OMTN, Volume *7*

Supplemental Information

The Formation of Nanoparticles between Small Interfering RNA and Amphipathic Cell-Penetrating Peptides

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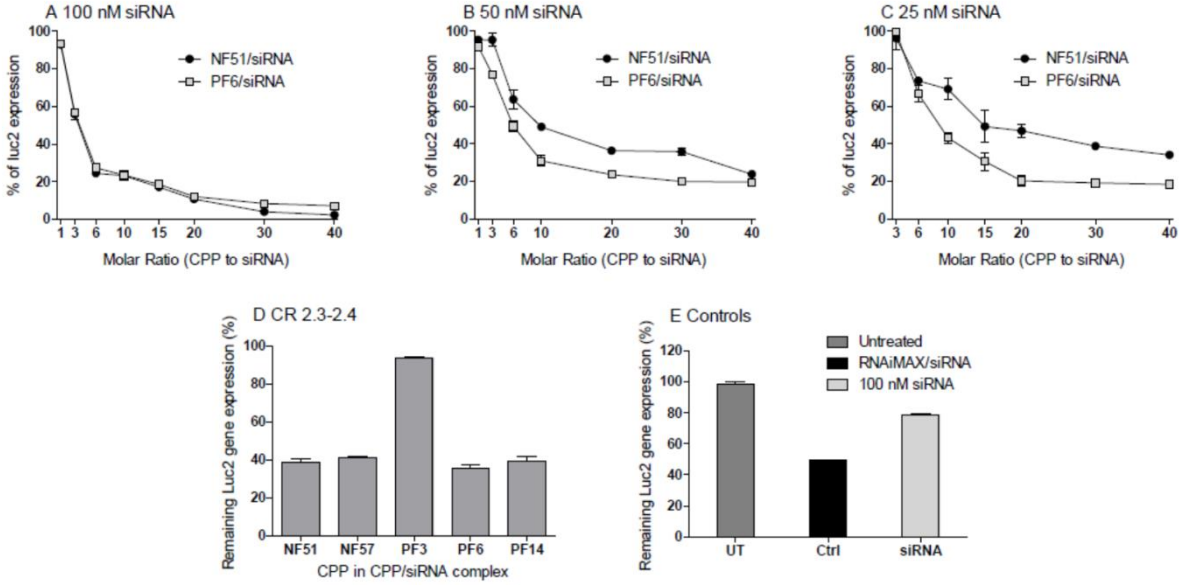
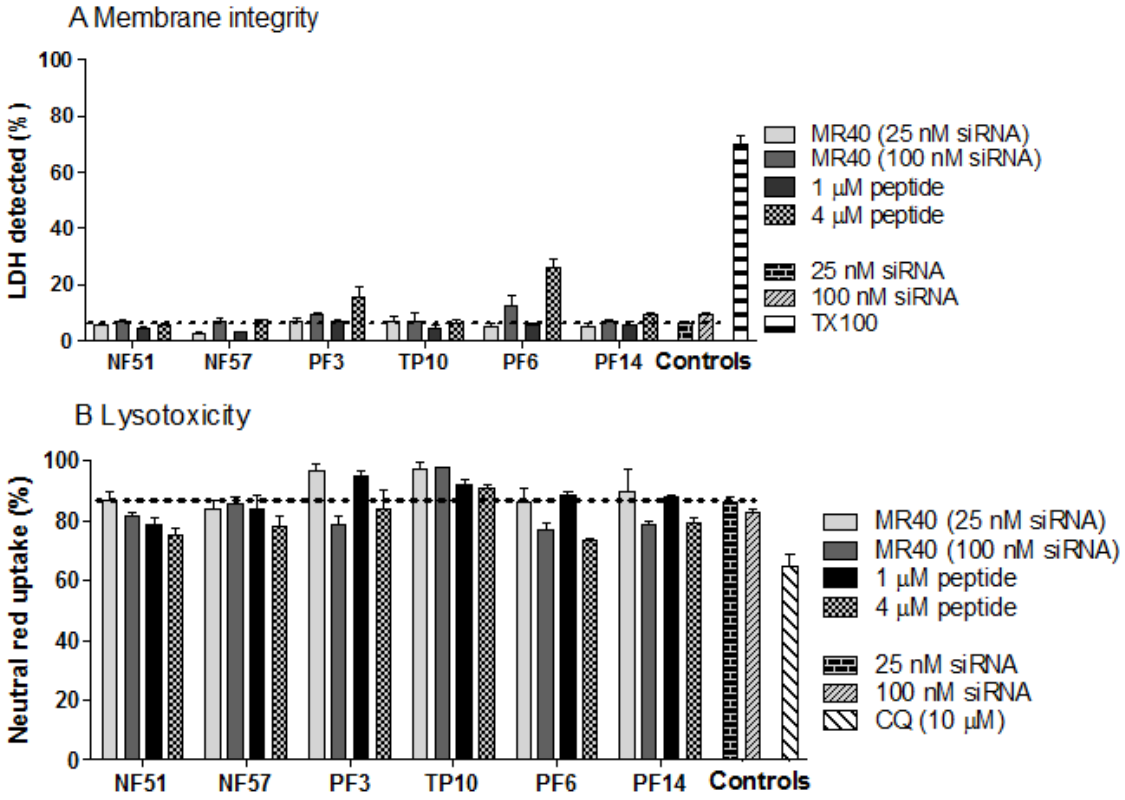
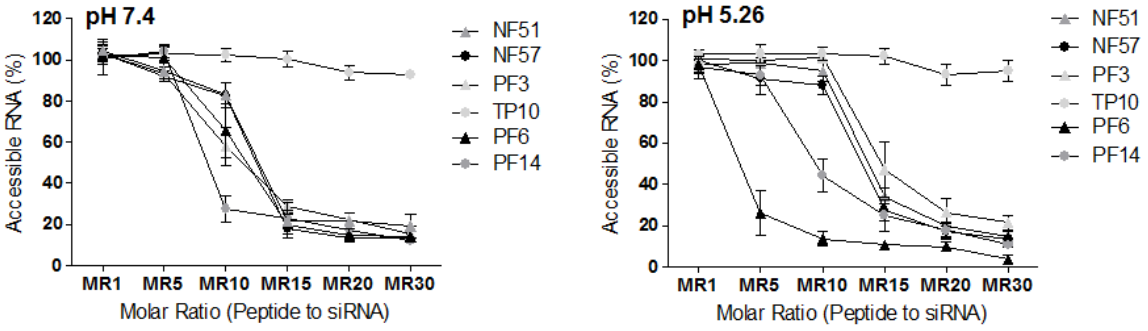
Supplementary Figure 1. Down-regulation of luc2 gene in stable U87-luc2 cells in serum and serum free conditions and toxicity of peptide or CPP/siRNA complexes at MR40. Molar ratios in the range of 1 to 40 (CPP excess to siRNA) were used to down-regulate luc2 gene. SiRNA final concentration 100 nM (**A**) 50 nM (**B**) or 25 nM (**C, D**) were used to form complexes with NF51 or PF6. Peptide concentration was dependent on molar ratio. Complexes were formed in 1/10 of final treatment volume. Results were normalised to untreated cells (0% downregulation). On graph **D** down-regulation is expressed charge ratio 2.3-2.4 with final siRNA concentration of 25 nM was used to form complexes with CPPs. The molar ratio corresponding to the charge ratio is as follows NF51/NF57/PF3 and TP10 complexes with siRNA at MR25, PF14/siRNA at MR20 and PF6/siRNA at MR10. **E** contains results with controls that were run in the same setting as experiments on figures A-D. RNAiMax was used according to manufacturer´s protocol, free siRNA was added at a final concentration 100 nM and for untreated cells only MQ was added instead of complexes.



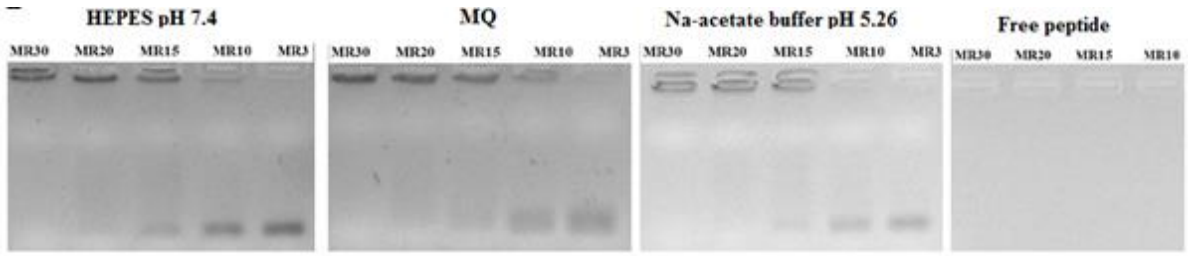
Supplementary Figure 2. Toxicity of CPP and CPP/siRNA complexes in serum free conditions. A Lactate dehydrogenase leakage detected from reduced serum media after 6 h incubation with complexes or free peptide. Dotted line runs from the same level as 25 nM siRNA treated cells. **B** Neutral red uptake reduction detected from cell lysate after 6 h incubation with complexes or free peptide. Dotted line runs from the same level as 25 nM siRNA treated cells. Results are shown as mean of at least 6 experiments with SEM.



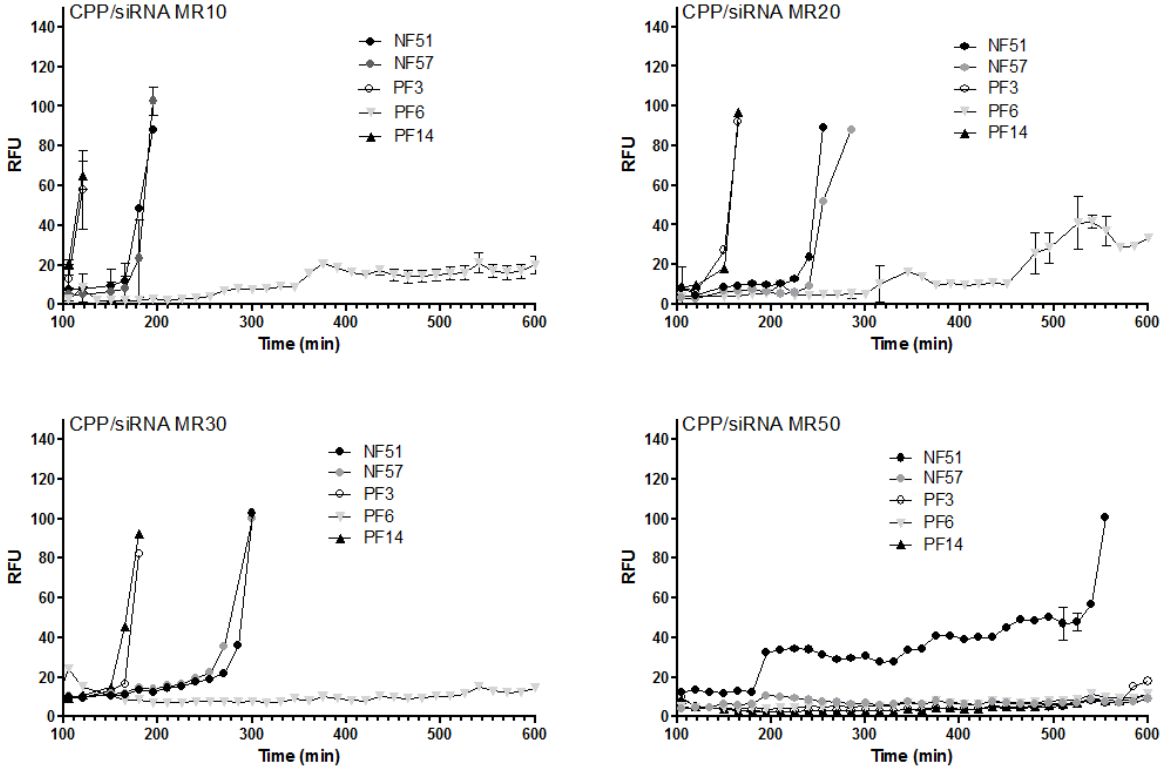
Supplementary Figure 3. Complex formation assessed by PicoGreen intercalation assay. Complexes were formed in HEPES buffer (pH 7.4) or Acetate buffer (pH 5.26), incubated at RT for 1 h and PicoGreen was added. The percent of accessible siRNA was measured after 5 min of incubation. Results are normalized to free siRNA in the same concentration as used in the complexes.



Supplementary Figure 4. Representative gel electrophoresis of pre-formed complexes and sizes of NF51/siRNA and PF/siRNA complexes in buffers. In panel is shown gel electrophoresis of complexes (NF51, siRNA) that are formed in different buffers (HEPES, Sodium acetate buffer) or in MQ. Formed at room temperature for 1 h and transferred to 1.8% agarose gel containing ethidium bromide (0.5 µg). After gel electrophoresis bands were visualized under UV-light. Lower panel shows gel electrophoresis of all tested peptides at selected MRs.



Supplementary Figure 5. Enzymatic degradation of pre-formed complexes. Resistance to degradation was measured for each peptide at the same MRs. To pre-formed complexes PicoGreen dye was added and accessible siRNA was measured. Proteinase K was added to pre-formed complexes and accessibility of siRNA was measured over a period of 10 h.



Supplementary Table 1. Complex packing and siRNA protection assessed by gel electrophoresis and fluorescent dye intercalation assay. Shown in table are the molar ratios (MR) needed to pack siRNA into a particle or MR at which most of siRNA is protected.

CPP	Gel electrophoresis*			PicoGreen assay†		
	pH 5.26	MQ	pH 7.4	pH 5.26	MQ	pH 7.4
NF51	10	10	15	15	15	15
NF57	10	10	15	15	15	15
PF3	10	10	15	15	15	15
TP10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PF6	3	6	10	6	10	15
PF14	10	10	10	10	10	10

*Molar ratio (MR) at which free siRNA band is not detected

†Molar ratio (MR) at which siRNA is packed and fluorescent dye can not intercalate into siRNA

Supplementary Table 2. Hydrodynamic diameters of PF14 and NF51 particles with siRNA by DLS at MR30. Results are shown as mean of three independent measurements with deviations.

CPP/siRNA	**milliQ water**	**HEPES, pH 7.4**	**MES, pH 6.3**	**Na-acetate buffer pH 5.3**
NF51/siRNA	115.4 ± 18.2	103.9 ± 3.8	101.5 ± 23.5	97.9 ± 1.5
PF6/siRNA	106.7 ± 3.7	156.3 ± 18.3	101.4 ± 0.7	179.1 ± 1.4