Supporting Information for:

A Definition of the Molecular Parameters for the Formation of Transfection-Effective siRNA-Cell-Penetrating Peptide Nanoparticles

Alexander H. van Asbeck[†,‡], Andrea Beyerle[‡], Hesta McNeill[‡], Petra H. Bovee-Geurts[†], Staffan Lindberg[§], Wouter P.R. Verdurmen[†], Mattias Hällbrink[§], Ülo Langel[§], Olaf Heidenreich[‡] and Roland Brock[†]

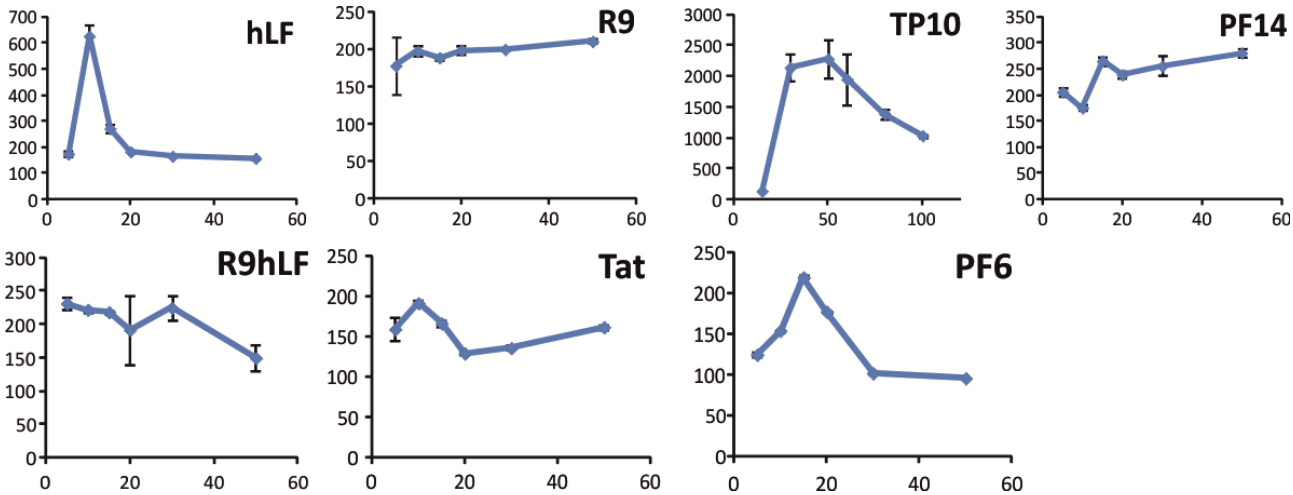
[†] Department of Biochemistry, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, 6525 GA, The Netherlands

[‡] Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, NE2 4HH, UK

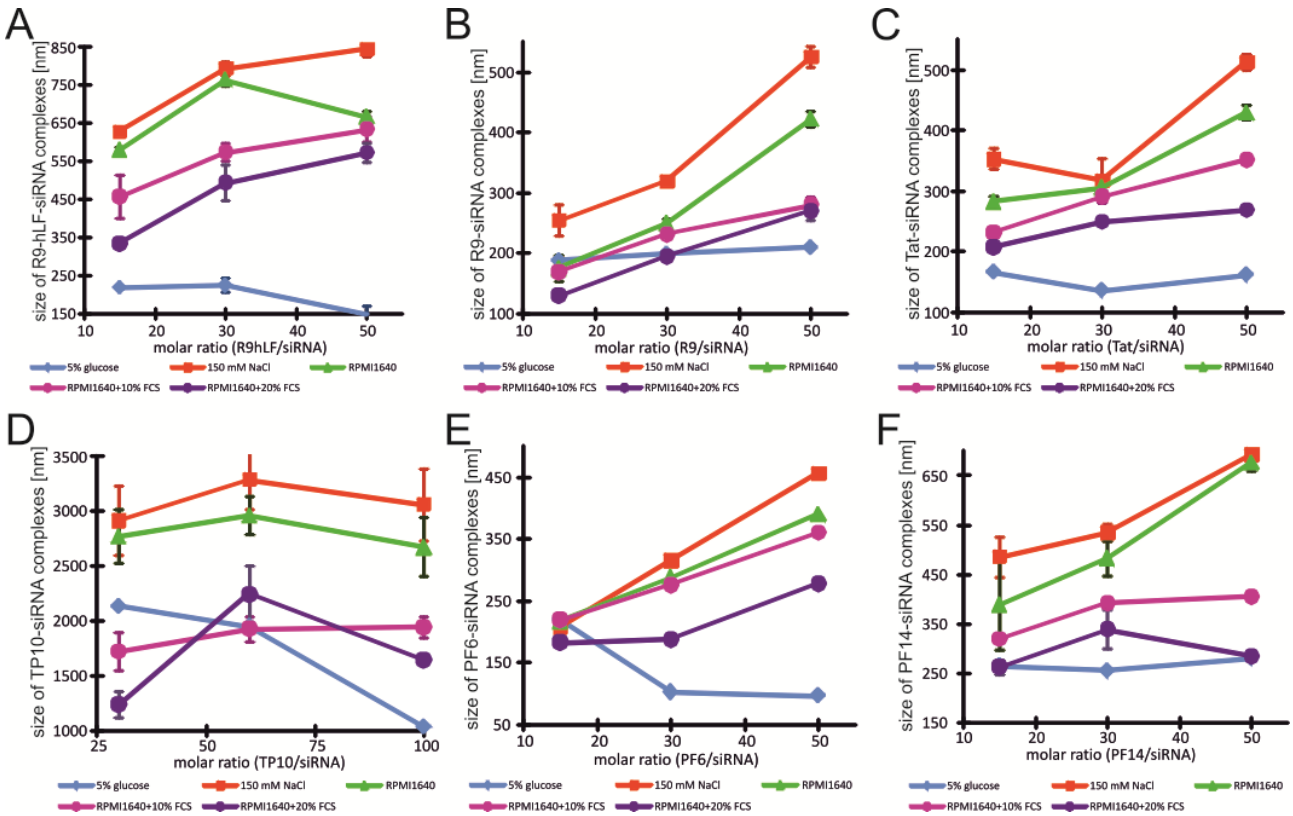
[§] Department of Neurochemistry, Stockholm University, S-10691, Stockholm, Sweden

Corresponding author: Prof. Dr. Roland Brock, Department of Biochemistry (286), Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Geert Grooteplein 28, 6525 GA Nijmegen; Ph.: +31-24-3666213; Fax: +31-24-3616413; E-mail: R.Brock@ncmls.ru.nl

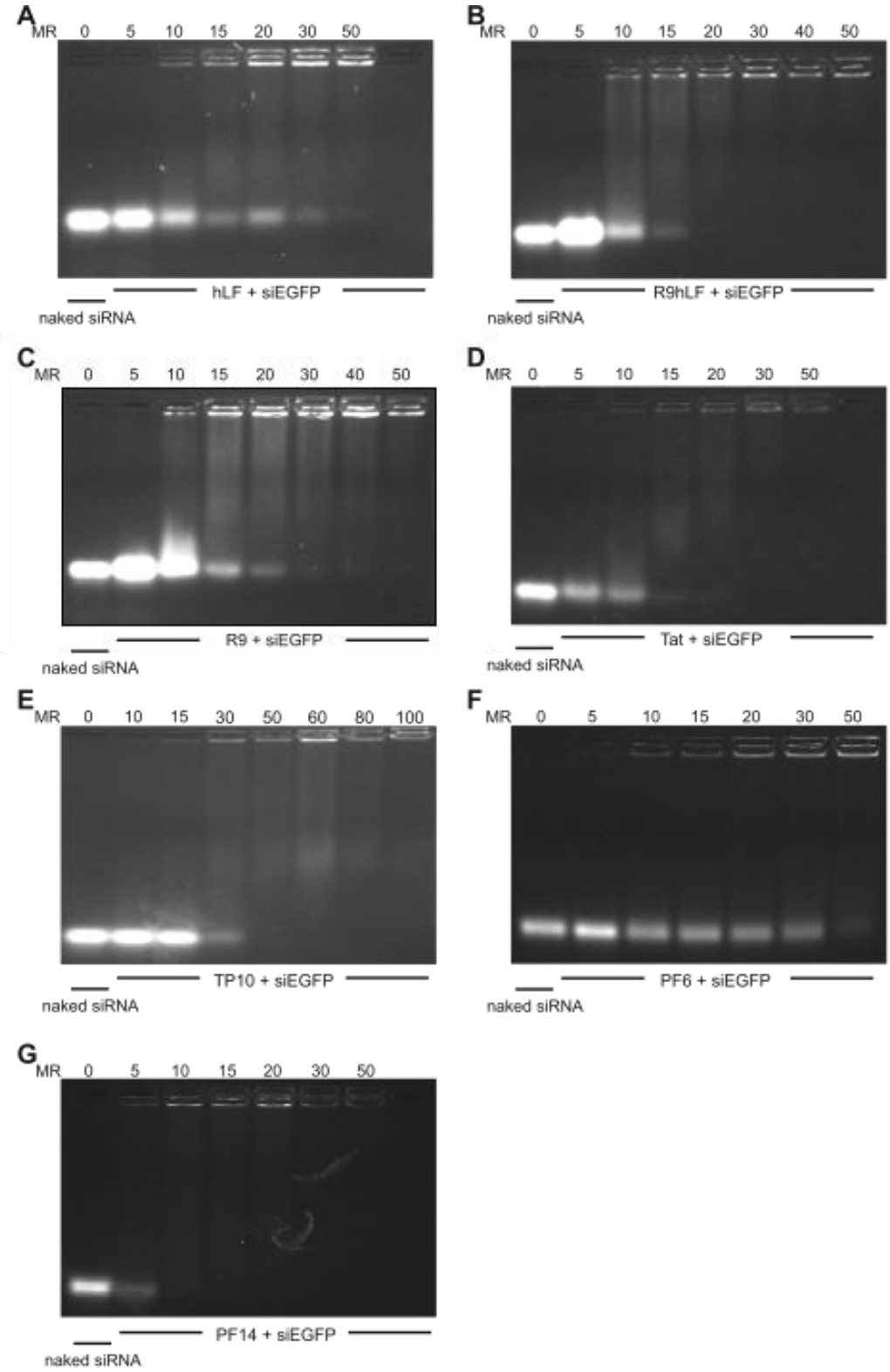
or Prof. Dr. Olaf Heidenreich, Northern Institute for Cancer Research, Paul O'Gorman Bld., Medical Faculty, Newcastle University, Framlington Pl., Newcastle upon Tyne, NE2 4AD, United Kingdom; E-mail: olaf.heidenreich@ncl.ac.uk



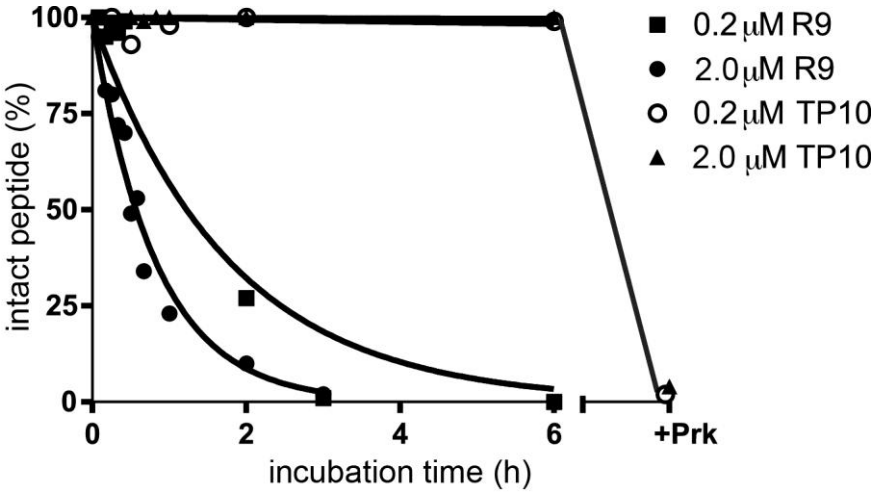
Supporting Information figure 1. Size dependence of CPP-siRNA complexes on molar ratio similar to figure 1, but depicted separately for clarity and correct depiction of standard deviation. The size of CPP-siRNA complexes, formed by mixing of CPP and siRNA (MR5, 10, 15, 20, 30, 50 for all, except TP10 = MR15, 30, 50, 60, 80, 100) in water supplemented with 5% glucose.



Supporting Information figure 2. Dependence of the size of CPP-siRNA particles on the presence of salts and proteins. The size of all peptide-siRNA combinations at various molar ratios was measured by DLS (n=3).

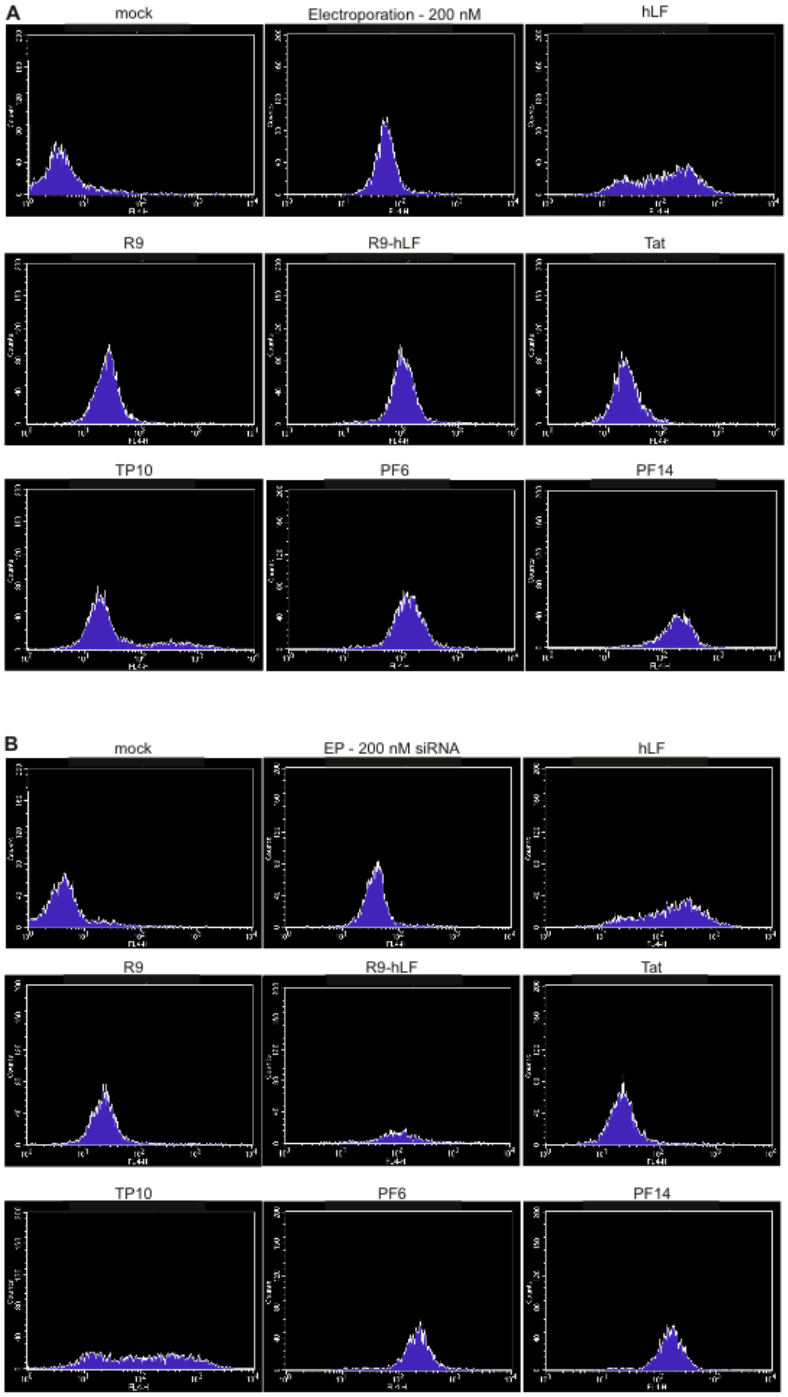


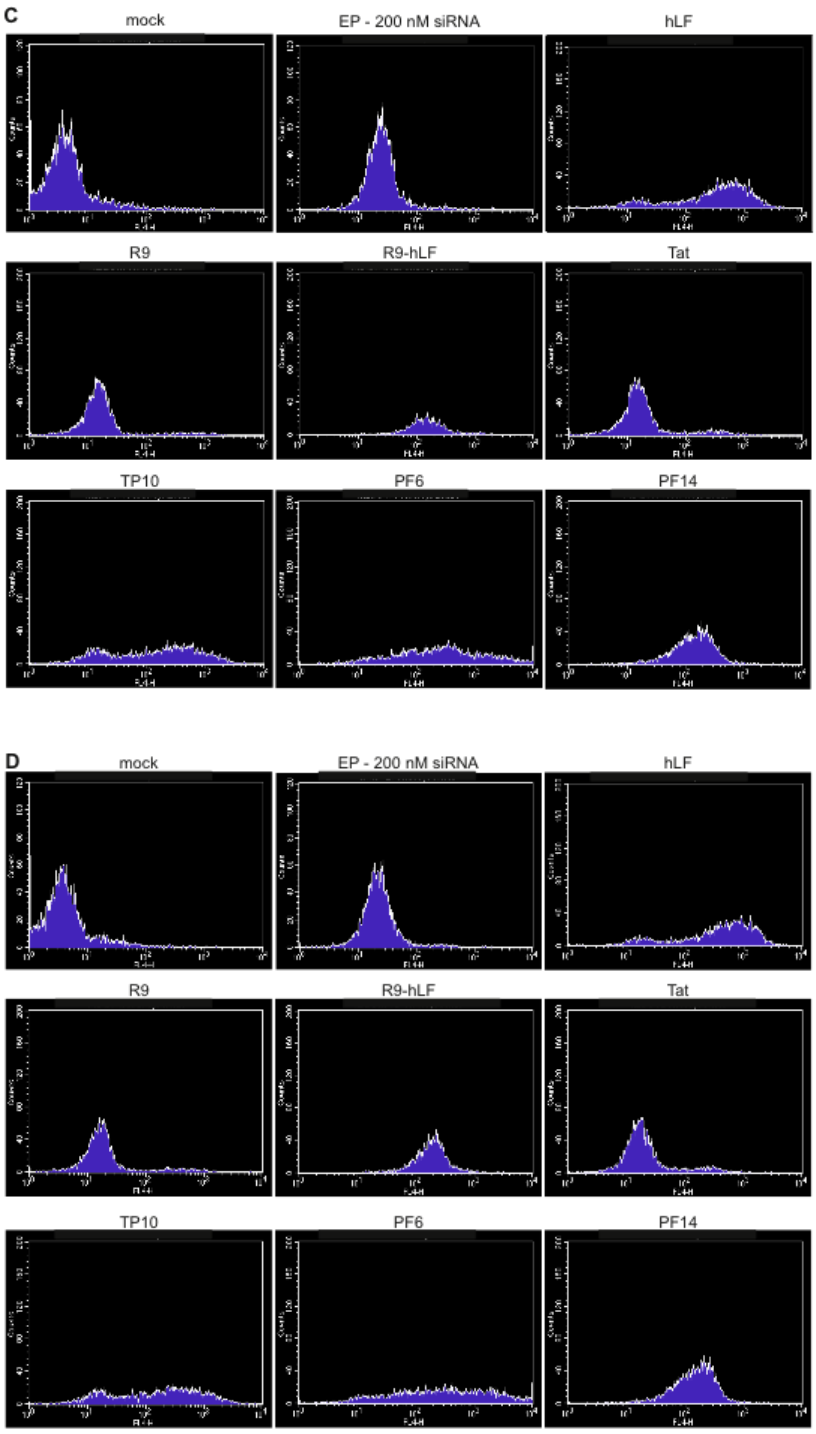
Supporting Information figure 3. Gel shift assays of CPP-siRNA nano-particles. CPP and siEGFP were incubated for 5h at room temperature and loaded on 3% agarose gel, with Gel Red (Biotium).



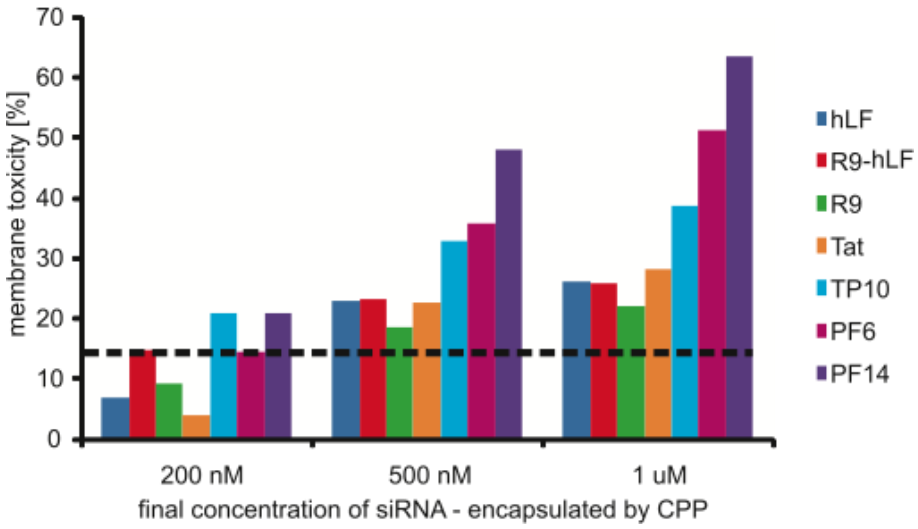
Supporting Information figure 4. Determination of peptide stability. Peptide degradation was determined by fluorescence correlation spectroscopy as described.[1] Peptides were diluted to final concentrations of 2 μM and 0.2 μM into ice-cold HEPES-buffered saline containing 10 % FCS at a total volume of 200 μl. Two different concentrations were tested in order to probe for the presence of aggregation. Immediately after addition of peptide an aliquot of 5 μl was transferred into 45 μl of a solution of protease inhibitor cocktail (Roche) and frozen. The peptide solution was incubated at 37 °C in a heating block and 5 μl aliquots were transferred into protease inhibitor cocktail at the indicated time points and frozen. After 6 h proteinase K (2 mg/ml, recombinant PCR grade, Roche) was added to fully degrade all peptide and the solution was incubated for a further 2 h at 37 °C. In preparation for fluorescence correlation spectroscopy measurements, wells of a 384 well plate (Sensoplate Black, Greiner), were blocked by incubation with 0.1 % BSA in PBS for 30 min and washed twice with PBS. Samples were thawed on ice and diluted with 200 μl of 100 mM Tris buffer pH 8.8 to obtain maximum fluorescein fluorescence. 20 μl of the sample solutions were transferred into the wells of the 384 wells plate and fluorescence correlation spectroscopy measurements performed with a Leica TCS SP5 confocal microscope with a dual channel correlation unit. Fluorescence was excited at 488 nm and detected at 500 - 550 nm. For each sample 5 measurements of 20 s each were performed. Autocorrelation functions were fitted with a model accounting for intact and degraded peptide using the ISS VISTA software. The diffusional autocorrelation time for the intact peptide was fixed to the time obtained for the undegraded sample,

the diffusional autocorrelation time for the degraded peptide fixed to the one obtained for the sample after proteinase K treatment.





Supporting Information figure 5. Association of Cy5 labeled siRNA, encapsulated by CPP, to SKNO-1 cells in RPMI1640 + 20% FCS after 20 min (A), 1h (B), 2h (C) and 3h (D), MR of hLF = 50, R9-hLF = 50, R9 = 50, Tat = 50, TP10 = 100, PF6 = 50, PF14 = 50).



Supporting Information figure 6. Membrane toxicity of CPP-siRNA complexes at high concentrations of siRNA, as measured by the release of LDH. 2% Triton X-100 solution was used as reference (100%; molar ratio of hLF = 50, R9-hLF = 50, R9 = 50, Tat = 50, TP10 = 100, PF6 = 50, PF14 = 50).

Supporting Information References

1. Ruttekolk, I. R.; Witsenburg, J. J.; Glauner, H.; Bovee-Geurts, P. H.; Ferro, E. S.; Verdurmen, W. P.; Brock, R. The intracellular pharmacokinetics of terminally capped peptides. *Mol. Pharm.* **2012**, *9*, 1077-1086