Electronic Supplementary Information

Surface coating of siRNA-peptidomimetic nano-self-assemblies with anionic lipid bilayers: Enhanced gene silencing and reduced adverse effects *in vitro*

Xianghui Zeng [*a], Anne Marit de Groot [b], Alice J.A.M Sijts [c], Femke Broere [b], Erik Oude Blenke [a,c], Stefano Colombo [a], Willem van Eden [b], Henrik Franzyk [d], Hanne Mørck Nielsen [a], Camilla Foged[*a]

[a]Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, Copenhagen, 2100-DK, Denmark

[b]Division of Immunology, Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, 3584 CL Utrecht, the Netherlands

[c]Department of Pharmaceutics, Utrecht Institute of Pharmaceutical Sciences, Faculty of Science, Utrecht University, Universiteitsweg 99, 3584 CG Utrecht, The Netherlands

[d]Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, Copenhagen, 2100-DK, Denmark

E-mail: xianghui.zeng@sund.ku.dk, camilla.foged@sund.ku.dk

Formulation at N/P = 1

EGFP siRNA-loaded complexes and liposomes were prepared at an N/P ratio of 1 by using methods as previously described [1]. Since cationic nanoparticles (and non-complexed cationic peptides) in general affect cell viability [2,3], we reduced the amount of peptidomimetics applied for complexation with EGFP siRNA (N/P ratio 1). These conditions effectively resulted in nanoscale complexes and liposomes (140-200 nm, Table S1). The results indicate that even though the lower N/P ratio may be advantageous in reducing cationic reagent-mediated cytotoxicity, this could be at the expense of reduced encapsulation efficiency.

Table S1. Physicochemical characteristics of P3-C complexes and P3-L liposomes encapsulating EGFP siRNA at an N/P = 1.

Sample	Size (nm)	PDI	Charge (mV)	EE (%)
Complexes	148.5±13.5	0.27±0.01	-28±0.9	77.5±6.2
Liposomes	182.5±4.8	0.27±0.02	-43±1.5	60.3±4.7

P3 denotes the sequence of the α-peptide/β-peptoid with the alternating lysine and arginine residues repeat three times. PDI, polydispersity index; EE, encapsulation efficiency. Values represent mean ± SD (n =3).

Coating with DPPC/CHEMS

In order to verify the effects of coating with fusogenic lipid mixture (DOPE/CHEMS) versus coating with a non-fusogenic lipid mixture, DOPE was replaced with the the zwitterionic lipid dipalmitoylphosphatidylcholine (DPPC), which was used in conjunction with CHEMS for the preparation of liposomes. DPPC/CHEMS liposomes loaded with EGFP siRNA were prepared using the same methods as for the P3-L system. Two different DPPC/CHEMS molar ratios were used (9:2 and 9:4). This resulted in nanoscale liposomes (200-250 nm, results not shown). However, the silencing efficiency of the DPPC/CHEMS-based liposomes was low (< 20% at 100 nM siRNA, Fig. S1), suggesting that the helper lipid DOPE is indeed needed for efficient transfection.

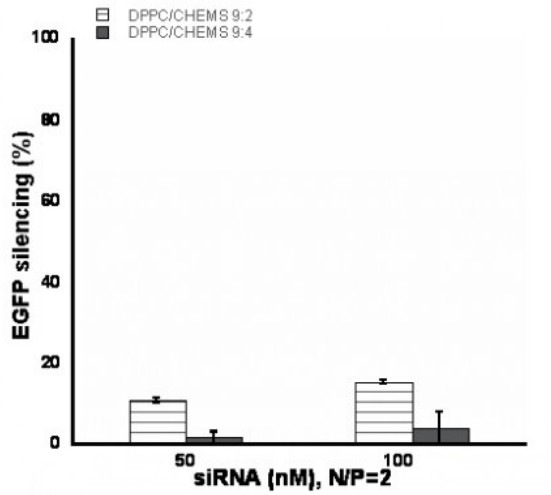

Fig. S1. Silencing effect of the DPPC/CHEMS liposomes at an N/P ratio of 2 in H1299 EGFP cells. Results are expressed as mean ± SD (n = 3).

Cell viability – siRNA + liposomal vector

As shown in Fig. S2, the cell viability was not significantly influenced after exposure to non-complexed EGFP siRNA for 48 h at concentrations ranging from 50 to 500 nM), confirming that EGFP siRNA is non-toxic *in vitro*. It also reveals that the empty liposomal vector without siRNA did not induce cell death at equivalent siRNA concentrations (200 to 500 nM).

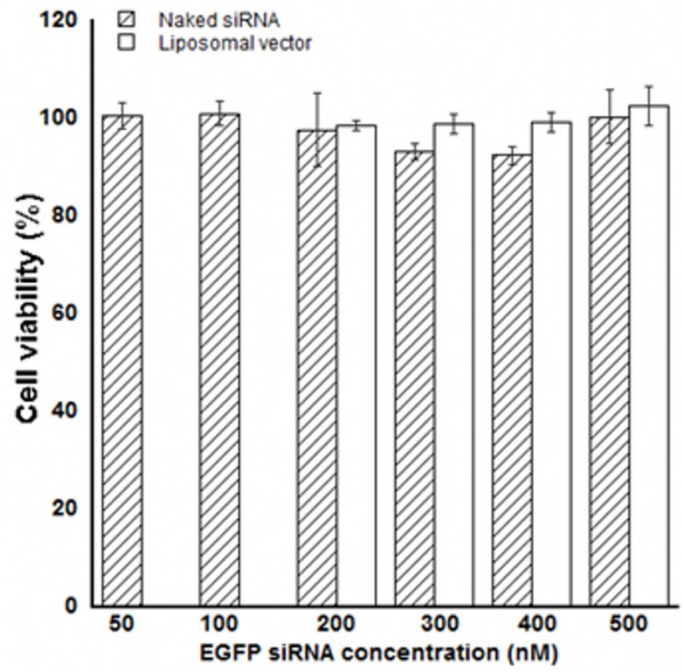

Fig. S2. The cell viability was assessed by using the MTS/PMS assay upon exposure of the human lung cancer cell line H1299 to non-complexed EGFP siRNA or empty liposomal vector at equivalent siRNA concentrations for 48 h. Values represent mean ± SD (n =3).

TLR activation at low concentrations

At lower concentrations of peptidomimetics (0.5 μM), P3-C and P3-L particles (equivalent to 50 nM siRNA), no Toll-like receptor (TLR) activation wasr observed (Fig. S3). The drug delivery systems may possibly inhibit the activation of

the TLRs *i)* by inhibiting the activation or binding to TLRs,. *ii)* by inhibiting the reporter pathway, and/or *iii)* by causing apoptosis. To exclude these possibilities, the positive control and the drug delivery systems were both added simultaneously and compared to the addition of the positive control + negative control, simultaneously. Not in all cases this possibility could be excluded. Therefore, some of the results of the TLR activation assay were invalid and these have been marked as n.a..

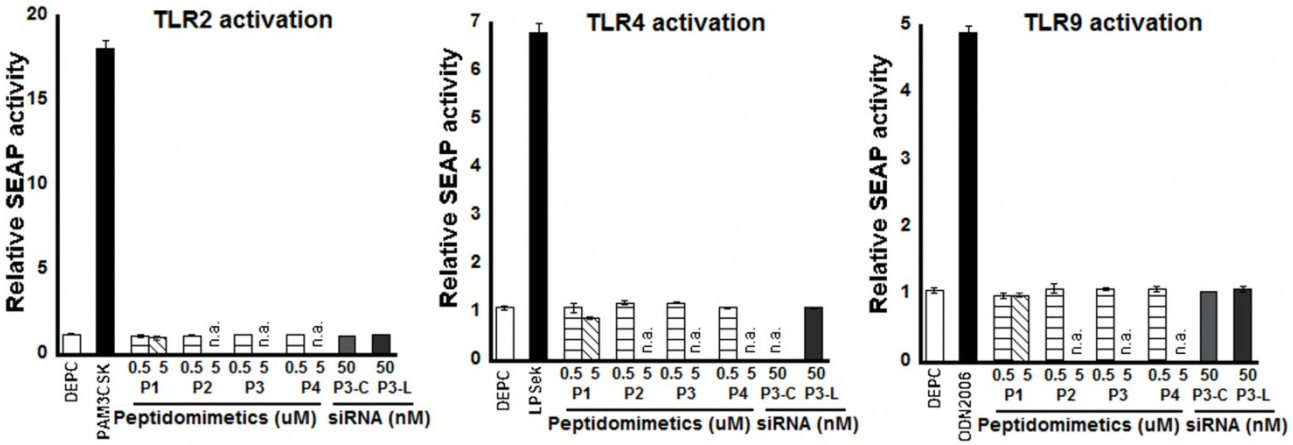

Fig. S3. Effect of the four peptidomimetics P1-P4, P3-C complexes and P3-L liposomes on the activation of Toll-like receptors (TLR2, TLR4 and TLR9), expressed as the relative SEAP activity. DEPC water and PAM3CSK/LPS/ODN were used as negative and positive controls, respectively. Results are expressed as means ± SD (n = 3) and data is representative for two independent experiments.

TEM and cryoTEM

From the overview images shown in Fig. S4, we found that the P3-L particles show a nuclear nanocomplex (Fig. S4, A) within a lipid envelope (Fig. S4, B), and cryo-TEM images confirmed the core-structure of the P3-L liposomes (Fig. S4, C).

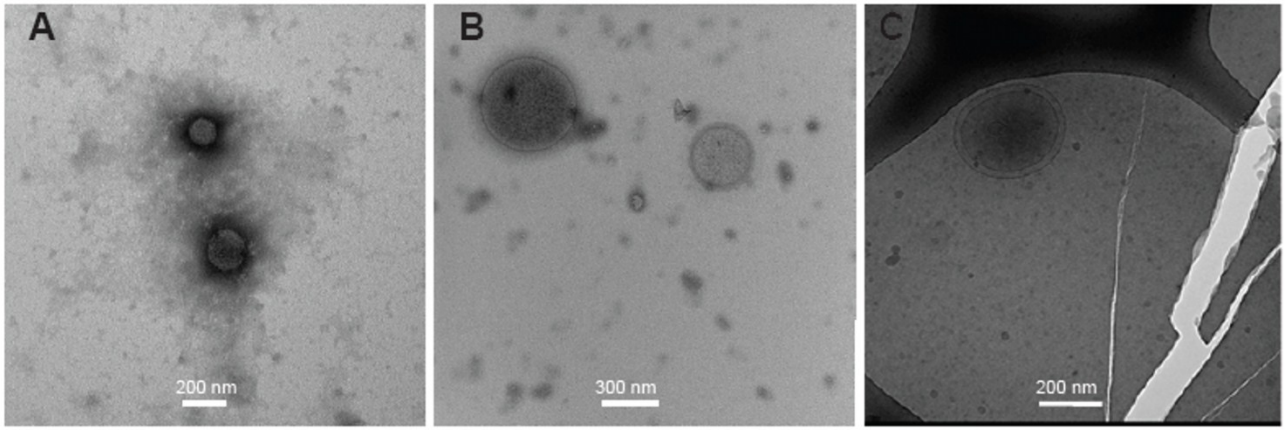

Fig. S4. (A) Representative transmission electron micrograph (TEM) image of the P3-C complexes. (B) Representative TEM image of the P3-L particles. (C) Representative cryo-TEM image of the P3-L liposomes.

Control DOPE/DPPC liposomes

In order to verify the effects of adding the acidsensitive lipid CHEMS versus adding a neutral stabilizer lipid in the liposomal system, CHEMS was replaced with the neutral stabilizer lipid DPPC, which was used in conjunction with DOPE for the preparation of liposomes. DOPE/DPPC liposomes loaded with EGFP siRNA were prepared using the same methods as for the P3-L particles. Two different DOPE/DPPC molar ratios were used (9:2 and 9:4). Thisresulted in liposomes (200-400 nm, results not shown). However, the silencing effect of these DOPE/DPPC-based liposomes was modest (< 50% at 100 nM siRNA, Fig. S5, A), and intracellular trafficking images displayed limited cytosolic

accumulation of siRNA (Fig. S5, B). The results thus indicate that the anionic CHEMS may be more advantageous for low pH-mediated endosomal/lysosomal escape, as compared to the neutral (zwitterionic) lipid DPPC.

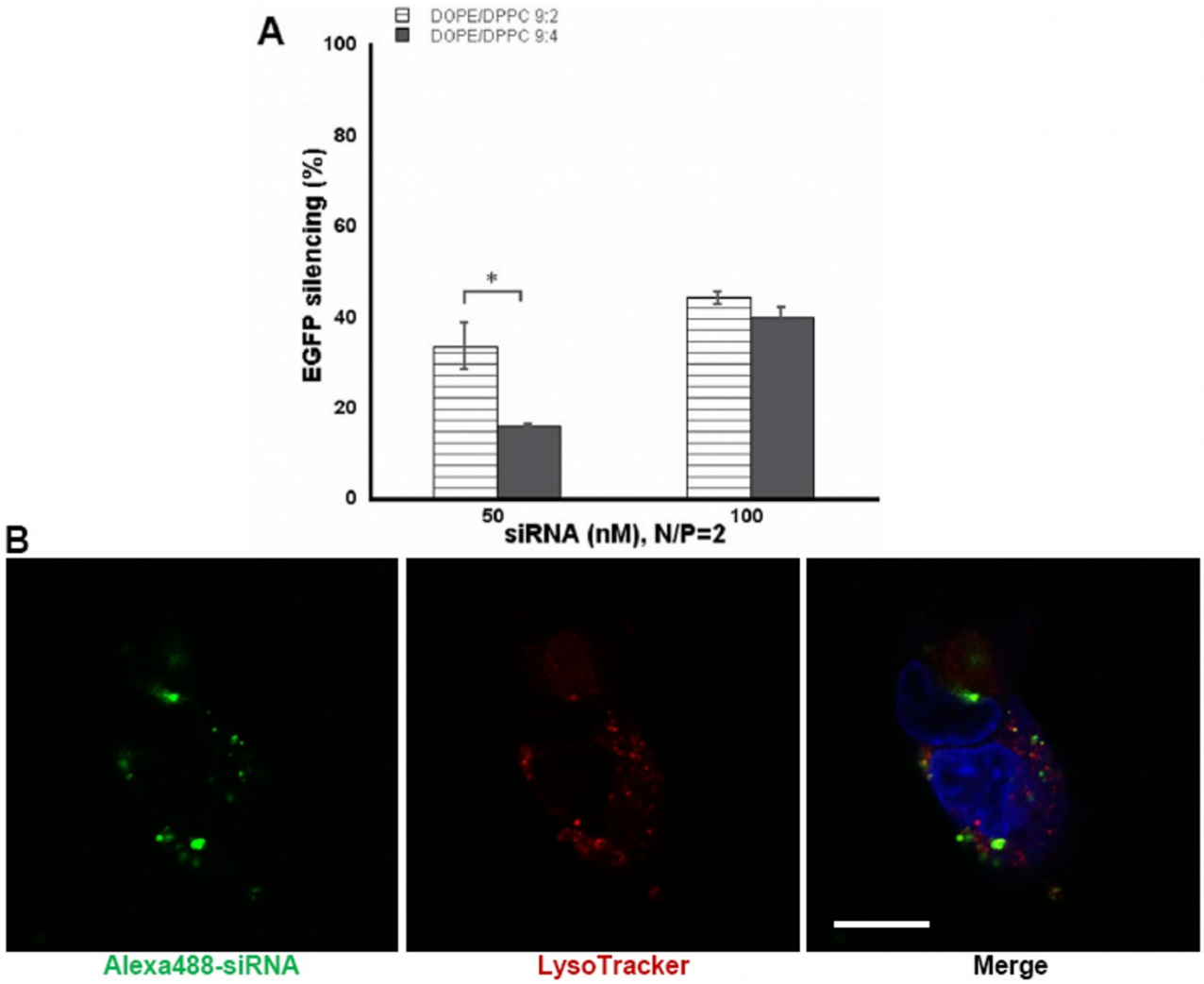

Fig. S5. (A) Silencing effect of the DOPE/DPPC liposomes at an N/P ratio of 2 in H1299 EGFP cells. Results are expressed as mean ± SD (n = 3). *p < 0.05. (B) Representative confocal laser scanning microscopy images of H1299 cells (blue: nuclei) transfected with DOPE/DPPC nanoparticles loaded with 100 nM Alexa488-siRNA. Co-localization of siRNA (green) with LysoTracker (red) 12 h after treatment with nanoparticles. Scale bar = 20 μm.

References

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