## Supplemental Data

### Preparation of NODAGA-RM1 and NODAGA-AMBA.

The G-4-aminobenzoyl-Gln-Trp-Ala-Val-Gly-His-Leu-Met-$NH_2$ (abbreviated as AMBA) and G-4-aminobenzoyl-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-$NH_2$ (abbreviated as RM1) peptides (Figure 1) were synthesized on a CS Bio CS036 Peptide Synthesizer (Menlo Park, CA) using Fmoc-based SPPS as previously reported (*1*). The peptide purity and molecular masses were determined by analytical scale RP-HPLC and matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS), respectively. Briefly, Rink amide resin was swollen in N,N-dimethylformamide (DMF) for 30 min. The Fmoc groups were removed using 20% piperidine in DMF. Aliquots of amino acids (1 mmol) were activated in a solution containing 1 mmol hydroxybenzotriazole (HOBt) and 0.5 M diisopropylcarbodiimide (DIC) in DMF. Following synthesis, side-chain deprotection and resin cleavage were achieved by the addition of a 92.5:2.5:2.5:2.5 (v/v) mixture of TFA:triisopropylsilane:ethanedithiol:water for 2-4 h at room temperature. Semi-preparative RP-HPLC was used for the purification.

The two peptides were conjugated with NODAGA-NHS to obtain NODAGA-AMBA and NODAGA-RM1, respectively. Specifically, the peptides (1 μmol each) and NODAGA-NHS (1 μmol) were dissolved in 50 μL of DMF, to which 1 μL of DIPEA was added. The reaction mixtures were stirred for 2 h at room temperature, followed by purification of the final products by the semi-preparative HPLC.

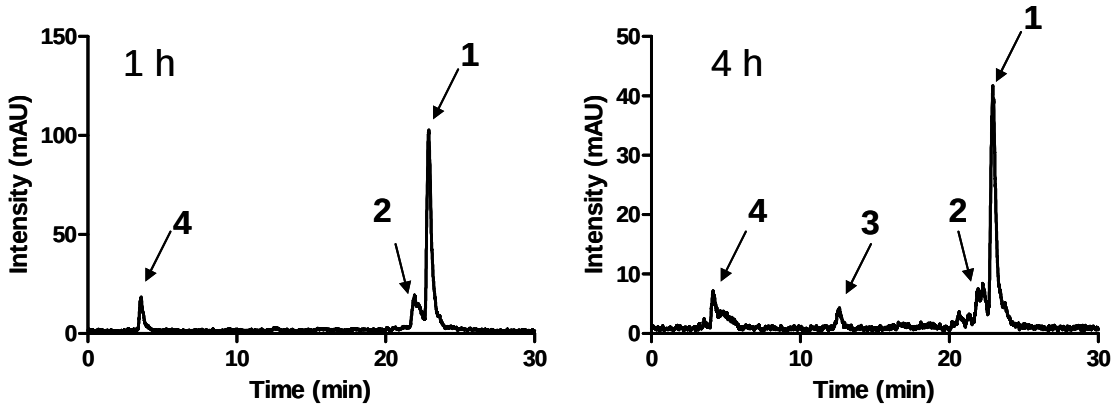
### Cell Culture and Cell-Binding Assays

The PC3 cells were cultured in RM1640 containing high glucose (GIBCO, Carlsbad, CA), 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. The cells were expanded in tissue culture dishes and maintained in a humidified atmosphere of 5% $CO_2$ at 37°C. The medium was changed every other day. Confluent monolayers were detached using 0.05% Trypsin-EDTA and 0.01 M PBS (pH 7.4) and dissociated into a single-cell suspension for further cell culture.

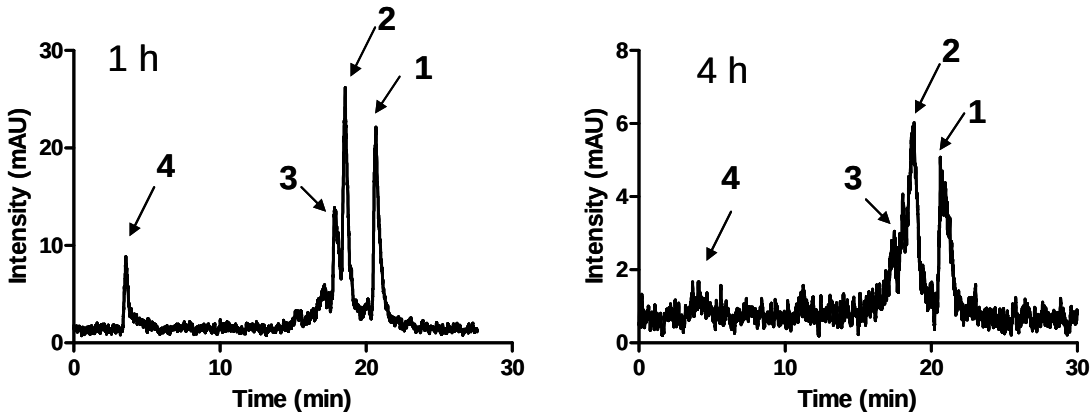
The cell-binding assay was performed similarly as previously reported (*2*, *3*). Briefly, the PC3 cells ($3 \times 10^4$) were incubated with 0.06 nM $^{125}$I-[Tyr4]BBN and varying concentrations of peptides in the binding buffer (RPMI 1640 + 2 mg/mL BSA + 5.2 mg/mL HEPES) at 37°C for 1 h. The cell-bound, residual radioactivity after washing was determined by gamma counting. The $IC_{50}$ values, the concentration of competitor required to inhibit 50% of the radioligand binding, were determined by non-linear regression using GraphPad Prism (GraphPad Software, Inc.). The experiments were performed in quadruplicate.

**Small Animal PET Imaging**

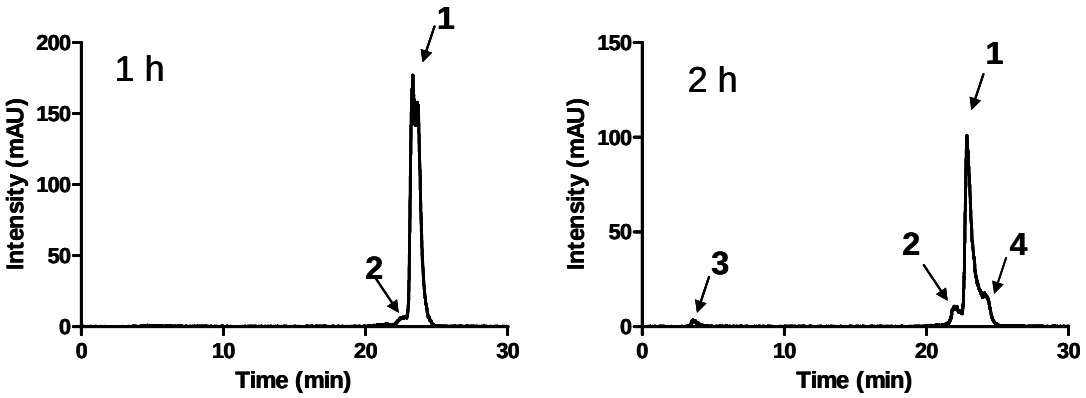
The animal procedures were performed according to a protocol approved by the Stanford University Institutional Animal Care and Use Committee. The small animal PET scans were performed using a microPET R4 rodent model scanner (Siemens Medical Solutions USA, Inc., Knoxville, TN). The scanner has a computer-controlled bed and 10.8-cm transaxial and 8-cm axial fields of view (FOVs). Approximately $3 \times 10^6$ cultured PC3 cells were suspended in PBS and subcutaneously implanted in one shoulder of male nude mice. The tumors were allowed to grow to a diameter of 0.6-1 cm (5-6 weeks).



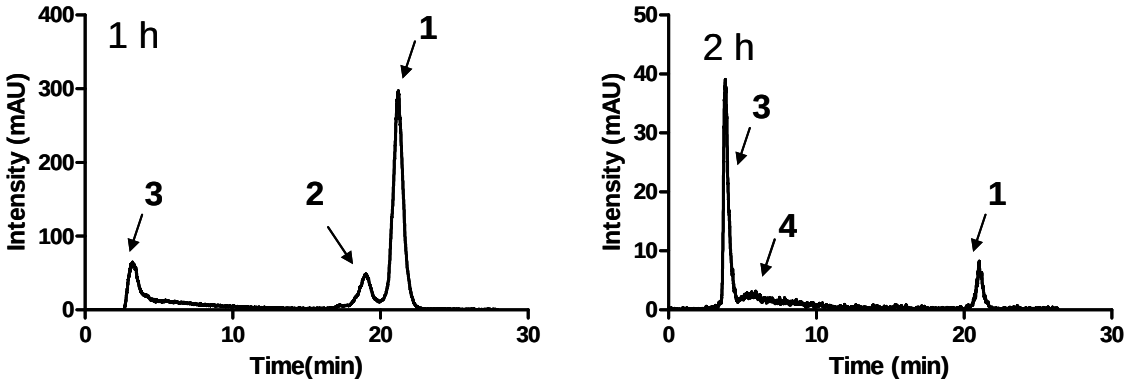

**SUPPLEMENTAL FIGURE 1** *In vitro* stability assay of $^{64}$Cu-NODAGA-RM1 in mouse serum after incubation at 37°C. Peak 1 is the intact probe. The retention times (rt) for the intact probe and metabolite are 22.9 min (peak 1), 22.0 min (peak 2), 12.6 min (peak 3), and 3.9 min (peak 4), respectively, and their percentages are 76.1 % and 61.3 % (for peak 1), 14.2 % and 14.1% (for peak 2), 0% and 5.3 % (for peak 3), 9.5% and 12.2% (for peak 4) at 1 h and 4 h, respectively. The radiometabolites were not further characterized and identified.



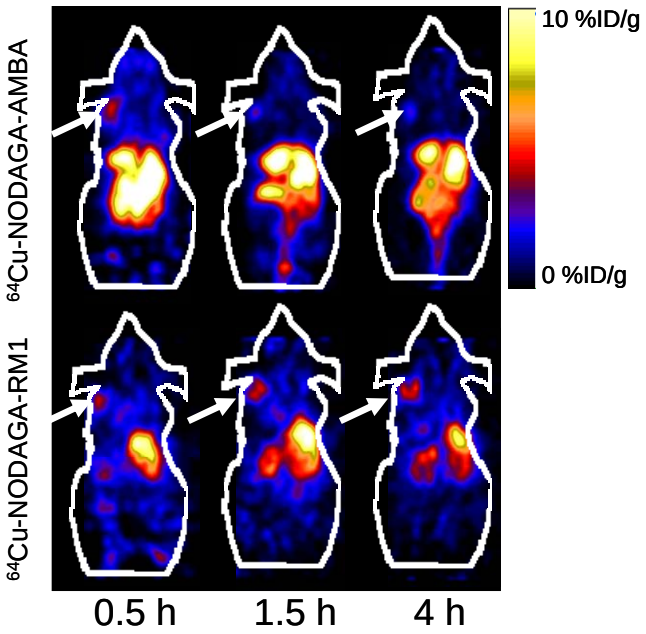

**SUPPLEMENTAL FIGURE 2** *In vitro* stability assay of $^{64}$Cu-NODAGA-AMBA in mouse serum after incubation at 37°C. Peak 1 is the intact probe. The retention times (rt) for the intact probe and metabolite are 20.7 min (peak 1), 18.6 min (peak 2), 17.8 min (peak 3), and 3.5 min (peak 4), respectively, and their percentages are 31.2 % and 29.7 % (for peak 1), 33.7 % and 39.4% (for peak 2), 18.5 % and 18.2 % (for peak 3), 11.4 % and 11.7 % (for peak 4) at 1 h and 4 h, respectively. The radiometabolites were not further characterized and identified.



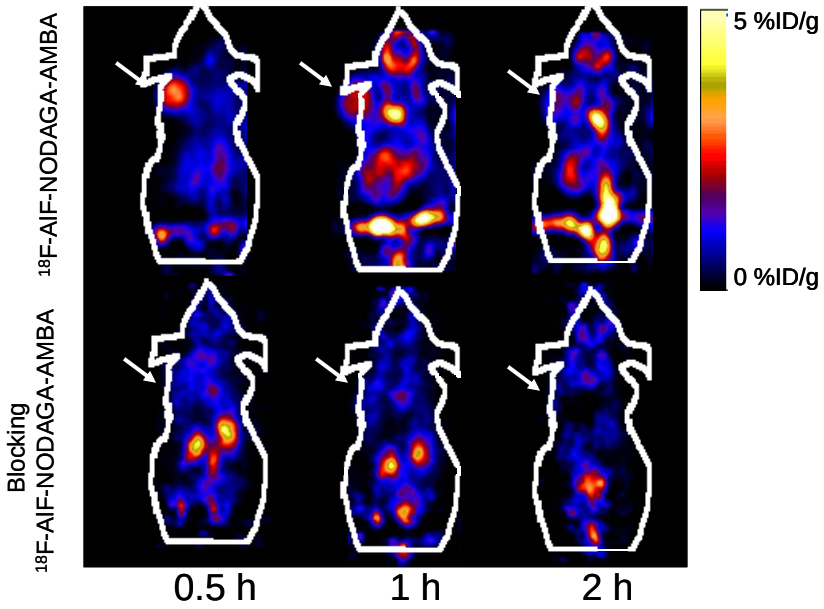

**SUPPLEMENTAL FIGURE 3** *In vitro* stability assay of $^{18}$F-AlF-NODAGA-RM1 in mouse serum after incubation at 37°C. Peak 1 is the intact probe. The retention times (rt) for the intact probe and metabolite are 23.0 min (peak 1), 22.5 min (peak 2), 3.4 min (peak 3), and 23.5 min (peak 4), respectively, and their percentages are 97.6 % and 86.7 % (for peak 1), 1.8 % ad 2.9 % (for peak 2), 0% and 1.1 % (for peak 3), 0% and 6.5 % (for peak 3), at 1 h and 2 h, respectively. The radiometabolites were not further characterized and identified.



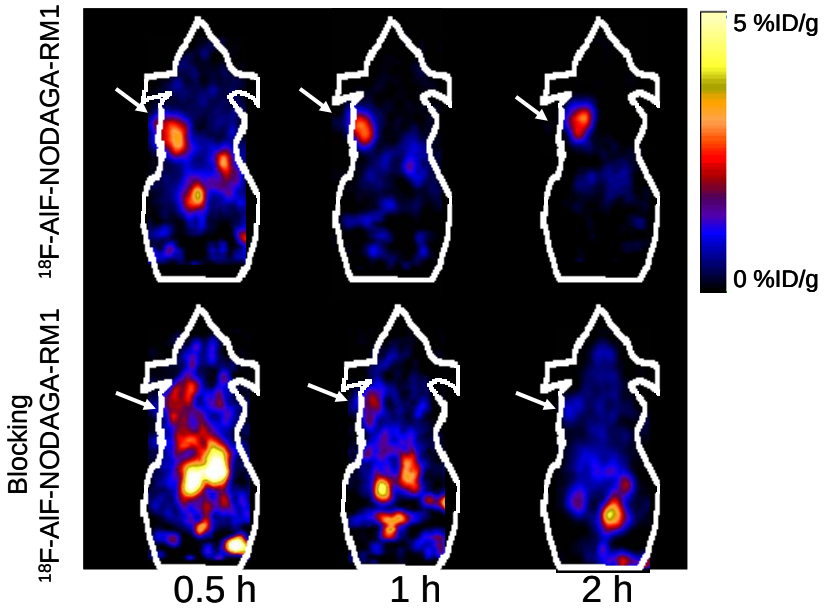

**SUPPLEMENTAL FIGURE 4** *In vitro* stability assay of $^{18}$F-AlF-NODAGA-AMBA in mouse serum after incubation at 37°C. Peak 1 is the intact probe. The retention times (rt) for the intact probe and metabolite are 21.2 min (peak 1), 18.8 min (peak 2), 3.3 min (peak 3), and 5.5 min (peak 4), respectively, and their percentages are 72.5 % and 13.4 % (for peak 1), 10.3 % and 0 % (for peak 2), 14.1 and 63.3 % (for peak 3), 0 and 15.4 % (for peak 4) at 1 h and 2 h, respectively. The radiometabolites were not further characterized and identified.



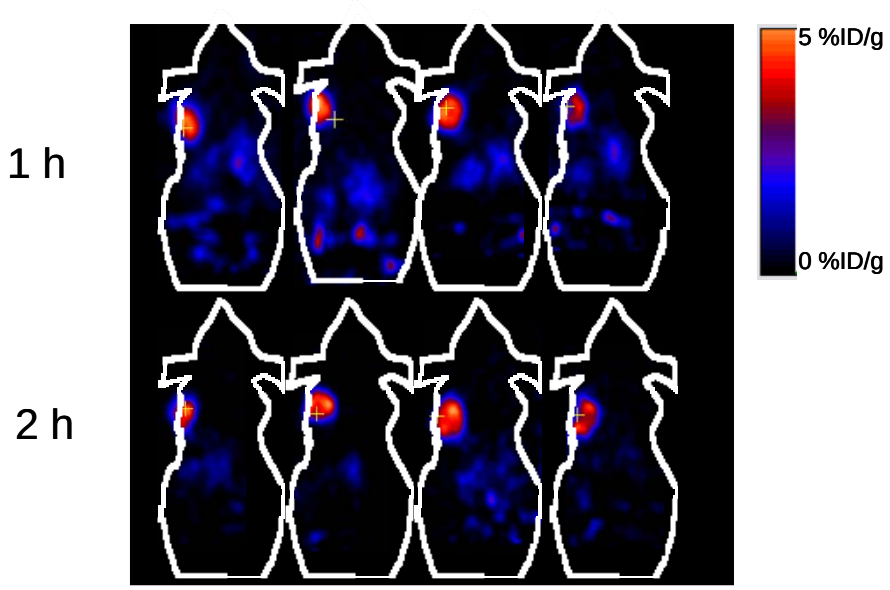

**SUPPLEMENTAL FIGURE 5** Decay-corrected whole-body coronal small animal iPET images of PC3 tumor-bearing male nude mice from a static scan at 0.5, 1.5 and 4 h after the injection of $^{64}$Cu-NODAGA-AMBA and $^{64}$Cu-NODAGA-RM1. The tumors are indicated by arrows.




**SUPPLEMENTAL FIGURE 6** Decay-corrected whole-body coronal small animal PET images of PC3 tumor-bearing male nude mice from a static scan at 0.5, 1 and 2 h after the injection of $^{18}$F-AlF-NODAGA-AMBA without (top) or with (bottom) the co-injection of AMBA as a blocking agent (10 mg/kg body weight). The tumors are indicated by arrows.




**SUPPLEMENTAL FIGURE 7** Decay-corrected whole-body coronal small animal PET images of PC3 tumor-bearing male nude mice from a static scan at 0.5, 1 and 2 h after the injection of $^{18}$F-AlF-NODAGA-RM1 without (top) or with (bottom) AMBA as a blocking agent (10 mg/kg body weight). The tumors are indicated by arrows.




**SUPPLEMENTAL FIGURE 8** Decay-corrected whole-body coronal small animal PET images from 4 nude mice bearing subcutaneous PC-3 tumor xenografts next to left shoulder. Mice were injected with $^{18}$F-AlF-NODAGA-RM1 and scanned at 1 h and 2 h after injection.

**1.** Miao Z, Ren G, Liu H, Qi S, Wu S, Cheng Z. PET of EGFR expression with an 18F-labeled affibody molecule. *J Nucl Med.* 2012;53:1110-1118.

**2.** Yang M, Gao H, Zhou Y, et al. (18)F-Labeled GRPR Agonists and Antagonists: A Comparative Study in Prostate Cancer Imaging. *Theranostics.* 2011;1:220-229.

**3.** Xu Y, Huang W, Ren G, et al. A Four-Arm Star-Shaped Poly(ethylene glycol) (StarPEG) Platform for Bombesin Peptide Delivery to Gastrin-Releasing Peptide Receptors in Prostate Cancer. *ACS Macro Letters.* 2012;1:753-757.