Electronic Supporting Information

Cell-compatible, integrin-targeted cryptophane-^{129}Xe NMR biosensors

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TABLE OF CONTENTS

Reagents

Organic reagents and solvents were used as purchased from the following commercial sources: *Sigma-Aldrich*, dimethyl sulfoxide (DMSO), methanol, 2,6-lutidine, piperidine, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT); *Fisher*, dichloromethane, potassium carbonate, sodium chloride, copper(II) sulfate; *Anaspec*, cyclo-Arg-Gly-Asp-D-Tyr-Lys (c[RGDyK]), cyclo-Arg-Ala-Asp-D-Phe-Lys (c[RADfK]); *Invitrogen*, RPMI medium 1640, Dulbecco's phosphate buffered saline (DPBS), anti-CD51 mouse antibody, Alexa Fluor 488 azide; *MP Biomedicals*, fibronectin, fibrinogen, vitronectin; *Pierce*, NeutrAvidin-HRP; *KPL, Inc.*, tetramethyl benzidine (TMB) substrate, milk solution; *Thermofisher*, EZlink biotin; *Acros*, trifluoromethanesulfonic anhydride, sodium ascorbate; *Pierce*, Ellman's reagent; *EMD Biosciences*, human integrin receptor $\alpha_{IIb}\beta_3$ (platelet membrane glycoprotein IIb/IIIa); Millipore, human integrin receptor $\alpha_v\beta_3$, human integrin receptor $\alpha_v\beta_5$. For biological assays, all solutions were prepared using deionized water (resistivity = 18 megaohm-cm) purified by Mar Cor Premium Grade Mixed Bed Service Deionization.

General Characterization Methods

All air- and moisture-sensitive reactions were performed under dinitrogen with glassware oven-dried and then flamed under partial vacuum. HPLC analysis was performed on a Varian Prostar 215 binary system equipped with a Varian Prostar 325 UV-vis Detector. HPLC purification was done on a Zorbax Rx-C8 semi-preparative (9.4 × 250 mm, 5 microns) or analytical column (4.6 × 150 mm, 5 microns). The gradient eluent was composed of two solvents: 0.1% aqueous TFA (solvent A) and a 0.1% solution of TFA in CH_3CN (solvent B). Mass identification of all peptide-containing compounds was performed on a Bruker Ultraflex MALDI-TOF mass spectrometer. The 96 well fluorescence and absorbance measurements were made using a Perkin-Elmer 2102 Envision Multilabel Microplate Reader. Flow cytometry measurements were taken on a BD FACSCalibur flow cytometer using 488 nm excitation and a 530 nm emission filter.

Synthesis of Azido-Lys c[RGDyK] Peptide 2

The c[RGDyK] peptide (25.0 mg, 0.0400 mmol) was dissolved in deionized H_2O (5 mL). Potassium carbonate (8.4 mg, 0.060 mmol, 1.5 equiv) and copper(II) sulfate (0.6 mg, 0.004 mmol, 0.1 equiv) were added and stirred until dissolution. The reaction vessel was then sealed by septum and placed under N_2 flow. Triflyl azide (3 mmol scale, roughly 0.5 g) was synthesized according to a previously published protocol,[1] isolated by extraction with dichloromethane and added directly by syringe to the peptide solution. The reaction vessel was placed in ice bath with stirring. Triflyl azide is barely soluble in water, thus methanol (5 mL) was added by syringe until homogeneous solution was achieved. After overnight reaction, the excess reactants were removed by three consecutive additions of 5 mL dichloromethane. The azido-Lys peptide product was isolated in the aqueous layer and dried under high vacuum. No further purification was required, as shown by HPLC trace (see Supporting Information, Figure S1). MALDI MS ($C_{27}H_{40}N_{11}O_8$) [$M+H^+$]: calcd, 646.67; found 646.86.

Cell Culture

HFL-1 human diploid lung fibroblasts and AsPC-1 human pancreatic carcinoma cell lines were obtained from the Cell Culture Core of the Center for Molecular Studies in Liver and Digestive Diseases (University of Pennsylvania Medical School, Philadelphia, PA). NCI-H1975 human lung carcinoma cells were from Dr. Intae Lee (UPenn). All cells were grown in 25 cm^2 tissue culture flasks in RPMI-1640 with 25 mM HEPES supplemented with 2 mM L-glutamine, 15% fetal calf serum, 100 units/mL of penicillin and 100 units/mL of streptomycin. Cells were subcultured weekly, or more frequently, as needed.

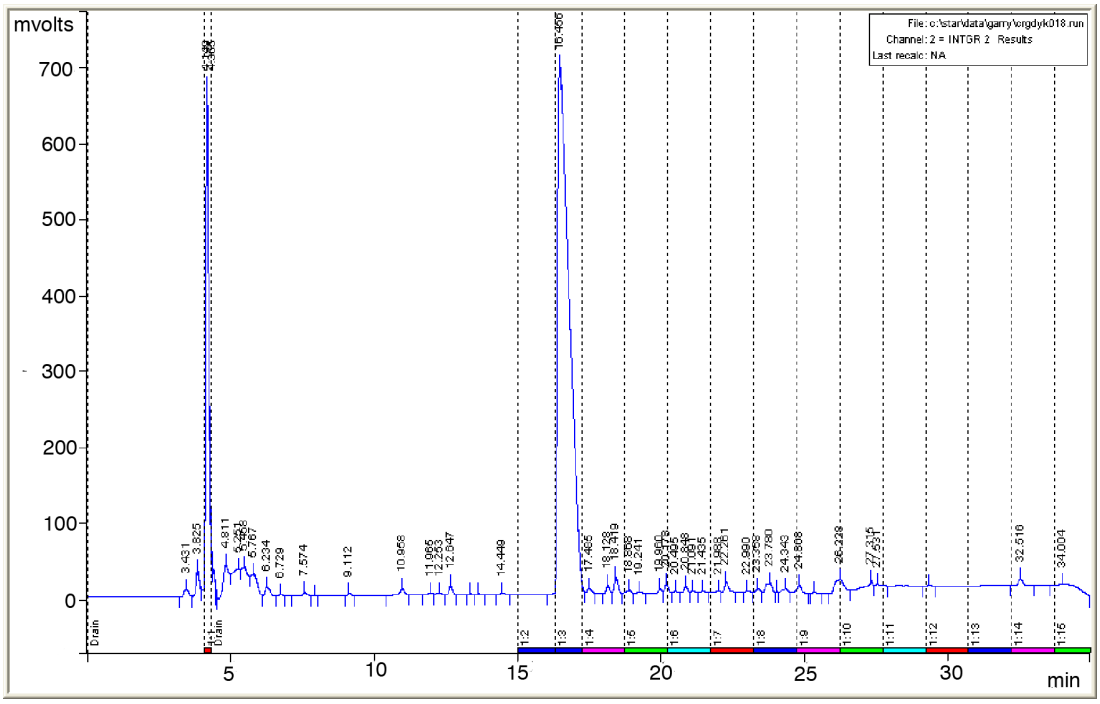

Fig. S1 HPLC trace of azido peptide c[RGDyK] **2**. Column: Zorbax Rx-C8, 80 Å, 5 µm, 9.4 mm x 250 mm (Agilent); flow rate: 3 mL/min; injected volume: 1 mL; wavelength detection, 215 nm; gradient: 0 to 25% buffer B. Buffer A: H_2O, 0.1% TFA. Buffer B: acetonitrile, 0.1% TFA. Retention time: 16.46 min.

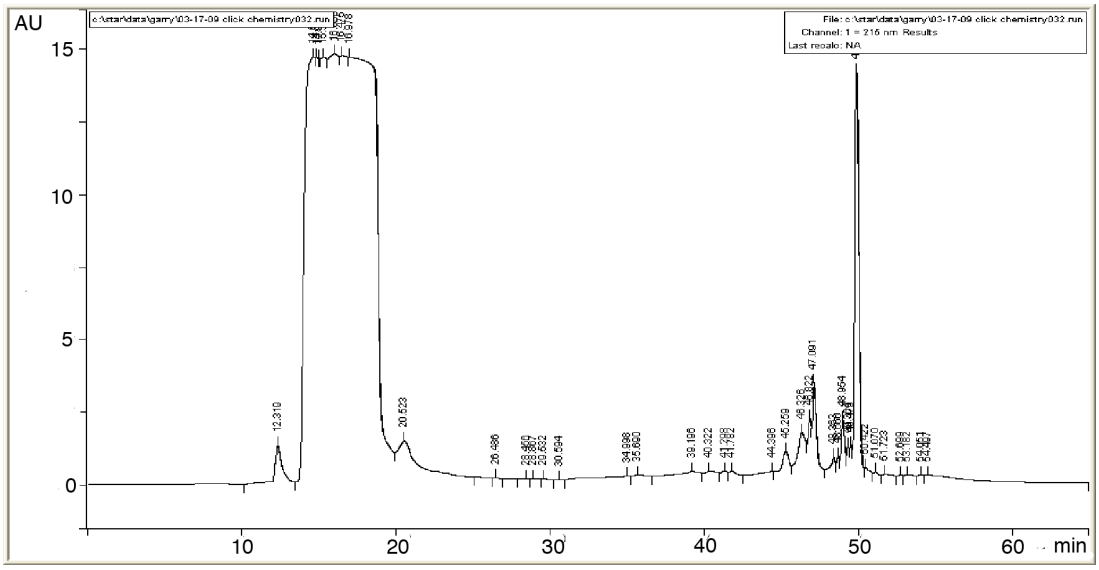

Fig. S2 HPLC trace of biosensor **3**. Column: Zorbax Rx-C8, 80 Å, 5 µm, 9.4 mm x 250 mm (Agilent); flow rate: 3 mL/min; injected volume: 1 mL; wavelength detection, 215 nm; gradient: 15 to 60% buffer B in 45 min, 50-80% in 2 min, 80% for 10 min. Buffer A: H_2O, 0.1% TFA. Buffer B: acetonitrile, 0.1% TFA. Retention time: 49.98 min.

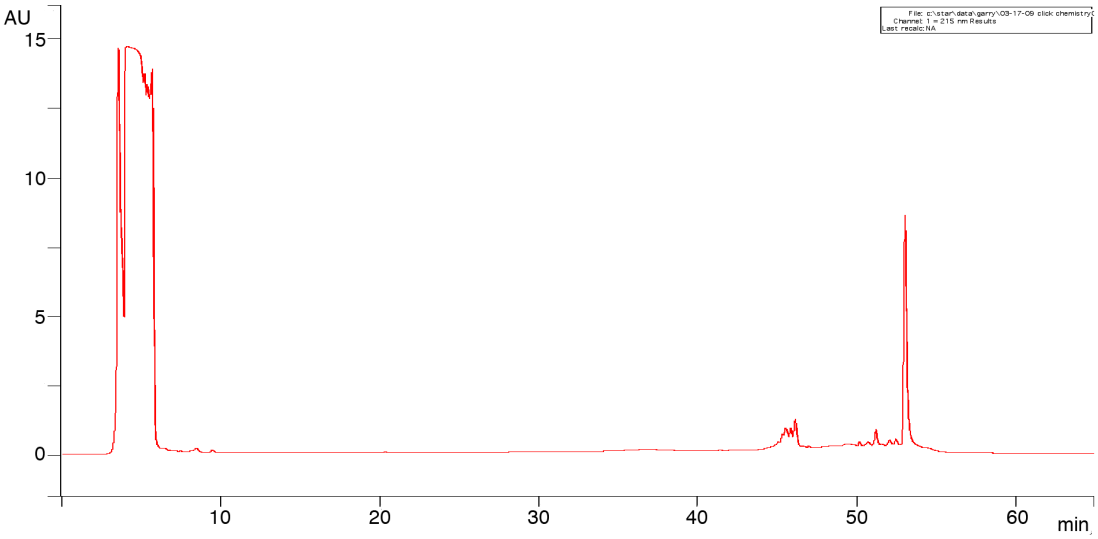

Fig. S3 HPLC trace of biosensor **4**. Column: Zorbax Rx-C8, 80 Å, 5 µm, 9.4 mm x 250 mm (Agilent); flow rate: 3 mL/min; injected volume: 1 mL; wavelength detection, 215 nm; gradient: 15 to 60% buffer B in 45 min, 60-80% in 2 min, 80% for 10 min. Buffer A: H_2O, 0.1% TFA. Buffer B: acetonitrile, 0.1% TFA. Retention time: 53.07 min.

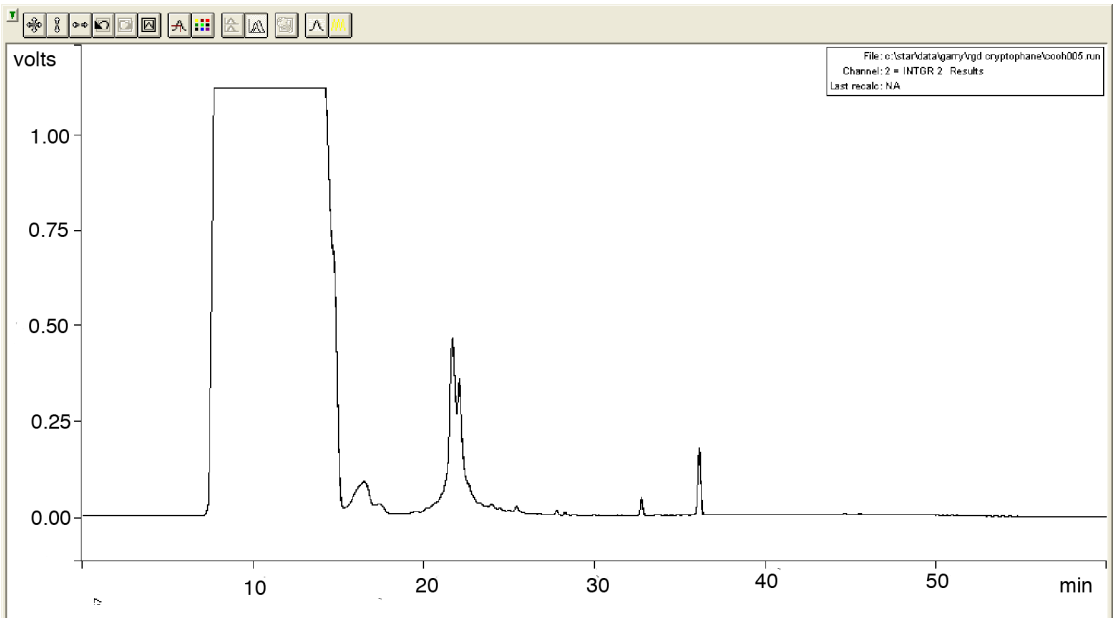

Fig. S4 HPLC trace of biosensor **5**. Column: Zorbax Rx-C8, 80 Å, 5 µm, 9.4 mm x 250 mm (Agilent); flow rate: 3 mL/min; injected volume: 1 mL; wavelength detection, 215 nm; gradient: 10% buffer B for 5 min, 10-50% buffer B in 40 min. 5-80% buffer B in 2 min, 80% buffer B for 10 min. Buffer A: H_2O, 0.1% TFA. Buffer B: acetonitrile, 0.1% TFA. Retention time: 22.76 min.

Table S1 Mean fluorescence intensities from flow cytometry assays, which measured uptake of fluorescently-labeled biosensor **4** in HFL-1 and AsPC-1 cell lines.

Cells	No treatment	Antibody block	RGDyK block	RADfK block	Biosensor **4**
AsPC-1	5	7	21	1800	2100
HFL-1	3	4	6	300	600

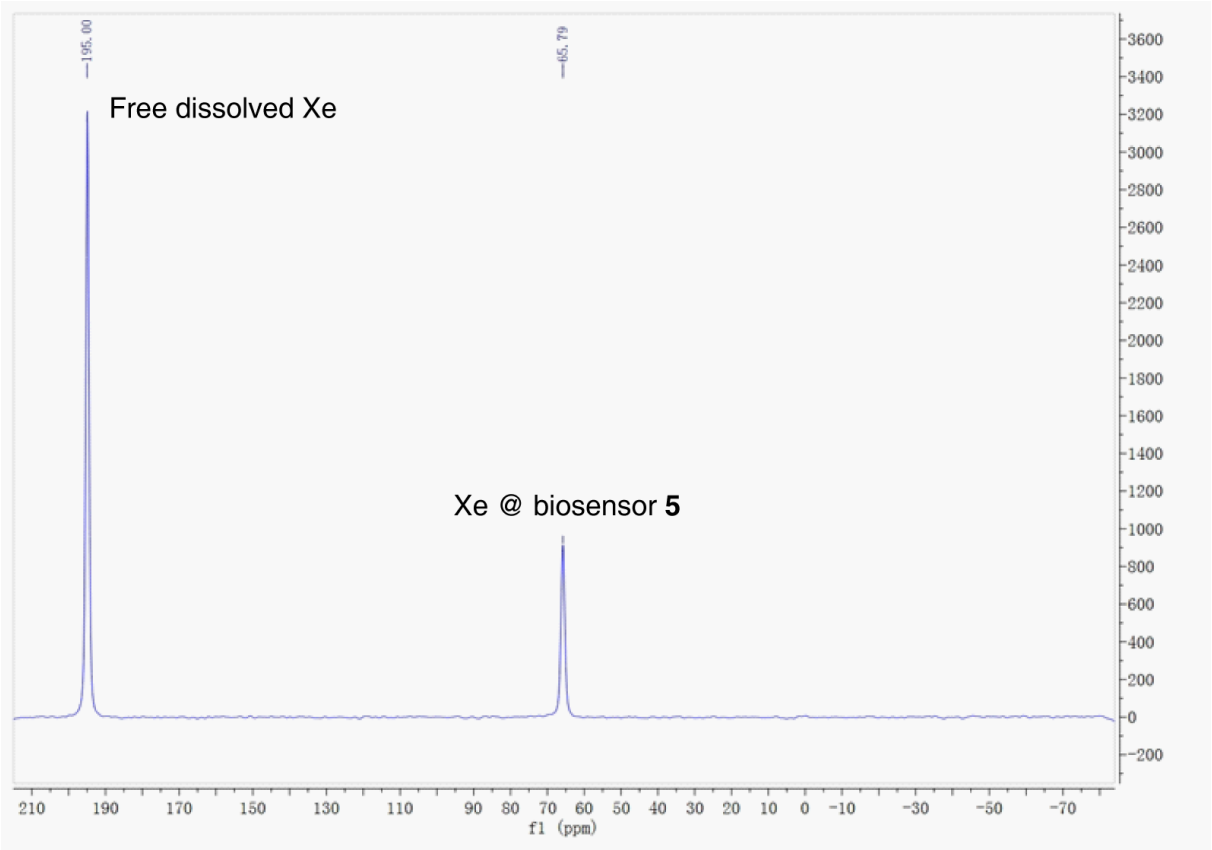

Fig. S5 Hyperpolarized ^{129}Xe NMR spectrum of biosensor **5** (100 μM in 1 mM Tris buffer, pH 7.2). The spectrum is calibrated by the solvent ^{129}Xe NMR peak at 195 ppm. Sample temperature was controlled by VT unit on NMR spectrometer to 27 ± 1 °C.

Cited Reference

1. P. B. Alper, S. C. Hung and C. H. Wong, *Tetrahedron Lett.*, 1996, **37**, 6029-6032.