Dissection of protein kinase pathways in live cells using photoluminescent probes: surveillance or interrogation?

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Table of Contents:

Table S1. Representatives of Class 1B probes 2
Table S2. Representatives of Class 3A probes 4
Table S3. Representatives of Class 3B probes 8
Supplementary References 10

Table S1. Representatives of Class 1B probes.

Probe name	Consensus sequence [1]	Target PK(s)	Affinity *in vitro* [2]	Mode of delivery [3]	Concentration and incubation time in cell imaging assay	Comments on reporter moiety and optical properties	Ref
Abltide-TAT	E-A-I-**Y**-A-A-P	Abl 1,2	NA	Covalently linked with CPP (TAT)	50 nM; 24 h	Labelled with Cy5 at Cys introduced into linker at the C-terminus of probe; fluorescence lifetime is changed in the presence of target PK	[1]
ASOR	A-R-K-R-E-R-A-Y-**S**-F-K	Akt/PKB	NA	Covalently linked with CPP (TAT)	20 μM; 20 min	Labelled with Cy5 at Lys; 32% enhancement of fluorescence upon phosphorylation	[2]
CDKACT	G-G-C-S-**T**-P-K-K-A-K-K-L	Cdk 1,2,4 (activated with cyclins)	NA	complexed with Pep1	1-3 μM; 1h	Labelled with Cy5 at Cys preceding phosphorylatable Thr; conjugated with PAAD and RFP; 60% enhancement of fluorescence upon phosphorylation	[3]
Probe 2 + Evans Blue	G-R-T-G-R-R-F-**S**-Y-amide	PKAc	K_M^{app} ca 0.35 μM	hypotonic dilution method	1 μM probe, 2 μM quencher; 40 min	N-terminally labelled with Atto633; unconjugated quencher used (Evans Blue); 30-fold enhancement of fluorescence upon phosphorylation in vitro, 3.9-fold in lysate; caged version of probe available	[4]
ARII	D-L-D-V-P-I-P-G-R-F-D-R-R-V-**S**-V-A-A-C	PKAc	NA	Cell-permeable	100 ug/mL; 30-60 min	Labelled with acrylodan at the C-terminus; 3.3% reduction of fluorescence upon phosphorylation; DRII version of probe with improved cell membrane penetrative properties available	[5]
Compound 1	**S**-F-R-R-R-R-K-amide	PKC α,β,γ	K_M^{app} = 5.0-9.0 μM k_{cat} = 180-380 min^{-1} (dependent on PK isoform)	micro-injection	200 μM; final estimated concentration of 20 μM in cells	N-terminally labelled with NBD; 1.5-fold enhancement of fluorescence upon phosphorylation; caged version of probe available	[6–8]
Sensor 8	Ac-E-E-Dab-I-**Y**-G-I-E-A-amide	Src	K_M^{app} = 300 μM	micro-injection	200 μM; final estimated concentration of 20 μM in cells	Labelled with Cascade Yellow via Dab; 2.7-fold enhancement of fluorescence upon phosphorylation; caged version of probe available	[9]

VSOR	G-R-R-R-A-A-P-E-D-L-**<u>Y</u>**-K	VEGFR2 (KDR)	NA	Covalently linked with CPP (TAT)	20 μM; 20 min	Labelled with 5-FAM at Lys; 28% enhancement of fluorescence upon phosphorylation	[2]

The rows in the table are arranged according to the target PKs presented in the alphabetic order, and then sorted by year of publication. [1] Phosphorylatable amino acid is shown in bold and is underlined; Dab, (L)-2,4-diaminobutanoic acid. [2] NA, not available in literature. [3] CPP, cell-penetrating peptide.

Table S2. Representatives of Class 3A probes .

Probe name	PK-sensing fragment	Target PK(s)	Affinity *in vitro* [1]	Major off-targets [1]	Concentration and incubation time in cell imaging assay	Comments on reporter moiety and optical properties	Ref
REVERSIBLE (NON-COVALENT) PROBES							
MLN8054-TCO + TR-Tz / CFDA-Tz	MLN8054	Aurora A	437 nM (probe labelled with Texas Red) 62 nM (unlabelled MLN8054-TCO) 1.5 nM (unlabelled MLN8054)	Aurora B, Flt4 (unlabelled MLN8054)	125-1500 nM MLN8054-TCO for 20-30 min (followed by 1 μM TR-TZ for 20 min or 190 nM CFDA-TZ for 30 min)	Labelled with Texas Red (TR) or carboxyfluorescein diacetate (CFDA) after introducing into cells as MLN8054-TCO; CFDA preferred for imaging	[10]
Vemurafenib-derived probes	Vemurafenib (PLX4720)	BRAF (including V600E mutant), c-RAF	250-730 nM (probe, dependent on the label) 110 nM (unlabelled vemurafenib)	SRMS, ACK1, MAP4K5 (KHS1), FGR (unlabelled vemurafenib)	1 μM; 1-2 h	Several fluorescent dyes tested; BODIPY 493/503 preferred for cellular studies	[11]
CDKSENS	peptide derived from CDC6 (HHAGPRK) and peptide derived from p107 (RRLFGE)	Cdk 1/2 complex with cyclin A/B	8…18 nM (probe with FITC label, dependent on target PK complex used)	Other CDKs and cyclins (probe)	3 μM; 1 h	Cy3 label used for cellular applications; detection performed relative to control peptide labelled with Cy5	[12]
ARC-1837	ARC-1836 (conjugate of ATP analogue and oligoaspartate)	CK2	3.0 nM (probe ARC-1836, not as prodrug)	TAK1, GSK3β (unlabelled analogue)	1-10 μM; 1-2 h	Labelled with BODIPY FL	[13]
Compound 2	GE11 peptide (YHWYGYTPQNVI)	EGFR	8.9 μM (probe) 22 nM (unlabelled GE11)	Not reported	1 μM; 1-6 h	Labelled with NIR fluorophore Cy5.5	[14]
QD-AZD4547	AZD4547	FGFR3	100 nM (probe) 7.6 nM (unlabelled AZD4547)	FGFR 1, 2, 4; VEGFR2 (KDR) (unlabelled AZD4547)	5 nM; 30 min	Labelled with Qdot 605 ITK; imaging performed only after fixation of cells	[15]

Probe 6	N-phenyl-4-aminoquinazoline scaffold	HER2	27 nM (probe)	Not reported	2 μM; 20 min	The PK-targeting fragment gains fluorescence upon binding to PK (max Ex 320 nm, Em 480 nm)	[16,17]
MERi-SiR	UNC2025	Mer	0.74 nM (unlabelled UNC2025)	Flt3, Axl, Tyro3 (unlabelled UNC2025)	3 μM; 24 h	Labelled with NIR fluorophore SiR, gains fluorescence upon binding to target PK	[18]
ARC-1185	conjugate of Se-containing ATP analogue and oligoarginine	PKAc, Rho kinase II, MSK1, Akt3, PKCδ	Less than 0.2 nM to 40 nM (dependent on the PK)	Basophilic PKs (generic probe)	10 μM; 1 h	Labelled with Texas Red; gains long-lifetime luminescence (τ > 20 μs) in complex with target PKs (Ex 365 nm, Em 610 nm)	[19]
Probe 1, 2	SBE13	Plk1	0.2 nM (unlabelled SBE13)	Selective within a small panel of mitotic PKs	20 μM; 20 h	Labelled with coumarin (probe 1) or NIR fluorophore 3-cyano-4-phenyl-2(5H)-furanon (probe 2)	[20]
BI-BODIPY	BI2536	Plk 1,2,3	5-19 nM (probe, dependent on Plk isoform) 4-11 nM (unlabelled BI2536, dependent on Plk isoform)	Highly selective	100 nM; 2h	Labelled with BODIPY FL	[21]; similar scaffold reported by [22]
dasatinib-BODIPY	Dasatinib (Sprycel, BMS-354825)	Src family	One-digit nanomolar (probe) Subnanomolar (unlabelled dasatinib)	Abl, BTK, EphB2 (probe) Abl, c-Kit, PDGFR (unlabelled dasatinib)	100 nM; 2 h	Labelled with BODIPY FL	[23]
Molecule 1	IY-IY (incorporates Ile and Tyr analogues)	TrkC	ca 0.2 μM (probe)	Not reported	1 μM; 12 h	Incorporates photosensitizer based on diiodo-BODIPY	[24]; similar compound reported in [25]

SP1	sunitinib	VEGFR2 (KDR)	2.2 μM (probe) 80 nM (unlabelled sunitinib)	Various Tyr PKs including PDGFR α, β, Kit, Flt3 (unlabelled sunitinib)	1 μM; 30 min	Labelled with pyrene; gains fluorescence upon binding to target PKs	[26]
			IRREVERSIBLE (COVALENT) PROBES				
DA-2 + rhodamine-N_3	dasatinib	Abl, Src family PKs	15 nM (probe; after 20 min incubation) 3.6 nM (unlabelled dasatinib)	See proteomics results in [27] (probe) c-Kit, PDGFRb (unlabelled dasatinib)	20 μM; 5 h	Labelled post-fix with rhodamine-N_3 after introducing into live cells as DA-2; photoactivatable	[27]
MLN-2 + rhodamine-N_3 / Cy5-N_3	MLN-8237	Aurora A	15 nM (probe; after 20 min incubation) 5.2 nM (unlabelled MLN-8237)	See proteomics results in [28] (probe); Aurora B, Blk, Yes1, Abl1 (unlabelled MLN-8237)	2 μM; 1 h	Labelled post-fix with rhodamine-N_3 or Cy5-N_3 after introducing into live cells as MLN-2; photoactivatable	[28]
Probe 1	Covalent inhibitor ibrutinib	Btk	ca 0.72 nM (unlabelled ibrutinib; after 1 h incubation)	Lyn, Lck (unlabelled ibrutinib)	1 μM; 25 min - 1 h	Labelled with BODIPY FL; dye fluorescence in free probe quenched by dinitrophenyl group, requires several hours for uncaging by target PK	[29]; similar ABPP probe reported by [30]
PU-1 + Cy5-N_3	Purvalanol B	Cdk1	34 nM (probe; after 20 min incubation) 21 nM (unlabelled purvalanol B)	See proteomics results in [28] (probe) Cdk2, Cdk5 (unlabelled purvalanol B)	2 μM; 1 h	Labelled post-fix with Cy5-N_3 after introducing into live cells as PU-1; photoactivatable	[28,31]

Cy3-RA1	Covalent inhibitor RA1	EGFR, HER2, JAK3	ca 340 nM (probe; after 30 min incubation) 22 nM (unlabelled RA1; after 30 min incubation)	MEK4, LKB1 (unlabelled RA1)	100 nM; 30 min	Labelled with Cy3	[32]
STS-1 + rhodamine-N_3	staurosporine	PKAc	62 nM (probe; after 20 min incubation) 35 nM (unlabelled staurosporine)	Ser/Thr PKs (generic probe)	20 μM; 1 h	Labelled post-fix with rhodamine-N_3 after introducing into live cells as STS-1; photoactivatable	[31,33,34]

The rows in the table are arranged according to the target PKs presented in the alphabetic order, and then sorted by year of publication.[1] Data on probe and/or non-labelled PK-sensing fragment provided (see information in brackets).

Table S3. Representatives of Class 3B probes.

Probe name	PK-targeting scaffold	Target PK(s)	Affinity *in vitro* [1]	Mode of delivery	Concentration and incubation time in cell imaging assay	Comments on reporter moiety and optical properties	Ref
			BINDING-RESPONSIVE				
GFP CaMKII FingR	Fibronectin intrabody generated with mRNA display	CaMK II	NA	Genetically encoded		Tagged with GFP	[35]
QD-EGF	Mono-biotinylated EGF coupled to streptavidin-(PEG)-QDs	EGFR	NA	Cells, tissues: extracellular	2 nM; 30 min	Labelled with 625QD, 655QD, or 705QD	[36]
QD-MAb (EGFR)	Mouse monoclonal antibodies 528 (IgG_{2a}), H-11 (IgG_1) and H199.12 (IgG_{2a}) against extracellular portion of EGFR	EGFR	NA	Cells, tissues: extracellular	5 μg/mL; 30 min	Labelled with 625QD, 655QD, or 705QD directly or *via* secondary antibody	[36]
Z(EGFR)-Green/Red	Affibody (7 kDa)	EGFR	20-28 nM (probe, dependent on FP tag)	Extracellular	100 μg/mL; 1 h	Tagged with EGFP or mCherry	[37]
EGFR biosensor	One or two SH2 domains derived from protein Grb2	EGFR	NA	Genetically encoded		Tagged with tagRFP; co-expressed with GFP that was utilized as a volume marker for ratiometric measurement	[38]
EGF-Atto532/Cy5	Recombinant mouse EGF	EGFR	NA	Extracellular	0.4 nM + 0.4 nM (mixture of differently labelled probes); no pre-incubation	Labelled with Atto532 or Cy5; super-resolution and oblique illumination achieved using uPAINT principle	[39]
Panitumumab-Atto647N	Panitumumab antibody	EGFR	NA	Extracellular	0.4 nM; no pre-incubation	Labelled with Atto647N; oblique illumination achieved using uPAINT principle	[39]
$Z_{EGFR:1907}$-FAP_{dL5**}-$Z_{EGFR:1907}$ (AFA)	Affibody $Z_{EGFR:1907}$	EGFR	37 nM (probe)	Extracellular	250 nM; 1 h	Labelled on cells with malachite green derivative MG-B-tau (100 nM, 5 min); fluorescence enhancement of MG occurs in complex with FAP	[40]

CNDC	Centyrin 83v2Cys (10 kDa)	EGFR	0.6 nM k_{off} = 2.9·10^{-5} s (unlabelled Centyrin)	Extracellular	100 nM; 1h	Labelled with NIR dye S0456	[41]
Z(HER2)-Green/Red	Affibody (7 kDa)	HER2	12-13 nM (probe, dependent on FP tag)	Extracellular	100 μg/mL; 1 h	Tagged with EGFP or mCherry	[37]
His$_6$-ZHER2:GS-Cys-DyLight750	Affibody (8.3 kDa)	HER2	NA	Cells: extracellular (recognizes extracellular part of HER2) Animals: injection	0.5 μg/mL; 1.5 h (cells) 10 μg (animals)	Labelled with NIR fluorophore DyLight750; labelling with DyLight488 was applied in pilot experiments; fluorescence lifetime imaging used	[42]
QD-Anti-HER2-Ab	Mouse anti-HER2 monoclonal antibody	HER2	NA	Extracellular	100 μg/mL; 1 h	Labelled with NIR QDs	[43]
SFK monobody biosensor	Fibronectin monobody 1F11 (95 residues) against SH3 domain	Src family PKs	0.69 μM (probe) 0.25 μM (unlabelled 1F11)	microinjection	40 μM; 30-60 min	Tagged with m-Cerulean and labelled with merocyanine dye mero-53; ratio of emission of the environmentally sensitive dye *vs* FP measured	[44]
				Extracellular			
PHOSPHORYLATION-RESPONSIVE							
Fab311-488 **Fab313-488**	Monoclonal antibodies against phospho-Ser10 in histone H3 (with different state of adjacent Lys9 methylation)	Aurora B [2]	NA	Cells: using 100 μM glass beads Animals: injection	500 μg/mL; over 4 h	Labelled with Alexa Fluor 488	[45]

The rows in the table are arranged according to the target PKs presented in the alphabetic order, and then sorted by year of publication. [1] NA, not available in literature; [2] possibly also reports activity of other PKs performing phosphorylation of phospho-Ser10 in histone H3.

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