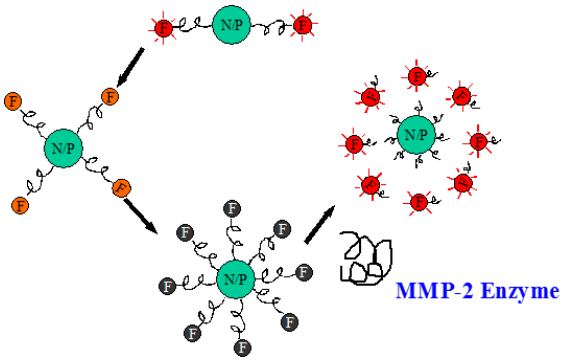
Self-quenched substrates, Silica-Cored Carrier Particle

KareBay™ BioChem Co.,Ltd

Protease peptide substrates labeled with self-quenching labile dyes, such as Cy7, are conjugated to a nanocarrier with such a high intensity that the dye molecules cause self-quenching among their vicinity neighbors. Enzyme cleavage of the peptide substrate releases the dye carrying fragment from nanoparticle surface into solution space, the self-quenching effect is released and the dye fluorescence intensity is recovered. This type of substrate can be used for cancer in-vivo imaging due to the nanoparticles beneficiary from EPR effect.

Principle Illustration

a. Figure 1



b. Figure 2

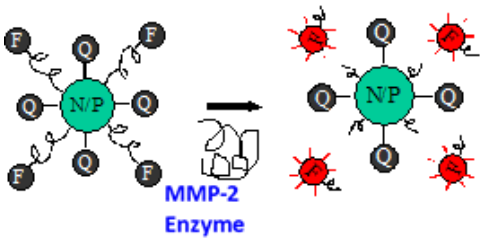
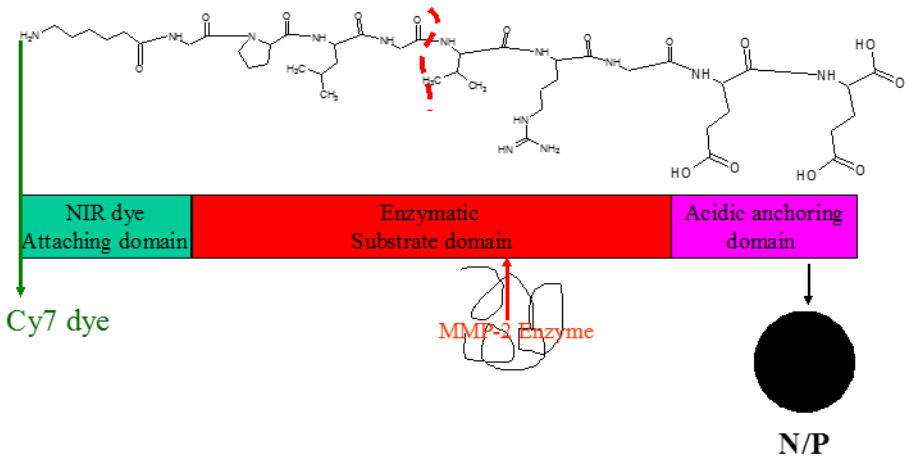
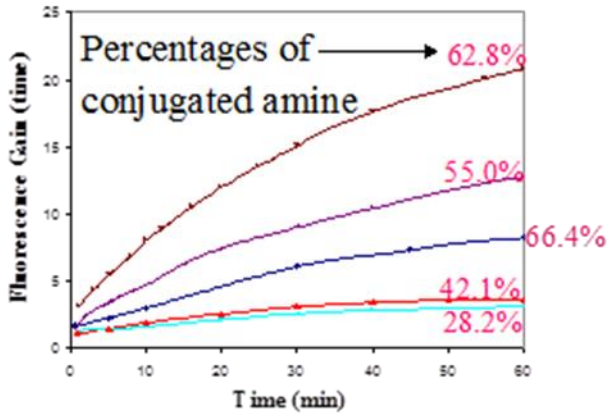


Illustration of two pathways utilized to construct activatable probes using polymer-grafted silicon nanoparticle as the platform. A) Self-quenching strategy. With a couple of peptide-dye conjugates loaded onto one nanoparticle, the distance between those fluorophores are too far to induce self-quenching (a); however, with the increasing of peptide-dye conjugation density, the fluorophore starts to quench each other (b) and finally arrive` at a maximum quenched status (c); upon MMP-2 enzyme cleavage of the peptide linker, the fluorophores along with peptide fragment are released into the solution space, leading to a dequenching state (d). B) FRET strategy. Fluorophores are attached to the nanoparticle via peptide linker, while quenchers are directly attached to the nanoparticles, absorbing partial of the emission by the fluorophores. After enzymatic cleavage of the peptide linker, fluorphores along with peptide fragments are released into solution

Example, MMP2 peptide substrate labeled with Cy7

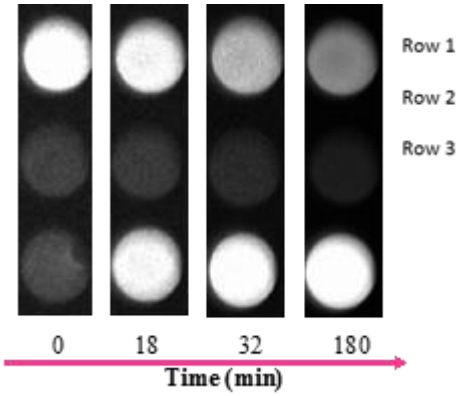
Cy7-AhxPLG-VRGEE:





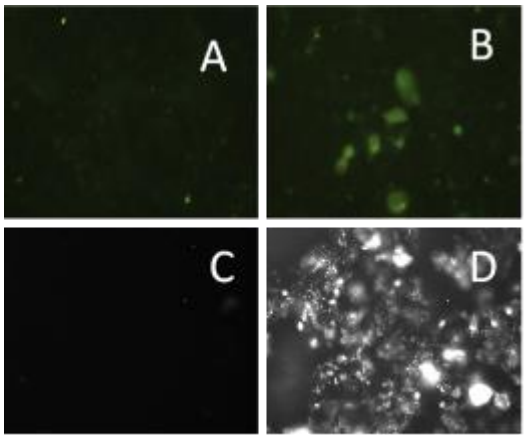
Enzymatic assay results peptide substrate design: MMP2 peptide substrate labeled with Cy7:

Effects of peptide density on signal amplification capability of nanoprobes. A) Nanoprobe signal amplification rate is tightly related to peptide-dye conjugate density on nanopartcile surface. With the increase percentage of reacted amine (with peptide-dye conjugates) on nanoparticle surface, nanoprobe signal amplification capability increases and achieves a maximum value at 62.8% of reacted amine; beyond this, such as for a 66.4% of reacted amine case, the amplification capability decreases instead.



96 plate-well analysis of activatable probe with various surface amine conjugated. Nanoparticle solutions are all

of 100 µl, but with different concentration: Row 1: 1.08mg/ml, row 2: 0.0108 mg/ml, row 3: 0.0108 mg/ml in the presence of 0.2 µg MMP2 enzyme.



Detection of Matrix Metalloproteinase-2 (MMP2) activity in Breast cancer MCF-7 cells (A and B) with Fluorescence Image and NIR imaging. (A and C). Cy7 is attached to silicon nanoparticle (A, C) and to Peptide-dye conjugate loaded nanoparticle (B, D).