Custom Synthesized Peptides with Fluorescent Dyes and Labels

Fluorescently labeled peptides are commonly used to investigate protein interactions such as those involving proteases.

Topics in this article:

Labeling Peptides

Mimotopes' range of fluorescent labels are suitable for labeling the N-Terminus of your peptides. Labeling at other positions within your sequence or at the C-terminus of your peptides can be achieved by adding your chosen fluorescent group to the side chain of a Lysine or Dap residue. A list of common fluorescent labels available is shown in Table 1. Contact Mimotopes to inquire about other labels.

Red Labels
5-carboxytetramethylrhodamine [5TMR]
5,6-carboxytetramethylrhodamine [5(6)TMR]
Lissamine Rhodamine B [LRhodB]
Green Labels
5-carboxyfluoresceine [5FAM]
5,6-carboxyfluoresceine [5(6)FAM]
Methoxy coumarin acetic acid
7-nitro-4-benzofurazanyl [NBD]

Table 1. Commonly used fluorescent labels available from Mimotopes

Applications Using FRET Peptides

Fluorescent Resonance Energy Transfer (FRET) is commonly used to investigate protein interactions such as those involving proteases. FRET peptides are typically labeled with a fluorescent donor and a non-fluorescent acceptor. When these molecules form part of the same short peptide, they are close enough for the acceptor to quench the signal from the donor and for there to be very little fluorescence emission. However, if the acceptor is removed - for example, by protease cleavage - the quenching effect is lost, leading to increased fluorescence from the donor at the appropriate excitation wavelength (see Figure 1).

FRET peptides can therefore be used to study any biochemical reaction, which changes the physical distance between donor and acceptor molecules. FRET assays offer a safer alternative to the use of radiolabelled isotopes and methods are quick, sensitive and easily automated.

FRET Peptide Design

Fluorescent donor and acceptor groups are quite large so spacer residues are usually incorporated into test sequences to ensure access to potential recognition sites. Hydrophilic spacers, such as b-Alanine, are preferred as these make the peptides easier to solubilize. If a hydrophobic spacer has to be used, peptide solubility can be improved by the addition of one or more Lysine residues at the C terminus.

Finding the best peptide analog is readily accomplished using FRET PepSets™ Peptide Libraries from Mimotopes. Using a unique parallel synthesis technology, PepSets peptide libraries can be prepared to individual specifications quickly and affordably using a range of labels suitable for use in FRET peptides (see Figure 2).

Synthesis procedures are monitored closely to minimize the incidence of peptides labelled with only one or other of the fluorophores.

FRET peptides can be designed with or without prior knowledge of target sequences. Where information about targets is limited, a replacement set of amino acids can be defined for each residue position and all possible

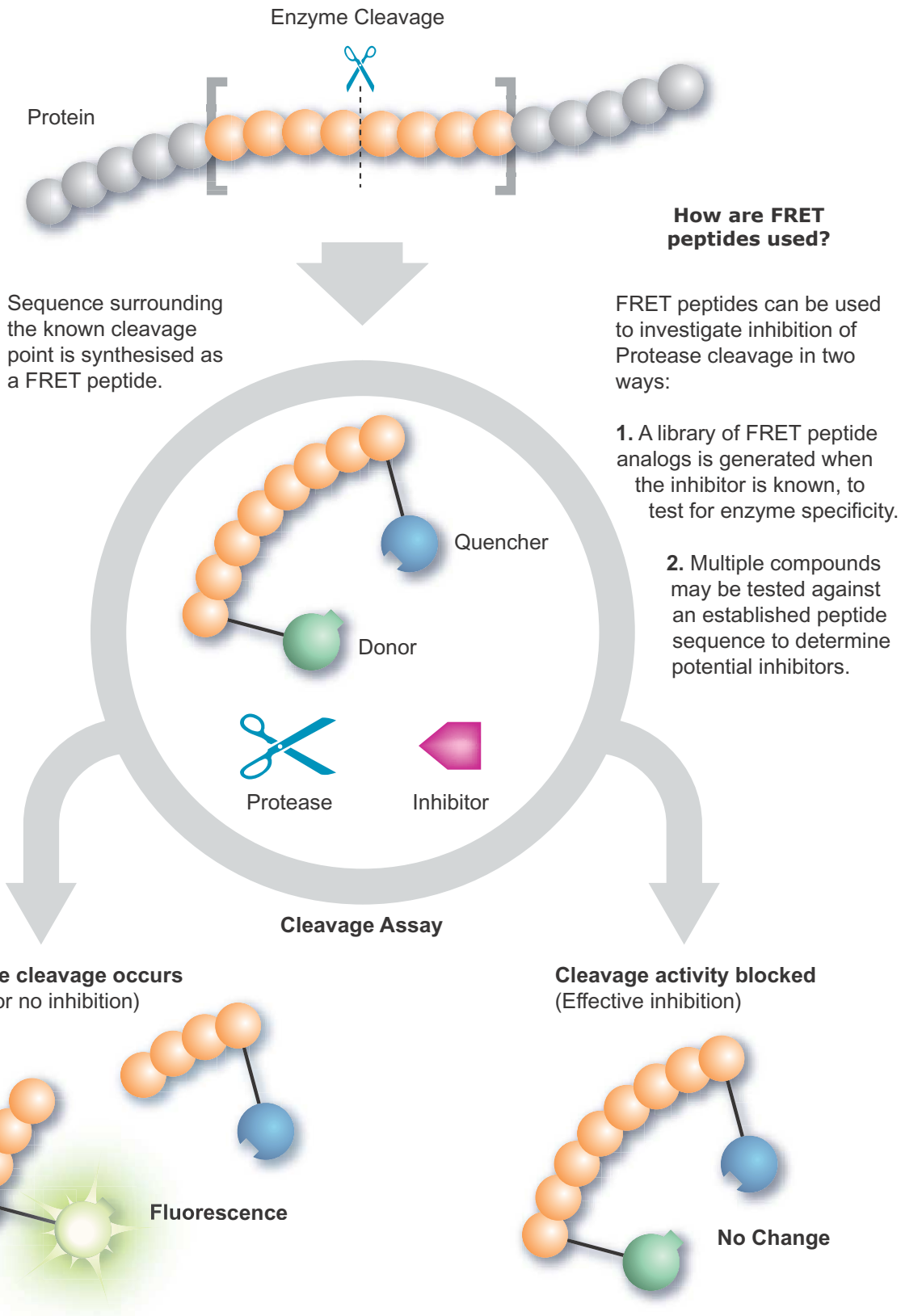
Enzyme Cleavage
Protein
How are FRET peptides used?
Sequence surrounding the known cleavage point is synthesised as a FRET peptide.
FRET peptides can be used to investigate inhibition of Protease cleavage in two ways:
1. A library of FRET peptide analogs is generated when the inhibitor is known, to test for enzyme specificity.
2. Multiple compounds may be tested against an established peptide sequence to determine potential inhibitors.
Quencher
Donor
Protease
Inhibitor
Cleavage Assay
Protease cleavage occurs
(Partial or no inhibition)
Cleavage activity blocked
(Effective inhibition)
Fluorescence
No Change
The Donor will fluoresce having been separated from the Quencher. The resulting fluorescence can be used to monitor protease activity.
Absence of fluorescence indicates successful inhibition of the protease.

Figure 1

Examples of common FRET pair combinations are shown below, but many others are possible.

Donor	Acceptor
ABZ	DNP
DANSYL	Tyr(NO_2), FAM
FAM	TMR
EDANS	DABCYL or DABSYL

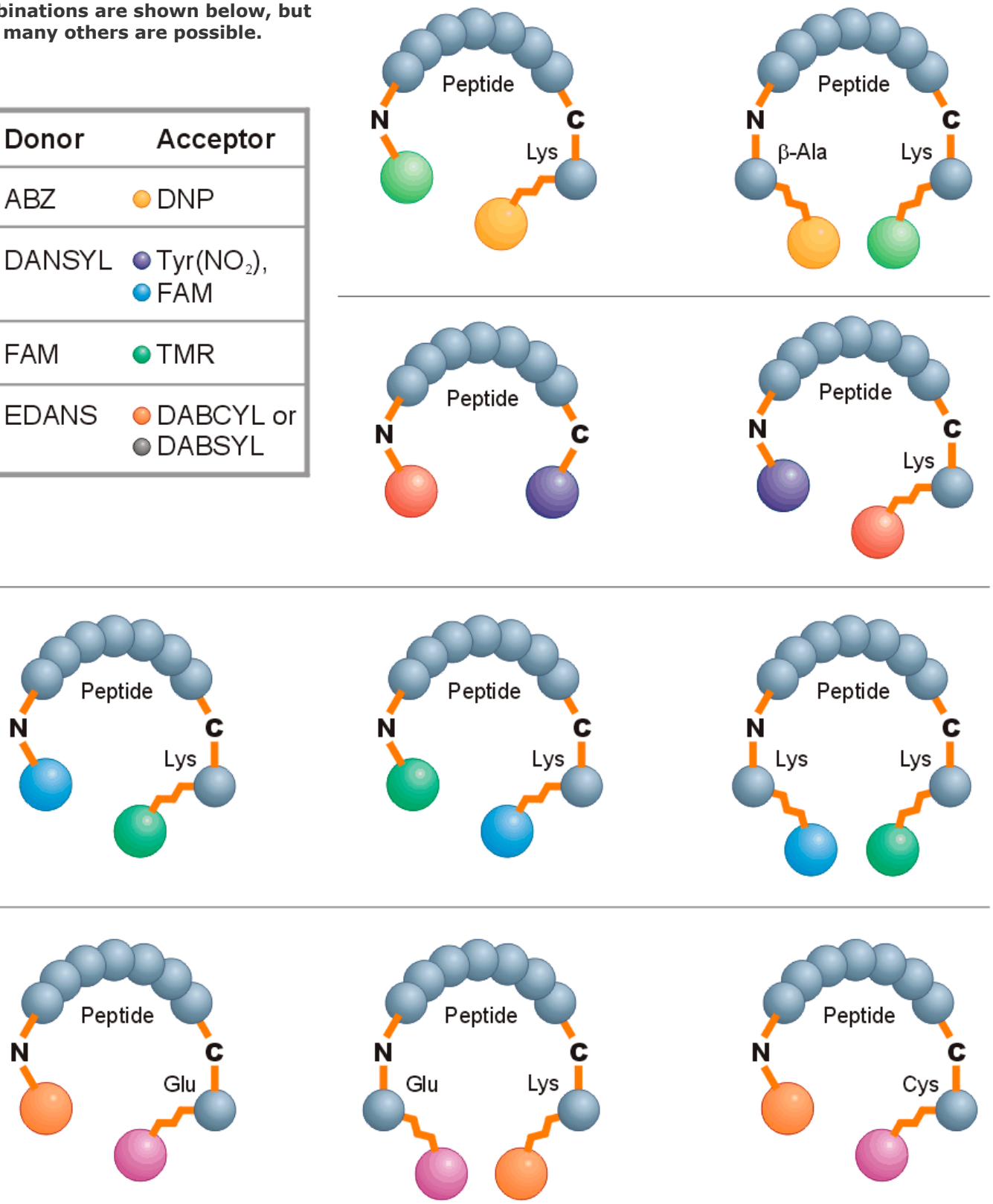

Figure 2

combinations prepared. If the replacement set is large or two or more residue positions are variable, mixtures can be used to reduce the number of peptides in the screening set.

Replacement sets can include L-amino acids, D-amino acids and any unusual amino acids, which are available commercially in an Fmoc-protected form.

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