Supporting Information

Combined probes strategy to increase enzymatic digestion rate and accelerate renal radioactivity clearance of peptide radiotracers

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Synthesis of NOTA-Exendin 4 and NOTA-MVK-Exendin 4

Exendin 4 (1 eq.) was dissolved in 1 mL 0.1% Na-ascorbate (w/v) in PBS and added with either Maleimide-NOTA or NOTA-MVK-Mal (1.5 eq) in 0.2 mL DMSO. The reaction was stirred at room temperature for 2 h and purified by HPLC to give the desired conjugated products in a chemical purity > 95% and a yield of 53-65%. LC-MS analysis confirmed mass of 4694 $[M+H]^+$ for NOTA-Exendin 4 and 5084 $[M+H]^+$ for NOTA-MVK-Exendin 4.

Synthesis of Boc-MVK-Dde and Boc-MFK-Dde

Boc-MVK-Dde was synthesized as the previous study reported.[1] The synthesis of Boc-MFK-Dde was performed with a peptide synthesizer by adding the amino acids in the sequence of Fmoc-Lys(Dde)-OH, Fmoc-Phe-OH and Boc-Met-OH. After the cleavage of resin with 20% hexafluoroisopropanol in dichloromethane, the crude product was precipitated with ether and further purified by HPLC. Boc-MFK-Dde was got as white solid with chemical purity > 95% and a yield of 74%. LC-MS analysis confirmed mass of 688 $[M-H]^-$.

Synthesis of NOTA-MVK-PEG5K

HS-PEG5K-NH_2 (1.0 eq.) was dissolved in 1 mL 0.1% Na-ascorbate (w/v) in PBS and added with NOTA-MVK-Mal (1.5 eq) in 0.2 mL DMSO. The reaction was stirred at room temperature for 2 h and purified by PD-10 desalting column (GE Healthcare). There were many groups of molecular ion peaks ($[M+H]^+$) around 5620 to 6050 for NOTA-MVK-PEG5K on LC-MS spectrum.

Synthesis of NOTA-Met-OH

NOTA-NHS ester (1 eq.) was dissolved in 0.5 mL DMSO, then L-NH2-Met-OH (1.05 eq.) was added, followed by addition of 5 eq. of tri ethylamine. The reaction was stirred at room temperature until full conversion into NOTA-Met-OH was observed by HPLC analysis. Then the mixture was purified on preparative HPLC to give NOTA-Met-OH in a chemical purity > 95% and a chemical yield of 77%. LC-MS analysis confirmed mass of 433 $[M-H]^-$.

Making Standard Curves for Both Model Compounds

0.352 mg (0.55 μmol) Boc-MVK-Dde or 0.988 mg (1.44 μmol) Boc-MFK-Dde was dissolved in 110 or 288 μL DMSO respectively, to obtain the stock solution A or B with the same concentration of 5000 μM. A series of standard solutions of Boc-MVK-Dde or Boc-MFK-Dde with different concentrations (5-3500 μM) were diluted from the stock solution A or B respectively. 10 μL from all the standard solutions of each model compound was subjected to HPLC, each injection was repeated three times. At the wavelength of 254 nm, the peak of the model compound was integrated to calculate the peak area. Plot the concentration with peak area to obtain the standard curve for each model compound.

The standard curve (Figure S1) indicated that, when the concentration of Boc-MVK-Dde varies from 0 to 4000 μM, there was an excellent correlation between its peak area and concentration, with the value of R square to be 0.9997. For Boc-MFK-Dde, the correlation between the peak area and concentration was extremely high when the concentration ranged from 0 to 1500 μM.

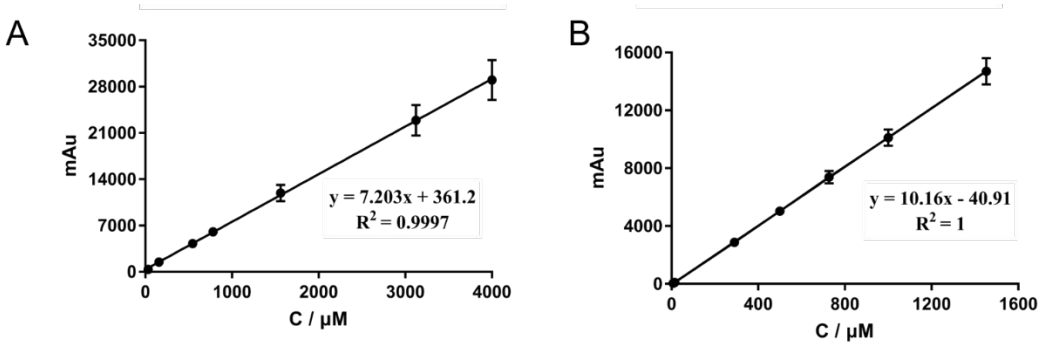

Figure S1. The standard curve of Boc-MVK-Dde (A) and Boc-MFK-Dde (B).

In Vitro Enzymatic Digestion Study

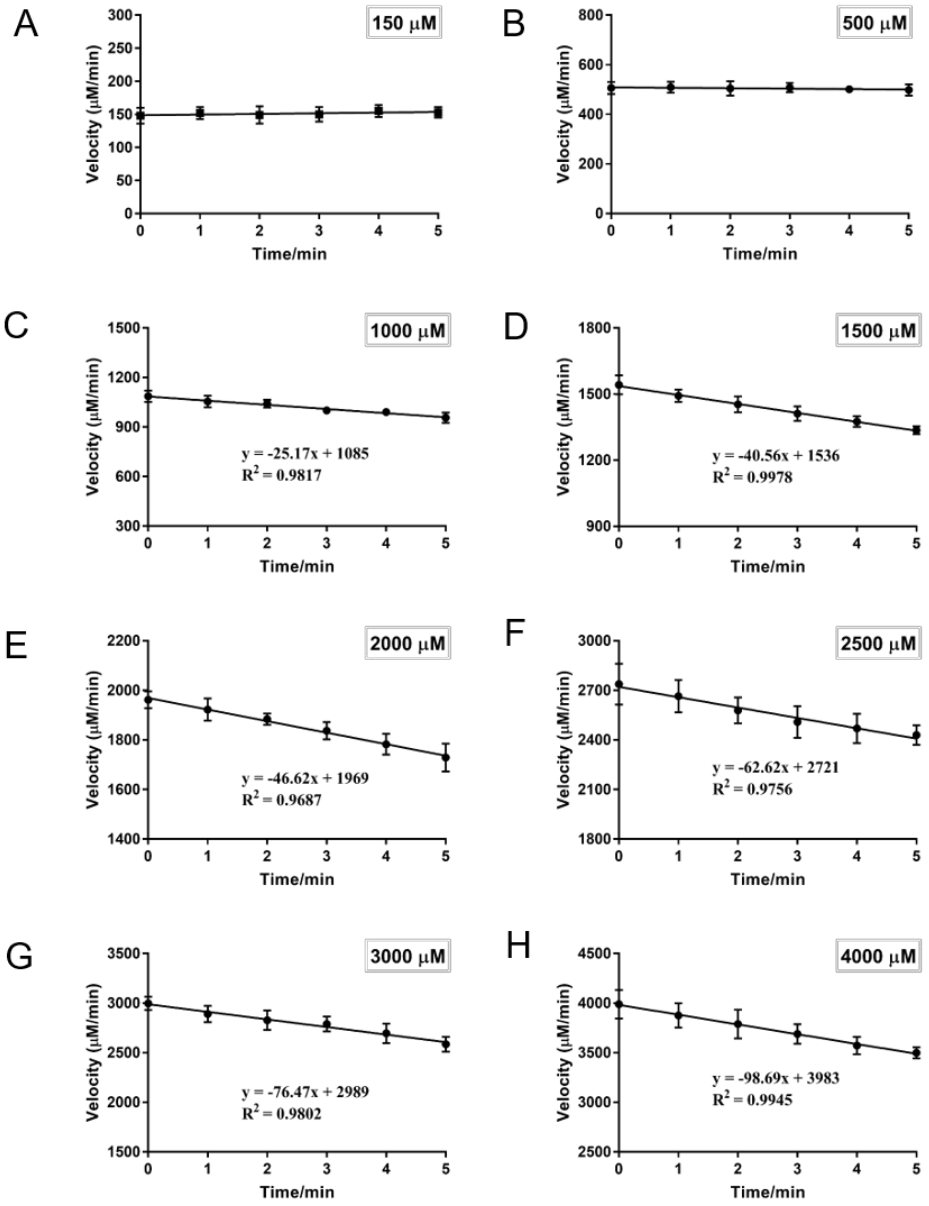

Figure S2. The enzymatic digestion results of Boc-MVK-Dde. The concentration of Boc-MVK-Dde ranges from 150 to 4000 μM (A-H).

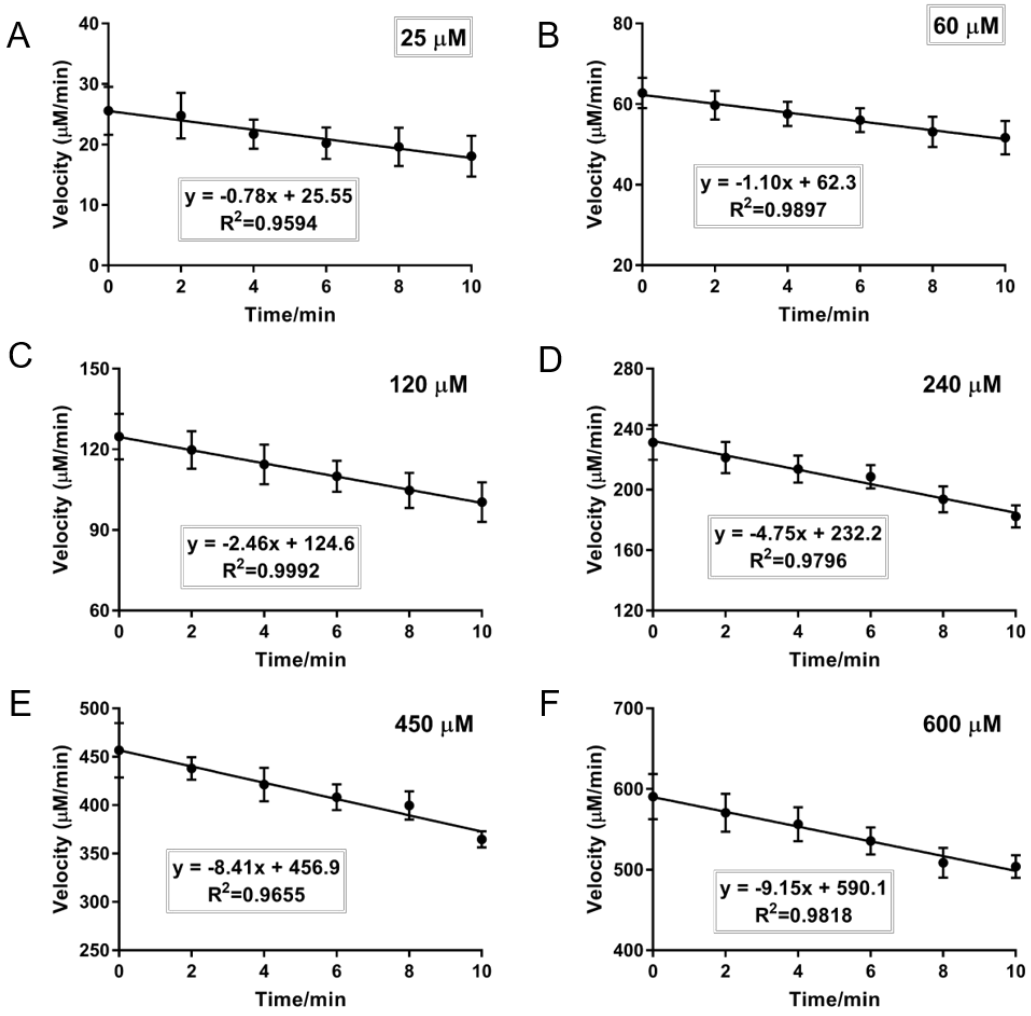

Figure S3. The enzymatic digestion results of Boc-MVK-Dde. The concentration of Boc-MFK-Dde ranges from 25 to 600 μM (A-F).

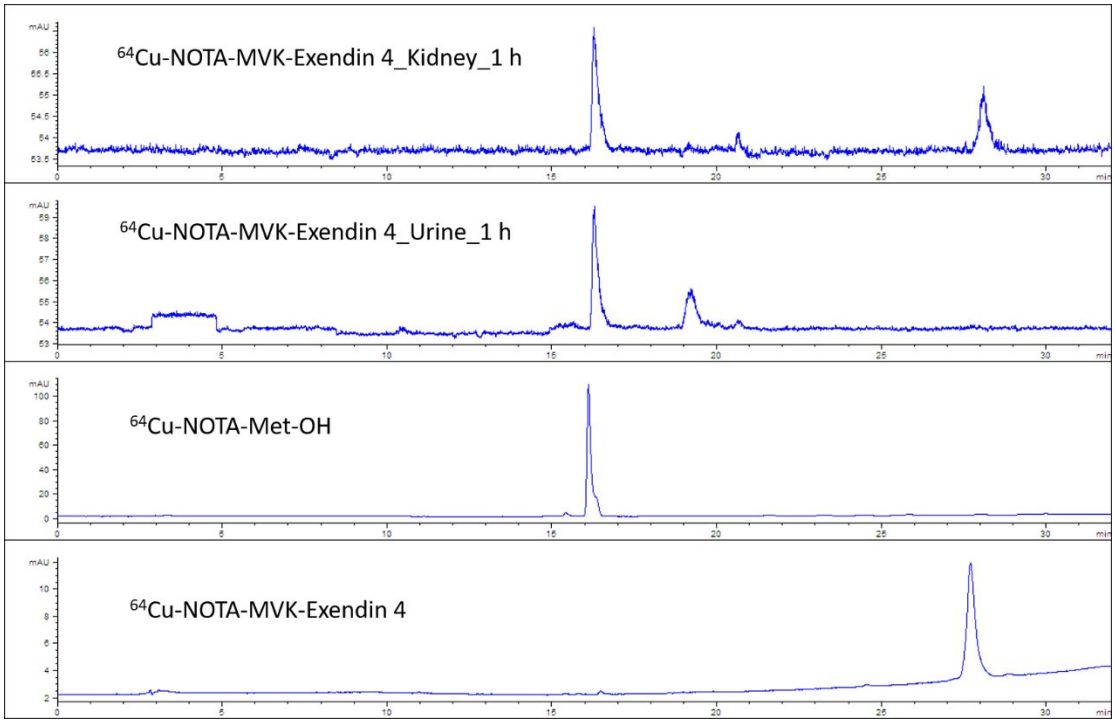

Figure S4. The *in vivo* metabolism study of ^{64}Cu-NOTA-MVK-Exendin 4 (0.2 nmol) in normal mice co-injected with NOTA-MVK-PEG5K (1.3 nmol) at 1 h.

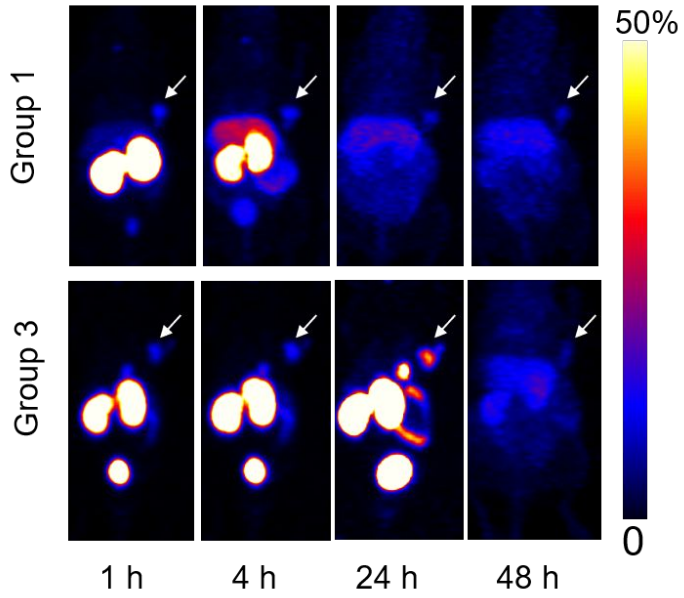

Figure S5. The PET images of INS-1 tumor bearing mice in Groups 1 and 3, which were injected with 0.2 nmol ^{64}Cu-NOTA-Exendin 4 and ^{64}Cu-NOTA-MVK-Exendin 4, respectively.

REFERENCES

(1) Zhang, M.; Jacobson, O.; Kiesewetter, D. O.; Ma, Y.; Wang, Z.; Lang, L.; Tang, L.; Kang, F.; Deng, H.; Yang, W.; et al. Improving the Theranostic Potential of Exendin 4 by Reducing the Renal Radioactivity through Brush Border Membrane Enzyme-Mediated Degradation. *Bioconjug Chem*. **2019**, 30(6), 1745-1753.