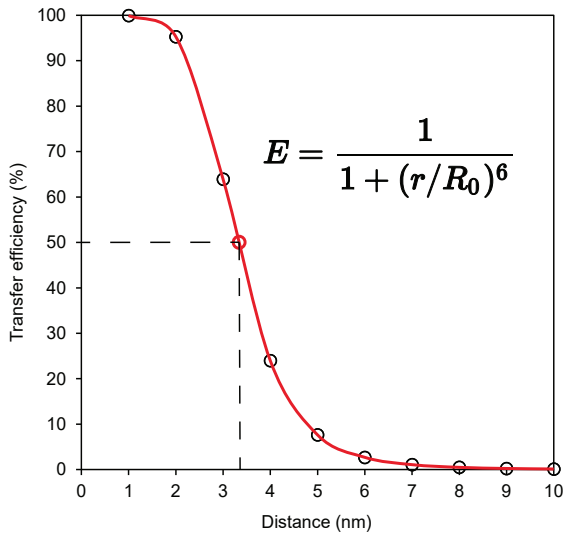
FRET Peptides

FRET Substrates for Research, Therapeutic, and Diagnostic Applications | FEBRUARY 1, 2022

FRET (Fluorescence Resonance Energy Transfer) is a distancedependent dipole-dipole interaction without the emission of a photon, which results in the transfer of energy from an initially excited donor molecule to an acceptor molecule. It allows the detection of molecular interactions in the nanometer range. FRET peptides are labeled with a donor molecule and an acceptor (quencher) molecule. In most cases, the donor and acceptor pairs are two different dyes. The transferred energy from a fluorescent donor is converted into molecular vibrations if the acceptor is a non-fluorescent dye (quencher). When the FRET is terminated (by separating donor and acceptor), an increase of donor fluorescence can be detected. When both the donor and acceptor dyes are fluorescent, the transferred energy is emitted as light of longer wavelength so that the intensity ratio change of donor and acceptor fluorescence can be measured. In order for efficient FRET quenching to take place, the fluorophore and quencher molecules must be close to each other (approximately 10-100 Å) and the absorption spectrum of the quencher must overlap with the emission spectrum of the fluorophore. While designing a donor-quencher FRET system, a careful comparison of the donor's fluorescence spectrum with the quencher's absorption spectrum is required.



Förster Equation.
According to the Förster equation, energy transfer efficiency = $1/(1+r^6/Ro^6)$ where r is the distance between the donor and acceptor groups and Ro is distance at which there is 50% energy transfer from donor to acceptor. Ro, also termed Förster radius, for the donor-acceptor pair EDANS-dabcyl is calculated at 3.3 nm.

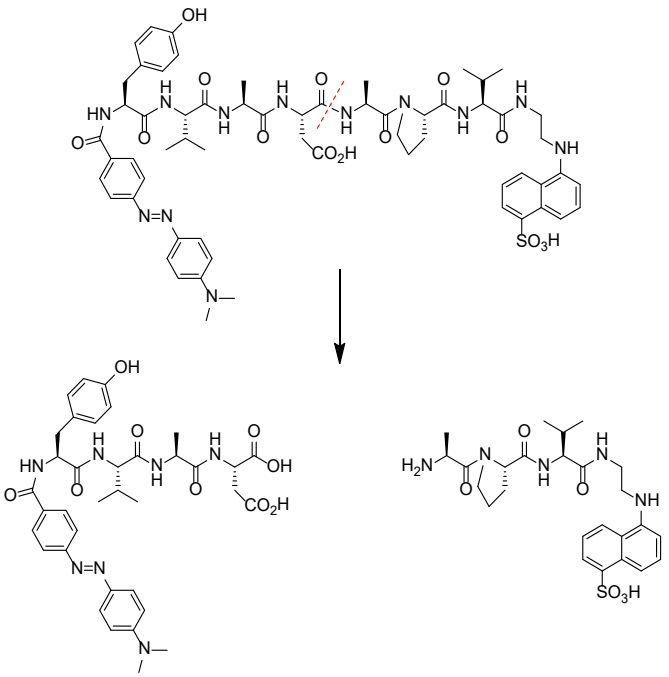


Figure 1.
FRET caspase-1 substrate, Dabcyl-Tyr-Val-Ala-Asp-Ala-Pro-Val-EDANS (CASP-023). This fluorogenic caspase-1 substrate enables a continuous assay of caspase-1 helpful in the screening of inhibitory compounds (Km = 11.4 µM, kcat = 0.79 s^{-1})

CPC Scientific has extensive knowledge in the design and synthesis of peptide FRET substrates. We offer a wide range of FRET substrates to suit your research needs as pre-manufactured FRET peptides or as custom FRET sequences. As part of our services, we provide a free consultation to help you design your FRET peptide and select the appropriate FRET pair (see Table of Common FRET Pairs). We often recommend our trade-marked highly efficient quencher, CPQ2™, to pair with the fluorescent donor 5-carboxyfluorescein (5-FAM). This efficient pair, 5-FAM/CPQ2,™ has been cited in a variety of publications in research areas spanning from cancer therapeutics to diabetes.

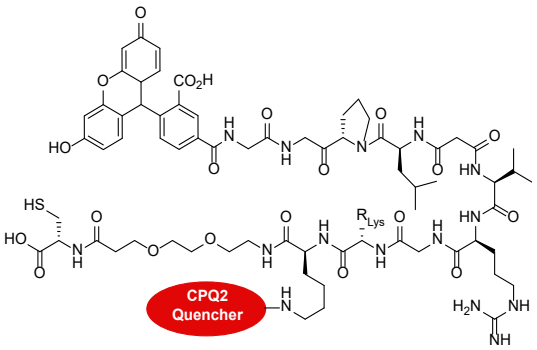


Figure 2a.
"FRET substrate 5-FAM–GGfPRSGGGK(CPQ2)–PEG2–C-OH with FAM (5-Carboxyfluorescein) as the donor and CPQ2 as the quencher.[1]

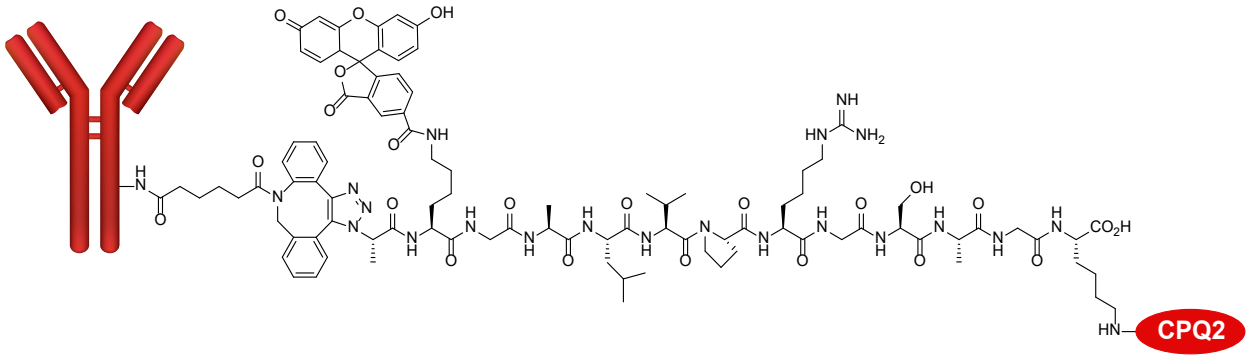


Figure 2b.
FRET substrate azidoacetyl-AK(5FAM)-GALVPRGSAGK(CPQ2)-NH2.[2]

FRET Substrate Design

The design and synthesis work at CPC for FRET and TR-FRET peptide substrates include modification of sequences, selection of donor/quencher pairs, improvement of FRET substrate solubility and quenching efficiency. CPC has experience with a wide range of protease peptide substrates including:

- Aggrecanase
- ADAMs
- ACE-2
- APCE
- 2A protease
- BACE1
- Calpains
- Caspases
- Carboxypeptidases
- Caspases
- Cathepsins
- Chymopapain
- Complement component C1s
- CMV protease
- ECE-1
- Factor Xa
- Furin
- Granzyme K
- HCV protease
- HIV protease HRV1
- Kallikreins
- Interferon-alpha A
- Lethal Factor Protease
- Malaria Aspartyl Proteinase
- MMPs
- Pepsin
- Plasmin
- Plasmepsin II
- Proteinases Protein Tyrosine Phosphatase
- Renin
- SARS
- TACE
- Thrombin
- TEV protease
- Trypsin
- West Nile Virus Protease

Time-resolved FRET (TR-FRET) Peptides

Time-resolved FRET (TR-FRET) has emerged as a method that utilizes long-lived fluorophores (characteristic of lanthanide elements) to delay measurements by 50–150 µs. Consequently, TR-FRET peptides are labeled with a well-defined fluorescent donor (a fluorophore) that delays the measurements by this timeframe. This time delay allows the signal to be cleared of most nonspecific short-lived emissions. TR-FRET eliminates background fluorescence resulting in better data quality. Eu(III) Chelate and QSY-7 (Ex/Em = 340/613 nm) is an ideal FRET pair for TR-FRET HTS assays.

Table of Common FRET Pairs

DONOR (FLUOROPHORE)	EX (nm)	EM (nm)	EM COLOR	EXTINCTION COEFFICIENT M^{-1} cm^{-1} (ε)	ACCEPTOR (QUENCHER)
Trp (Tryptophan)	280	360		5,600	Dnp (2,4-Dinitrophenyl)
Trp (Tryptophan)	280	360		5,600	4-Nitro-Z (4-Nitro-benzyloxycarbonyl)
Mca (7-Methoxycoumarin-4-yl)acetyl)	325	392		11,820	Dnp (2,4-Dinitrophenyl)
Abz (2-Aminobenzoyl)	320	420			pNA (para-Nitroaniline)
Abz (2-Aminobenzoyl)	320	420			3-Nitro-Tyr (3-Nitro-tyrosine)
Abz (2-Aminobenzoyl)	320	420			4-Nitro-Phe (4-Nitro-phenylalanine)
EDANS (5-[(2-Aminoethyl) amino] naphthalene-1-sulfonic acid)	340	490		5,900	Dabcyl (4-(4-Dimethylaminophenylazo)benzoyl)
5-FAM (5-Carboxyfluorescein)	492	518		83,000	CPQ2™ (proprietary structure)
CP488	495	519			CPQ2™ (proprietary structure)
Lucifer Yellow	430	520		11,000	Dabsyl (4-(4-Diethylaminophenylazo)-benzenesulfonyl)
FITC (Fluorescein isothiocyanate)	490	520		73,000	Dnp (2,4-Dinitrophenyl)
Dansyl (5-(Dimethylamino)naphthalene-1-sulfonyl)	342	562		3300	4-Nitro-Phe (4-Nitro-phenylalanine)
5-TAMRA (Carboxytetramethylrhodamine)	547	573		90,000	QSY7
Eu(III) Chelate	340	613			QSY7
Cy5	647	665			QSY21
Cy5.5	678	701			QSY21

References

1. Kwon, Ester J., Jaideep S. Dudani, and Sangeeta N. Bhatia. *Nature Biomedical Engineering* 1 (**2017**): 0054.
2. Zhu, Shu, et al. *Blood* 126.12 (**2015**): 1494-1502.

FRET Peptide Citations

All peptides were synthesized by CPC Scientific [..] and reconstituted in dimethylformamide (DMF) [..] Sequences [..] in Supplementary Table S1.

S14-Q: (5FAM)-GLAQAPhe(homo)RSG-K(CPQ2)-(PEG2)-GC-NH2
S14-Z: U-eeeeeeee-X-GLAQAPhe(homo)RSG-rrrrrrrrr-X-K(Cy3)-NH2
S16-QZ: (QSY21)-eeeeeeeee-c-o-GPVPLSLVMG-rrrrrrrrr-K(Cy5)-NH2
polyR: rrrrrrrrr-X-K(Cy7)-NH2

Soleimany, Ava P., [..] Sangeeta N. Bhatia. "Activatable zymography probes enable in situ localization of protease dysregulation in cancer." *Cancer Research* 81, no. 1 (**2021**)**:** 213-224.

A synthetic peptide of the following composition was used as a substrate: Dabcyl-HPHPHLSFMAIPK (5-FAM) KK-NH2 (Dabcyl = 4-(dimethylaminoazo) benzene-4-carboxylic acid, 5-FAM = 5-carboxyfluorescein) (CPC Scientific, USA) containing the Chn-sensitive region of bovine κ-casein.

Belenkaya, S. V., A. A. Bondar, T. A. Kurgina, V. V. Elchaninov, A. Yu Bakulina, E. A. Rukhlova, O. I. Lavrik, A. A. Ilyichev, and D. N. Shcherbakov. "Characterization of the Altai Maral Chymosin Gene, Production of a Chymosin Recombinant Analog in the Prokaryotic Expression System, and Analysis of Its Several Biochemical Properties." *Biochemistry* (Moscow), **2020**.

Calpain substrate peptide (QSY21-QEVYGAMP-K(Cy5)-PEG2-GC- NH2) was synthesized by CPC Scientific Inc. (Sunnyvale, CA ...

Kudryashev, Julia A., Lauren E. Waggoner, Hope T. Leng, Nicholas H. Mininni, and Ester J. Kwon. "An Activity-Based Nanosensor for Traumatic Brain Injury " *ACS Sensors* (**2020**).

"FITC-labelled GzmB substrate peptides with internal quencher ((5-FAM)aIEFDSGK(CPQ2)kkc) were synthesized by CPC Scientific and used for all in vitro activity assays."

Mac, Quoc D., Dave V. Mathews, Justin A. Kahla, Claire M. Stoffers, Olivia M. Delmas, Brandon Alexander Holt, Andrew B. Adams, and Gabriel A. Kwong. "Non-invasive early detection of acute transplant rejection via nanosensors of granzyme B activity." *Nature Biomedical Engineering* 3, no. 4 (**2019**): 281.

"The FRET substrates used in this project are: neutrophil elastase substrate ((Z-AAAA)2Rh110 Abs/Em:497/520 nm, Anaspec), Granzyme B substrate (5TAMRA-VGPDFGR-K(QSY7)-NH2 Abs/Em: 546/580, CPC scientific), and metalloproteinase substrate (QSY21-HGDQMAQKSK(Cy5)-NH2 Abs/Em:649/666 nm, CPC Scientific)."

Zeming, K. K.; Lu, R.; Woo, K. L.; Sun, G.; Quek, K. Y.; Cheow, L. F.; Chen, C.-H.; Han, J.; Lim, S. L. "Multiplexed Single-Cell Leukocyte Enzymatic Secretion Profiling from Whole Blood Reveals Patient-Specific Immune Signature. " *Analytical Chemistry* **2021**.

All peptides were chemically synthesized by CPC Scientific, Inc. [..] K(N3)-ANP-GPVPLSLVMGGC [..] 5FAM-GGf-Pip-KSGGGK(CPQ2)-PEG2-GC

Hao, Liangliang, Renee T. Zhao, Chayanon Ngambenjawong, Heather E. Fleming, and Sangeeta Bhatia. "CRISPR-Cas-amplified urine biomarkers for multiplexed and portable cancer diagnostics." *bioRxiv* (**2020**).

The peptide sequences of the four FRET-based substrates ([..] CPC Scientific) are as follows: UV: AlexaFluor405-Leu-Ala-Gln-Ala-HompheArg-Ser-Lys (QSY35)-NH2; Blue: Dabcyl-Gly-Pro-Leu-Gly-Met-Arg-Gly-Lys (5-FAM)-NH2; Green: QSY7-Ala-Pro-Phe-Glu..

Ng, Ee Xien, Myat Noe Hsu, Guoyun Sun, and Chia-Hung Chen. "Single-cell assays using integrated continuous-flow microfluidics." *Methods in Enzymology* 628 (**2019**): 59-94.

The substrates QSY21-Gly-Asp-Asp-Asp-Lys-Ile-Val-Gly-Gly-Lys(Cy5) and 5FAM-Abu-Gly-Asp-Asp-Asp-Lys-Ile-Val-Gly-Gly-Lys(CPQ2)-Lys-Lys-NH2 were purchased from CPC Scientific (Sunnyvale, CA).

Sasaki, Masako, Ken-ichi Hamagami et al. "Discovery and characterization of a small-molecule enteropeptidase inhibitor, SCO-792." *Pharmacology Research & Perspectives* 7, no. 5 (**2019**): e00517.

"ANP FAP and aP NIRF were synthesized by CPC Scientific (Sunnyvale, CA)... Another internally-quenched FRET peptide substrate (ANPFAP) for FAP has recently been reported and demonstrated for use as an activity-based, in vivo imaging tool. The peptide sequence contains two internal Gly-Pro dipeptide motifs, susceptible to FAP cleavage and a Cy5.5/QSY21, quenched-FRET pair."

Bainbridge, Travis W., et al. "Selective Homogeneous Assay for Circulating Endopeptidase Fibroblast Activation Protein (FAP)." *Scientific Reports* 7.1 (**2017**): 12524.

"All peptides were synthesized by CPC Scientific. For recombinant enzyme studies and ABNz, intramolecularly quenched peptides were used: MMP substrate, 5-FAM–GGPLGVRGKK(CPQ2)–PEG2–C; thrombin substrate, 5-FAM–GGfPRSGGGK(CPQ2)–PEG2–C; where 5-FAM is the 5-carboxyfluorescein fluorophore, CPQ2 is the quencher, PEG2 is the linker polyethylene glycol, and lower case letters [...]

Kwon, Ester J., Jaideep S. Dudani, and Sangeeta N. Bhatia. "Ultrasensitive tumor-penetrating nanosensors of protease activity." *Nature Biomedical Engineering* 1 (**2017**): 0054.

"Internally quenched peptides of the sequences of IFFDTWDNE, TWDNEAYVH, EAYVHDAPV, and HDAPVRSLN, corresponding to amino acids 103 to 111, 107 to 115, 111 to 119, and 115 to 123 of the reference human pro–IL-1b sequence (UniProt: P01584), were labeled on the N terminus with Mca and on the C terminus with Lys-Dnp (CPC Scientific)."

LaRock, Christopher N., et al. "IL-1b is an innate immune sensor of microbial proteolysis." *Science Immunology* 100.200: 300 (**2016**).

"Cysteine-terminated peptides (Q1 = 5FAM-GGPLGVRGKK(CPQ-2)-PEG2-C, CPC Scientific.."

Kwong, Gabriel A., et al. "Mathematical framework for activity-based cancer biomarkers." *Proceedings of the National Academy of Sciences* 112.41 (**2015**): 12627-12632.

"The custom-made thrombin-sensitive peptide azidoacetyl-AK(5FAM)-GALVPRGSAGK(CPQ2)-NH2 was obtained from CPC Scientific (Sunnyvale, CA) for click reactions to anti-CD61, as previously described."

Zhu, Shu, et al. "FXIa and platelet polyphosphate as therapeutic targets during human blood clotting on collagen/tissue factor surfaces under flow." *Blood* 126.12 (**2015**): 1494-1502.

"... Dabcyl-HPHPHLSFMAIPK(5-FAM)KK-NH 2 (98% purity; Figure 1) was purchased from CPC Scientific (Sunnyvale, CA). ..."

Jensen, Jesper Langholm, et al. "The function of the milk-clotting enzymes bovine and camel chymosin studied by a fluorescence resonance energy transfer assay." *Journal of Dairy Science* 98.5 (**2015**): 2853-2860.

"Fusolisin's restriction specificity was verified using the FRET substrate CPQ2-Gly-Phe-Ile-Thr-Ala-Phe-Pro-Lys-(5FAM)-Arg-Arg-NH2 that was custom synthesized by CPC Scientific (Sunnyvale, CA, USA)."

Welsh, J. D., et al. "Identification and Characterization of Fusolisin, the Fusobacterium nucleatum Autotransporter Serine Protease." *Journal of Thrombosis and Haemostasis* 10.11 (**2012**): 2344-2353.

"A custom peptidewith the sequence 5-carboxymethylfluorescein-Thr-Gln-Thr-Val-Ala-Ala-Gly-Ser-Lys(CPQ2)-D-Arg-D-Arg was obtainedcommercially (CPC Scientific)"

Hershey, David M., et al. "Magnetite biomineralization in Magnetospirillum magneticum is regulated by a switch-like behavior in the HtrA protease MamE." *BioRxiv* (**2016**): 047555.

"Protease activity in regurgitant from three replicate tanks were compared using a mixture of 7 internally quenched fluorescent substrates available from CPC Scientific, Sunnyvale, California (Table 1) [AMYD-112, AMYD-114, AMYD-109, MMPS-024, SUBS-017, AMYD-111, CASP-060]. These substrates were chosen based on their diverse sequence composition to enable detection of multiple protease classes."

Goupil, Louise S., et al. "Cysteine and Aspartyl Proteases Contribute to Protein Digestion in the Gut of Freshwater Planaria." *PLoS Negl Trop Dis* 10.8 (**2016**): e0004893.

"Peptides corresponding to the substrate sequences L/VP4.1 and non-hydrolysable linker (NHL) were synthesized between a 5-carboxyfluorescein (5-FAM) fluorophore/CPC Quencher (CPQ2) quencher pair (CPC Scientific). Additional lysines were added for increased solubility giving final peptide sequences of CPQ2-IVYELQGP-K(5FAM)-KK-NH2 and CPQ2-GGSGGS-K(5FAM)-KK-NH2 for L/VP4.1 and NHL, respectively. The identity and sequence of the L/VP4.1 and NHL peptides were confirmed by CPC Scientific [...]

Miles, Linde A., et al. "Seneca Valley Virus 3C pro Substrate Optimization Yields Efficient Substrates for Use in Peptide-Prodrug Therapy." *PloS One* 10.6 (**2015**): e0129103.

"The cleavage preference of AGP was tested using an E(Edans)-AAXAAK-(Dabcyl)-NH 2 fluorescent substrate kit (CPC Scientific Inc., Calif., USA) with "A" representing alanine residues, "K" lysine residues, and "X" different individual amino acid residues as specified by their one ..."

Shi, Jing, et al."Properties of Hemoglobin Decolorized with a Histidine-Specific Protease." *Journal of Food Science* 80.6 (**2015**): E1202-E1208.

"Thrombin-sensitive peptide (ThS-P) azidoacetyl alanine-K(5FAM)GALVPRGSAGK(CPQ2) was custom synthesized (2143 MW, > 95% purity; CPC scientific, Sunnyale, CA, USA) and dissolved in DMSO (20 mm ThS-P)."

Welsh, J. D., et al. "Platelet-targeting sensor reveals thrombin gradients within blood clots forming in microfluidic assays and in mouse." *Journal of Thrombosis and Haemostasis* 10.11 (**2012**): 2344-2353.

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