SETA Fluorescence Lifetime Probes & Labels

Dyes with Environment-Sensitive and Environment-Insensitive Fluorescence Lifetimes (FLTs)

SETA BioMedicals offers Square, Seta, and SeTau dyes, probes and labels for environment-sensitive or environment-insensitive fluorescence lifetimes applications. They are excitable with UV, visible, and near-infrared (NIR) light sources. These products are available exclusively from SETA BioMedicals.

The table below summarizes lifetime data for these proprietary dyes, including the observed changes upon analyte-interactions. A dye is listed as having an environment-sensitive FLT when a change in its microenvironment produces an at least 1.5-fold lifetime change. The change in lifetime of a fluorescently labeled analyte mainly depends on the properties and size of its binding partner.

Product Number (Click for Specs Sheet)	Product Name (Click for Product Info)	Target Group	Environment Sensitive FLT	Excitation Wavelength (nm)												Medium	Unbound (Free) Dye τ_{Free} [ns]	Dye Bound to Analyte			FLT‡ change: Increase (×) or Decrease (÷) τ_{Bound} / τ_{Free}
				380	405	436	488	532	594	635	650	680	700	750	780			Analyte	Dye/Analyte Ratio	τ_{Bound} [ns]	
K1-204	SeTau-380-NHS	NH_2	No	•	•											PB 7.4	32.5	BSA	1	30.6	÷1.1
K4-205	Seta-375-NHS	NH_2	No	•												PB 7.4	3.1	IgG	1	4.0	×1.3
																			5	3.5	×1.1
K6-207	3-DAB	—[§]	Yes		•	•	•									PB 7.4	1.5	BSA	6 mg/mL	6.5	×4.3
																Ethanol	4.0	–	–	–	–
K7-544	SeTau-425-Maleimide	SH	n.d.*	•	•	•										PB 7.4	26.2	–	n.d.	n.d.	n.d.
K7-545	SeTau-425-NHS	NH_2	n.d.*	•	•	•										PB 7.4	26.2	–	n.d.	n.d.	n.d.
K7-547	SeTau-405-NHS	NH_2	No	•	•											PB 7.4	9.3	IgG	2	8.9	÷1.04
K7-548	SeTau-405-Maleimide	SH	No[†]	•	•											PB 7.4	9.1[#]	–	n.d.	n.d.	n.d.
K7-567	SeTau-405-Azide	C≡C	No[†]	•	•											PB 7.4	9.1[#]	–	n.d.	n.d.	n.d.
K8-3002	Seta-470-NHS	NH_2	Yes, Viscosity		•	•	•									PB 7.4	0.21	–	–	–	–
																Glycerol / Water (4:1)	–	18 cP	+50 °C	0.7	–
																		770 cP	-6 °C	2.7	–
K8-1341	Seta-670-Maleimide	SH	Yes[†]	•	•					•	•					PB 7.4	0.42[#]	–	n.d.	n.d.	n.d.
K8-1342	Seta-670-NHS	NH_2	Yes	•	•					•	•					PB 7.4	0.42[#]	BSA	1.5	2.43	×5.8
																		IgG	5	0.85	×2.0
K8-1346	Seta-670-Azide	C≡C	Yes[†]	•	•					•	•					PB 7.4	0.42[#]	–	n.d.	n.d.	n.d.
K8-1350	Square-670-Carboxy	—[§]	Yes[†]	•	•					•	•					PB 7.4	0.29	–	n.d.	n.d.	n.d.
K8-1351	Square-660-Carboxy	—[§]	Yes	•	•					•	•					PB 7.4	0.27	BSA	2 mg/L	3.56	×13.2
K8-1352	Square-660-NHS	NH_2	Yes	•	•					•	•					PB 7.4	0.27[#]	BSA	1.2	3.32	×12.3
																		IgG	0.7	2.73	×10.1
																		ssOligo	1	3.0	×11.1
																		dsOligo	1	1.8	×6.7
K8-1405	Square-650-pH-Carboxy	—[§]	Yes, pH[§]				•	•		•	•					PB 5.6	1.17	UB 9.0[§]	–	0.53	÷2.2
K8-1407	Square-650-pH-NHS	NH_2	Yes, pH[§]				•	•		•	•					PB 5.6	1.17[#]	UB 9.0[§]	–	0.52	÷2.3
																		IgG/UB 9.0	0.8	0.89	÷1.3
																		IgG/UB 2.0	0.8	1.52	×1.3
K8-1662	Seta-640-NHS	NH_2	Yes							•						PB 7.4	0.7	IgG	3	1.2	×1.7
																		BSA	1	3.0	×4.3
																			2	2.7	×3.9

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				380	405	436	488	532	594	635	650	680	700	750	780			Analyte	Dye/Analyte Ratio	τ_{Bound} [ns]	
K8-1663	Seta-633-NHS	NH_2	Yes						•	•						PB 7.4	0.25	BSA	0.9	2.81	×11.2
																		IgG	1.1	1.49	×6.0
																		Biotin	1	0.66	×2.6
K8-1667	Seta-635-pH-di-NHS	NH_2	Yes, pH							•						PB 7.4	1.80	IgG	4	1.41	÷1.3
																		BSA	2	1.37	÷1.3
K8-1682	Seta-660-di-NHS	NH_2	n.d.*							•	•					PB 7.4	0.9	–	n.d.	n.d.	n.d.
K8-3402	Zy3-NHS	NH_2	No†					•								PB 7.4	< 0.3#	–	n.d.	n.d.	n.d.
K8-3403	Zy3-Maleimide	SH	No†					•								PB 7.4	< 0.3#	–	n.d.	n.d.	n.d.
K8-5402	Zy5-NHS	NH_2	No†							•						PB 7.4	1.0#	–	n.d.	n.d.	n.d.
K8-5403	Zy5-Maleimide	SH	No†							•						PB 7.4	1.0#	–	n.d.	n.d.	n.d.
K8-5422	Zy5.5-NHS	NH_2	No†							•	•	•				PB 7.4	1.0#	–	n.d.	n.d.	n.d.
K8-5423	Zy5.5-Maleimide	SH	No†							•	•	•				PB 7.4	1.0#	–	n.d.	n.d.	n.d.
K8-7402	Zy7-NHS	NH_2	No†									•	•	•		PB 7.4	0.57#	–	n.d.	n.d.	n.d.
K9-3152	SeTau-488-NHS	NH_2	Yes				•									PB 7.4	1.2#	IgG	1	2.4	×2.0
																			2	2.3	×1.9
K9-3153	SeTau-488-Maleimide	SH	Yes†				•									PB 7.4	1.2#	–	n.d.	n.d.	n.d.
K9-3162	SeTau-500-NHS	NH_2	Yes				•									PB 7.4	1.7	IgG	1	2.7	×1.6
																			2	2.6	×1.5
																		BSA	1	2.9	×1.7
K9-4119	SeTau-665-NHS	NH_2	n.d.*								•	•				PB 7.4	3.1	–	n.d.	n.d.	n.d.
K9-4142	SeTau-647-di-NHS	NH_2	No							•	•					PB 7.4	3.2#	IgG	2	3.1	÷1.03
K9-4148	SeTau-647-Maleimide	SH	No†							•	•					PB 7.4	3.2	–	n.d.	n.d.	n.d.
K9-4149	SeTau-647-NHS	NH_2	No							•	•					PB 7.4	3.2	IgG	1	3.3	×1.03
																			2	3.1	÷1.03
K9-4150	SeTau-647	–§	No†							•	•					PB 7.4	3.1	–	–	–	–
K9-4159	SeTau-660-NHS	NH_2	Yes							•	•					PB 7.4	3.36	IgG	3	2.47	÷1.4
																			4	2.23	÷1.5
K9-4169	SeTau-670-NHS	NH_2	No							•	•	•				PB 7.4	1.6	IgG	4	1.75	×1.1
K9-4179	SeTau-680-NHS	NH_2	No							•	•	•				PB 7.4	2.86	IgG	1	2.94	×1.03
																			2	2.91	×1.02
																			4	2.84	÷1.01
KS-370	330–405 nm FLT Standard	–§	n.d.*	•	•											PB 7.4	3.18	–	–	–	–
KS-405	370–420 nm FLT and FP Standard	–§	n.d.*	•	•											PB 7.4	9.07	–	–	–	–
KS-470	436–490 nm FP Standard	–§	n.d.*			•	•									PB 7.4	0.21	–	–	–	–
KS-650	590–650 nm FLT Standard	–§	n.d.*							•						PB 7.4	0.98	–	–	–	–
KS-660	550–670 nm FP Standard	–§	n.d.*							•	•					PB 7.4	0.27	–	–	–	–

‡ Acquired with ISS Chronos FD at 25 °C
† Anticipated based on the chemical structure.
# For free acid
* Not determined
§ Fluorescent probe for non-covalent binding
§ pH-sensitive dye, excitable at 635 nm and 650 nm under acidic conditions and at 532 nm under basic conditions, emitting at 660–670 nm under all conditions.

Other environment-sensitive and environment-insensitive FLT dyes can be developed upon request.

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1. Environment-sensitive lifetime dyes

Selected Square, Seta, and SeTau dyes exhibit shorter fluorescence lifetimes (FLTs) in aqueous media when conjugated to low-molecular-weight analytes (drugs or small ligands) but exhibit several times longer lifetimes after direct interaction with the binding partner (protein, DNA, peptide or oligonucleotides). These environment-sensitive dyes are therefore well-suited as tracers in FLT-based assays (see, e.g., https://www.ddw-online.com/fluorescence-lifetime-assays-953-201310/), where the FLT dye is initially conjugated, e.g., to a small antigen (ligand) exhibiting a short lifetime but after binding to a larger protein (antibody or receptor) substantially increases its FLT.

Note: In FRET- or quencher-based enzyme substrates, a change in fluorescence lifetime reflects an assay-induced change in energy transfer or quenching. It should not necessarily be interpreted as an intrinsic response of the fluorophore to its environment.

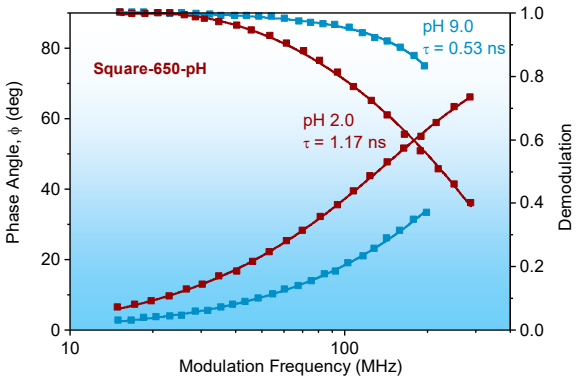
1.1. FLT probes

FLT probes bind reversibly to a target or accumulate in a cell compartment, and their lifetime changes due to the alteration their chemical or physical environment. These probes can be used for:

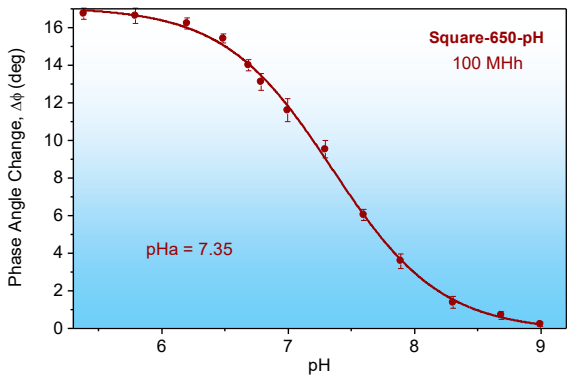
- **Immunoassays**: a small antigen or ligand conjugated to an environment-sensitive FLT dye can show a short lifetime free in solution and a substantially longer lifetime after binding to a large antibody or receptor; the lifetime shift enables homogeneous, wash-free immunoassays.
- **Aminopeptidase activity measurements**: an aminopeptidase substrate bearing an environment-sensitive fluorophore can undergo a lifetime change upon enzymatic cleavage or product release, allowing continuous kinetic monitoring of exopeptidase activity.
- **Caspase-activity measurements**: caspase-cleavable peptide substrates containing FRET donor–acceptor or fluorophore–quencher pairs report cleavage through an assay-induced increase in donor FLT. Cleavage reduces FRET or quencher interaction, thereby lengthening the donor lifetime; the change can be measured in bulk, by flow cytometry, or by FLIM.
- **pH sensing** in live cells, organelles, biofilms, and tissues by FLIM.
- **Oxygen sensing** through collisional quenching, including hypoxia, tissue oxygenation, and photodynamic-therapy monitoring.
- **Ion sensing**: Ca^{2+}, Cl^{-}, Zn^{2+}, and other cations/anions in intracellular signaling and physiology.
- **Membrane polarity, viscosity, and phase-state sensing**: lipid-domain mapping, membrane-order measurements, and organelle-membrane characterization by FLIM.
- **Nucleic-acid binding and structure sensing**: DNA/RNA stains and G-quadruplex probes whose lifetime reports binding, packing, or conformation.
- **Protein-binding and conformation sensing**: affinity probes whose lifetime changes upon binding an activated protein conformation; useful for live-cell FLIM biosensors.
- **Metabolic-state imaging**: lifetime changes of endogenous NADH/FAD report free versus protein-bound cofactors and cellular metabolic status.
- **Biomolecule-concentration sensing**: for example, albumin-sensitive squaraine dyes used in FLIM of tumor biopsies.
- **Temperature and polarity mapping** in cells and materials.

Fluorescent probes developed by SETA BioMedicals such as Square-660-Carboxy show large changes in FLT upon binding to proteins or cell surfaces. This probe is water-soluble but has a strong affinity for the hydrophobic regions of biomolecules and cells.

Square-650-pH is a NIR probe that exhibits a pKa around 7 and is therefore useful for measuring pH changes across the physiologically relevant pH-range of 6–8. Importantly, both forms (protonated as well as deprotonated) of this probe are fluorescent, and they exhibit distinct lifetimes, which makes it useful for as a specific lifetime-probe (see figure below).



Frequency responses of Square-650-pH at acidic and basic pH



Phase angle changes vs. pH for Square-650-pH measured at 100 MHz

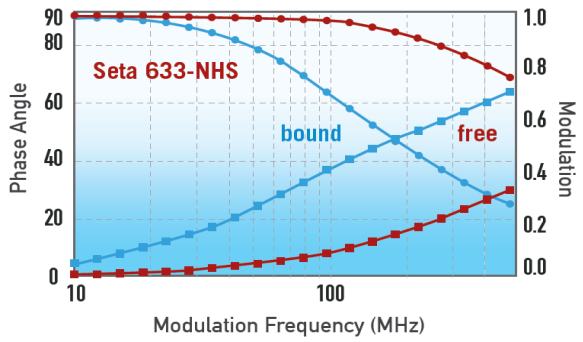
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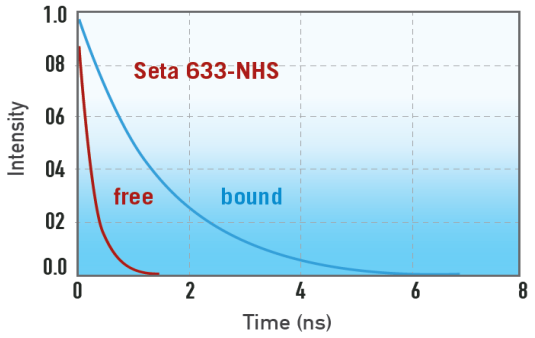
1.2. Hydrophilic FLT labels

These are reactive labels that show changes in FLT upon permanent (covalent) attachment to either proteins, antibodies, oligonucleotides, DNA, cell surfaces, or other species. In general, these labels are conjugated to a low-molecular-weight binding partner, and the interaction with a high-molecular-weight protein or receptor leads to a change in FLT. Generally, FLT labels are used for:

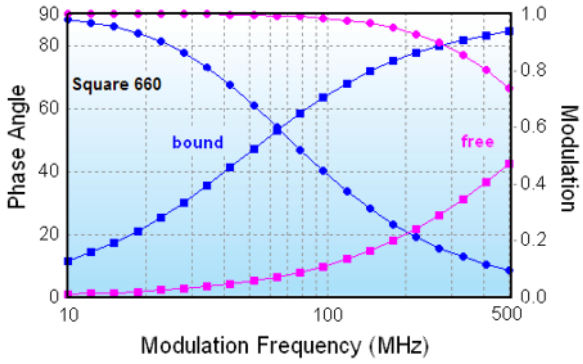
- **Immunoassays and receptor-binding assays**: covalent FLT labels on antigens, haptens, peptides, or antibodies provide lifetime-based detection of antibody–antigen or ligand–receptor binding; binding to a larger biomolecule can substantially increase the lifetime of the label.
- **Aminopeptidase activity measurements**: covalent donor–acceptor or fluorophore–quencher peptide substrates can be designed for aminopeptidases. Enzymatic cleavage separates the donor dye from the quencher/acceptor, increasing the donor's FLT; the donor dye's intrinsic lifetime may be relatively insensitive to its environment.
- **Caspase-activity measurements and apoptosis imaging**: FRET or quenched peptide probes containing a caspase-cleavable sequence (e.g., DEVD) report caspase activation via an increase in donor FLT after cleavage; FLIM maps caspase activation in individual live cells.
- **Targeted pH, ion, ROS, and oxygen sensing**: SNAP-tag or similar chemistry can attach Ca^{2+}, Zn^{2+}, H_2O_2, or other indicators to a chosen protein, tracking and reporting these analytes near that protein.
- **Membrane-protein microenvironment mapping**: covalent attachment of solvatochromic dyes reports local polarity and membrane state around a defined protein based on observed lifetime.
- **FLIM-FRET biosensors**: donor lifetime shortens upon FRET, enabling spatially resolved measurement of protein–protein interactions, conformational changes, and binding events.
- **Enzyme-activity and signaling assays**: covalently labeled substrates or sensors report protease, kinase/phosphatase, and other enzymatic activity via lifetime changes.
- **Protein-conformation biosensing**: an environment-sensitive dye covalently attached to an affinity reagent can report the activated conformation of proteins by FLIM.
- **In situ activity-dependent covalent labeling**: probes that react only after recognizing a biomarker can covalently label nearby proteins for precise imaging in complex biological environments.
- **Tissue FLIM research**: targeted environment-sensitive probes can map disease-associated microenvironments, including albumin-rich tumor regions.



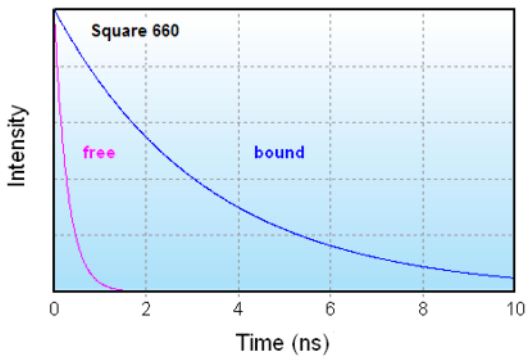
Comparison of the frequency responses of Seta-633 before and after binding to protein or oligos (τ_{Free} = 250 ps; $\tau_{IgG\ bound}$ = 1.49 ns)



Comparison of the intensity decays of Seta-633 before and after binding to protein or oligos (τ_{Free} = 250 ps; $\tau_{IgG\ bound}$ = 1.49 ns)



Comparison of the frequency responses of Square-660-NHS before and after binding to protein or oligos (τ_{free} = 290 ps; $\tau_{BSA\ bound}$ = 3.32 ns)

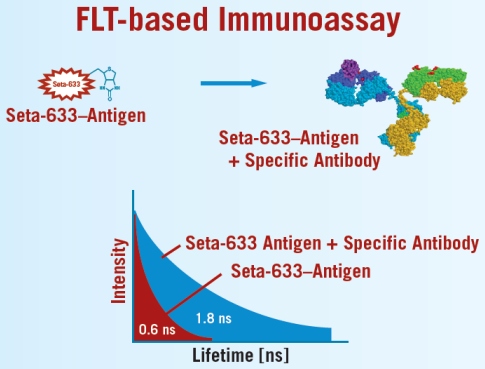


Comparison of the intensity decays of Square-660-NHS before and after binding to protein or oligos (τ_{free} = 290 ps; $\tau_{BSA\ bound}$ = 3.32 ns)

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Application: Lifetime Labels for Low-Molecular-Weight Analytes[1]

In general Seta and Square dye labeled lifetime tracers (labeled low-molecular weight antigens) bind to antibodies with high specificity and this interaction is accompanied by a several-fold change in fluorescent lifetime. This behavior is contrary to cyanine-type tracers (Cy or Alexa dyes) which exhibit much smaller lifetime changes and are therefore not suitable as reporters for lifetime-based assays.



[1] Povrozin Y. et al., Bioconjugate Chem. 20, 1807-1812 (2009).

2. Environment-insensitive lifetime dyes

2.1. FLT probes

Non-covalent, environment-insensitive FLT probes are chosen for their ability to keep lifetimes changes as small as possible despite changes in, e.g., analyte binding, concentration, solvent polarity, or photobleaching.

- **Cell tracking and lineage tracing**: long-lived, stable-lifetime particles or dyes distinguish labeled cells from autofluorescence over time.
- **Multiplexed FLIM**: spectrally overlapping probes can be separated by lifetime rather than emission color.
- **Autofluorescence rejection**: a stable-lifetime label can be distinguished from tissue autofluorescence, improving contrast in label-free or weakly labeled samples.
- **Reference channels for ratiometric lifetime measurements**: a stable probe normalizes instrument response, illumination variation, and local probe concentration.
- **Flow cytometry and particle tracking**: lifetime-stable labels support identification of cells, beads, or nanoparticles independently of brightness.
- **Material and polymer tracing**: stable fluorophores track diffusion, mixing, coating uniformity, or particle distribution where lifetime should not report the medium.

Examples of these environment-insensitive FLT probes are Seta-635-pH-di-Carboxy and SeTau-647.

2.2. FLT labels

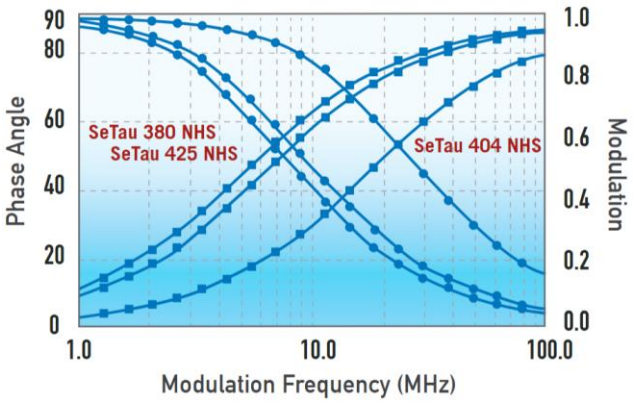
Covalent, environment-insensitive FLT labels are designed to retain a reproducible lifetime after attachment, making them suitable for localization and quantification rather than sensing.

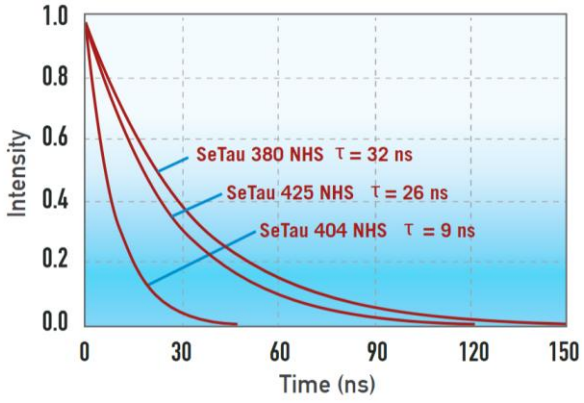
- **Immunoassays**: environment-insensitive covalent labels are useful as stable tracers or reference labels in competitive or sandwich-type immunoassays, especially where the readout should not be affected by changes in buffer composition or the local binding environment.
- **Caspase and protease assays**: covalently attached, stable-lifetime labels can serve as donor or acceptor-type tags in FRET substrates. In these assays, a donor-lifetime change reports cleavage-induced changes due to FRET or quenching, rather than instability of the free label or intrinsic environmental sensitivity of the dye.
- **Aminopeptidase assays**: stable covalent labels can be incorporated into peptide substrates as reference or FRET tags, enabling reproducible time-resolved activity measurements.
- **Protein, peptide, antibody, and receptor tracking**: covalent labels provide stable localization for trafficking, turnover, degradation, and pulse-chase studies.
- **DNA/RNA labeling**: fluorescent primers, FISH probes, single-molecule RNA/DNA imaging, microarrays, and sequencing-related assays.
- **FLIM-FRET distance measurements**: a fixed donor lifetime can be used to obtain a reliable baseline for calculating the FRET efficiency and molecular distances.
- **Multiplexed lifetime imaging**: covalent labels with distinct, stable lifetimes enable simultaneous imaging of several targets even when their spectra overlap.
- **Single-molecule and super-resolution microscopy**: stable lifetimes support species separation, localization, and time-resolved imaging.
- **Drug and lipid tracking**: covalent red/NIR labels can follow drugs, lipids, and drug–target interactions without the label lifetime being strongly affected by the medium.
- **Instrument calibration and phasor-plot references**: stable-lifetime standards calibrate FLIM systems and define reference positions in phasor analysis.

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2.2.1. Long-lifetime, short-wavelength FLT labels

These proprietary long-lifetime labels with lifetimes of 9–32 ns in aqueous media are excitable in the UV or blue region. They are utilized as labels in lifetime-based applications and as polarization labels for the measurement of high-molecular-weight analytes, including small- and medium-sized proteins. For instance, the measurement of BSA (MW 65 kDa) with SeTau-425-NHS in a fluorescence polarization (FP) assay demonstrates that a suitable dynamic range can be achieved even for large antigens with this long-lifetime polarization dye. Importantly, the FLT of these labels is largely insensitive to labeling and binding to proteins or other analytes.

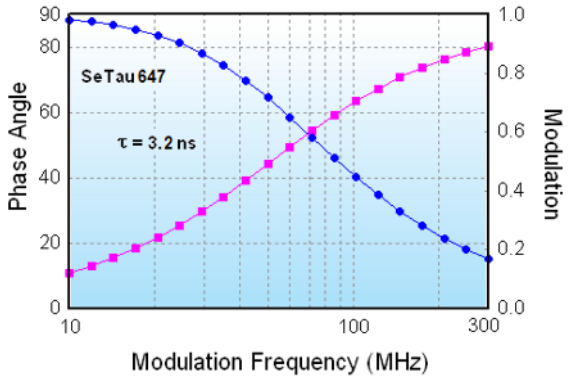




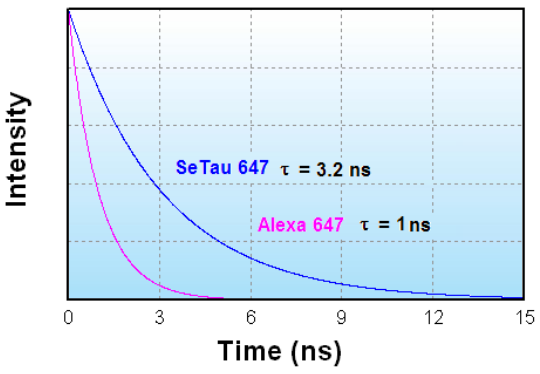
2.2.2. Long-wavelength, squaraine-rotaxane-based FLT labels

SeTau-647-NHS, SeTau-500-NHS, SeTau-665-NHS, and SeTau-680-NHS are examples of some of our environment-insensitive fluorescence lifetime (FLT) labels based on rotaxanes. Their average lifetime is approximately 3 ns when labeled to biomolecules, compared to approximately 1 ns for Cy3, Alexa 555, Cy5 or Alexa 647 in water. This longer lifetime makes them, in particular, useful as lifetime or polarization probes and as FRET donors, offering a wider measurable range of donor-lifetime changes in some applications. At very high FRET efficiencies, however, the donor lifetime will still be substantially shortened.

SeTau-647-type dyes have been successfully used in the studies of the mechanism of muscle contractions using fluorescence correlation spectroscopy (FCS), specifically fluorescence lifetime correlation spectroscopy (FLCS), fluorescence lifetime, and time-resolved fluorescence polarization methods [2], while the novel, recently added SeTau-500-NHS is a new, unique label with almost no cited applications. It has an exceptionally large Stokes shift of >60 nm (much larger than Alexa 488 (23 nm), Alexa 555 (15 nm), or Cy3 (15 nm). The larger Stokes shift enables the use of wider filter sets and thereby improving measurement sensitivity. The high fluorescence quantum yield of 40–70% in aqueous media and ~60% for protein and antibody conjugates, which is much higher than Cy3 (QY ~ 4% or Alexa 555 (QY ~ 10% in PBS) also demonstrates the potential of this dye as a sensitive label. Here we invite our experienced, open-minded customers to try it for some new applications.



Frequency responses of SeTau-647-NHS in aqueous buffer solution pH 7.4



Intensity decays of SeTau-647-NHS in aqueous buffer solution pH 7.4 in comparison to Alexa 647

[2] Midde K. et al., J. Biol. Chem. 288:10, 7012–7023 (2013).

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