Electronic Supplementary Material (ESI) for RSC Advances.

Electronic supplementary information

Covalent Conjugation of Cysteine-engineered scFv to PEGylated Magnetic Nanoprobes for Immunotargeting of Breast Cancer Cells

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        10         20         30         40         50         60
QVQLQESGGG LVQPGGSLRL SCAASGFNIK DTYIHWVRQA PGKGLEWVAR IYPTNGYTRY

        70         80         90        100        110        120
ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCSRWG GDGFYAMDYW GQGTLVTVSS

       130        140        150        160        170        180
GGGGSGGGGS GGGGSDIQMT QSPSSLSASV GDRVTITCRA SQDVNTAVAW YQQKPGKAPK

       190        200        210        220        230        240
LLIYSASFLY SGVPSRFSGS RSGTDFTLTI SSLQPEDFAT YYCQQHYTTP PTFGQGTKVE

       250
IKAHHHHHHG GSSGSGC
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Figure S1. Amino acids sequence of scFv 4D5-Cys. VH and VL are colored in green and in purple respectively. Linker $(Gly_4Ser)_3$ is colored in blue. The C-terminus flag is underlined. Hexahistidine tag is colored in pink whereas terminal cysteine is colored in red.

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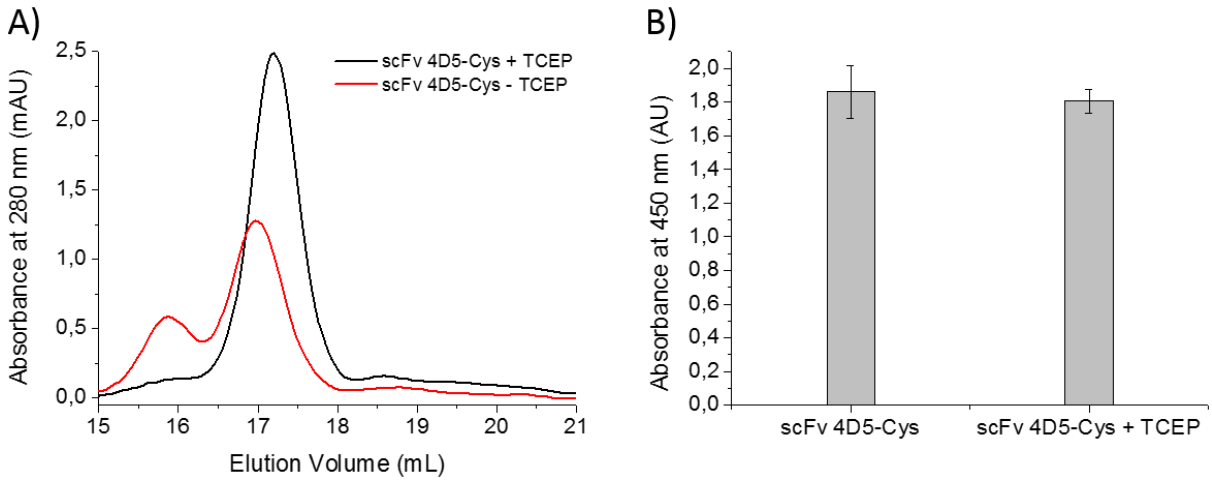

Figure S2. (A) Size-exclusion chromatography (FPLC, Superdex 200 10/300 GL column) of purified scFv 4D5-Cys after incubation during 2 hours at room temperature in absence (red curve) or in presence (black curve) of TCEP (40–50 equivalents per scFv molecule). (B) Immunoreactivity assessment by indirect ELISA assay of purified scFv 4D5-Cys after incubation during 2 hours at room temperature in absence or in presence of TCEP (~40 equivalents per scFv molecule). The amount of HER2 protein immobilized in each well was 0.1 µg. scFv solutions (with or without TCEP) was then added at the concentration of 7 $\mu g.mL^{-1}$.

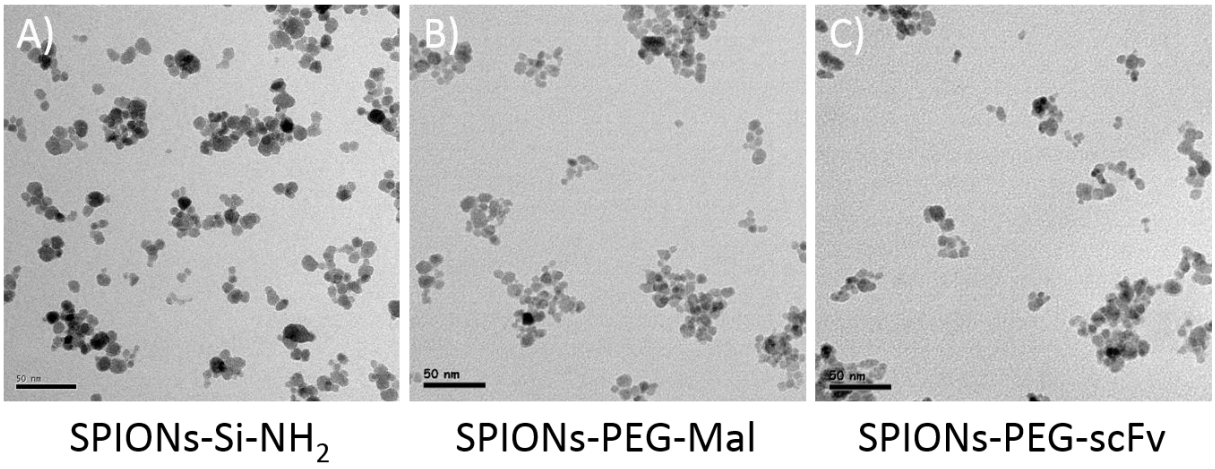

Figure S3. TEM images of aqueous colloidal suspensions of (A) silanized SPIONS (SPIONs-Si-NH_2), (B) PEGylated SPIONs (SPIONs-PEG-Mal) and (C) scFv-functionalized PEGylated SPIONs (SPIONs-PEG-scFv). The scale bar is 50 nm.

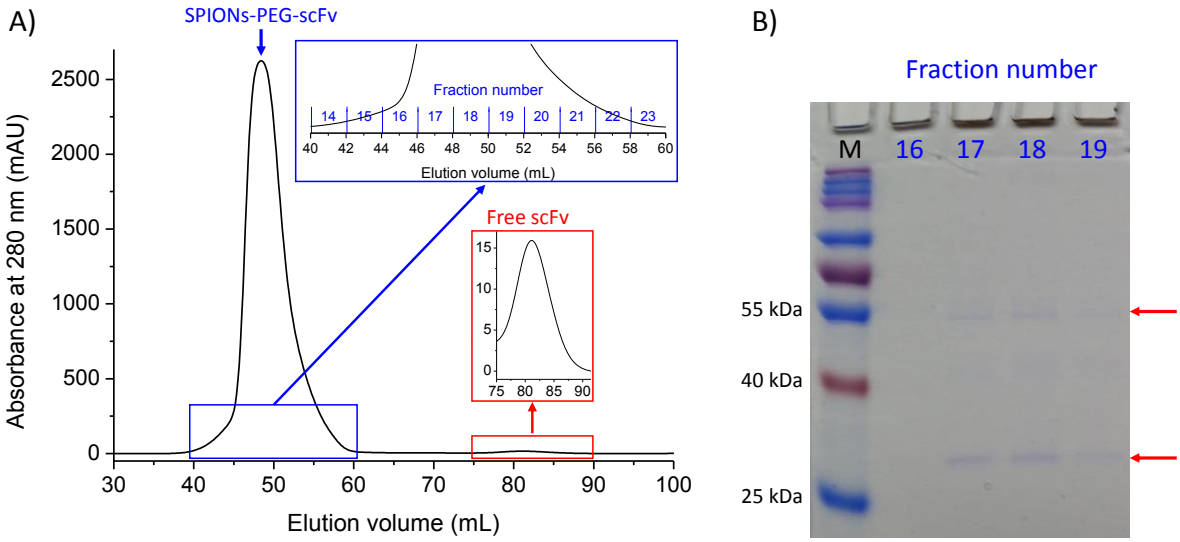

Figure S4. Control of presence of unconjugated scFv in SPIONs-PEG-scFv batches purified by size exclusion chromatography (SEC). A: the free scFv peak was efficiently separated from that of the SPIONs-PEG-scFv. (B) SPIONs-PEG-scFv peak was collected into separate elution fractions and analyzed by SDS-PAGE. The fractions 17-19 showed a minor presence of some unconjugated scFv with a molecular weight of monomer and dimer.

The SEC purification of SPIONs-PEG-scFv nanoparticles was made using an ÄKTApurifier FPLC system equipped with a prepacked Superdex 75 column (flow: 1.6 mL.min^{-1}; eluent: PBS, pH 7.4). The detection of bound and free scFv was performed with a UV absorption detector at 280 nm. During the purification process, fractions of 2 mL were collected and analyzed by SDS-PAGE as described in the experimental section.

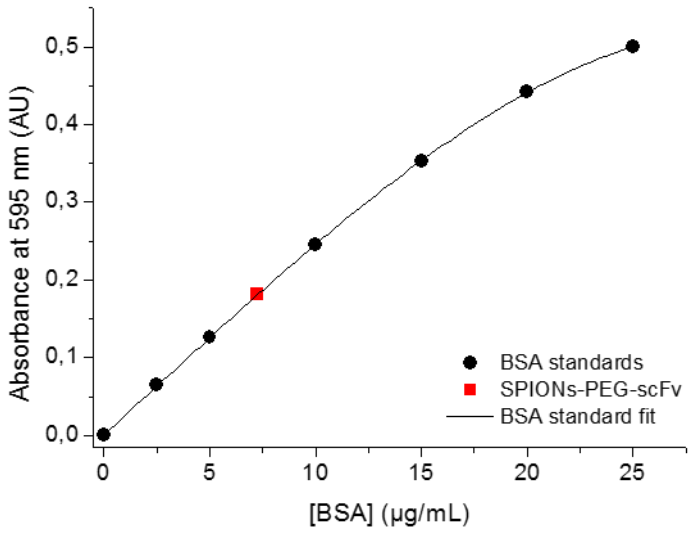

Figure S5. Quantification of scFv concentration in a colloidal suspension of SPIONs-PEG-scFv 4D5 nanoparticles ([Fe] = 55 mg.L^{-1}) by the Bradford assay.

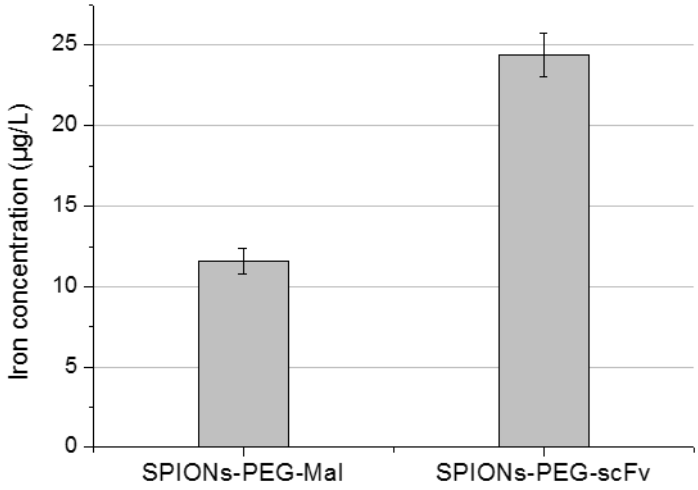

Figure S6. ICP-AES quantitation of nanoparticles uptaken in 1 hour by HER2-overexpressing BT-474 cancer cells.