Peptide PEGylation

Peptide PEGylation Improves Solubility, Reduces Renal Clearance and Immunogenicity

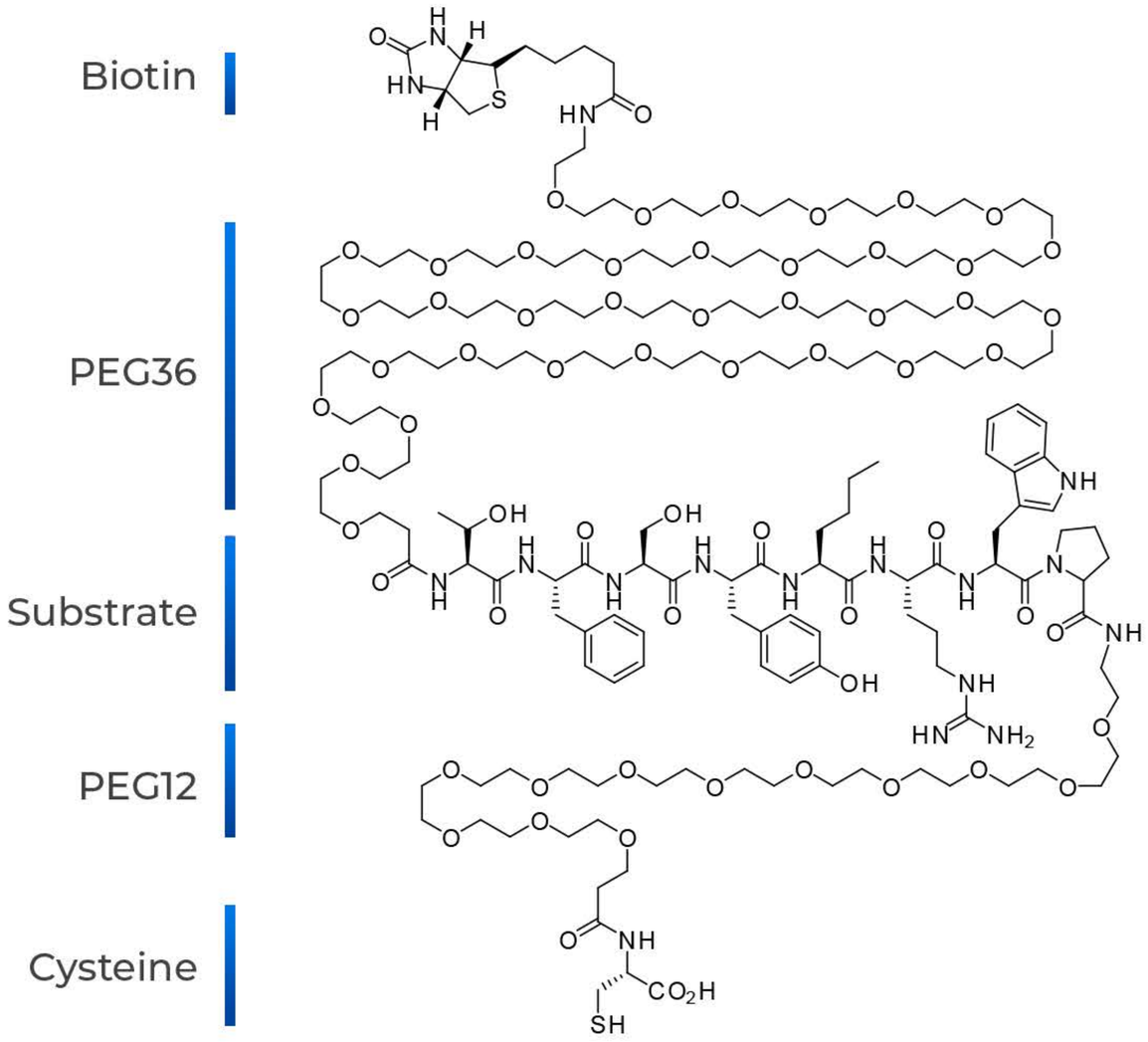
FEBRUARY 1, 2022

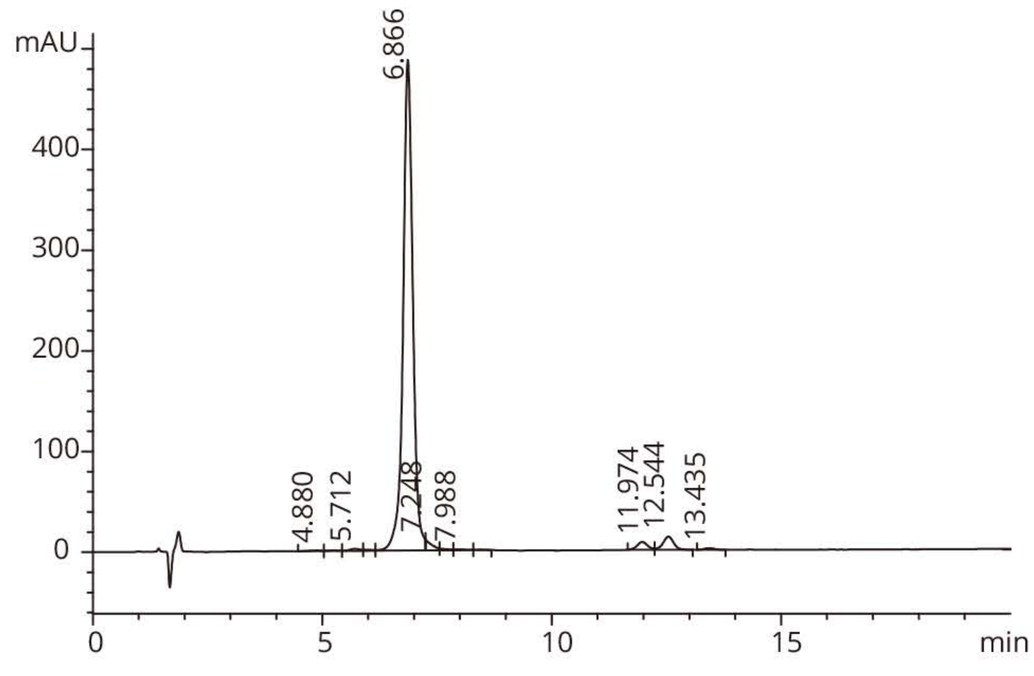
Peptides play a key role in the pharmaceutical industry and drug therapeutic development; however, their in vivo applications are sometimes limited due to fast degradation by proteases, poor solubility, antigenic responses, and glomerular filtration in the kidney. The covalent attachment of polyethylene glycol (PEG) chains to peptides is one approach that can reduce immunogenicity, improve solubility, and reduce renal clearance. The addition of monodispersed PEG chains may be a factor in the development of safe and effective PEGylated peptide therapeutics and can be crucial for achieving optimal therapeutic results.

PEGylation Chemistry

PEGylation is now a well-established technique in the field of targeted drug delivery systems. As more PEGylated therapeutic agents receive FDA approval, more attention has been given to site-specific PEGylation of discrete or monodispersed PEG chains. Polydispersed PEG chains conjugated to biological molecules can have variations in dispersity and be difficult to characterize creating additional hurdles for FDA approval.

CPC Scientific can perform site-specific PEGylation at a variety of sites on the peptide. N-terminal PEGylation can be accomplished by direct PEG carboxylic acid coupling or native chemical ligation with PEG thioester and a cysteine residue. C-terminal is more complicated, but can be achieved through a thiocarboxylic acid modification and sulfone-azide PEG reagent. Hydrazide modifications combined with a pyruvoyl PEG reagent is also a useful approach to C-terminal PEGylation. In addition to the N- and C-terminal, PEGlyation is also possible at virtually any amino acid side chain bearing the appropriate functional group.





Mobile Phase A: 0.1%TFA in H_2O
Buffer B: 0.09%TFA in (80% ACN + 20% H_2O)
Flow:1.0 mL/min 43.0% - 53.0% B buffer in 20 min

Monodisperse PEG36 and PEG12 Linkers

Giant magnetoresistive biosensors for real-time quantitative detection of protease activity. Adem, Sandeep, Sonal Jain, Michael Sveiven, Xiahan Zhou, Anthony J. O'Donoghue, and Drew A. Hall. "Giant magnetoresistive biosensors for real-time quantitative detection of protease activity." *Scientific Reports* 10, no. 1 (**2020**): 1-10

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Table of Common PEG Abbreviations:

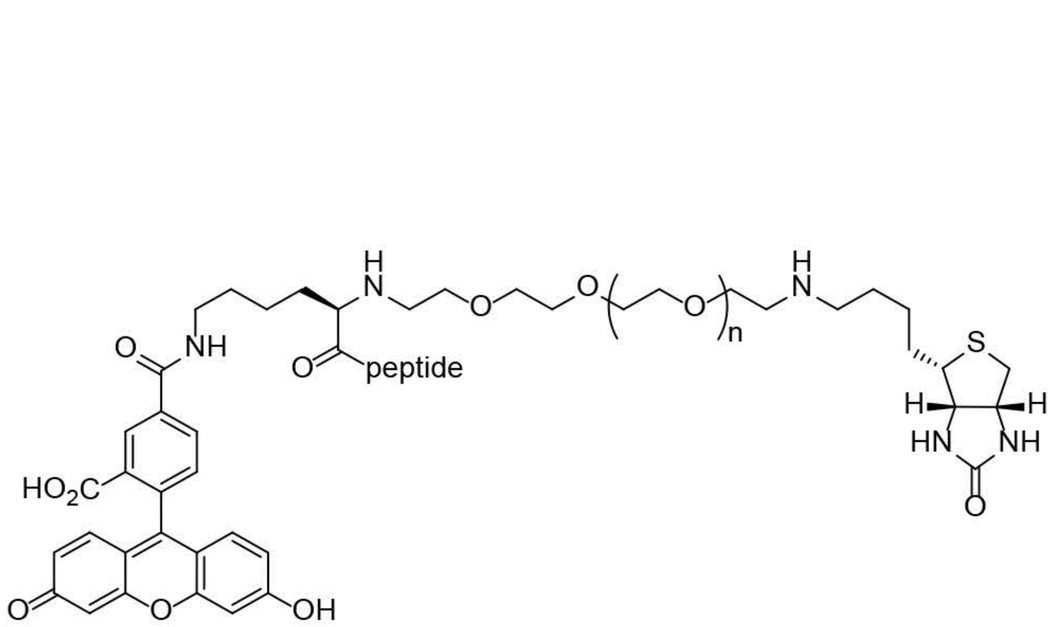
In this context, three methodologies are often used for site-specific PEGylations:

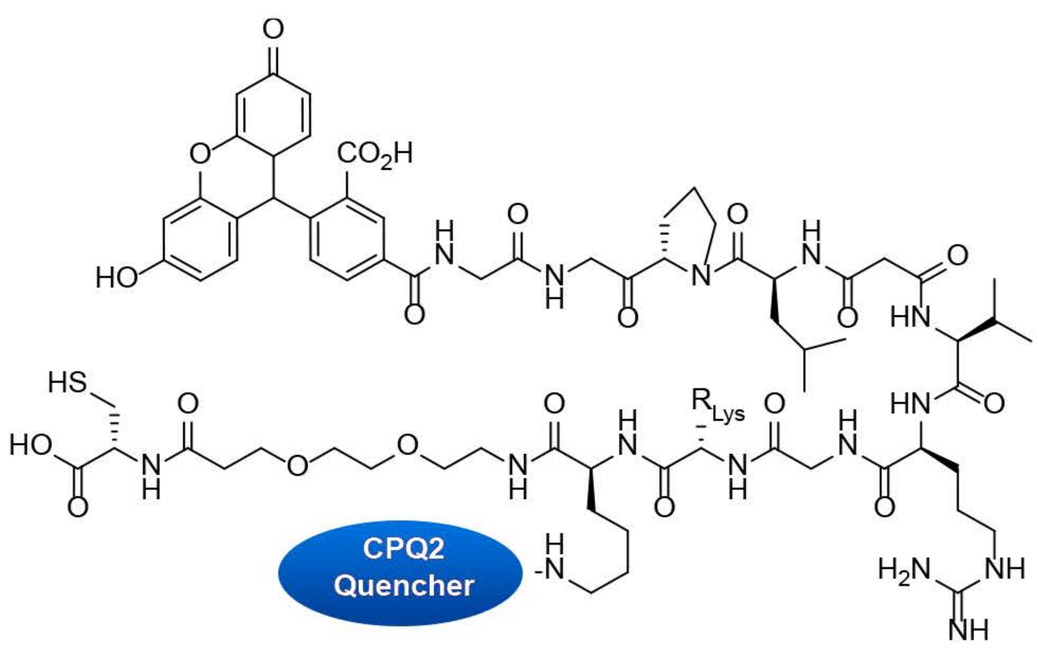
- Click Chemistry, which takes place between an azide group of the PEG reagent and an alkyne group of the peptide, or vice versa.
- Suzuki-Miyaura Coupling, which takes place between the iodophenyl group of the PEG reagent and an aryl boronic acid group of peptide, or vice versa.
- Sonogashira Coupling, which takes place between an iodophenyl group of the PEG reagent and an alkyne group of the peptide, or vice versa.

Abbrev.	Full PEG Chain Name
PEG	monoethylene glycol
PEG_2	diethylene glycol
PEG_3	triethylene glycol
PEG_4	tetraethylene glycol
mini-PEG_2	8-amino-3,6-dioxaoctanoic acid
mini-PEG_3	amino-3,6,9-trioxaundecanoic acid
NH_2-PEG_2-acid	3-(2-(2-Aminoethoxy)ethoxy)-propanoic acid
PEG750	Poly(ethylene glycol) methyl ether (average Mn 750)
PEG1000	Poly(ethylene glycol) methyl ether (average Mn 1000)
PEG2000	Poly(ethylene glycol) methyl ether (average Mn 2000)
PEG5000	Poly(ethylene glycol) methyl ether (average Mn 5000)

PEGylation and Peptide Bioavailability

PEGylation offers multiple physiochemical and pharmacokinetic benefits to peptide-based therapeutics. Following covalent attachment of a PEG chain to a peptide, the PEG-peptide conjugate has longer blood circulation times, increased solubility, and reduced immunogenicity. Longer circulation times (i.e., enhanced bioavailability) also results in a lower frequency of dosings and lower dosing amounts. The increased steric hindrance from the PEG chain can hinder much of the non-specific protease interactions. N-terminal PEGylation can specifically block endopeptidases and provide steric hindrance to proteolytic enzymes. In a similar fashion, PEG chains can block epitope sites on the peptide from antibody binding.





Biotin-PEG5kDa-Lys(5FAM) Polydisperse PEG Linker.
Warren, Andrew D., et al. *Journal of the American Chemical Society* 136.39 (**2014**): 13709-13714.

Monodisperse PEG2 Linker.
Dvir, Hay, et al. *Proceedings of the National Academy of Sciences* 109.18 (**2012**): 6916-6921.

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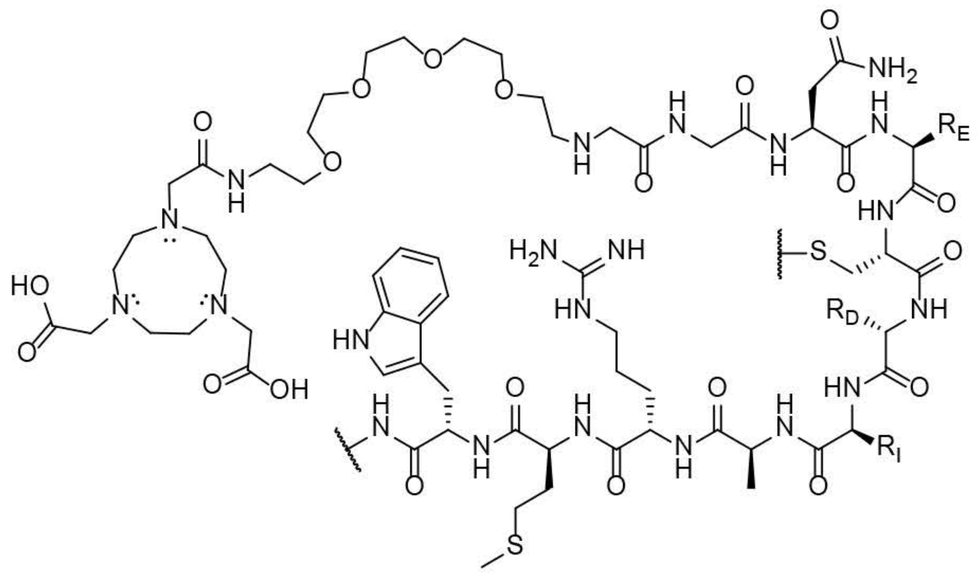
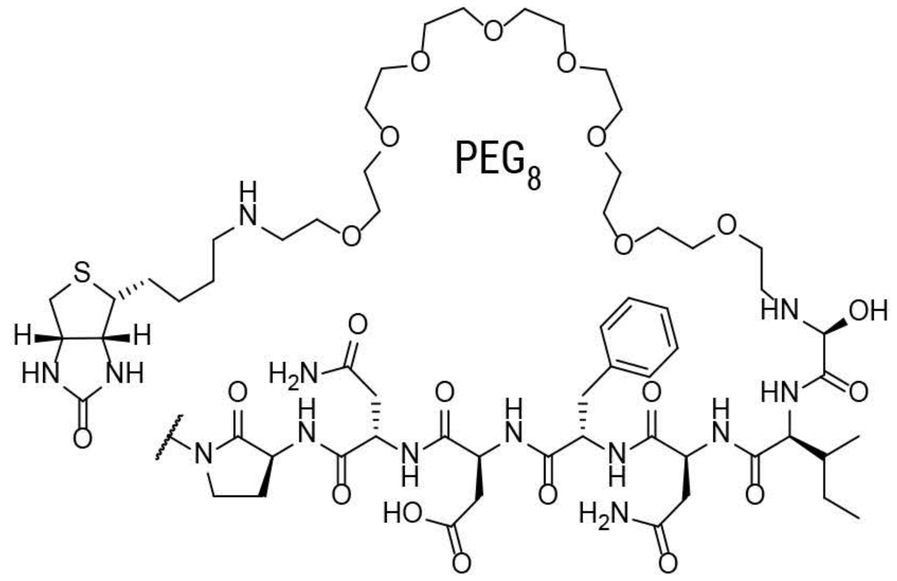


Monodispersed PEG4 Linker.
Marquez, B. V., Ikotun, O. F., Parry, J. J., Rogers, B. E., Meares, C. F., & Lapi, S. E., *Nuclear Medicine*, 55(6), (**2014**)
1029-1034.

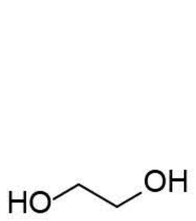


Monodispersed PEG8 Linker.
Dvir, Hay, et al. *Proceedings of the National Academy of Sciences* 109.18 (**2012**): 6916-6921.

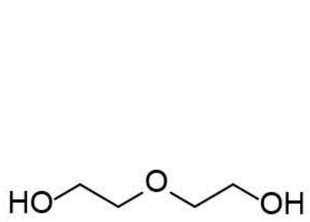
With the advancement of more sensitive and non-invasive imaging techniques (e.g. PET, SPECT), more interest has focused on the drugability of targeting peptide-chelate conjugates. Spacers between the peptide and chelate (e.g., DOTA, NOTA, etc) are often incorporated to improve the peptide binding affinity. PEG spacers offer advantages over hydrocarbon spacers due to their increased hydrophilicity. Profound increases in tumor uptake and retention have been observed in PEGylated RGD-based probes.[1]

Imaging peptide-based probes can be easily modified to improve and extend the duration of target uptake. Vascular endothelial growth factor (VEGF) is an important target for imaging probes because it is over-expressed in cerain cancer cells that stimulate angiogenesis. Anti-VEGF monoclonal antibody, bevacizumab, successfully targets VEGF and has been approved by the FDA for noninvasive PET and SPECT imaging of VEGF. The v107 peptide also binds to VEGF, but only with micromolar affinity that is insufficient for targeted molecular imaging. Marquez and co-workers redesigned the peptide by substituting leucine-19 for a lysine residue and incorporating a chelating moiety, 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA), at the N-terminus separated by a PEG4 spacer (figure on right). The resultant sequence, NOTA-PEG4- GGNECDIARMWEWECFERK-NH2, was then cross-linked with 5-fluoro-2,4-dinitrobenzene to form a covalent bond with lysine-19 (L19K-FDNB). This modification increases binding affinity because it enables the peptide probe to irreversibly bind to VEGF by a covalent attachment to a site inside the binding pocket of the protein. The inert and flexible characteristic of the PEG linker provides distance between the peptide cargo carrier (e.g., NOTA) for optimal binding to the receptor.

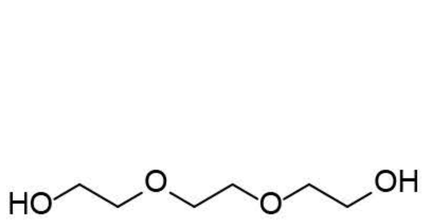
Common PEG Linkers for Peptide Conjugation



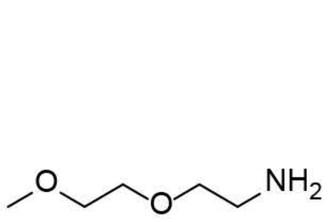
PEG



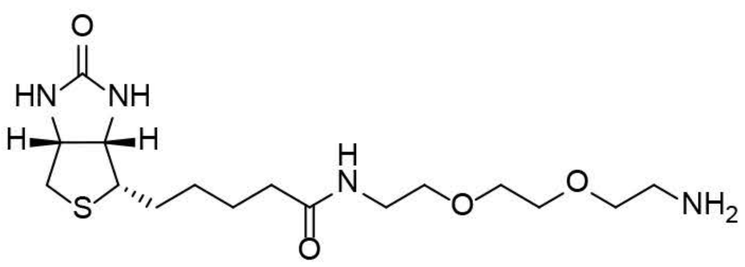
PEG_2



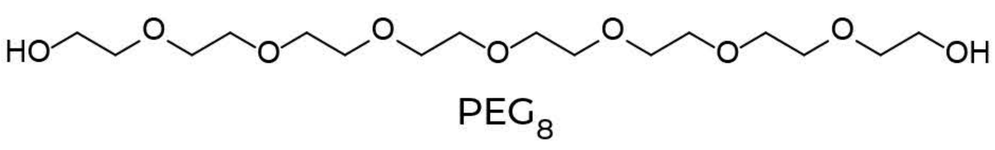
PEG_3



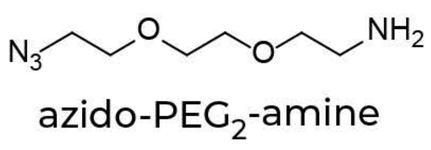
m-PEG_2-amine



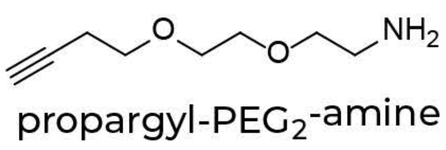
biotin-PEG_2-amine



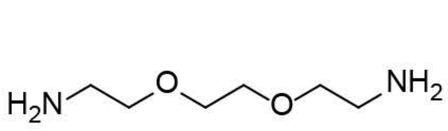
PEG_8



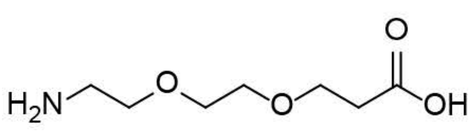
azido-PEG_2-amine



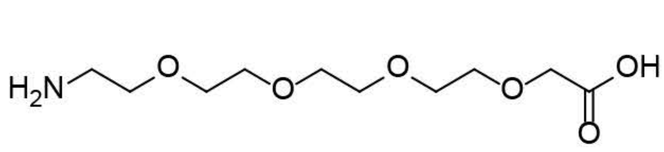
propargyl-PEG_2-amine



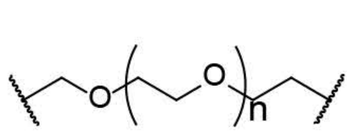
amino-PEG_2-amine



amino-PEG_2-acid



mini-PEG_3



custom PEGs

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PEGylated 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-NH2S14-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.

"The D-VEGF-A polypeptide chain (COOH acid, residues 8-109 (1)) was chemically synthesized using solid phase peptide synthesis (SPPS) and native chemical ligation, and [...]

Paul S. Marinec, Kyle E. Landgraf, Maruti Uppalapati, Gang Chen, Daniel Xie, Qiyang Jiang, Yanlong Zhao, Annalise Petriello, Kurt Deshayes, Stephen B. H. Kent, Dana Ault-Riche*, and Sachdev S. Sidhu.* "A Non-immunogenic Bivalent D-Protein Potently Inhibits Retinal Vascularization and Tumor Growth" *ACS Chem*. Biol. (**2021**), 16, 3, 548–556.

HFA-modifed peptides were synthesized by CPC Scientifc (>95% purity). Briefy, the peptide substrate, Ac-CKKK(Cy5)-PEG4-Nle(O-Bzl)-Met(O)2-Oic-Abu-OH, was synthesized on Fmoc-Abu-CTC resin via standard Fmoc solid phase peptide synthesis.

Chan, Leslie W., Melodi N. Anahtar, Ta-Hsuan Ong, Kelsey E. Hern, Roderick R. Kunz, and Sangeeta N. Bhatia. "Engineering synthetic breath biomarkers for respiratory disease." *Nature Nanotechnology* (**2020**): 1-9.

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**).

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**).

Tandem Peptides. Tandem peptides were purchased from CPC Scientific.

Sequences are:
mTP-TAMRA-LyP1 (Myr-GWTLNSAGYLLGKINLKALA-ALAKKILGGGGK(5TAMRA)-CGNKRTRGC (C-C bridge));
mTP-TAMRA-CRV (Myr-GWTLNSAGYLLGKINLKALA-ALAK-KILGGGGK(5TAMRA)-CRVLRSGSC (C-C bridge));
mTP-TAMRA-iRGD (Myr-GWTLNSAGYLLGKINLKALA-ALA-KKILGGGGK(5TAMRA)-CRGDRGPDC (C-C bridge));
mTP-TAMRA-ARAL (Myr-GWTLNSAGYLLGKINLKAL-AALA-KKILGGGGK(5TAMRA)-ARALPSQRSR).
PEGylated formulations of these peptides were synthesized as previously described (49). The sequences are:
mTP-PEG-LyP1 (Myr-GWTLNSAGYLLGKINLKALA-ALAKKILC-PEG5K-GGGCGNKRTRGC (C-C bridge));
mTP-PEG-CRV (Myr-GWTLNSAGYLLGKINLKALA-ALAKKILC-PEG5K-GGGCRVLRSGSC (C-C bridge));
mTP-PEG-iRGD (Myr-GWTLNSAGYLLGKINLKALA-ALAKKILC-PEG5K-GGGCRGDRGPDC (C-C bridge));
mTP-PEG-ARAL (Myr-GWTLNSAGYLLGKINLKALA-ALAKKILC-PEG5K-GGGARALPSQRSR).

Buss, Colin G., and Sangeeta N. Bhatia. "Nanoparticle delivery of immunostimulatory oligonucleotides enhances response to checkpoint inhibitor therapeutics" *Proceedings of the National Academy of Sciences* (**2020**).

Thio-peptide: NH2-Cys-PEG4-Sar-YNLYRVRS-NH2, MW 1,490 g/mol was custom synthesized, via solid state methods by CPC Scientific (Sunnyvale, CA, USA).

Melgar-Asensio, Ignacio, et al. "Extended intravitreal rabbit eye residence of nanoparticles conjugated with cationic arginine peptides for intraocular drug delivery: in vivo imaging." *Investigative Ophthalmology & Visual Science* 59.10 (**2018**): 4071-4081.

"Our previously reported PLZ4-PEG 5k -CA 8 telodendrimer was synthesized by the conjugation of alkyne-derivatized bladder cancer targeting ligand PLZ4 (CPC Scientific, Sunnyvale, CA)"

Lin, Tzu-Yin, et al. "Novel theranostic nanoporphyrins for photodynamic diagnosis and trimodal therapy for bladder cancer." *Biomaterials* 104 (**2016**): 339-351.

"antipeptide (EGVYVHPV), angiotensin II, human (DRVYIHPF), and isotopically (13C) labeled heptapeptide (AAAAHAA-NH2 [where "A" indicates a carbon thirteen (13C) "Synthesis was conducted at CPC Scientific Inc. Ac-Asp(OtBu)-Thr(tBu)-His(Trt)-Phe-Pro-Ile-Cys(Trt)-Ile-PhePEG3-Arg(Pbf)-Arg(Pbf)-Lys(Boc)-wang resin (2)... Ac-Asp-Thr-His-Phe-Pro-Ile-Cys-Ile-Phe-PEG3-Arg-Arg-Lys(BODIPY_TMR_C6)."

Skerratt, Sarah, et al. "Identification of a novel BODIPY minihepcidin tool for the high content analysis of Ferroportin (SLC40A1) pharmacology. " *MedChemComm* (**2016**).

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"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.

Biotin-PEG36-Thr-Phe-Ser-Tyr-Nle-Arg-Trp-Pro-PEG12-Cys (known as peptide) was synthesized by CPC Scientific..

Adem, Sandeep, Sonal Jain, Michael Sveiven, Xiahan Zhou, Anthony J. O'Donoghue, and Drew A. Hall. "Giant magnetoresistive biosensors for real-time quantitative detection of protease activity." *Scientific Reports* 10, no. 1 (**2020**): 1-10

"The biotinylated synthetic LDLR peptide [Biotin-PEG8-SINFDNPVYQKT (CPC Scientific)] was captured to ≈20–50 RU, whereas ≈200 RU of the J.D. mutant peptide (Biotin-PEG8-SINFDNPVCQKT) was required for proper detection of ARH binding."

Dvir, Hay, et al. "Atomic structure of the autosomal recessive hypercholesterolemia phosphotyrosine-binding domain in complex with the LDL-receptor tail." *Proceedings of the National Academy of Sciences* 109.18 **(2012)**: 6916-6921.

"Thrombin-sensitive reporter 1 (R1) was synthesized by CPC Scientific, with the sequence Biotin-PEG5kDa-Lys(5FAM)-Gly-Gly-DPhe-Pro-Arg-Ser-Gly-Gly-Gly-Cys, where PEG5kDa is 5 kDa poly(ethylene glycol)."

Warren, Andrew D., et al. "Disease detection by ultrasensitive quantification of microdosed synthetic urinary biomarkers." *Journal of the American Chemical Society* 136.39 (**2014**): 13709-13714.

"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.

"L19K was synthesized by CPC Scientific and comprised the sequence NO2A-PEG4-GGNECDIARMWEWECFERK-CONH2, with Cys-Cys disulfide bridge and polyethylene glycol (PEG4) as a spacer between peptide and chelator. "

Marquez, B. V., Ikotun, O. F., Parry, J. J., Rogers, B. E., Meares, C. F., & Lapi, S. E., "Development of a radiolabeled irreversible peptide ligand for PET imaging of vascular endothelial growth factor. " *Nuclear Medicine*, 55(6), (**2014**) 1029-1034.

"...protease activity are focused on functionalizing synthetic peptide substrates with reporters that emit ... In vivo, veiled nanosensors are selectively activated at the ..... sized by CPC Scientific, Inc. (V1: Biotin-PEG(5 kDa)-(KFAM)-..."

Dudani, Jaideep S., et al. "Photoactivated Spatiotemporally-Responsive Nanosensors of in Vivo Protease Activity." *ACS Nano* 9.12 (**2015**): 11708-11717.

"Two versions of the peptide L19K were synthesized by CPC Scientific (Sunnyvale, CA) consisting of the sequence DO3A- or NO2A-PEG4-GGNECDIARMWEWECFERK-CONH2, with a Cys-Cys disulfide bridge and polyethylene glycol (PEG) as a spacer between peptide and chelate."

Mastren, Tara, et al. "Cyclotron Production of High–Specific Activity 55 Co and In Vivo Evaluation of the Stability of 55 Co Metal-Chelate-Peptide Complexes." *Molecular Imaging* 14.10 (**2015**): 11-22.

"...4 -c(GX1) 774.9 μM or HYNIC-E-[c(RGDfk)-c(GX1)] 569.5 μM (μg/mL) (CPC Scientific Inc., CA ... 5 /well) were seeded into well culture plates, and it was added the radiotracers 99m Tc-HYNIC-PEG 4 -c ... For nonspecific binding assays, cold conjugate (1 mmol/L/well) was also ..."

Oliveira, E. A., and B. L. Faintuch. "Radiolabeling and biological evaluation of the GX1 and RGD-GX1 peptide sequence for angiogenesis targeting." *Nuclear Medicine and Biology* 42.2 (**2015**): 123-130.

"The peptides, shown in Figure 1, were designed and synthesized. MPER (RRRNEQELLELDKWASLWNWFDITNWLWYIRRRR), TT peptide (FNNFTVSFWLRVPKVSASHLE), T10HE peptide (FNNFTVSFWLRVPKVSASHLE-PEG2-LWNWF-S5-ITN-S5-LWYIR-PEG2-KK), and T10E peptide (FNNFTVSFWLRVPKVSASHLEPEG2-LWNWFDITNWLWYIR-PEG2-KK) were purchased from CPC Scientific Inc. (Sunnyvale, CA, USA)."

Yu, Yang, et al. "10E8-like neutralizing antibodies against HIV-1 induced using a precisely designed conformational peptide as a vaccine prime." *Science China Life Sciences* 57.1 (**2014**): 117-127.

"The peptides P20A (Ac-AAASGINAEWPLWPGEAGWGRLEGRRTYEAEI-NH 2) and biotin-P20A (biotin-PEG4-AAASGINAEWPLWPGEAGWGRLEGRRTYEAEI-NH2) were synthesized by CPC Scientific."

Fu, Shushu, et al. "P20A inhibits HIV-1 fusion through its electrostatic interaction with the distal region of the gp41 fusion core." *Microbes and Infection* 17.9 (**2015**): 665-670.

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US Headquarter

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