SETA BioMedicals
http://www.setabiomedicals.com
e-mail: info@setabiomedicals.com

Lifetime Labels for Biomedical Applications

A study has shown that unlike open-chain polymethines dyes (such as **Cy5™** or **Alexa 647**) **Square** and **Seta** dyes exhibit shorter fluorescence lifetimes (FLTs) in aqueous media but increased brightness and up to **ten-fold** longer FLTs when bound to proteins (***Table 1***). Seta and Square labels are therefore perfect tracers in FLT-based biomedical applications, where e.g. the FLT-tracer would be labeled to a small antigen (ligand) displaying a short lifetime but showing a substantial FLT-increase upon binding to an antibody (receptor).

Table 1. Fluorescence decay times (τ) of selected **Seta** and **Square** dyes and dye-conjugates in water in comparison with Cy5™. Also listed is the change in lifetime (Δτ) between the free and protein-bond dyes.

Dye		Dye — BSA Conjugate		Δτ [ns] (Dye–conj. – Dye)
Number	τ_{mean} [ns]	D/P ratio[1]	τ_{mean} [ns]	
K8-1300	0.46	0.96	3.26	**2.80**
K8-1250	0.26	0.58	2.44	**2.18**
K8-1365	2.00	—	—	-
K8-1255	0.17	2.6	1.30	**1.13**
K8-1351	0.29	0.9	2.61	**2.32**
K8-1352	0.29	1.2	3.32	**3.09**
K8-1626	0.7	1.0	3.0	**2.70**
K8-1665	1.80	—	—	-
Cy5™	1.10	0.94	1.87	**0.77**

[1] Dye-to-protein ratio.

The frequency response curves (***Fig. 1***) also show that the FLTs of a series of SETA and Square dyes increases in the order: di-sulfto (**1**), tetra-sulfo (**10**), di-sulfo NH (**11**) squaraines.

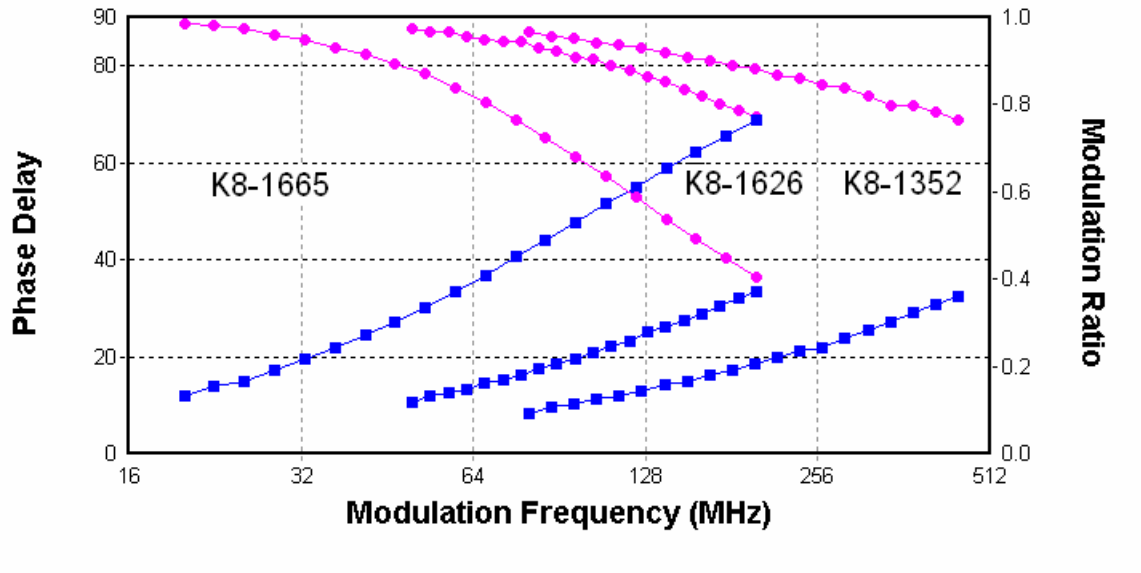

Fig. 1. Frequency response curves with FLTs of representative examples of **Square** or **Seta** dyes

Dyes such as K8-1665 or K8-1365 have exceptionally long lifetimes for cyanine dyes (1.8 – 2 ns in water), which make them good candidates as donors in energy transfer based assays assuring that even with large energy transfer the lifetimes of these probes are not too short to be measured accurately. The typical lifetimes of water-soluble cyanine dyes in water are in the order of 1 ns (**Cy5** or **Alexa 647**).

Example: **Lifetime-based Hybridization Assays**

We have investigated the performance of our labels for use in a fluorescence *in-situ* hybridization assay (***Fig. 2***). A 15-mer oligonucletide was labeled at the amino-modified 5-end with the NHS-ester **K8-1352** and the lifetime of this labeled oligo was determined to be 3.2 ns. Upon hybridization with the complementary strand the lifetime changes to 2.0 ns, a 40 % change in lifetime. These results demonstrate that these labels are very promising for use in lifetime-based assays and would lead to very robust assays requiring only one label.

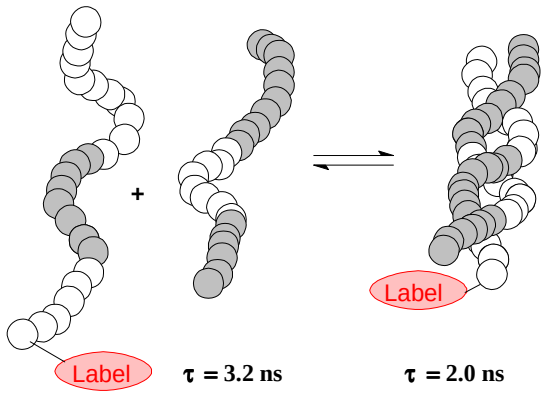

Fig. 2. Lifetime-based hybridization assays