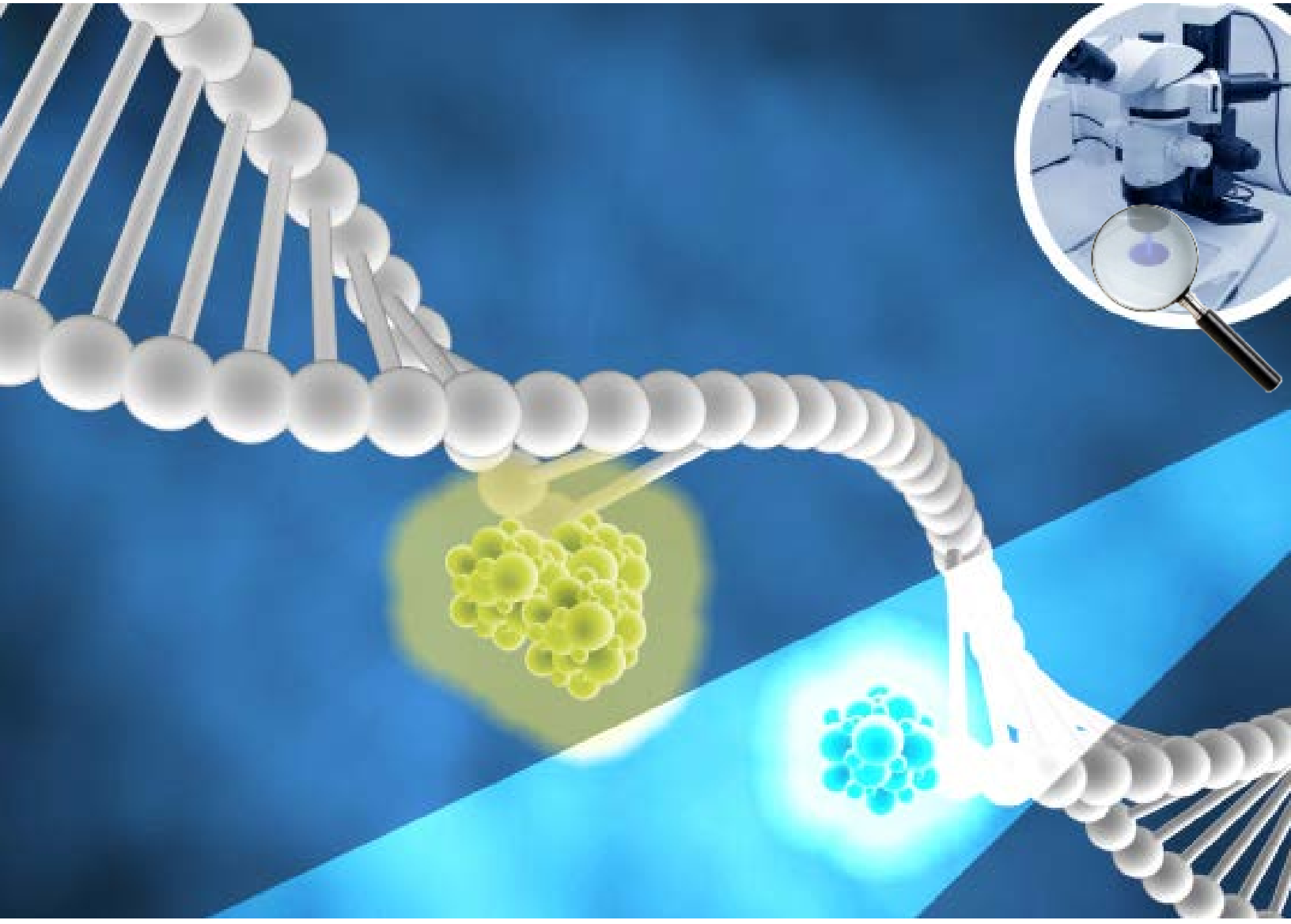
FRET Building Blocks & Probes



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AAT Bioquest®

Our Mission

AAT Bioquest® is committed to constantly meet or exceed its customer's requirements by providing consistently high quality products and services, and by encouraging continuous improvements in its long-term and daily operations. Our core value is Innovation and Customer Satisfaction.

Our Story

AAT Bioquest®, Inc. develops, manufactures and markets bioanalytical research reagents and kits to life sciences research, diagnostic R&D and drug discovery. We specialize in photometric detections including absorption (color), fluorescence and luminescence technologies. The Company's superior products enable life science researchers to better understand biochemistry, immunology, cell biology and molecular biology. AAT Bioquest offers a rapidly expanding list of enabling products. Besides the standard catalog products, we also offer custom services to meet the distinct needs of each customer. Our current services include custom synthesis of biological detection probes, custom development of biochemical, cell-based and diagnostic assays and custom high throughput screening of drug discovery targets.

It is my greatest pleasure to welcome you to AAT Bioquest. We greatly appreciate the constant support of our valuable customers. While we continue to rapidly expand, our core value remains the same: Innovation and Customer Satisfaction. We are committed to being the leading provider of novel biological detection solutions. We promise to extend these values to you during the course of our service and to continue to support you with our new products and services. It is our greatest honor to receive valuable feedbacks and suggestions from you so that we can better serve your projects.

Very truly yours,

Zhenjun Diwu, Ph.D.
President

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Fluorescence Resonance Energy Transfer (FRET)

Fluorescence

Fluorescence is a three-stage, luminescent process which occurs in molecules commonly referred to as fluorophores. This process requires an external stimulus, such as a light source, that produces a photon of energy which is absorbed by the fluorophore. As a fluorophore absorbs this energy, valence electrons are boosted to a higher energy level creating an excited singlet state. This excited state is relatively short-lived, typically lasting 1-10 nanoseconds.

During this time the fluorophore undergoes conformational changes to partially dissipate some of the energy it absorbed until it adopts the lowest energy level in the excited state. This relaxed singlet excited state is where fluorescence emission emanates. As the fluorophore returns to its ground state it emits a lower energy photon, indicative of a longer wavelength, than it initially absorbed. This relaxation process from a higher to lower energy state classifies fluorescence as one possible 'de-excitation' pathway for excited molecules.

Adaptation of fluorescence for its use in various applications such as microscopy, offers researchers a unique and powerful tool for investigating complex biological samples. In microscopy, introduced fluorophores successfully targeted to their appropriate cell structure undergo fluorescence to produce a detectable and quantifiable signal. This photometric data has the potential to provide qualitative and quantitative data elucidating to the structure and functionality of cells and organelles. More recently, increasingly elaborate techniques involving fluorescence have been developed enabling the visualization and analysis of highly complex mechanisms in cells, organelles and subcellular organelles. One such technique is referred to as fluorescence resonance energy transfer or FRET.

Fluorescence Resonance Energy Transfer

Fluorescence or Förster resonance energy transfer (FRET) is a distance-dependent mechanism describing the transfer of energy between two chromophores, a donor and an acceptor. When in close physical proximity, a donor chromophore in its electronic excited state may transfer energy to an acceptor chromophore without the emission of a photon. This non-radiative transfer of energy is accomplished through long-range dipole-dipole coupling interactions between the donor and acceptor chromophores. As a result, the transfer of energy quenches the fluorescence intensity of the donor, reduces its excited state lifetime and causes an increase in the fluorescence intensity of the acceptor's signal.

In biological research, investigating spatial relationships of molecular species can provide a better understanding of their cellular mechanisms. With conventional fluorescence microscopy, localization of fluorescently targeted compounds can be approximated to an optical spatial resolution limit of 200 nm. However, with FRET, the relative proximity of two molecules can be determined within several nanometers of each other making it a sensitive tool for probing molecular interactions.

Although there are many factors which may influence FRET, it is imperative that the following conditions are satisfied in order for FRET to develop:

1. Spectral overlap of donor and acceptor chromophores
2. Physical proximity of donor and acceptor chromophores
3. Dipole orientation of chromophore pairs

Relaxation Pathways For Excited Molecules

- **Internal Energy Conversions:** The rapid return of electrons from the second (or higher) excited state to the first excited state is termed internal conversion. Energy is released through rapid solvent relaxation.
- **Light Emissions:** Light is released by either the transition from S1 to S0 (fluorescence emission) or transition from T1 to S0 (phosphorescence emission).
- **Collisional Quenching:** Collisions between an excited-state fluorophore and other molecules will sometimes quench the fluorophore, returning it to the ground state without emission of a photon. Collisional quenchers include molecular oxygen and electron scavengers such as Cu^{2+}, Mn^{2+}, halides, isothiocyanate, nitrite and nitrate ions. Collisional quenching primarily occurs when these ions are present in the millimolar range or higher. Therefore, under most experimental conditions, collisional quenching is usually negligible.
- **Fluorescence Resonance Energy Transfer:** FRET occurs when donor and acceptor molecules are within a specified range, usually within 10 to 100 Å. In the process of FRET, the excited-state energy of a donor is transferred to an acceptor molecule. Photons of light aren't involved in this transfer. The pathway leading from the S1 level of the donor to the S1 level of the acceptor represents FRET. Once excited, the acceptor can return to the ground state by the same pathway as described for the donor. If the acceptor molecule is also fluorescent, it can emit light at its characteristic wavelength, which is always longer than the emission wavelength of the donor.

Donor-Acceptor Spectral Overlap

The fluorescence emission spectrum of the donor chromophore must overlap the absorption or excitation spectrum of the acceptor chromophore. The degree to which the donor and acceptor fluorophore overlap is commonly referred to as the spectral overlap integral J(λ).

J(λ) is in units of $M^{-1}cm^{-1}nm^4$ and is defined by the following equation:

$$J(\lambda) = \int F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d(\lambda)$$

Physical Proximity of Donor-Acceptor Pair

Of the necessary FRET parameters, it is paramount that the donor and acceptor chromophores are in close proximity of each other, approximately 10-100 Å (1-10 nm). Satisfying this FRET requirement is beneficial in two ways. First, this distance range is optimal for resonance energy transfer to occur between chromophore pairs. Secondly, this FRET requirement significantly improves the spatial resolution of conventional fluorescence microscopy from 200 nm to below 10 nm.

Orientation of Transition Dipoles

The fundamental mechanism of FRET relies on the non-radiative transfer of energy between the donor and acceptor chromophores. Through long-range dipole-dipole coupling interactions, the donor chromophore can transfer its excitation energy to the acceptor chromophore without emitting a photon. This dipole-dipole interaction operates through an oscillating electric field produced by an excited donor chromophore.

Stimulus by an external light source causes excited electrons of the donor chromophore to undergo intervals of harmonic oscillation at a specific natural frequency. This creates an oscillating electric dipole which generates an electric field surrounding the excited donor chromophore. If the oscillating electric field generated is sufficient and in close proximity to an acceptor chromophore with a corresponding resonance frequency, the acceptor chromophore will oscillate. This donor-acceptor oscillation at matching frequencies allows for resonance energy transfer.

FRET Efficiency

FRET efficiency (E), which is the quantum yield of the energy transfer transition, indicates the percentage of the excitation photons that contribute to FRET. It is dependent upon the inverse sixth power of the distance between the donor and acceptor chromophore (denoted as *r*) and is expressed as follows:

$$E = R_0^6 / R_0^6 + r^6$$

Where, R_0 is the Förster radius at which half (50%) of the excitation energy of the donor is transferred to the acceptor chromophore (typically in the range of 20 – 60 Å). The FRET efficiency of a theoretical chromophore pair is graphically demonstrated in **Figure 1.2**. The sharp decline of the FRET efficiency curve is attributed to the inverse

Donor-Acceptor Spectral Overlap

$$J(\lambda) = \int F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d(\lambda)$$

Where:

- **$F_D(\lambda)$** is the corrected fluorescence intensity of the donor in the wavelength range **λ to λ+Δλ** with the total intensity (area under the curve) normalized to unity.
- **ε_A** is the extinction coefficient of the acceptor at **λ** in units of $M^{-1}cm^{-1}$.
- **λ** is the wavelength in nm

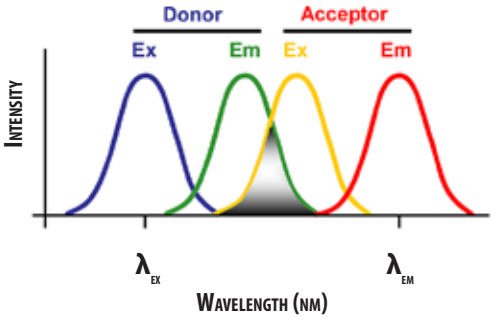

Figure 1.1 Schematic representation of the FRET spectral overlap integral (shown in gray shadow)

Dipole-Dipole Orientation Factor

Dipole-dipole orientation factor, ***k²,*** can range between 0 to 4:

- 0 : dipoles are perpendicular
- 4 : dipoles are collinear

1. For randomly oriented donors and acceptors **$k^2 = 2/3$**.
2. When **$k^2 = 1$**, the donor and acceptor transition dipoles are parallel to each other. This orientation is optimal for resonance energy transfer.

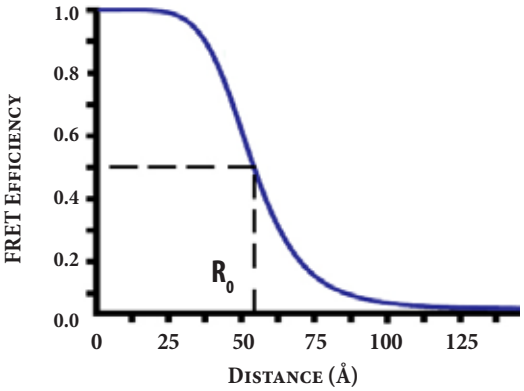

Figure 1.2 Schematic representation of the FRET efficiency dependence on the distance between donor and acceptor.

Förster Radius

R_0 is also dependent on a number of factors including:

1. **Φ_D :** fluorescence quantum yield of donor in the absence of acceptor.
2. **n :** refractive index of the solution.
   - The refractive index is generally known from the solvent composition or estimated for macromolecules such as proteins and nucleic acids.
   - **n** is often assumed to be that of water **(n = 1.33)** for aqueous solutions, or to be that of small organic molecules **(n = 1.39)** for organic solutions.
3. **k^2 :** dipole angular orientation of each molecule
   - **k^2 = 2/3** for randomly oriented donors and acceptors
4. **J(λ) :** spectral overlap integral of donor and acceptor

Therefore, R_0 can also be determined by the following equation:

$$R_o^6 = 8.79 \times 10^{23}[k^2 n^{-4} \Phi_D J(\lambda)]$$

Table 1.1 Some typical R_0 values of D/A pairs*

Donor	Acceptor	Ro (Å)
B-Phycoerythrin	Cy5	79
Dansyl	Fluorescein	33-41
EDANS	DABCYL	33
Fluorescein	Fluorescein	44
Fluorescein	Tetramethylrhodamine	49-56
IAEDANS	5-(Iodoacetamido)fluorescein	49
IAEDANS **	FITC	49
Naphthalene	Dansyl	22
Pyrene	Coumarin	39
Tryptophan	Dansyl	21-24
Tryptophan	Pyrene	28
Tryptophan	IAEDANS	22

* The value may change under different conditions; **IAEDANS = 5-((Iodoacetyl) amino)naphthalene-sulfonic acid.

sixth power dependence of the distance between the two chromophores.

FRET efficiency is notably dependent of the intermolecular distance between the donor and acceptor chromophores. For distances less than R_0, the efficiency is close to maximal, whereas for distances greater than R_0 the efficiency quickly approaches zero. Exploitation of FRET's dependence on donor-acceptor proximity makes it a highly sensitive spectroscopic ruler for investigating distances between molecules during complex cellular mechanisms.

Selecting Donor and Acceptor FRET Pairs

Selecting the appropriate donor and acceptor chromophores pair is essential for improving FRET efficiency. As aforementioned, optimal FRET pairs should satisfy these specifications:

1. Select FRET pairs which maximize the spectral overlap of donor emission and acceptor excitation.
2. Donor and acceptor pair must have a sufficient spectral difference such that they are distinguishable from each other.
3. Acceptor fluorophores should have minimal direct excitation at the absorbance maximum of the donor fluorophore, to minimize any potential cross-talk.

Additionally, parameters such as donor quantum yield and the extinction coefficient of the acceptor can affect FRET efficiency. To address this concern, select donor and acceptor chromophores that are complementary to one another. Selecting a donor with the highest quantum yield and pairing it with the highest absorbing acceptor, assuming sufficient spectral overlap between the pair, will maximize the FRET signal. In situations of low FRET efficiency, a more efficient FRET pair will significantly improve the detectable FRET interaction.

Donor-acceptor pair may consist of the same or different chromophores. In most applications, the donor and acceptor chromophores are different. In these scenarios, FRET is identified by the presence of sensitized fluorescence of the acceptor chromophore or the fluorescence quenching of the donor chromophore. The later method is independent of the acceptor chromophores fluorescence capabilities. If the acceptor is fluorescent, FRET can be also be detected by the intensity ratio change of the donor/acceptor.

A number of R_0 values for various donor-acceptor chromophore pairs have been identified and published. FRET pairs with larger R_0 values are indicative of a higher FRET efficiency. A commonly used chromophore pair for developing FRET-based assays is fluorescein and tetramethlyrhodamine. Together this FRET pair has an R_0 value ranging between 49-56 Å. R_0 values of common donor-acceptor pairs are listed in **Table 1.1**. For a more extensive assortment of AAT Bioquest's, Tide Fluor™ (TF) and Tide Quencher™ (TQ) FRET building blocks designed for developing FRET probes refer to **Appendix A**. Our recommended FRET pairs demonstrate superior sensitivity and

low background interference in protease and nucleic acid detection assays.

Detecting FRET

FRET detection and quantitation can be calculated in a variety of different manners depending upon the donor-acceptor pair chosen. Sensitized emission is perhaps the most common and simplest method for quantifying FRET. This approach is well-suited for fluorescent and non-fluorescent acceptor chromophores. When using fluorescent acceptor chromophores, FRET results in a simultaneous decrease of donor emission and increase in fluorescence intensity of the acceptor. Such technique is advantageous because a ratio metric calculation of the two signals can be made independent of the absolute concentration of the sensor.

Non-fluorescent acceptor chromophores are utilized for their quenching properties. In this approach, the fluorescence intensity of the donor is determined by its relative position to the acceptor. Interactions establishing close proximity of the donor to the acceptor will result in fluorescence quenching of the donor. Conversely, interactions removing donor and acceptor proximity will restore or increase fluorescence of the donor.

Proper filter selection is critical for the detection of FRET. When selecting a donor excitation filter it must be able to selectively excite the donor chromophore while simultaneously reducing any direct excitation of the acceptor chromophore. Large amounts of direct excitation to the acceptor will contaminate any data. However, under appropriate controls some direct excitation of the acceptor can be accounted for.

Another key concern regarding FRET detection involves analyte concentration. Only interacting donors and acceptors will result in FRET. If there is an abundance of both molecules present, but they are not interacting with one another, then the amount of FRET occurring would be insufficient to detect. In reference to FRET, the term "analyte" refers to the measurement of the donor-acceptor complex rather than the individual components. For example, cellular localization can affect FRET outcome. If a sufficient concentration of the donor and acceptor chromophores are introduced into a system, but localize in spatially distant organelles, interactions between the two chromophores will not occur impeding FRET.

Applications for FRET Probes

Various combinations of donor and acceptor pairs have been developed for investigating distinct cellular processes of biomolecules. Probes incorporating fluorescent donors and non-fluorescent acceptors are well-suited for FRET-based assays detecting proteolysis and nucleic acid hybridization. For example, EDANS/DABCYL chromophore pair has been intensively used to develop FRET-based nucleic acid probes and protease substrates. MCA (7-methoxycoumarin-4-acetic acid) and DNP (2, 4-dinitroaniline) are another donor-acceptor pair for developing FRET-based fluorescent probes. Compared to EDANS/DABCYL, MCA/DNP pairs usually have a shorter and weaker wavelength of fluorescence signal. However, the pair often demonstrates better affinity or turnover rate due to their smaller sizes. Incorporation of fluorescent donors and non-fluorescent acceptor combinations can also be adapted for other FRET-based assays investigating receptor-ligand interactions, distribution and transport of lipids, membrane potential sensing and cyclic AMP (cAMP) detection.

FRET Peptides for Detecting Proteases

Proteases or peptidases are enzymes that catalyze the hydrolysis of peptide bonds which break down proteins and facilitate their

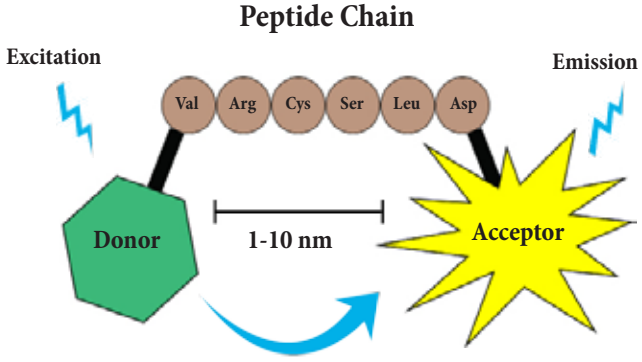

Figure 1.3 Mechanism of FRET detection using a fluorescent donor and acceptor chromophore pair.

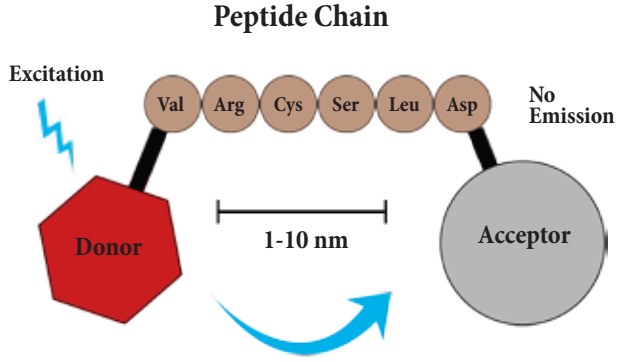

Figure 1.4 Mechanism of FRET detection using a fluorescent donor and a non-fluorescent acceptor (quencher).

Table 1.2 Tide Fluor™ 2/Tide Quencher™ 2-based FRET peptide for high throughput screening of HIV-1 protease inhibitors and continuous quantification of HIV-1 protease activity

Substrate	Peptide Sequence
1	TQ2-Gaba-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-5-FAM
2	Arg-Glu(TF2)-Val-Ser-Phe-Asn-Phe-Pro-Gln-Ile-Thr-Lys(TQ2)-Arg
3	Arg-Glu(5-FAM)-Ser-Gln-Asn-Tyr-Ile-Val-Gln-Lys(TQ2)-Arg
4	Arg-Arg-Glu(5-FAM)-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-Lys(5-FAM)-Arg-Arg
5	Ac-Arg-Glu(5-FAM)-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-Lys(5-FAM)-Arg-NH_2

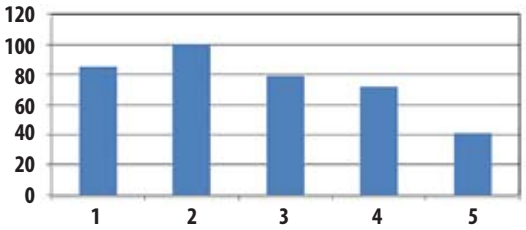

Figure 1.5 HIV protease cleavage of TF2/TQ2-derived FRET peptides. All the substrates were used @10 µM. The reaction rate is set at 100% with Sub #2.

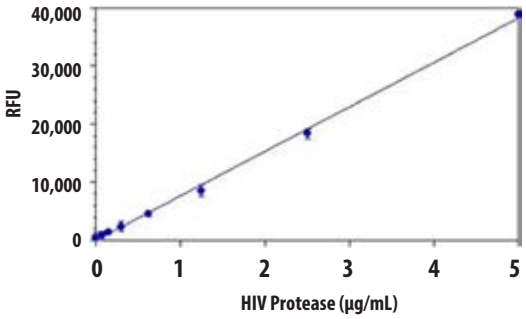

Figure 1.6 HIV protease cleavage of Arg-Glu(5-FAM)-Val-Ser-Phe-Asn-Phe-Pro-Gln-Ile-Thr-Lys(TQ2)-Arg. The substrate was incubated with HIV protease. Upon HIV protease cleavage, the fluorescence of 5-FAM was recovered and monitored at Ex/Em = 490 /520 nm.

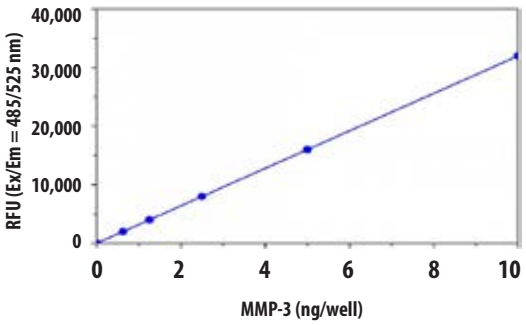

Figure 1.7 Dose response of MMP-3 enzyme activity was measured with Amplite™ MMP-3 Activity Assay Kit using a NOVOStar microplate reader (BMG Labtech).

digestion. Proteases are essential in several physiological processes such as signal transduction, digestion of food proteins, protein turnover and cell division. In drug discovery, detection of proteases and screening of specific protease inhibitors aid in assessing the effectiveness of a potential drug at treating and managing protease-related diseases. Various combinations of donor and acceptor chromophores can be adapted to develop FRET-based assays for the detection of protease activity.

In FRET-based protease assays, non-fluorescent acceptors are utilized for their quenching properties. In this technique, the donor and acceptor chromophores are placed on each side of the scissile bond on a specific substrate. Location of the donor-acceptor pair is selected as to not compromise the integrity of the amino acid sequences adjacent to the scissile bond and close enough for FRET to occur. Cleavage of this scissile bond by protease hydrolysis spatially separates the donor from the acceptor chromophore disassembling the substrate and restoring donor fluorescence. This spectrophotometric method can be well adapted for use in high throughput screening assays of protease activities and inhibitors.

HIV Protease

Identification of HIV protease has promoted the development of effective treatments against AIDS. This aspartic HIV protease, 10-12 kD of human immunodeficiency virus-1 (HIV-1), is essential for the post-translational cleavage of HIV precursor polyproteins, Pr^{gag} and $Pr^{gag\text{-}pol}$. Since cleavage of these proteins is necessary for the maturation of HIV, it has become a key target for the development of AIDS-drugs.

AAT Bioquest has developed donor and acceptor chromophores well suited for high throughput screening of HIV-1 protease inhibitors and continuous quantification of HIV-1 protease activity (**Table 1.2**). Most notably is the donor-acceptor pair, Tide Fluor™ 2 and Tide Quencher™ 2. In the FRET peptide, the fluorescence of Tide Fluor™ 2 is quenched by Tide Quencher™ 2 until the peptide is cleaved into two separate fragments by the HIV-1 protease. The sequences of AAT Bioquest's FRET peptides are derived from the native p17/p24 cleavage site on Pr^{gag} for HIV-1 protease.

Matrix Metalloproteinases (MMPs)

Matrix metalloproteinases (MMPs) are comprised of zinc-dependent endopeptidases responsible for the degradation and turnover of extracellular matrix components. MMPs are associated with various physiological processes such as the breakdown of connective tissue, tissue damage repair and bone remodeling. However, the exact contribution of MMPs to specific pathological processes is difficult to assess. For example, MMPs appear to play a key role in the development of arthritis as well as in the invasion and metastasis of cancer.

MMPs tend to have multiple substrates, with the ability to degrade various types of collagen along with elastin, gelatin and fibronectin. Therefore, it can prove difficult to find a substrate selective to a single MMP enzyme. AAT Bioquest has identified X-X-X-TQ2WS-GABA-Pro-Cha-Abu-Smc-His-Ala-Dab(TF2)-Ala-Lys-X-X as a generic FRET substrate for most of MMP enzymes. Tide Fluor™ and Tide Quencher™ dyes

conjugated to this substrate have been utilized in FRET-based protease assays for detecting MMP activities and screening MMP inhibitors. For example, TF2-Arg-Pro-Lys-Pro-Val-Glu-Nva-Trp-Arg-Lys(TQ2WS)-X-X-X has relatively good selectivity for MMP-3 enzyme.

FRET Assays for Nucleic Acid Detection and Molecular Diagnostics

Oligonucleotides containing DNA backbones conjugated to fluorophores generate DNA-based FRET probes. Depending on experimental design strategy, the resulting probes will either be a binary probe or a molecular beacon. These synthetic DNA/RNA probes are well-suited for a variety of *in vivo* and *in vitro* applications monitoring DNA and RNA interactions including PCR, DNA hybridization, cleavage or ligation.

Binary Probes

Binary probes, used mainly in hybridization assays, utilize a dual oligonucleotide probing system. The two oligonucleotide probes, complementary to the target oligonucleotide, are respectively labeled such that one probe is labeled with a single donor and the other with an acceptor. In the absence of its DNA target, the intermolecular FRET signal will not be observed. A detectable signal is only generated when both parts of the binary probe hybridize to the analyte bringing the donor and acceptor chromophore within close proximity of each other. A benefit to this approach is the elimination of false positive or nonspecific FRET signals.

Molecular Beacons

A Molecular Beacon is a unique single-stranded oligonucleotide sequence labeled with two fluorophores and held in a 'hairpin' like configuration. The 5' and 3' terminal ends of the oligonucleotide sequence are labeled with a fluorescent reporter and quencher molecule. In the absence of the target the 'hairpin' structure of the Molecular Beacon, attributed by the probe's self-complementary stem structure, brings these two molecules within a close proximity of each other such that the fluoresce signal is suppressed. When the Molecular Beacon hybridizes to its target, separation of the fluorescent reporter and quencher occur, resulting in the reporter emitting at its characteristic wavelength.

Molecular Beacons have enhanced specificity for their targets, making them superior in hybridization-based investigations of single nucleotide polymorphisms (SNPs). They have been used in hybridization-based assays to identify rifampin-resistant Mycobacterium and to detect virus replication in HIV type 1-infected individuals. Additionally, they have also been widely used for nuclease detection. Dual-labeled FRET probes are commonly used to detect the 5' exonuclease activity of Taq polymerase. The two labels on the FRET oligonucleotide can be separated by as many as 25-30 bases. If the oligonucleotide is intact, the donor molecule's signal is quenched. During the course of the assay, the cleavage event separates the donor molecule from its acceptor, restoring the donor's characteristic emission. If a dark quencher is used as the acceptor molecule, multiple FRET probes can be used, each labeled with a unique fluorophore, making these probes amenable to multiplex assays.

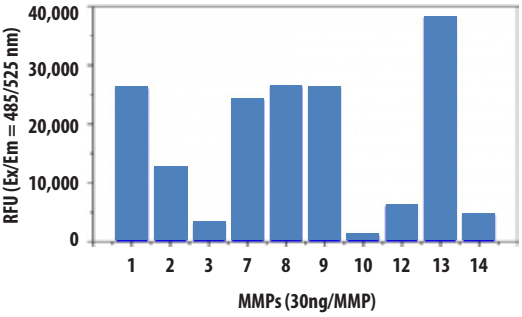

Figure 1.8 Detection of MMPs activity using Amplite™ Universal Fluorimetric MMP Activity Assay Kit. The APMA-activated MMPs, 30 ng each, were mixed with MMP Green™ substrate. The fluorescence signal was monitored one hour after starting the the reaction by using a NOVOStar microplate reader (BMG Labtech) with a filter set of Ex/Em = 490/525 nm. The reading from all wells was subtracted with the reading from substrate control, which contains MMP Green™ substrate but no MMPs. The MMP Green™ substrate can detect the activity of sub-nanogram of all MMPs (n=3)

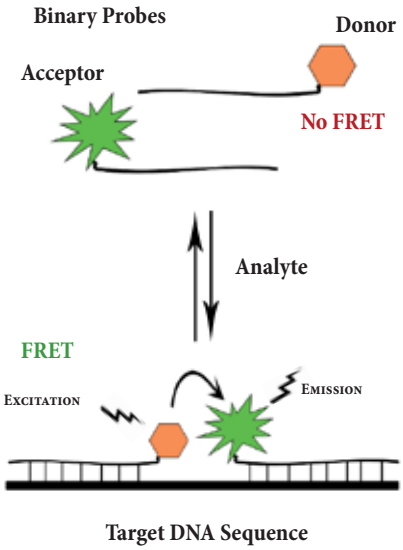

Figure 1.9 Schematic representation of fluorogenic binary probes.

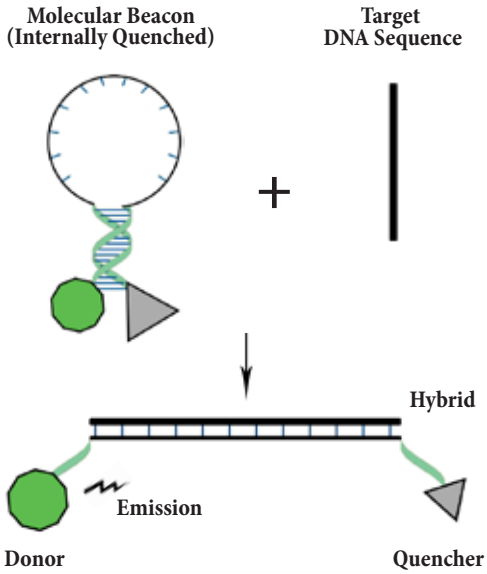

Figure 1.10 Schematic representation of fluorogenic Molecular Beacon probes.

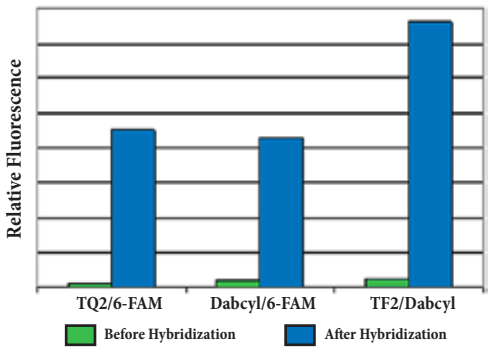

Figure 1.11 Hybridization-induced fluorescence enhancement of Molecular Beacon oligonucleotide probes that contain Tide Fluor™ dyes as donors or Tide Quencher™ dyes as acceptors.

Applications of FRET Probes:

- Protease detection and molecular diagnostics
- Structure and conformation of proteins
- Spatial distribution and assembly of proteins
- Receptor/ligand interactions
- Immunoassays
- Structure and conformation of nucleic acids
- SNP detection
- Nucleic acid sequencing
- Distribution and transport of lipids
- Membrane fusion assays
- Membrane potential sensing
- cyclic AMP

AAT Bioquest offers a complete set of dye phosphoramidites and dye CPG supports for preparing FRET oligonucleotides. This set includes the classic dyes such as FAM, HEX, TET and JOE, as well as superior oligo-labeling dyes such as Tide Fluor™ and Tide Quencher™ dyes. Our Tide Fluor™ dyes, TF1through TF8, have enhanced fluorescence and higher photostability than typical fluorophores such as fluorescein, rhodamine and cyanine as described below. Our TF2 has nearly identical excitation and emission wavelengths compared to carboxyfluorescein (FAM) making it a superior replacement in biological applications utilizing fluorescein because of its improved performance. Compared to FAM probes, TF2 has much stronger fluorescence at physiological conditions, and it is much more photostable. Compared to other fluorescein and cyanine dye alternatives, such as Alexa Fluor® and Cy3®, Cy5® and Cy7® dyes, Tide Fluor™ dyes are much more cost-effective with comparable or better performance for your desired biological applications.

In addition to our Tide Fluor™ donor dyes, AAT Bioquest has also engineered robust Tide Quencher™ acceptor dyes. Tide Quencher™ dark FRET acceptors, TQ1 through TQ7, are optimized to pair with our Tide Fluor™ dyes and classic fluorophores, such as AMCA, EDANS, FAM, TAMRA, HEX, JOE, TET, ROX, Cy3®, Cy5® and Cy7®. Like Tide Fluor™, our Tide Quencher™ acceptor dyes are much more cost-effective with comparable or better performance for your desired biological applications than other similar products available on the market.

To facilitate your research, AAT Bioquest offers both our Tide Fluor™ and Tide Quencher™ dyes in a variety of reactive forms. For in-synthesis labeling of oligonucleotides, we provide phosphoramidites of Tide Fluor™ and Tide Quencher™ dyes as well as their CPG supports. For post labeling of oligonucleotides, we offer both amino-reactive and thiol-reactive Tide Fluor™ and Tide Quencher™ dyes that are water-soluble.

Product Ordering Information For Matrix Metalloproteinases (MMP) Assay Reagents and Kits

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
13512	Amplite ™ Fluorimetric MMP-3 Activity Assay Kit *Green Fluorescence*	100 tests	498	520
13510	Amplite ™ Universal Fluorimetric MMP Activity Assay Kit *Green Fluorescence*	100 tests	494	521
13511	Amplite ™ Universal Fluorimetric MMP Activity Assay Kit *Red Fluorescence*	100 tests	545	576
13520	MMP Green™ Substrate	1 mg	498	520
13521	MMP Red™ Substrate	1 mg	545	576
13528	MMP-3 Green™ Substrate	1 mg	498	520

TIDE FLUOR™ DYES

Key Features of Tide Fluor™ Dyes:

- **Optimized** to pair with Tide Quencher™ and other common dark acceptors
- **Stronger fluorescence** intensity to enhance assay sensitivity
- **pH-insensitive** and environment-insensitive fluorescence
- **Higher photostability** to improve fluorescence assay quality
- **Adjustable** water solubility
- **A variety** of reactive forms available for preparing conjugations

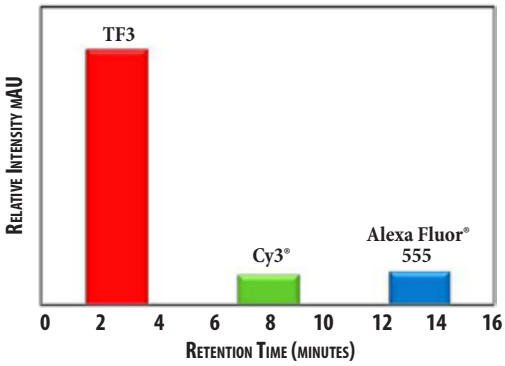

Figure 2.1 Fluorescence quantum yield comparison of TF3, Cy3® and Alexa Fluor® 555 on the peptide of Dye-PLSRTLSVAAKK-NH2.

Tide Fluor™ Dyes: Fluorescent Dyes For Labeling Peptides

EDANS, FAM, TAMRA, ROX, Cy3® and Cy5® are commonly adopted for developing various peptide probes. However, there are still some limitations associated with their use. For instance, EDANS weak absorption and environment-sensitive fluorescence has severely limited its sensitivity making it unsuitable for developing protease assays and nucleic acid detection probes. When compared to EDANS, fluorescein-based probes like FAM, HEX, JOE and TET-based probes have a stronger absorption and fluorescence. Unfortunately, the fluorescence of fluorescein-based probes is strongly pH dependent. At high pH levels, these probes exhibit their strongest fluorescence. For assays requiring low pH levels, fluorescein-based fluorescent probes should be avoided. Additionally, fluorescein-based probes exhibit low photostability limiting their applications in fluorescence imaging.

Among cyanine dyes, non-sulfonated Cy3® and Cy5® are occasionally used for developing peptide probes, but they exhibit fairly low fluorescence quantum yields in aqueous media. The sulfonated Cy3® and Cy5® dyes have improved fluorescence quantum yields, and even some Alexa Fluor™ dyes are sulfonated cyanine dyes. However, they are extremely expensive making them unpractical to use when preparing peptide conjugates.

AAT Bioquest has addressed these limitations by developing Tide Fluor™ donor dyes that have nearly identical spectral properties and comparable performance to Alexa Fluor® dyes at a fraction of the cost. Tide Fluor™ dyes are optimized building blocks for developing FRET-based oligonucleotides and peptides for a use in various biological applications.

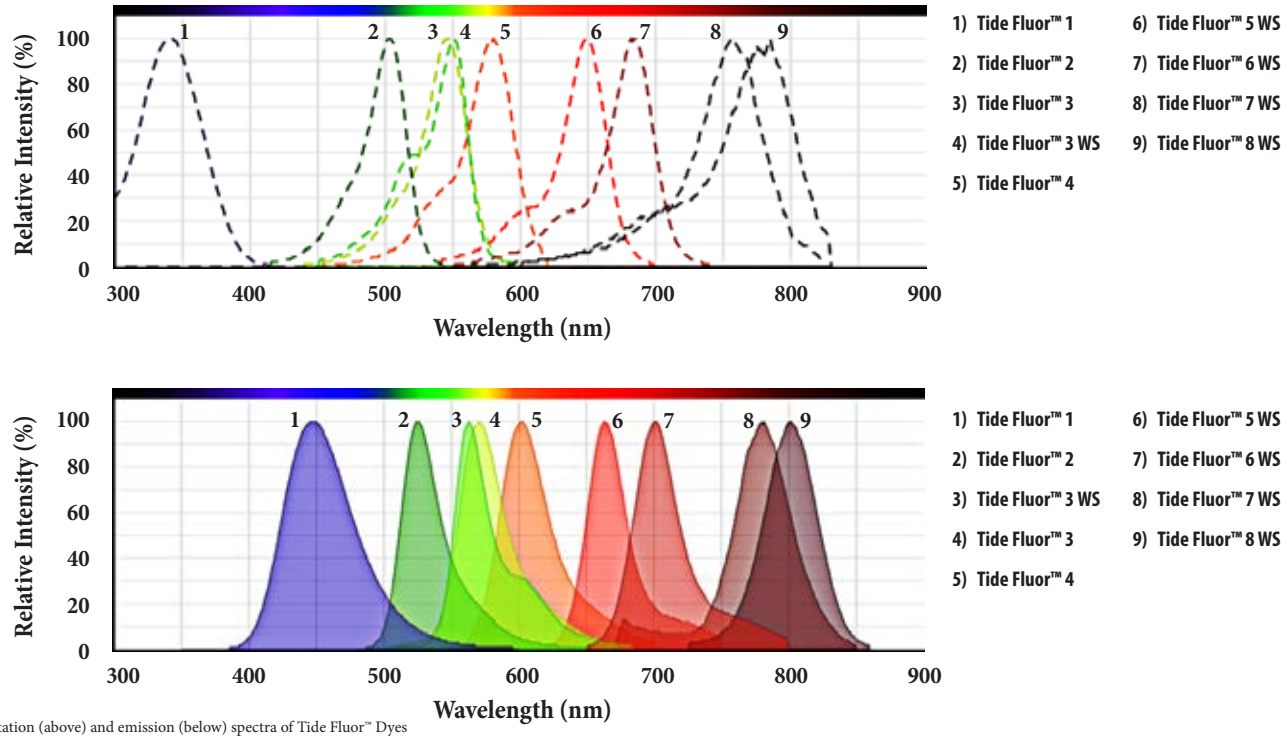

Figure 2.2 Excitation (above) and emission (below) spectra of Tide Fluor™ Dyes

Our Tide Fluor™ dyes, TF1 through TF8, exhibit stronger fluorescence and higher photostability than the typical fluorophores such as fluorescein, rhodamines and cyanine described previously. Our TF2 has the similar excitation and emission wavelengths to those of carboxyfluoresceins (FAM), making it a suitable replacement in biological applications that are done with fluorescein. Compared to FAM probes, TF2 has a much stronger fluorescence at physiological conditions with improved photostability. On peptides, TF3 has a stronger fluorescence and photostability than Cy3®, Alexa Fluor® 555 and DyLight™ 555, with nearly identical spectral properties to all three dyes (refer to **Figure 2.3)**. Compared to other fluorescent dyes alternative to fluorescein and Cy® dyes, like Alexa Fluor™ and DyLight®, Tide Fluor™ dyes are much more cost-effective with comparable or better performance for your desired biological applications.

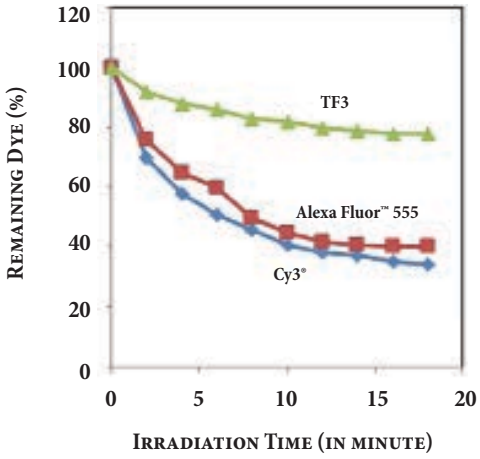

Figure 2.3 Photostability comparison of TF3 vs. Alexa Fluor® 555 and Cy3®

Key Features of Tide Fluor™ Dyes:

Tide Fluor™ Donor	Extinction Coefficient ($cm^{-1}M^{-1}$)	Excitation (nm)	Emission (nm)	Features	Replaces
Tide Fluor™ 1 (TF1)	20,000	345	442	• Much stronger absorption • Much stronger fluorescence • Less environmental sensitivity	EDANS
Tide Fluor™ 2 (TF2)	75,000	500	527	• pH-insensitive fluorescence • Good photostability	FAM, FITC and Alexa Fluor® 488
Tide Fluor™ 2WS (TF2WS)	75,000	495	518	• pH-insensitive fluorescence • Good photostability	Alexa Fluor® 488
Tide Fluor™ 3 (TF3)	85,000	555	584	• Strong fluorescence • Good photostability	Cy3® and Alexa Fluor® 555
Tide Fluor™ 3WS (TF3WS)	150,000	555	565	• Strong fluorescence • Good photostability	Cy3® and Alexa Fluor® 555
Tide Fluor™ 4 (TF4)	90,000	590	618	• Strong fluorescence • Good photostability	ROX, Texas Red® and Alexa Fluor® 594
Tide Fluor™ 5WS (TF5WS)	250,000	649	664	• Strong fluorescence • Good photostability	Cy5® and Alexa Fluor® 647
Tide Fluor™ 6WS (TF6WS)	220,000	676	695	• Strong fluorescence • Photostable	Cy5.5®, IRDye® 700 and Alexa Fluor® 680
Tide Fluor™ 7WS (TF7WS)	275,000	749	775	• Strong fluorescence • Good photostability	Cy7® and Alexa Fluor® 750
Tide Fluor™ 8WS (TF8WS)	250,000	775	807	• Stronger fluorescence • Higher Photostability	IRDye® 800

Product Ordering Information For Tide Fluor™ Labeling Dyes

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
2238	Tide Fluor™ 1 Acid [TF1 Acid] *Superior Replacement for EDANS*	100 mg	345	442
2237	Tide Fluor™ 1 Alkyne [TF1 Alkyne] *Superior Replacement for EDANS*	5 mg	345	442
2239	Tide Fluor™ 1 Amine [TF1 Amine] *Superior Replacement for EDANS*	5 mg	345	442
2236	Tide Fluor™ 1 Azide [TF1 Azide] *Superior Replacement for EDANS*	5 mg	345	442
2242	Tide Fluor™ 1 Maleimide [TF1 Maleimide] *Superior Replacement for EDANS*	5 mg	345	442
2244	Tide Fluor™ 1 Succinimidyl Ester [TF1 SE] *Superior Replacement for EDANS*	5 mg	345	442
2245	Tide Fluor™ 2 Acid [TF2 Acid] *Superior Replacement for Fluorescein*	25 mg	500	527
2253	Tide Fluor™ 2 Alkyne [TF2 Alkyne] *Superior Replacement for Fluorescein*	1 mg	500	527
2246	Tide Fluor™ 2 Amine [TF2 Amine] *Superior Replacement for Fluorescein*	1 mg	500	527
2252	Tide Fluor™ 2 Azide [TF2 Azide] *Superior Replacement for Fluorescein*	1 mg	500	527
2247	Tide Fluor™ 2 Maleimide [TF2 Maleimide] *Superior Replacement for Fluorescein*	1 mg	500	527
2248	Tide Fluor™ 2 Succinimidyl Ester [TF2 SE] *Superior Replacement for Fluorescein*	5 mg	500	527
2350	Tide Fluor™ 2WS Maleimide [TF2WS Maleimide] *Superior Replacement for FITC*	1 mg	495	518
2349	Tide Fluor™ 2WS, Succinimidyl Ester [TF2WS, SE] *Superior Replacement for FITC*	5 mg	495	518
2268	Tide Fluor™ 3 Acid [TF3 Acid] *Superior Replacement for Cy3®*	25 mg	555	584
2255	Tide Fluor™ 3 Alkyne [TF3 Alkyne] *Superior Replacement for Cy3®*	1 mg	555	584
2269	Tide Fluor™ 3 Amine [TF3 Amine] *Superior Replacement for Cy3®*	1 mg	555	584
2254	Tide Fluor™ 3 Azide [TF3 Azide] *Superior Replacement for Cy3®*	1 mg	555	584
2270	Tide Fluor™ 3 Maleimide [TF3 Maleimide] *Superior Replacement for Cy3®*	1 mg	555	584
2271	Tide Fluor™ 3 Succinimidyl Ester [TF3 SE] *Superior Replacement for Cy3®*	5 mg	555	584
2345	Tide Fluor™ 3WS Acid [TF3WS Acid] *Superior Replacement for Cy3®*	10 mg	555	565
2344	Tide Fluor™ 3WS Maleimide [TF3WS Maleimide] *Superior Replacement for Cy3®*	1 mg	555	565
2346	Tide Fluor™ 3WS, Succinimidyl Ester [TF3WS, SE] *Superior Replacement for Cy3®*	5 mg	555	565
2285	Tide Fluor™ 4 Acid [TF4 Acid] *Superior Replacement for ROX and Texas Red®*	10 mg	590	618
2301	Tide Fluor™ 4 Alkyne [TF4 Alkyne] *Superior Replacement for ROX and Texas Red®*	1 mg	590	618
2286	Tide Fluor™ 4 Amine [TF4 Amine] *Superior Replacement for ROX and Texas Red®*	1 mg	590	618
2300	Tide Fluor™ 4 Azide [TF4 Azide] *Superior Replacement for ROX and Texas Red®*	1 mg	590	618
2287	Tide Fluor™ 4 Maleimide [TF4 Maleimide] *Superior Replacement for ROX and Texas Red®*	1 mg	590	618
2289	Tide Fluor™ 4, Succinimidyl Ester [TF4, SE] *Superior Replacement for ROX and Texas Red®*	5 mg	590	618
2278	Tide Fluor™ 5WS Acid [TF5WS Acid] *Superior Replacement for Cy5®*	10 mg	649	664
2276	Tide Fluor™ 5WS Alkyne [TF5WS Alkyne] *Superior Replacement for Cy5®*	1 mg	649	664
2275	Tide Fluor™ 5WS Azide [TF5WS Azide] *Superior Replacement for Cy5®*	1 mg	649	664
2280	Tide Fluor™ 5WS Maleimide [TF5WS Maleimide] *Superior Replacement for Cy5®*	1 mg	649	664
2281	Tide Fluor™ 5WS, Succinimidyl Ester [TF5WS, SE] *Superior Replacement for Cy5®*	5 mg	649	664
2291	Tide Fluor™ 6WS Acid [TF6WS Acid] *Superior Replacement for Cy5.5®*	10 mg	676	695
2303	Tide Fluor™ 6WS Alkyne [TF6WS Alkyne] *Superior Replacement for Cy5.5®*	1 mg	676	695
2302	Tide Fluor™ 6WS Azide [TF6WS Azide] *Superior Replacement for Cy5.5®*	1 mg	676	695
2293	Tide Fluor™ 6WS Maleimide [TF6WS Maleimide] *Superior Replacement for Cy5.5®*	1 mg	676	695
2294	Tide Fluor™ 6WS, Succinimidyl Ester [TF6WS, SE] *Superior Replacement for Cy5.5®*	1 mg	676	695
2330	Tide Fluor™ 7WS Acid [TF7WS Acid] *Superior Replacement for Cy7®*	10 mg	749	775
2305	Tide Fluor™ 7WS Alkyne [TF7WS Alkyne] *Superior Replacement for Cy7®*	1 mg	749	775
2304	Tide Fluor™ 7WS Azide [TF7WS Azide] *Superior Replacement for Cy7®*	1 mg	749	775
2332	Tide Fluor™ 7WS Maleimide [TF7WS Maleimide] *Superior Replacement for Cy7®*	1 mg	749	775
2333	Tide Fluor™ 7WS, Succinimidyl Ester [TF7WS, SE] *Superior Replacement for Cy7®*	1 mg	749	775
2335	Tide Fluor™ 8WS Acid [TF8WS Acid] *Near Infrared Emission*	10 mg	775	807
2307	Tide Fluor™ 8WS Alkyne [TF8WS Alkyne] *Near Infrared Emission*	1 mg	775	807
2306	Tide Fluor™ 8WS Azide [TF8WS Azide] *Near Infrared Emission*	1 mg	775	807
2337	Tide Fluor™ 8WS Maleimide [TF8WS Maleimide] *Near Infrared Emission*	1 mg	775	807
2338	Tide Fluor™ 8WS, Succinimidyl Ester [TF8WS, SE] *Near Infrared Emission*	1 mg	775	807

TIDE QUENCHER™ DYES

Table 3.1 Tide Quencher™ Dye Equivalents of Common Dyes

If you are using	Try this Tide Quencher™ dye
DABCYL, BHQ®-0, QSY®35	TQ1 [Tide Quencher™ 1]
BHQ®-1, QXL™ 520, QSY®35	TQ2 [Tide Quencher™ 2]
BHQ®-2	TQ3 [Tide Quencher™ 3]
BHQ®-3, QSY®7, QSY®9	TQ4 [Tide Quencher™ 4]
QSY®21	TQ5 [Tide Quencher™ 5]
IRDye® QC-1	TQ6 [Tide Quencher™ 6]
IRDye® QC-1	TQ7 [Tide Quencher™ 7]

The Advantages of Tide Quencher™ Dyes:

- TQ dyes enable you to explore the FRET potentials that might be impossible with other quenchers.
- Versatile reactive forms are convenient for self-constructing your desired FRET biomolecules.
- Perfectly match your desired fluorescent donors.
- Competitive price with better performance.

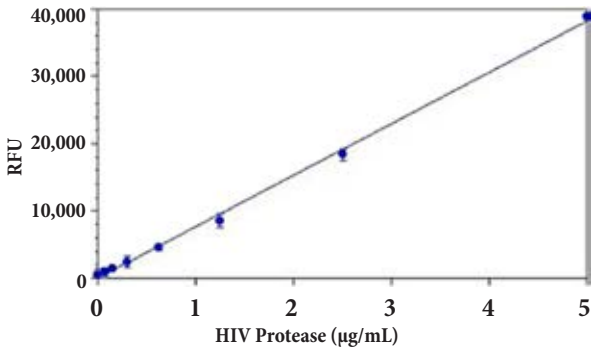

Figure 3.1 HIV protease cleavage of Arg-Glu(5-FAM)-Val-Ser-Phe-Asn-Phe-Pro-Gln-Ile-Thr-Lys(TQ2)-Arg. The substrate was incubated with HIV protease . Upon HIV protease cleavage, the fluorescence of 5-FAM was recovered and monitored at Ex/Em = 490 /520 nm.

DABCYL A Non-Fluorescent Quencher

DABCYL exhibits a broad and intense absorption without a fluorescence emission making it a suitable dark quencher when preparing FRET peptides. AAT Bioquest offers a variety of DABCYL derivatives for developing FRET peptide substrates. However, for longer wavelength fluorescent dyes such as rhodamine and cyanine, DABCYL has a lower quenching efficiency making them ineffective. Additionally, the absorption spectrum of DABCYL is environment-sensitive. To address this concern, we recommend Tide Quencher™ acceptor dyes optimized for preparing FRET peptides.

Tide Quencher™ Dyes: Optimized To Maximize FRET Efficiency

AAT Bioquest has developed robust Tide Quencher™ acceptor dyes for synthesizing long wavelength FRET probes. Tide Quencher™ acceptor dyes are superior alternatives for eliminating the limitations associated with using classic quenchers. Our Tide Quencher™ series of non-fluorescent dyes spans the full visible spectrum with unusually high efficiency. For example, TQ2 has absorption maximum perfectly matching the emission of FAM while TQ3, TQ5 and TQ7 are proven to be the best quenchers for Cy3®, Cy5® and Cy7®.

Tide Quencher™ dyes are excellent dark quenchers that are individually optimized to pair with popular fluorescent dyes such as fluorescein, rhodamine and cyanine. These Tide Quencher™ dark FRET acceptors, TQ1 through TQ7, are perfectly matched to be paired with our Tide Fluor™ dyes. Additionally, Tide Quenchers are paired will with classic fluorophores, such as AMCA, EDANS, FAM, TAMRA, HEX, JOE, TET, ROX, Cy3®, Cy5® and Cy7®. Like our Tide Fluor™ donor dyes, our Tide Quencher™ acceptor dyes are much more cost-effective with comparable or better performance for your desired biological applications than other similar products.

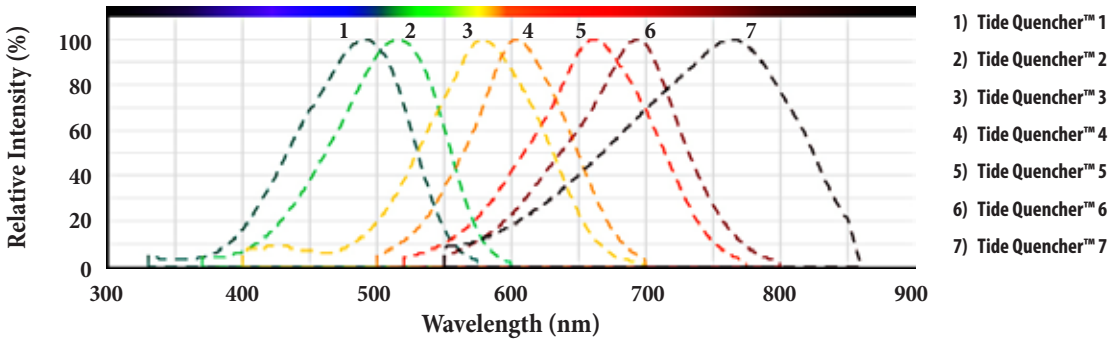

Figure 3.2 Excitation Spectrum of Tide Quencher™ Dyes

Key Features of Tide Quencher™ Dyes:

Tide Quencher™ Donor	Extinction Coefficient ($cm^{-1}M^{-1}$)	Excitation (nm)	Features	Replaces
Tide Quencher™ 1 (TQ1)	20,000	510	• Best paired with Tide Fluor™ 1 (TF1) • Excellent FRET efficiency with coumarins	DABCYL, QSY® 35 and BHQ®-0
Tide Quencher™ 2 (TQ2)	21,000	531	• Best paired with Tide Fluor™ 2 (TF2) • Better matched with FAM, FITC and Alexa Fluor® 488 than other commercial quenchers	BHQ®-1
Tide Quencher™ 2WS (TQ2WS)	19,000	539	• Best paired with Tide Fluor™ 2 (TF2) • Better matched with FAM, FITC and Alexa Fluor® 488 than other commercial quenchers	BHQ®-1
Tide Quencher™ 3 (TQ3)	22,000	598	• Best paired with Tide Fluor™ 3 (TF3) • Excellent FRET efficiency with Cy3®, Alexa Fluor® 555 and TAMRA than other commercial quenchers	QSY® 7, QSY® 9 and BHQ®-2
Tide Quencher™ 3WS (TQ3WS)	90,000	576	• Best paired with Tide Fluor™ 3 (TF3) • Excellent FRET efficiency with Cy3®, Alexa Fluor® 555 and TAMRA than other commercial quenchers	QSY® 7, QSY® 9 and BHQ®-2
Tide Quencher™ 4 (TQ4)		603	• Strong absorption • Best paired with Tide Fluor™ 4 (TF4) • Better FRET efficiency with ROX, Texas Red® and Alexa Fluor® 594 than other commercial quenchers	
Tide Quencher™ 4WS (TQ4WS)	90,000	601	• Strong absorption • Best paired with Tide Fluor™ 4 (TF4) • Better FRET efficiency with ROX, Texas Red® and Alexa Fluor® 594 than other commercial quenchers	
Tide Quencher™ 5 (TQ5)		670	• Best paired with Tide Fluor™ 5 (TF5) • Excellent FRET efficiency with Cy5®, DyLight® 649 and Alexa Fluor® 647	QSY® 21 and BHQ®-3
Tide Quencher™ 5WS (TQ5WS)	130,000	662	• Best paired with Tide Fluor™ 5 (TF5) • Excellent FRET efficiency with Cy5®, DyLight® 649 and Alexa Fluor® 647	QSY® 21 and BHQ®-3
Tide Quencher™ 6WS (TQ6WS)	130,000	702	• Stronger absorption • Best paired with Tide Fluor™ 6 (TF6) • Better FRET efficiency with Cy5.5®, IRDye® 700 and Alexa Fluor® 680 than other commercial quenchers	
Tide Quencher™ 7WS (TQ7WS)	140,000	763	• Stronger absorption • Best paired with Tide Fluor™ 7 (TF7) • Better FRET efficiency with Cy7® and Alexa Fluor® 750 than other commercial quenchers	

Product Ordering Information For Tide Quencher™ Labeling Dyes

Cat #	Product Name	Unit Size	Excitation (nm)	EC ($cm^{-1}M^{-1}$)
2189	Tide Quencher™ 1 Alkyne [TQ1 Alkyne]	5 mg	490	20,000
2192	Tide Quencher™ 1 Amine [TQ1 Amine]	5 mg	490	20,000
2188	Tide Quencher™ 1 Azide [TQ1 Azide]	5 mg	490	20,000
2196	Tide Quencher™ 1 Maleimide [TQ1 Maleimide]	5 mg	490	20,000
2199	Tide Quencher™ 1, Succinimidyl Ester [TQ1, SE]	25 mg	490	20,000
2200	Tide Quencher™ 2 Acid [TQ2 Acid]	100 mg	515	21,000
2212	Tide Quencher™ 2 Alkyne [TQ2 Alkyne]	100 mg	515	21,000
2202	Tide Quencher™ 2 Amine [TQ2 Amine]	5 mg	515	21,000
2211	Tide Quencher™ 2 Azide [TQ2 Azide]	5 mg	515	21,000
2206	Tide Quencher™ 2 Maleimide [TQ2 Maleimide]	5 mg	515	21,000
2210	Tide Quencher™ 2, Succinimidyl Ester [TQ2, SE]	25 mg	515	21,000
2058	Tide Quencher™ 2WS, Succinimidyl Ester [TQ2WS, SE]	5 mg	515	19,000
2220	Tide Quencher™ 3 Acid [TQ3 Acid]	100 mg	570	22,000
2232	Tide Quencher™ 3 Alkyne [TQ3 Alkyne]	5 mg	570	22,000
2222	Tide Quencher™ 3 Amine [TQ3 Amine]	5 mg	570	22,000
2231	Tide Quencher™ 3 Azide [TQ3 Azide]	5 mg	570	22,000
2226	Tide Quencher™ 3 Maleimide [TQ3 Maleimide]	5 mg	570	22,000
2230	Tide Quencher™ 3, Succinimidyl Ester [TQ3, SE]	25 mg	570	22,000
2229	Tide Quencher™ 3WS, Succinimidyl Ester [TQ3WS, SE]	1 mg	578	90,000
2060	Tide Quencher™ 4WS Acid [TQ4WS Acid]	5 mg	603	90,000
2069	Tide Quencher™ 4WS Alkyne [TQ4WS Alkyne]	1 mg	603	90,000
2061	Tide Quencher™ 4WS Amine [TQ4WS Amine]	1 mg	603	90,000
2068	Tide Quencher™ 4WS Azide [TQ4WS Azide]	1 mg	603	90,000
2064	Tide Quencher™ 4WS Maleimide [TQ4WS Maleimide]	1 mg	603	90,000
2067	Tide Quencher™ 4WS, Succinimidyl Ester [TQ4WS, SE]	1 mg	603	90,000
2083	Tide Quencher™ 5WS Alkyne [TQ5WS Alkyne]	1 mg	661	130,000
2076	Tide Quencher™ 5WS Amine [TQ5WS Amine]	1 mg	661	130,000
2082	Tide Quencher™ 5WS Azide [TQ5WS Azide]	1 mg	661	130,000
2079	Tide Quencher™ 5WS Maleimide [TQ5WS Maleimide]	1 mg	661	130,000
2081	Tide Quencher™ 5WS, Succinimidyl Ester [TQ5WS, SE]	1 mg	661	130,000
2090	Tide Quencher™ 6WS Acid [TQ6WS Acid]	5 mg	704	130,000
2098	Tide Quencher™ 6WS Alkyne [TQ6WS Alkyne]	1 mg	704	130,000
2091	Tide Quencher™ 6WS Amine [TQ6WS Amine]	1 mg	704	130,000
2097	Tide Quencher™ 6WS Azide [TQ6WS Azide]	1 mg	704	130,000
2094	Tide Quencher™ 6WS Maleimide [TQ6WS Maleimide]	1 mg	704	130,000
2096	Tide Quencher™ 6WS, Succinimidyl Ester [TQ6WS, SE]	1 mg	704	130,000
2113	Tide Quencher™ 7WS Alkyne [TQ7WS Alkyne]	1 mg	763	140,000
2106	Tide Quencher™ 7WS Amine [TQ7WS Amine]	1 mg	763	140,000
2112	Tide Quencher™ 7WS Azide [TQ7WS Azide]	1 mg	763	140,000
2109	Tide Quencher™ 7WS Maleimide [TQ7WS Maleimide]	1 mg	763	140,000
2111	Tide Quencher™ 7WS, Succinimidyl Ester [TQ7WS, SE]	1 mg	763	140,000

trFluor™ Dyes

Features of trFluor™ Dyes:

- **trFluor™ dyes** are available in a variety of reactive forms.
- **trFluor™ dyes** are much easier to be conjugated to proteins and other biomolecules, giving much higher conjugation yield than other europium and terbium dyes.
- **trFluor™ conjugates** are maximally excited by the common light sources at ~350 nm.
- **To maximize** its TR-FRET potential, the trFluor™ Eu dyes are optimized to pair with APC, iFluor™ 647, TF5, Cy5®, DyLight™ 650 or Alexa Fluor® 647.
- **To maximize** its TR-FRET potential, the trFluor™ Tb dyes are optimized to pair with FITC, iFluor™ 488, TF2, DyLight™ 488 or Alexa Fluor® 488.
- **No fluoride addition is required.**
- **No enhancing solution is required.**

INTRODUCTION

Many biological compounds present in cells, serum or other biological fluids are naturally fluorescent. Thus, the use of conventional, prompt fluorophores leads to serious limitations in assay sensitivity due to the high background caused by the autofluorescence of the biological molecules to be assayed. Utilizing long-lived fluorophores combined with time-resolved detection, a delay between excitation and emission detection, minimizes prompt fluorescence interferences. Our trFluor™ probes enable time-resolved fluorometry (TRF) for the assays that require high sensitivity. These trFluor™ probes have large Stokes shifts and extremely long emission half-lives when compared to traditional fluorophores such as Alexa Fluor® or cyanine dyes. Compared to other TRF compounds, our trFluor™ probes have relatively high stability, high emission yield and ability to be linked to biomolecules. Moreover, our trFluor™ Eu probes are insensitive to fluorescence quenching when conjugated to biological polymers such as antibodies.

Table 4.1 Typical acceptors for the time -resolved luminescent probes

trFluor™ Donors	Recommended Acceptors
trFluor™ Eu	iFluor™ 647, TF5, APC
trFluor™ Tb	iFluor™ 488, TF2, FITC

trFLUOR™ Tb DYES

An Excellent Building Block for Developing TR-FRET Assays

Our trFluor™ Tb probes enable TRF for the assays that require high sensitivity. The trFluor™ Tb dye has large Stokes shifts and extremely long emission half-lives when compared to more traditional fluorophores such as Alexa Fluor® or cyanine dyes. Compared to other TRF compounds, our trFluor™ Tb probes have relatively high stability, high emission yield and ability to be linked to biomolecules with higher conjugation yield. Moreover, our trFluor™ Tb probes are insensitive to fluorescence quenching when conjugated to biological polymers such as antibodies. To maximize its TR-FRET potential, trFluor™ Tb dye is optimized to pair with FITC, iFluor™ 488, TF2, DyLight™ 488 and Alexa Fluor® 488.

tr-FRET Mechanism

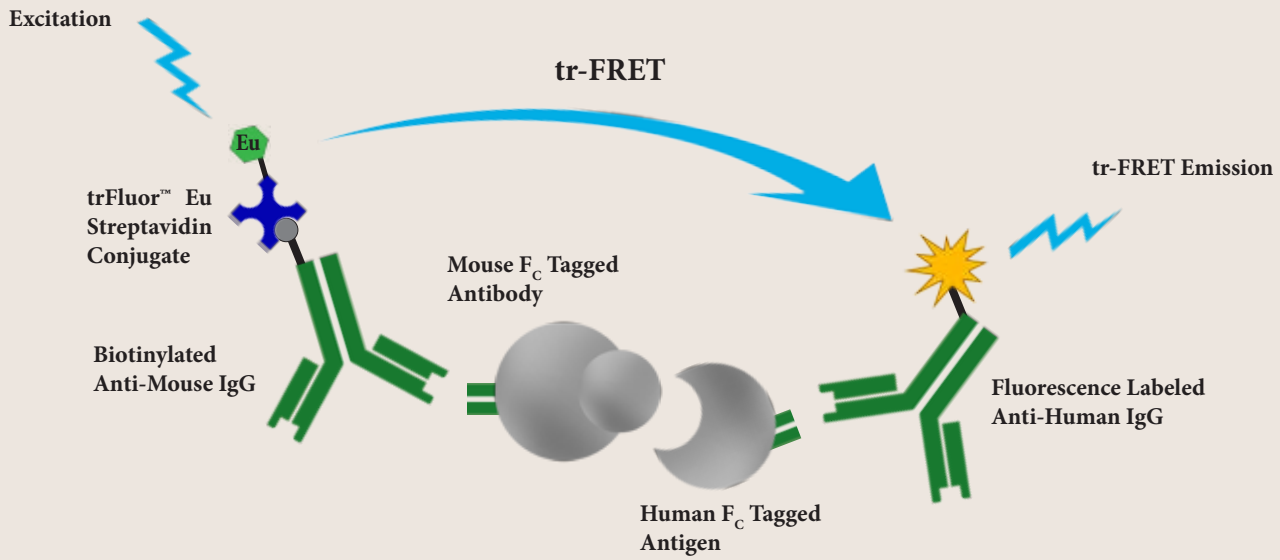

Figure 4.1 tr-FRET assay principle using trFluor™ Eu - labeled streptavidin conjugates.

trFLUOR™ Eu DYES

An Excellent Building Block for TR-FRET Assays

Our trFluor™ Eu probes enable TRF for the assays that require high sensitivity. The trFluor™ Eu dye has large Stokes shifts and extremely long emission half-lives when compared to more traditional fluorophores such as Alexa Fluor® or cyanine dyes. Compared to the other TRF compounds, our trFluor™ Eu probes have relatively high stability, high emission yield and ability to be linked to biomolecules with higher conjugation yield. Moreover, our trFluor™ Eu probes are insensitive to fluorescence quenching when conjugated to biological polymers such as antibodies. To maximize its TR-FRET potential, trFluor™ Eu dye is optimized to pair with APC, iFluor™ 647, TF5, Cy5®, DyLight™ 650 and Alexa Fluor® 647.

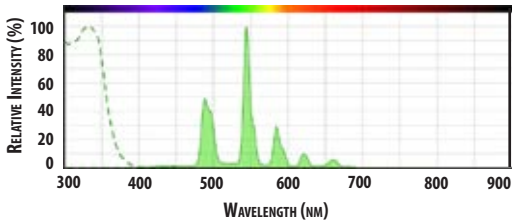

Figure 4.2 Excitation and Emission spectra of trFluor™ Tb.

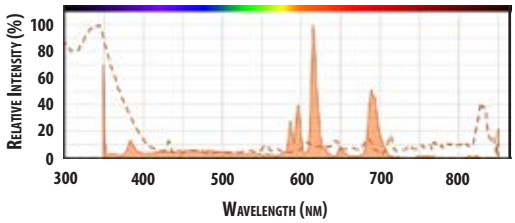

Figure 4.3 Excitation and Emission spectra of trFluor™ Eu.

Product Ordering Information For trFluor™ Labeling Dyes

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
1300	ReadiLink™ trFluor™ Eu protein labeling kit	1 kit	346	617
1305	ReadiLink™ trFluor™ Tb protein labeling kit	1 kit	330	544
16518	trFluor™ Eu goat anti-mouse IgG (H+L)	100 µg	346	617
16668	trFluor™ Eu goat anti-rabbit IgG (H+L)	100 µg	346	544
1434	trFluor™ Eu maleimide	100 µg	346	617
16925	trFluor™ Eu-streptavidin conjugate	100 µg	346	617
1433	trFluor™ Eu succinimidyl ester	1 mg	346	617
16519	trFluor™ Tb goat anti-mouse IgG (H+L)	100 µg	330	544
16669	trFluor™ Tb goat anti-rabbit IgG (H+L)	100 µg	330	544
1444	trFluor™ Tb maleimide	100 µg	330	544
16926	trFluor™ Tb-streptavidin conjugate	100 µg	330	544
1443	trFluor™ Tb succinimidyl ester	1 mg	330	544

Appendix A

FRET Dark Quenchers (Acceptors)

Fluorescent Dyes (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/FITC, Cy2®	□	■	□				
Helix Fluor™ 555 HEX TET JOE VIC		□	■	□			
Tide Fluor™ 3 (TF3) iFluor™ 555 Helix Fluor™ 575 Alexa Fluor® 546 Alexa Fluor® 555 ATTO 550 & ATTO 565 DyLight Fluor™ 550 Cy3®, NED, TAMRA		□	■	□			
Tide Fluor™ (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™ 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®						□	■

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Table Legend:

■ **Best to Use**

□ **OK to Use**

□ **Not Recommended for Use**

Alphabetical Index

Alphabetical Index

Catalog Number Index

International Distributors

Austria:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

Australia:
Assay Matrix Pty Ltd.
Email: info@assaymatrix.com
Website: http://www.assaymatrix.com

Life Research Pty Ltd.
Email: info@liferesearch.com
Website: http://www.liferesearch.com

Belgium:
Gentaur BVBA
Email: info@gentaur.com
Website: http://www.gentaur.com

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Website: http://www.cedarlanelabs.com

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Website: http://www.biosite.fi

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Website: http://www.bcls.in

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Website: http://www.biosite.se

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Website: http://www.biosite.se

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Website: http://www.vitro.ro

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Website: http://www.csbiotech.com.tw

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Website: http://www.rainbowbiotech.com.tw

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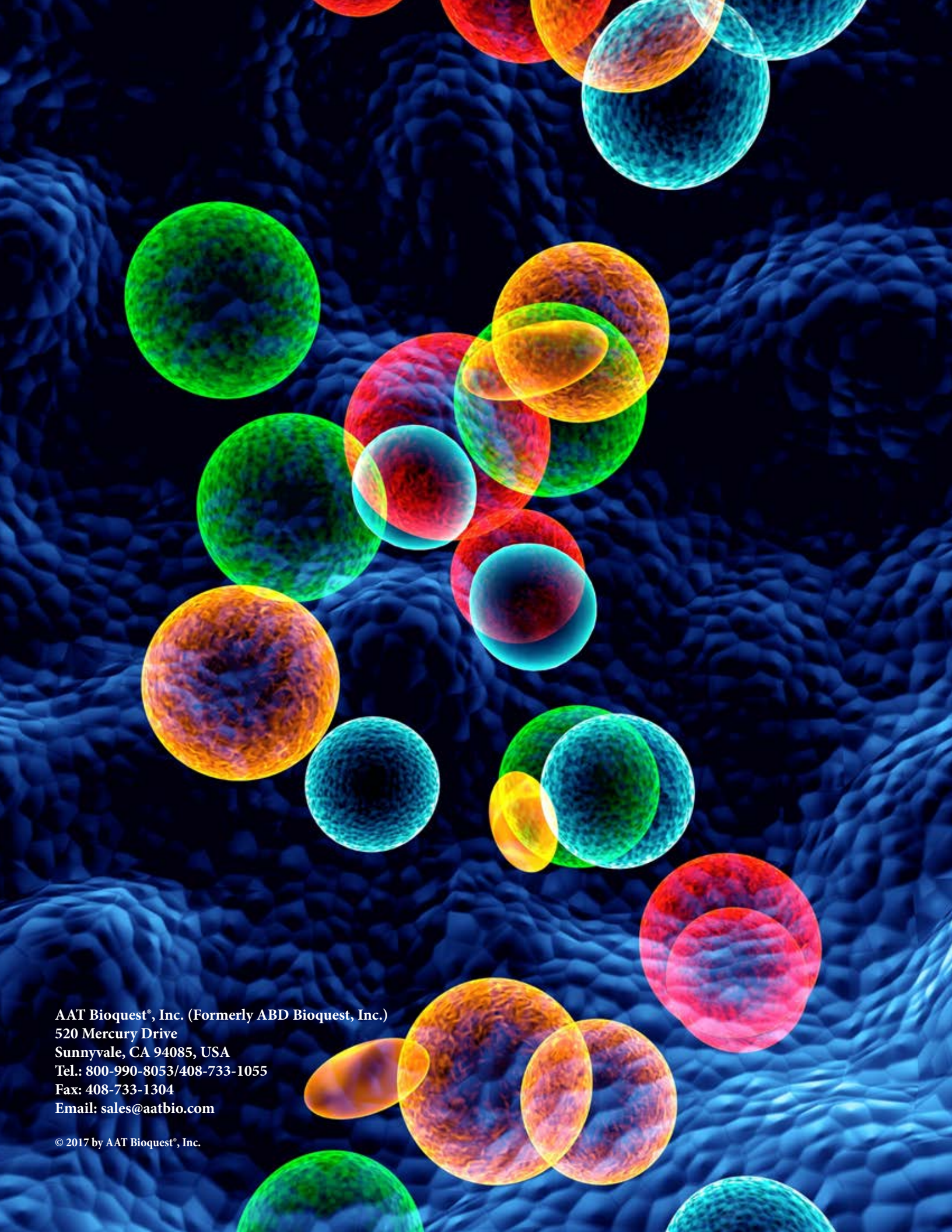
Email: info@biomol.de
Website: http://www.biomol.de

United Kingdom:
Stratech Scientific Ltd.
Email: info@stratech.co.uk
Website: http://www.stratech.co.uk



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