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Preparation and Characterization of Dentin Phosphophoryn-Derived Peptide-Functionalized Lignin Nanoparticles for Enhanced Cellular Uptake

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Table S1. Summary of the reaction ratios used for the carboxylation of the original lignin polymer.

Sample	Original Lignin (mg)	Succinic anhydride (mg)
Carboxylated Lignin (1:2)	100.0	200.0 (42.4 mmol)
Carboxylated Lignin (1:1)	100.0	100.0 (21.2 mmol)
Carboxylated Lignin (2:1)	100.0	50.0 (10.6 mmol)
Carboxylated Lignin (3:1)	100.0	33.3 (7.06 mmol)
Carboxylated Lignin (4:1)	100.0	25.0 (5.3 mmol)
Carboxylated Lignin (5:1)	100.0	20.0 (4.2 mmol)

Table S2. Description of the main lignin functional groups: IR absorption bands and respective type of vibration.[1, 2]

IR band (cm^{-1})	**Type of vibration**
3500–3100	Stretching vibrations of alcohol and phenol –OH groups involved in hydrogen bonds
2920–2850	Stretching vibrations of C–H bonds in methoxy group
1720	Stretching vibrations of C=O bonds at β location and in unconjugated –COOH group
1600	Stretching vibrations of C=O bonds at α- and γ-locations
1512	Aromatic ring vibrations
1465	
1427	
1269	Vibrations of guaiacyl rings and stretching vibrations of C–O bonds
1215	
1150	Deformation vibrations of C–H bonds in guaiacyl rings
1083	Deformation vibrations of C–O bonds in secondary alcohols and aliphatic ethers
1033	Deformation vibrations of C–H bonds in the aromatic rings and deformation vibrations of C–O bonds in primary alcohols
858	Deformation vibrations of C–H bonds in the aromatic rings
819	

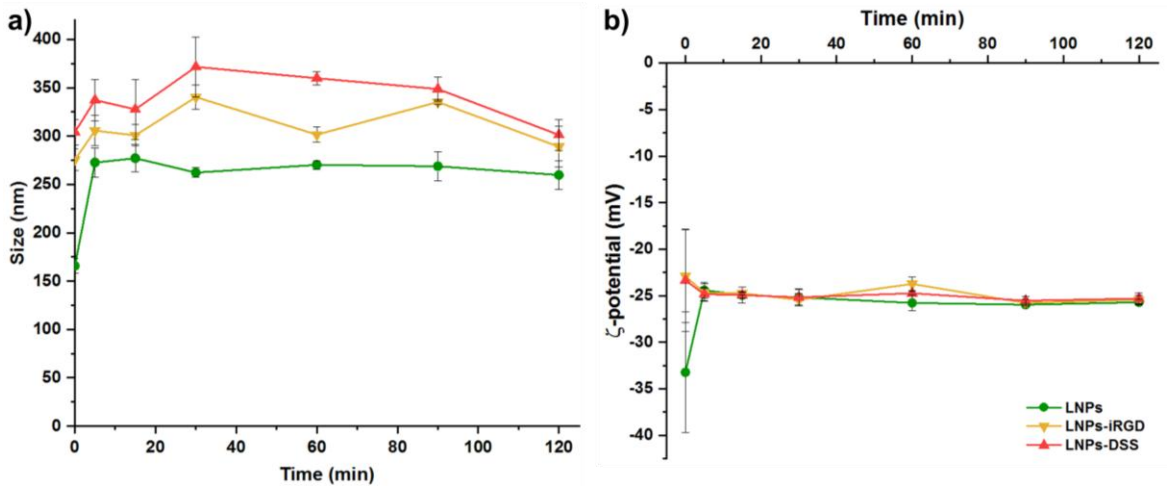

Figure S1. Stability of bare and peptide-decorated LNPs in DMEM supplemented with 10% FBS: effect on the size (a) and ζ-potential (b) of LNPs, LNPs-iRGD and LNPs-DSS after 2 h incubation at 37 °C. Errors bars represent mean ± s.d. (n = 3).

Table S3. Inhibitory Concentration (IC) values by 50% (IC_{50}), 80% (IC_{80}) and 90% (IC_{90}), after incubation of BZL and BZL-loaded LNPs, LNPs-iRGD and LNPs-DSS with a 2D model of PC3-MM2, MDA-MB-231 and A549 for 6 and 24 h at 37 °C. These values were obtained by means of a concentration response curve by non-linear regression using OriginPro 2018.

		PC3-MM2				MDA-MB-231				A549			
		BZL	**BZL@ LNPs**	**BZL@ LNPs-iRGD**	**BZL@ LNPs-DSS**	**BZL**	**BZL@ LNPs**	**BZL@ LNPs-iRGD**	**BZL@ LNPs-DSS**	**BZL**	**BZL@ LNPs**	**BZL@ LNPs-iRGD**	**BZL@ LNPs-DSS**
IC_{50} (μM)	**6h**	56.4	-	-	50.7	-	-	-	-	18.2	27.0	27.4	24.1
	24h	11.4	10.8	8.5	9.3	12.7	5.4	10.2	9.4	12.5	15.4	15.9	16.1
IC_{80} (μM)	**6h**	-	-	-	-	-	-	-	-	29.4	35.2	34.4	37.4
	24h	17.3	17.2	15.5	16.7	20.1	22.4	26.0	22.0	25.2	29.4	29.3	28.9
IC_{90} (μM)	**6h**	-	-	-	-	-	-	-	-	41.5	41.1	43.5	49.8
	24h	22.5	22.5	22.3	23.6	29.8	31.9	35.5	31.4	31.9	36.5	36.0	35.2

Table S4. Inhibitory Concentration (IC) values by 50% (IC_{50}), 80% (IC_{80}) and 90% (IC_{90}), after incubation of BZL and BZL-loaded LNPs, LNPs-iRGD and LNPs-DSS with a 3D tumor spheroids of PC3-MM2, MDA-MB-231 and A549 for 6, 24 and 48 h at 37 °C. These values were obtained by means of a concentration response curve by non-linear regression using OriginPro 2018.

		PC3-MM2				MDA-MB-231				A549			
		BZL	**BZL@ LNPs**	**BZL@ LNPs-iRGD**	**BZL@ LNPs-DSS**	**BZL**	**BZL@ LNPs**	**BZL@ LNPs-iRGD**	**BZL@ LNPs-DSS**	**BZL**	**BZL@ LNPs**	**BZL@ LNPs-iRGD**	**BZL@ LNPs-DSS**
IC_{50} (μM)	**6h**	-	33.1	45.4	31.5	11.5	18.8	19.9	18.3	-	42.6	53.7	.
	24h	-	45.4	22.1	21.1	17.0	17.0	15.7	19.5	20.9	20.3	20.1	24.5
	48h	-	31.5	20.1	20.9	15.9	20.1	20.4	21.0	21.1	18.9	20.2	24.3
IC_{80} (μM)	**6h**	-	-	-	-	34.9	38.9	42.6	33.7	-	-	-	-
	24h	-	49.3	44.0	41.7	38.2	33.6	32.4	33.4	45.5	61.7	54.6	62.7
	48h	-	59.4	41.8	42.4	37.6	40.7	38.5	36.7	40.9	50.5	44.4	47.6
IC_{90} (μM)	**6h**	-	-	-	-	50.6	49.7	54.9	41.3	-	-	-	-
	24h	-	-	-	-	50.1	42.2	41.2	39.9	-	-	-	-
	48h	-	-	53.3	53.7	50.1	51.5	47.5	44.2	51.0	70.2	57.7	60.1

Materials: Tetrahydrofuran (THF), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and 2-(*N*-morpholino)ethanesulfonic acid (MES) were purchased from Sigma-Aldrich®, USA. Culture flasks were purchased from Corning Inc., USA. Dulbecco's Modified Eagle medium (DMEM), Roswell Park Memorial Institute 1640 medium (RPMI), heat inactivated fetal bovine serum (FBS), L-glutamine (200 mM), non-essential amino acids (NEAA), penicillin (100 IU/mL), streptomycin (100 mg/mL) and trypsin (2.5%) were acquired from HyClone Waltham, USA. Dulbecco's phosphate buffer saline (10× PBS) and Hank's balanced salt solution (10× HBSS) were purchased from Life Technologies Gibco® (Carlsbad, CA, USA).

Cell Culturing: Human prostate cancer (PC3-MM2), human mammary carcinoma (MDA-MB-231) and human lung carcinoma (A549) cell lines were obtained from American Type Culture Collection (ATCC), USA. The PC3-MM2 and A549 cells were incubated in DMEM, the MDA-MB-231 cells were incubated with RPMI, supplemented with 10% FBS, 1% NEAA, 1% L-glutamine and 1% penicillin-streptomycin in 75 cm^2 flasks. Cells were maintained in an incubator (BB 16 gas incubator, Heraeus Instruments GmbH) at 37 ºC, 5% CO_2 and 95% relative humidity.

Preparation of Lignin Nanoparticles and experimental conditions for BZL quantification

Table S5. HPLC conditions used in this study for quantification of the loaded and released BZL.

	Benzazulene
Mobile Phase (v/v)	Solution A: 0.1% Trifluoroacetic acid (pH 2.0) Solution B: Acetonitrile (35:65)
Column	Kinetex® C_{18}, 75 mm × 4.6 mm, 2.6 µm (Phenomenex, USA)
Flow Rate (mL/min)	1.0
Detection (UV, nm)	295
Injection Volume (µL)	15
Temperature (°C)	25

Conjugation of the peptides to LNPs:

Table S6. Relevant information of the peptides for the conjugation reactions.

	DSS	**iRGD**
Length	22 aa	10 aa
Sequence	DSSDSSDSSDSSDSSDSSKKKK	CCRGDKGPDC
Molecular weight	2267.15	1053.34
250 µM of peptide (mg/mg of LNPs)	0.283 mg	0.132 mg

References

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