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Supplementary information

Ruthenium dendrimers as carriers for anticancer siRNA

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Structures of siRNA

Three different siRNAs and one scrambled siRNA were synthesized (Darmacon, Inc). All siRNA were labelled with fluorescein for electrophoresis runs. The sequences of the siRNA were:

Bcl-xl,
Sense: 5'-CAG GGA CAG CAU AUC AGA Gtt 3'
Antisense: 5'-C UCU GAU AUG CUG UCC CUGtt 3'

Bcl-2,
Sense: 5'-G CUG CAC CUG ACG CCC UUCtt 3'
Antisense: 5'-GAA GGG CGU CAG GUG CAG Ctt 3'

Mcl-1,
Sense: 5'-GGACUUUUAUACCUGUUAUtt 3'
Antisense: 5'-AUAACAGGUAUAAAAGUCCtg 3'

Scramble,
Scrl a: 5'-ACUCUAGCGGCACCAUCGUGCCtt 3'
Scrl b: GGCACGAUGGUGCCGCUAGAGUtt3'

Computer modeling details (Experimental section)

3D computer model of CRD13 dendrimer (first generation) structure was created using dendrimer builder, as implemented in the Materials Studio software package from BIOVIA (formerly Accelrys). The RESP technique1 was used for calculation of dendrimer atoms partial charges. For this charge parameterization the R.E.D.-IV tools were used. The necessary Quantum Mechanics (QM) calculations (QM structure minimisations, molecular electrostatic potential (MEP) calculations) were done using GAMESS. The DFT method wB97X-D together with 3-21G(d) basis set was used for all charge-related QM calculations and the MEP potential was fitted on Connolly molecular surface. GAFF force field (Generalized Amber Force Field), was used for parameterization of dendrimers. 5 Missing force field parameters were fitted by minimizing the differences between QM and force field based relative energies of properly chosen molecular fragments. QM energies were calculated using DFT method wB97X-D and 3-21G(d, p) basis set. These QM calculations were again done using GAMESS software. Fitting was accomplished using paramfit routine from Amber16 software. Slightly adjusted Van der Waals (VDW) parameters for Si atoms from MM3 force field and exact Ru VDW parameters form MM3* force field were used in this study. The siRNA (Bcl-xl) computer model was created using NAB module, which is a part of simulation package Amber16, and parameterized using ff99OL3 (A, C, G, U) and OL15 (T) force fields. First the individual components (dendrimer, siRNA) were solvated in cuboid box with explicit water (TIP3P model) with the proper number of Na+ and Cl− ions to preserve neutrality of the system and to ensure the correct ionic strength (0.15 M). The minimum distance between the solute atoms and the wall of the periodic box was set to 19 Å and 24 Å in case of single components and complexes, respectively. These stand-alone systems were minimized (5000 steps with 5 kcal/(mol $Å^2$) restraint + 5000 steps without restraint), heated (100 ps NVT) to 295 K and equilibrated (siRNA 2 ns and dendrimer 20 ns, NPT). From this equilibrated components the initial dendrimer/siRNA systems were created using UCSF Chimera software, which was also used for final visualizations. Two initial dendrimer/siRNA systems were created. The first one (system A) was composed of 2 dendrimers and 1 siRNA (+/- charge ratio 0.2), the second one (system B) contained 20 dendrimers and 1 siRNA (+/- charge ratio 2). The same steps as in the case of individual components were done with complexes up to heating which was followed by 150 ns (system **A**), 70 ns (system **B**) molecular dynamics simulation in NPT ensemble. Hydrogens were constrained with the SHAKE algorithm to allow 2 fs time step and Langevin thermostat with collision frequency 2 ps^{-1} was used for all MD runs. Particle mesh Ewald method (PME) was used to treat long range electrostatic interactions under periodic conditions with a direct space

cutoff of 10 Å. The same cutoff was used for van der Waals interactions. The pmemd.cuda module from Amber16 package was employed for Molecular Dynamics simulations. In the case of the system **A** the last 10 ns of the whole simulation were used for energetic analyses (enthalpic contribution (dH) calculated with 0.1 ns sampling step (i.e. 100 frames used), entropic contribution (TdS) calculated using 0.5 ns sampling step (i.e. 20 frames used)) by using the molecular mechanics/Poisson–Boltzmann surface area (MM/PBSA) methodology as implemented in Amber16 routine MMPBSA.py to obtain estimates of free energies of binding. PBSA calculations were done using Adaptive Poisson-Boltzmann Solver sander.APBS from Amber16. The probe radius used for calculation of solvent accessible surface area (SASA) was 1.4 Å. Default APBS value a = 0.02508 kcal*mol-1*Å^{-2} of cavity surften parametr for calculation of the non-polar solvent contribution ENP = a*SASA was used. The dielectric constant of the solute was set to one and in the case of solvent to 80. Normal mode analysis was made taking into account solvent using HCT Generalized Born implicit solvation model.

Table 1. MMPBSA estimate of dendrimer/siRNA free energy of binding (dG) together with all important energetic components (mean values). The units are [kcal/mol]. VDW is van der Waals contribution to free energy of binding (f.e.b.); ENP is an estimate for non-polar solvation contribution to f.e.b.; EPB is the polar (electrostatic) solvation contribution to f.e.b. (desolvation penalty) calculated by Poisson-Boltzmann equation solver; dH is total enthalpic part of f.e.b.; dS is change in entropy due to binding and T is absolute temperature. dH=VDW+EEL+EPB+ENP, dG=dH-TdS.

WDV	-54.29
EEL	-2173.30
EPB	2171.96
ENP	-25.93
dH	-81.57
TdS	-24.20
dG	**-57.37**

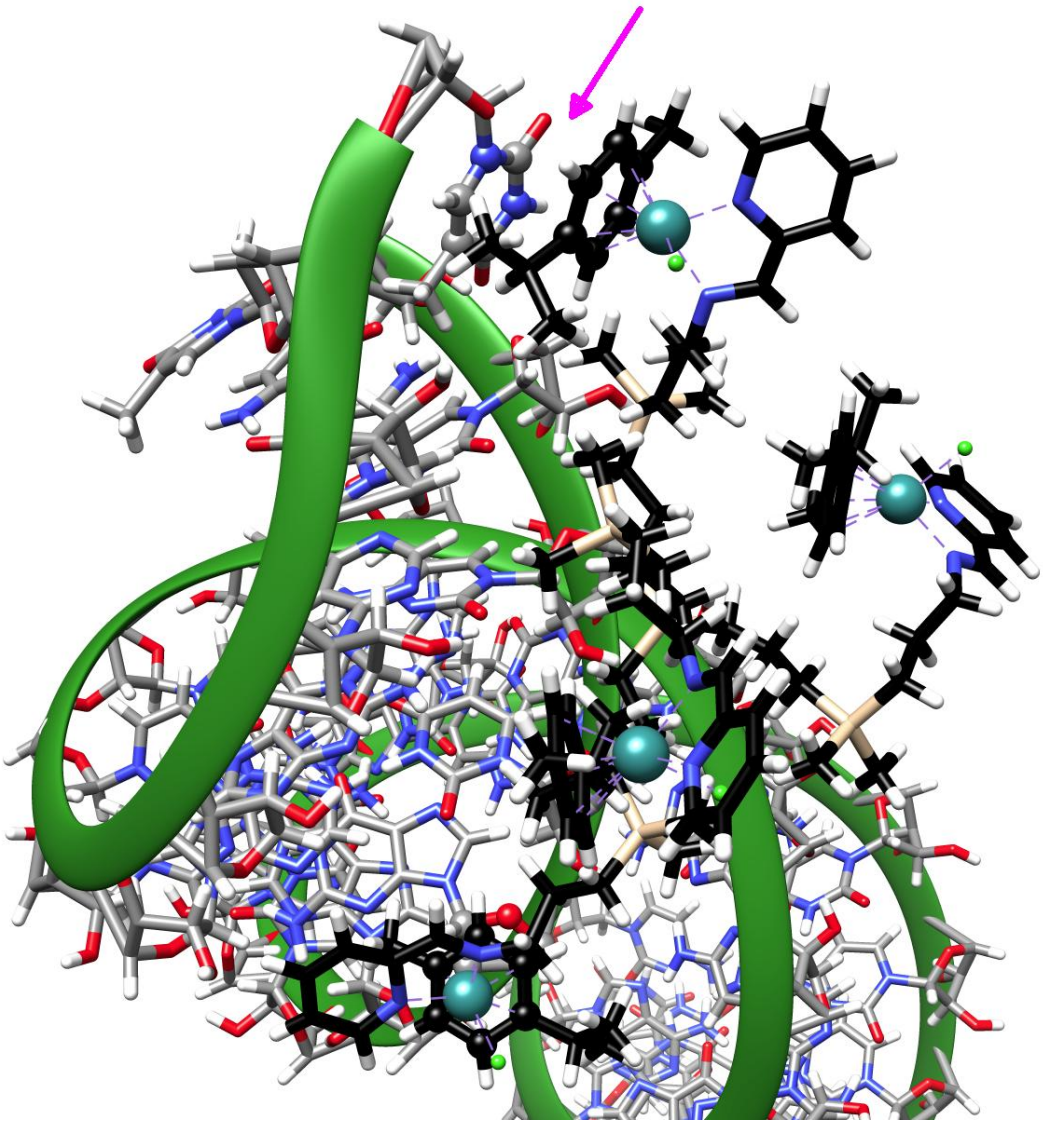

Figure 1. Detailed view of siRNA/CRD13 complex (1 siRNA + 2 CRD13 system). Colors: C - gray (siRNA) or black (dendrimer), O - red, H - white, N - blue, Si - beige and the ruthenium atoms are presented as turquoise colored balls. The magenta arrow indicates siRNA/CRD13 ring-ring interaction.

Gel electrophoresis

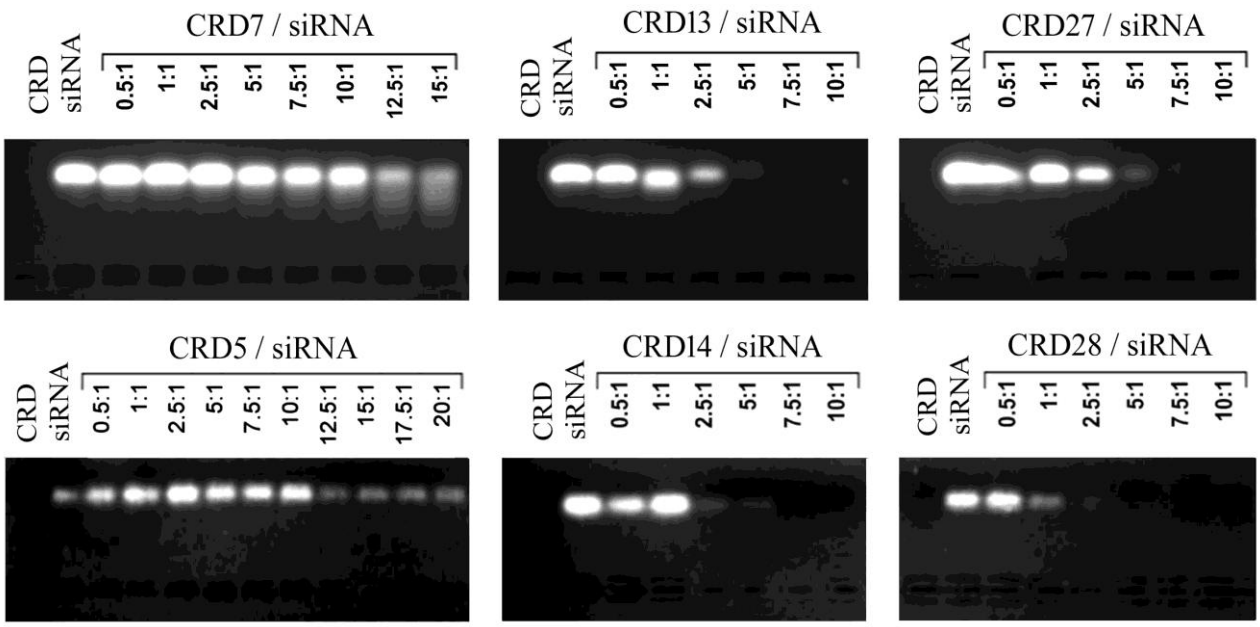

Figure 2. Analysis of the formation of dendrimer/siRNA complexes. 2 μM fluorescein-labeled siRNA (**siMcl-1**) were complexed with carbosilane ruthenium-terminated dendrimers in 10 mM Na-phosphate buffer, pH 7.4. Dendriplexes were prepared at different dendrimer/siRNA charge ratios. The first lane shows the migration of dendrimer, and the second reflects the migration of non-complexed siRNA.

Transmission Electron Microscopy (TEM)

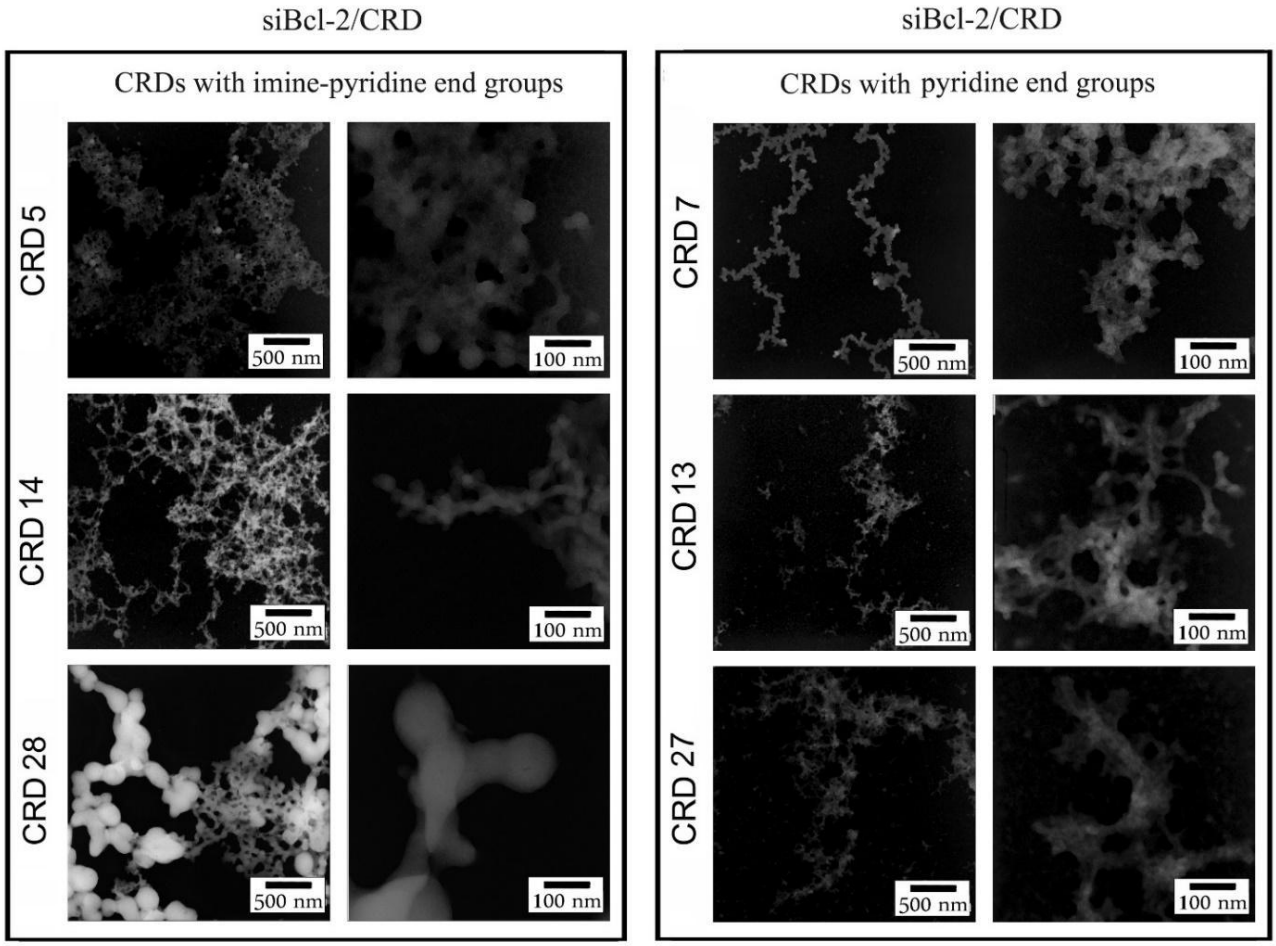

Figure 3. TEM microimages of dendrimer/siBcl-2 complexes. The co-ordination of ruthenium: (left panels) – imine-pyridine groups, CRD7, CRD13, CRD27; (right panels) -pyridine groups CRD5, CRD14, CRD28. The following charge ratios used to form dendrimer/siRNA complexes were 10:1 (generation 0) and 5:1 (generations 1 and 2). The concentration of siRNA was 3 μM. Complexes were formed in 10 mM Na-phosphate buffer, pH-7.4. Magnifications of 5,0000 and 100,000x were used to take the images. Bar = 100 nm and 500 nm. The colour of the microphotographs has been inverted.

Circular dichroism

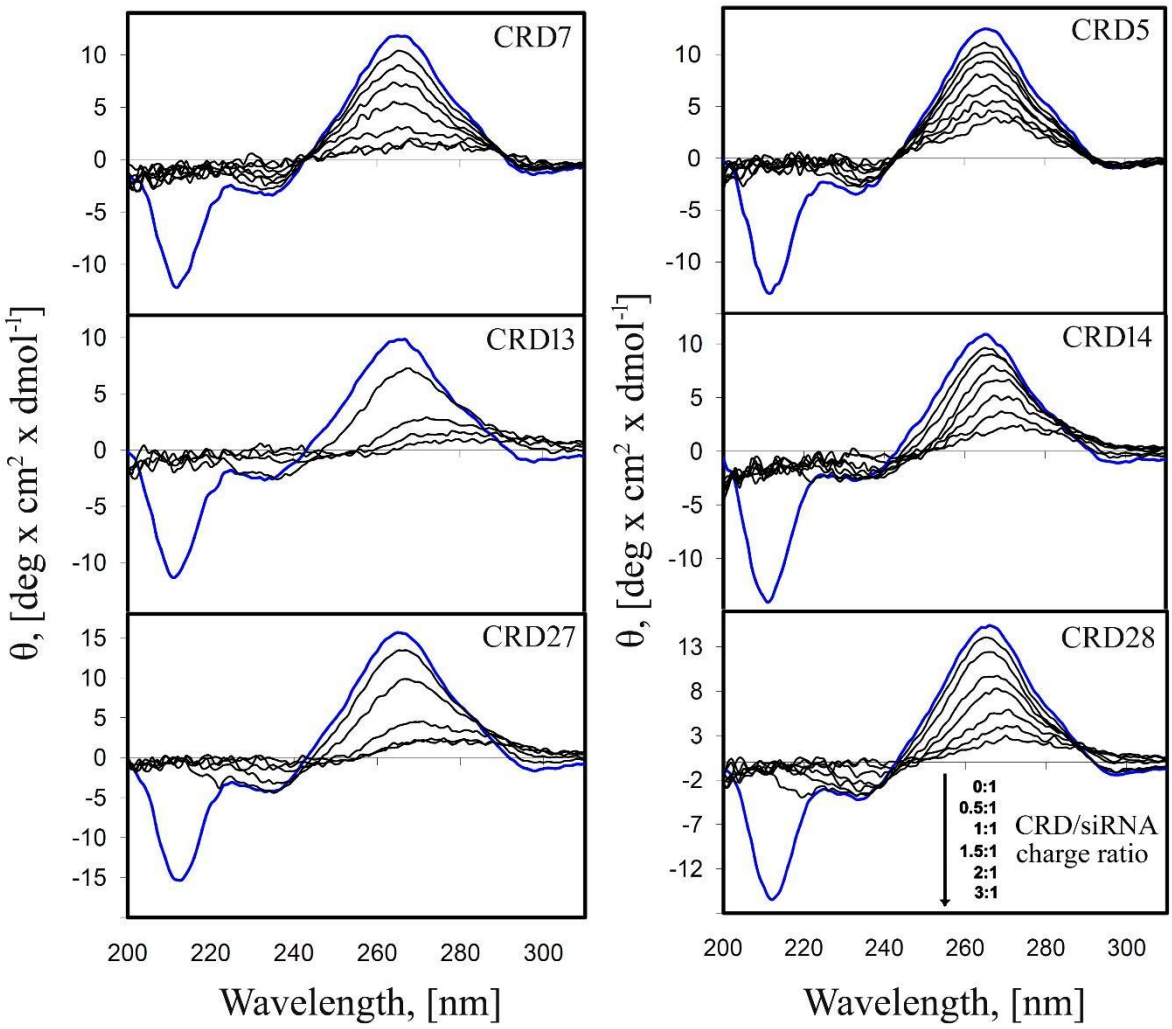

Figure 4. CD spectra of siBcl-2 in the presence of: CRD dendrimers ending with imine-pyridine groups, CRD7, CRD13, CRD27 (left panels); CRD dendrimers ending with pyridine groups CRD5, CRD14, CRD28 (right panels). siRNA concentration 2.5 μM, wavelength 200-310 nm, scan speed 50 nm/min, step resolution 0.5 nm, bandwidth 1.0 nm, response time 4 s, Na-phosphate buffer 10 mM, pH 7.4.