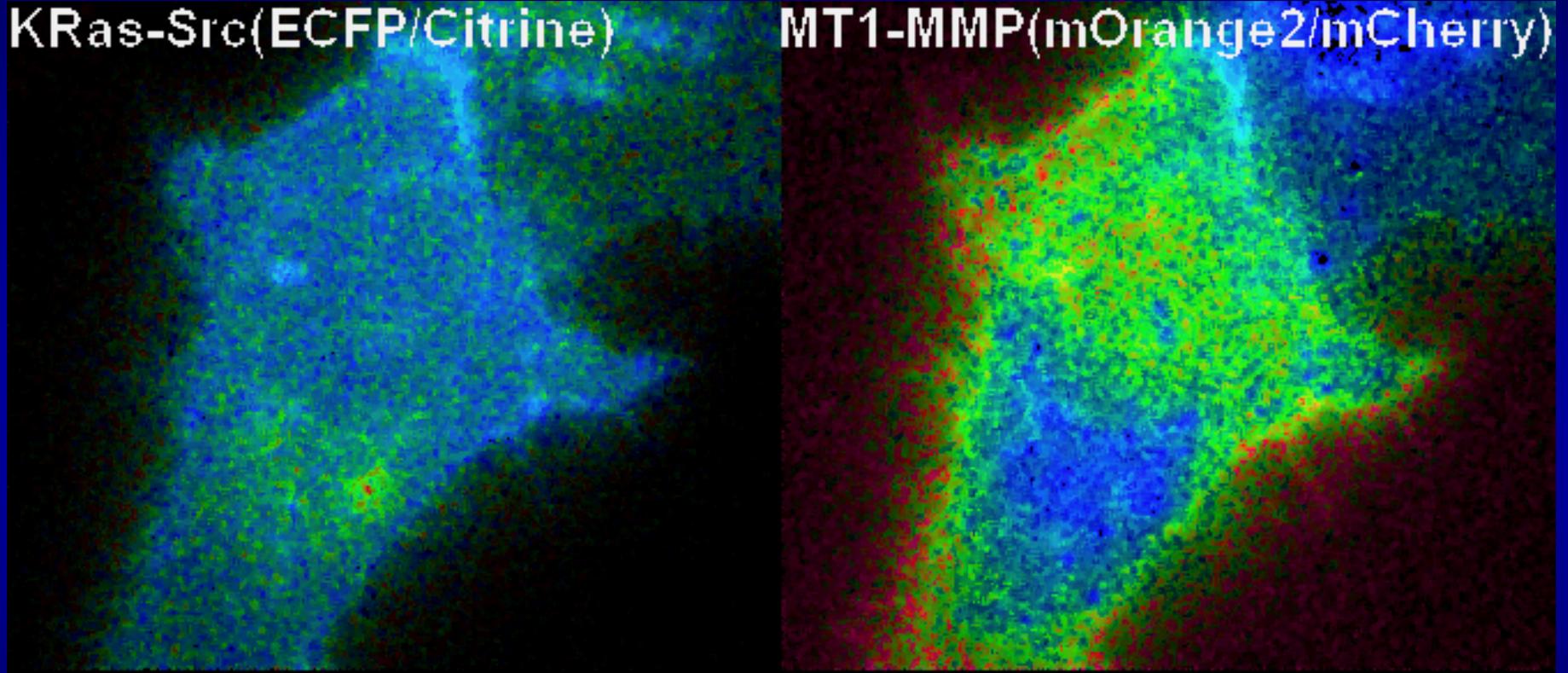
A New FRET Biosensor with Red Color



Simultaneous imaging of Src and MT1-MMP activities in a single cell

KRas-Src(EGFP/Citrine)

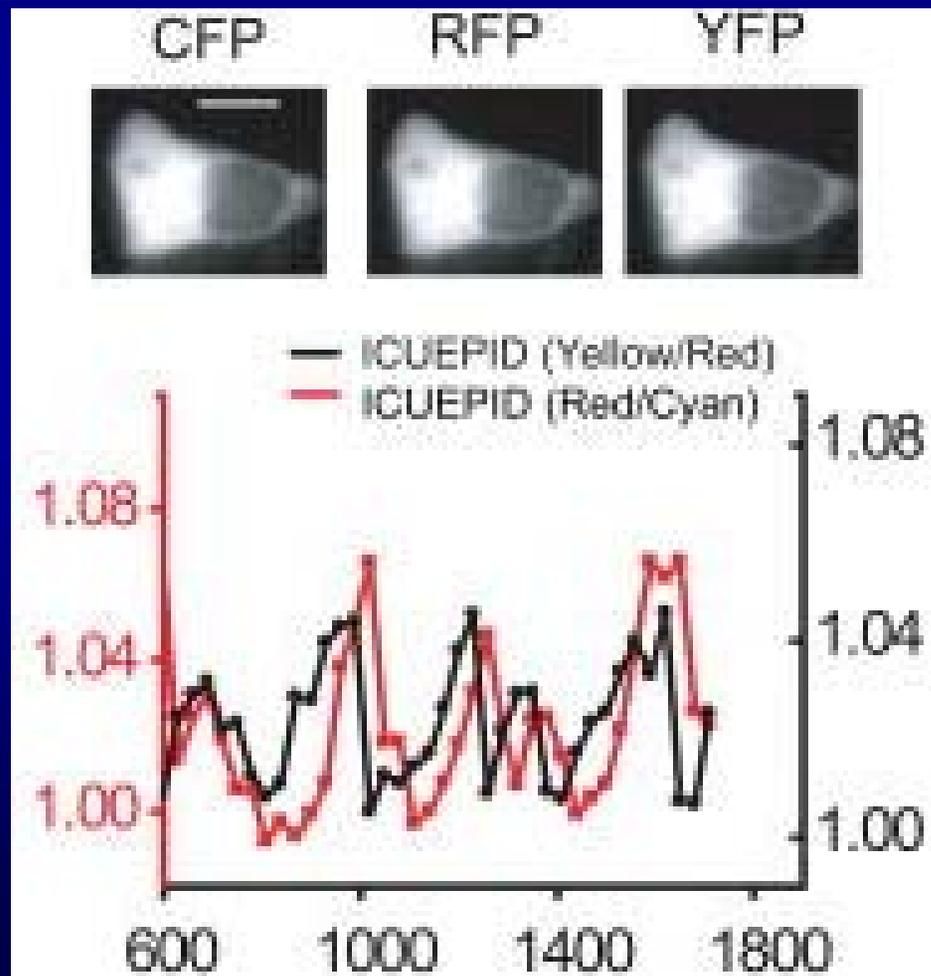
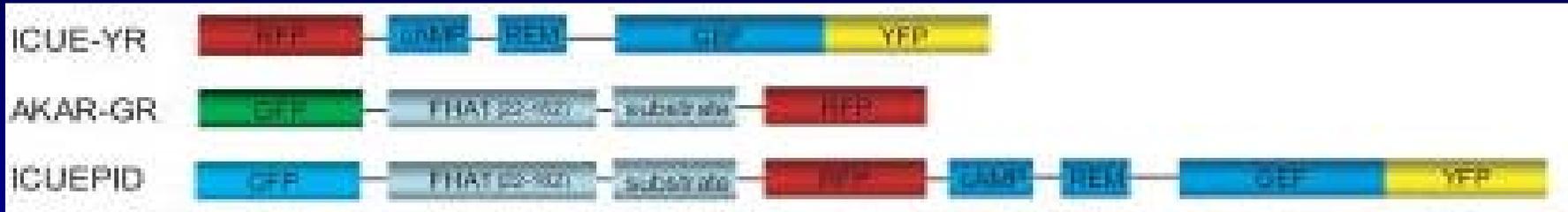
MT1-MMP(mOrange2/mCherry)



Ouyang M., et al. Cancer Research, 2010

Multi-Color FRET Biosensors

Red/Cyan, PKA; Yellow/Red, cAMP

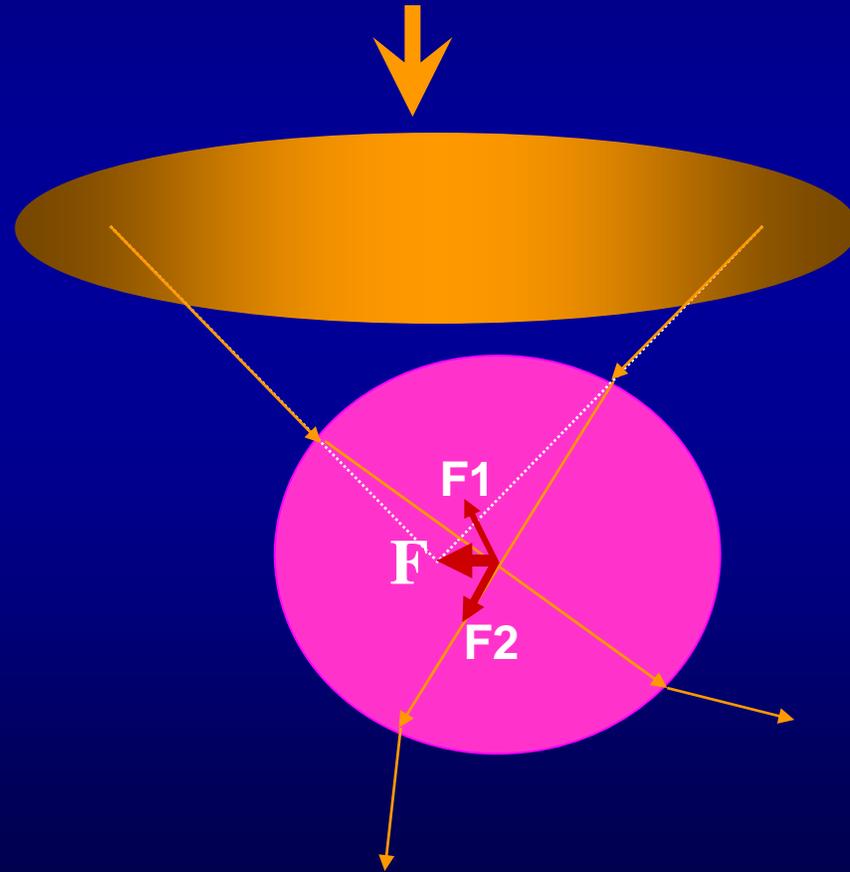
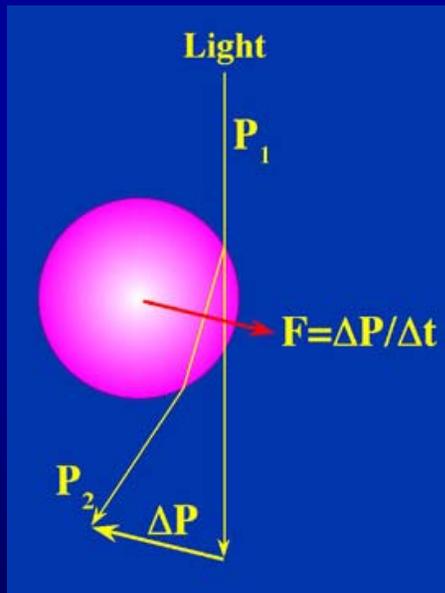


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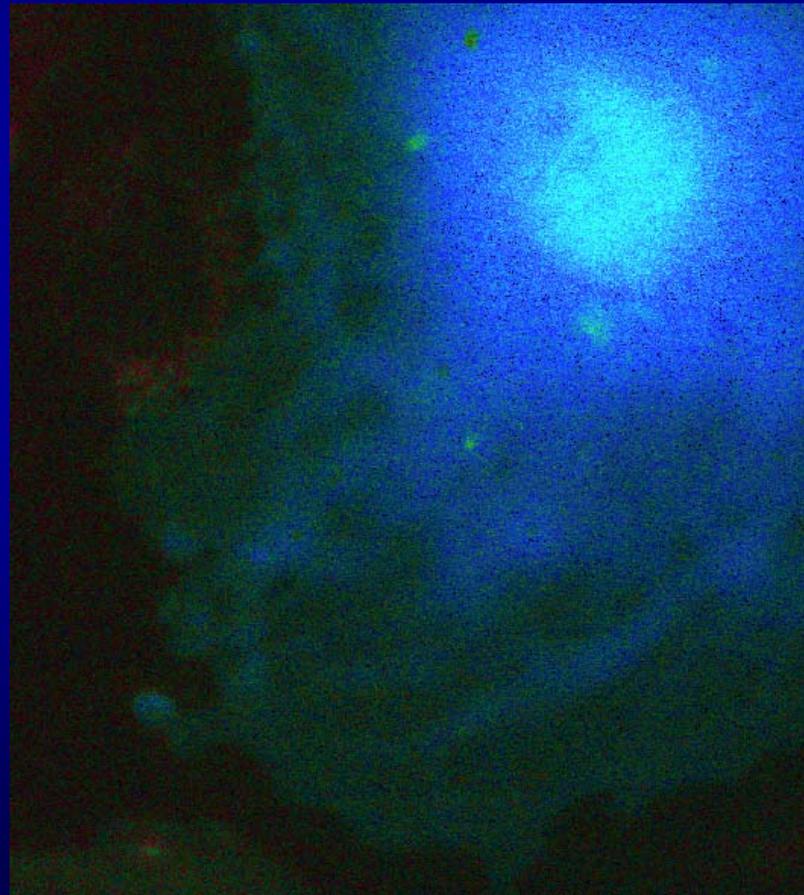
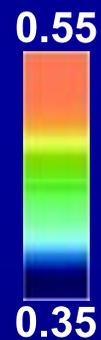
Application of Mechanical Stimulation by Using Laser Tweezers

Physical Principle of Laser Tweezers

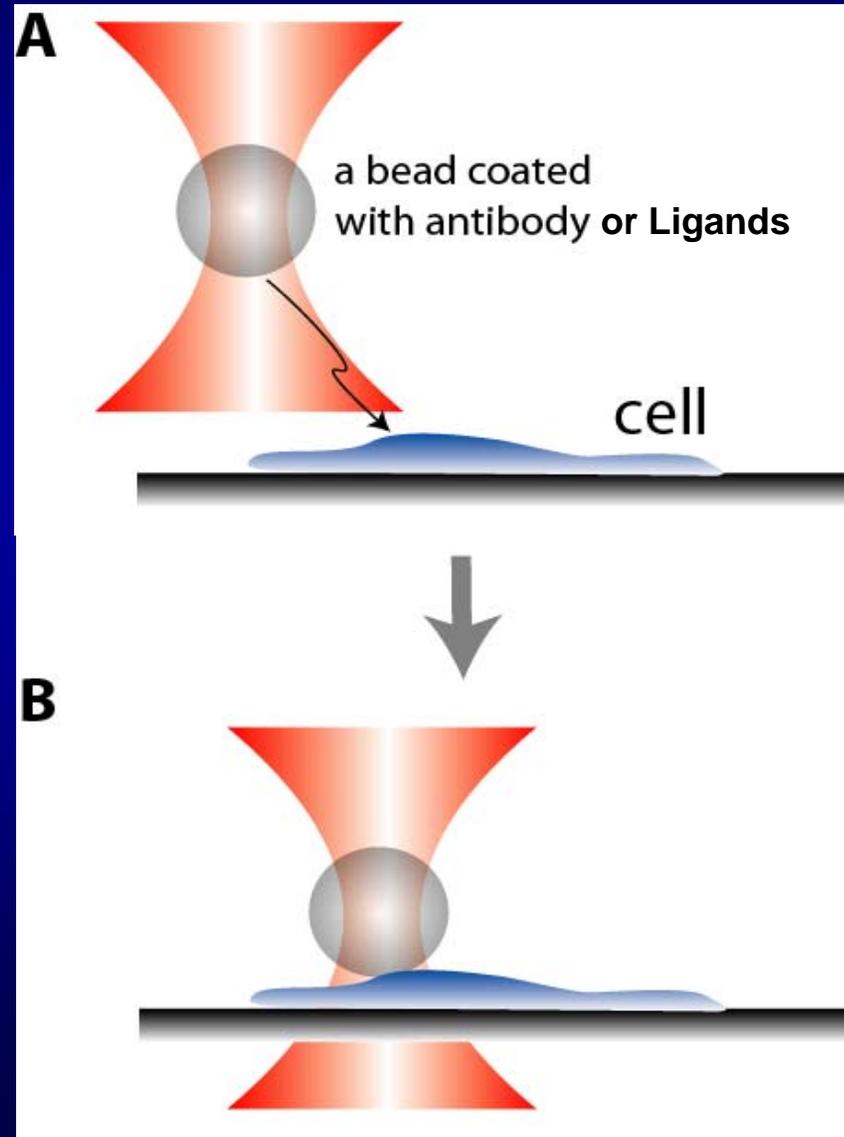


***Pulling Polylysine-coated beads
did not have significant effects on FRET***

FRET

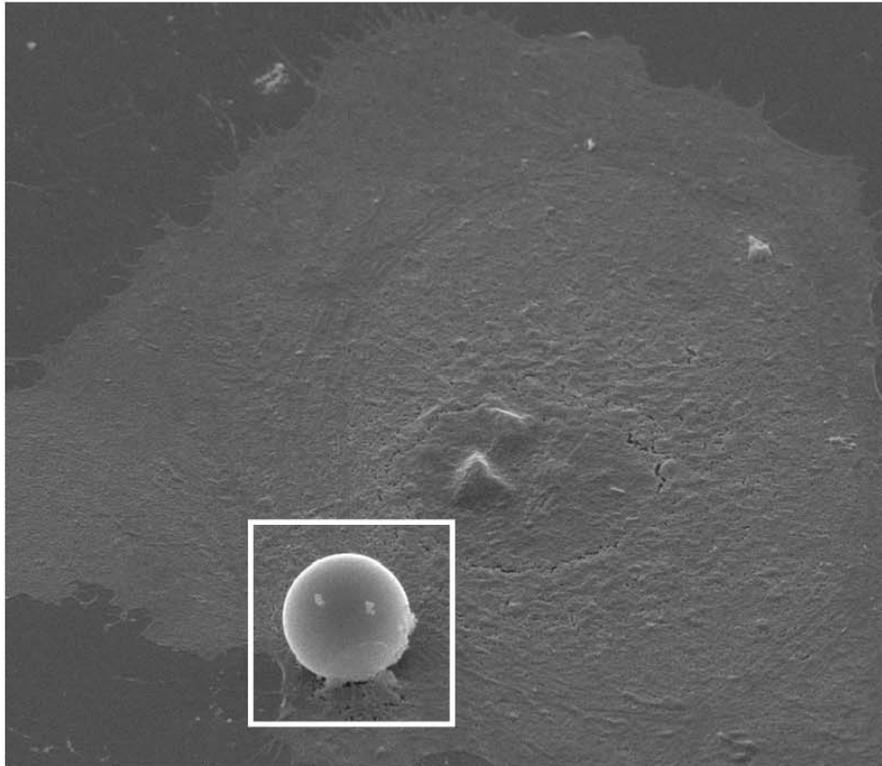


Laser-Tweezer to Localize the stimulation



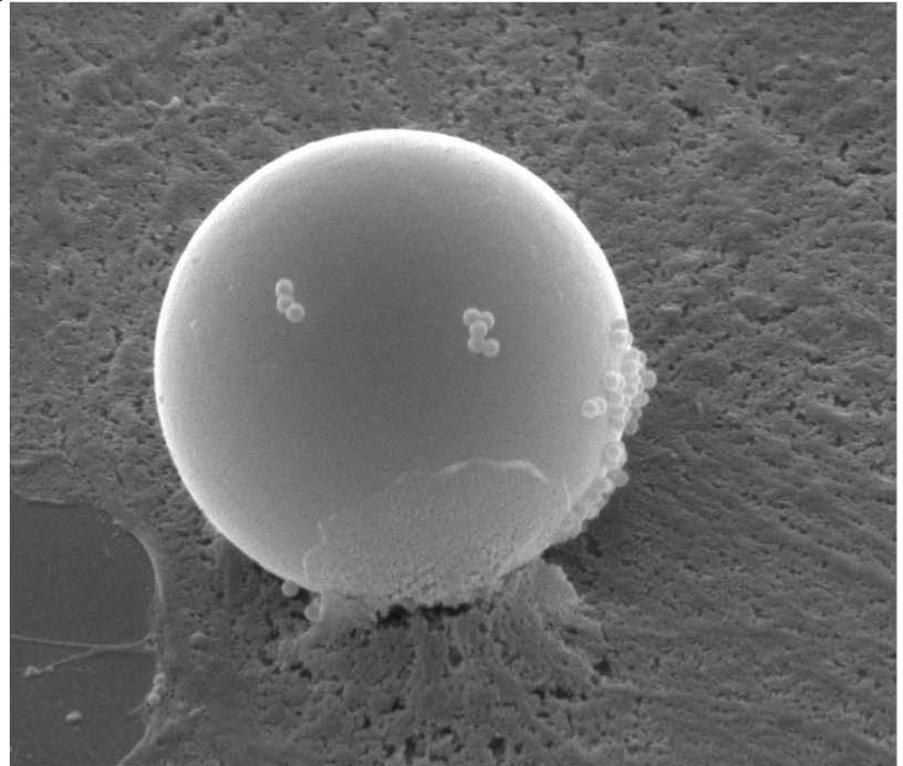
Polystyrene beads were coated with fibronectin and positioned on cells

a



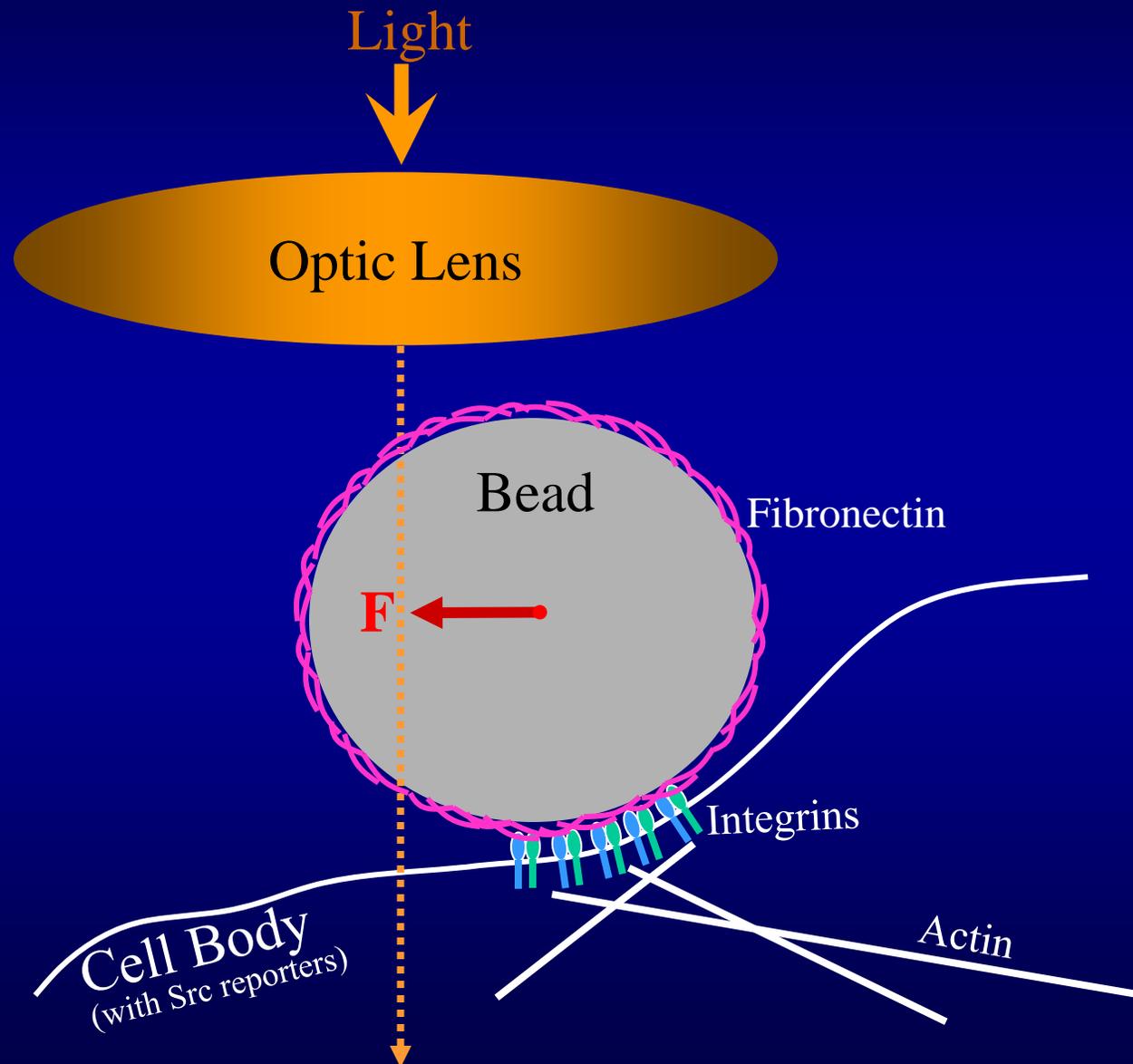
HFW	HV	WD	Sig	Spot	Mag	
64.00 μm	20.0 kV	13.61 mm	SE	2.5	4000x	—10 μm —

b

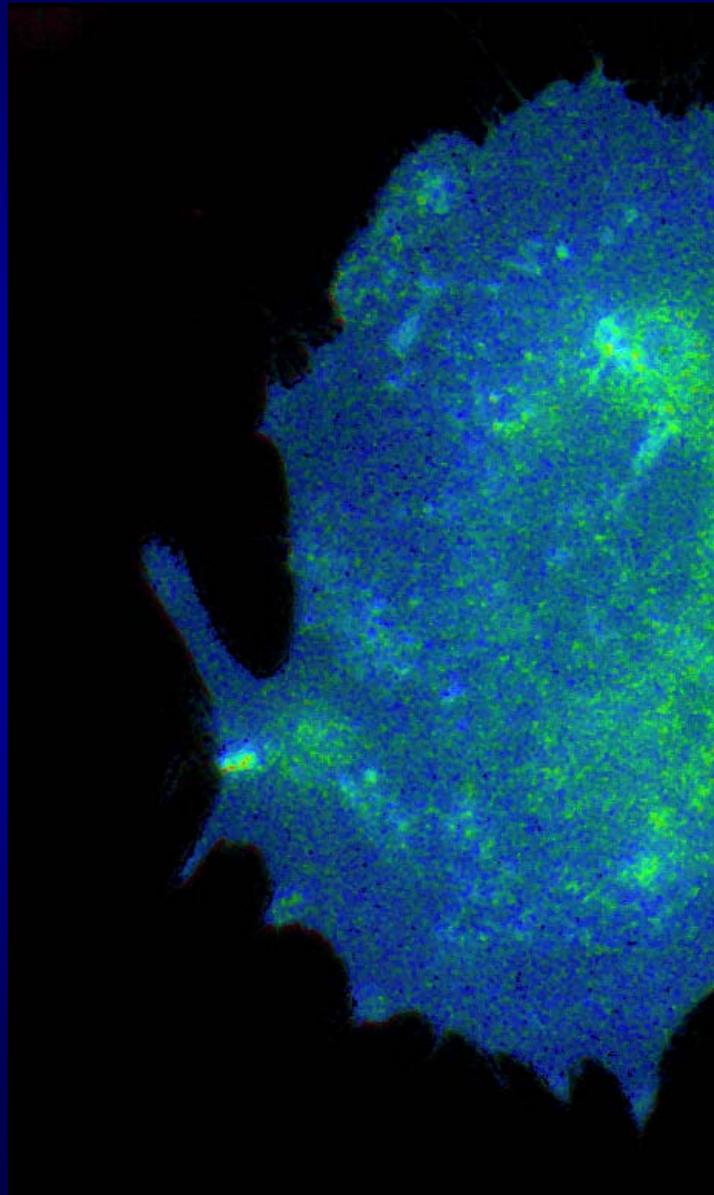
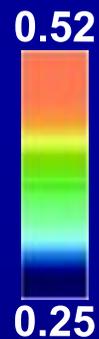


HFW	HV	WD	Sig	Spot	Mag	
17.07 μm	20.0 kV	13.59 mm	SE	2.5	15000x	—2 μm —

Polystyrene beads were coated with fibronectin and positioned on cells



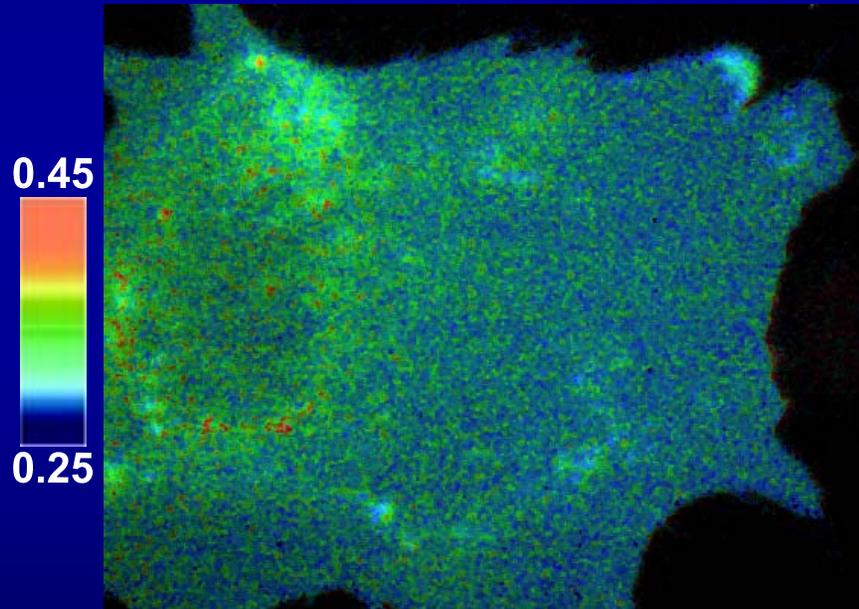
Pulling Fibronectin-coated Beads induced a directed propagation of Src activation



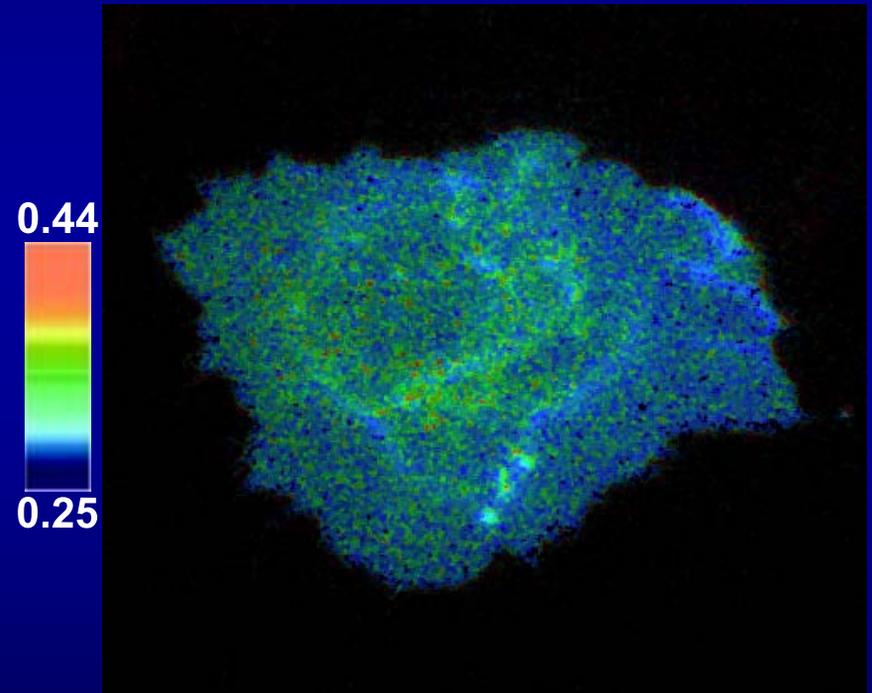
Wang, et al. Nature, 2005

The directed and long-range activation of Src is dependent on cytoskeleton-integrity

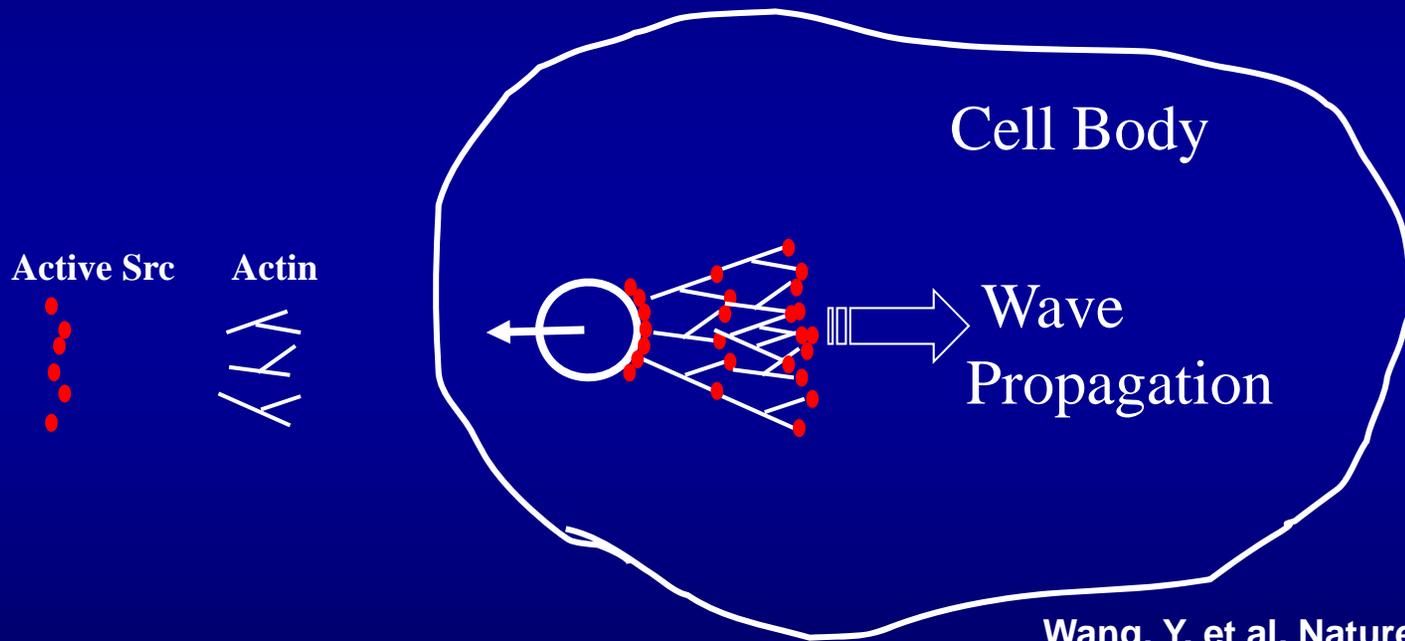
Cytochalasin D Treated



Nocodazole Treated



The Proposed Model of Src Activation and Actin Dynamics



Summary

- 1. The molecular engineering and fluorescence biosensors can provide powerful tools for live-cell imaging to study molecular activities and interactions, address important biological questions.**
- 2. The molecular activities and hierarchy inside live cells are largely dependent on the sub-cellular locations.**
- 3. Multi-color FRET biosensors and automated correlation analysis can allow the simultaneous visualization of molecular activities in a single live cell and the elucidation of molecular hierarchy.**

Acknowledgment

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Dr. Taejin Kim

Dr. Jie Sun

Ping Zhang

Dr. John Eichorst

Kaiwen Zhang



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Dr. Roger Y. Tsien, UCSD

Dr. Ning Wang, UIUC

Dr. Zong-Lai Jiang, SJTU

Dr. Yingxin Qi, SJTU

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Thank you!