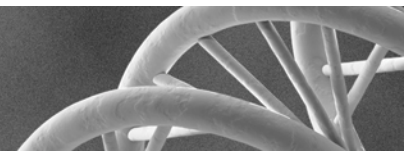


Multiplex Tagging Tools for the Study of Protein Dynamics in Living Cells and beyond

SNAP-Tag Technology

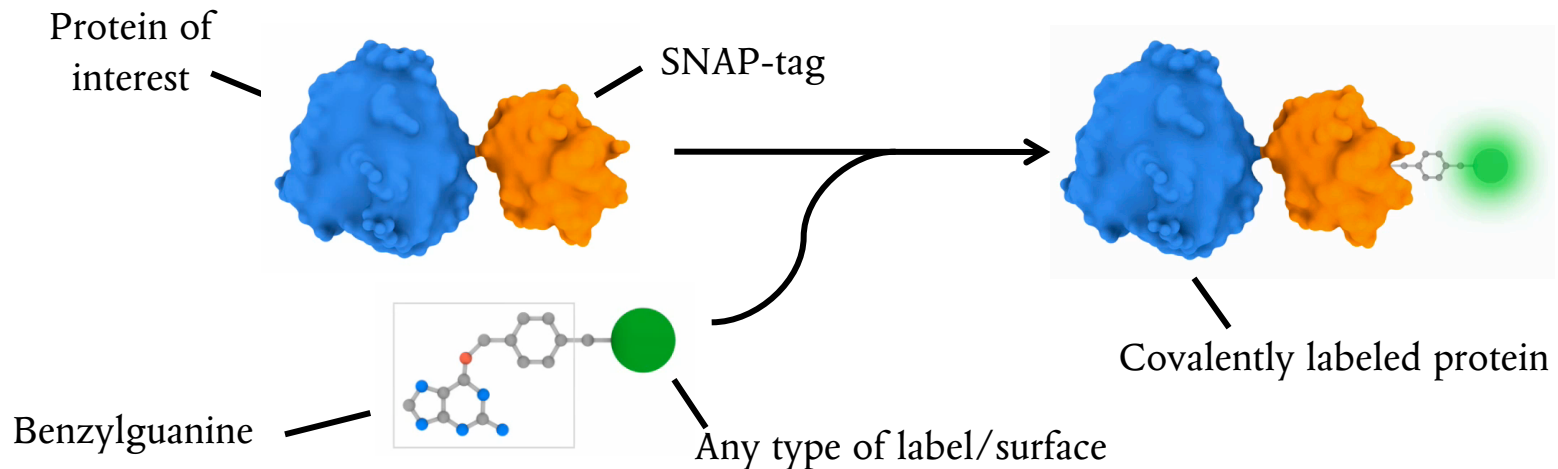
Spanning the Big, Little & Tiny...

- Introduction to SNAP-tag and related technologies
- How does it differ from other approaches (ie. FPs)?
- Applications: Spanning the Big, Little & Tiny
- Conclusions and Questions



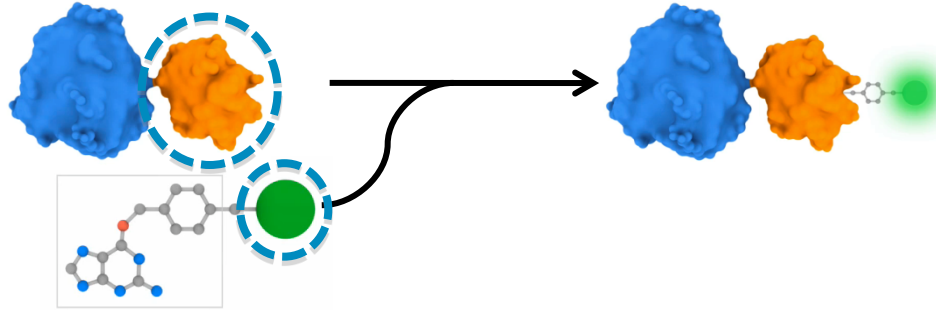
Overview of SNAP-tag Technologies

- **Overview** – fusion protein technology based on orthogonal protein tags that can covalently self-label in a variety of contexts including: in live cells, fixed cells and in cell lysates.
- **Applications** – wide range of validated applications including: cellular imaging, biochemical assays, pull-downs, etc.

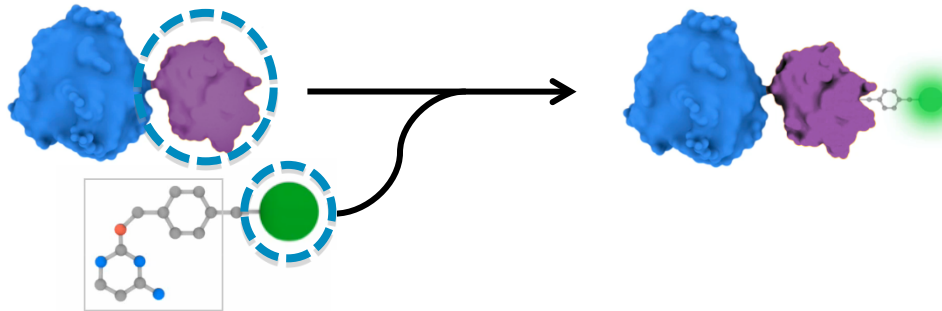


Four Tags Available – infinite possibilities

SNAP-tag
(app 20kDa)

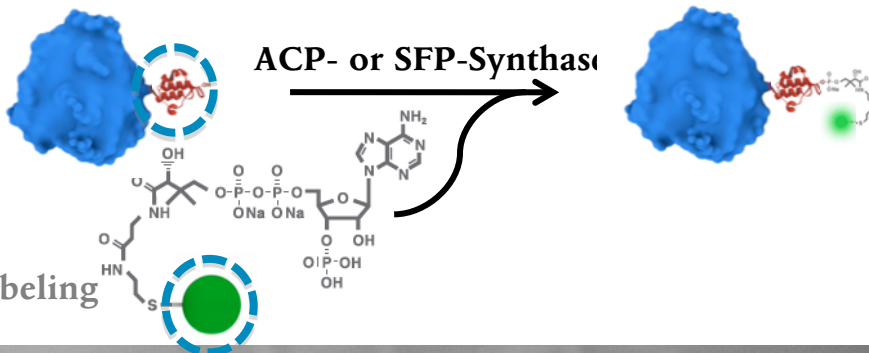


CLIP-tag
(app 20kDa)



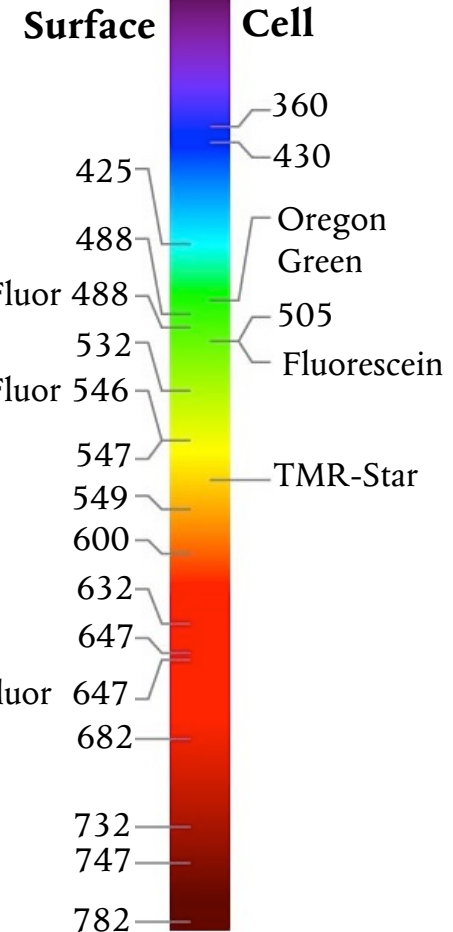
Self-labeling

ACP-tag
MCP-tag
(app 8kDa)


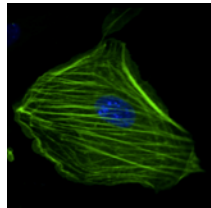

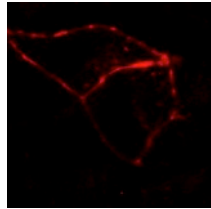

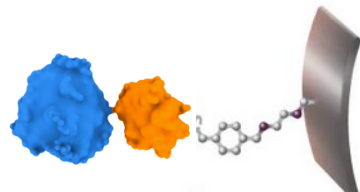


Enzyme-mediated labeling

Fluorophores

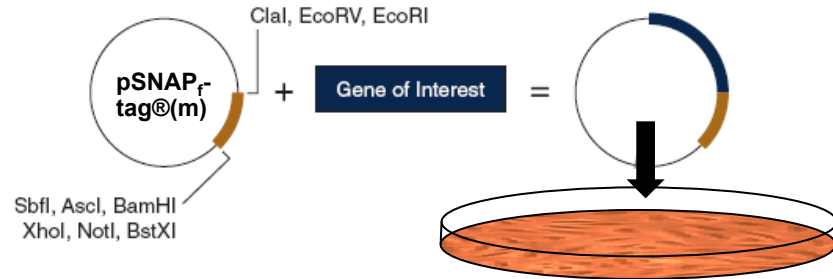


Functionalities “Encoded” by Each Label

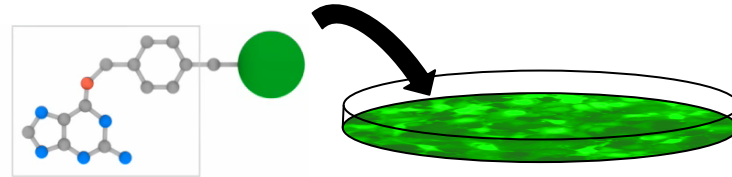
“Label”		Product Name	Function/Application	
Cell-permeable fluorophores		SNAP-Cell CLIP-Cell	Visualizing proteins inside or on the surface of living or fixed cells	 <p>3T3 Cells SNAP-Actin SNAP-Cell 505</p>
Non cell-permeable fluorophores		SNAP-Surface CLIP-Surface CoA Labels	Visualizing proteins on the surface of living or fixed cells (ex. receptor internalization)	 <p>COS7 Cells SNAP-ADRB2 SNAP-Surface Alexa Fluor® 546</p>
Magnetic and non-magnetic beads		SNAP-Capture	Protein pull-downs	

Live Cell Imaging Protocol Overview

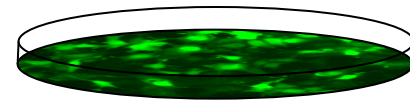
Clone gene of interest into NEB expression vector, transfect fusion into cells



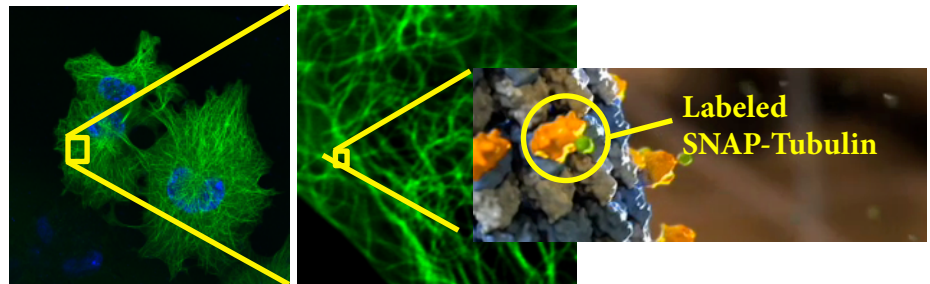
Re-suspend label in DMSO and dilute in media; add to cells, incubate



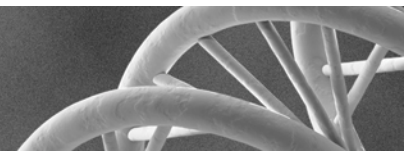
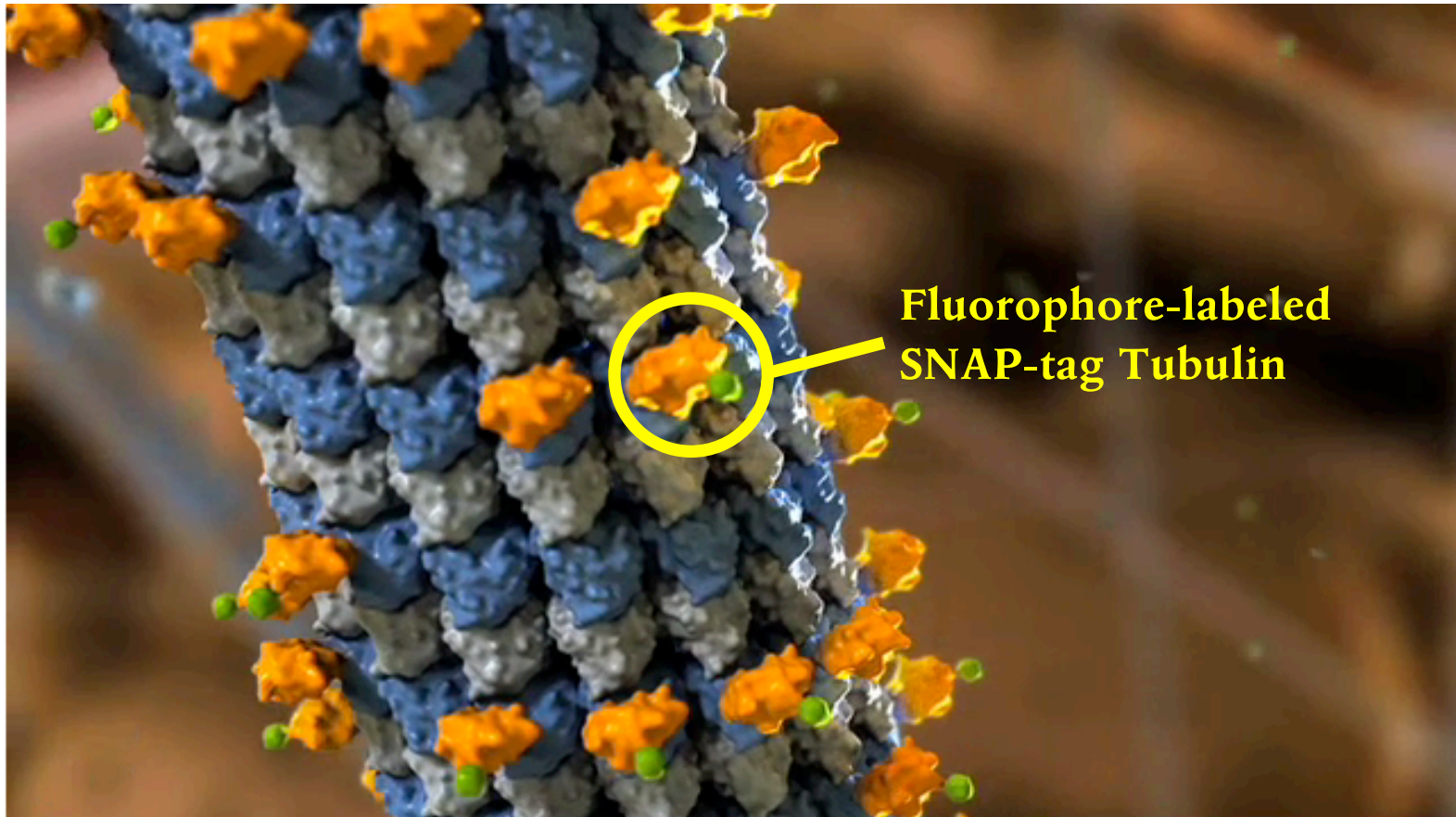
Covalent modification occurs, labeling protein; un-bound label is removed, cells rinsed



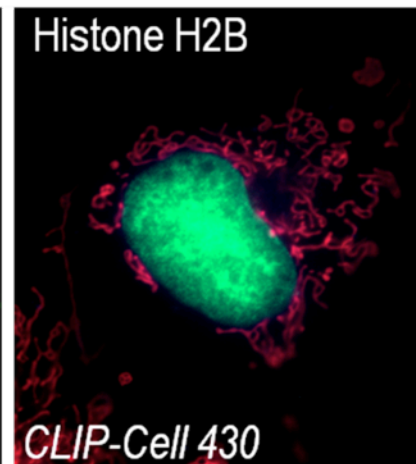
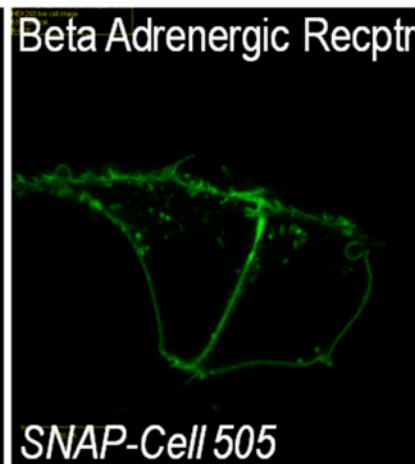
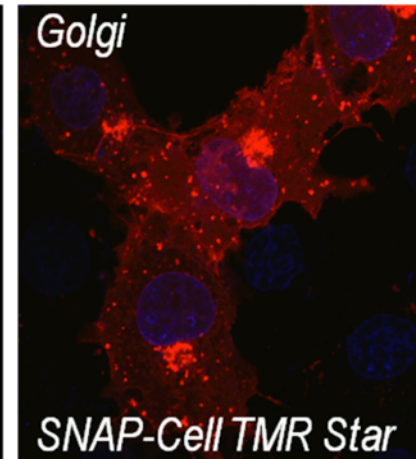
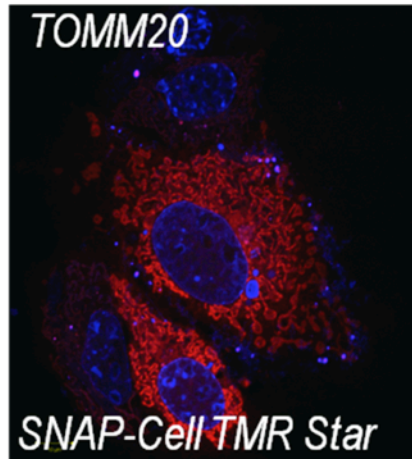
Visualize & Study



Labeling of SNAP-Tubulin in Living Cells



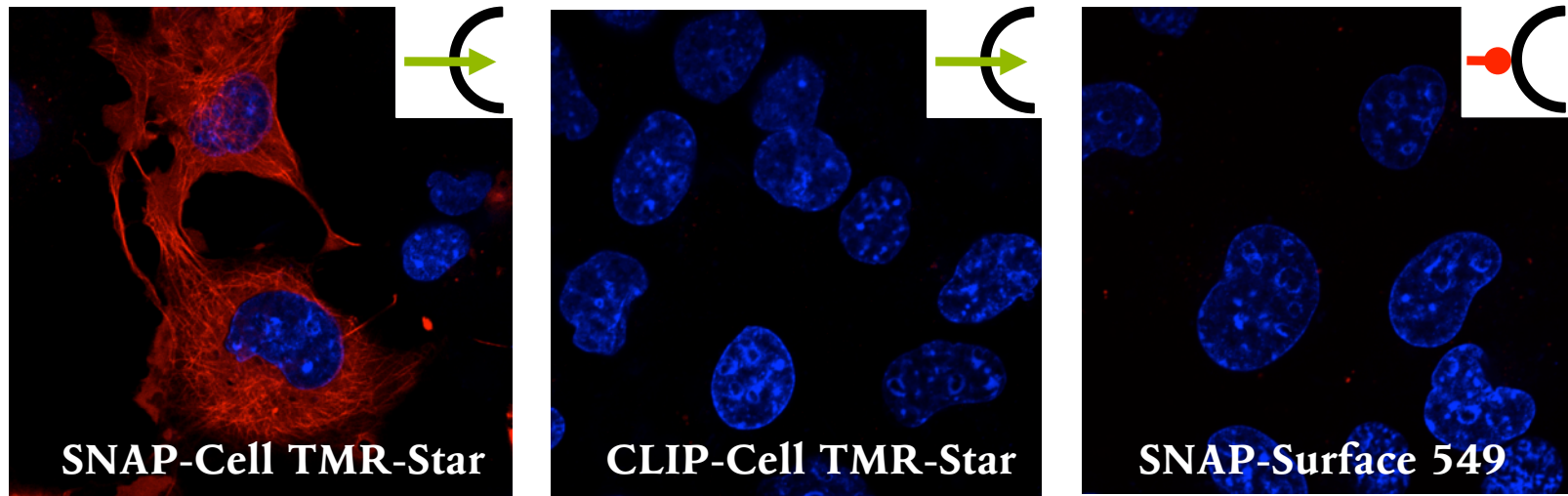
Tags Localize Appropriately with Fusion Partner



No Background Labeling in Live Cells

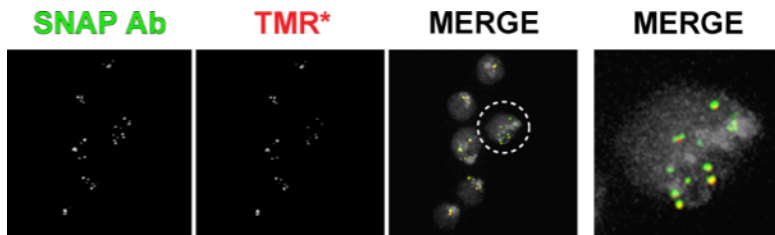
- **No Background Staining** – Exclusively labels fusion tag
- **No Crosstalk** – Highly Specific

Live COS7 Cells Transiently Expressing SNAP-Tubulin



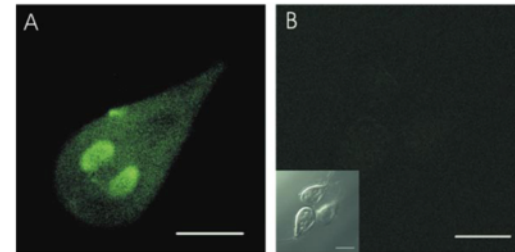
Live Cell Labeling of Different Organisms

Drosophila melanogaster Kc cells
SNAP-Cell TMR Star labeling at centromeres

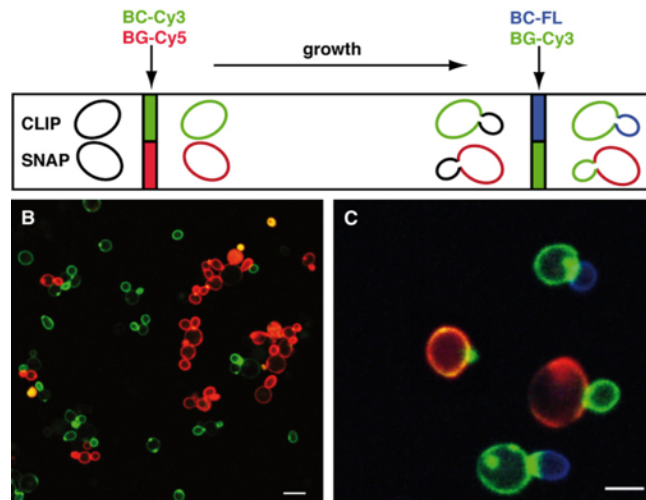


Erhardt, J Cell Biol, 2008, 183, 805

Flagellated protozoan parasite Giardia lamblia
SNAP-Cell Fluorescein staining of two nuclei of Giardia



Regoes, *Biotechniques* 2005, 39, 809



Gautier, *Chem. Biol.* 2008, 15, 128

Gram-positive bacterium Bacillus subtilis
SNAP-Cell 430 labeling of flotillin-like protein YuaG



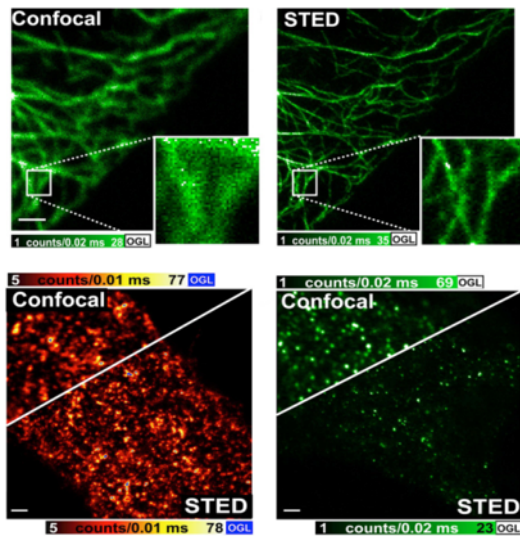
Donovan, *Microbiology* 2009, 155, 1786

Saccharomyces cerevisiae
Double pulse-chase experiments of SNAP
and CLIP fusion proteins

Super-resolution microscopy

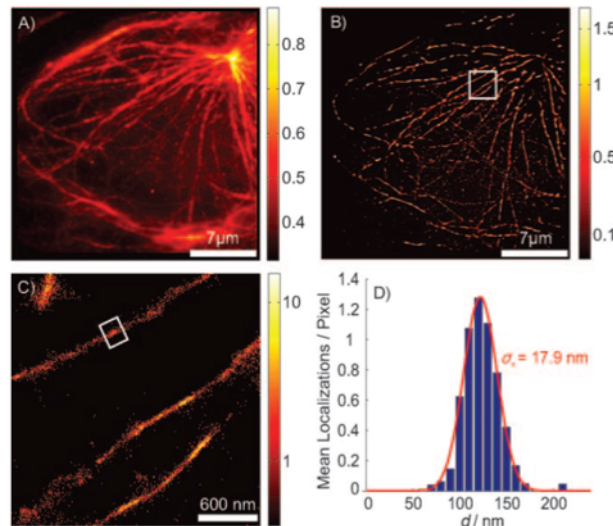
- SNAP-tag targeted probes for nanoscopy of biological structures

STED

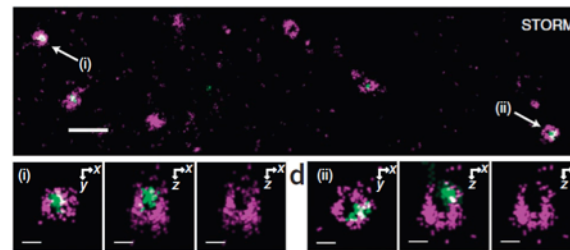


Hein, *Biophys J* 2010, 98, 158

STORM

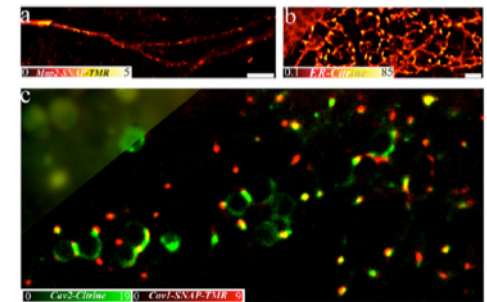


Dellagiacoma, *ChemBioChem* 2010, 11, 1361



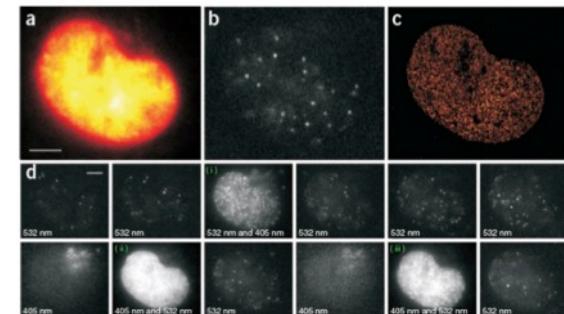
Jones, *Nat. Methods* 2011, 8, 499

GSDIM



Testa, *Biophys J* 2010, 99, 2686

dSTORM



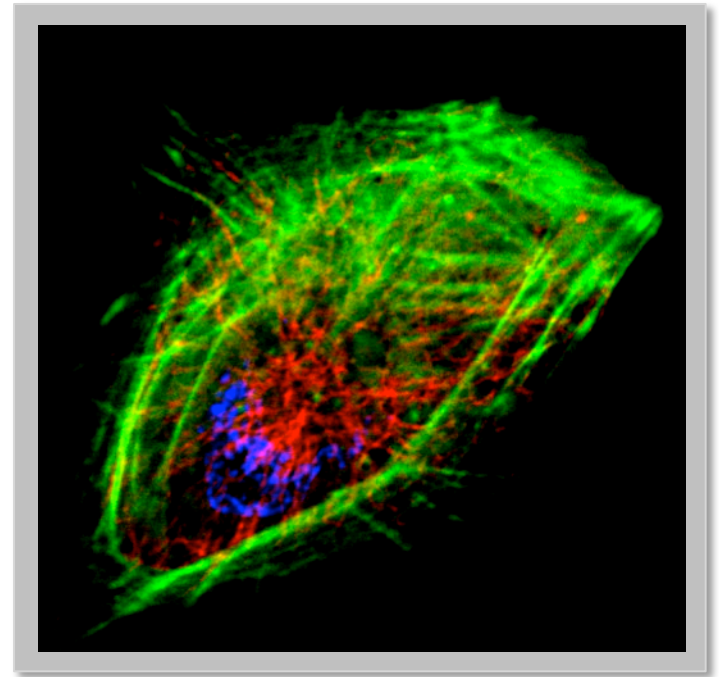
Klein, *Nat. Methods* 2011, 8, 7

Simultaneous Two-Color Live Cell Labeling

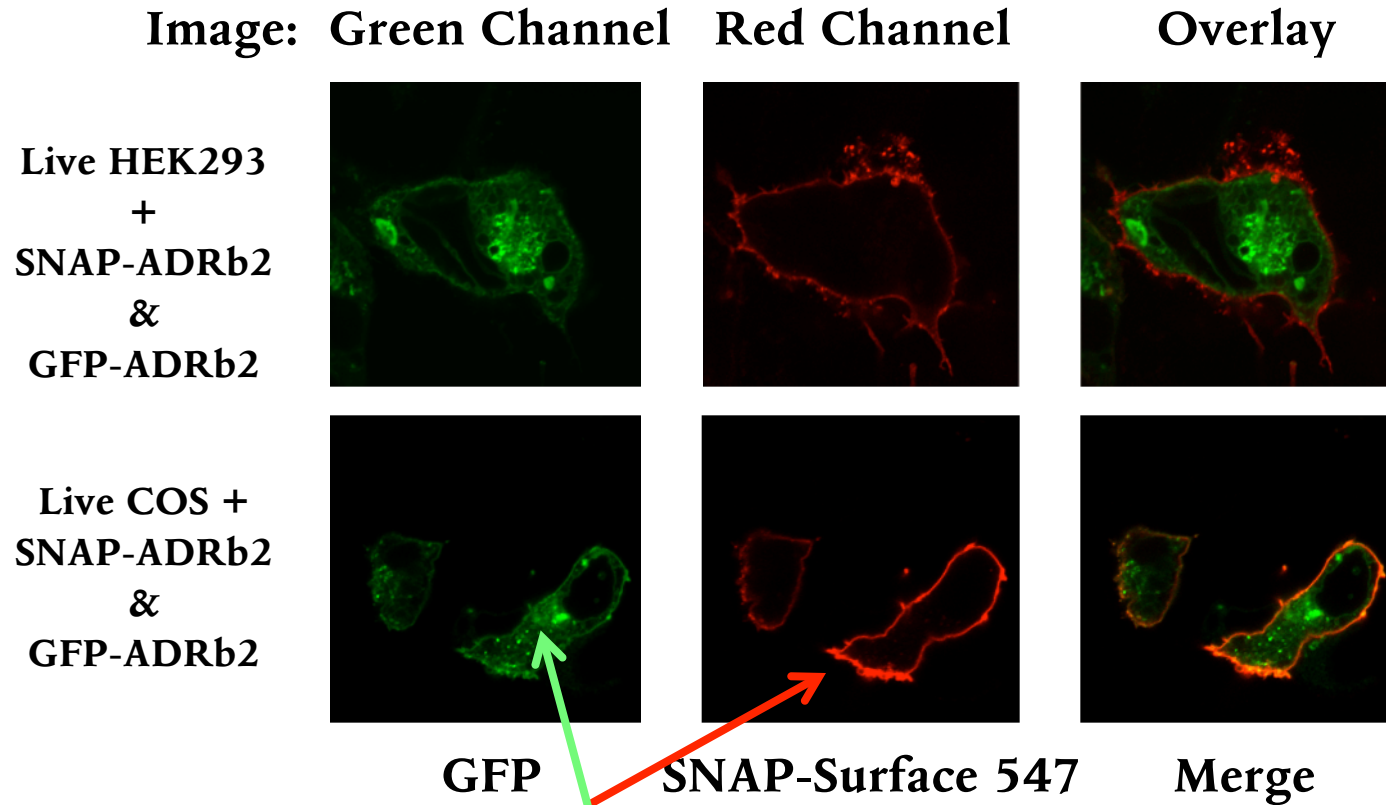
- **No Background Staining** – Exclusively labels fusion tag
- **No Crosstalk** – Highly Specific

Live U20S Cells Expressing SNAP-Actin and CLIP-Tubulin

SNAP-Cell Oregon Green & CLIP-Cell TMR-Star

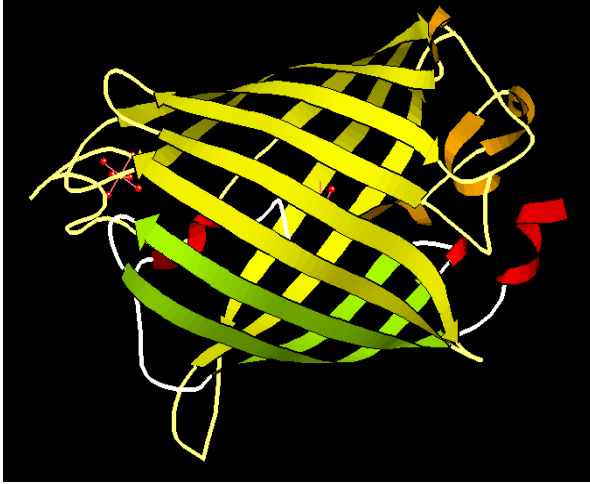


Visualize Proteins Only on the Surface of Live Cells



— SNAP-ADRB2 can be visualized **exclusively** on the surface of cells while GFP-ADRB2 is found near the surface and inside cells

How do SNAP-tag and GFP Differ?



GFP (27kDa)

- Major use is live cell imaging
- Color is genetically encoded
- “Always” fluorescent
- Must re-clone to change color
- Not fluorescent after fixation
- “No” blocking agents available

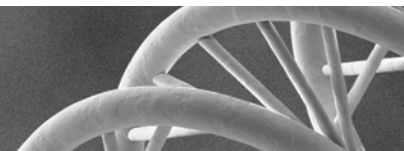


SNAP-tag (20kDa)

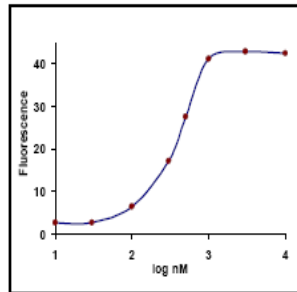
- Multifunctional tagging tool
- Color from added substrate/label
- Fluorescent upon addition of label
- Different substrate = Different color
- Fixation does not affect fluorescence
- Blocking agents available

How Does SNAP-tag Differ from GFP?

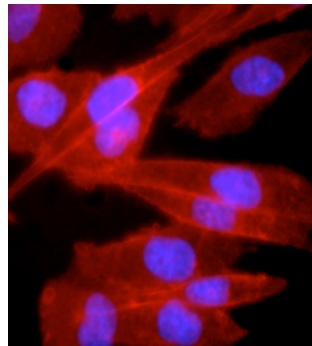
	SNAP-tag Advantages	GFP Characteristics
Mode of Fluorescence	Takes full advantage of a panoply of chemical fluorophores , allowing access to distinct spectra, photostabilities and other enabling characteristics	New “colors” rely on protein engineering; fluorescence generally “biologically” defined
“Time-resolved” labeling	Fluorescence initiated upon addition of label	Color is genetically encoded, protein is “always” expressed and “on”
Ability to change colors	A single construct can be used with different dye substrates to label with multiple colors, including in the near-IR	Requires separate cloning and expression for each color
Surface specific labeling	Can specifically label subpopulation of target protein expressed on cell surface using non-cell permeable substrates	Surface subpopulation cannot be specifically visualized
Visualizing fixed cells	Resistant to fixation ; strong labeling	Labile to fixation; weak labeling
Pulse-chase analysis	Labeling of newly synthesized proteins can be turned off using available blocking reagents (e.g., SNAP-Cell™ Block)	Fluorescence of newly synthesized proteins difficult to quench
Customizable	Readily customizable with “ building blocks ”	Not readily tunable/customizable



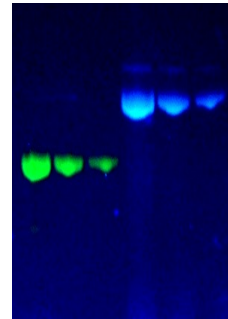
Many Applications, One Construct



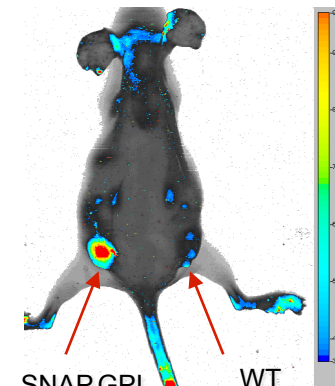
in vitro and live cell
“biochemical” assays



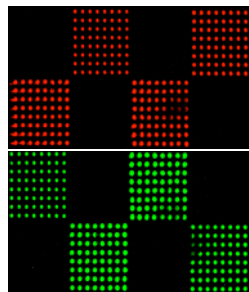
Labeling in cells



Labeling in gels

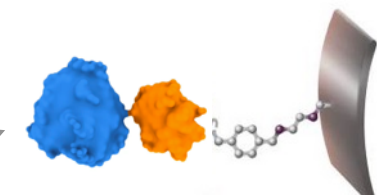


Live animal imaging



Immobilization

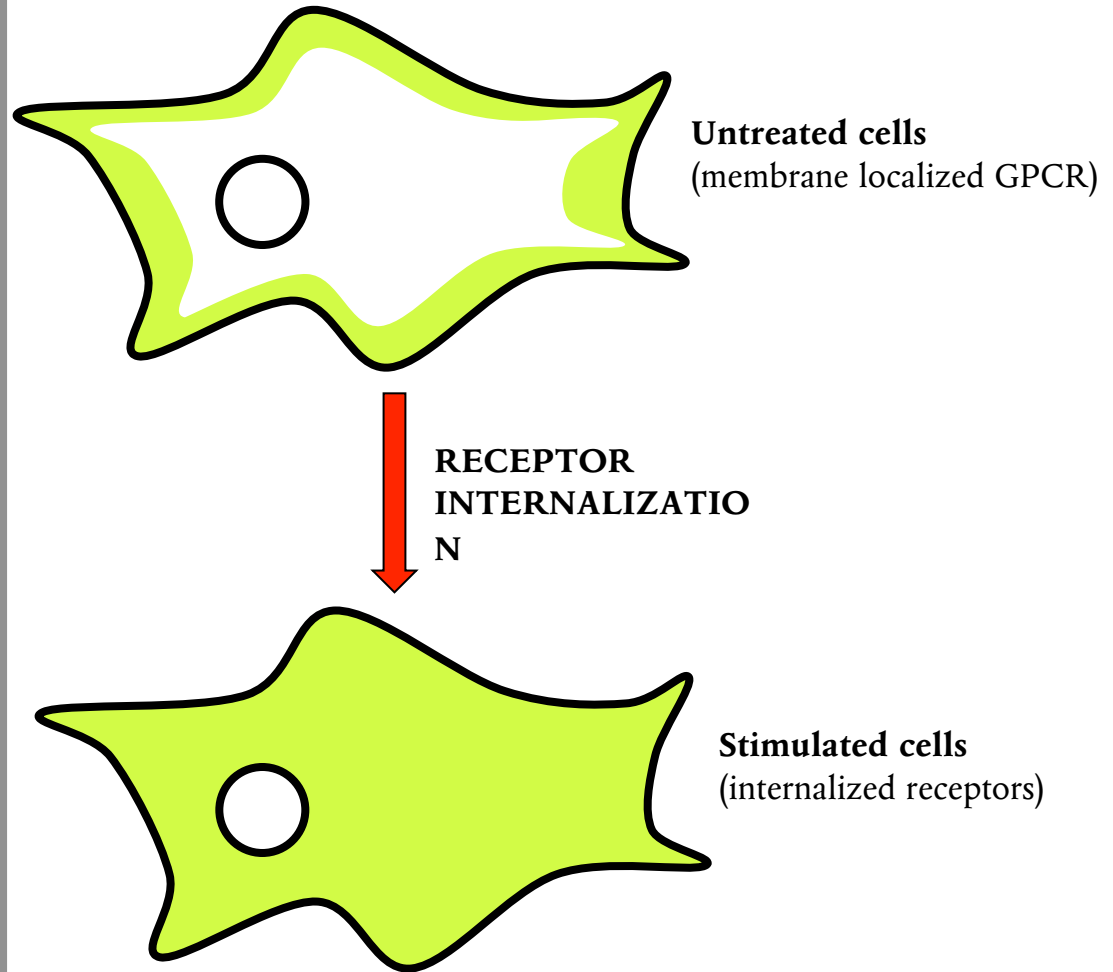
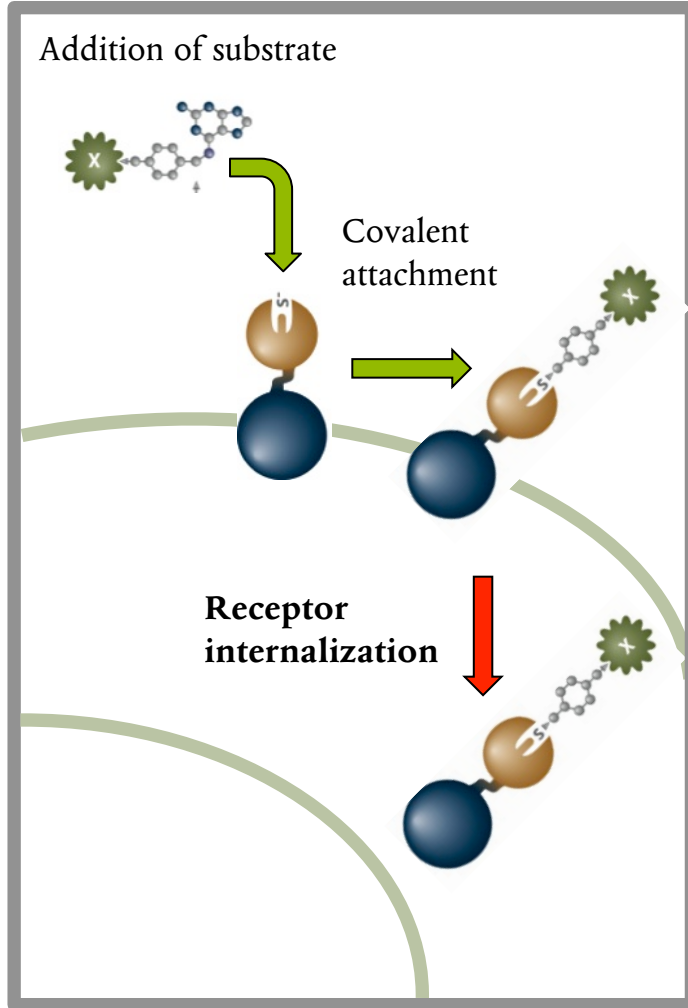
SNAP-tag
Technologies



Protein pull-downs

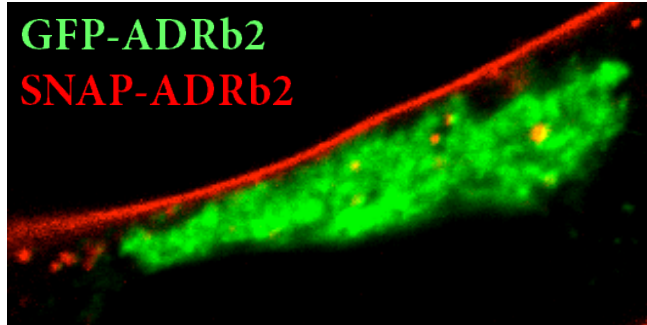
Labeling on the Surface of Cells

– Receptor Internalization

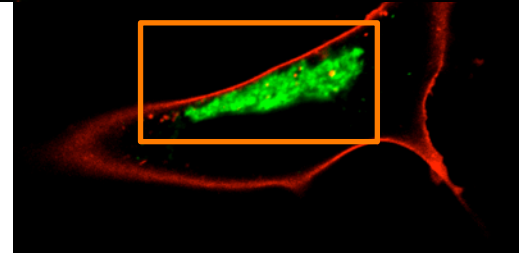


Label Proteins Exclusively on the Surface of Cells

5 minutes after labeling

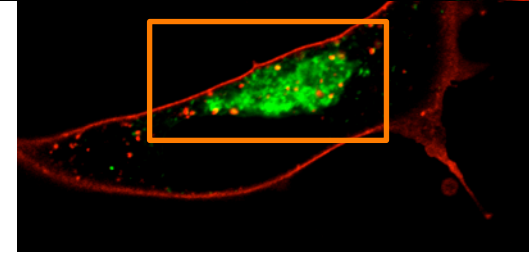
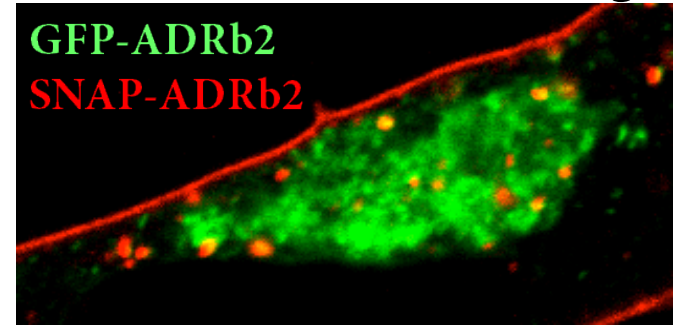


Live HEK293
+
SNAP-ADRB2
&
GFP-ADRB2




Few receptor complexes
inside the cell

25 minutes after labeling



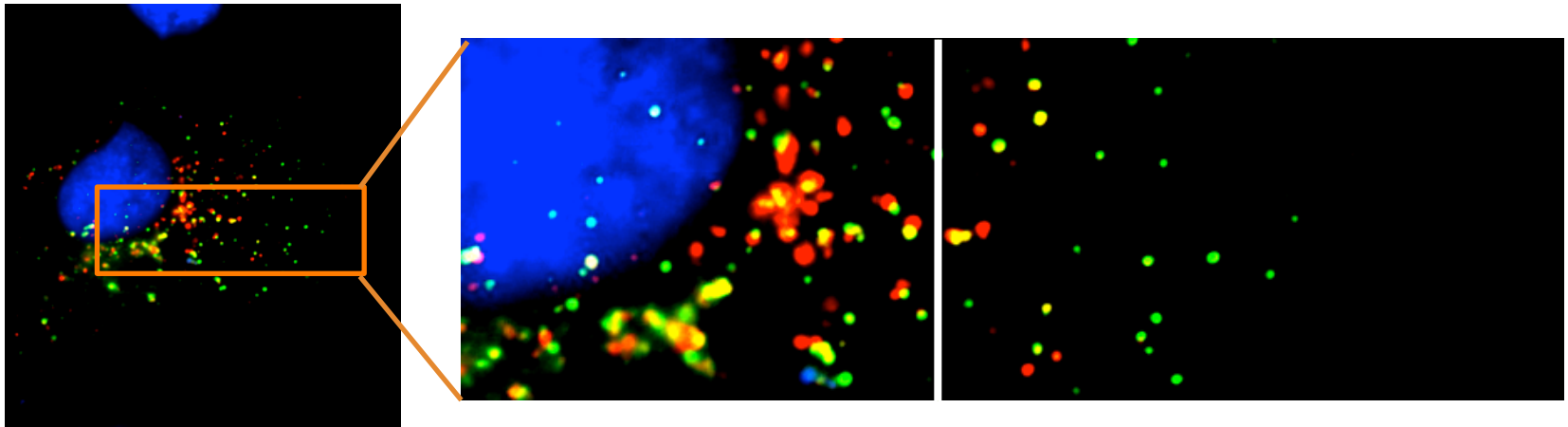
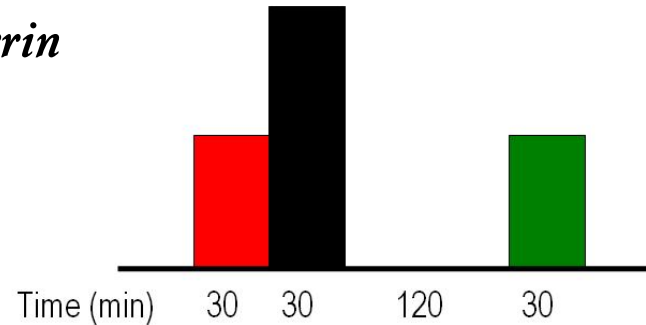
Increase in internalized
receptor complexes

 *HEK293 cells expressing SNAP-ADRB2 and GFP-ADRB2 treated with SNAP-Surface 549. SNAP-ADRB2 labeled with SNAP-Surface 549 becomes readily internalized.*

Pulse-Chase Receptor Internalization Application

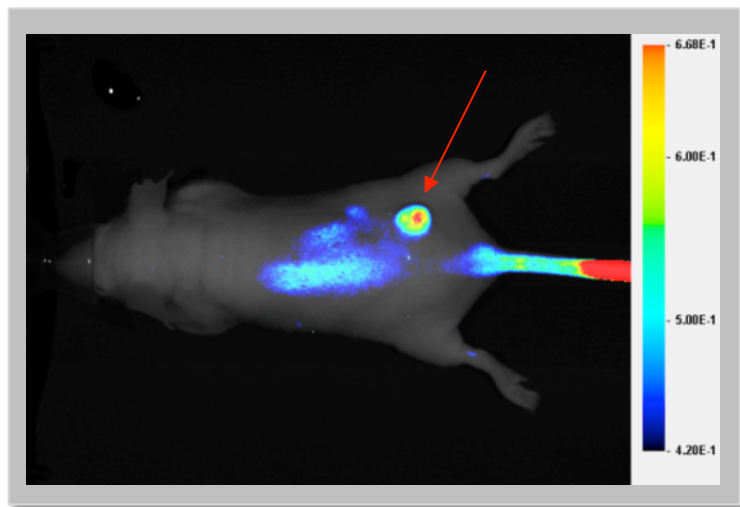
Pulse-chase using SNAP-Transferrin

1. SNAP-Surface 547
2. SNAP-Surface Block
3. SNAP-Surface 488



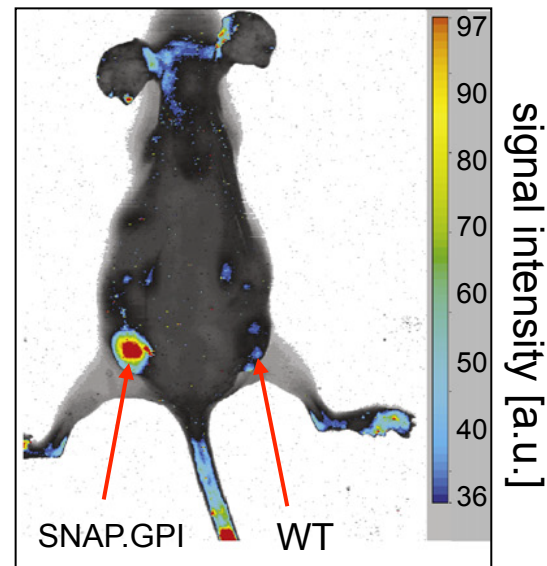
—(Live-cell labeling and imaging of COS-7 cells transiently expressing SNAP-Transferrin receptor (TfR). Differences in the localization of internalized SNAP-TfR can be monitored in **real time**.

Imaging in Small Animal Models



24h mouse image of SNAP-EGF-800CW: A431 tumor cells were implanted subcutaneously on the right flank of a nu/nu mouse and grown to 0.5 cm. Imaging was performed with a Pearl Imager at LI-COR Biosciences at 24 hours post injection of **IRDye800CW-SNAP-EGF**.

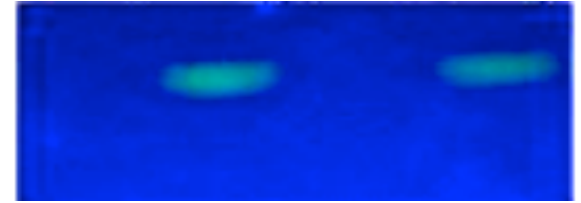
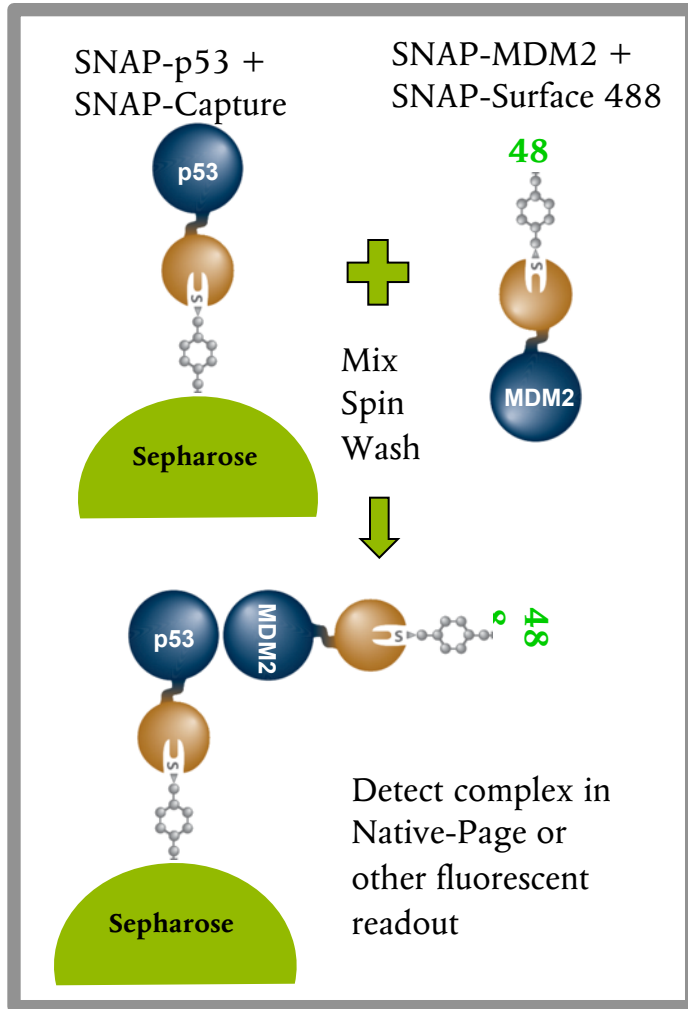
(Collaboration between NEB and LI-COR)



in vivo imaging of a nude mouse bearing TSA SNAP-GPI (left) and WT (right) tumors 24 hr after an intravenous injection of SNAP-Surface 782.

(Bojkowska et al., *Chemistry & Biology* **2011**, 18, 805–815)

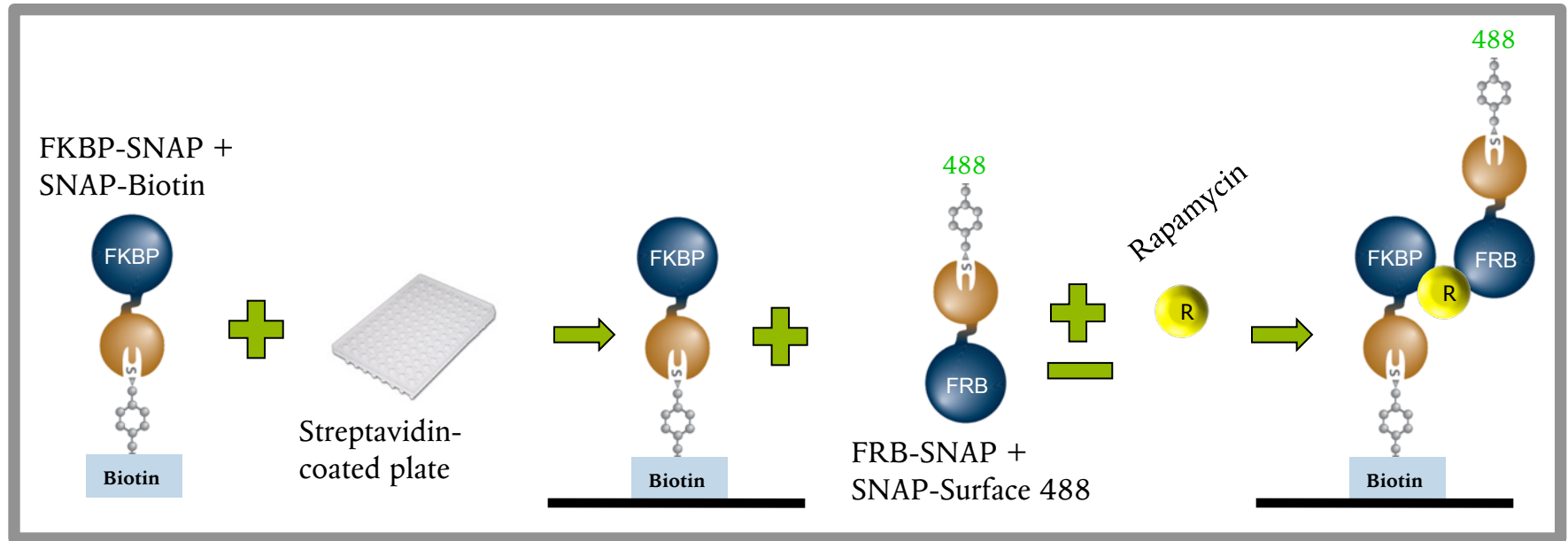
Protein Pull-downs Using SNAP-Capture Products



SNAP-Cell Block	<div>+ -</div>		<div>+ -</div>	
	MDM2		MDM2	
	(Lysate)		(Purified)	

Use of SNAP-p53 conjugated to sepharose beads (**SNAP-Capture Pull Down Beads**) to pull down SNAP-MDM2 labeled with **SNAP-Surface 488** from a lysate or cocktail of purified proteins.

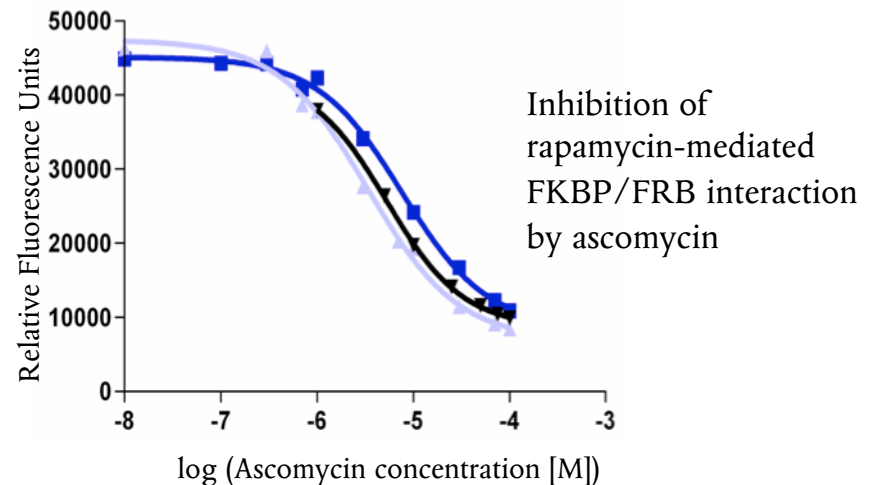
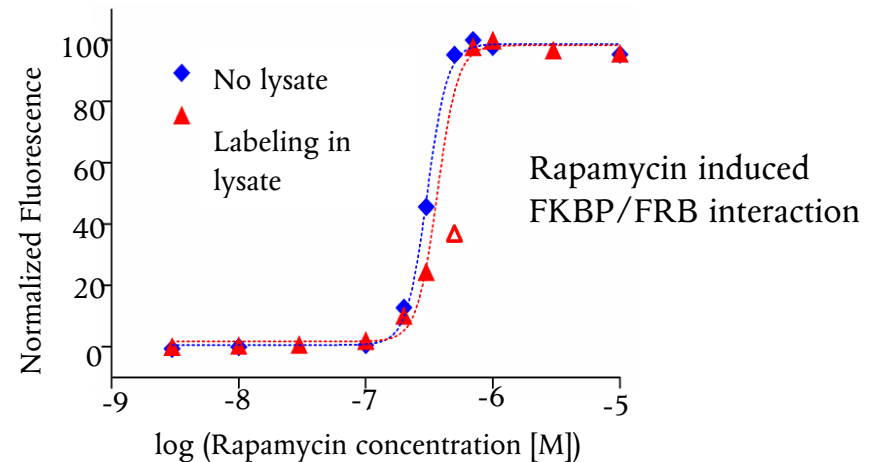
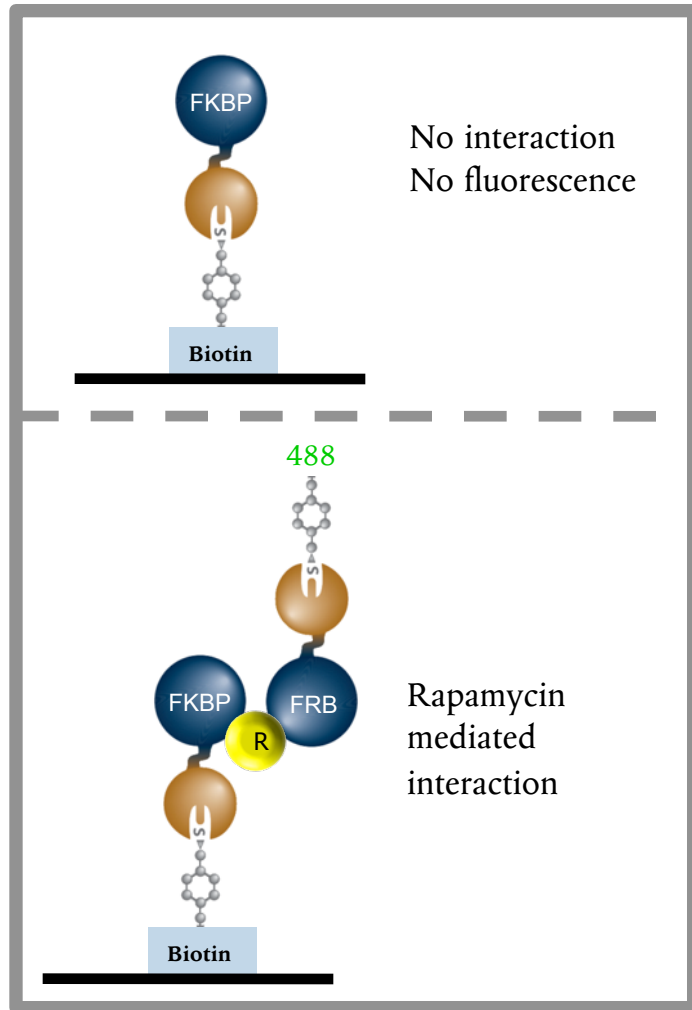
HTS Binding Assays Using SNAP-tag Technologies

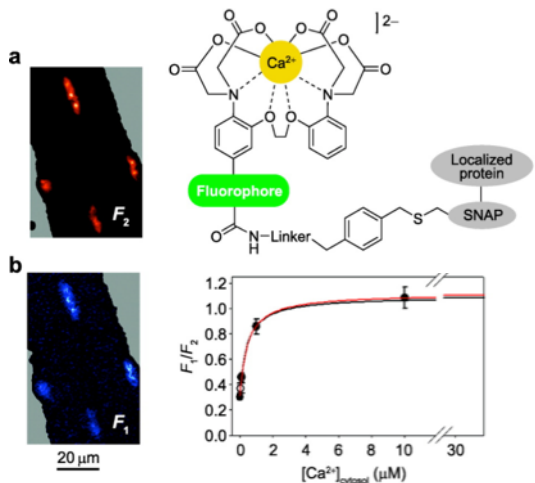


High throughput plate based biochemical assay to study the kinetics of **rapamycin** regulated interaction between **SNAP-FKBP** and **SNAP-FRB**

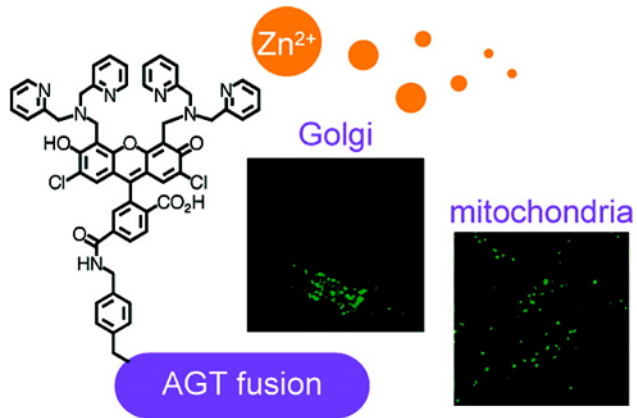
*FKBP, or FK506 binding protein, is a family of proteins that have prolyl isomerase activity and are related to the cyclophilins in function, though not in amino acid sequence.
FK506-rapamycin binding protein (FRB)*

HTS Binding Assays Using SNAP-tag Technologies

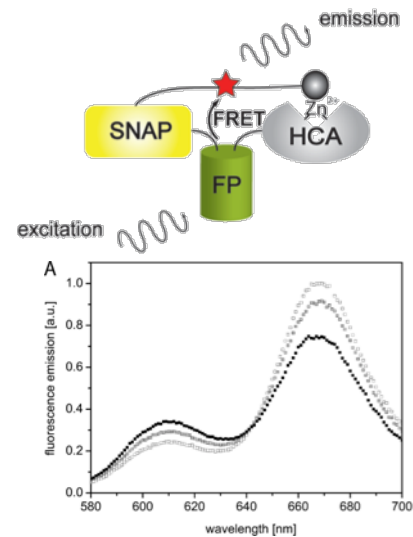




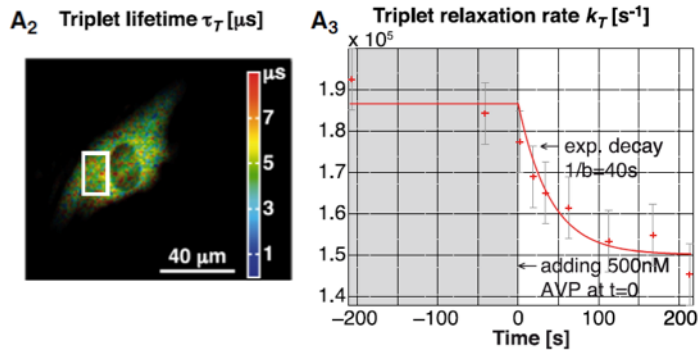
Bannwarth, *ACS Chem Biol* 2009, 4, 179



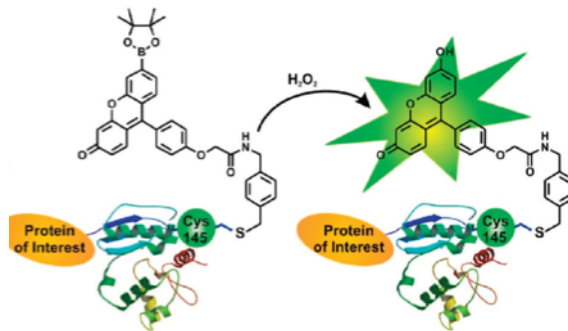
Tomat, *JACS* 2008, 130, 15776



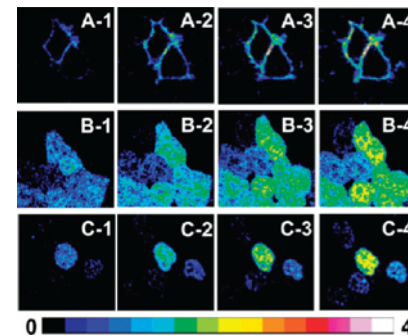
Brun, *JACS* 2009, 131, 5873



Geissbuehler, *Biophys J* 2010, 98, 339-349

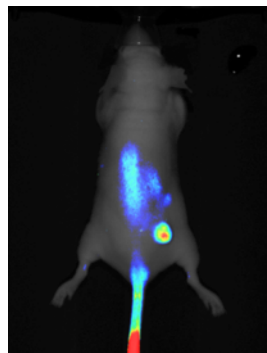


Srikun, *JACS* 2010, 132, 4455



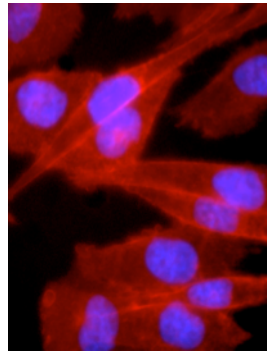
In Conclusion...

- **Enabling** – unique with advantages vs. other technologies
- **Multiplex** – 4 tags available with a wide range of labels
- **Seamless** – Move between living and fixed cells (and cell lysates)



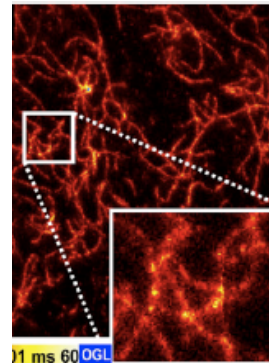
Whole Animals

cm



Living & Fixed Cells

μm



Super-Resolution



Single Molecule

nm

Acknowledgements

Chris Provost
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Anastasiya Masharina

Elissa Maunus

John Buswell
Brenda Baker

Jack Benner
Casey Swaim
Shelley Cushing

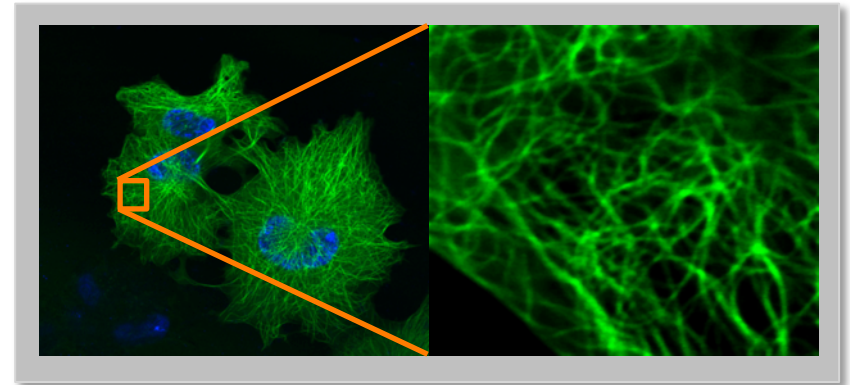
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Salvatore Russello
Ted Davis

Bill Jack
Rich Roberts

Jim Ellard
Don Comb
(all from NEB Inc.)

Kai Johnsson,
Ecole Polytechnique
Fédérale de Lausanne



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