Fluorescent Dyes for Labeling Peptides

Dye-labeled peptides are important tools in biochemical and cellular studies. Fluorescent peptides have been extensively used in fluorescence fluorimetry, fluorescence microscopy, fluorescence polarization spectroscopy, time-resolved fluorescence (TRF) and fluorescence resonance energy transfer (FRET). Fluorescent peptides participating in peptide-receptor interactions can be monitored to determine the location of receptors in cells or tissues, to allow quantification of receptors, to determine receptor affinity for various unknown ligands (drug screening), and to identify various populations of cells endowed with peptide receptors. FRET peptides are widely used for detecting the activities of proteases and protein kinases. Other applications include receptor sorting using fluorescence-associated cell sorting and the measurement of serum peptide levels using fluorescent immunoassays either *in vivo* or *in vitro* for research and diagnostic purposes. The most important characteristics of fluorescent peptides are high sensitivity and non-radioactive detection.

Selection of a Fluorescent Dye for Labeling Peptides

A fluorescent dye can be attached to a peptide at a specific point through a covalent bond depending on the sequence of peptide. The linkage between dye and peptide is a covalent bond, which is stable and not destructive under most biological conditions. In some cases, a functional linker is introduced between dye and peptide to minimize the alteration of peptide biological activity. For all the peptide labelings, the dye need be attached at a defined position: N-terminus, C-terminus, or in the middle of sequence.

In general, the preferred fluorescent labels should have high fluorescence quantum yields and retain the biological activities of the unlabeled biomolecules. AAT Bioquest offers a variety of fluorescent labeling reagents for facilitating the conjugation of dyes to peptides that are used for a variety of biological studies.

Coumarin Dyes

Coumarin dyes are predominantly used for preparing blue fluorescent peptides. Among them, 7-hydroxy, 7-alkoxy and 7-aminocoumarin dyes are more frequently used. AMCA might be the best blue fluorescent dye for labeling peptides due to its high fluorescence quantum yield and pH-insensitivity. Our Tide Flour™ 1 (TF1) is a water-soluble derivative of AMCA, and can be used for improving peptide water solubility if the labeling of a peptide by AMCA significantly decreases its water solubility. AMCA and TF1 have almost identical spectral properties. Besides AMCA and TF1, AAT Bioquest also offers other coumarin dyes for labeling peptides.

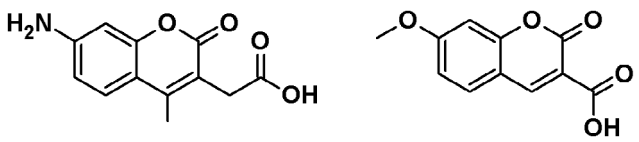

Figure 1.1. The chemical structures of AMCA acid (Cat# 501, left) and 7-methoxycou-marin-3-carboxylic acid (Cat# 560, right), the two most common coumarin dyes for labeling peptides.

Table 1.1 Coumarin Dyes for Labeling Peptides

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1} M^{-1}$)	CF @280 nm
501	AMCA Acid	25 mg	353	455	18,000	0.153
507	AMCA Alkyne	1 mg	353	455	19,000	0.153
508	AMCA Azide	1 mg	353	455	19,000	0.153
503	AMCA C2 Maleimide	5 mg	353	455	19,000	0.153
504	AMCA Ethylenediamine	5 mg	353	455	19,000	0.153
502	AMCA, Succinimidyl Ester	10 mg	353	455	19,000	0.153
505	DEAC [7-Diethylaminocoumarin-3-carboxylic acid]	100 mg	427	478	40,000	Not Determined
506	DEAC, SE [7-Diethylaminocoumarin-3-carboxylic acid, Succinimidyl Ester]	25 mg	427	478	40,000	Not Determined
554	7-Hydroxy-4-methylcoumarin-3-acetic Acid	100 mg	360	455	20,000	0.100
556	7-Hydroxy-4-methylcoumarin-3-acetic Acid, Succinimidyl Ester	25 mg	364	458	25,000	0.100
550	7-Hydroxycoumarin-3-carboxylic Acid	250 mg	387	448	25,000	0.0815
551	7-Hydroxycoumarin-3-carboxylic Acid, Succinimidyl Ester	50 mg	419	447	35,000	0.0815
552	7-Hydroxycoumarin-4-acetic Acid	100 mg	360	450	18,000	0.100
553	7-Hydroxycoumarin-4-acetic Acid, Succinimidyl Ester	25 mg	360	450	18,000	0.100
557	MCA [7-Methoxycoumarin-4-acetic Acid]	1 g	322	390	15,000	0.300
558	MCA Succinimidyl Ester [7-Methoxycoumarin-4-acetic Acid, Succinimidyl Ester]	25 mg	322	390	15,000	0.300
560	7-Methoxycoumarin-3-carboxylic Acid	1 g	336	402	20,000	0.171
563	7-Methoxycoumarin-3-carboxylic Acid, Succinimidyl Ester	100 mg	358	410	25,000	0.171

Fluorescein Dyes

Fluorescein derivatives are the most common fluorescent derivatization reagents for covalently labeling peptides. In addition to their high absorptivity, excellent fluorescence quantum yields and good water solubility, fluorescein derivatives have an excitation maximum (494 nm) that closely matches the 488 nm spectral line of the argon-ion laser, making them important fluorophores for fluorescence microscopy and flow cytometry applications. 5-FAM derivatives have been predominantly used for labeling peptides. AAT Bioquest offers the most complete product line of FAM for labeling peptides.

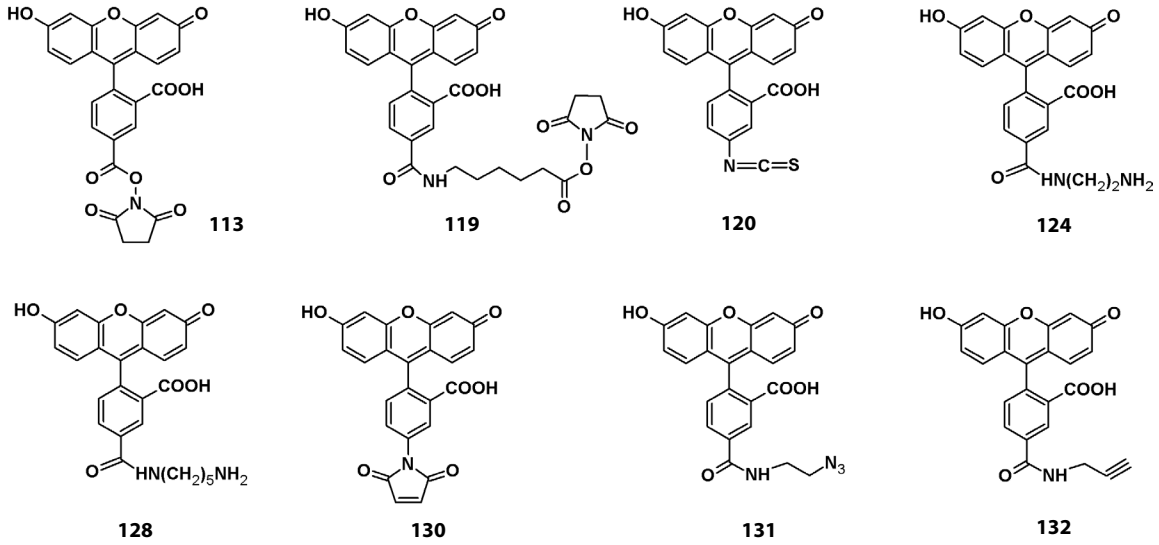

Figure 1.2. The chemical structures of typical fluorescein dyes for labeling peptides

Table Fluorescein Dyes for Labeling Peptides

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1}M^{-1}$)	CF @280 nm
103	5-FAM [5-Carboxyfluorescein] *Single Isomer*	100 mg	494	521	75,000	0.178
105	5-FAM [5-Carboxyfluorescein] *Validated for Labeling Peptides*	5 g	494	521	75,000	0.178
113	5-FAM, SE [5-Carboxyfluorescein, Succinimidyl Ester] *Single Isomer*	10 mg	494	521	75,000	0.178
115	5-FAM, SE [5-Carboxyfluorescein, Succinimidyl Ester] *Validated for Labeling Peptides*	1 g	494	521	75,000	0.178
132	5-FAM Alkyne	10 mg	494	521	75,000	0.178
131	5-FAM Azide	10 mg	494	521	75,000	0.178
128	5-FAM Cadaverine	100 mg	494	521	75,000	0.178
124	5-FAM Ethylenediamine	100 mg	494	521	75,000	0.178
119	5-FAM-X, SE	5 mg	494	521	75,000	0.178
100	5(6)-FAM [5-(and-6)-Carboxyfluorescein] *Mixed Isomers*	1 g	494	519	75,000	0.172
101	5(6)-FAM [5-(and-6)-Carboxyfluorescein] *Validated for Labeling Peptides and Oligos*	10 g	494	519	75,000	0.172
110	5(6)-FAM, SE [5-(and-6)-Carboxyfluorescein, Succinimidyl Ester] *Mixed Isomers*	25 mg	494	519	75,000	0.172
112	5(6)-FAM, SE [5-(and-6)-Carboxyfluorescein, Succinimidyl Ester] *Validated for Labeling Peptides and Oligos*	1 g	494	519	75,000	0.172
127	5(6)-FAM Cadaverine	100 mg	494	519	75,000	0.172
123	5(6)-FAM Ethylenediamine	100 mg	494	519	75,000	0.172
120	5-FITC [FITC Isomer I; Fluorescein-5-isothiocyanate] *UltraPure Grade*	100 mg	492	515	76,000	0.254
121	5-FITC [FITC Isomer I; Fluorescein-5-isothiocyanate] *UltraPure Grade*	1 g	492	515	76,000	0.254
130	Fluorescein-5-maleimide	25 mg	491	514	74,000	0.275

Rhodamine Dyes

Rhodamine dyes are supplements to fluoresceins as they offer longer wavelength emission maxima and provide opportunities for multicolor labeling and staining. Rhodamines exhibit much higher photostability than fluoresceins and coumarins. Carboxytetramethylrhodamine (TAMRA) is a common fluorophore for preparing peptide conjugates. Sulforhodamine 101 sulfonyl chloride (Texas Red®) is another popular peptide labeling dye. However, Texas Red® is unstable and gives much lower coupling yield than other carboxyrhodamine dyes (such as 5-TAMRA, SE). AAT Bioquest has recently developed California Red™, a replacement superior to Texas Red®. California Red™ has the spectral properties almost identical to those of Texas Red® and similar water solubility. However, California Red™ is much more stable and gives much higher conjugation yield than Texas Red® (See Table 1.4).

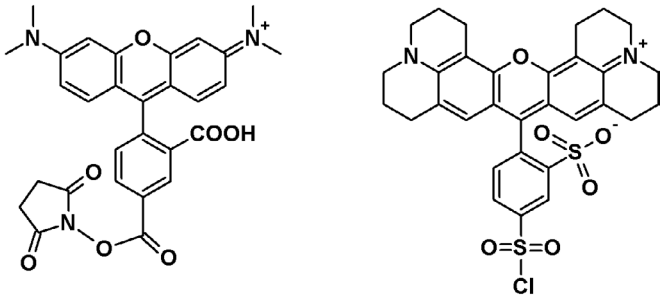

Figure 1.3. The chemical structures of 5-TAMRA, SE (Cat# 373, left) and Texas Red® (Cat# 480, right).

Table 1.3 Rhodamine Dyes for Labeling Peptides

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1} M^{-1}$)	CF @280 nm
473	California Red™, SE	5 mg	583	603	100,000	0.360
322	5-CR110 [5-Carboxyrhodamine 110] *Single Isomer*	5 mg	498	521	75,000	0.091
351	5-CR110, SE [5-Carboxyrhodamine 110, Succinimidyl Ester] *Single Isomer*	5 mg	498	521	80,000	0.091
331	5-CR6G [5-Carboxyrhodamine 6G] *Single Isomer*	10 mg	518	544	80,000	0.214
341	5-CR6G, SE [5-Carboxyrhodamine 6G, Succinimidyl Ester] *Single Isomer*	1 mg	524	556	80,000	0.214
320	5(6)-CR110 [5-(and 6)-Carboxyrhodamine 110] *Mixed Isomers*	100 mg	498	521	75,000	0.091
350	5(6)-CR110, SE [5-(and 6)-Carboxyrhodamine 110, succinimidyl ester] *Mixed isomers*	5 mg	498	521	78,000	0.091
330	5(6)-CR6G [5-(and 6)-Carboxyrhodamine 6G]	25 mg	519	544	80,000	0.214
340	5(6)-CR6G, SE [5-(and 6)-Carboxyrhodamine 6G, Succinimidyl Ester] *Mixed Isomers*	10 mg	522	550	86,000	0.214
470	Lissamine Rhodamine B Sulfonyl Chloride [Sulforhodamine B Sulfonyl Chloride]	100 mg	568	583	85,000	0.171
381	5-ROX [5-Carboxy-X-rhodamine] *Single Isomer*	5 g	567	591	86,000	0.168
391	5-ROX, SE [5-Carboxy-X-rhodamine, Succinimidyl Ester] *Single Isomer*	100 mg	573	602	95,000	0.168
380	5(6)-ROX [5-(and 6)-Carboxy-X-rhodamine] *Mixed Isomers*	100 mg	568	591	85,000	0.168
390	5(6)-ROX, SE [5-(and-6)-Carboxy-X-rhodamine, Succinimidyl Ester]	25 mg	576	601	93,000	0.168
480	Sulforhodamine 101 Sulfonyl Chloride [Also known as *Texas Red®*]	10 mg	588	601	100,000	0.360
363	5-TAMRA [5-Carboxytetramethylrhodamine] *Single Isomer*	10 mg	541	568	75,000	0.178
365	5-TAMRA [5-Carboxytetramethylrhodamine] *Validated for Labeling Peptides*	1 g	541	568	75,000	0.178
373	5-TAMRA, SE [5-Carboxytetramethylrhodamine, Succinimidyl Ester] *Single Isomer*	5 mg	547	575	78,000	0.178
375	5-TAMRA, SE [5-Carboxytetramethylrhodamine, Succinimidyl Ester] *Validated for Labeling Peptides*	1 g	547	575	78,000	0.178
360	5(6)-TAMRA [5-(and-6)-Carboxytetramethylrhodamine] *Mixed Isomers*	100 mg	541	565	75,000	0.187
362	5(6)-TAMRA [5-(and-6)-Carboxytetramethylrhodamine] *Mixed Isomers*	5 g	541	565	75,000	0.187
370	5(6)-TAMRA, SE [5-(and-6)-Carboxytetramethylrhodamine, Succinimidyl Ester] *Mixed Isomers*	25 mg	546	575	77,000	0.187
372	5(6)-TAMRA, SE [5-(and-6)-Carboxytetramethylrhodamine, Succinimidyl Ester] *Mixed Isomers*	1 g	546	575	77,000	0.187
485	Texas Red® Alkyne [TR Alkyne]*Single Isomer*	5 mg	588	601	95,000	0.360
484	Texas Red® Azide [TR Azide]*Single Isomer*	5 mg	588	601	95,000	0.360

California Red™ and SunRed™, Superior Replacements for Texas Red® and Texas Red®-X for Labeling Peptides

Although sulforhodamine 101 acid chloride (also called Texas Red®) is the most popular labeling reagent of sulfonyl chloride, it is quite unstable in water, especially at the higher pH required for reaction with aliphatic amines. Texas Red® reacts with both aliphatic amines and aromatic amines indiscriminately. In addition, the labeling efficiency of Texas Red® is extremely low compared to dye succinimidyl esters. California Red™ SE is a succinimidyl ester. It is an excellent replacement for Texas Red®. California Red™ reacts with amine compounds such as amino acids, peptides and proteins to give bright red fluorescent conjugates that are extremely stable. Compared to Texas Red®, California Red™ has much higher labeling efficiency, and more importantly, the resulted conjugates are more fluorescent than the corresponding Texas Red® conjugates for long peptides. The conjugates of California Red™ have the identical excitation and emission wavelengths to those of Texas Red®. Our in-house studies indicated that California Red™ is more stable than Texas Red® under the same labeling conditions.

SunRed™ has even better water solubility than Texas Red®, Texas Red®-X and California Red™. It is extremely useful for labeling hydrophobic peptides that are often poorly labeled by Texas Red® or Texas Red®-X. The conjugates of hydrophobic peptides with Texas Red® are difficult to use for measuring biological activity assays due to their poor water solubility.

Features and Benefits of California Red™ and SunRed™

- *Spectral properties almost identical to those of Texas Red®*
- *Fluorescence less-quenched on proteins than Texas Red®*
- *More stable than Texas Red®*
- *Higher conjugation yield*

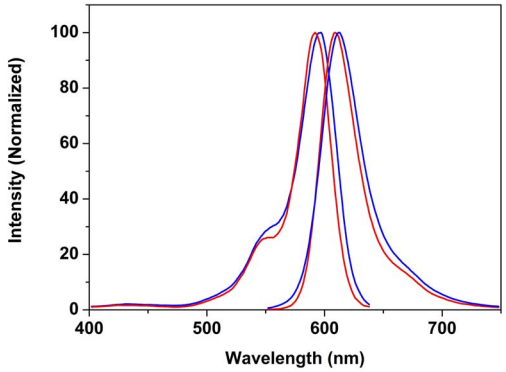

Figure 1.4. Spectral comparison of California Red™ and Texas Red® conjugated to Gly-Gly-Sor-Ser-Arg-Trp (Red: Texas Red®; Blue: California Red™).

Table 1.4 Comparison of California Red™ and SunRed™ with Texas Red®

Dye Properties	California Red™	SunRed™	Texas Red®
Maximum Absorption Wavelength (nm)	595	595	594*
Maximum Fluorescence Wavelength (nm)	615	615	613*
Extinction Coefficient ($cm^{-1}M^{-1}$)	100,000	100,000	100,000
Purity	Single Isomer	Single Isomer	Mixture of 3 Isomers
Reactive Group	NHS Ester	NHS Ester	Sulfonyl Chloride
Water Solubility (pH 7.0)	<1 mg/mL	>10 mg/mL	<1 mg/mL
Conjugation Yield (after HPLC Purification)**	76%	71%	26%

* Glycine conjugate; ** Based on the reaction with Gly-Gly-Ser-Ser-Arg-Trp.

Table 1.5 Superior Replacement Dyes for Texas Red®

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1}M^{-1}$)	CF @280 nm
473	California Red™, SE	5 mg	583	603	100,000	0.360
480	Sulforhodamine 101 Sulfonyl Chloride [also known as Texas Red®]	10 mg	588	601	100,000	0.360
472	SunRed™, SE	5 mg	583	603	98,000	0.366
485	Texas Red® Alkyne *Single Isomer*	5 mg	588	601	95,000	0.360
484	Texas Red® Azide *Single Isomer*	5 mg	588	601	95,000	0.360
482	Texas Red® Cadaverine *Single Isomer*	5 mg	582	602	95,000	0.360
481	Texas Red® Hydrazide *Single Isomer*	5 mg	582	602	95,000	0.360
483	Texas Red® Maleimide *Single Isomer*	5 mg	588	601	95,000	0.360

Cyanine Dyes

As fluorescent dyes, cyanine dyes have many uses, particularly in biomedical imaging. Depending on the structures, they cover the visible and IR portion of the spectrum. Cy3®, Cy5® and Cy7® are the most popular cyanine dyes. Cy3® has orange fluorescence (~550/570 nm), while Cy5® is fluorescent in the red region (~650/670 nm). Cy3® and Cy5® are typically combined for 2- color detection. They are usually synthesized with reactive groups on either one or both of the nitrogen side chains so that they can be chemically linked to either nucleic acids or protein molecules. Labeling is done for visualization and quantification purposes. Cy3® and Cy5® are used in a wide variety of biological applications including comparative genomic hybridization and gene chips, which are used in transcriptomics. They are also used to label proteins and nucleic acids for various studies including proteomics and RNA localization.

Caution must be exercised when selecting a cyanine dye. In general, Cy3®, Cy5®, Cy5.5® and Cy7® are all referred as the sulfonated cyanine dyes (Figure 1.6) in the literature. However, some vendors offer the less expensive non-sulfonated cyanines (Figure 1.5) as replacements for the Cy3®, Cy5®, Cy5.5® and Cy7® (sulfonated) cited in the literature. The widely used sulfonated cyanine dyes (known as Cy3®, Cy5®, Cy5.5® and Cy7®) should *not* be exchanged with the less expensive non-sulfonated cyanine dyes due to the drastically different properties. The sulfonated cyanine dyes have much higher fluorescence quantum yield than the non-sulfonated cyanines (Table 1.6) in aqueous solutions. The sulfonated cyanine dyes are highly water-soluble while the non-sulfonated cyanines are difficult to be dissolved in water.

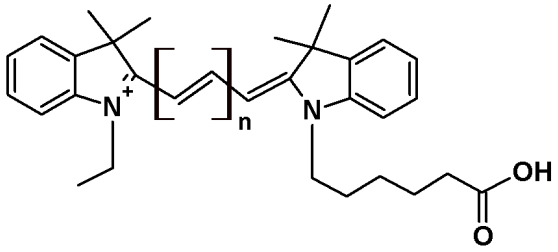

Figure 1.5a. The chemical structures of typical non-sulfonated cyanine dyes for labeling peptides (Cy3NS: n=1; Cy5NS: n=2; Cy7NS: n=3).

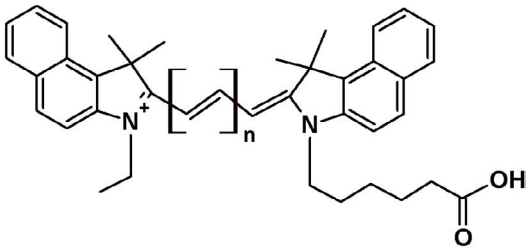

Figure 1.5b. The chemical structures of typical non-sulfonated cyanine dyes for labeling peptides (Cy3.5NS: n=1; Cy5.5NS: n=2; Cy7.5NS: n=3).

Table 1.6 Spectral Comparison of Cyanine Dyes

Product Name	Ex (nm)	Em (nm)	EC ($cm^{-1} M^{-1}$)	Quantum Yield
Cy3® (Sulfonated)	555	565	150,000	0.10
Cy3NS (Non-Sulfonated)	549	565	145,000	0.07
Cy5® (Sulfonated)	649	664	250,000	0.25
Cy5NS (Non-Sulfonated)	644	665	230,000	0.16
Cy5.5® (Sulfonated)	676	695	250,000	0.18
Cy5.5NS (Non-Sulfonated)	675	697	230,000	Not Determined
Cy7® (Sulfonated)	749	775	275,000	0.12
Cy7NS (Non-Sulfonated)	750	780	250,000	0.02

Table 1.7 Non-Sulfonated Cyanine Dyes for Labeling Peptides

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1} M^{-1}$)	CF @280 nm
190	Cy3NS Acid	100 mg	549	565	145,000	0.073
191	Cy3NS, Succinimidyl Ester	25 mg	549	565	145,000	0.073
194	Cy5NS Acid	100 mg	644	665	230,000	0.030
195	Cy5NS, Succinimidyl Ester	25 mg	644	665	230,000	0.030
197	Cy7NS Acid	100 mg	750	780	250,000	0.036
198	Cy7NS, Succinimidyl Ester	25 mg	750	780	250,000	0.036
181	ICG-ATT [3-ICG-acyl-1,3-thiazolidine-2-thione]	1 mg	780	800	240,000	0.076
182	ICG-OSu	1 mg	780	800	240,000	0.076
180	ICG-Sulfo-OSu	1 mg	780	800	240,000	0.076

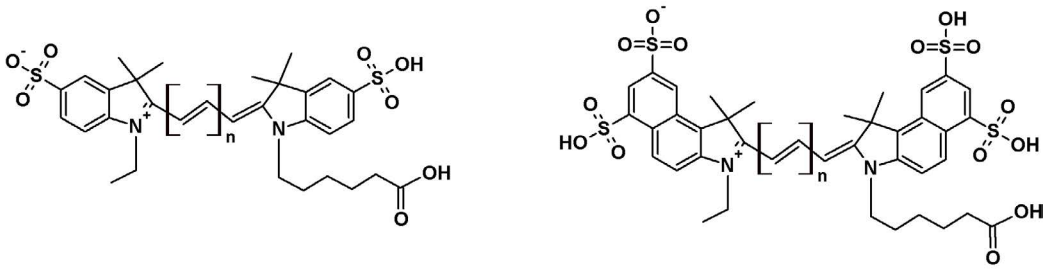

Figure 1.6. The chemical structures of sulfonated cyanine dyes for labeling peptides. (Left: Cy3: n=1; Cy5: n=2; Cy7: n=3. Right: Cy3.5: n=1; Cy5.5: n= 2; Cy7.5: n=3).

Table 1.8 Sulfonated Cyanine Dyes for Labeling Peptides

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1} M^{-1}$)	CF @280 nm
144	Cyanine 3 Alkyne [equivalent to Cy3® Alkyne]	1 mg	555	565	150,000	0.073
145	Cyanine 3 Amine [equivalent to Cy3® Amine]	1 mg	555	565	150,000	0.073
143	Cyanine 3 Azide [equivalent to Cy3® Azide]	1 mg	555	565	150,000	0.073
146	Cyanine 3 Hydrazide [equivalent to Cy3® Hydrazide]	1 mg	555	565	150,000	0.073
142	Cyanine 3 Maleimide [equivalent to Cy3® Maleimide]	1 mg	555	565	150,000	0.073
140	Cyanine 3 Monoacid [equivalent to Cy3® Acid]	5 mg	555	565	150,000	0.073
141	Cyanine 3, Monosuccinimidyl Ester [equivalent to Cy3® NHS Ester]	1 mg	555	565	150,000	0.073
139	Cyanine 3.5 Amine [equivalent to Cy3.5® Amine]	1 mg	581	596	125,000	0.178
149	Cyanine 3.5 Maleimide [equivalent to Cy3.5® Maleimide]	1 mg	581	596	125,000	0.178
148	Cyanine 3.5, Monosuccinimidyl Ester [equivalent to Cy3.5® NHS Ester]	1 mg	581	596	125,000	0.178
154	Cyanine 5 Alkyne [equivalent to Cy5® Alkyne]	1 mg	649	665	250,000	0.030
155	Cyanine 5 Amine [equivalent to Cy5® Amine]	1 mg	649	665	250,000	0.030
153	Cyanine 5 Azide [equivalent to Cy5® Azide]	1 mg	649	665	250,000	0.030
156	Cyanine 5 Hydrazide [equivalent to Cy5® Hydrazide]	1 mg	649	665	250,000	0.030
152	Cyanine 5 Maleimide [equivalent to Cy5® Maleimide]	1 mg	649	665	250,000	0.030
150	Cyanine 5 Monoacid [equivalent to Cy5® Acid]	5 mg	649	665	250,000	0.030
151	Cyanine 5, Monosuccinimidyl Ester [equivalent to Cy5® NHS Ester]	1 mg	649	665	250,000	0.030
179	Cyanine 5.5 Alkyne [equivalent to Cy5.5® Alkyne]	1 mg	678	701	230,000	0.101
176	Cyanine 5.5 Amine [equivalent to Cy5.5® Amine]	1 mg	678	701	230,000	0.101
178	Cyanine 5.5 Azide [equivalent to Cy5.5® Azide]	1 mg	678	701	230,000	0.101
177	Cyanine 5.5 Hydrazide [equivalent to Cy5.5® Hydrazide]	1 mg	678	701	230,000	0.101
175	Cyanine 5.5 Maleimide [equivalent to Cy5.5® Maleimide]	1 mg	678	701	230,000	0.101
173	Cyanine 5.5 Monoacid [equivalent to Cy5.5® Acid]	5 mg	678	701	230,000	0.101
174	Cyanine 5.5, Monosuccinimidyl Ester [equivalent to Cy5.5® NHS Ester]	1 mg	678	701	230,000	0.101
164	Cyanine 7 Alkyne [equivalent to Cy7® Alkyne]	1 mg	749	776	275,000	0.036
165	Cyanine 7 Amine [equivalent to Cy7® Amine]	1 mg	749	776	275,000	0.036
163	Cyanine 7 Azide [equivalent to Cy7® Azide]	1 mg	749	776	275,000	0.036
166	Cyanine 7 Hydrazide [equivalent to Cy7® Hydrazide]	1 mg	749	776	275,000	0.036
162	Cyanine 7 Maleimide [equivalent to Cy7® Maleimide]	1 mg	749	776	275,000	0.036
160	Cyanine 7 Monoacid [equivalent to Cy7® Acid]	5 mg	749	776	275,000	0.036
161	Cyanine 7, Monosuccinimidyl Ester [equivalent to Cy7® NHS Ester]	1 mg	749	776	275,000	0.036

Tide Fluor™ Dyes Optimized for Labeling Peptides

Although EDANS, FAM, TAMRA, ROX, Cy3® and Cy5® have been widely used to develop a variety of peptide probes, there are still some limitations in the use of these dyes. For example, the weak absorption and environment-sensitive fluorescence of EDANS have severely limited its sensitivity for developing protease assays and nucleic acid detection probes. Compared to EDANS, fluorescein-based probes (such as FAM, HEX, JOE and TET) have stronger absorption and fluorescence. However, the fluorescence of fluorescein-based probes is strongly pH dependent. They only exhibit the strongest fluorescence at higher pH. The pH dependence makes the fluorescein-based fluorescent probes inconvenient for the assays that require low pH. In addition, most of fluorescein-based probes have quite low photostability, which limits their applications in fluorescence imaging.

Table 1.9 Tide Fluor™ Dye Equivalents of Common Dyes

If you are using	Try this Tide Fluor™ dye
Alexa Fluor® 350, AMCA, DyLight™ 350	TF1 [Tide Fluor™ 1]
Alexa Fluor® 488, Cy2®, FITC, DyLight™ 488	TF2 [Tide Fluor™ 2]
Alexa Fluor® 555, Cy3®, DyLight™ 550, TRITC	TF3 [Tide Fluor™ 3]
	TF3WS [Tide Fluor™ 3WS]
Alexa Fluor® 594, DyLight™ 594, Texas Red®	TF4 [Tide Fluor™ 4]
Alxea Fluor® 647, Cy5®, DyLight™ 650	TF5WS [Tide Fluor™ 5WS]
Alexa Fluor® 680, Cy5.5®, IRDye® 700, DyLight™ 680	TF6WS [Tide Fluor™ 6WS]
Alexa Fluor® 750, Cy7®, DyLight™ 750	TF7WS [Tide Fluor™ 7WS]
Alexa Fluor® 790, DyLight™ 800, IRDye® 800	TF8WS [Tide Fluor™ 8WS]

Among cyanine dyes, non-sulfonated Cy3® and Cy5® are occasionally used for developing a variety of peptide probes, but they have quite low fluorescence quantum yields in aqueous media. The sulfonated Cy3® and Cy5® have improved fluorescence quantum yields. Some Alexa Fluor™ dyes (e.g. Alexa Fluor® 555, 647, 680, 700 and 750) are sulfonated cyanine dyes. However, they are extremely expensive. It's unpractical to use them for preparing peptide conjugates in some cases.

To address these limitations, AAT Bioquest has developed Tide Fluor™ donor dyes that have almost identical spectral properties to those of Alexa Fluor® dyes. They are optimized as building blocks for developing FRET oligonucleotides and peptides for a variety of biological applications. We recommend you try our Tide Fluor™ dyes at much lower cost with comparable performance.

Our Tide Fluor™ dyes (such as TF1, TF2, TF3, TF4, TF5, TF6, TF7 and TF8) have stronger fluorescence and higher photostability than the typical fluorophores such as fluoresceins, rhodamines and cyanines described above. Our TF2 has the similar excitation and emission wavelengths to those of carboxyfluoresceins (FAM), making them readily used for the biological applications that are done with fluoresceins. Compared to FAM probes, TF2 has much str onger fluorescence at physiological conditions, and it is much more photostable. Compared to other fluorescent dyes alternative to fluoresceins and Cy® dyes (such as Alexa Fluor™ and DyLight® dyes), Tide Fluor™ dyes are much more cost-effective with comparable or even better performance for your desired biological applications. On peptides, TF3 is much brighter and more photostable than Cy3®, Alexa Fluor® 555 and DyLight™ 555, although TF3 has almost identical spectra to those of the three dyes (Figure 1.7).

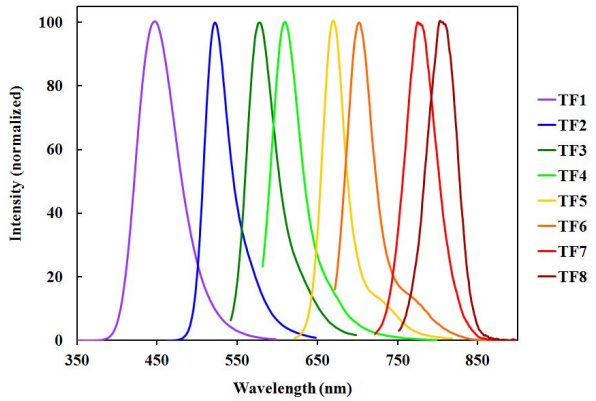

Figure 1.7. The normalized fluorescence spectra of Tide Fluor™ dyes.

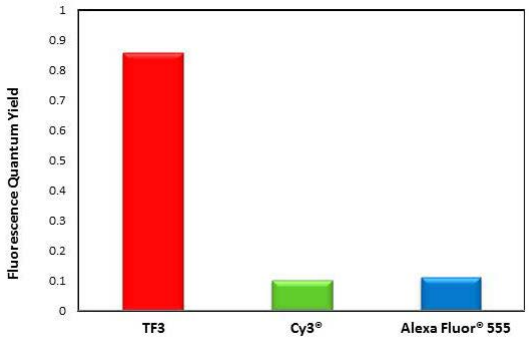

Figure 1.8. Fluorescence quantum yield comparison of TF3, Cy3® and Alexa Fluor® 555 on the peptide of Dye-PLSRTLSVAAKK-NH_2.

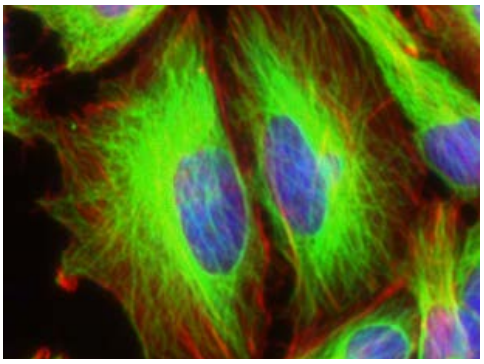

Figure 1.9. Image of HeLa cells. Tublins were stained with mouse anti-tubulin followed with iFluor™ 488 Goat Anti-Mouse IgG (green, Cat# 16448), actin filaments were stained with TF3- Phalloidin Conjugate (red, Cat# 23119), and nuclei were stained with Hoechst 33342 (blue).

Table 1.10 Tide Fluor™ Dyes, a Full Spectrum of Fluorescent Dyes to Replace Alexa Fluor® Dyes

Tide Fluor™ Donor	Ex (nm)	Em (nm)	Features and Benefits	Ordering Information
Tide Fluor™ 1 (TF1)	345	442	Alternative to EDANS • *Much stronger absorption* • *Much stronger fluorescence* • *Less environmental sensitivity*	Cat# 2236 (TF1 azide, click chemistry) Cat# 2237 (TF1 alkyne, click chemistry) Cat# 2238 (TF1 acid) Cat# 2239 (TF1 amine) Cat# 2242 (TF1 maleimide, SH-reactive) Cat# 2244 (TF1 SE, NH_2-reactive)
Tide Fluor™ 2 (TF2)	500	527	Alternative to FAM, FITC and Alexa Fluor® 488 • *pH-insensitive fluorescence* • *Good photostability*	Cat# 2245 (TF2 acid) Cat# 2246 (TF2 amine) Cat# 2247 (TF2 maleimide, SH-reactive) Cat# 2248 (TF2 SE, NH_2-reactive) Cat# 2252 (TF2 azide, click chemistry) Cat# 2253 (TF2 alkyne, click chemistry)
Tide Fluor™ 2WS (TF2WS)	502	525	Alternative to Alexa Fluor® 488 • *pH-insensitive fluorescence* • *Good photostability*	Cat# 2348 (TF2WS acid) Cat# 2349 (TF2WS SE, NH_2-reactive)
Tide Fluor™ 3 (TF3)	555	584	Alternative to Cy3® and Alexa Fluor® 555 • *Strong fluorescence* • *Good photostability*	Cat# 2254 (TF3 azide, click chemistry) Cat# 2255 (TF3 alkyne, click chemistry) Cat# 2268 (TF3 acid) Cat# 2269 (TF3 amine) Cat# 2270 (TF3 maleimide, SH-reactive) Cat# 2271 (TF3 SE, NH_2-reactive)
Tide Fluor™ 3WS (TF3WS)	555	565	Alternative to Cy3® and Alexa Fluor® 555 • *Strong fluorescence* • *Good photostability*	Cat# 2345 (TF3WS acid) Cat# 2346 (TF3WS SE, NH_2-reactive)
Tide Fluor™ 4 (TF4)	590	618	Alternative to ROX, Texas Red® and Alexa Fluor® 594 • *Strong fluorescence* • *Good photostability*	Cat# 2285 (TF4 acid) Cat# 2286 (TF4 amine) Cat# 2287 (TF4 maleimide, SH-reactive) Cat# 2289 (TF4 SE, NH_2-reactive) Cat# 2300 (TF4 azide, click chemistry) Cat# 2301 (TF4 alkyne, click chemistry)
Tide Fluor™ 5WS (TF5WS)	649	664	Alternative to Cy5® and Alexa Fluor® 647 • *Strong fluorescence* • *Good photostability*	Cat# 2275 (TF5WS azide, click chemistry) Cat# 2276 (TF5WS alkyne, click chemistry) Cat# 2278 (TF5WS, acid) Cat# 2279 (TF5WS amine) Cat# 2280 (TF5WS maleimide, SH-reactive) Cat# 2281 (TF5WS SE, NH_2-reactive)
Tide Fluor™ 6WS (TF6WS)	676	695	Alternative to Cy5.5®, IRDye® 700 and Alexa Fluor® 680 • *Strong fluorescence* • *Photostable*	Cat# 2291 (TF6WS acid) Cat# 2292 (TF6WS amine) Cat# 2293 (TF6WS maleimide, SH-reactive) Cat# 2294 (TF6WS SE, NH_2-reactive) Cat# 2302 (TF6WS azide, click chemistry) Cat# 2303 (TF6WS alkyne, click chemistry)
Tide Fluor™ 7WS (TF7WS)	749	775	Alternative to Cy7® and Alexa Fluor® 750 • *Strong fluorescence* • *Good photostability*	Cat# 2304 (TF7WS azide, click chemistry) Cat# 2305 (TF7WS alkyne, click chemistry) Cat# 2330 (TF7WS acid) Cat# 2331 (TF7WS amine) Cat# 2332 (TF7WS maleimide, SH-reactive) Cat# 2333 (TF7WS SE, NH_2-reactive)
Tide Fluor™ 8WS (TF8WS)	775	807	Alternative to IRDye® 800 • *Stronger fluorescence* • *Higher Photostability*	Cat# 2306 (TF8WS azide, click chemistry) Cat# 2307 (TF8WS alkyne, click chemistry) Cat# 2335 (TF8WS acid) Cat# 2336 (TF8WS amine) Cat# 2337 (TF8WS maleimide, SH-reactive) Cat# 2338 (TF8WS SE, NH_2-reactive)

Table 1.11 Tide Fluor™ Dyes for Labeling Peptides

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1} M^{-1}$)	CF @280 nm
2238	Tide Fluor™ 1 Acid [TF1 Acid] *Superior Replacement for EDANS*	100 mg	345	442	20,000	0.187
2237	Tide Fluor™ 1 Alkyne [TF1 Alkyne] *Superior Replacement for EDANS*	5 mg	345	442	20,000	0.187
2239	Tide Fluor™ 1 Amine [TF1 Amine] *Superior Replacement for EDANS*	5 mg	345	442	20,000	0.187
2236	Tide Fluor™ 1 Azide [TF1 Azide] *Superior Replacement for EDANS*	5 mg	345	442	20,000	0.187
2348	Tide Fluor™ 2WS Acid [TF2WS Acid] *Superior Replacement for FITC*	10 mg	502	525	75,000	0.091
2349	Tide Fluor™ 2WS, Succinimidyl Ester [TF2WS, SE] *Superior Replacement for FITC*	5 mg	502	525	75,000	0.091
2345	Tide Fluor™ 3WS Acid [TF3WS Acid] *Superior Replacement for Cy3®*	10 mg	555	565	150,000	0.079
2346	Tide Fluor™ 3WS, Succinimidyl Ester [TF3WS, SE] *Superior Replacement for Cy3®*	5 mg	555	565	150,000	0.079
2285	Tide Fluor™ 4 Acid [TF4 Acid] *Superior Replacement for ROX and Texas Red®*	10 mg	590	618	90,000	0.436
2301	Tide Fluor™ 4 Alkyne [TF4 Alkyne] *Superior Replacement for ROX and Texas Red®*	1 mg	590	618	90,000	0.436
2286	Tide Fluor™ 4 Amine [TF4 Amine] *Superior Replacement for ROX and Texas Red®*	1 mg	590	618	90,000	0.436
2300	Tide Fluor™ 4 Azide [TF4 Azide] *Superior Replacement for ROX and Texas Red®*	1 mg	590	618	90,000	0.436
2287	Tide Fluor™ 4 Maleimide [TF4 Maleimide] *Superior Replacement for ROX and Texas Red®*	1 mg	590	618	90,000	0.436
2289	Tide Fluor™ 4, Succinimidyl Ester [TF4, SE] *Superior Replacement for ROX and Texas Red®*	5 mg	590	618	90,000	0.436
2278	Tide Fluor™ 5WS Acid [TF5WS Acid] *Superior Replacement for Cy5®*	10 mg	649	664	250,000	0.027
2276	Tide Fluor™ 5WS Alkyne [TF5WS Alkyne] *Superior Replacement for Cy5®*	1 mg	649	664	250,000	0.027
2279	Tide Fluor™ 5WS Amine [TF5WS Amine] *Superior Replacement for Cy5®*	1 mg	649	664	250,000	0.027
2275	Tide Fluor™ 5WS Azide [TF5WS Azide] *Superior Replacement for Cy5®*	1 mg	649	664	250,000	0.027
2280	Tide Fluor™ 5WS Maleimide [TF5WS Maleimide] *Superior Replacement for Cy5®*	1 mg	649	664	250,000	0.027
2281	Tide Fluor™ 5WS, Succinimidyl Ester [TF5WS, SE] *Superior Replacement for Cy5®*	5 mg	649	664	250,000	0.027
2291	Tide Fluor™ 6WS Acid [TF6WS Acid] *Superior Replacement for Cy5.5®*	10 mg	676	695	220,000	0.101
2303	Tide Fluor™ 6WS Alkyne [TF6WS Alkyne] *Superior Replacement for Cy5.5®*	1 mg	676	695	220,000	0.101
2292	Tide Fluor™ 6WS Amine [TF6WS Amine] *Superior Replacement for Cy5.5®*	1 mg	676	695	220,000	0.101
2302	Tide Fluor™ 6WS Azide [TF6WS Azide] *Superior Replacement for Cy5.5®*	1 mg	676	695	220,000	0.101
2293	Tide Fluor™ 6WS Maleimide [TF6WS Maleimide] *Superior Replacement for Cy5.5®*	1 mg	676	695	220,000	0.101
2294	Tide Fluor™ 6WS, Succinimidyl Ester [TF6WS, SE] *Superior Replacement for Cy5.5®*	1 mg	676	695	220,000	0.101
2330	Tide Fluor™ 7WS Acid [TF7WS Acid] *Superior Replacement for Cy7®*	10 mg	749	775	275,000	0.049
2305	Tide Fluor™ 7WS Alkyne [TF7WS Alkyne] *Superior Replacement for Cy7®*	1 mg	749	775	275,000	0.049
2331	Tide Fluor™ 7WS Amine [TF7WS Amine] *Superior Replacement for Cy7®*	1 mg	749	775	275,000	0.049
2304	Tide Fluor™ 7WS Azide [TF7WS Azide] *Superior Replacement for Cy7®*	1 mg	749	775	275,000	0.049
2332	Tide Fluor™ 7WS Maleimide [TF7WS Maleimide] *Superior Replacement for Cy7®*	1 mg	749	775	275,000	0.049
2333	Tide Fluor™ 7WS, Succinimidyl Ester [TF7WS, SE] *Superior Replacement for Cy7®*	1 mg	749	775	275,000	0.049
2335	Tide Fluor™ 8WS Acid [TF8WS Acid] *Near Infrared Emission*	10 mg	775	807	250,000	0.109
2307	Tide Fluor™ 8WS Alkyne [TF8WS Alkyne] *Near Infrared Emission*	1 mg	775	807	250,000	0.109
2336	Tide Fluor™ 8WS Amine [TF8WS Amine] *Near Infrared Emission*	1 mg	775	807	250,000	0.109
2306	Tide Fluor™ 8WS Azide [TF8WS Azide] *Near Infrared Emission*	1 mg	775	807	250,000	0.109
2337	Tide Fluor™ 8WS Maleimide [TF8WS Maleimide] *Near Infrared Emission*	1 mg	775	807	250,000	0.109
2338	Tide Fluor™ 8WS, Succinimidyl Ester [TF8WS, SE] *Near Infrared Emission*	1 mg	775	807	250,000	0.109

Other Dyes

EDANS dyes are quite useful for preparing fluorescent peptides. They might be the first group of fluorescent dyes used for preparing FRET peptides to analyze a broad range of protease activities. AAT Bioquest offers a variety of EDANS derivatives for preparing FRET peptide substrates.

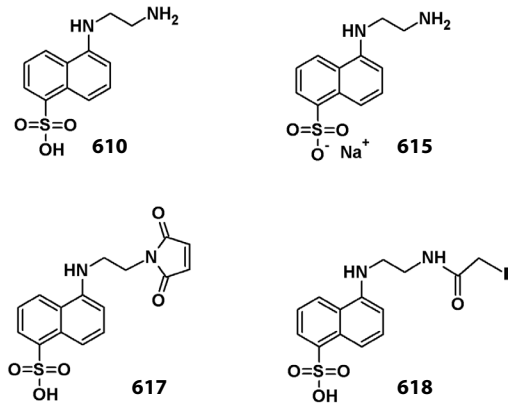

Figure 1.10. The chemical structures of EDANS dyes.

Besides the popular fluorescent dyes such as coumarins, fluoresceins, rhodamines, cyanines and EDANS dyes, AAT Bioquest also offers some fluorescent dyes that are ocassionally used for labeling peptides. Our Bodi Fluor™ dyes are chemically the same as the Bodipy® dyes offered by Invitrogen, e.g., Bodi Fluor™ 488 is equivalent to Bodipy® FL. Bodi Fluor™ 488 is highly fluorescent, and often used as an alternative to fluorescein since its fluorescence is not pH-dependent. The sharper excitation and emission peaks of Bodi Fluor™ 488 make it better suitable for multi-color fluorescence imaging applications.

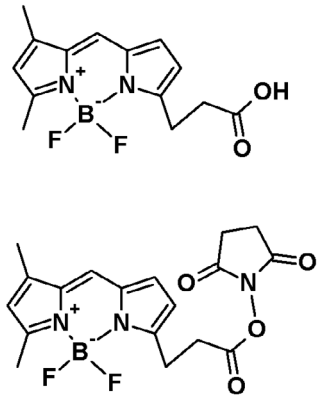

Figure 1.11. The chemical structures of Bodi Fluor™ 488 acid (Cat# 700, top) and its succinimidyl ester (Cat# 701, bottom).

Dansyl dyes are usually used to label peptides for biophysical investigations due to their small sizes and little interference to the binding affinity of peptides. The environmental sensitivity of Dansyl dyes is often used to probe biological microenvironments.

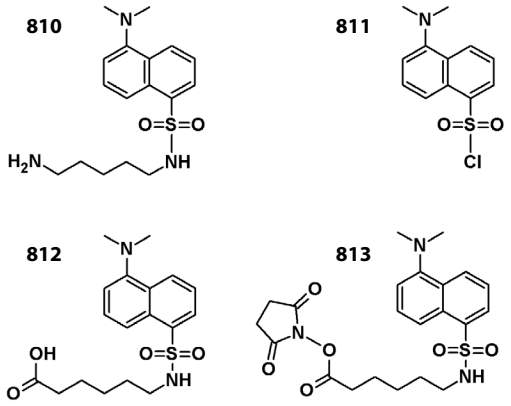

Figure 1.12. The chemical structures of typical Dansyl dyes.

Table 1.12 Bodi Fluor™, Dansyl and EDANS Derivatives for Labeling Peptides

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1} M^{-1}$)	CF @280 nm
700	Bodi Fluor™ 488 Acid	10 mg	505	512	70,000	0.018
701	Bodi Fluor™ 488, SE	5 mg	505	511	70,000	0.018
810	Dansyl Cadaverine	25 mg	333	518	4,000	0.387
811	Dansyl Chloride	100 mg	335	518	4,000	0.387
812	Dansyl-X Acid	5 g	335	518	4,000	0.387
813	Dansyl-X, SE	1 g	335	518	4,000	0.387
610	EDANS Acid	1 g	335	493	5,500	0.107
617	EDANS C2 Maleimide	25 mg	335	493	5,500	0.107
618	EDANS Iodoacetamide	25 mg	335	493	5,500	0.107
615	EDANS, Sodium Salt	1 g	335	493	5,500	0.107

Dye Selection Guide for Preparing Fluorescent Peptides with Desired Excitation and Emission Properties*

Emisson Color / Excitation Wavelength	Blue	Green	Yellow	Orange	Red	Far Red	Infra-Red
355 nm	Alexa Fluor® 350 AMCA **EDANS** iFluor™ 350 **Tide Fluor™ 1**	Dansyl Dyes					
405 nm	Alexa Fluor® 405 **iFluor™ 405** mFlour™ Violet 450	mFlour™ Violet 510	mFlour™ Violet 540				
436 nm	Alexa Fluor® 430 iFluor™ 405 **mFlour™ Violet 450**	mFlour™ Violet 510	mFlour™ Violet 540				
488 nm		Alexa Fluor® 488 Cy2® **FAM** FITC iFluor™ 488 **Tide Fluor™ 2** **Tide Fluor™ 2WS**			mFlour™ Blue 570		
532 nm				Alexa Fluor® 532 iFluor™ 532 **Rhodamine 6G**	mFlour™ Green 620		
561 nm					Alexa Fluor® 555 **California Red™** Cy3® iFluor™ 555 mFlour™ Yellow 630 ROX **TAMRA** Texas Red® Texas Red®-X **Tide Fluor™ 3** **Tide Fluor™ 4**		
633 nm						Alexa Fluor® 647 Cy5® iFluor™ 647 **Tide Fluor™ 5**	mFlour™ Red 780
647 nm						Alexa Fluor® 647 Cy5® iFluor™ 647 **Tide Fluor™ 5**	mFlour™ Red 780
670 nm						Alexa Fluor® 680 Cy5.5® iFluor™ 680 **Tide Fluor™ 6**	mFlour™ Red 780
745 nm							Alexa Fluor® 750 Cy7® iFluor™ 750 IRDye® 800 **Tide Fluor™ 7**

* Notes: 1). Excitation sources: 355 nm: UV laser/mercury arc lamp; 405 nm: violet diode laser; 436 nm: mercury arc lamp; 488 nm: argon laser; 532 nm: Nd: YAG laser; 561 nm: yellow diode laser; 633 nm: He-Ne laser; 647 nm: krypton laser; 670 nm: NIR laser; 745 nm: NIR laser.
2). Recommended dyes are bolded in green based on the cost and performance.

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Dye Selection Guide for Preparing FRET Peptides

Acceptors / Donors	TQ1 BHQ®-0 QSY®35 DABCYL	TQ2 BHQ®-1	TQ3 BHQ®-2 QSY®7 QSY®9	TQ4 BHQ®-3 QSY®21	TQ5	TQ6	TQ7
Tide Fluor™ 1 (TF1) **iFluor™ 350** Alexa Fluor® 350 DyLight™ Fluor 350 EDANS MCA	☺						
Tide Fluor™ 2 (TF2) **iFluor™ 488** Alexa Fluor® 488 ATTO 488 DyLight™ Fluor 488 FAM/TITC Cy2®		☺					
Helix Fluor™ 555 **iFluor™ 532** Hex TET JOE VIC			☺				
Tide Fluor™ 3 (TF3) **iFluor™ 555** **Helix Fluor™ 575** Alexa Fluor® 546 & 555 ATTO 550 & 565 DyLight™ Fluor 550 Cy3®/NED/TAMRA			☺				
Tide Fluor™ 4 (TF4) **iFluor™ 594** **California Red™** **SunRed™** Alexa Fluor® 594 DyLight™ Fluor 594 ROX Texas Red® Texas Red®-X				☺			
Tide Fluor™ 5 (TF5) **iFluor™ 647** Alexa Fluor® 647 DyLight™ Fluor 650 Cy5®					☺		
Tide Fluor™ 6 (TF6) **iFluor™ 680** IRDye® 700 Alexa Fluor® 680 DyLight™ Fluor 680 Cy5.5®						☺	
Tide Fluor™ 7 (TF7) **iFluor™ 750** Alexa Fluor® 750 DyLight™ Fluor 755 IRDye® 800 Cy7®							☺

Best to use | OK to use | Not recommended

AAT Bioquest®