Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Dual-Receptor Targeted Strategy in Nanoparticle Design Achieves Tumor Cell Selectivity Through Cooperativity

Jared F. Stefanick[†], David Omstead[†], Tanyel Kiziltepe[†‡§], and Basar Bilgicer[†‡§‖*]

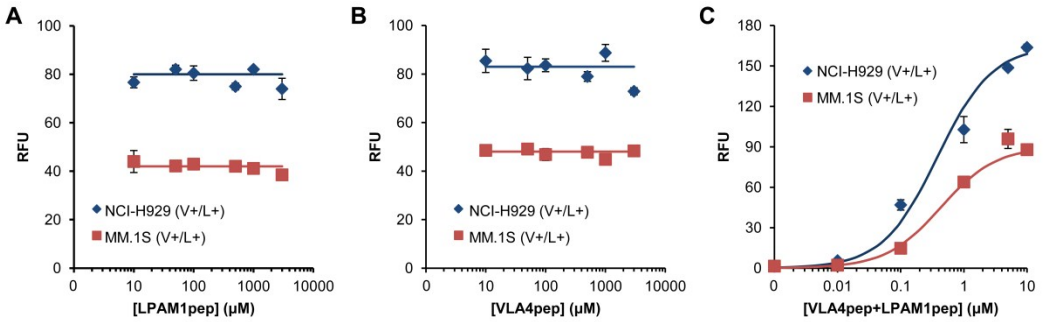
[†]Department of Chemical and Biomolecular Engineering, University of Notre Dame, Notre Dame, IN 46556
[‡]Harper Center Research Institute, University of Notre Dame, Notre Dame, IN 46556
[§]Advanced Diagnostics and Therapeutics, University of Notre Dame, Notre Dame, IN 46556
[‖]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556

Supplementary Table 1. Mass spectrometry data for peptide conjugates.

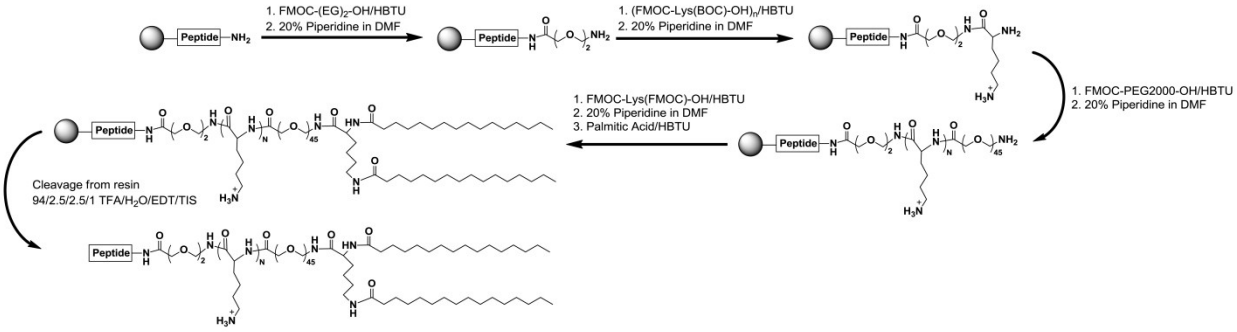
Molecule	Expected Mass (Da)	Found Mass (Da)
VLA4pep	597.17	598.489
LPAM1pep	979.39	980.400
VLA4pep-FITC	1403.48	1404.365
LPAM1pep-FITC	1785.70	1786.024
VLA4pep-lipid	3975.73	3975.894
LPAM1pep-lipid	4358.96	4359.903

Supplementary Table 2. Particle size and zeta potential of $NP_{non\text{-}targeted}$, NP_{VLA4}, NP_{LPAM1}, and $NP_{VLA4+LPAM1}$.

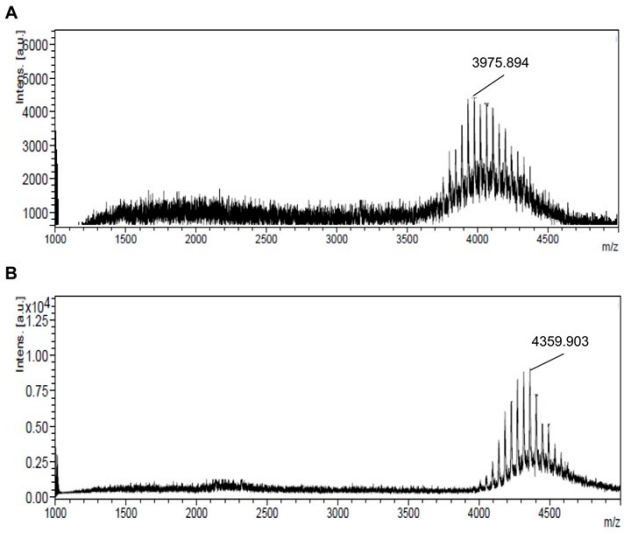
	Formulation	Particle Size (nm)	Zeta-potential (mV)
Liposome			
$NP_{non\text{-}targeted}$	95:10:5 HSPC:CHOL:PEG2000	101.5 ± 3.5	-21.1 ± 3.2
NP_{VLA4}	94:10:5:1 HSPC:CHOL:PEG2000:VLA4pep	103.2 ± 1.4	-21.4 ± 2.3
NP_{LPAM1}	94:10:5:1 HSPC:CHOL:PEG2000:LPAM1pep	101.5 ± 1.7	-19.6 ± 4.2
$NP_{VLA4+LPAM1}$	93:10:5:1:1 HSPC:CHOL:PEG2000:VLA4pep:LPAM1pep	99.1 ± 2.3	-20.2 ± 3.1



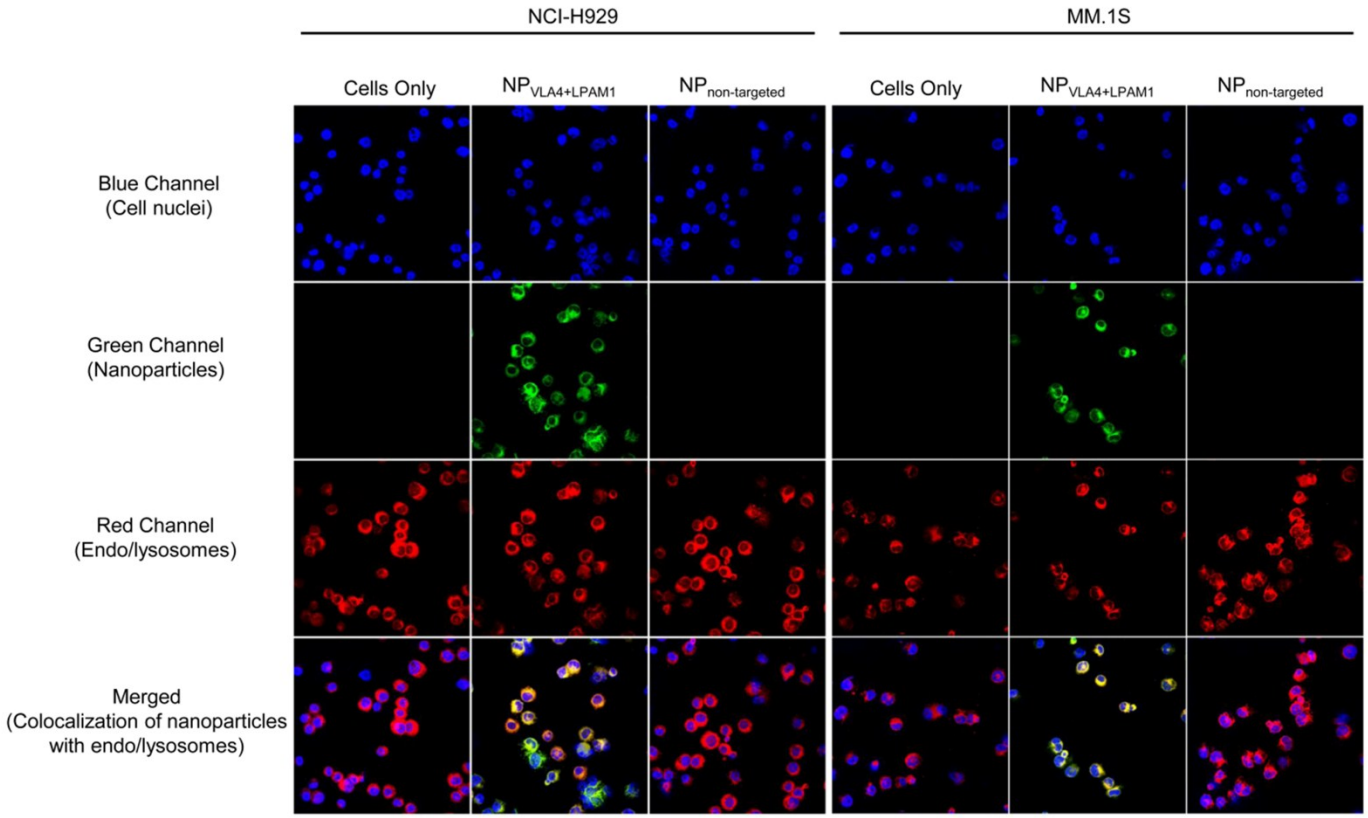
Supplementary Figure 1. Competitive binding experiments. (A) Fluorescein labeled VLA4pep was incubated simultaneously with unlabeled LPAM1pep at 1-300 fold molar excess on ice. (B) Fluorescein labeled LPAM1pep was incubated simultaneously with unlabeled VLA4pep at 1-300 fold molar excess on ice. (C) Fluorescein labeled VLA4pep and LPAM1pep were incubated simultaneously at 0-10 μM on ice. Cellular binding was evaluated using NCI-H929 (V+/L+) and MM.1S (V+/L+) cells. All experiments were repeated in triplicates and data represents means (±s.d.)



Supplementary Figure 2. Synthesis of peptide conjugated lipid amphiphilic molecules. Schematic of the synthetic steps for the synthesis of the peptide-lipid conjugates.



Supplementary Figure 3. **Mass spectra of VLA4pep-lipid and LPAM1pep-lipid conjugates.** (A) MALDI-TOF (linear) spectrum of VLA4pep-lipid. Expected mass 3975.73 Da. Found mass 3975.894 Da. (B) MALDI-TOF (linear) spectrum of LPAM1pep-lipid. Expected mass 4358.96 Da. Found mass 4359.903 Da.



Supplementary Figure 4. Determination of cellular uptake *via* confocal microscopy. Fluorescein labeled, dual-receptor targeted liposomes were incubated with NCI-H929 (V+/L+) or MM.1S (V+/L+) cells for 3 h at 37 °C. The cells were counterstained with Lysotracker Red and Hoechst dyes. Merged images reveal colocalization. Internalization of nanoparticles was determined with a Nikon A1R confocal microscope using a 40x oil lens. Image acquisition was performed by Nikon Elements Ar software.