ELECTRONIC SUPPLEMENTARY MATERIAL

Single chain variable fragment fused to maltose binding protein: A modular nanocarrier platform for the targeted delivery of antitumorals

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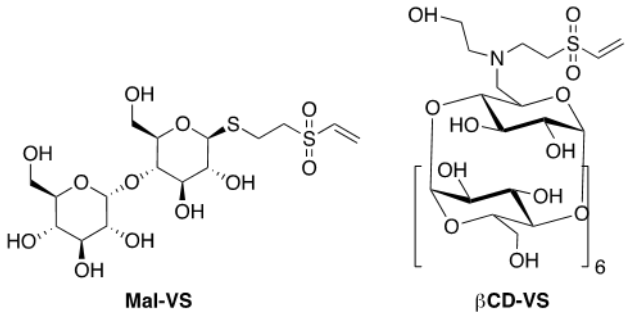
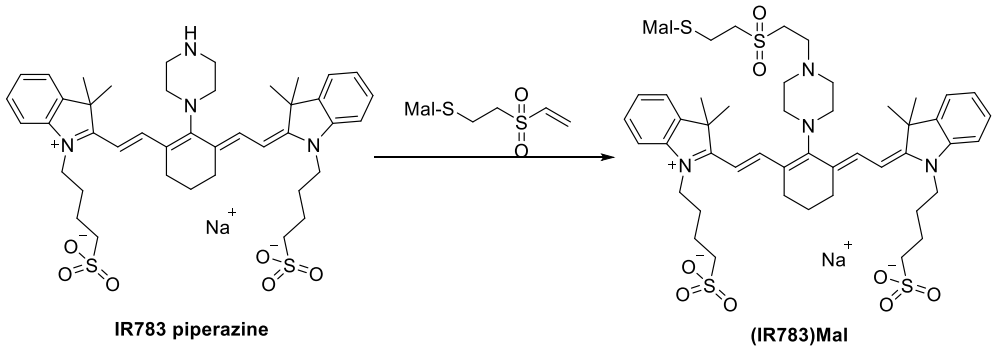
1. Vinyl sulfone-based reagents

The following reported vinyl sulfone-based reagents were prepared following reported procedures and used in the present work: 2-(ethenylsulfonyl)ethyl 4-*O*-β-D-galactopyranosyl-1-thio-β-D-glucopyranoside (**Mal-VS**),[1] and6-deoxy-6-(2-hydroxyethyl) (vinylsulfonyl)methyl)amino-β-cyclodextrin (β**CD-VS**).[2]



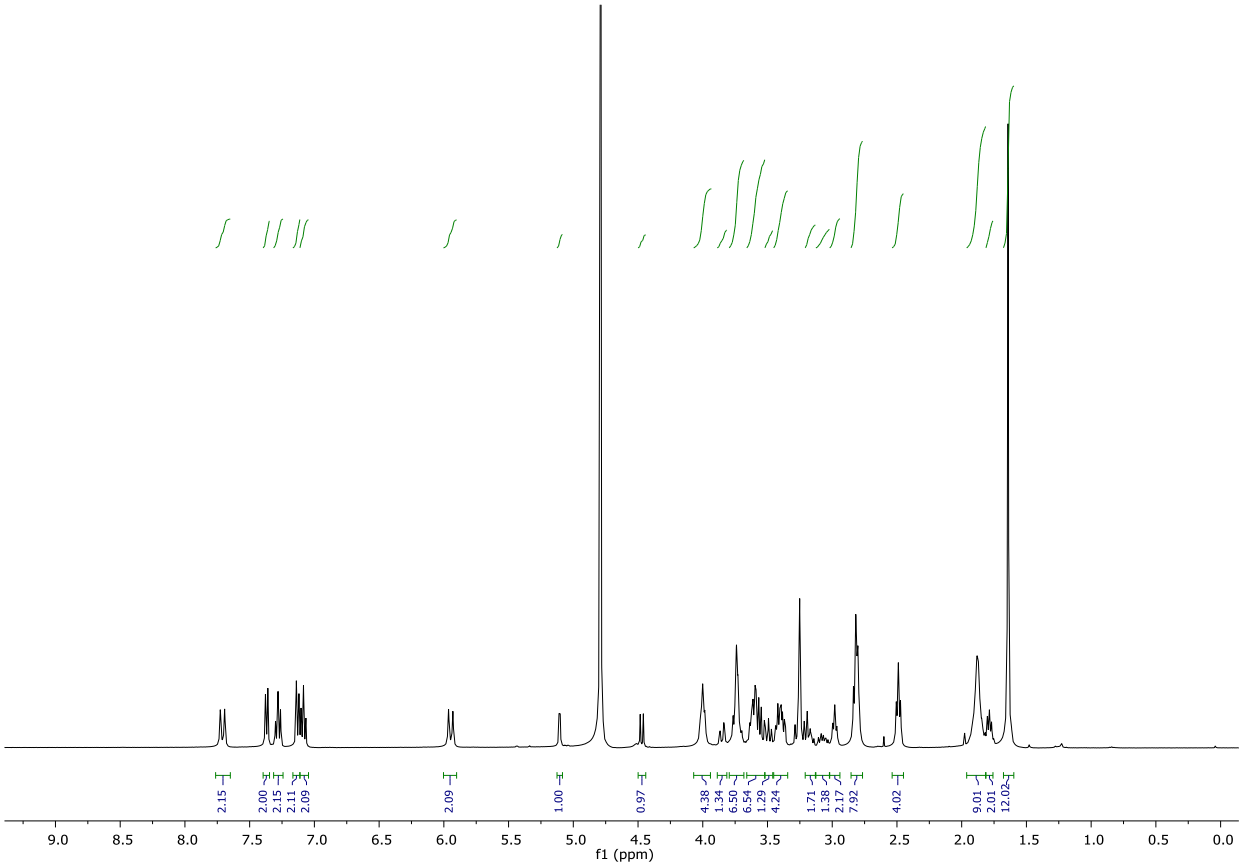
2. Chemical synthesis of maltosylated ligands

2.1. Synthesis of (IR783)Mal imaging reagent:

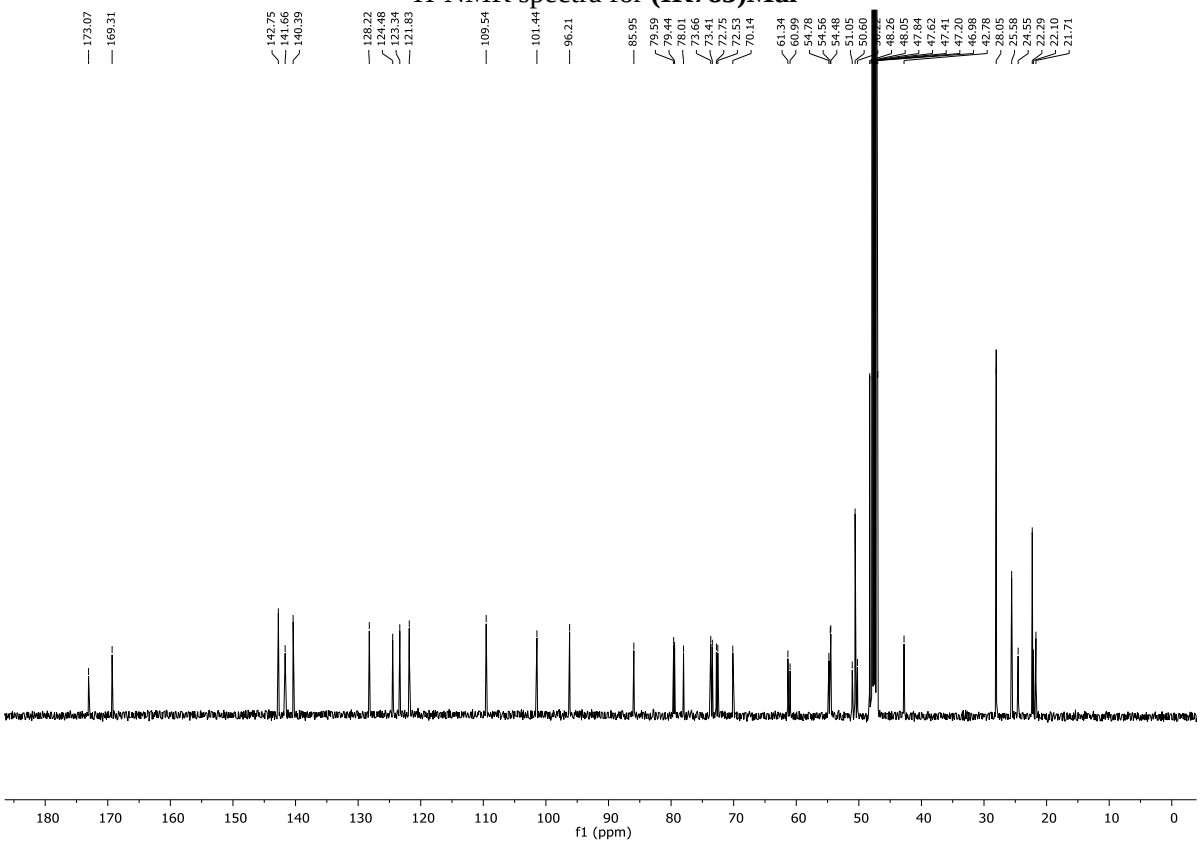


Scheme 1

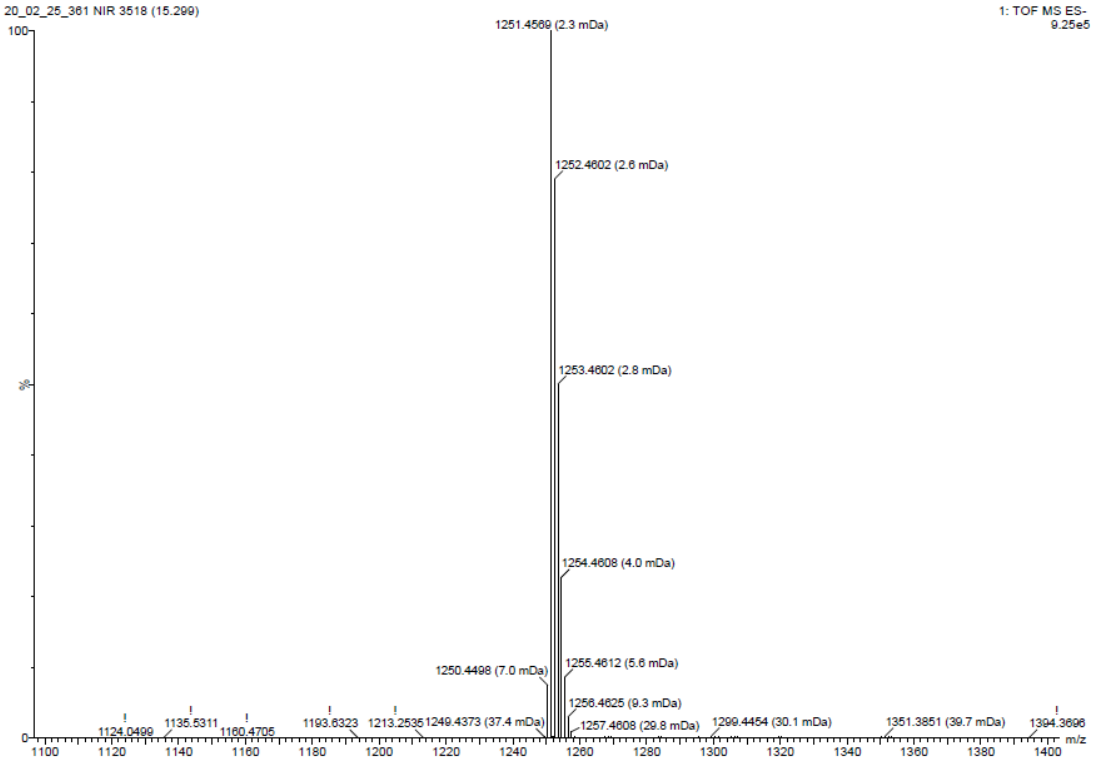
To a solution of the **IR-783** piperazine derivative[3](47 mg , 0.059 mmol) in MeOH (5 mL), a solution of **Mal-VS** (27 mg, 0.057 mmol) in water (2 mL) and triethylamine (20µl, 0.14 mmol) were successively added. The reaction mixture was kept under stirring overnight. After this time, evaporation of the solvents under reduced pressure produced a crude product that was purified by column chromatography (acetonitrile:water 8:1) yielding **(IR783)Mal**(35 mg, 0.027mmol, 47%):Mp: 196°C. IR: ν=1550.95, 1511.03, 1454.7, 1380.15, 1347.31, 1286.69, 1256.71, 1144.05, 1127.20, 1107.12, 1089.84, 1033.81, 1018.65, 929.16, 795.08, 753.55 cm^{-1}. ^{1}H NMR (400 MHz, methanol-d_4): δ 7.71 (d, *J* = 13.4 Hz, 2H), 7.37 (d, *J* = 7.4 Hz, 2H), 7.28 (t, *J* = 7.7 Hz, 2H), 7.13 (d, *J* = 8.0 Hz, 2H), 7.08 (t, *J* = 7.5 Hz, 2H), 5.95 (d, *J* = 13.4 Hz, 2H), 5.11 (d, *J* = 3.8 Hz, 1H), 4.47 (d, *J* = 9.7 Hz, 1H), 4.06 – 3.95 (m, 4H), 3.85 (dd, *J* = 12.3, 1.9 Hz, 1H), 3.80 – 3.68 (m, 6H), 3.61 (dt, *J* = 9.2, 4.5 Hz, 5H), 3.58 – 3.52 (m, 2H), 3.49 (t, *J* = 9.2 Hz, 1H), 3.40 (dq, *J* = 13.4, 5.1, 3.8 Hz, 4H), 3.20 – 3.14 (m, 1H), 3.07 (ddd, *J* = 13.8, 9.1, 6.5 Hz, 1H), 2.98 (t, *J* = 6.5 Hz, 2H), 2.82 (t, *J* = 6.9 Hz, 8H), 2.49 (t, *J* = 6.5 Hz, 4H), 1.87 (dq, *J* = 10.8, 5.2 Hz, 10H), 1.82 – 1.76 (m, 2H), 1.64 (s, 12H).^{13}C NMR (101 MHz, MeOD):δ 173.07, 169.31, 142.75, 141.66, 140.39, 128.22, 124.48, 123.34, 121.83, 109.54, 101.44, 96.21, 85.95, 79.59, 79.44, 78.01, 73.66, 73.41, 72.75, 72.53, 70.14, 61.34, 60.99, 54.78, 54.56, 54.48, 51.05, 50.60, 50.22, 48.26, 48.05, 47.84, 47.62, 47.41, 47.20, 46.98, 42.78, 28.05, 25.58, 24.55, 22.29, 22.10, 21.71.HR-MS (ESI^-): m/z= found 1251.4569, calcd. for $[M-H]^-$ $C_{58}H_{83}N_4O_{18}S_4$: 1251.4591



^{1}H-NMR spectra for **(IR783)Mal**

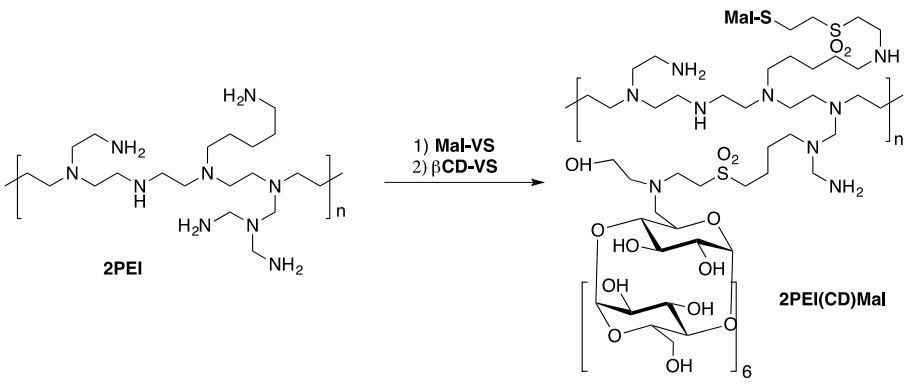


^{13}C-NMR spectra for **(IR783)Mal**



HR-MS (ESI) spectra for **(IR783)Mal**

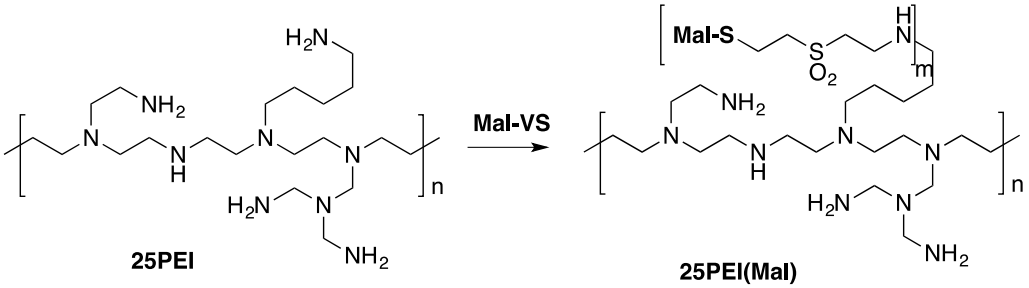
2.2. Synthesis of 2PEI(CD)Mal



Scheme 2

To a solution of previously lyophilized 2PEI (200mg, 0.1 mmol) in water (2.5 mL) a solution of **Mal-VS** (47.6mg, 0.1mmol) in water (2.5 mL) was added. The mixture was stirred overnight until the disappearance of **Mal-VS** was detected by TLC. Then, a solution of β**CD-VS**(518mg, 0.4mmol) in DMSO (3 mL) was added and the reaction mixture was kept at room temperature for one day. Dialysis (3.5kD membrane) was followed by lyophilization, yielding **2PEI(CD)Mal**(535mg) that was used without any additional purification.

2.3. Synthesis of 25PEI(Mal)



To a solution of 25PEI (250 mg, 0.05 mmol) in water (4 mL), a solution of **Mal-VS**(23.8mg, 0.05 mmol) in water (1 mL) was added. The mixture was stirred overnight until the disappearance of **Mal-VS** was followed by lyophilization, yielding **25PEIMal** that was used without any additional purification.

3. **Table S1.** *List of oligonucleotides*

Table S1. *Oligonucleotides used in this article*

Name	Sequence	Purpose
ScFv-Forward	ggatCCATGGCCCAGGTGCAGCTGGTG	Cloning ScFv in pMAL-TEV-His
ScFv-Reverse	aagcTTAATGGTGGTGGTGATGATGAGATCCTCC	Cloning ScFv in pMAL-TEV-His
MBP_{I334W}-Forward	CAGAAAGGTGAAATCATGCCGAACTGGCCGCAGATG TCCGCTTTCTGG	Site directed mutagenesis MBP, underlined mutations
MBP_{I334W}-Reverse	CCAGAAAGCGGACATCTGCGGCCAGTTCGGCATGATT TCACCTTTCTG	Site directed mutagenesis MBP, underlined mutations

4. **Fig. S1.** *Expression and purification of MBP*-ScFv*

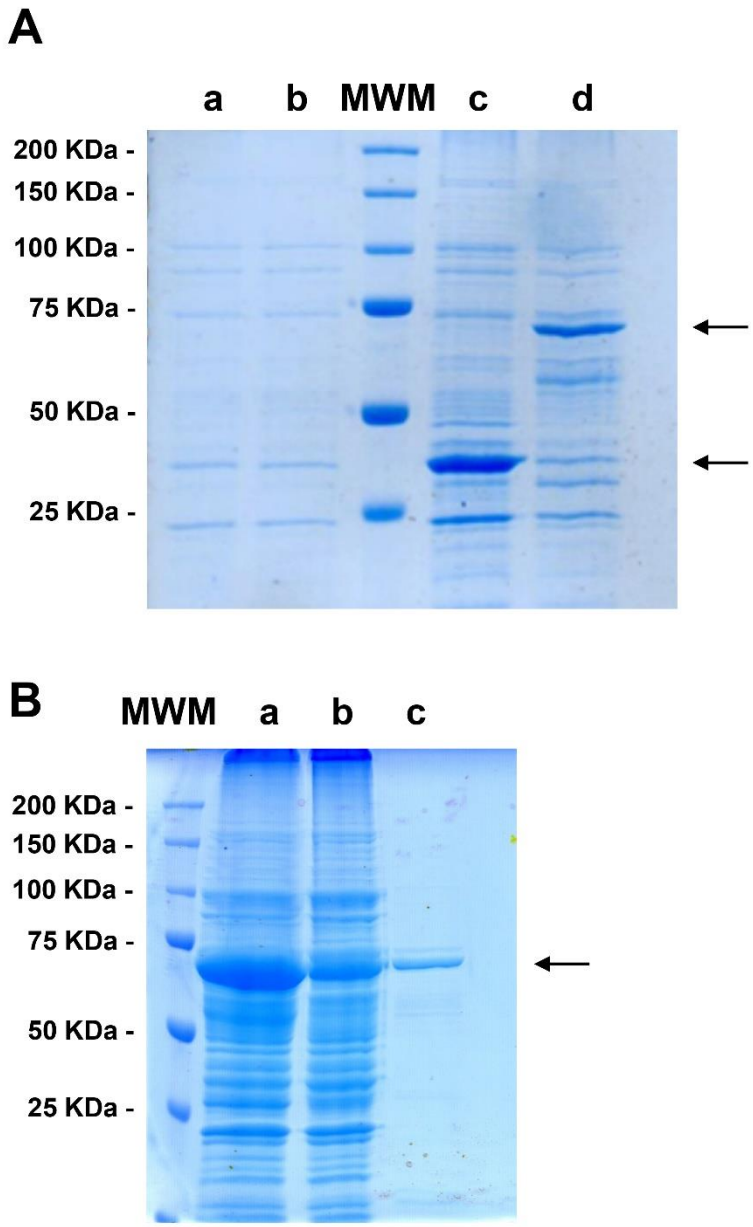
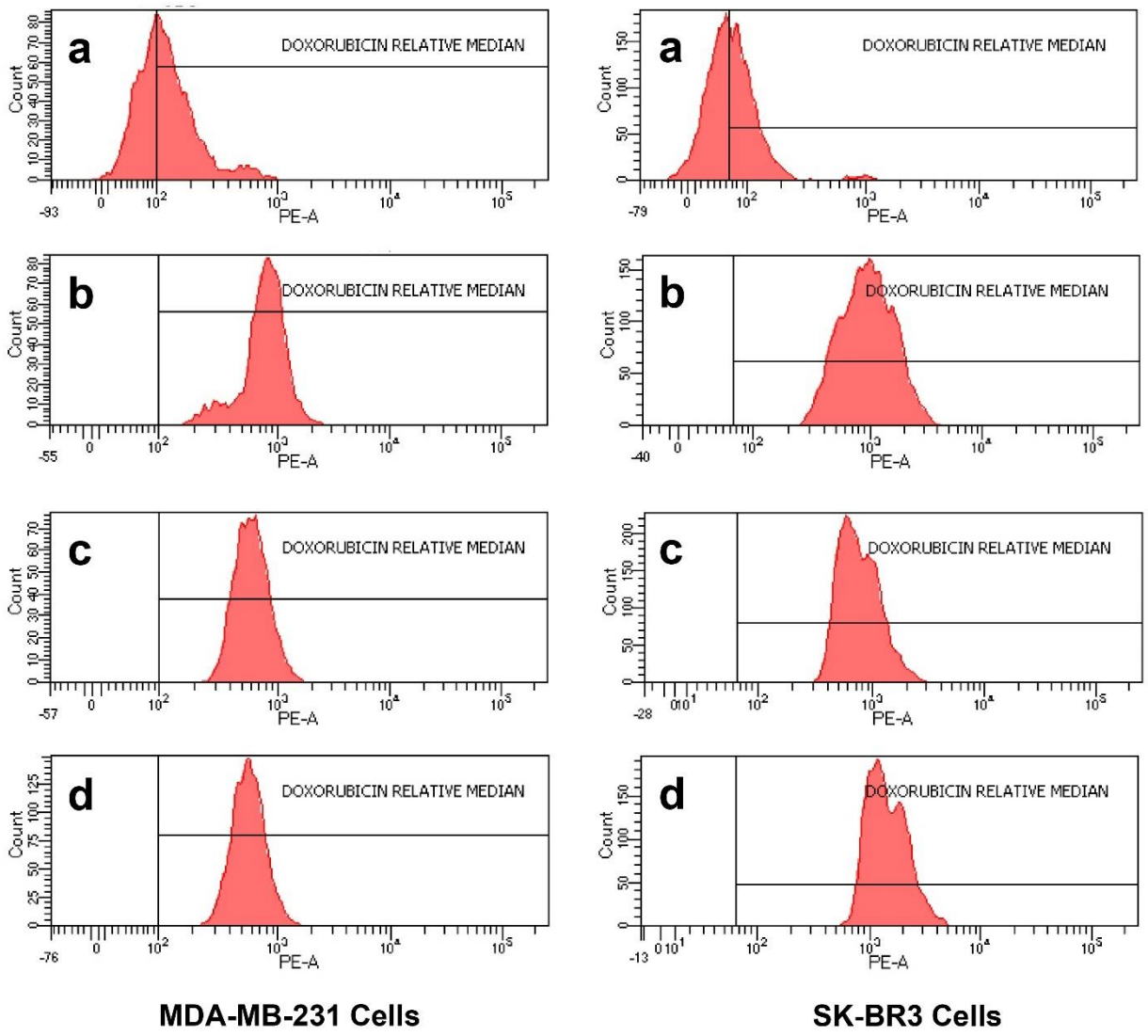


Fig. S1. *Expression and purification of MBP*-ScFv.* **A.-** Novagen's Rosetta™ 2 cells bearing plasmids pMALI334W-TEV-His (lanes a and c) or pMALI334W-TEV-ScFvHER2-His (lanes b and d) were grown in LB Broth medium until OD600 = 0.5 (lanes a and b) and then, cells were incubated with 0.5mM IPTG to induce the protein expression at 30°C for an additional period of 6h (lanes c and d). Samples corresponding to non-induced and induced cells were analysed by SDS-PAGE. MWM, protein molecular weight marker. Arrows mark the expected MBP* and MBP*-ScFv molecular weights. **B.-** MBP*-ScFv protein expression and purification. SDS-PAGE: MWM, protein molecular weight marker; (a) induced crude extracts corresponding of pMALI334W-TEV-ScFvHER2-His transformed bacteria, (b) soluble fraction from crude induced extract, (c) purified affinity chromatography fraction. Arrow marks the expected MBP*-ScFv molecular weight.

5. **Fig. S2.** *Flow cytometry analysis of targeted DOX delivery mediated by a MBP*-ScFv binding complex.*



	PEA-Mean	
Sample	***MDA-MB-231 cells***	***SK-BR3 cells***
Control	**141**	**89**
2kPEI(DOX⊂CD)Mal	**788**	**1026**
2kPEI(DOX⊂CD)Mal~[MBP*]	**609**	**787**
2kPEI(DOX⊂CD)Mal~[MBP*-ScFv]	**566**	**1477**

Fig. S2. *Flow cytometry analysis of targeted DOX delivery mediated by a MBP*-ScFv binding complex.* Fluorescence histograms of MDA-MB-231 or SKBR3 cells were incubated for 1h in the absence (a) or presence of 1μM DOX (b), 2kPEI(DOX⊂CD)Mal (b), [2kPEI(DOX⊂CD)Mal]~[MBP*] (c), or [2kPEI(DOX ⊂ CD)Mal] ~[MBP*-ScFv] (d). PEA-mean value is shown.

6. **Fig. S3.** *Gene delivery capabilities of MBP*based/pDNA polyplexes*

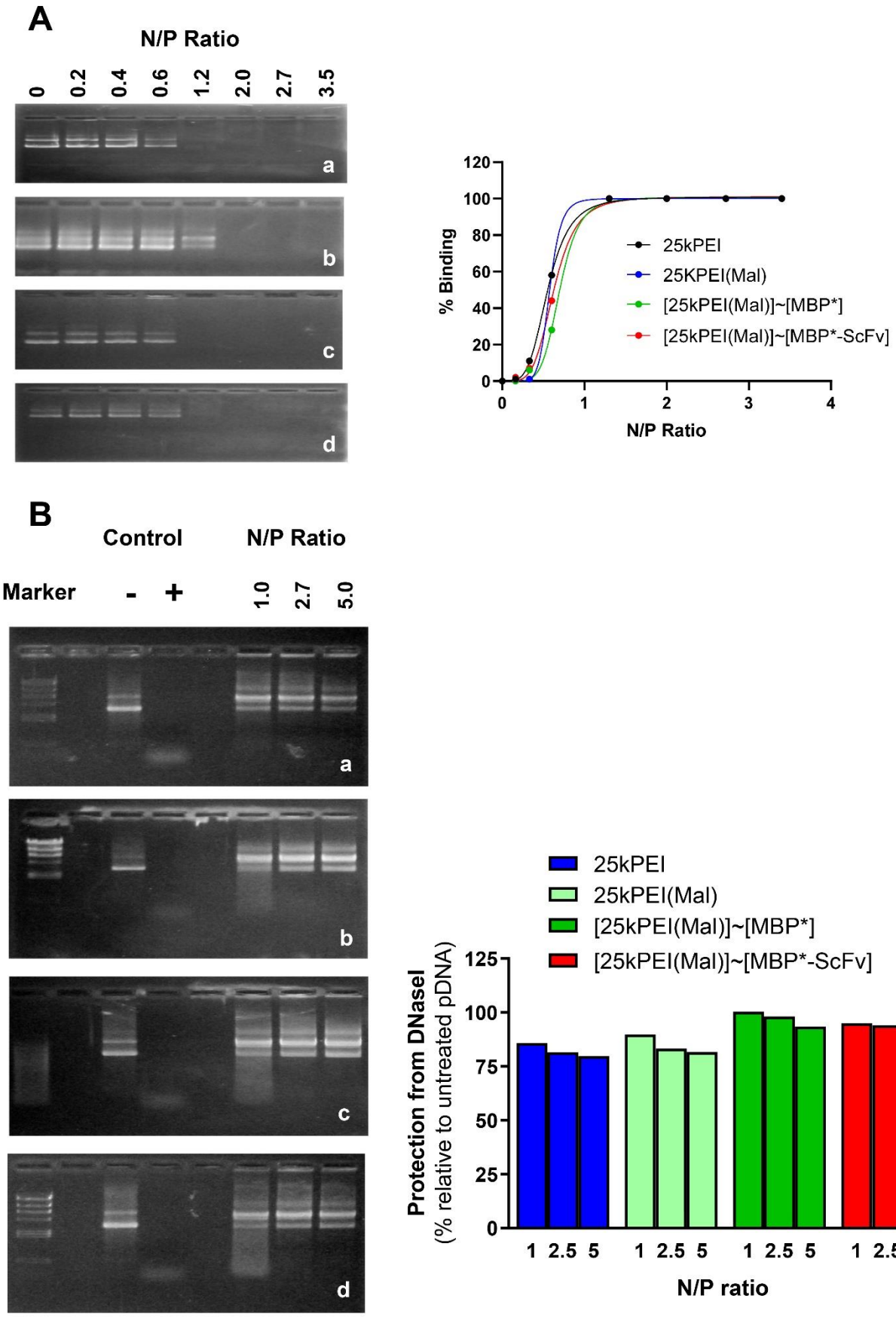


Fig. S3. *Gene delivery capabilities of MBP* derived polyplexes*. **(A)** Gel electrophoresis shift assay at different N/P ratios. The relative percentage of binding (mean values of three independent experiments) was calculated by quantification of the intensity of the plasmid bands. **(B)** DNase I protection experiments: Quantification of the relative intensity (untreated pEGFP-N3 value equal to 100) of the sum of relaxed and supercoiled electrophoretic plasmid bands treated with DNase I. For both experiments (a) 25kPEI, (b) 25kPEI(Mal), (c) [25kPEI(Mal)]~[MBP*], (d) [25kPEI(Mal)]~[MBP*-ScFv]. Results are expressed as means of three independent experiments.

7. References

1. F. J. Lopez-Jaramillo, M. Ortega-Munoz, A. Megia-Fernandez, F. Hernandez-Mateo and F. Santoyo-Gonzalez, *Bioconjugate Chem.*, 2012, **23**, 846-855.

2. T. del Castillo, J. Marales-Sanfrutos, F. Santoyo-Gonzalez, S. Magez, F. J. Lopez-Jaramillo and J. A. Garcia-Salcedo, *ChemMedChem*, 2014, **9**, 383-389.

3. E. De los Reyes-Berbel, R. Salto-Gonzalez, M. Ortega-Munoz, F. J. Reche-Perez, A. B. Jodar-Reyes, F. Hernandez-Mateo, M. D. Giron-Gonzalez and F. Santoyo-Gonzalez, *Bioconjugate Chem.*, 2018, **29**, 2561-2575.