Supporting Information for “Imaging Deferoxamine-bearing PEG-like Nanoprobes in Disease Models”

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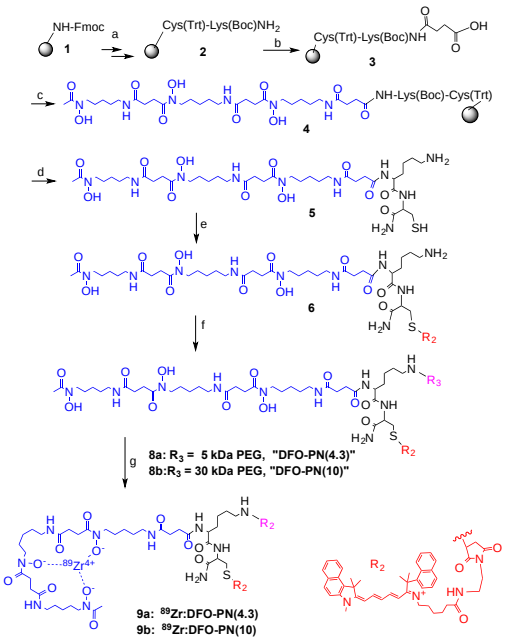
Materials and Methods-detailed syntheses

Fmoc-protected L-amino acids, PyBOP and Rink Amide MBHA resin were from Novabiochem (EMD Biosciences). Other special chemicals were from other sources: Deferoxamine mesylate salt (DFO mesylate) (Sigma), succinic anhydride (Aldrich), mPEG-NHS ester (5 kDa and 30 KDa; Creative PEGworks). Cy5.5-maleimide was from Lumiprobe. All the other solvents and chemicals were from Sigma-Aldrich. Lower molecular weights were obtained by MS-ESI Micromass (Waters) and the higher molecular weights were obtained by MALDI-TOF analyses at the Tufts University Core Facility. RP-HPLC (Varian ProStar detector and delivery modules) employed an eluant A (0.1% TFA /water) and eluant B (0.1% TFA and 9.9% water in acetonitrile).

The synthesis of DFO-PNs proceeds as in scheme 1 given in the main text but repeated below. It involves three basic steps: (i) the synthesis of the (DFO)Lys-Cys peptide or compound (5), (ii) synthesis of the (DFO)Lys-Cys(Cy5.5) peptide or compound 6 and, (iiia) the reaction of the 5 kDa PEG (7a) to yield DFO-PN(4.3) or compound 8a, or (iiib) reaction of the 30 kDa PEG (7b) to yield DFO-PN(10) or compound 8b. DFO-PN(4.3) and DFO-PN(10) are then reacted with ^{89}Zr.



Scheme 1 (from text)

(i) Synthesis of (DFO)Lys-Cys or (5)

The (DFO)Lys(Boc)-Cys(Trt) peptide was manually synthesized on Rink Amide MBHA resin (0.15

mmol) with an Fmoc/*t*-Bu strategy using a polypropylene 5 mL disposable syringe fitted with a sintered frit. Coupling reactions employed 2 equiv. (relative to resin) of Fmoc-protected amino acid activated in situ with 2 equiv. of PyBOP and 4 equiv. of DiPEA in DMF (10 mL/g resin) for 1-1.5 hrs. Coupling efficiency was assessed with picrylsulfonic acid. Fmoc groups were removed with a piperidine/DMF solution (1:4) for 4x10 min (10 mL/g resin). Before the coupling of DFO, a succinic acid linker was added in between DFO and N-terminal of the peptide by incubating the mixture of succinic anhydride (8eq.) and DIPEA (8eq) in DMF (3ml) at room temperature for 1hr. The coupling of DFO was carried out by firstly activating the acid on the resin with PyBOP (4eq) and DIPEA (16eq) in DMSO (anhydrous, 0.5ml) for 1h, then incubating with DFO mesylate (4eq) in DMSO (2.25ml) for overnight. (DFO)-Succ-Lys-Cys was released from the solid support with TFA/H_2O/TIS/EDT 88:2:5:5 (twice, 3 h, 20 mL/g resin). The solution was directly triturated with cold diethyl ether. An off-white solid could be obtained by centrifuge. The solid was purified further by HPLC with polymer based C18 column (Agilent, PLRP-S, 100A, 8uM, 300X7.5MM, PL1112-6800); gradient: 15% - 60% buffer B in 10min, back to 15%B in 5 min, and isocratic for 5 min. A white powder of compound (DFO)-Succ-Lys-Cys was obtained after lyophilization with a yield of 40%. MS: $C_{38}H_{70}N_{10}O_{12}S$: theoretical 890.49; found = 891.5 (M+1).

(ii) Synthesis of (DFO)Lys-Cys(Cy5.5) or (6)

(DFO)Lys-Cys (51mg, 56.7umol) and Cy5.5-Maleimide (41.97mg, 56.7umol) was mixed in DMSO (3 ml) for 1 h in the presence of DIPEA (4eq, 226.8umol, 39.6ul) at room temperature under N_2. HPLC was employed to purify the compound with a polymer based C18 column (Agilent, PLRP-S, 100A, 8uM, 300X7.5 mm, PL1112-6800); gradient: 20-100% buffer B in 7min, back to 20% in 2 min, and isocratic for 3min; flow: 4ml/min detected at 675 nm. A blue powder was obtained after lyophilization. Yield: 30mg, 33%. MS: $C_{84}H_{119}N_{14}O_{15}S^+$: theoretical 1595.87; found: 1596.0 (M^+), 798.6 $(M+H)^{2+}$.

(iiia) Synthesis of DFO-PN(4.3) or 8a

A mixture of the solution of (DFO)Lys-Cys(Cy5.5) (13.5mg, 8.45umol)) and m-PEG-5K-NHS (127mg, 25.35μmol, 3eq), and DiPEA (44μL**,** 253umol, 30eq to MSAP**)** in DMSO (2ml) was incubated under room temperature for 7 days. HPLC was employed to purify the compound with a polymer based C18 column (Agilent, PLRP-S PLRP-S 100A 8um 250x4.6mm, PL1512-5800): gradient: 20-100% buffer B in 10 min, back to 20%B in 2min, then isocratic for 3min; flow: 1.5ml/min; detected at 675nm. Yield: 30%. MS: theoretical: 6595, found: 6674 (peak center)

(iiib) Synthesis DFO-PN(10) or 8b

A mixture of the solution of (DFO)Lys)-Cys(Cy5.5) (13.5mg, 8.45umol), m-PEG-30K-NHS (760mg, 25.35μmol, 3eq), and DiPEA (44μL**,** 253umol, 30eq to MSAP**)** in DMSO (12ml) was incubated under 37^oC for 2 days. HPLC was employed to purify the compound with a polymer based C18 column (PLRP-S, 100A, 8uM, 300X7.5MM, PL1112-6800): gradient: 20-100% buffer B in 8min, back to 20%B in 2min, and isocratic for 2.5min; flow: 4ml/min; detected at 675nm. Yield: 27.8%. MS: theoretical: 31595,

found: 30817

Reactions of (DFO)-PN(4.3) or (DFO)-PN(10) with ^{89}Zr

A solution of ^{89}Zr oxalic acid (3.0 mCi, 100 μl) was neutralized by a solution of Na_2CO_3 (1 M in chelexed water, 90 μl) up to pH 8. (DFO)-PN(4.3) (60nmol in chelexed water 102μl) was added to the solution of ^{89}Zr. After incubation for 2 h at room temperature, the mixture was loaded onto a PD-10 column eluted by 0.9% saline (sterilized). The fraction at 2.7-4.3ml was the labeled product, which was concentrated with a centrifuge by an Amicon Ultra filter (MC 10K). RCY: 72%, specific activity: 0.036 Ci/μmol.

^{89}Zr labeling of (DFO)-PN(10) used 80 nmol and ^{89}Zr (4.8mCi). RCY: 74%, specific activity: 0.0446 Ci/umol.[8]

Purity of radiolabeled PN's was demonstrated by TLC with chelex treated Whatman paper or silica with a running buffer of 50 mM DTPA at pH 7-8. Here the ^{89}Zr:DTPA complex runs at about 90 mm, see Figure S1.

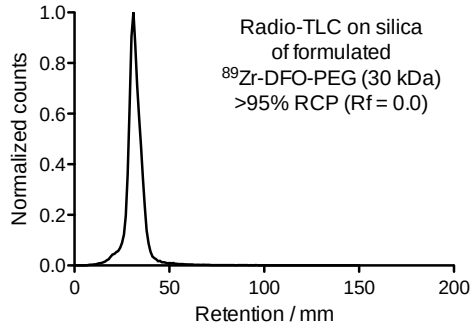


Figure S1: TLC of purified [^{89}Zr]:DFO-PN(10).