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Bright Tide Fluor™-Based Fluorescent Peptides and Their Applications In Drug Discovery and Disease Diagnosis

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Introduction

Fluorescent peptides are widely used as important tools in drug discovery and disease diagnosis. Although EDANS, FAM and TAMRA are widely used to label peptides, their short absorption wavelengths, low extinction coefficients and pH-sensitive fluorescence have limited their use in the development of sensitive fluorescent peptide probes. Alexa Fluor® (AF) dyes (Invitrogen) demonstrated improved fluorescence properties. However, the high cost of Alexa Fluors make the AF dyes unaffordable for labeling peptides.

The strongly fluorescent and affordable Tide Fluor™ (TF) dyes eliminate the above-mentioned limitations of the classic dyes. They are individually optimized for labeling peptides and nucleotides. As the Alexa Fluors, the TF dyes are bright pH-insensitive and photostable. Compared to Alexa Fluors, TF dyes are more stable to the peptide deprotecction conditions.

We have used TF 2 and TF5 dyes to develop new fluorescent peptides for high throughput analysis of protease and protein kinase activities and screening of protease and protein kinase inhibitors. Excellent spectral properties and labeling efficiencies have also been observed for TF3, 4, 6, 7 and 8. Among them TF 6, 7 and 8 have been used for developing fluorescent peptides that have emission in the near infrared or infrared range. These NIR and IR TF-based peptides have been successfully used in in-vivo imaging.

Materials and Methods

All the peptides were synthesized using the standard FMOC chemistry by American Peptide Company. Some of the TF peptides were prepared using FMOC-Lys(TF)-OH, FMOC-Asp(TF)-OH or FMOC-Glu(TF)-OH amino acids. For the post-labeling of the premade peptides, TF NHS esters were used for labeling N-terminal amino or ε–amino group of lysine residue. TF maleimides or iodoacetamides were used for labeling the SH group of cysteine residues.

TF1, TF2, TF3, TF4, TF5, TF6, TF7 and TF8 acids, maleimides and NHS esters, FMOC-Lys(TF2-Boc)-OH, FMOC-Lys(TF3)-OH, FMOC-Asp(TF2-Boc)-OH, FMOC-Asp(TF3)-OH, FMOC-Asp(TF2-Boc)-OH, FMOC-Glu(TF3)-OH, FMOC-Glu(TF2-Boc)-OH and FMOC-Glu(TF3)-OH are now commercially available from ABD Bioquest.

All the enzyme assays were done with purified enzymes that are commercially available from Sigma, R&D Systems, EMD Chemicals. Absorption spectra were taken with Hitachi U-3010. Endpoint fluorescence assays were run on either BMG NovoStar or Gemini SpectraMax (Molecular Devices). Enzyme kinetic assays were run on FlexStation Molecular Devices).

Spectral Properties of Tide Fluor™ (TF) Dyes

Our TF series of fluorescent labeling dyes cover the full visible spectrum with unusually high labeling efficiency. For example, TF2 has spectral properties essentially identical to AF 488, FAM and FITC. It is 3 times brighter and 4 times more photostable than Fluorescein under the physiological conditions or in cells. TF dyes have fluorescence that is insensitive to pH fluctuation from pH 4 to pH 10 while the fluorescence intensity of FAM and FITC is strongly dependent on pH in the same range.

TF Dyes	Excitation	Emission	Replacement for
TF1	353 nm	442 nm	AF350, AMCA, EDANS
TF2	498 nm	520 nm	AF488; DL488, FITC, FAM, Cy2
TF3	560 nm	575 nm	AF546, AF555, Cy3, DL549, TAMRA
TF4	585 nm	605 nm	AF594, DL594, Texas Red®, ROX
TF5	650 nm	670 nm	AF633, AF647, DL633, DL649, Cy5
TF6	685 nm	700 nm	AF680, DL680, Cy5.5
TF7	755 nm	780 nm	AF750, DL750, Cy7
TF8	785 nm	800 nm	DL800, IRDye CW800

Legends: AF = Alexa Fluor® (Invitrogen); DL = DyLight™ (Thermo Fisher); IRDye CW800 (Li-Cor); TF = Tide Fluor™ (ABD Bioquest)

Detection of Matrix Metalloproteinase (MMP) Activities Using TF-Based FRET Substrates

Matrix metalloproteinases (MMPs) are involved in the degradation of components of extracellular matrix and play an important role in apoptosis, embryogenesis, reproduction tissue remodeling and repair. The following TF-based FRET substrates were synthesized and screened for detecting MMP activities.

#Sub	Peptide Sequence	Excitation	Emission
1	TQ2-Gaba-Pro-Cha-Abu-Smc-His-Ala-Dab(5-FAM)-Ala-Lys-NH_2	492 nm	514 nm
2	TQ2-Gaba-Pro-Cha-Abu-Smc-His-Ala-Dab(TF2)-Ala-Lys-NH_2	495 nm	520 nm
3	TQ3-Gaba-Pro-Cha-Abu-Smc-His-Ala-Lys(TF3)-Ala-Lys-NH_2	560 nm	575 nm
4	TQ4-Gaba-Pro-Cha-Abu-Smc-His-Ala-Lys(TF4)-Ala-Lys-NH_2	585 nm	605 nm
5	TQ5-Gaba-Pro-Cha-Abu-Smc-His-Ala-Lys(FT5)-Ala-Lys-NH_2	650 nm	670 nm
6	TQ6-Gaba-Pro-Cha-Abu-Smc-His-Ala-Lys(FT6)-Ala-Lys-NH_2	685 nm	700 nm
7	TQ7-Gaba-Pro-Cha-Abu-Smc-His-Ala-Lys(TF7)-Ala-Lys-NH_2	755 nm	780 nm
8	TQ8-Gaba-Pro-Cha-Abu-Smc-His-Ala-Lys(TF8)-Ala-Lys-NH_2	785 nm	800 nm

Detection of MMP Activities (Continued)

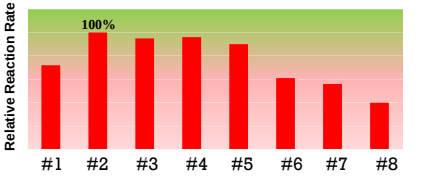

Figure 1. TF-based MMP substrates were incubated with MMP-1 (50 units/ml) at room temperature for 15 min. All the substrates were used at 5 uM, and the reaction rate is set at 100% with Sub #2. The fluorescence increase were measured with the excitation and emission wavelengths respectively listed in the above table.

Detection of Generic Protease Activities Using TF-Labeled Casein Substrates

Monitoring of various protease activities has become a routine task for many biological laboratories. TF dyes-labeled casein conjugates are proven to be generic substrates for a broad spectrum of proteases. In the intact substrates, casein is heavily labeled with a TF dye, resulting in significant fluorescence quenching. Protease-catalyzed hydrolysis relieves its quenching effect, yielding brightly fluorescent TF dye-labeled peptide fragments. The increase in fluorescence intensity of TF dye-labeled peptide is directly proportional to the protease activity.

#Sub	Peptide Substrate	Ex/Em	Rel. K_{cat}/K_m
1	TF1-Casein (~6 Dyes/Casein)	492/514 nm	98%
2	TF2-Casein (~6 Dyes/Casein)	495/520 nm	92%
3	TF3-Casein (~6 Dyes/Casein)	560/575 nm	100%
4	TF4-Casein (~5 Dyes/Casein)	585/605 nm	73%
5	TF5-Casein (~5 Dyes/Casein)	650/670 nm	81%
6	TF6-Casein (~4 Dyes/Casein)	685/700 nm	76%
7	TF7-Casein (~4 Dyes/Casein)	755/780 nm	56%
8	TF8-Casein (~4 Dyes/Casein)	785/800 nm	43%

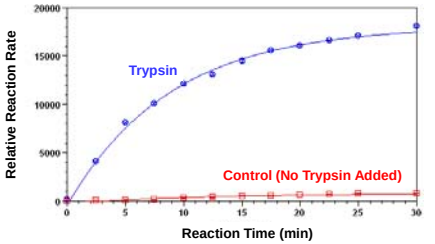

Figure 2. Proteolytic cleavage of Substrate #3 trypsin. Substrate #3 was incubated with trypsin at room temperature The control wells had protease substrate only without trypsin. The fluorescence signal was measured starting from Time 0 (when trypsin was added) using FlexStation.