Electronic Supplementary Information (ESI)

Leucine zipper pair-based lipid vesicle for image-guided therapy in breast cancer

Eun-Kyung Lim[a], Panki Bae[b], Haeran Kim[b] and Juyeon Jung[*a,c]

[a] BioNanotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology,
[b] BioNano Health Guard Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), 305-806, Daejeon, Republic of Korea.
[c] Nanobiotechnology Major, School of Engineering, University of Science and Technology, 125 Gwahangro, Yuseong-gu, Daejoen 305-806 Republic of Korea

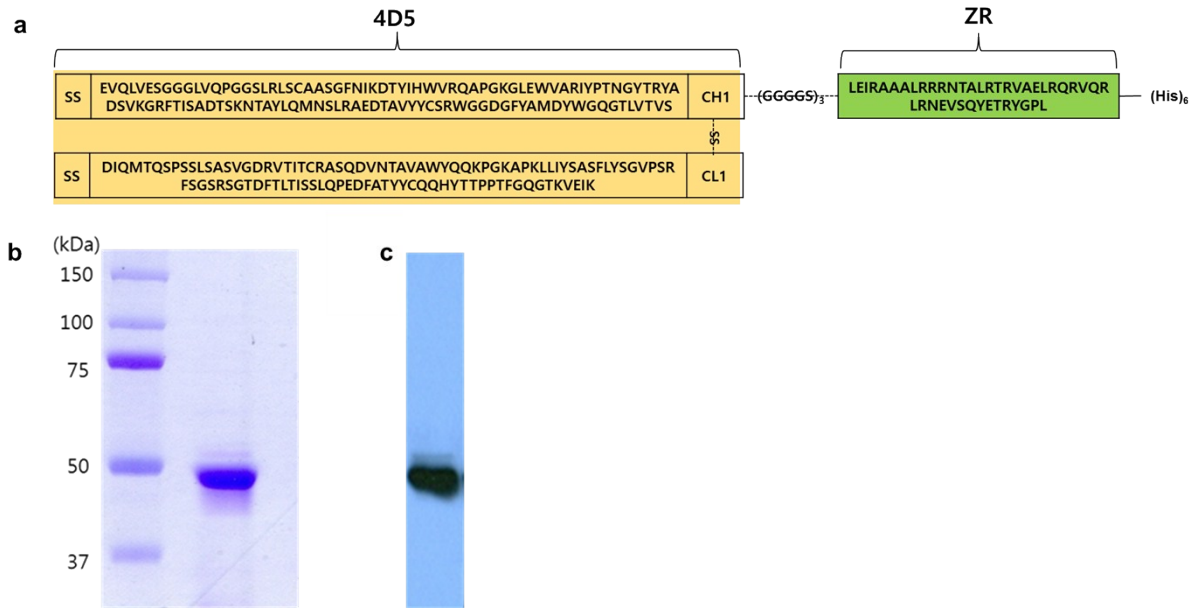

Fig. S1 Construction and expression of a ZR-4D5 fusion protein. (a) ZR-4D5 Fab fusion gene was constructed (from N-to-C-terminus) from a secretion signal peptide, 4D5 VH-CH, a $(GGGGS)_3$ flexible linker, ZR, a hexahistidine tag, a secretion signal peptide, and 4D5 VL-CL. (b) SDS-PAGE and (c) western blot using an anti-His tag antibody of the expressed and purified ZR-4D5 fusion protein are shown.

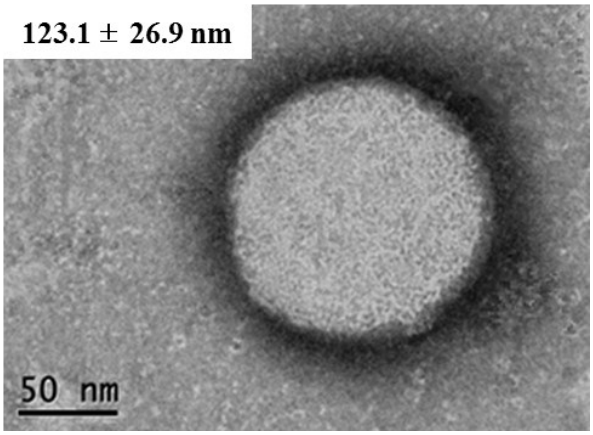

Fig. S2 The morphology of ZE-PFC/doxorubicin nanoemulsion. TEM image obtained after negative staining with 1% phosphotungstic acid solution.

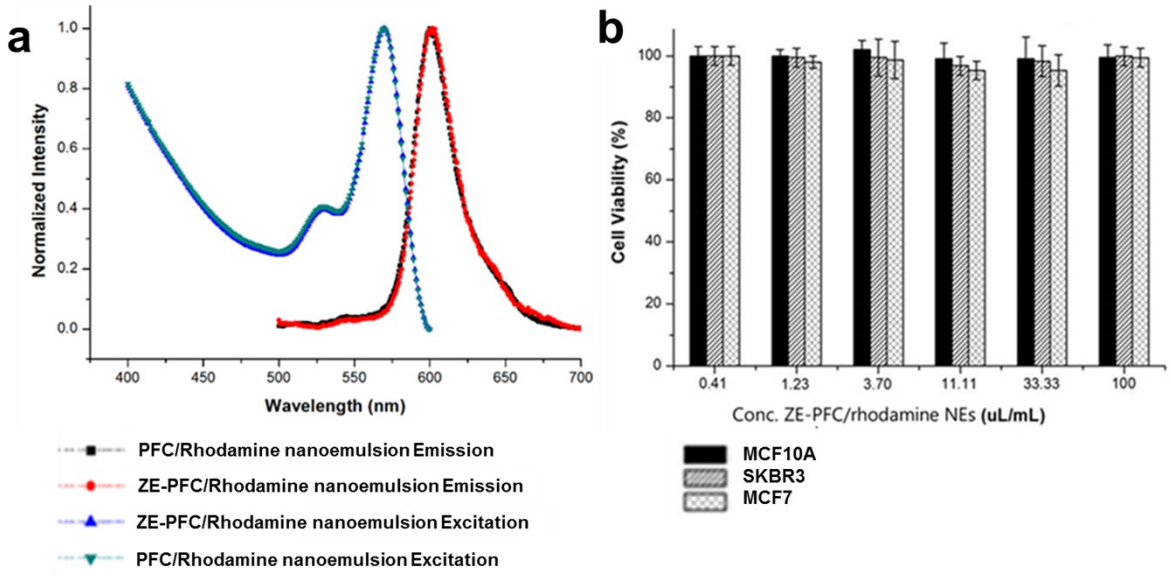

Fig. S3 Characterization of ZE-PFC/rhodamine nanoemulsions. (a) The excitation and emission spectra of the nanoemulsions. (b) In vitro cytotoxicity of the ZE-PFC/rhodamine nanoemulsions in SKBR3, MCF10A, and MCF7 cells. Cells were incubated with the ZE-PFC/rhodamine nanoemulsions for 48 h at 37°C, and the viability of the cells was evaluated with increasing concentrations of the nanoemusions using an MTT assay (sample size, n=6).

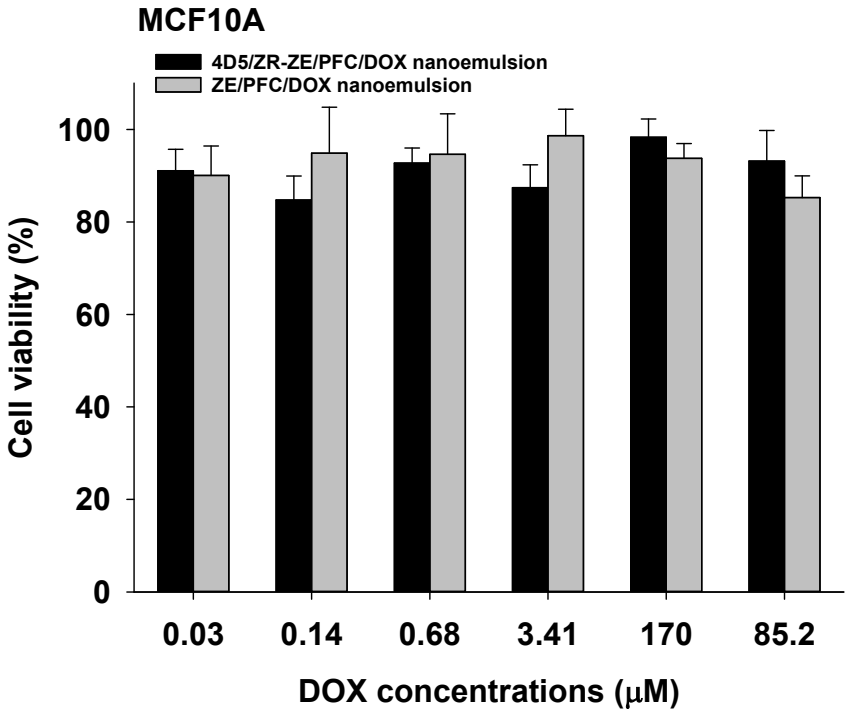

Fig. S4 Cell viability assay results of MCF-10A cells incubated with ZR-4D5 followed by incubation with various concentrations of ZE-PFC/DOX nanoemulsion (4D5/ZR-ZE/PFC/DOX nanomeulsion) or ZE-PFC/DOX nanoemulsion in the absence of ZR-4D5 (ZE/PFC/DOX nanomeulsion).

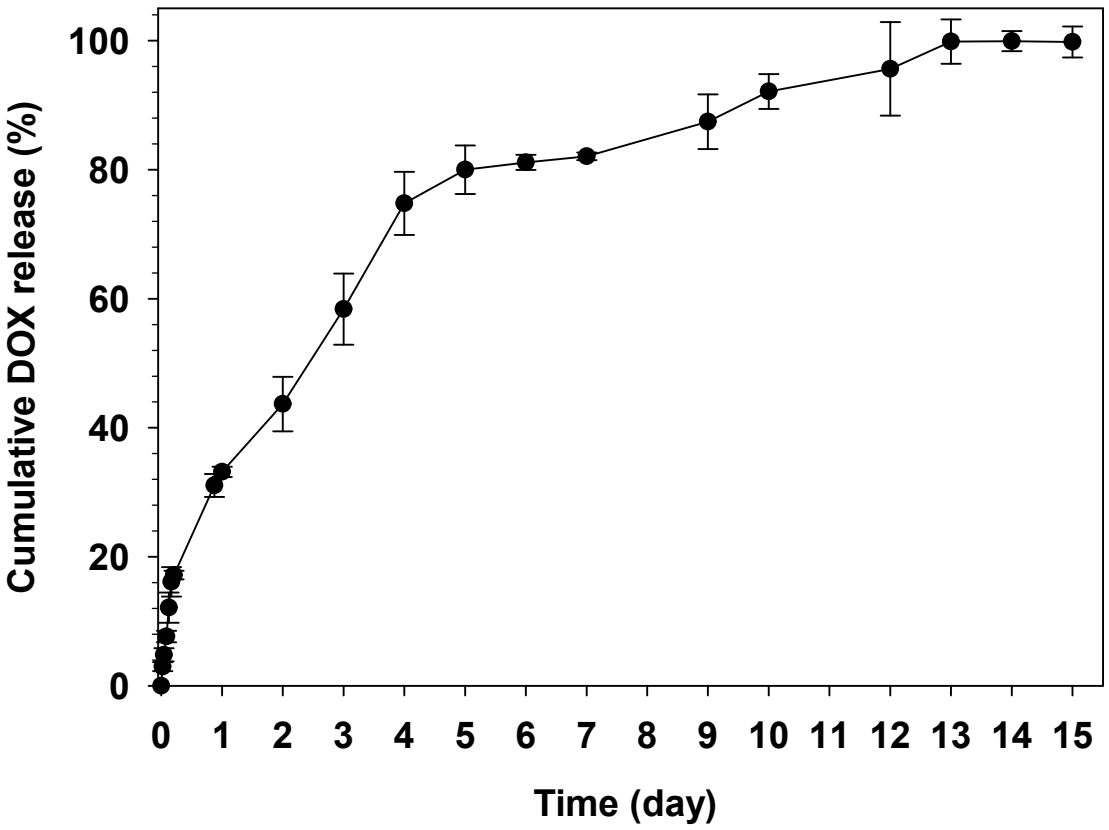

Fig. S5 Doxorubicin release profile from ZE-PFC/doxorubicin nanoemulsions in PBS (10 mM, pH 7.4) at 37°C with weak magnetic agitation. The amount of released DOX was measured for 15 days at λ_{ex}=490 nm and λ_{em}=590 nm using a fluorescence spectrometer.

Preparation of PFC/doxorubicin nanoemulsions

To synthesize the PFC/doxorubicin nanoemulsions, Perfluoro-15-crown ether (PFCE) liquids were emulsified in an aqueous solution using a lipid mixture. The lipid compositions of the PFC/doxorubicin nanoemulsions were EggPC/cholesterol/DSPE-mPEG2000/DSPE-PEG3400-NH2/ 1,2-Dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE) in a molar ratio of 73.4:20:3:2.6:1, respectively. The lipid mixture was dissolved in chloroform and was evaporated with a rotary evaporator to ensure the production of a thin lipid film, and dried in a vacuum oven (25°C) for 24 h. Doxorubicin (1mg/mL) in sterile normal saline was added to the dried lipid film. The lipid film was rehydrated with phosphate-buffered saline (PBS), and the resulting solution was sonicated in a bath sonicator followed by five cycles of freezing and thawing. The rehydrated lipid mixture (2% w/v) and PFCE solution (20% v/v) were mixed for 4 min using a homogenizer, followed by microfluidisation. A M-110S microfluidiser (Microfluidics Inc., Newton, MA) operating at a liquid pressure of approximately 20,000 psi was used for nanoemulsion preparations. The PFC/ doxorubicin nanoemulsions were stored at 4˚C.[1,2]

Preparation of ZE-PFC/doxorubicin nanoemulsions

For targeting the breast cancer cells, the ZE peptide was conjugated to the PFC/ doxorubicin nanoemulsions. The ZE peptide was reacted with the NHS ligand of PFC/ doxorubicin nanoemulsions for 2 h at room temperature. To quench the reaction, add 2-mercaptoethanol to a final concentration of 10 mM. The ZE-coated PFC/doxorubicin nanoemulsions were purified by size-exclusion with sepharose 4B column and concentrated using ultrafiltration for 30 min. Loaded DOX concentrations (0.62 mg/mL) in ZE-PFC/DOX nanoelumsions was measured by fluorescence at 490 nm (excitation) and 590 nm (emission) using a fluorescence spectrometer. Loading efficiency (%) of ZE-PFC/DOX nanoelumsions for DOX was 62 % [loading efficiency (%) = amount of DOXloading /amount of DOXinput].[1,2]

References

1. P. K. Bae and B. H. Chung, *Mol. Imaging Biol.* 2013, **15**, 401-410.
2. P. C. Gokhale, B. Radhakrishnan, S. R. Husain, D. R. Abernethy, R. Sacher, A. Dritschilo, and A. Rahman, *Br. J. Cancer.*, 1996, **74**, 43-48