Physical Exam for the Cell

Class	Subclass	Probe Name	Biosensing mechanism	Reference
Structure				
Nuclear				
	Envelope			
	Nucleoli			
	Nuclear bodies			
		eGFP C2-sp100	Sp100 is a marker of the PML nuclear bodies	
		eGFP-PML	PML is a marker of the PML nuclear bodies.	
	Chromatin			
ER				
	Endoplasmic reticulum			
		ER-mGFP A206K	“Inert” ER localized protein (crowdedness) with no known interaction partners, predicted to rapidly sample the ER lumen.	Snapp et al., PNAS, 2006
		ER-RFP	“Inert” ER localized protein (crowdedness) with no known interaction partners, predicted to rapidly sample the ER lumen.	Snapp et al., PNAS, 2006
		ER-sfGFP	Erik Snapp says that this version has a dimmer signal, but it is a better protein and folds correctly. On 10/16, he said that he would have this construct to send "in a few weeks."	
		Calreticulin-GFP	Lectin chaperone calreticulin, which is sensitive to acute changes in subsrate load	Snapp et al., PNAS, 2006
		pEYFP-ER	The endoplasmic reticulum (ER) targeting sequence of calreticulin (1), which is cloned at the 5' end; and the sequence encoding the ER retrieval sequence, KDEL (2, 3), which is cloned at the 3' end.	
		sec24-YFP and sec24 mCherry	Component of COPII coat that labels ER exit sites	Horton et al., J Neurosci, 2003
	Smooth ER			
	Rough ER			
Golgi				
	Golgi			

		blue Golgi marker	Erik Snapp, on 10/16, said he will have blue and green Golgi complex markers to distribute "in a few weeks."	
		green Golgi marker	Erik Snapp, on 10/16, said he will have blue and green Golgi complex markers to distribute "in a few weeks."	
		RFP-Golgi		
		EYFP-Golgi	pEYFP-Golgi encodes a fusion protein consisting of enhanced yellow fluorescent protein (EYFP) and a sequence encoding the N-terminal 81 amino acids of human beta 1,4-galactosyltransferase (GT; 1).	
	Trans Golgi			
		CFP-Galtase	Galtase is a trans-Golgi marker and is found in dendrites	Horton et al., J Neurosci, 2003
		ER-Golgi	VSVG-GFP misfolded and retained in ER at 39.5C. At 32C folds correctly and exits ER and traffics to Golgi. Horton et al., 2003 J Neurosci	
Endosomes				
		Dsred-Rab7	Implicated in transport from early to late endosomes	
		pmCherryC1-Rab5	Involved in early endosome fusion, may label endocytic vesicle fusion	
Lysosomes				
		GFP-Lamp1	Lysosomal marker	
		Lamp1-RFP	Lysosomal marker	
Peroxisomes				
		DD-EGFP-PTS1	This is a Shield1 based system that depends on stabilizing the construct in the cytosol with Shield until enough can be imported into peroxisomes to visualize them. Then Shield1 is removed and the protein left outside peroxisomes but inside the cytoplasm is rapidly degraded	Noguchi et al., Genes Cells, 2013
Mitochondria				
		GFP mito		
		mRFP-mito		
		mitoDendra2		
		Eos2 mito		
		mito mKeima pIND (SP1)	Keima is a coral-derived acid-stable fluorescent protein that emits different-colored signals at acidic and neutral pHs.	Katayama et al., Chem Biol, 2011
Cytoskeleton				

	(+) ends of microtubules			
		EB3-GFP	Clever image analysis approach to being able with two images to deduce 4 features of MT dynamics. Might be more broadly applicable (e.g., synapses, myelination, etc…)	Garrison et al., PLoS ONE, 2012
		Actin GFP	Actin fused to GFP to visualize synaptic remodeling	Colicos et al., Cell, 2001
		GFP Actin	beta-actin promoter, GFP fused to gamma cyto	
		CFP-Actin	CFP-actin from Y. Goda.	
		GFP-MAP2	beta-actin promoter, GFP fused to MAP2c	
Ribosomes				
Plasma membrane				
Spines (neurons)				
		Synapsin	Cherry-synaptophysin, BPF2 synaptophysin	
		PSD95-GFP		
		PSD-95	Sensitive measure of X-ray damage	Shirai et al., Radiat Res, 2013
Axons				
		NFH-GFP	C-terminal tail of neurofilament H deleted and the remaining piece (rod, head and tail) fused to GFP	LeTournel et al., Neurosci, 2006
Function				
Electrical Activity				
	Membrane potential/action potential firing			
		ElectricPk – an order of magnitude faster (t ~1-2 ms) than any currently published FP-based voltage probe. Linear response to hyperpolarizing and depolarizing steps. Signal size sucks, however (-0.7% DF/F)	Voltage-sensitive portion (S1-S4) of *Ciona intestinalis* voltage sensitive phosphatase (CiVSP) fused to circular permuted eGFP	Barnett et al., PLoS ONE, 2012

		pN1-R-CaMP1.07	Though a Ca-sensitive protein, it reportedly can linearly follow action potential firing up to 50 Hz.	Ohkura et al., PLoS ONE, 2012
		GCaMP6s & GCaMP6f (pGP-CMV-GCaMP)	Reportedly the best Ca sensor yet with temporal responsiveness sufficient to record action potential spiking.	Chen et al., Nature, 2013
	Synaptic activity			
	Glutamate release/uptake/synaptic spillover	SuperGluSnFr	GltI domain inserted between Citrine and ECFP. Increased FRET upon glutamate binding when expressed on the cell surface	Hires et al., PNAS, 2008
Gene expression				
	DNA methylation			
		mCpG-SEER	Basically, it uses a split GFP approach with one half fused to a sequence-specific DNA binding domain and another with a methylation binding domain. If there is local DNA methylation around the DNA binding site, fluorescence occurs	Stains et al., JACS, 2006
	Transcription			
		GRE-GFP	Glucocorticoid receptor response element driven GFP	Voss et al., Mol Endocrinol, 2006
	mRNA stability			
		GFP-IL3 ARE, deltaARE and TNFa ARE	A reporter that places GFP under the control of the IL3 promoter and places an ARE from the 3'UTR of IL3 in the GFP 3' region. The ARE is an AU-rich sequence that mediates RNA destabilization such that signaling to that ARE lead to lower levels of GFP. The ARE is found in a number of mRNAs of cytokines	Benjamin et al., Mol Biosystems, 2006
	mRNA trafficking			
		MS2-GFP		
	Splicing			
		RG6	Kind of a cool plasmid design that expresses either GFP or dsRED depending on the splicing event	Orengo et al., NAR, 2006
			This group described a coupled system to amplify the signal which is often low in splicing type assays. It basically involved a splicing reporter that generated a powerful transcriptional activator (VP16) co-transfected with a UAS reporter system for amplification	Levinson et al., RNA, 2006
			FGFR2 exon IIIb splicing	Bonano et al., RNA, 2006
	Translation			

		Main, alt (1-4), TTR, None reporter plasmids	Plasmids designed to detect alternative translation initiation sites. Unfortunately, the reporter they describe is for a yeast gene, but the approach could be adapted to create a mammalian reporter gene.	Ben-Yehezkel et al., Genomics, 2013
		D2EGFP	They just used a destabilized version of GFP to measure the effects of shiga toxin on protein synthesis inhibition. Effectively, its like a pulse chase	Quinones et al., App Environ Microbiol, 2009
Secretion				
Proteostasis				
	UPS -- proteasome			
		FP-CL	Fluorescent protein coupled to CL, a degron that is targeted to proteasome	
		Dendra2-CL	Dendra2 fused to CL	
		Ub-FP	N-terminal fusion of Ub to GFP (Ub-R)	
		ODC-Venus	Ornithine decarboxylase – Venus (Ub-independent proteasome degradation)	
		Ub-G76V-GFP	N-terminal fusion of a non-cleavable Ub to GFP (Ub-G76V)	
		Ub-G76V-Dendra2	N-terminal fusion of a non-cleavable Ub to Dendra2 (Ub-G76V)	
	UPS -- ubiquitylation			
		pEGFP-C1 NEMO UBAN and pcDNA3-GFP-TAB2 NZF	Live cell sensors that bind ubiquitinated proteins in a linkage-specific manner, capable of distinguishing linear (M1 or head-to-tail linked Ubs) from K63-polyUb changes	Van Wijk et al., Nat Protocols, 2013
		Vx3(A7)-EGFP / Vx3K0-EGFP (specific for lys63-Ub4 linkages) NLS-Vx3-EGFP Vx3NB-EGFP – (binding deficient control)	Good for studying Lys63 linkages dynamically. The paper shows the process during mitophagy and the NLS construct was used to track DSBs.	Sims et al., Nat Methods, 2012
	UPR			
		ERAI		Iwawaki and Akai, 2006

	Autophagy			
		Initiation foci	WIPI	
			FP3	
	Lysosomes			
	Conformation sensor		In cell fluorine NMR	
			Unnatural amino acid incorporation	
	iPOD			
	/JNK (Hsp42)			
	Hsp90	GR binding / Src		
	Hsp70	?	A FRET-based reporter that might sense a conformational switch upon binding the Hsp	
		HSP70_GFP	A reporter gene that places GFP expression under the Hsp70 promoter	
	Dumb chaperone	Binding non-native sites	Bilirubin	
	Small Hsp	N-terminus	May bind	
	GroEL			
Proteolysis				
	Caspase 3,7 proteolysis			
		pCAGFP (GFP-DEVD-i)	This construct contains a consensus caspase cleavage site (DEVD) fused to a hydrophobic 27 amino acid peptide on the C-terminus that inhibits the maturation of the chromophore unless its cleaved off	Wu et al., Biophysical J, 2013
		pT-CAGFP-IRES-mLumin	Seems to be similar to above, but in an IRES construct with mLumin	-
	Caspase 6 proteolysis			
		C6A-GFPe in PBB75 (GFP-VEIDG-i)	Similar mechanism, but a different linker to confer specificity for Caspase 6. ****bacterial expression only**	Wu et al., Biophysical J, 2013
	Caspase 3 proteolysis			
		CaspeR3	TagGFP and TagRFP linked by a linker with a caspase-3 consensus cut site. Caspase-3 cleavage results in loss of FRET but preservation of donor and acceptor signals	Shcherbo et al., BMC 2009

Signal transduction				
	Calcium			
		GCAMP3		
		GECO		
		R-GECO		
	CaMKII			
		CaMKII-GFP	Translocation to puncta	
	PKD			
		Ca-dependent PKD1 reporter	FRET	Kunkel et al., JBC 2007
	IP3 Signaling			
		FIRE – Type I, II, III	CFP/YFP FRET with ligand binding regions of each receptor in between	Remus et al., JBC, 2006
	cAMP			
				Nikolaev et al., J Biol Chem, 2004
				Tian et al., J Biomol Screen, 2012
	NFKb			
		RelA-EGFP & ILbeta GFP	RelAGFP is a fusion protein whose translocation can be traced upon activation. ILbeta is a reporter gene with GFP under the control of ILbeta activation pathways	
	Toxin-induced			
		Cyp1a or Cyp1b reporter genes (promoter of Cyp driving expression of an FP)	AhR is a ligand-activated nuclear transcription factor that responds to exogenous ligands that are known toxins such as dioxin (TCDD), benzo[a]pyrene (B[a]P), 3-methylcholanthrene (3-MC) and b-napthoflvone (b-NF). Ligand-bound AhR translocates to the nucleus, binds to the AhR nuclear translocator (Arnt) and then associates with dioxin-response elements (DREs) of TCDD-responsive genes, one of which is cyp1a, a gene that encodes P4501A, a xenobiotic metabolizing enzyme.	Kim et al., Aquatic Toxicology, 2013

	Rac activation; cell migration			-
		FRET pair – Arf6-CyPET & YPET-GGA3	FRET upon Arf activation	Hall et al., Anal Biochem, 2008
	Ras activation			
		GFP RBD 3 R59A N64D	Oligomerized Ras binding domain	Augsten et al., EMBO, 2005
	Foxo3a activation			
		GFP-Foxo3a nuclear translocation		
	Calcineurin activation			
		CaNAR1	FRET based reporter based on NFAT phosphorylation/dephosphorylation	Newman and Zhang, Mol Biosystems, 2008
	Farnesylation, geranylation			
		GFP-tagged GTPases (H-Ras, K-Ras, Rac1, and Rab5)	Lipidation changes the subcellular localization of GTPase	Keller et al., Methods, 2005
	Palmitoylation			
		GAP43-YFP	Translocation reporter	Mikic et al., Methods Enzymol, 2006
Receptor trafficking/clustering				
		FCS on GFP-tagged EGFRs	Kind of a clever assay for EGFR oligomerization as well as an assay for the cholesterol content of membranes (which can be used to sense cholesterol depletion with cyclodextrin) because EGFR partitions into cholesterol rich membranes.	Saffarian et al., Biophys J, 2007
Axonal/microtubule-based trafficking				
		NFH-GFP	See details above under axonal structure	

Cell proliferation				
Bioenergetics				
	ATP metabolism			
		Various sensors (only in vitro)	Sensors were described for ATP, ADP and P. Unfortunately, they were only demonstrated in vitro	Webb, Mol Biosystems, 2007
	ROS			
		roGFP	A redox sensitive GFP, ratiometric to allow for correction of bleaching	
		roGFP-p47^{phox} (p47-roGFP)	Redox sensitive protein to assess NADPH oxidase (Nox) activity. Nox activity has been associated with ROS-mediated damage and found in phagocytes and cardiac myocytes)	Pal et al., PLoS ONE, 2013
		Mito-roGFP	Mitochondrially targeted ROS sensor	
		IMM-roGFP	IMM-targeted ROS sensor	
		ARE-FP	The antioxidant response element driving the expression of a fluorescent protein of choice. ARE is the consensus binding site for Nrf2, a transcription factor that senses a variety of cell stresses, especially ROS and protein misfolding.	
		MERO-GFP	Redox-sensitive ratiometric GFP targeted to mammalian ER. Emission taken at 510nm. The ratio of emission from excitation at 488nm versus 405nm is a read out of redox state of ER.	Kanekura et al., Laboratory Investigation, 2013
Cell type				
	Smooth muscle/osteoprogenitor cells			
		Alpha-smooth muscle actin GFP		Kalajzic et al., Bone, 2008
Miscellaneous				
	Transfection marker			
		p1xEGFP, p3xEGFP, p6xEGFP, p1xVenus, p3xVenus	They showed that multimerizing the FPS pretty much generated a proportional amount of signal. It raises some interesting possibilities for both short and long wavelength indicators.	Genové et al., Biotechniques, 2005

		Flavin reporter proteins (e.g., iLOV – [monomer - QY:0.34], PbFbFP – [dimer - QY:0.17], EcFbFP – [dimer - QY:0.34]). 450 nm excitation -> 495 nm emission	Small size (100-140 aa – almost half the size of GFP - 12 kD) and oxygen independent maturation	Mukherjee et al., PLoS ONE, 2013
		SNAP tags based on ATTO or HALO tags	Even smaller and 16-20 fold brighter than flavin-based tags. But cytotoxicity and permeability can be an issue.	
		A clever multiplexing strategy that involves rationing different wavelengths and would be feasible with standard optical filters and a CCD but would also be compatible with spectral detection.		Krylova et al., PLoS ONE, 2013