Advancing Biological Research with Fluorescent Peptides: Illuminating Cellular Processes for Enhanced Understanding

White Paper

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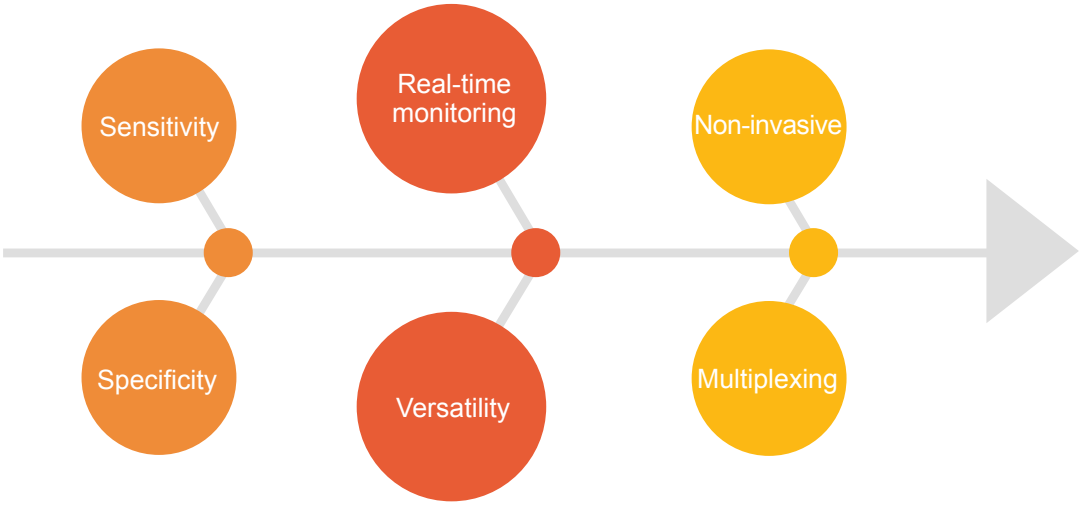
Fluorescent Peptide

Introduction

Fluorescent peptides are short amino acid sequences modified to incorporate a fluorescent molecule. These peptides are designed to emit light of a specific wavelength when excited by an external light source, such as ultraviolet (UV) or blue light. The fluorescence emitted by these peptides can be used for various applications, including biological imaging, protein-protein interaction studies, and drug discovery[1].

Modifying peptides with fluorescent molecules allows researchers to track and visualize specific cellular processes or molecules of interest in real-time. Scientists can study the fluorescent peptide's localization, movement, and interactions within living cells or organisms by attaching the fluorescent peptide to a target protein or molecule.

WHY FLUORESENCE MODIFICATION IS NEEDED?



The choice of the fluorescent molecule and its specific attachment site on the peptide can influence the fluorescence properties, such as brightness, photostability, and spectral characteristics. Different fluorescent dyes, such as fluorescein, rhodamine, or cyanine dyes, can label peptides, each with unique fluorescence properties[2].

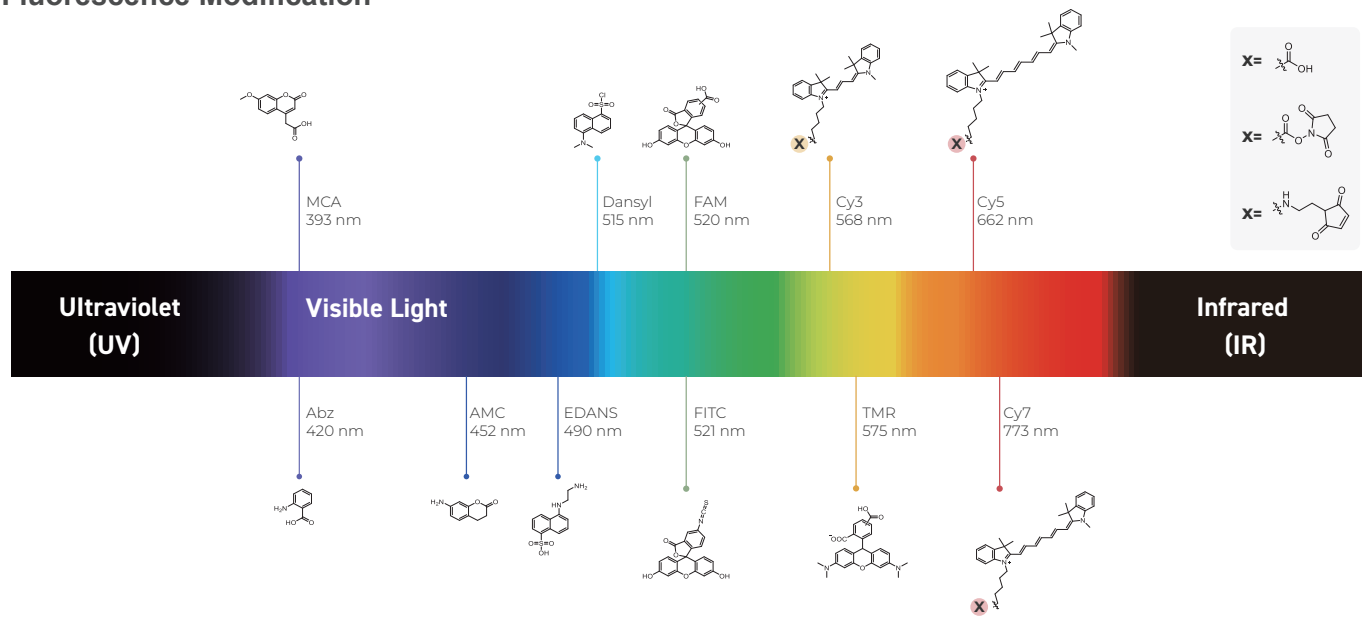
Fluorescent peptides have become valuable tools in molecular biology and biotechnology research due to their ability to provide visual information about cellular processes. They are widely used in fluorescence microscopy, flow cytometry, and fluorescence resonance energy transfer (FRET) assays.

The use of fluorescent amino acids, such as tryptophan (Trp), tyrosine (Tyr), and phenylalanine (Phe), has gained significant attention in recent years. These natural fluorescent amino acids possess specific fluorophores that undergo π–π transitions when excited by light of a particular wavelength, resulting in fluorescence emission. This

property has made them valuable building units for enhancing the fluorescence properties of other molecules[3].

In summary, fluorescent peptides are modified amino acid sequences that emit fluorescence when excited by external light sources. They are used to visualize and study various biological processes, contributing to advancements in biological research, drug discovery, and medical diagnostics.

Fluorescence Modification



Donor			Acceptor		
Name	**Excitation (nm)**	**Emission (nm)**	**Name**	**Excitation (nm)**	**Emission (nm)**
Cy2	490	510	Cy3	555	570
FITC	494	521	TRITC	557	576
FAM	495	520	Cy3	555	570
FAM	495	520	Texas Red	589	615
FAM	495	520	Cy5	646	662
Cy3	555	570	Cy5	646	662
EDANS	335	493	DABCYL	453	-
Glu(EDANS)-NH2	335	493	DABCYL	453	-
MCA	328	393	DNP	348	-
Abz	330	420	DNP	348	-
Abz	330	420	Tyr (3-NO2)	360	-

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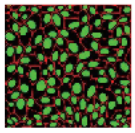
BASIC APPLICATION OF FLUORESCENT PEPTIDE

Fluorescent peptides have a wide range of applications in various fields. Here are some

01 In vivo biomedical imaging

Angiography- Fluorescent peptide is conjugated to a fluorescent dye or fluorophore and injected into the bloodstream to specifically target endothelial cells lining the blood vessels, allowing for visualization and imaging of the vasculature.

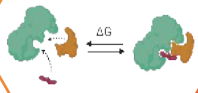
Cellular imaging-Fluorescent peptides can be designed to specifically target and bind to specific cell types or cellular structures to track cells in real-time within living organisms to study the cellular behaviors, interactions, and migration in their native environment.



Stimulated emission depletion microscopy (STED)- Allows for imaging at a resolution beyond the diffraction limit of light where researchers can label and visualize specific targets with high precision and detail to study the cellular structures at the nanoscale level, providing valuable insights into cellular organization and function.

02 Protein binding and localization studies

Immunoassays-Fluorescent peptides are utilized in immunoassays as a probe to bind to a specific target protein or antigen. The fluorescence signal emitted by the bound probe is measured to determine the presence and concentration of the target protein.



03 Fret Pairs

Fluorescence lifetime imaging- GFP & RFP, CFP & YFP, Fluorescein & Rhodamine, BODIPY & Fluorescein are frequently used FRET pairs in protein-protein interaction studies where the change in FRET efficiency allows for the real-time monitoring of enzymatic activity.

GenScript's case study:

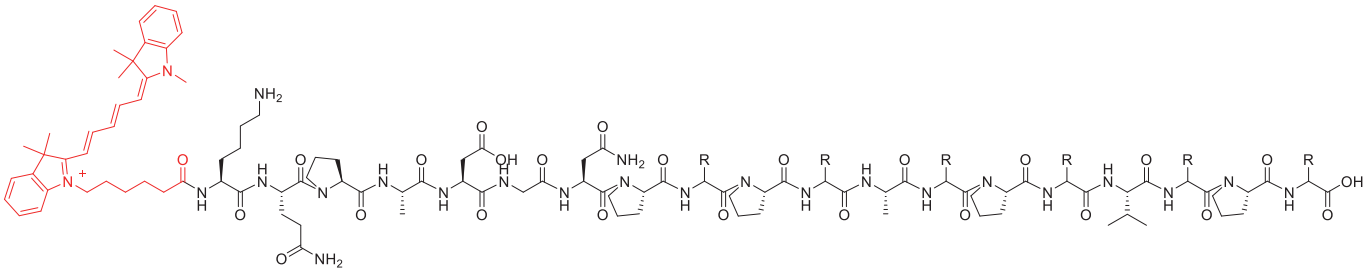
Case 1: N-terminal modification Cy5-COOH

Challenges:

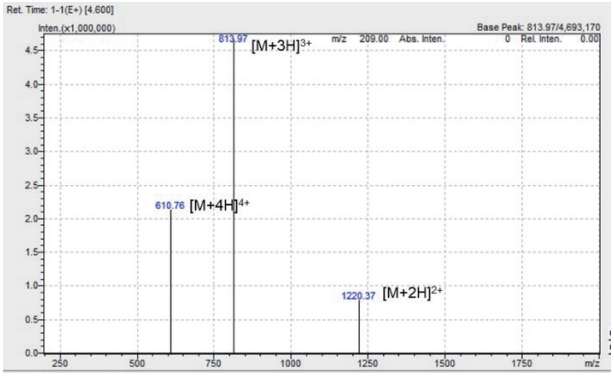
a) 19AA, 42% hydrophobic AA, hard to achieve high purity in both synthesis and purification steps.

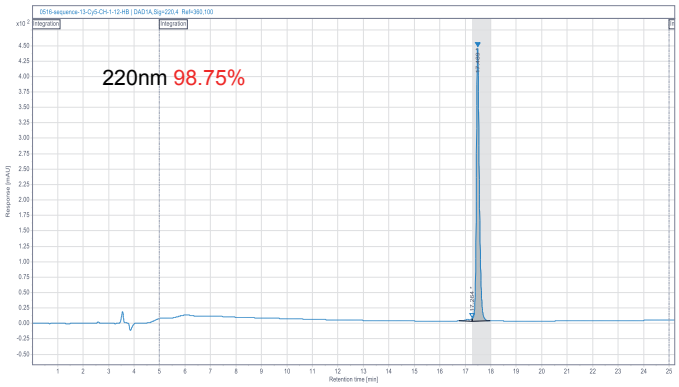
b) ≥95% purity with fluorescence test;

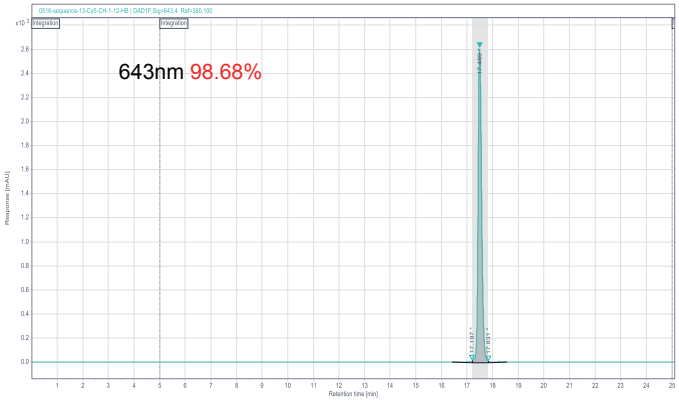
Results: Item is delivered as a high-purity peptide with a strong fluorescence response.



{Cy5}KQPADGNPXPXAXPXVXPX







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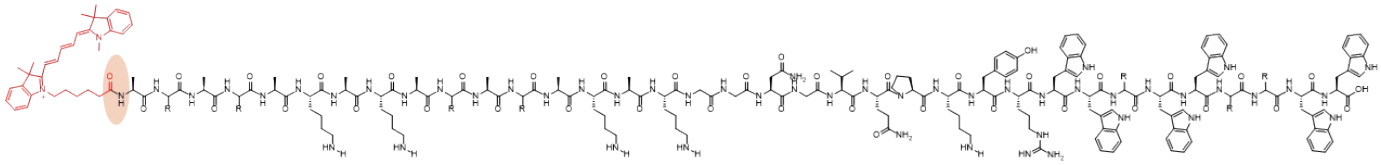
Case 2: N-terminal modification Cy5-COOH

Challenges:

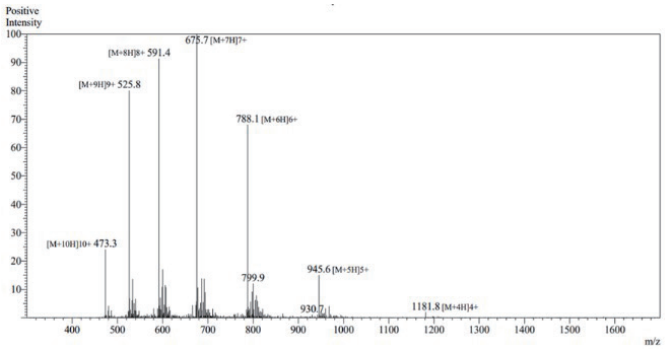
1) 35AA, 46% hydrophobic AA;

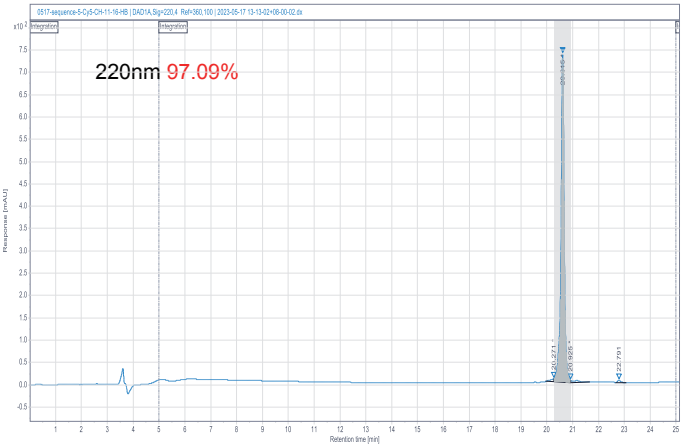
2) ≥95% purity，39mg required

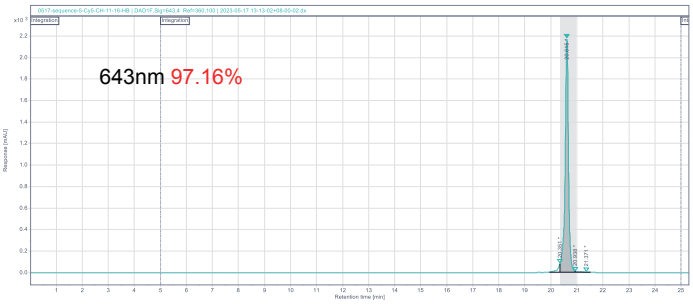
Results: All items were successfully synthesized and delivered in 1 round.



{Cy5}AXAXAKAKAXAXAKAKGGNGVQPKYRWWXWWXXWW







Case 3: Solid phase modification on the side chain of Lys

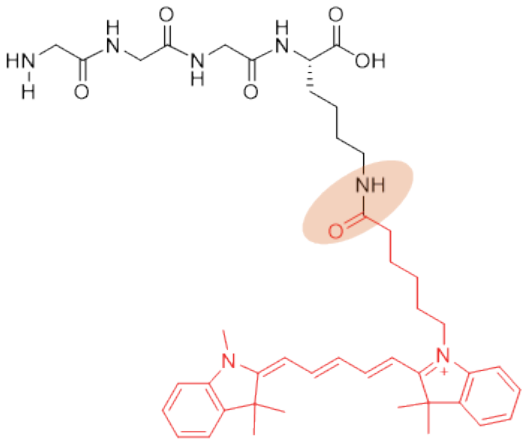
Challenges:

1) Specific protection group removal, resin terminal amino side chain modification.

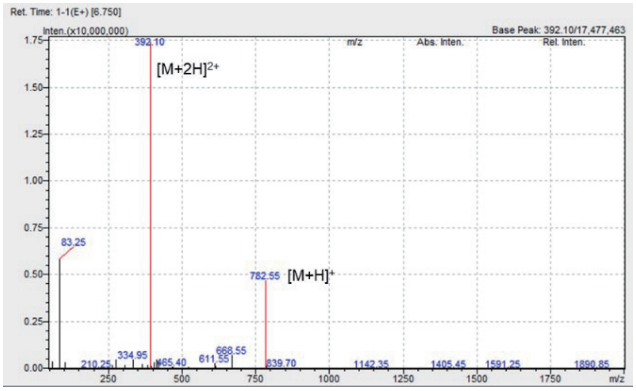
2) It is difficult to obtain hydrophilic short sequences during precipitation.

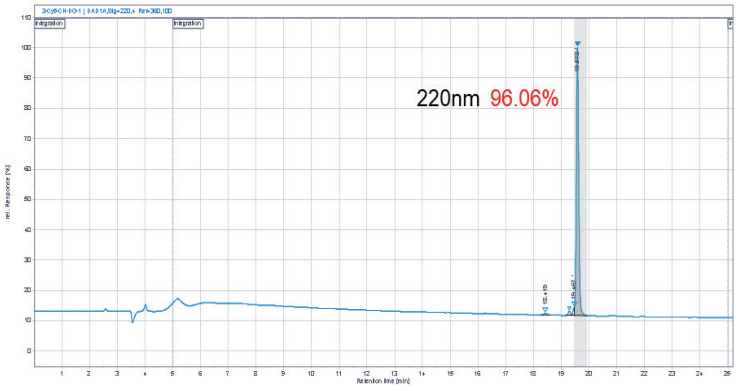
3) ≥95% purity

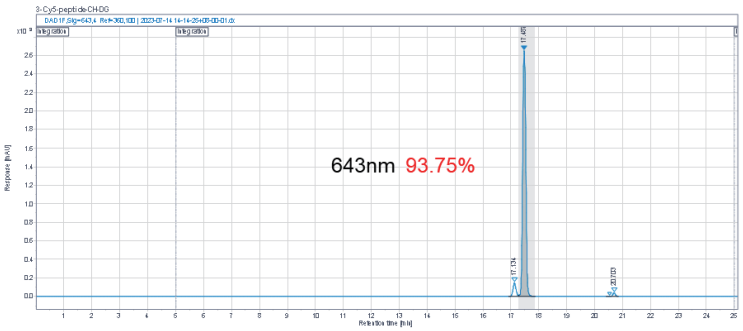
Results: All items were successfully synthesized and delivered.



GGG{Lys(Cy5)}







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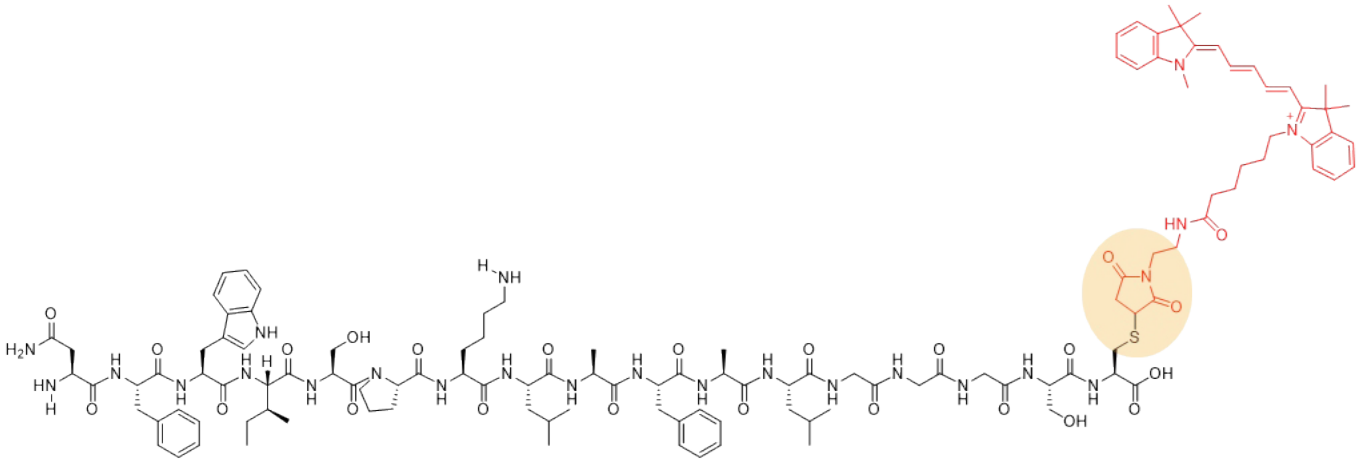
Case 4: Liquid phase modification on the side chain of Cys

Challenges:

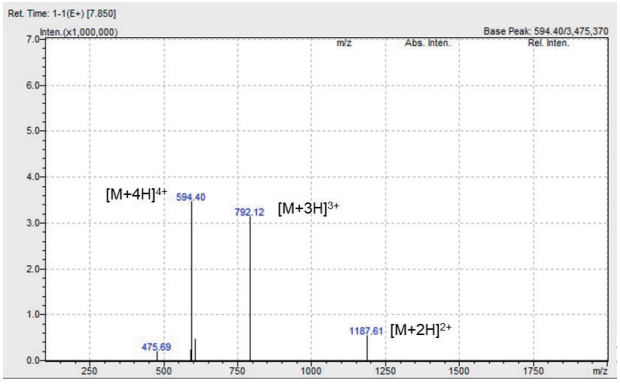
1) Hydrophobic peptide, 17 amino acids, with a hydrophobic amino acid content of 53%.

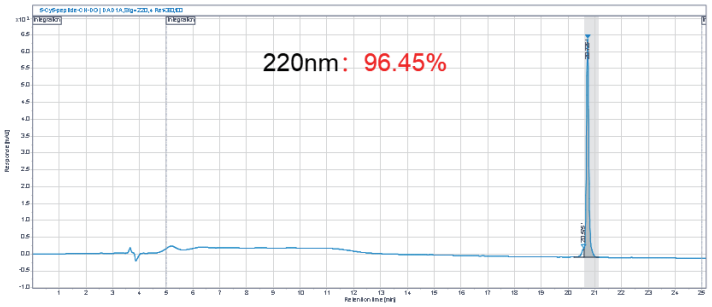
2) ≥90% purity

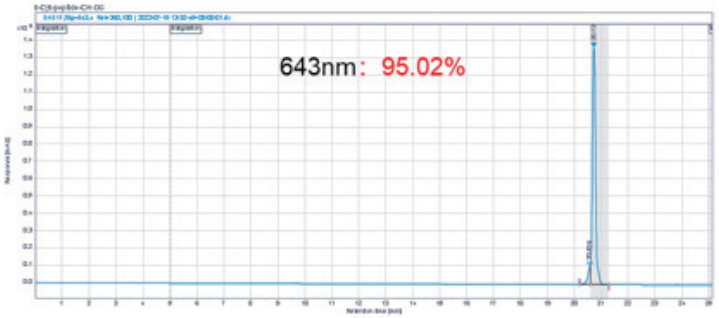
Results: All items were successfully synthesized in 1 round and delivered.



NFWISPKLAFALGGGS{Cys(Cy5)}







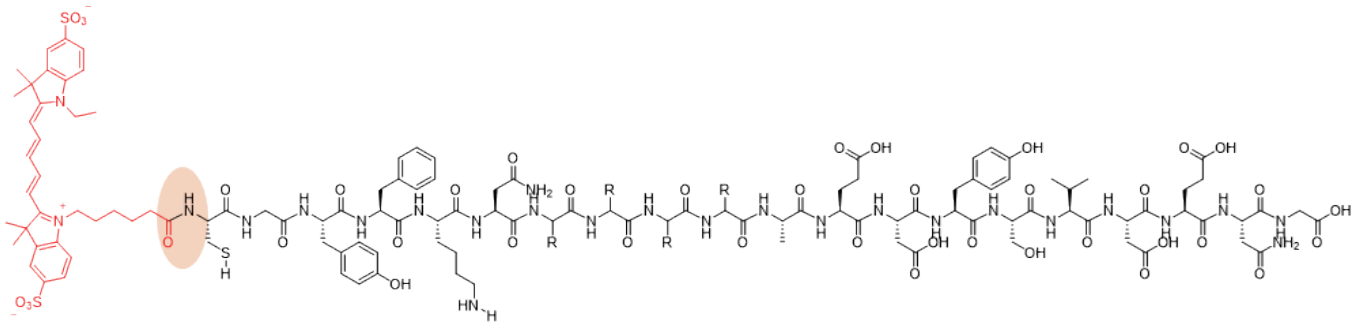
Case 5: N-terminal modification sul-Cy5-COOH (Water soluble Cy Dye)

Challenges:

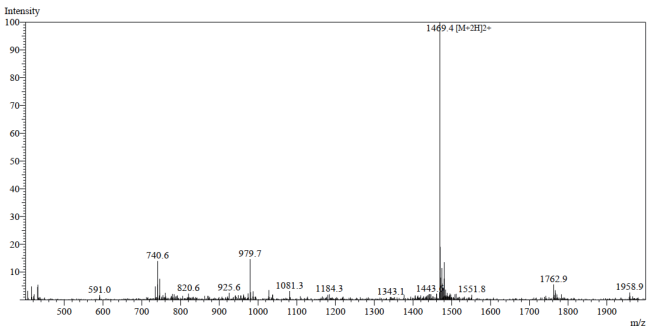
1) The fluorescence quantum yield of Sul-Cy5-COOH is higher than that of Cy5-COOH, resulting in better fluorescence performance. It also improves the water solubility of peptides. However, it poses challenges for solid-phase synthesis. (In reality, testing can be done for solid-phase modification).

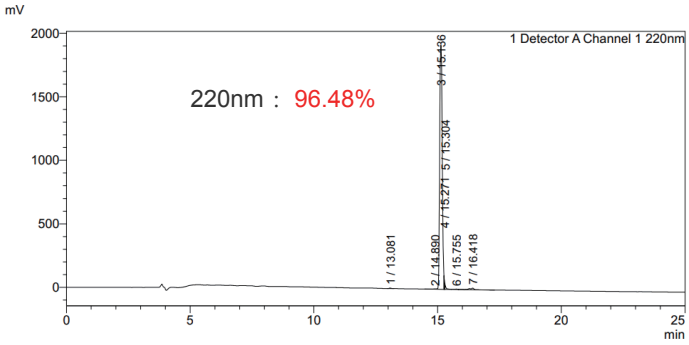
2) ≥95% purity

Results: All items were successfully synthesized in 1 round and delivered.



{Sul-Cy5}CGYFKNXXXXAEDYSVDENG





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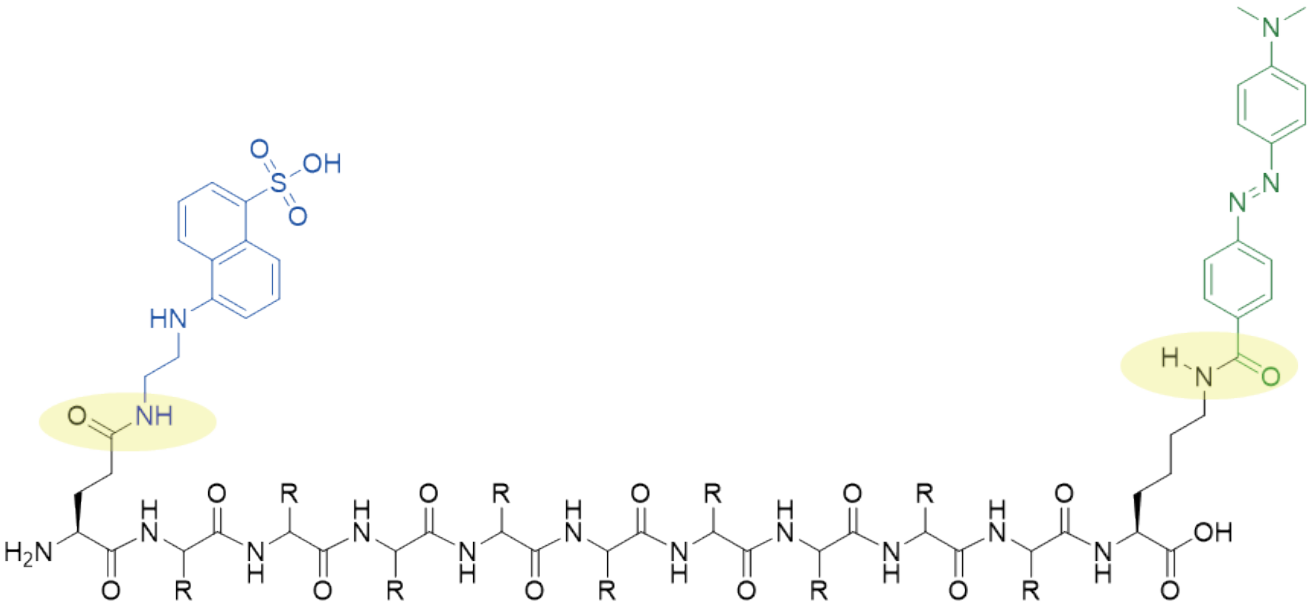
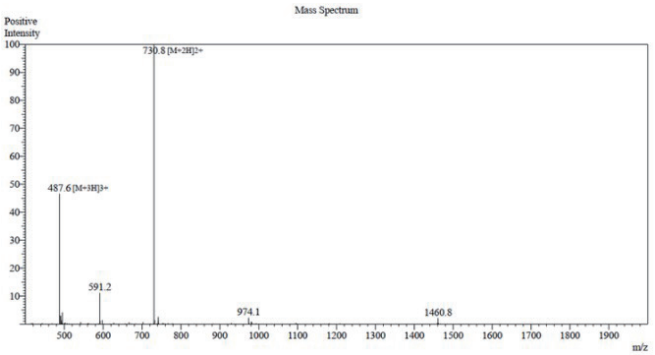
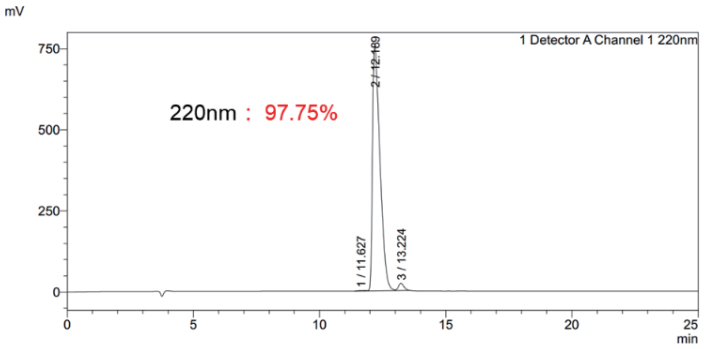
Case 6: EDANS (Donor) & DABCYL (Acceptor) FRET Pairs



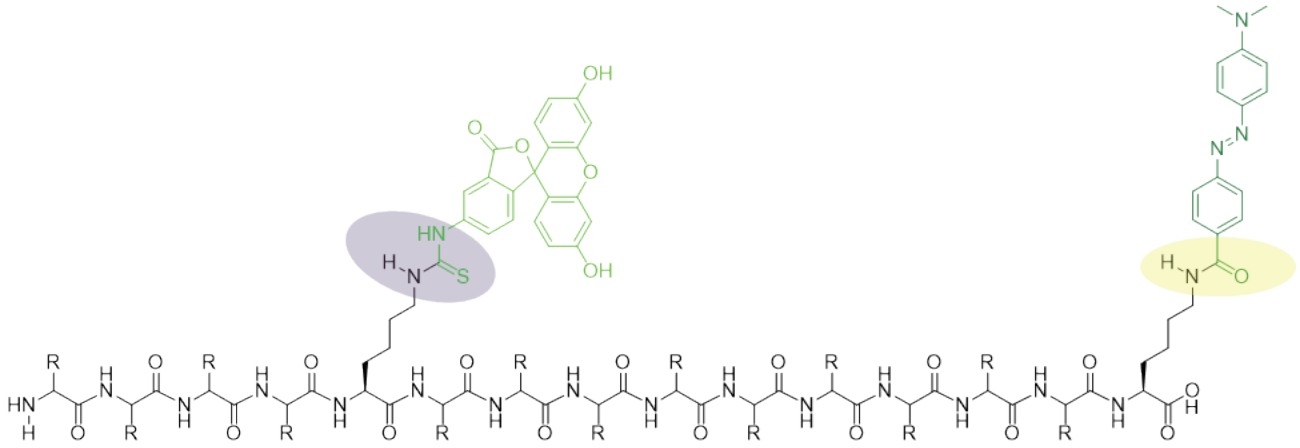
{GLU(EDANS)}XXXXGGGGG{LYS(DABCYL)}

X-Natural amino acids, Non-Natural amino acids



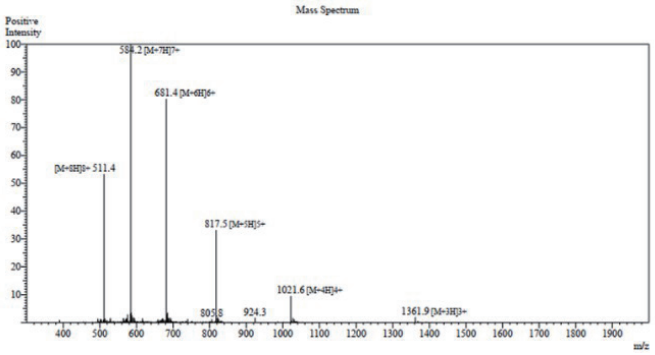


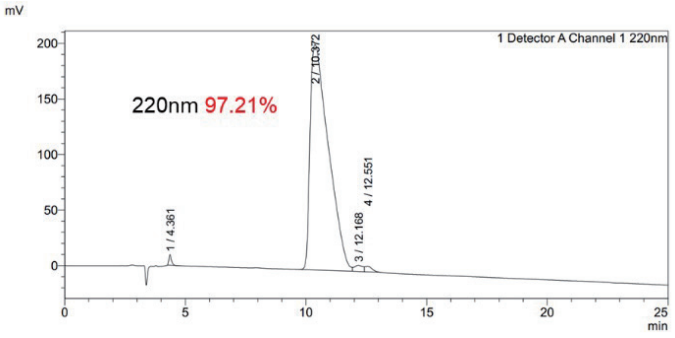
Case 7: FITC & DABCYL-Double Fluorescence modification-FRET Pairs



XXXXX{PEGn}{LYS(FITC)}XXXXGGGGG{LYS(DABCYL)}

X-Natural amino acids, Non-Natural amino acids





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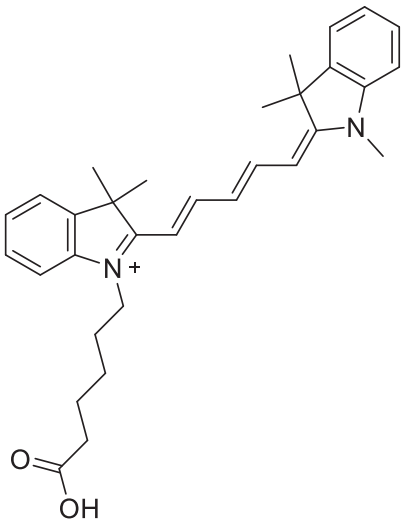
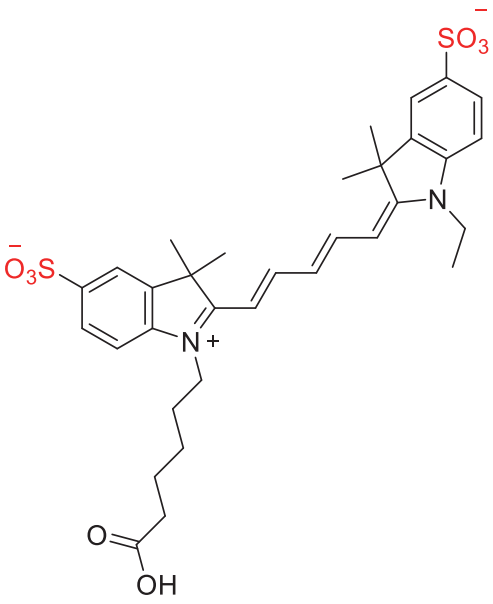
High fluorescence-signal modification available:



Cy5-COOH



sul-Cy5-COOH

Fluorescent compound		**Cy5-COOH**	**sul-Cy5-COOH**
Concentration		mg/mL	
FL	Em	660-665 nm	
	Signal	＞8000	＞14500

Contact our professional team if you have any inquiries on Fluorescent peptides!

References

1.Cheng, Z., Kuru, E., Sachdeva, A. et al. Fluorescent amino acids as versatile building blocks for chemical biology. Nat Rev Chem 4, 275–290 (2020)
2.Mendive-Tapia, L, Wang, J, Vendrell, M. Fluorescent cyclic peptides for cell imaging. Peptide Science. 2021; 113:e24181 (2021)
3.Xiong et al. A review on recent advances in amino acid and peptide-based fluorescence and its potential applications. New J. Chem. 1144-0546 (2021)
4.Ladokhin, A. S. Fluorescence Spectroscopy in Peptide and Protein Analysis. Encyclopedia of Analytical Chemistry. (2006)
5.Cheng, Z., Kuru, E., Sachdeva, A. et al. Fluorescent amino acids as versatile building blocks for chemical biology. Nat Rev Chem 4, 275–290. (2020)

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