Supporting Information

General Procedures for Peptide Synthesis, Purification and Characterization:

Monomeric peptide synthesis was performed on a Symphony[™] Synthesizer (Rainin Instruments, Protein Technologies, Inc. Woburn, MA) by Fmoc solid-phase peptide synthesis on a Rink Amide AM resin (substitution level 0.71 mmol/g) using standard protocols. Fmoc-protected amino acids were coupled at a 5fold excess using HBTU, HOBt and NMM coupling (45 min). Piperidine in DMF (20%) was employed to remove N-terminal Fmoc protecting groups. Fmoc-NH-(PEG)₁₁-COOH (C₄₂H₆₅NO₁₆, Polypure, Oslo, Norway) was coupled in the same fashion. Upon completion of the synthesis, peptides were cleaved from the resin using a TFA: triisopropylsilane: H₂O cocktail (95%:2.5%:2.5%) and precipitated in cold diethyl ether.

Crude peptides were purified by reverse phase HPLC using a Vydac PR-C18 column (250mm ×22mm, 10 μ m) on a BreezeTM HPLC (Water Inc.) with eluents of H₂O/0.1% TFA (eluent A) and acetonitrile/0.1%TFA (eluent B). The following elution profile (referred to as Method A) was utilized: 0-1 minute, 90%A,10%B; 1-61 minutes, eluent B was increased from 10-40% at a flow rate of 10 mL /minute. Elution of the peptides was monitored by UV absorbance at 220 nM.

The purified peptides were analyzed by RP-HPLC (Varian, PR-C18 column, 250mm ×4.6mm, 5 µm) using the following elution profile (Method B): 0-1 minute, 90%A, 10%B; 1-51 minutes, eluent B was increased from 10-60% at a flow rate of 1 mL /minute. The purity of the peptides was determined to be greater than 95% by HPLC (Figures 1SB-3SB). The peptide mass was confirmed by matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI MS) in reflector mode using a Voyager DETM Pro instrument (Applied Biosystems Inc.) using α -cyano-4-hydroxycinnamic acid as the matrix.

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Table 1S. H2009 Peptides Synthesized for Cell Binding Experiments						
Peptide Sequence ^a	Elemental	Expected	MH+	Purity ^b		
			2054 22	059/		
RGDLATLRQLAQEDGVVGVR(PEG)11C	C ₁₂₀ П ₂₁₆ N ₃₄ O ₄₃ S ₁	2003.00	2004.20	95%		
		1843 02	1844 18	08%		
NODEATERQE(I EO)HO	078111461 2002801	1040.02	1044.10	3070		
RGDLATL(PEG)11C	$C_{61}H_{115}N_{13}O_{24}S_1$	1445.78	1446.73	96%		
	Table 1S. H2009 Peptides S Peptide Sequence ^a RGDLATLRQLAQEDGVVGVR(PEG) ₁₁ C RGDLATLRQL(PEG) ₁₁ C RGDLATL(PEG) ₁₁ C	Table 1S. H2009 Peptides Synthesized for (Peptide Sequence*Peptide Sequence*Elemental CompositionRGDLATLRQLAQEDGVVGVR(PEG)11CC120H216N34O43S1RGDLATLRQL(PEG)11CC78H146N20O28S1RGDLATL(PEG)11CC61H115N13O24S1	Table 1S. H2009 Peptides Synthesized for Cell Binding Experi Peptide Sequence ^a Elemental Composition Expected Monoisotopic Mass RGDLATLRQLAQEDGVVGVR(PEG)11C C120H216N34O43S1 2853.55 RGDLATLRQL(PEG)11C C78H146N20O28S1 1843.02 RGDLATL(PEG)11C C61H115N13O24S1 1445.78	Table 1S. H2009 Peptides Synthesized for Cell Binding Experiments Peptide Sequence ^a Elemental Composition Expected Monoisotopic Mass MH+ RGDLATLRQLAQEDGVVGVR(PEG)11C C120H216N34O43S1 2853.55 2854.23 RGDLATLRQL(PEG)11C C78H146N20O28S1 1843.02 1844.18 RGDLATL(PEG)11C C61H115N13O24S1 1445.78 1446.73		

^aPeptide sequences are given in single letter code in standard N-terminal to C-terminal notation. The Fmoc-NH-(PEG)₁₁-COOH (C₄₂H₆₅NO₁₆) was purchased from Polypure (Oslo, Norway). All peptides were synthesized as C-terminal amides. ^bAs determined by analytical RP HPLC monitoring at 220 nM.



A. Preparative HPLC of peptide

B. Analytical HPLC of peptide

C. MALDI MS of peptide.

Figure 1S:Purification and Characterization of Peptide 1, RGDLATLRQLAQEDGVVGVR(PEG)11C



A. Preparative HPLC of peptide

B. Analytical HPLC of peptide

C. MALDI MS of peptide.





Figure 3S:Purification and Characterization of Peptide 3, RGDLATL (PEG)₁₁C

Table 2S. H1299 peptides synthesized for cell binding experiments

Compound Name	Peptide Sequence ^a	Elemental Composition	Expected Monoisotopic Mass	MH+	Purity⁵
Compound 4	VSQTMRQTAVPLLWFWTGSL(PEG) ₁₁ C	$C_{78}H_{146}N_{20}O_{28}S_1$	3021.56	3022.10	98%
Compound 5	YAAWPASGAWTGTAPC(Acm)SAGT(PEG)11C	$C_{119}H_{183}N_{26}O_{41}S_2$	2696.23 ^c	2719.72	98%

^aAll peptides were synthesized as C-terminal amides. ^bAs determined by analytical RP HPLC monitoring at 220 nM. ^c M Na+ adduct was observed.



A. Preparative HPLC of peptide

B. Analytical HPLC of peptide

C. MALDI MS of peptide.

Figure 4S : Purification and Characterization of Peptide H1299.1, compound 4



Figure 5S : Purification and Characterization of Peptide H1299.2(Acm), compound 5

The deletion peptides used to map the critical binding domain were synthesized as above. The PEG linker was not included and all peptides were synthesized a C-terminal amides. The characteristics of these peptides are listed in Table 3S.

Table 3S. H2009 Monomeric Deletion Peptide Synthesized for Cell Binding Experiments

Peptide Name	Peptide Sequence	Elemental Composition	Expected Monoisotopic Mass	MH+	Purity
H2009.1 10-mer	RGDLATLRQL-NH ₂	$C_{48}H_{88}N_{18}O_{14}$	1140.67	1141.35	98%
H2009.1 9-mer	RGDLATLRQ-NH ₂	$C_{42}H_{77}N_{17}O_{13}$	1027.59	1028.96	98%
H2009.1 8-mer	RGDLATLR-NH ₂	$C_{37}H_{69}N_{15}O_{11}$	899.53	900.65	98%
H2009.1 7-mer	RGDLATL-NH ₂	$C_{31}H_{57}N_{11}O_{10}$	743.43	745.02	98%
H2009.1 6-mer	RGDLAT-NH₂	$C_{25}H_{46}N_{10}O_9$	630.34	631.48	98%

Synthesis, Purification, and Characterization of Maleimido Activated Cores (compounds 6-10):

The maleimido tetrameric core was synthesized on $Fmoc_4$ -Lys₂-Lys- β -Ala-CLEARTM Acid Resin, $Fmoc_4$ -Lys₂-Lys-Lys(Biotin-PEG)- β -Ala-CLEARTM Acid Resin and $Fmoc_4$ -Lys₂-Lys-Cys(Acm)- β -Ala-CLEARTM Acid Resin respectively as described in the text. The compounds were purified by reverse phase HPLC using method A and characterized by analytical HPLC (method B). MALDI MS were obtained using α -cyano-4-hydroxycinnamic acid as the matrix. The structure and characterization of the compounds are shown in Figure 6S and Table 4S.

The maleimido dimeric core was obtained by Fmoc solid-phase peptide synthesis on a Rink Amide AM resin (substitution level 0.71 mmol/g) using standard protocols. Fmoc- β -Ala-OH was coupled at a 5-fold excess using HBTU, HOBt and NMM coupling (45 min). Piperidine in DMF (20%) was employed to remove N-terminal Fmoc protecting groups. Fmoc-Lys(Fmoc)-OH and 3-maleimidopropionic acid was coupled in the same fashion. Upon completion of the synthesis, the maleimido dimeric core was cleaved from the resin, purified by reverse phase HPLC using method A and characterized by analytical HPLC (method B). MALDI MS were obtained using α -cyano-4-hydroxycinnamic acid as the matrix.

The maleimido trimeric core was synthesized on Fmoc-β-Ala-CLEAR[™] Acid Resin, (substitution level 0.52 mmol/g) using standard protocols. Fmoc-Lys(Mtt)-OH was coupled at a 5-fold excess using HBTU, HOBt and NMM coupling (45 min). Piperidine in DMF (20%) was employed to remove N-terminal Fmoc protecting groups. Fmoc-Lys(Fmoc)-OH was coupled in the same fashion. The Mtt protecting group was then removed by treating

the resin with 1%TFA in DCM for 2 min, 5 times¹. After thoroughly washing the resin with DCM and DMF, 3maleimidopropionic acid was coupled using standard protocols. Upon completion of the synthesis, the maleimido trimeric core was cleaved from the resin, purified by reverse phase HPLC using method A and characterized by analytical HPLC (method B). MALDI MS were obtained using α -cyano-4-hydroxycinnamic acid as the matrix.

Table 45. Characteristics of Maleinindo Activated Cores					
Compound Name	Elemental Composition	Expected Mass (monoisotopic)	MNa ^{+ a}	Purity ^b	
Compound 6	$C_{49}H_{63}N_{11}O_{17}$	1077.44	1099.83	96%	
Compound 7	$C_{71}H_{100}N_{16}O_{23}S_1$	1576.69	1599.79	95%	
Compound 8	$C_{55}H_{73}N_{13}O_{19}S_1$	1251.49	1274.27	95%	
Compound 9	$C_{23}H_{30}N_6O_8$	518.21	541.28	98%	
Compound 10	$C_{36}H_{46}N_8O_{13}\\$	798.32	821.30	96%	

 Table 4S. Characteristics of Maleimido Activated Cores

^aThe sodium adduct, not the MH+, was the observed parent ion for compounds 3-6. ^bAs determined by analytical RP HPLC monitoring at 220 nM.

Synthesis of Compound 11: 8µmol of compound **1** and 1µmol of compound **6** was dissolved in 1.5 mL of Arpurged 1 × PBS/0.01M EDTA. The reaction mixture was stirred at RT for 2 hrs (Scheme I). The products were then purified with PR-HPLC using the Method A. The purified peptide was analyzed by analytical HPLC using the Method B. MALDI Mass Spectra was obtained in linear mode using sinapinic acid as matrix. HPLC and MALDI MS data are shown in (Table 5S and Figure 7S). The purities are based on analytical HPLC data monitoring elution at 220nM. Surprisingly, while only tetrameric peptide is observed by analytical HPLC, some monomeric peptide is observed by MALDI MS. This may be trace amounts of material that is not observed by HPLC but is detected by MS. However, it should be noted that MALDI MS is not quantitative, and the intensity ratios of tetrameric product and monomeric peptide cannot be used as a measure of peptide purity. This is especially true as the monomeric peptide is expected to ionize better than the branched tetrameric version. Alternatively, we cannot rule out the possibility that the peptide conjugate is unstable under the MS conditions.

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Compound 6

Compound 7







Compound 9

Compound 10

Figure 6S: Structure of Activated Maleimido Cores, Compounds 6-10



Scheme 1. Convergent Synthesis of Tetrameric Peptides

Compound Name	Elemental Composition	Expected Mass (average)	MH⁺ (average)	Purity ^a
Compound 11	$C_{529}H_{927}N_{147}O_{189}S_4$	12499.15	12497.91	95%
Compound 12	$C_{361}H_{647}N_{91}O_{129}S_4$	8454.79	8451.98	98%
Compound 13	$C_{179}H_{322}N_{46}O_{64}S_2$	4206.87	4205.42	98%
Compound 14	$C_{383}H_{684}N_{96}O_{135}S_5$	8954.42	8952.21	95%
Compound 15	$C_{367}H_{657}N_{93}O_{131}S_5$	8629.01	8626.77	95%
Compound 16	$C_{364}H_{652}N_{92}O_{130}S_5$	8557.94	8556.88	98%
Compound 17	$C_{270}H_{484}N_{68}O_{97}S_3$	6331.32	6334.04	95%
Compound 18	$C_{299}H_{533}N_{65}O_{115}S_5$	7039.12	7040.81	95%

Table 5S: Characteristics of Multimeric H2009 Peptides

^aAs determined by analytical RP HPLC monitoring at 220 nM.



f1: monomer; f2: tetramer

B. Analytical HPLC of peptide

C. MALDI MS of peptide Note: both singly charged and doubly

charged parent ions are observed.

6249.63

12497.91

9649 35

Figure 7S: Purification and Characterization of Compound 11

Synthesis of compounds 12-15 and 17-18: The synthesis of compounds 12-15 and 17-18 is similar to that of

compound **11**, but that the following combinations of activated lysine cores and peptides were used:

Compound 2 + Compound 6 = Compound 12 Compound 2 + Compound 9 = Compound 13 Compound 2 + Compound 7 = Compound 14 Compound 2 + Compound 8 = Compound 15 Compound 2 + Compound 10 = Compound 17 Compound 3 + Compound 8 = Compound 18

The structures of the compounds 11-18 are shown in Figure 8S. Characteristics of the peptides are given in

Table 5S and Figures 7S and 9S-15S.

Synthesis of Compound 16: The acetamidomethyl group of the cysteine was removed according to published procedure ². Specifically, 1 µmol of compound **15** was dissolved in 1 mL of TFA/anisole (99:1) to which 28 mg (167 µmol) of silver acetate was added. The solution was stirred for 2 hrs at 4°C and then concentrated under Argon to 0.5 mL. The peptide was precipitated by the addition of 5 mL of cold ether and the precipitate was collected by centrifugation. The precipitate was then treated with 1 mL of dithiothreitol (0.2 M) in 1 M acetic acid at RT for 3 hrs. The solution was filtered and purified by HPLC (Method A). HPLC traces and MALDI MS are shown in figure 13S.



Compound 11: Peptide Sequence H2009.1 RGDLATLRQLAQEDGVVGVR Compound 12: Peptide Sequence H2009.1 RGDLATLRQL







Compound 13: Peptide Sequence H2009.1 RGDLATLRQL



Compound 17: Peptide Sequence H2009.1 RGDLATLRQL



Compound 15: Peptide Sequence H2009.1 RGDLATLRQL Compound 18: Peptide Sequence H2009.1 RGDLATL

Figure 8S: Chemical Structures of Compounds 11-15 and 17-18.



Note: both singly charged and doubly charged parent ions are observed.

Figure 9S: Purification and Characterization of Compound 12



A. Preparative HPLC of peptide f1: monomer; f2: dimer

f1: monomer; f2: tetramer

B. Analytical HPLC of peptide

C. MALDI MS of peptide

Figure 10S:Purification and Characterization of Compound 13



Figure 11S:Purification and Characterization of Compound 14

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A. Preparative HPLC of peptide f1: monomer; f2: tetramer



C. MALDI MS of peptide Note: both singly charged and doubly charged parent ions are observed.







A. Preparative HPLC of peptide

B. Analytical HPLC of peptide

C. MALDI MS of peptide Note: both singly charged and doubly charged parent ions are observed.

Figure 13S: Purification and Characterization of Compound 16





B. Analytical HPLC of peptide

C. MALDI MS of peptide



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B. Analytical HPLC of peptide

C. MALDI MS of peptide

Figure 15S : Purification and Characterization of Compound 18

Synthesis of the Tetrameric 10-mer H2009.1 Peptide by Linear SPPS, Compounds 19-20

Synthesis of the tetrameric 10-mer H2009.1 peptide (compound **19**) by linear synthesis was performed on a Symphony[™] Synthesizer (Rainin Instruments, Protein Technologies, Inc. Woburn, MA) by Fmoc solid-phase peptide synthesis on a Fmoc₄-Lys₂-Lys-Cys(Acm)-β-Ala-CLEAR[™] Acid Resin (substitution level 0.21 mmol/g) using standard protocols. Fmoc-protected amino acids were doubly coupled at a 5-fold excess using HBTU, HOBt and NMM coupling (60 min). Piperidine in DMF (20%) was employed to remove N-terminal Fmoc protecting groups. A single coupling of Fmoc-NH-(PEG)₁₁-COOH was performed overnight. Upon completion of the synthesis, peptide was cleaved from the resin, purified by reverse phase HPLC using method A and characterized by analytical HPLC (method B). MALDI MS were obtained in linear mode using sinapinic acid as matrix. Removal of the Acm protecting group from the cysteine to reveal a free thiol (compound **20**) was performed in the same manner as described above for the synthesis of compound **16**.

Table 6S: Characteristics of Tetrameric H2009 Peptides Synthesized by Linear Fmoc SPPS

Compound Name	Elemental Composition	Expected Mass (average)	MH⁺ (average)	Purity
Compound 19	$C_{327}H_{606}N_{82}O_{114}S_1$	7542.86	7543.03	95%
Compound 20	$C_{324}H_{601}N_{81}O_{113}S_1$	7471.78	7473.63	95%

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A. Preparative HPLC of peptide B. Analytical HPLC of peptide

C. MALDI MS of peptide.

Figure 16S :Purification and Characterization of H2009.1 10-mer tetramer linear synthesis(Acm), compound 19



A. Preparative HPLC of peptide

B. Analytical HPLC of peptide

C. MALDI MS of peptide.

Figure 17S :Purification and Characterization of H2009.1 10-mer tetramer linear synthesis after removal of the Acm protecting group, Compound 20



Figure 18S. The H2009.1 Peptide can be Truncated. Four alanine scanning derivatives of the H2009.1 peptide were synthesized. The ability of the peptides to block uptake of the H2009.1 phage on H2009 cells was determined. Values are normalized to the output/input ratio with no peptide included. **Peptide Blocking Experiments**: Peptide solutions were prepared in phosphate buffered saline, pH7.4 and concentrations were confirmed by Edman sequencing. The ability of the peptides to block their cognate phage was determined as previously described.^{3,4} Peptide and phage clones were added to the cells simultaneously. No prior incubation of the peptide with the target cells was performed. Phage uptake was determined by standard bacterial titering. **Cellular uptake of the peptide-Qdot conjugate**: The biotin labeled tetrameric peptide (compound 14, 4000 nM) was incubated with Streptavidin-Qdot 605 made in house (SAQdot605, 200 nM) for 2 hrs at RT. The peptide-SAQdot605 conjugate solution were diluted with PBS⁺/0.1%BSA to a final Qdot concentration of 20 nM before overlaying onto cells as previously described.³ A sequence scrambled control peptide was used to assure that the uptake was specific (sequence: DALRLQGTLR). H2009.1 scrambled-10-mer tetrameric peptide-on biotin containing trilysine core (C₃₈₃H₆₈₄N₉₆O₁₃₅S₅): expected mass (average): 8954.42; MH+(average): 9021; Purity: 95%. Fluorescent microscopy images are shown in Figure 19S and 20S.



Figure 19S: The Tetrameric 10-mer H2009.1 Peptide Mediates Cellular Uptake of a Qdot in the Human Lung Adenocarcinoma Cell Line, H2009. A: The peptide was conjugated to the Qdot via a streptavidin-biotin interaction. Cells were incubated with 20 nM SAQdot605 conjugated to the tetrameric H2009.1 10-mer peptide and visualized with 400-fold magnification using a fluorescent microscope. B: A scrambled H2009.1 peptide was conjugated to the Qdots as described above. No uptake of the fluorescent Qdots was observed. Overlay of the DAPI nuclear stain is shown so that cells can be observed. **C**: Cells were incubated with 20 nM SAQdot605 that had been quenched with a 20X excess of free biotin prior to incubating with H2009 cells. No accumulation of Qdots is observed confirming that the uptake of the fluorescent nanoparticle is mediated by the cell binding peptide. **D:** The corresponding nuclear staining with Hoechst 33342 confirms the presence of cells in the microscopic field shown in panel C.



Figure 20S: Cellular uptake of the peptide-Qdot conjugate: The biotin labeled tetrameric peptide (4000 nM) was incubated with Streptavidin-Qdot 605 made in house (SAQdot605, 200 nM) for 2 h at RT. The peptide-SAQdot605 conjugate solution were diluted with PBS⁺/0.1%BSA to a final Qdot concentration of 20 nM before overlaying onto cells as previously described.³ Cells were incubated with peptide-labeled Qdots for 10 minutes at 37°C before washing 4 times with PBS⁺/0.1%BSA and once with pH2.2 glycine buffer at room temperature. Cells were washed 2 times with PBS⁺/0.1%BSA to remove acid. Vectashield containing nuclear stain was added before sealing the coverslip and visualization on the microscope. A sequence scrambled control peptide-labeled Qdot was used to assure that the uptake was specific. Images were captured on a Leica TCS SP5 confocal microscope at 400x magnification with 7x optical zoom. Images are presented as an overlay series of 26 frames from the Z-stack acquisition as well as an individual slice from the middle of the stack. Similar data was collected from distinct areas of the slide.

Synthesis of Tetrameric Peptide- Alexa Fluor 488 C₅-Maleimide Dye Conjugates, H2009.1 10-mer

tetrameric peptide-dye and H2009.1 10-mer scrambled peptide-dye:

A solution of 0.015 µmol H2009.1 tetrameric-SH peptide (compound 16) was prepared in 500 µL of Ar-purged

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Confocal Microscopy Analysis: H2009 cells were incubated for 10 minutes at 37°C with 1 µM peptide-dye conjugate prior to washing and microscopic observation as detailed for Qdots. The scrambled sequence peptide-dye conjugate was used as a control. Z-stack images are shown in Figure 21S.



Figure 21S: Cellular uptake of H2009.1 10mer tetramer-Alexa Fluor 488 conjugate. H2009 cells were incubated for 10 minutes at 37°C with 1 µM peptide-dye conjugate prior to washing and microscopic observation as detailed for Qdots. Images were captured on a Leica TCS SP5 confocal microscope at 400x magnification with 7x optical zoom. Images are presented as an overlay series of 18 frames from the Z-stack acquisition. Similar data was collected from distinct areas of the slide.

Prediction of Helical Content of the Monomeric H2009.1 peptides

The helical propensity of the H2009.1 20-mer (compound 1) 10-mer (compound 2) and 7-mer (compound 3) was determined using AGADIR, a freely available web-based program from to predict helical propensity of peptides at the amino acid level (software update August 6th 2003, http://www.embl-

heidelberg.de/Services/serrano/agadir/agadir-start.html). The helical propensity of the peptides is shown in Figure 22S.



Amino Acid Residue

Figure 22S: Helical propensity of the H2009.1 monomeric peptide increases with length.

References:

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