Supplemental Material

Dynamic PET and Optical Imaging and Compartment Modeling using a Dual-labeled Cyclic RGD Peptide Probe

Lei Zhu1,2, Ning Guo2,3, Quanzheng Li4, Ying Ma2, Orit Jacobson2, Seulki Lee2, Hak Soo Choi5, James R. Mansfield6, Gang Niu2*, and Xiaoyuan Chen2*

1. Center for Molecular Imaging and Translational Medicine, Xiamen University, Xiamen, Fujian, China, 361005

2. Laboratory of Molecular Imaging and Nanomedicine (LOMIN), National Institute of Biomedical Imaging and Bioengineering (NIBIB), National Institutes of Health, Bethesda, MD, 20892

3. Department of Biomedical Engineering, Huazhong University of Science and Technology, Wuhan, Hubei, China, 430074

4. Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Cambridge, MA 02138

5. Division of Hematology/Oncology, Department of Medicine and Department of Radiology, Beth Israel Deaconess Medical Center, 330 Brookline Avenue, SLB-05, Boston, MA 02215

6. Caliper Life Sciences, Inc., 68 Elm Street, Hopkinton, MA 01748

Lei Zhu and Ning Guo contributed equally.

For correspondence or reprints contact the following:

Xiaoyuan Chen, E-mail: shawn.chen@nih.gov

Or Gang Niu, E-mail: gang.niu@nih.gov
MATERIAL AND METHODS

Stability test

1 mL of RPMI1640 supplemented with 25% (v/v) of mouse serum was allocated into a 1.5 Eppendorf tube and temperature-equilibrated at 37°C for 15 min before adding of c(RGDyK)-C(DOTA)-ZW-1. Thereafter, 100 µL of the reaction solution was removed immediately and added into 200 µL of 96% ethanol for precipitation of serum proteins. The cloudy reaction sample is cooled at 4°C for 15 min and centrifuged at 14,000 rpm for 2 min to pellet the precipitated serum proteins. The reaction supernatant is then analyzed by RP-HPLC on C18 column (5 µm, 120Å, 250 × 4.6 mm) using 5% to 65% acetonitrile containing 0.1% TFA versus distilled water containing 0.1% TFA over 30 minutes at a flow rate of 1 mL/min. Sample after 60 minutes incubation with serum was taken out for HPLC test under the same condition as above.

Figure S1. Chemical structures of compounds involved in the synthesis of c(RGDyK)-C(DOTA)-ZW-1.
Figure S2. Mass spectrum of c(RGDyK)-C(DOTA)-ZW-1. m/z calculated/found: 724.33/725.534 [M + H]^3+.

Figure S3. HPLC analyze of $^{64}$Cu labeled c(RGDyK)-C(DOTA)-ZW-1. A. Radiologic signal from $^{64}$Cu labeled c(RGDyK)-C(DOTA)-ZW-1. B. Fluorescent signal from $^{64}$Cu labeled c(RGDyK)-C(DOTA)-ZW-1. C. TLC analyze of Copper labeled c(RGDyK)-C(DOTA)-ZW-1.
**Figure S4.** RP-HPLC analysis of c(RGDyK)-C(DOTA)-ZW-1 stability in mouse serum after 60 min of incubation. A. HPLC of c(RGDyK)-C(DOTA)-ZW-1 at starting time point. B. HPLC of c(RGDyK)-C(DOTA)-ZW-1 at 60 min after incubation with mouse serum. C. HPLC of c(RGDyK)-C(DOTA)-ZW-1 dissolved in H₂O. Retention time of c(RGDyK)-C(DOTA)-ZW-1 is 19.4 min.