Supporting Information for the Article

Characterization of iRGD-ligand modified arginine-histidine-rich peptides for nucleic acid therapeutics delivery to αvβ3 integrin-expressing cancer cells

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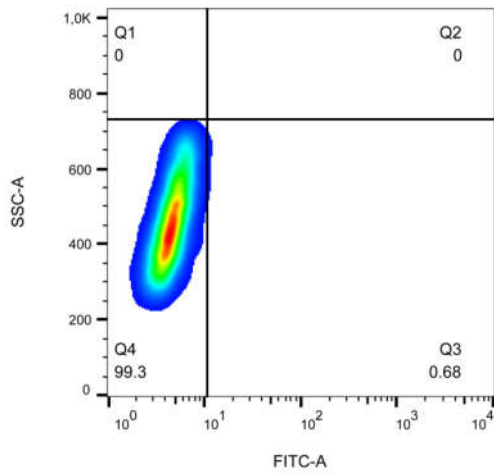
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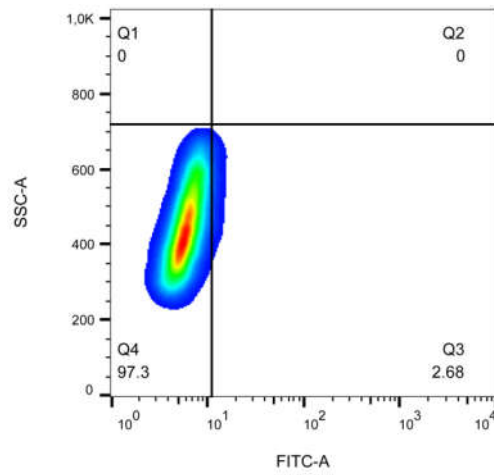
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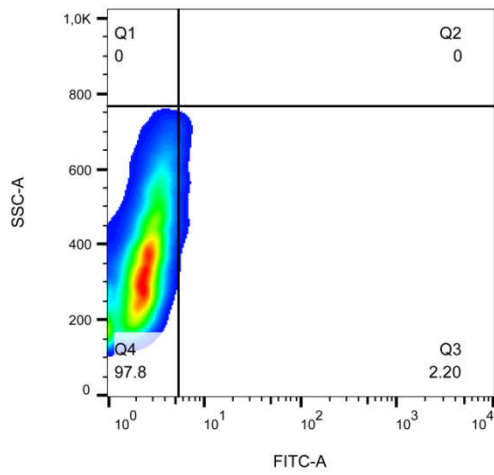
Contents

Figure S1. Detection of αvβ3 integrins on the surface of 293T **(a,b)**, HeLa **(c,d)**, PANC-1 **(e,f)** cell lines by flow cytometry analysis. **(a,c,e)** - unstained cells; **(b,d,f)** - CD51/CD61 antibody staining. The FACS data were processed by FlowJo software (FlowJo, LLC).
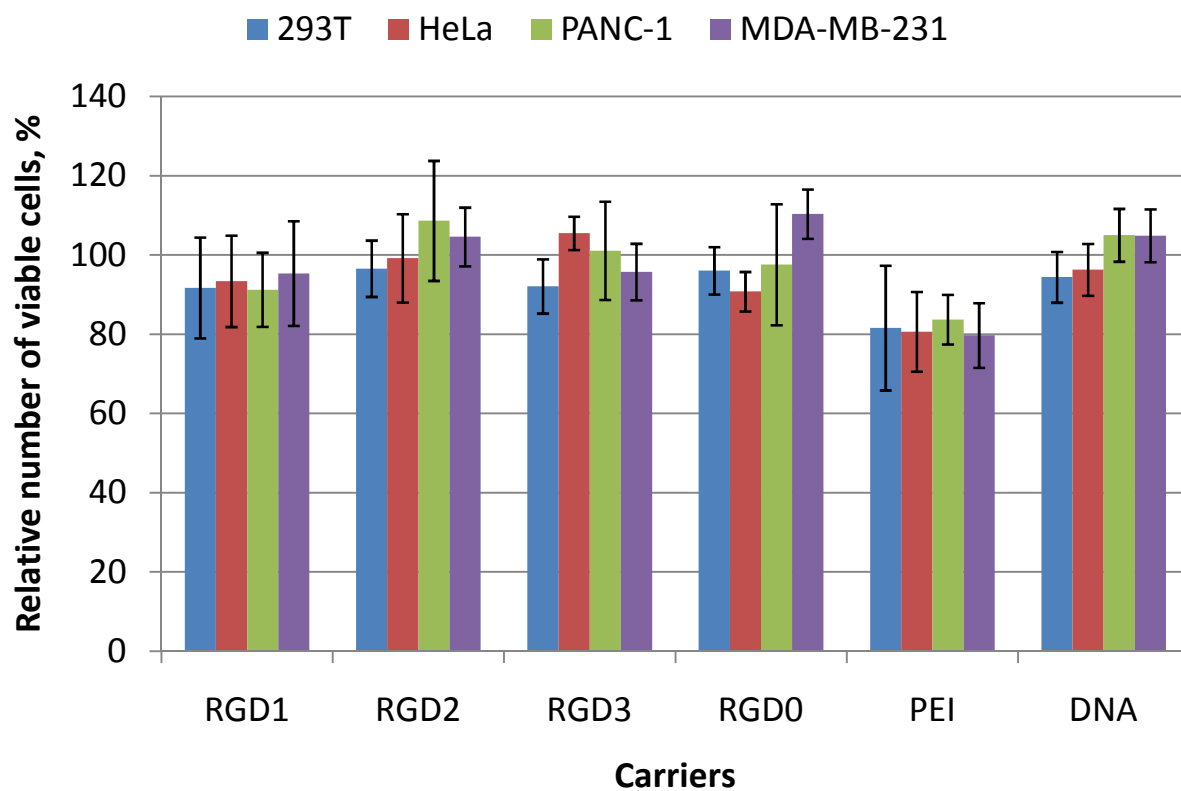
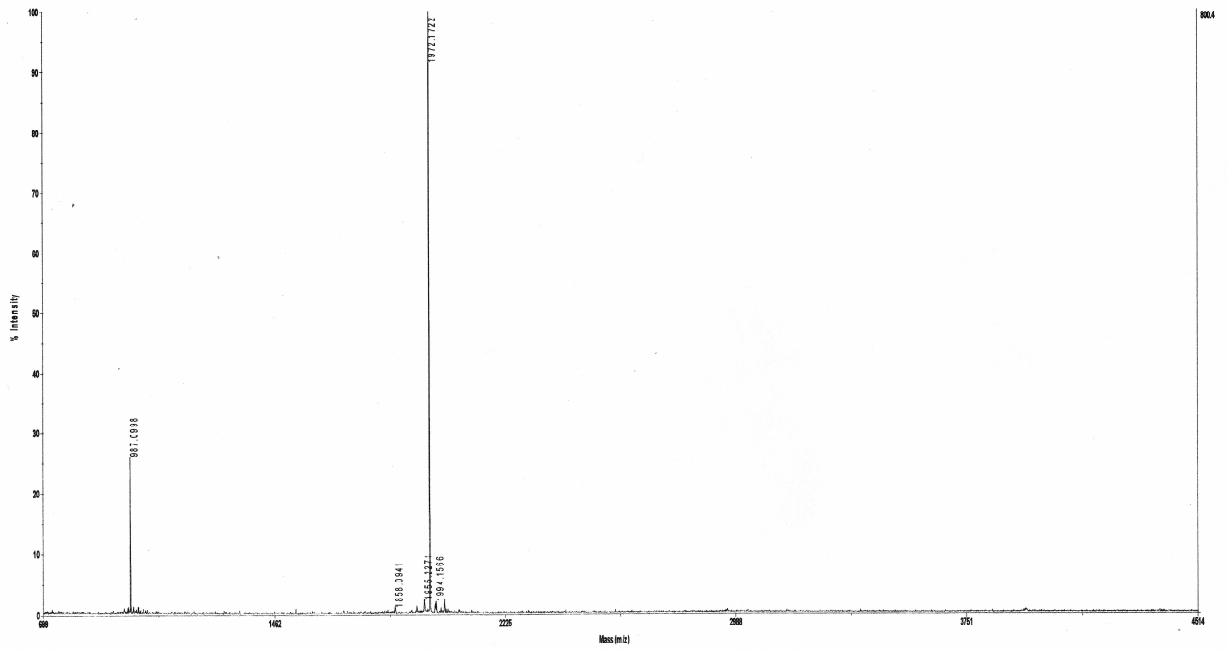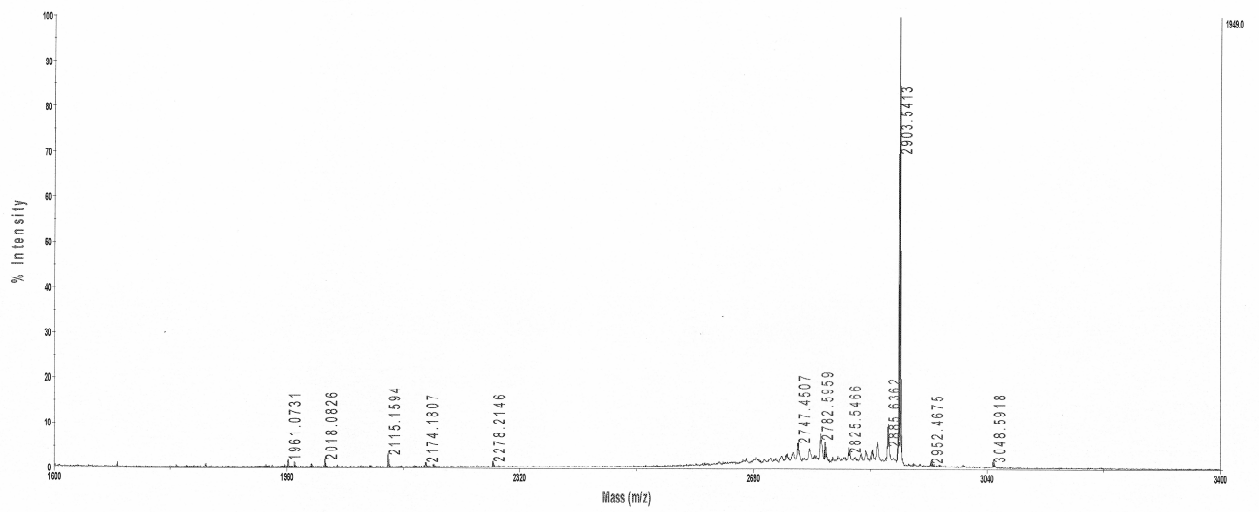**Figure S2.** Cytotoxicity evaluation of free carriers in 293T, HeLa, PANC-1 **,** and MDA-MB-231 cells by the Alamar blue assay. Values are the mean ± SD of the mean of triplicates. Amounts of carriers tested correspond to those applied for formation of NA/carriers complexes at N/P ratio 12/1.

(a)

(b)

Figure S3. Typical MALDI TOF mass spectra of RGD0 **(a)** and RGD1 **(b)** peptides.

Table S1. Experimental and theoretical molecular masses of RGD0 and RGD1 peptides.

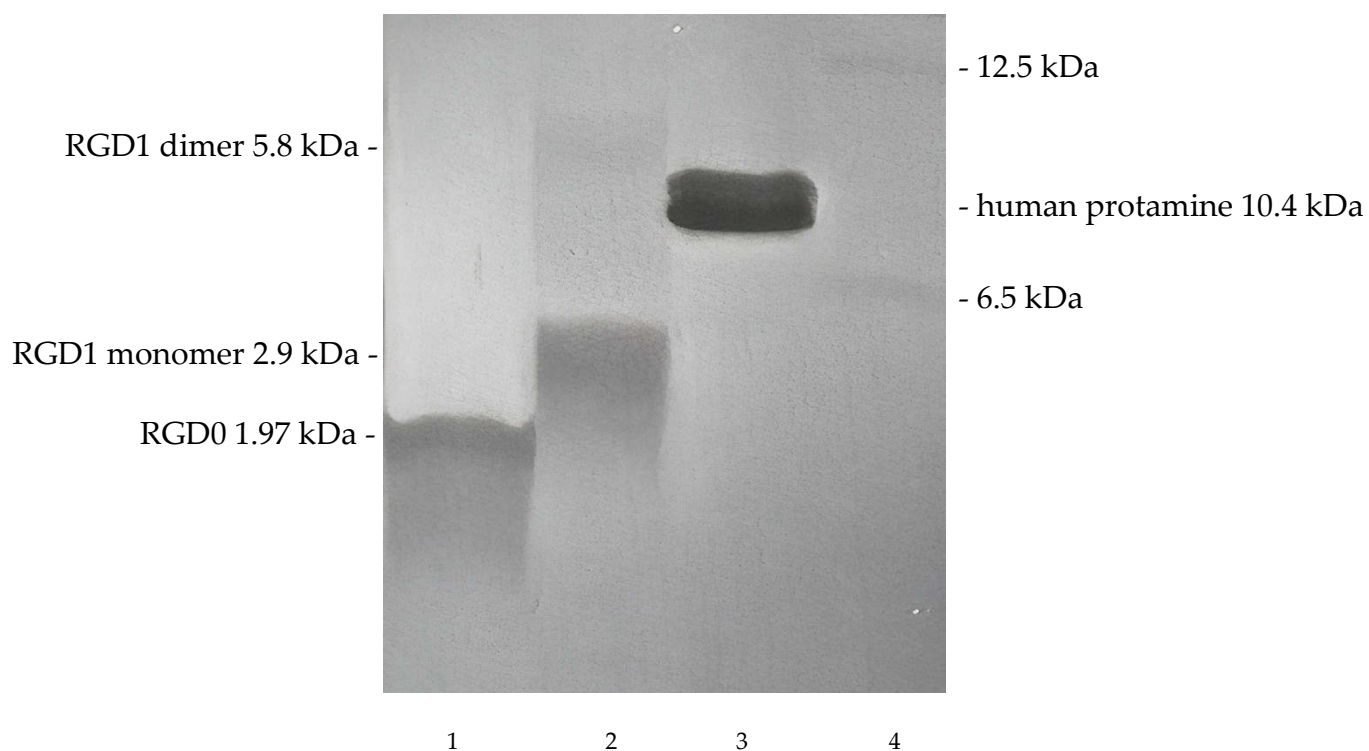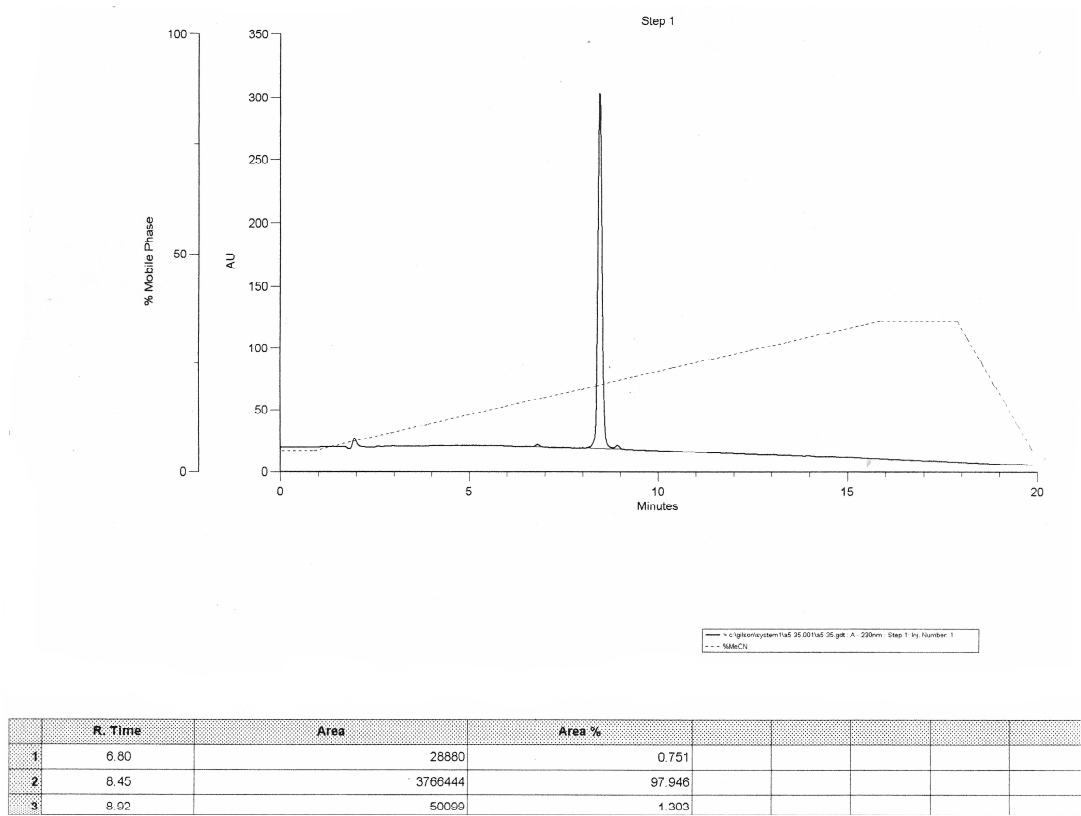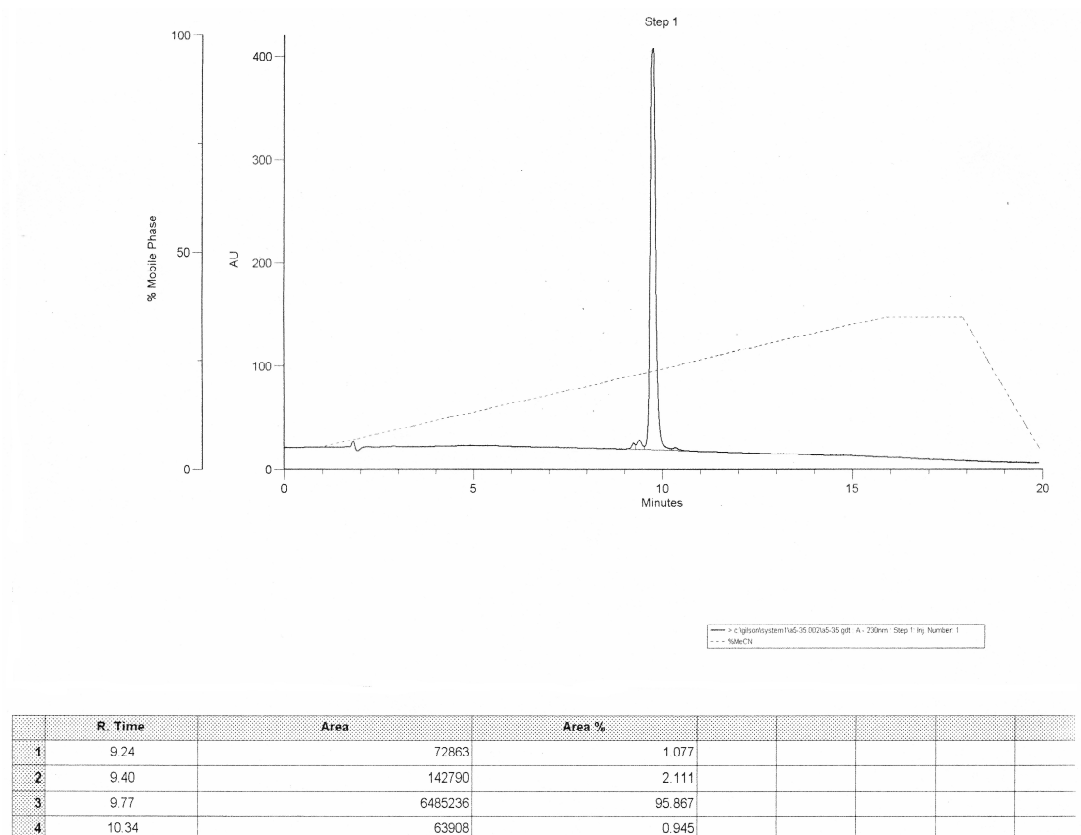| Peptide analyzed	Calculated value of molecular mass, g/mole	Experimental value of molecular mass, g/mole
RGD0	1972.28	1972.17
RGD1	2904.33	2903.54

Figure S4. SDS-PAGE results for RGD0 **(1)** and RGD1 **(2)** peptides; **(3)** - human protamine 1 protein; **(4)** - protein test mixture 6 for SDS PAGE (SERVA).

Note 1: RGD1 dimer cannot be fully denatured by SDS and possesses changed electrophoretic mobility.

Note 2: Tricine-sodium dodecyl sulphate-polyacrylamide gel electrophoresis 4%/16.5% (tricine-SDS-PAGE) was performed to separate low-molecular-weight peptides. Human protamine 1 protein (10.4 kDa) and protein test mixture 6 for SDS PAGE (SERVA) were used as molecular weight controls. Coomassie Brilliant Blue was used for the gel staining.

	R. Time	Area	Area %				
1	6.80	28880	0.751				
2	8.45	3766444	97.946				
3	8.92	50099	1.303				

(a)

	R. Time	Area	Area %				
1	9.24	72863	1.077				
2	9.40	142790	2.111				
3	9.77	6485236	95.867				
4	10.34	63908	0.945				

(b)

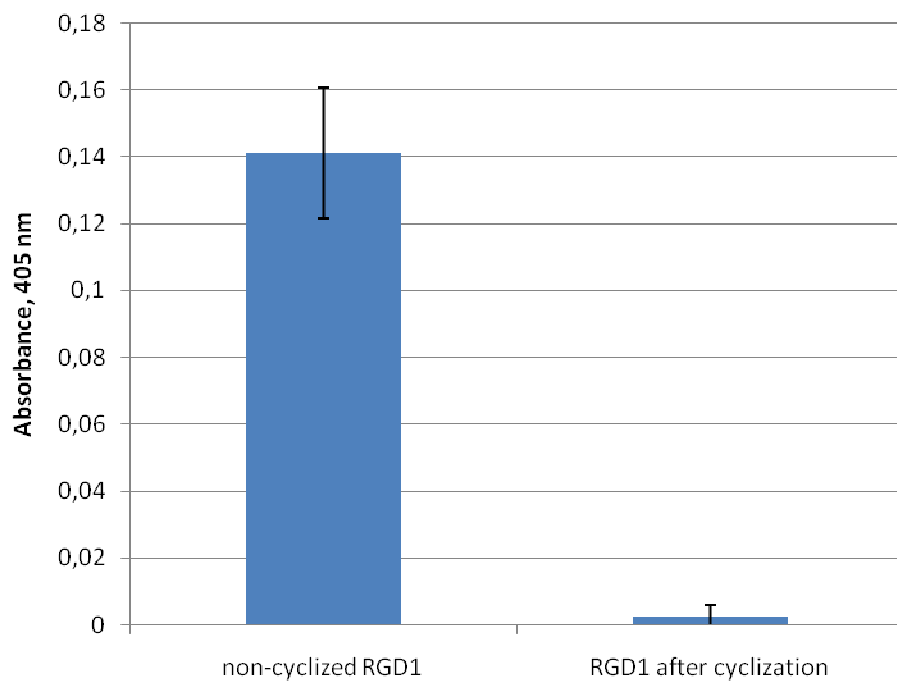**Figure S5.** Typical HPLC results for RGD0 **(a)** and RGD1 **(b)** peptides.

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Figure S6. Free thiol groups measurement by Ellman's assay after 24 h of RGD1 peptide cyclization.