Supporting information

Developing PEGylated Reversed D-Peptide as a Novel HER2-Targeted SPECT Imaging Probe for Breast Cancer Detection

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This file includes:

The procedures for synthesis of HYNIC-PEG_4-H6/DH6/RDH6 conjugates, synthesis of HYNIC-PEG_{12}/PEG_{24}-RDH6 conjugates, determination of Log $P_{o/w}$ values and surface plasmon resonance imaging (SPRi), and the Figure S1-S6 and Table S1.

Experimental Section

Synthesis of HYNIC-PEG$_4$-H6/DH6/RDH6 Conjugates

NH_2-PEG$_4$-COOH and sodium succinimidyl 6-(2-(2-sulfonatobenzaldehyde) hydrazono)nicotinate (HYNIC-NHS) was dissolved in 500 μL DMF and mixed with 3 μL DIEA. After stirring for 8 h at room temperature, conjugate HYNIC-PEG$_4$-COOH was isolated by semi-preparative HPLC. Fractions containing the product was collected and lyophilized.

HYNIC-PEG$_4$-COOH was dissolved in 500 μL DMF and mixed with EDC and NHS. After stirring for 8 h at room temperature, conjugate HYNIC-PEG$_4$-NHS was isolated by semi-preparative HPLC. Fractions containing the product was collected and lyophilized.

HYNIC-PEG$_4$-NHS and peptide (H6/DH6/RDH6) was dissolved in 500 μL DMF and mixed with 3 μL DIEA. After stirring for 8 h at room temperature, conjugate HYNIC-PEG$_4$-H6/DH/RDH6 was isolated by semi-preparative HPLC. Fractions containing the product was collected and lyophilized. The purities of products were >95%. The identity of the product was confirmed by mass spectrometry (m/z, 1670.6 for $[M+H]^+$).

Synthesis of HYNIC-PEG$_{12}$/PEG$_{24}$-RDH6 Conjugates

HYNIC-PEG$_{12}$-RDH6 and HYNIC-PEG$_{24}$-RDH6 are synthesized in the same way. Take HYNIC-PEG$_{24}$-RDH6 as an example.

Fmoc-PEG$_{24}$-COOH was dissolved in 500 μL DMF and mixed with EDC and NHS. After stirring for 8 h at room temperature, conjugate Fmoc-PEG$_{24}$-NHS was isolated by semi-preparative HPLC. Fractions containing the product was collected and lyophilized.

Fmoc-PEG$_{24}$-NHS and RDH6 was dissolved in 500 μL DMF and mixed with 3 μL DIEA. After stirring for 8 h at room temperature, conjugate Fmoc-PEG$_{24}$-RDH6 was isolated by semi-preparative HPLC. Fractions containing the product was collected

and lyophilized.

Fmoc-PEG_{24}-RDH6 was dissolved in 200 μL 20% Piperidine-DMF and stirring for 20 min at room temperature. Conjugate NH_2-PEG_{24}-RDH6 was isolated by semi-preparative HPLC. Fractions containing the product was collected and lyophilized.

NH_2-PEG_{24}-RDH6 and HYNIC-NHS was dissolved in 500 μL DMF and mixed with 3 μL DIEA. After stirring for 8 h at room temperature, conjugate HYNIC-PEG_{24}-RDH6 was isolated by semi-preparative HPLC. Fractions containing the product was collected and lyophilized. The purity of product was >95%. The identity of the product was confirmed by mass spectrometry (m/z, 2551.9 for $[M+H]^+$).

The identity of HYNIC-PEG_{12}-RDH6 was confirmed by mass spectrometry (m/z, 1509.7 for $[M+H]^+$).

Determination of Log $P_{o/w}$ Values

After radiolabeling, 2 μL of ^{99m}Tc-PEG_4-H6, ^{99m}Tc-PEG_4-DH6, ^{99m}Tc-PEG_4-RDH6, ^{99m}Tc-PEG_{12}-RDH6 and ^{99m}Tc-PEG_{24}-RDH6 was taken respectively and diluted to 50 μL with PBS. 50 μL diluent of ^{99m}Tc radiotraces were mixed with 500 μL octanol and 450 μL PBS (0.1 M, pH = 7.4), and stirred for 4 h at 4 ℃. After centrifugation, 100 μL liquid of organic phase and aqueous phase was taken respectively, and radioactivity was measured by γ-counter. The above experiment was repeated three times. The log P values were reported as the average of three independent measurements plus the standard deviation.

Calculation formula: Log $P_{o/w}$ = log (CPM of octanol/CPM of PBS)

Surface Plasmon Resonance Imaging

Surface plasmon resonance imaging (SPRi) analysis was performed on a Plexera PlexArray HT system (Plexera LLC, Bothell, WA) using a bare gold SPR chip (Nanocapture gold chips, with a gold layer of 47.5 nm thickness). All Sulfydryl-peptides (Cys-H6, Cys-DH6 and Cys-RDH6) were printed onto the gold

chip surface by the thiol group of the cysteine residue. The printed chip was then incubated in 4 ℃ overnight in a humid box. The SPR chip was washed and blocked using 5% nonfat milk in PBS overnight before use. The SPR analysis procedure follows the following cycle of injections: running buffer (PBST, baseline stabilization); sample (six concentrations of the protein, binding); running buffer (PBST, washing); and 0.5% (v/v) H_3PO_4 in deionized water (regeneration). HER2 protein was diluted with PBST to concentrations of 704 nM, 352 nM, 176 nM, 88 nM, 44 nM, 22 nM. Real-time binding signals were recorded and analyzed by PlexArray HT software.

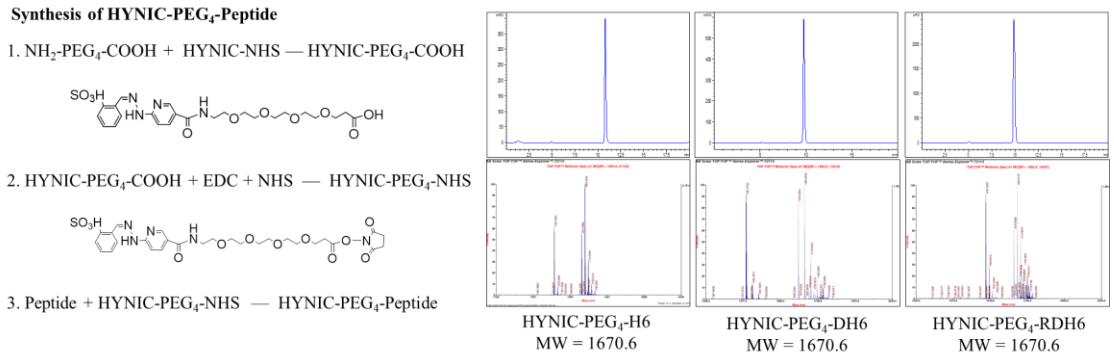

Figure S1. The synthesis process, typical HPLC chromatogram and MALDI-TOF mass spectrometry of HYNIC-PEG4-peptide.

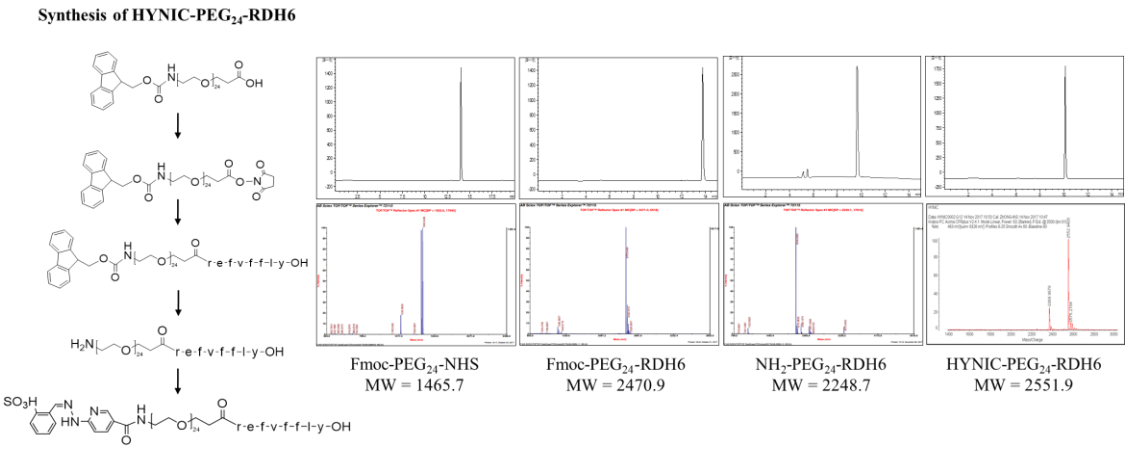

Figure S2. The synthesis process, typical HPLC chromatogram and MALDI-TOF mass spectrometry of HYNIC-PEG_{24}-RDH6.

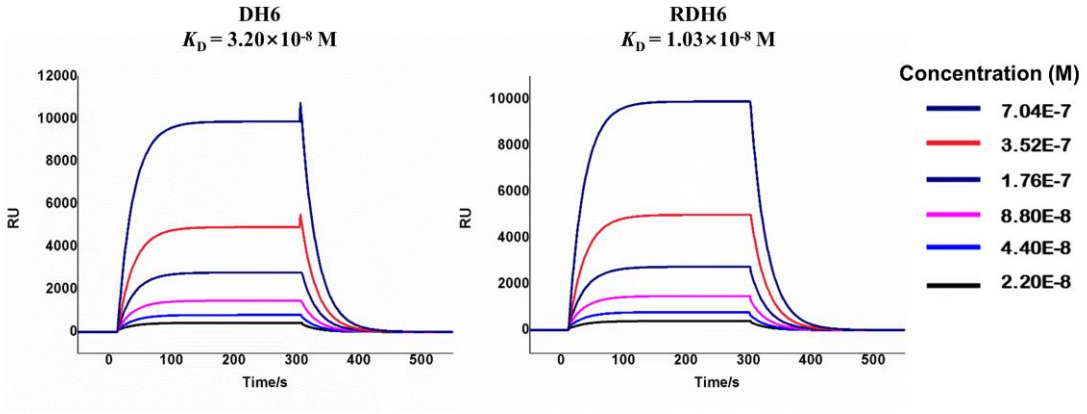

Figure S3. SPR detection of the binding affinity of DH6 and RDH6 toward HER2, respectively.

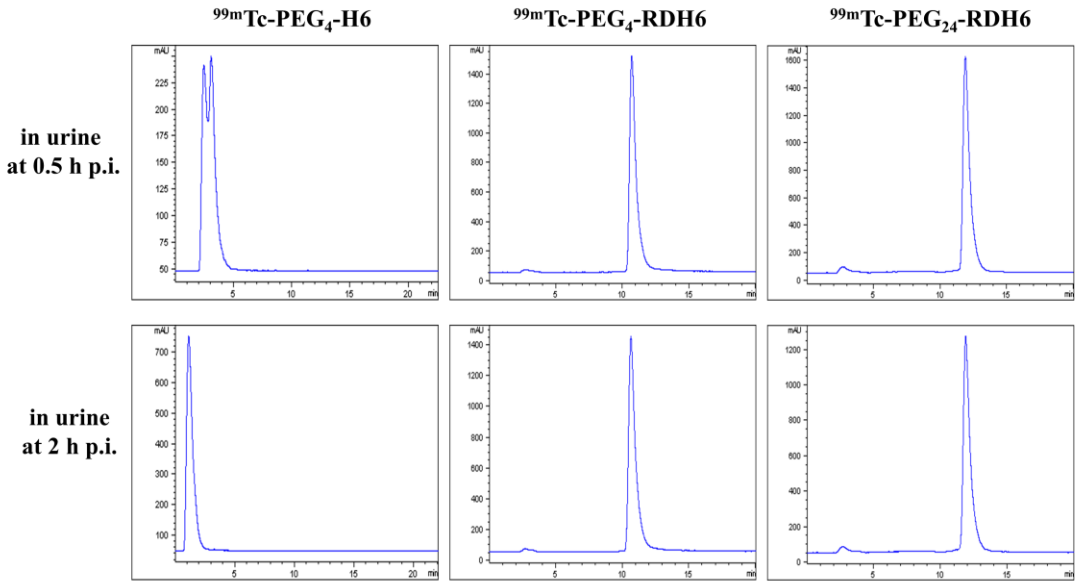

Figure S4. Radio-HPLC chromatograms of ^{99m}Tc-PEG$_4$-H6, ^{99m}Tc-PEG$_4$-RDH6 and ^{99m}Tc-PEG$_{24}$-RDH6 in urine at 0.5 and 2 h p.i. Two normal mice were used, and each was administered with ~10 MBq of tracer, respectively.

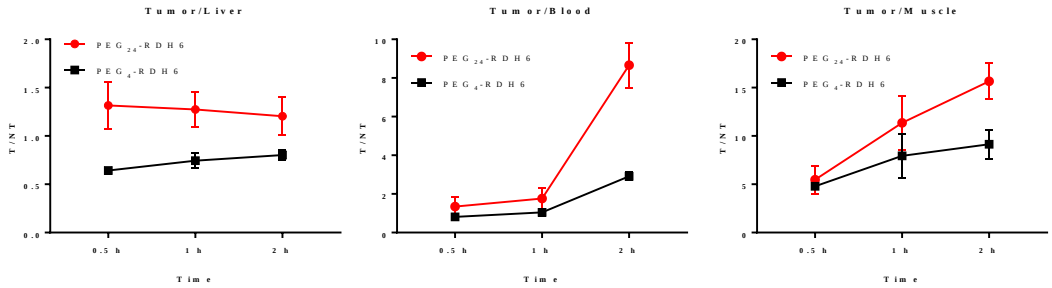

Figure S5. The tumor/non-tumor ratios of ^{99m}Tc-PEG$_{24}$-RDH6 and ^{99m}Tc-PEG$_4$-RDH6.

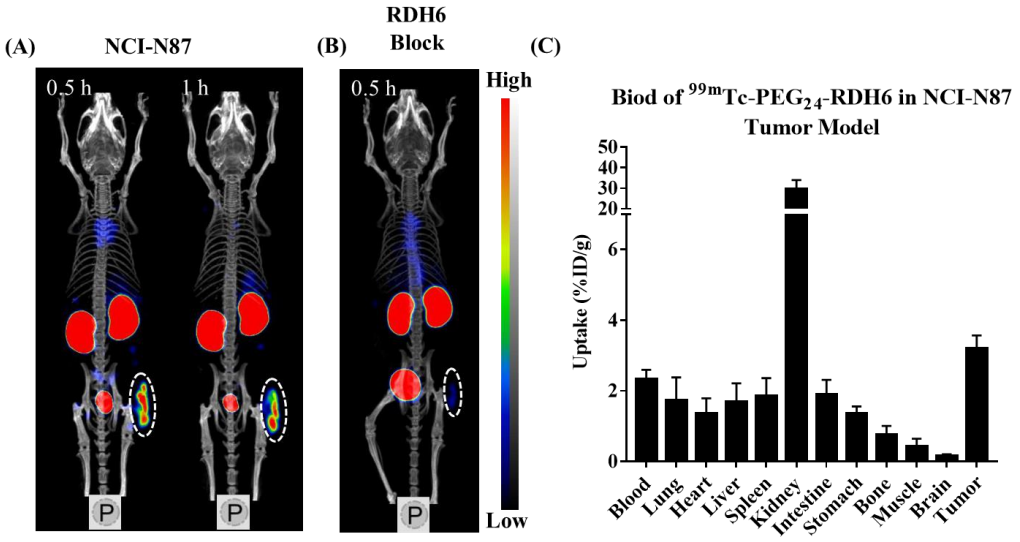

Figure S6. Representative nanoScanSPECT/CT images of ^{99m}Tc-PEG$_{24}$-RDH6 in NCI-N87 (HER2^{+}) gastric cancer model (A) at 0.5 and 1 h post-injection, (B) blocking with excess cold RDH6 peptide at 0.5 h post-injection. (C) Biodistribution of ^{99m}Tc-PEG$_{24}$-RDH6 in NCI-N87 gastric cancer model at 0.5 h post-injection.

Table S1. Lipo-hydro partition coefficient of ^{99m}Tc-PEG$_n$-H6/RDH6 (n = 4, 12, 24).

Peptide	**Log $P_{o/w}$**
^{99m}Tc-PEG$_4$-H6	2.56 ± 0.03
^{99m}Tc-PEG$_4$-DH6	2.69 ± 0.02
^{99m}Tc-PEG$_4$-RDH6	2.61 ± 0.02
^{99m}Tc-PEG$_{12}$-RDH6	1.95 ± 0.01
^{99m}Tc-PEG$_{24}$-RDH6	-0.93 ± 0.02