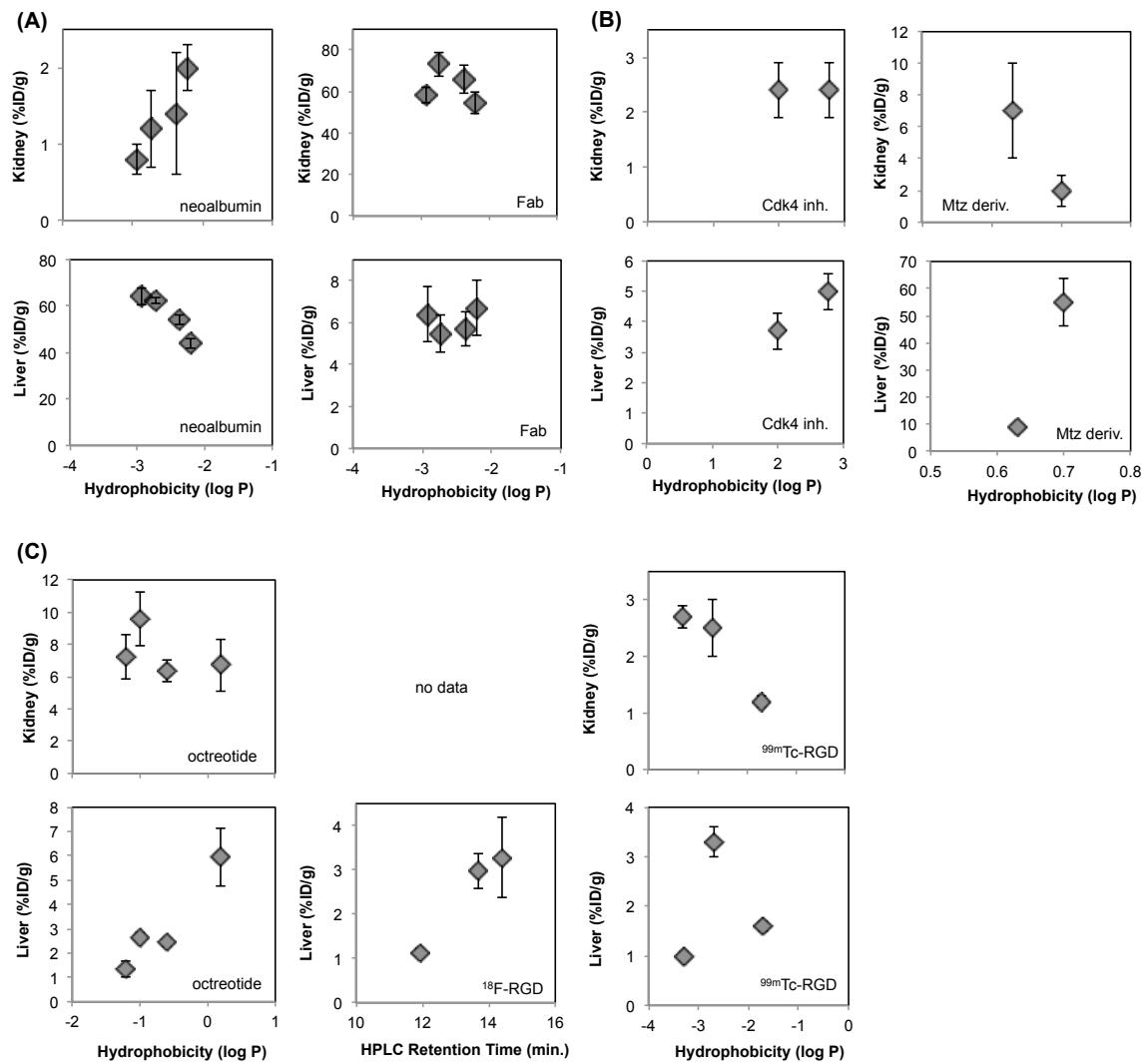


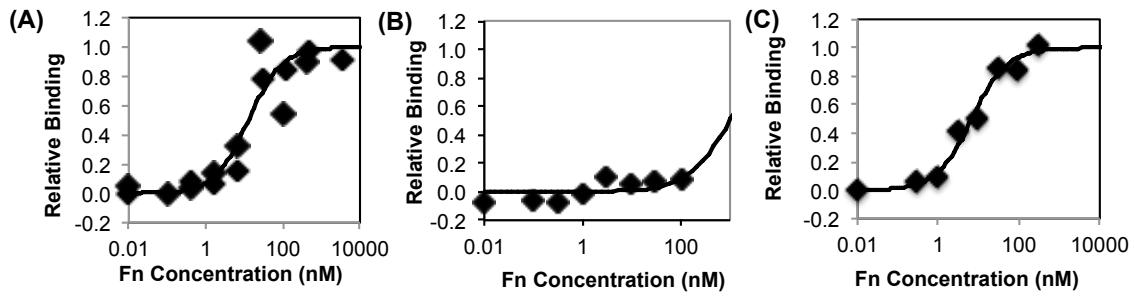
Supplemental

Supplemental Table 1. Charge mutant analysis. Mutants of A' were produced in BL21(DE3), purified, and analyzed by RP-HPLC using a 10-90% acetonitrile gradient over 30 min. The yield and retention time shift, both relative to A', are indicated.

Mutant	Yield	Δt_R (min.)
D3S	59%	0.0
R6N	326%	0.4
D7T	114%	0.0
E9Q	65%	-0.4
E9S	45%	0.0
R33Q	<10%	-
R33N	<10%	-
R33S	<10%	-
E38P	90%	-0.1
E38S	<10%	-
E47Q	48%	-0.1
E47T	78%	0.2
N63K	244%	-0.3
D67Q	67%	-0.2
D67T	64%	0.2
R93T	114%	0.0



Supplemental Figure 1. Published studies of the impact of hydrophobicity on renal and hepatic uptake. (A) Protein data for 99m Tc-labeled neoalbumin and 99m Tc-labeled Fab fragments with different coligands at 1 h post-injection(1). (B) Small molecule data for 124 I-labeled cyclin-dependent kinase inhibitors at 1 h post-injection(2) and 99m Tc-labeled metronidazole derivatives at 4 h post-injeciton(3). (C) Peptide data for glycosylation variants of 125 I-labeled octreotide at 0.5 h post-injection(4), and 18 F-labeled cyclic RGD peptide variants at 2 h post-injection(5), and 99m Tc-labeled cyclic RGD peptide with different coligands at 1 h post-injection(6).



Supplemental Figure 2. Affinity titrations. Cells were labeled at the indicated concentrations with His₆-tagged fibronectin domains and fluorescein-conjugated anti-His₆ antibody and were analyzed by flow cytometry. (A) Balb3T3 murine fibroblasts labeled with DOTA-conjugated A', (B) A431 human epidermoid carcinoma labeled with A' with a scrambled four amino acid segment in the FG loop, and (C) A431 human epidermoid carcinoma labeled with DOTA-conjugated D' R54A .

Supplemental References

1. Ono M, Arano Y, Mukai T, et al. Control of radioactivity pharmacokinetics of ^{99m}Tc -HYNIC-labeled polypeptides derivatized with ternary ligand complexes. *Bioconjug Chem* 2002; 13: 491-501.
2. Koehler L, Graf F, Bergmann R, Steinbach J, Pietzsch J, Wuest F. Radiosynthesis and radiopharmacological evaluation of cyclin-dependent kinase 4 (Cdk4) inhibitors. *Eur J Med Chem* 2010; 45: 727-37.
3. Giglio J, Fernández S, Rey A, Cerecetto H. Synthesis and biological characterisation of novel dithiocarbamate containing 5-nitroimidazole (^{99m}Tc)-complexes as potential agents for targeting hypoxia. *Bioorg Med Chem Lett* 2011; 21: 394-7.
4. Schottelius M, Wester H-J, Reubi JC, Senekowitsch-Schmidtke R, Schwaiger M. Improvement of pharmacokinetics of radioiodinated Tyr(3)-octreotide by conjugation with carbohydrates. *Bioconjug Chem* 2002; 13: 1021-30.
5. Glaser M, Morrison M, Solbakken M, et al. Radiosynthesis and biodistribution of cyclic RGD peptides conjugated with novel [^{18}F]fluorinated aldehyde-containing prosthetic groups. *Bioconjug Chem* 2008; 19: 951-7.
6. Kunstler J-U, Seidel G, Bergmann R, et al. Novel ^{99m}Tc '4 + 1' peptide conjugates: tuning the biodistribution by variation of coligands. *Eur J Med Chem* 2010; 45: 3645-55.