

Electronic supplementary information for

Strategies for organelle targeting of fluorescent probes

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Nucleus

Table S1: Targeting groups for genetically expressed fluorescent cargo to the nucleus

Structure of targeting group	Class of targeting group	Mechanism	Cargo / Examples	Selectivity
PKKKRKV, NLS from SV40 T antigen	Nuclear localisation sequence (NLS)	Signal peptide is recognised by nuclear import proteins (importins).	Fluorescent proteins, including GFP, ¹ RFP, ² CFP. ³	Selective for nucleus, also used in commercial products
Histone 2B	Protein	Cargo is attached to native nucleus-targeted protein.	Fluorescent proteins, including GFP, ⁴ RFP, ⁵ GFP 11. ⁶	Selective for nucleus, also used in commercial products
Hoescht	Minor groove binder	Localisation of eDHFR-GFP can be altered after subsequent binding to a Hoechst labelled trimethoprim (hoeTMP).	Genetically expressed eDHFR-GFP. ⁷	Selective for nucleus.

Table S2: Targeting groups for exogenous fluorescent cargo to the nucleus

Structure of targeting group	Class of targeting group	Mechanism	Cargo / Examples	Selectivity
Hoescht	Minor groove binder	Binds to double-stranded DNA	Small molecule fluorophores <i>e.g.</i> sulforhodamine, ⁸ various xanthenes, ⁹ rhodamine, ¹⁰ fluorescein, ¹⁰ BODIPY. ¹⁰	Selective for nucleus
(pyridin-4-yl)piperazine	Minor groove binder	Hoechst mimic. Binds to double-stranded DNA.	Coumarin. ¹¹	Selective for nucleus in red channel. Staining of LDs was seen in blue channel due to ratiometric emission of the probe.
Pyrrole polyamide	Minor groove binder	Binds to double-stranded DNA	Fluorescein. ¹²	Nucleus strongly stained; some spots outside nucleus also stained.
KKKRK (SV40 T antigen)	NLS	Signal peptide is recognised by nuclear import proteins (importins).	BODIPY. ¹³	Plasma membrane must be punctured for the molecule to reach the nucleus.
VQRKRQKLMP (NLS50)	NLS	Signal peptide is recognised by nuclear import proteins (importins).	Carboxyfluorescein, ¹⁴ naphthalimide. ¹⁵	Generally selective for nucleus.
YKQCHKKGGK KGSG	Nucleolus targeting peptide NrTP	Unknown, although clathrin mediated uptake is involved.	Carboxyfluorescein. ¹⁴	Generally selective for nucleolus.
R ₈	NLS peptide mimic	Positively charged peptides are likely to be recognized by importins.	PyTPE. ¹⁶	Generally selective for nucleus.
RRRRKR	NLS peptide mimic	Positively charged peptides are likely to be recognized by importins.	PyTPE. ¹⁷	Generally selective for nucleus.
polyethylenimine	Multivalent polyamine	Multivalent polyamines bind to and neutralise the negatively charged DNA backbone.	FITC. ¹⁸	Nuclear localisation and weak cytoplasmic localisation in digitonin permeabilised cells

Mitochondria

Table S3: Targeting groups for endogenous fluorescent cargo to the mitochondria

Structure of targeting group	Class of targeting group	Mechanism	Cargo / Examples	Selectivity
N-terminal 12 aa of pre-sequence of COX IV	MTS	Pre-sequence interacts with mitochondrial import receptors and is cleaved after import into mitochondria.	Fluorescent proteins, including ECFP, EYFP. ¹⁹	Selective for mitochondria
Pre-sequence of COX VIII	MTS	Pre-sequence interacts with mitochondrial import receptors and is cleaved after import into mitochondria.	GFP. ²⁰	Selective for mitochondria
Mff	Protein	Cargo is attached to Mff, a native mitochondrial outer membrane protein	GFP. ²¹	Selective for mitochondria

COX = cytochrome c oxidase, Mff = Mitochondrial fission factor, MTS = mitochondrial targeting sequence.

Table S4: Targeting groups for exogenous fluorescent cargo to the mitochondria

Structure of targeting group	Class of targeting group	Mechanism	Cargo / Examples	Selectivity
Rhodamine	DLC	Passive diffusion and accumulation to negatively charged mitochondrial membrane.	Rhodamine 123. ²²	Selective for mitochondria
Rosamine	DLC	Passive diffusion and accumulation to negatively charged mitochondrial membrane.	MitoTracker Orange, ²³ MitoTracker Red. ^{24, 25}	Selective for mitochondria
Cyanine	DLC	Passive diffusion and accumulation to negatively charged mitochondrial membrane.	MitoTracker Deep Red. ^{25, 26}	Selective for mitochondria
TPP	DLC	Passive diffusion and accumulation to negatively charged mitochondrial membrane.	Small molecule fluorophores including naphthalimide, ²⁷ BODIPY, ²⁸ fluorescein, ²⁹ flavin, ³⁰ etc. Nanomaterials include quantum dots, ³¹ nanoparticles, ³² liposomes ³³ etc.	Selective for mitochondria
F16	DLC	Passive diffusion and accumulation to negatively charged mitochondrial membrane.	BODIPY. ³⁴	Selective for mitochondria
Methyl-substituted TPP	DLC	Passive diffusion and accumulation to negatively charged mitochondrial membrane.	Hexachloro-fluorescein. ³⁵	Selective for mitochondria
CF ₃ -substituted TPP	DLC	Passive diffusion and accumulation to negatively charged mitochondrial membrane.	TAMRA. ³⁶	Selective for mitochondria
SS peptide (4 aa, alternating aromatic/cationic)	MPP	Not fully elucidated, but some variants target cardiolipin. Others via mechanism not involving receptor mediated endocytosis.	[Dmt ¹ ,Dap(atn) ⁴]DALDA, [Dmt ¹ ,Dap(dns) ⁴]DALDA; ³⁷ [aladan]SS-31. ³⁸	Selective for mitochondria, can penetrate inner mitochondrial membrane.
MPP (alternating hydrophobic and cationic)	MPP	Not fully elucidated, but it is possible to target different compartments of the mitochondria.	Thiazole orange, 2-aminobenzoic acid; ³⁹ TAMRA. ⁴⁰	Selective for mitochondria

TPP = triphenylphosphonium, TAMRA = carboxytetramethylrhodamine, F16 = ((E)-4-(1 H-indol-3-ylvinyl)-N-methylpyridinium iodide, SS = Szeto-Schiller, MPP = mitochondrial penetrating peptide, DLC delocalised lipophilic cation.

Lysosome

Table S5: Targeting groups for endogenous fluorescent cargo to the lysosome

Structure of targeting group	Class of targeting group	Mechanism	Cargo / Examples	Selectivity
LAMP1	Protein	Cargo is attached to a native lysosomal membrane protein	Fluorescent proteins, including GFP, ⁴¹ RFP, ⁴² YFP. ⁴³	Generally selective for lysosomes, may be found in other digestive organelles
LAMP2	Protein	Cargo is attached to a native lysosomal membrane protein	GFP, mCherry. ^{44, 45}	Generally selective for lysosomes, may be found in other digestive organelles

LAMP = Lysosome-associated membrane glycoprotein

Table S6: Targeting groups for exogenous fluorescent cargo to the lysosome

Structure of targeting group	Class of targeting group	Mechanism	Cargo / Examples	Selectivity
Morpholines	Tertiary amine	Passive diffusion and protonation in acidic lumen of lysosome.	Naphthalimide (LysoSensor Green). ²⁵	Selective for acidic lysosomes.
Dimethylamine	Tertiary amine	Passive diffusion and protonation in acidic lumen of lysosome.	BODIPY (LysoTracker Red and LysoTracker Green). ²⁵	Selective for acidic lysosomes.
Diethylamine	Tertiary amine	Passive diffusion and protonation in acidic lumen of lysosome.	Rhodamine. ⁴⁶	Selective for acidic lysosomes.
DAMP	Tertiary amine	Passive diffusion and protonation in acidic lumen of lysosome.	Nonfluorescent-activity based probes that later reacted with AlexaFluor 488. ⁴⁷	Selective for acidic lysosomes.
Histamine	Tertiary amine	Passive diffusion and protonation in acidic lumen of lysosome.	BODIPY FL histamine. ²⁵	Selective for acidic lysosomes.
N-linked glycans	Glycosylation	Unknown	Rhodamine spirolactam derivatives. ^{48, 49}	Selective for lysosomes (coloc with LysoTracker)
Methylcarbitol	Neutral	Unknown	Rhodamine spirolactam, ^{50, 51} carbazole-benzimidazole, ⁵² ethanoylcarbazole and terephthalaldehyde, ⁵³ rhodamine fused with tetrahydroquinoxaline. ⁵⁴	Selective for lysosomes.
CPP + epoxysuccinyl	Cathepsin targeting	CPP improves permeability; epoxysuccinyl scaffold forms covalent bond with cysteine cathepsin.	5(6)-carboxyfluorescein, rhodamine B; ⁵⁵ , Alexa Fluor 647, Atto 565, and Atto 488; ⁵⁶ FITC, rhodamine B, photo-activatable rhodamine B derivative. ⁵⁷	Selective for lysosomes.
Albumin / dextran	Macromolecules	Macromolecules cannot diffuse in the cell and are uptaken via endocytosis.	FITC-dextran. ⁵⁸	Tracks progress of endocytosis.

DAMP = (N-(3-((2,4-dinitrophenyl)amino)propyl)-N-(3-aminopropyl)methylamine, dihydrochloride), CPP = cell penetrating peptide, FITC = fluorescein isothiocyanate

Digestive organelles

Table S7: Targeting groups for endogenous fluorescent cargo to endosome related vesicles.

Structure of targeting group	Class of targeting group	Mechanism	Cargo / Examples	Selectivity
Rab5	Protein	Cargo is attached to a native early endosome protein.	GFP, ⁵⁹ RFP. ⁶⁰	Selective for early endosomes.
Rab7	Protein	Cargo is attached to a native late endosome protein.	GFP, ⁶¹ RFP. ⁶²	Generally selective for late endosomes, but also found in other compartments.
Rab11	Protein	Cargo is attached to a native recycling endosome protein.	GFP, ⁶³ EGFP. ⁶⁴	Selective for recycling endosomes.
EEA1	Protein	Cargo is attached to a native early endosome protein.	GFP, photoactivatable GFP. ⁶⁵	Selective for early endosomes.
Transferrin receptor	Protein	Cargo is attached to a native recycling endosome protein.	mCherry. ⁶⁶	Selective for recycling endosomes.
Arf6	Protein	Cargo is attached to a native recycling endosome protein.	GFP. ⁶⁷	Selective for recycling endosomes.

Rab = Ras analog in brain, EEA = early endosome antigen, Arf = ADP-ribosylation factor.

Table S8: Targeting groups for exogenous fluorescent cargo to endosome related vesicles.

Structure of targeting group	Class of targeting group	Mechanism	Cargo / Examples	Selectivity
Weakly acidic benzyl substituted amines	Modified tertiary amine	Passive diffusion and protonation in less acidic lumen of endosomes.	Fluorescent 'flipper' probe. ⁶⁸	Variants with higher pK_a values in the less acidic early endosomes; those with lower pK_a values in more acidic late endosomes
macrocyclic di(cyclo-squaramides).	Endocytosis related	Bind to phosphates on the outer cell membrane and are then internalised through the cell <i>via</i> receptor-mediated endocytosis	BODIPY, fluorescein. ⁶⁹	Selective for late endosomes
naphthylmethyl piperazine	Tertiary amine	pH partitioning; can stain pH flux from late endosome to lysosome	BODIPY. ⁷⁰	Sensitive to flux in pH from late endosomes to the lysosome
DiIC ₁₆ (3)	Lipid stain	Unknown	Silicon-rhodamine. ⁷¹	Selective for late endosomes

Table S9: Targeting groups for endogenous fluorescent cargo to autophagy related vesicles

Structure of targeting group	Class of targeting group	Mechanism	Cargo / Examples	Selectivity
WIPI1/2	Protein	Cargo is attached to an ATG protein, associated before autophagosome closes.	GFP. ⁷²	Selective for open phagophores, useful for differentiating stages of autophagy.
ATG12-ATG5-ATG16	Protein	Cargo is attached to an ATG protein, associated before autophagosome closes.	GFP, mCherry. ⁷³	Selective for phagophores
ULK1	Protein	Cargo is attached to an ATG protein, associated before autophagosome closes.	GFP. ⁷⁴	Selective for phagophores
LC3	Protein	Cargo is attached to an ATG protein, associated with most autophagy stages.	GFP, mRFP-GFP-LC3. ⁷⁵	Selective for most stages of autophagy. Note that GFP is quenched in acidic conditions
STX17	Protein	Cargo is attached to an ATG protein, associated with the closed autophagosome.	GFP, ⁷⁶ Turquoise2. ⁷⁷	Selective for autophagosomes – autolysosomes.

WIPI = WD repeat domain phosphoinositide-interacting protein 1, ATG = autophagy related protein, ULK1 = Unc-51 Like Autophagy Activating Kinase 1, LC3 = Microtubule-associated protein 1A/1B-light chain 3, STX = syntaxin

Table S10: Targeting groups for exogenous fluorescent cargo to autophagy related vesicles

Structure of targeting group	Class of targeting group	Mechanism	Cargo / Examples	Selectivity
Unknown	Cationic amphiphilic tracer	Unknown	Cyto-ID. ⁷⁸	Selective for autophagic compartments.
Piperazine	Amphiphilic detergent	Unknown; alkyl chain with terminal amino may contribute to uptake	DALGreen (naphthalimide). ⁷⁹	Selective for all autophagic compartments.
Aminopentyl	Amphiphilic detergent	Unknown; alkyl chain with terminal amino may contribute to uptake	DAPGreen (naphthalimide). ⁷⁹	pH dependent; stains flux and is more fluorescent in late-stage autophagy
Morpholine	Tertiary amine	Passive diffusion and protonation in acidic lumen of lysosomes. Fluorescence response to pH flux after fusion with autophagosomes.	Coumarin, ⁸⁰ p-methoxyphenylacetylene-substituted carbazole. ⁸¹	Sensitive to autophagic flux from lysosome to autolysosome.

Peroxisomes

Table S11: Targeting groups for endogenous fluorescent cargo to peroxisomes

Structure of targeting group	Class of targeting group	Mechanism	Cargo / Examples	Selectivity
PTS-1 (AKL or SKL)	Peptide	Active transport by peroxin 5.	GFP, ^{82, 83} fluorescent calcium sensor. ^{83, 84}	Selective for peroxisomes (including use in commercial products).

PTS = peroxisome targeting signal

Table S12: Targeting groups for exogenous fluorescent cargo to peroxisomes

Structure of targeting group	Class of targeting group	Mechanism	Cargo / Examples	Selectivity
PTS-1 (C-terminal AKL)	Peptide	Active transport by peroxin 5.	fluorescein, BODIPY, pH-sensor SNAFL-2, C2-BODIPY. ⁸⁵	Selective for peroxisomes

Endoplasmic reticulum

Table S13: Targeting groups for endogenous fluorescent cargo to the endoplasmic reticulum.

Structure of targeting group	Class of targeting group	Mechanism	Cargo / Examples	Selectivity
Upstream prolactin secretory sequence and KDEL retention sequence	Signal peptide and ER retention signal	Signal sequence targets the ER lumen whilst KDEL prevents the protein moving to the Golgi apparatus	GFP. ⁸⁶	Selective for ER
signal sequence of calreticulin and KDEL retention sequence	Signal peptide and ER retention signal	Uses endogenous protein targeting mechanism whilst KDEL prevents the protein moving to the Golgi apparatus	GFP, ⁸⁷ RFP. ⁸⁸	Selective for ER

Table S14: Targeting groups for exogenous fluorescent cargo to the endoplasmic reticulum.

Structure of targeting group	Class of targeting group	Mechanism	Cargo / Examples	Selectivity
Signal peptide (KDEL)	Peptide	The fluorescent peptide passively enters the cell and KDEL retains the protein inside the endoplasmic reticulum	BODIPY. ¹³	Selective to ER
Glibenclamide	Sulfonylurea	Binding to the sulfonylurea receptor in the ER	naphthalimide-based Zn sensor, ⁸⁹ BODIPY (ER-Tracker™ Red). ²⁵	Selective to ER
methyl sulphonamide (i.e. <i>p</i> -toluene-sulfonamide, sulfanilamide)	Sulfonylamide	Analogue of sulfonylurea compounds that likely bind to the K ⁺ channel related proteins which are mainly located in ER	Amino-NBD conjugated to diethylamino coumarin, ⁹⁰ 1,8-naphthalimide conjugated to benzothiazoline, ⁹¹ 4-nitro-1,8-naphthamimide, ⁹² 4-azido-1,8 naphthalimide, ⁹³ 4-methylthio naphthalimide, ⁹⁴ ER-Tracker Blue-White DPX (also has PFP). ²⁵	Selective to ER
Ethylene glycol	Lipophilic chain	Thought to take advantage of tendency of lipophilic or glycosylated compounds to localise in ER.	Naphthalimide ⁹⁵	Similar structure used for other organelles
Alkyl chain linker	Lipophilic chain	Thought to take advantage of tendency of lipophilic or glycosylated compounds to localise in ER	BODIPY-coumarin, ⁹⁶ BODIPY-Nile Red ⁹⁷	Similar structure used for other organelles
Pentafluorophenyl (PFP)	Covalent bond	Reacts with thiols in ER proteins, but often used with other moieties or probe structures that may be more directing to the ER.	ER Tracker Blue White (contains sulfonamide), Nile Red derivative (+ ethylene glycol chain) ⁹⁸ fluorescent flipper (+ ethylene glycol chain) ⁹⁹ , BODIPY. ¹⁰⁰	Often used with other moieties
chlorine (propyl chloride)	Covalent bond	Chlorine is suspected to bind to the chlorine pump in the ER	Fluorene derivative, ¹⁰¹ Nile Red derivative. ⁹⁸	Selective to ER

Fluorinated hydrophobic rhodols	Fluorinated hydrophobic rhodols (inherently fluorescent)	Unknown, suggested that hydrophobic and amphipathic properties allow localisation in cholesterol-poor ER membranes	p-aminophenol; ¹⁰² electrophilic nitrofurans; ¹⁰³ coumarin-3-carboxylic acid; ¹⁰⁴ chloroalkane, sulfonylfluoride, thioester. ¹⁰⁵	Selective to ER
Fluorescent dansyl group	N/A	Targeting mechanism of the dansyl group is not explained, but sulfonamide could play a role. Self-assembled NPs were observed to localize into the ER of HeLa cervical cancer cells after internalisation via macropinocytosis	Nanoparticle. ¹⁰⁶	Some mitochondrial localisation also observed
l-homoarginine	Membrane interaction	Crescent shaped peptide assemblies interact with synthetic lipid membranes. As ER is the largest membranous structure in cells, design allows ER targeting.	Nanoparticle. ¹⁰⁷	Some green fluorescence in HeLa cell membrane
Ceramide	Lipid	Lipid inserts into the membrane of the ER (and Golgi)	Trans-cyclooctene (to undergo tetrazine click reaction with silicon rhodamine). ¹⁰⁸	Also targets the Golgi

Golgi apparatus

Table S15: Targeting groups for endogenous fluorescent cargo to Golgi apparatus

Structure of targeting group	Class of targeting group	Mechanism	Cargo / Examples	Selectivity
N-acetylgalactos-aminyltransferase	Protein	Cargo is attached to a native Golgi enzyme.	GFP, ¹⁰⁹ RFP. ¹¹⁰	Selective for Golgi (used in commercial stains).
B4GALT1	Protein	Cargo is attached to a native Golgi enzyme.	GFP. ¹⁹	Selective for Golgi
SXYQRL	Peptide	Cargo is attached to sequence from native Golgi protein TGN38.	Glycophorin A (confirmed via immunofluorescence)	Selective for Golgi; note that cargo and TGN38 are both integral membrane proteins.

B4GALT1 = Beta-1,4-Galactosyltransferase 1; TGN38 = trans-Golgi network integral membrane protein 38.

Table S16: Targeting groups for exogenous fluorescent cargo to Golgi apparatus

Structure of targeting group	Class of targeting group	Mechanism	Cargo / Examples	Selectivity
Ceramide / sphingosine	Lipid	Stains the Golgi apparatus of fixed and live cells, most likely through interaction(s) with endogenous lipids and cholesterol. Fluorescence emission maxima strongly dependent upon the molar density of the probes in the membrane. (Primary part of sphingolipids that composes the skeleton targeting motif.)	BODIPY, ¹¹¹ 4-nitrobenzo-2-oxa-1,3-diazole, ¹¹² silicon-rhodamine, ¹¹³ rhodamine B. ¹¹⁴	Generally selective staining of Golgi apparatus, though some labeling of other intracellular membranes may also occur.
SDYQRL	Trans golgi network localising peptide	The sequence SXYQRL is a retention signal for proteins residing in the trans-Golgi network	BODIPY. ¹³	Selective staining of Golgi
Indomethacin	Drug	Indomethacin binds to COX-2 which is overexpressed in cancer cell lines and accumulates significantly in the Golgi apparatus.	acenaphtho[1,2-b]quinoxaline. ¹¹⁵	Selective staining of Golgi in cancer cell lines but no staining was observed in COS-7 cells and HEK 293 cells
L-Cysteine	Amino acid	Mimicking cysteine residues in Golgi apparatus localized galactosyltransferase and protein kinase D; while the mechanism has not been fully elucidated, the authors noted the necessity of free thiol groups for targeting and suggested they may bind to the sulfhydryl receptor site via formation of disulphide bonds.	Fluorescein, <i>meso</i> -tetra(4-carboxyphenyl)porphine, silica nanoparticles, quantum dots; ¹¹⁶ Near infra-red hemicyanine. ¹¹⁷	Selective staining of Golgi.

COX-2 = Cyclooxygenase-2, COS-7 = CV-1 (simian) in origin and carrying SV40 genetic material cell line, HEK 293 = human embryonic kidney 293

Lipid droplets

Table S17: Targeting groups for endogenous fluorescent cargo to lipid droplets

Structure of targeting group	Class of targeting group	Mechanism	Cargo / Examples	Selectivity
Perilipin-3	Protein	Cargo is attached to a LD membrane protein.	GFP. ¹¹⁸	Selective for LD membrane only.

Table S18: Targeting groups for exogenous fluorescent cargo to lipid droplets

Structure of targeting group	Class of targeting group	Mechanism	Cargo / Examples	Selectivity
N/A	Highly conjugated lipophilic structures	Solvatochromic shift and/or fluorogenic turn on in non-polar hydrophobic environments compared to aqueous environments.	Nile Red, ¹¹⁹ BODIPY 493/503. ¹²⁰	May also stain intracellular and plasma membranes.
Cyclohexyl rings	Alkyl rings	Improves affinity for LD core compared to the membrane.	Merocyanine, ¹²¹ Nile Red. ⁹⁸	Selective staining for LD core.

Plasma membrane

Table S19: Targeting groups for endogenous fluorescent cargo to the plasma membrane

Structure of targeting group	Class of targeting group	Mechanism	Cargo / Examples	Selectivity
myristoylation/ palmitoylation sequence from Lck tyrosine kinase	Palmitoylation sequence	Palmitoylation is a common post-translational modification used to attach fluorescent proteins to the plasma membrane.	GFP, ^{122, 123} RFP. ¹²⁴	Selective to plasma membrane.
palmitoylation sequence from GAP43	Palmitoylation sequence	Palmitoylation is a common post-translational modification used to attach fluorescent proteins to the plasma membrane.	GFP. ¹²⁵	Selective to plasma membrane.

GAP43 = Growth Associated Protein 43

Table S20: Targeting groups for exogenous fluorescent cargo to the plasma membrane

Structure of targeting group	Class of targeting group	Mechanism	Cargo / Examples	Selectivity
Long hydrocarbon tail and polar heads	Membrane lipid mimic	Can insert into plasma membrane due to similarity in structure to existing phospholipids.	Laurdan, ¹²⁶ Prodan, ¹²⁷ DiI. ¹²⁸	Selective to plasma membrane.
Cholesterol	Membrane lipid mimic	Fluorescent analogue of membrane lipids will have similar activity to the unmodified lipid	Chloestatrienol. ¹²⁹	May be incorporated into other compartments.
methotrexate	Ligand-directed	Small molecule ligand (methotrexate) can bind to plasma membrane protein (folate).	Fluorescein. ¹³⁰	Selective to plasma membrane.
carbetocin	Ligand-directed	Small molecule ligand (carbotoxin) can bind to plasma membrane protein (oxytocin).	Nile Red. ¹³¹	Selective to plasma membrane.
aptamer sgc8 and optimised forms	DNA activatable aptamer	DNA aptamer (sgc8) can interact with membrane protein (tyrosine kinase-7)	6-carboxyfluorescein, Cy5. ¹³²	Selective to plasma membrane.
DPA-Zn	Phospholipid binder	DPA-Zn will selectively bind externalised phosphatidylserine via intramolecular indicator displacement.	Coumarin. ¹³³	Selectively stains membrane during apoptosis only.

DPA-Zn = bis(zinc-dipicolylamine).

Cytoskeleton

Table S21: Targeting groups for endogenous fluorescent cargo to the cytoskeleton

Structure of targeting group	Class of targeting group	Mechanism	Cargo / Examples	Selectivity
α -tubulin	Protein	Labelled protein monomers are incorporated into microtubules	GFP1. ¹³⁴	Selective for α -tubulin
β -tubulin	Protein	Labelled protein monomers are incorporated into microtubules	GFP 11, ¹³⁵ GFP, ¹³⁶ RFP. ¹³⁷	Selective for β -tubulin
Talin	Protein	Protein associated with focal adhesions	GFP, ¹³⁶ RFP ¹³⁸	Selective for talin
phospho-deficient version of the microtubule-binding domain of Tau (mTMBD)	Cytoskeletal binding protein	Protein binds to microtubules	mCherry. ¹³⁹	Selective for microtubules
β -actin	Cytoskeletal protein	Labelled protein monomers are incorporated into actin fibres	GFP, ^{140, 141} RFP. ¹⁴²	Selective for actin

Table S22: Targeting groups for exogenous fluorescent cargo to the cytoskeleton

Structure of targeting group	Class of targeting group	Mechanism	Cargo / Examples	Selectivity
jasplakinolide	F-Actin-binding ligand	Jasplakinolide binds to and stabilises actin dimers	Silicon-rhodamine, ¹⁴³ rhodamine derivative (MaP55). ¹⁴⁴	For SiR, selective in 5/10 cell lines from 5 organisms and 4/10 showed improved staining after verapamil addition. For MaP55, selective in U2OS cells.
docetaxel	Tubulin binding ligand	docetaxel binds to a hydrophobic pocket on β -tubulin	Silicon-rhodamine, ¹⁴³ rhodamine derivative (MaP55), ¹⁴⁴ Tubulin Tracker Deep Red. ²⁵	Generally selective for tubulin.
paclitaxel / taxol	Tubulin binding ligand	Targets tubulin	Oregon Green 488, BODIPY 564/570, Tubulin Tracker Green. ²⁵	Generally selective for actin.
phalloidin	F-Actin-binding ligand	Phalloidin binds specifically at the interface between F-actin subunits, locking adjacent subunits together	TRITC. ¹⁴⁵	Generally selective for actin.

Cytosol

Table S23: Targeting groups for endogenous fluorescent cargo to the cytosol

Structure of targeting group	Class of targeting group	Mechanism	Cargo / Examples	Selectivity
SELQNKLEELDLDSYK	Nuclear export sequence (NES)	NES is appended onto proteins for export through the NPC, thus preventing passive diffusion of small FPs.	mScarlet. ¹⁴⁶	Allows cytosolic retention, prevents nuclear uptake.

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