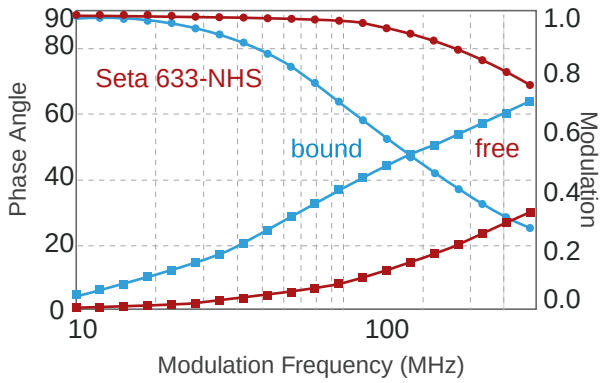
Fluorescence Lifetime

Environment-Sensitive Lifetime Dyes

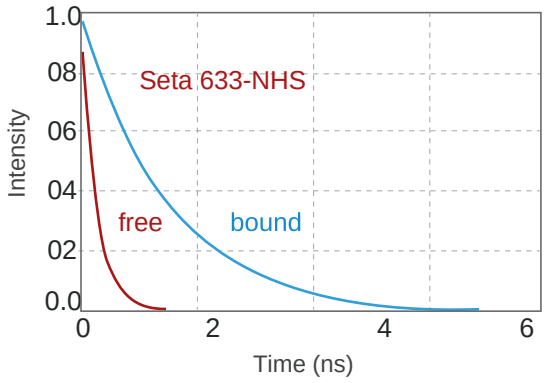
Selected Square and Seta dyes exhibit shorter fluorescence lifetimes (FLTs) in aqueous media when labeled to low molecular weight analytes (drugs or small ligands) but increased brightness and several-fold longer FLTs when bound to proteins or oligonucleotides. The environment sensitive Square and Seta labels are therefore well-suited as tracers in FLT-based assays, where the FLT-tracer would be labeled to a small antigen (ligand) displaying a short lifetime but showing a substantial FLT-increase upon binding to a larger protein (antibody or receptor).

The table below provides data of the expected changes in lifetime for some FLT dyes. It is important to note that the observed lifetime change of a fluorescently labeled ligand will strongly depend on the nature and size of the biomolecule it binds to:

Product	Dye	Dye-BSA Conjugate		[ns]
	τ_{mean} [ns]	D/P Ratio	τ_{mean} [ns]	$\Delta\tau$ (Dye–conj.) – τ (Dye)
Seta-670-NHS (K8-1342)	0.42	1.5	2.43	2.01
Square-660-NHS (K8-1352)	0.27	1.2	3.32	3.05
Seta-633-NHS (K8-1663)	0.25	0.9	2.81	2.56



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)

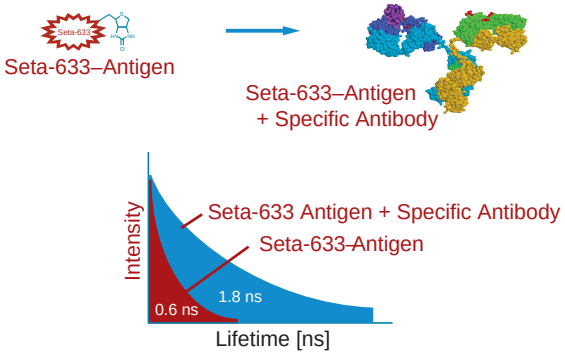


Comparison of the intensity decays of Seta-633 before and after binding to protein or oligos
(τ_{free} = 250 ps; $\tau_{IgG\ bound}$ = 1.49 ns)

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 changesand are therefore not suitable as reporters for lifetime-based assays.

FLT-based Immunoassay

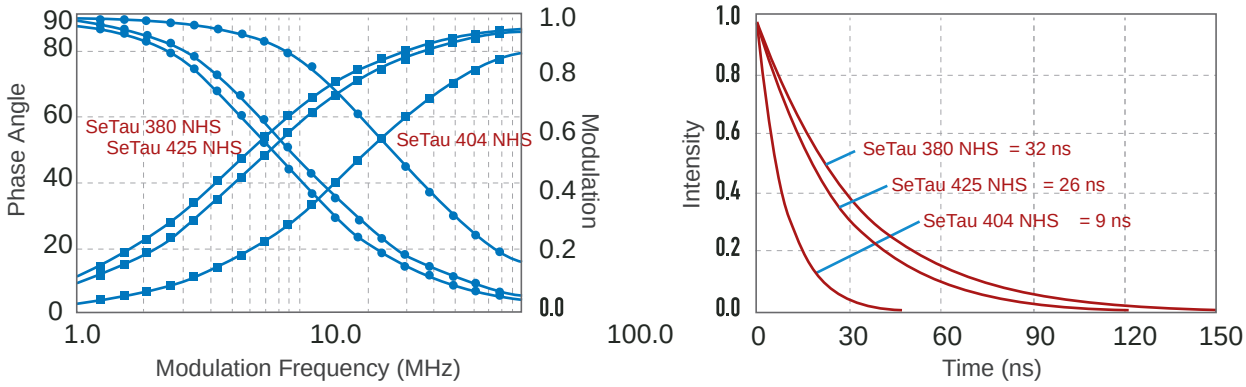


[1] Povrozin Y. et al., Bioconjugate Chem. 20, 1807-1812 (2009).

Environment-Insensitive Lifetime D

a) Blue Excitable, Long-lifetime Labels

These proprietary long-lifetime labels with lifetimes between to 32 and 9 ns in water are excitable in the UV or blue region. They are utilized as labels in lifetime based applications and as polarization probes for the measurement of high MW analytes (small - medium size proteins). The measurement of BSA (MM of 65 K) with SeTau-425-NHS in an FP assay demonstrates that a suitable dynamic range can be achieved even for large antigens with this long-lifetime polarization probe. Importantly the FLT of these labels does not change upon labeling or binding to proteins or other analytes (see table below).



b) Red Excitable Lifetime Labels

SeTau-647-NHS, SeTau-647-Maleimide and SeTau-647-di-NHS are FLT labels with lifetimes that are insensitive to the environment. The average lifetime of these labels is in the order of 3 ns which make them superior lifetime or polarization probes over Cy5 or Alexa dyes (Cy5, Alexa 647: FLT ~ 1 ns in water). The longer lifetimes make them also more suitable donors in energy-transfer-based assays assuring that even at higher energy transfer rates the lifetime of these labels is not too short to be accurately measured with inexpensive instrumentation.

SeTau-647-maleimide has also been successfully used in the studies of the mechanism of muscle contractions using FCS, specifically fluorescence lifetime correlation spectroscopy (FLCS), fluorescence lifetime and time-resolved fluorescence polarizations methods.[2]

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

Product Number	Product Name	Target Group	Medium	abs [nm]	[M^{-1}. cm^{-1}]	em [nm]	QY [%]	FLT [ns]
K1-204	SeTau-380-NHS	NH_2	BSA,D/P=1	383	23800	468	44	30.6
K7-544	SeTau-425-Maleimide	SH	water	425	4200	545	39	26.2
K7-545	SeTau-425-NHS	NH_2	BSA, D/P=1	427	4200	528	16	26.2
K7-547	SeTau-405-NHS	NH_2	IgG, D/P=8	405	13800	518	19	8.4
K7-548	SeTau-405-Maleimide	SH	EtOH	391	15000	498	32	8.5
K7-567	SeTau-405-Azide	triple-CC	EtOH	391	15000	498	55	8.5
K8-1342	Seta-670-NHS	NH_2	BSA, D/P=1.5	681	180000	696	27	2.4
K8-1352	Square-660-NHS	NH_2	BSA, D/P=1.2	676	182000	695	13	3.2
K8-1626	Seta-633-di-NHS	NH_2	IgG, D/P=1	639	192000	650	33	3.0
K8-1663	Seta-633-NHS	NH_2	IgG, D/P=1	637	250000	647	26	1.5
K9-4142	SeTau-647-di-NHS	NH_2	IgG, D/P=0.5	649	200000	691	58	3.2
K9-4148	SeTau-647-Maleimide	SH	PB 7.4	648	200000	692	45	3.2
K9-4149	SeTau-647-NHS	NH_2	IgG, D/P=1	648	200000	694	59	3.3
K9-4150	SeTau-647		PB 7.4	647	211000	693	59	3.1

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