Electronic Supplementary Material (ESI) for Nanoscale.
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Supporting Information for "***Steered Molecular Dynamics Simulations Reveal Self-Protecting Configuration of Nanoparticles during Membrane Penetration***"

Yousef Nademi,[†] Tian Tang,[§] and Hasan Uludağ[†, ¶, ‡]

[†] Department of Chemical and Materials Engineering,
[§] Department of Mechanical Engineering,
[¶] Department of Biomedical Engineering, and
[‡] Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada

S1: Simulation of CA-NP with a Different Initial Configuration

The initial structure of CA-NP discussed in the main text was generated from a previous study[1] where the original system contained 4 siRNAs and 18 PEI molecules. Two siRNAs and 6 PEIs were selected from the original system (already equilibrated) and the COM distance between the 2 siRNA molecules was 32.5 Å. In Section 3.1 of the main text, it was observed that the CA-NP had a much looser structure compared with native NP and LA-NP. To ensure that this is not caused by the specific initial configuration, we conducted a separate simulation with a more compact initial CA-NP structure. This structure also contained 2 siRNAs and 6 PEIs, but the initial COM distance between the siRNAs was much closer (27 Å).

Fig. S1 shows R_g of the CA-NP and COM distance between the 2 siRNAs as a function of simulation time for this new simulation. The horizontal time axis starts at 5 ns since the NP is subjected to restrained simulations with little structural changes during the first 5 ns. It is observed that R_g has an initial increase and decrease during the first 15 ns of the simulation, but ultimately reaches a stable value of 25.45±0.338 Å (data collected from the last 20 ns). The COM distance has a stable value during the first 15 ns of the simulation followed by an increasing trend, indicating siRNA molecules are moving apart compared to their starting configuration. The COM distance averaged over the last 20 ns is 29.33±0.556 Å. The final equilibrium values of R_g and COM distance are close to those given in the main text which resulted from a very different initial configuration. Therefore, it can be concluded that the looser structure of CA-NP, compared with native NP and LA-NP, is not caused by the specific initial configuration but rather due to the steric hindrance of the bridging PEIs.

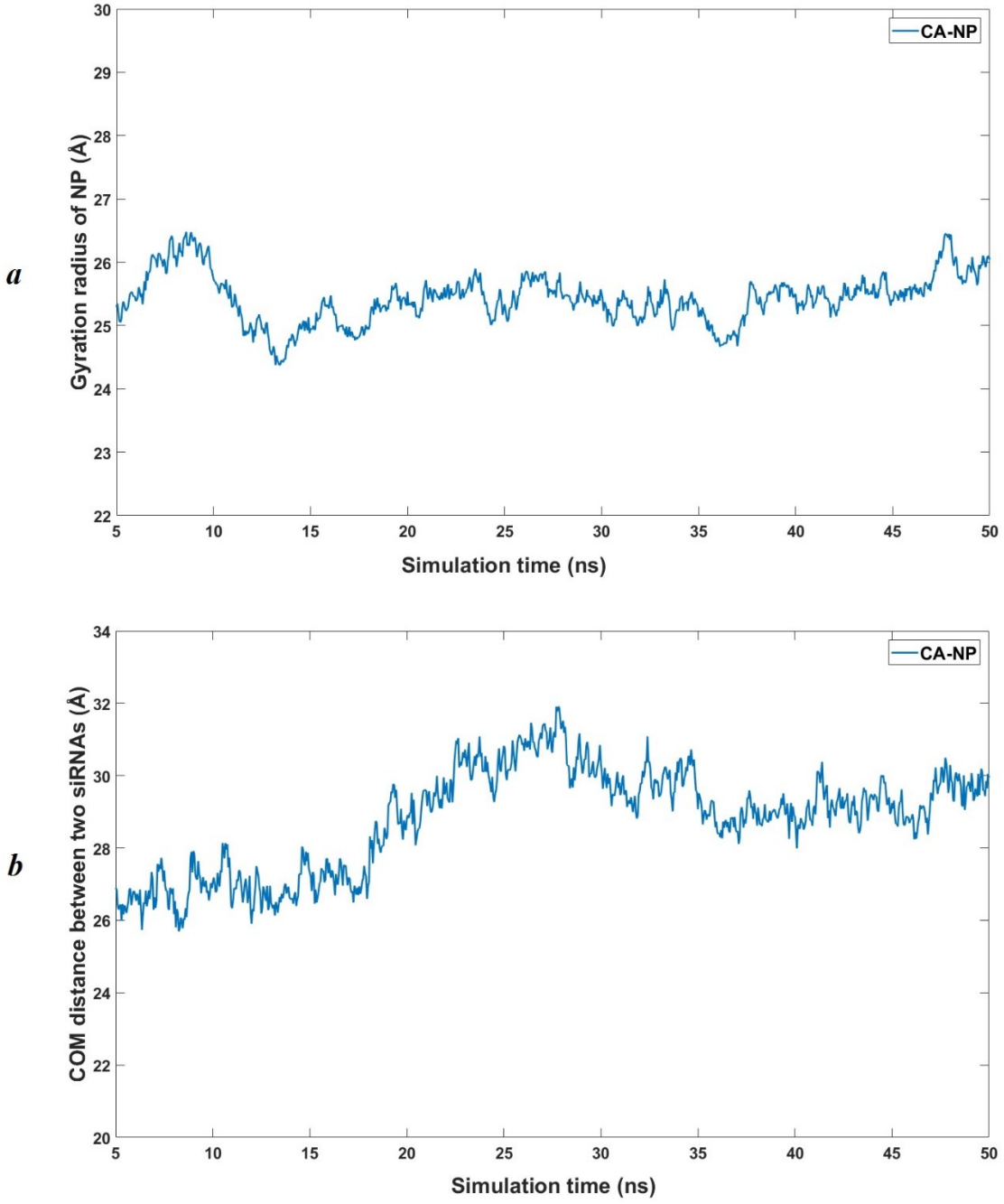

Figure S1. Time evolution of (a) gyration radius (b) COM distance between the two siRNA molecules in CA-NP with the new initial configuration.

S2: Number of Water-Membrane HB and Water-NP HB

In Section 3.2 of the main text, the number of HB between NPs and membrane have been discussed. Here we show the number of HB between water and the membrane, and between water and the NPs (Fig. S2). Number of HB between the membrane and water has an almost constant value during approach and attachment stages followed by an increase during embedment and detachment stages (Fig. S2a). The increasing trend indicates that initially the membrane surface was not saturated with the water due to the NP above the membrane. As the NP is pulled through the membrane to create a pore, the membrane molecules become more exposed to water, leading to formation of more HB between them.

Number of HB between water and the NP (Fig. S2b) also has an almost constant value during approach stage, followed by a decrease during attachment and embedment stages, and ultimately an increasing trend during detachment stage. The initial constant value is caused by the lack of interaction between the NP and the membrane so that the NP can maintain its interaction with surrounding waters. As the NP interacts with the membrane, increase in HB between the NP and the membrane causes the HB between water and the NP to decrease. During detachment, number of HB between water and the NP increases again, which is consistent with the decrease of HB between the membrane and NP.

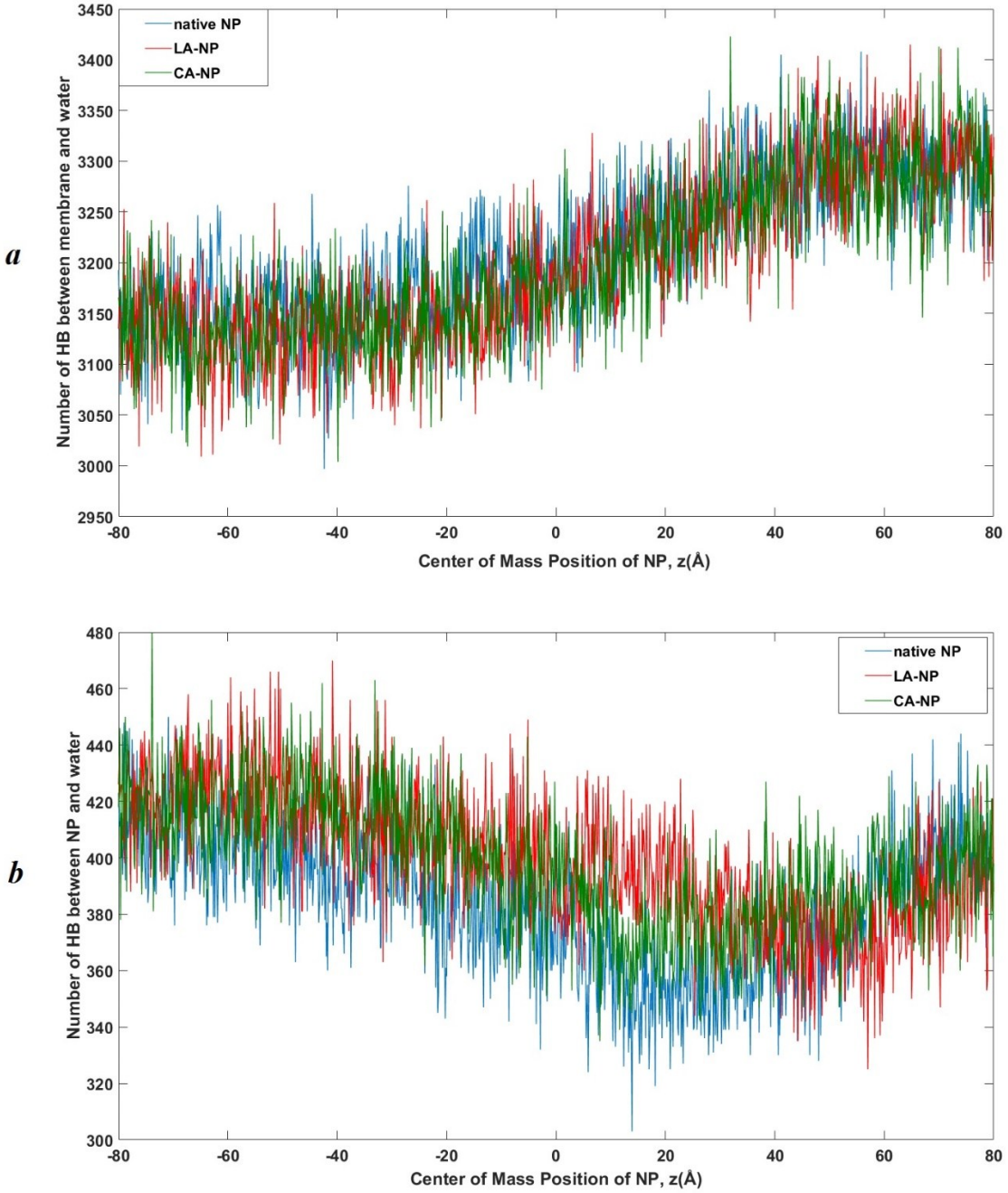

Figure S2. Number of HB (***a***) between water and the membrane, and (***b***) between water and the NPs as functions of COM position of the NP.

S3: Membrane Deformation during NP Penetration

Fig. S3 shows the *z* and *x* positions of phosphate (P) atoms of the membrane at selected time during the penetration. For clarity the position of other membrane atoms are not shown. Similar trends of membrane deformation are observed for native NP, CA-NP and LA-NP, where the P atoms rearranged themselves to interact with the polar amine groups of the NPs, and this led to pore formation that allowed the NP and water to cross the membrane. At the end of the penetration process (Fig. S3c), the LA-NP has pulled out some lipids which can be seen from the presence of some P atoms at very low locations along the *z*-axis. This phenomenon is consistent with Fig. 4b in the main texts where HB counts between NP and the membrane is higher for the LA-NP indicating stronger contact between LA-NP and the membrane.

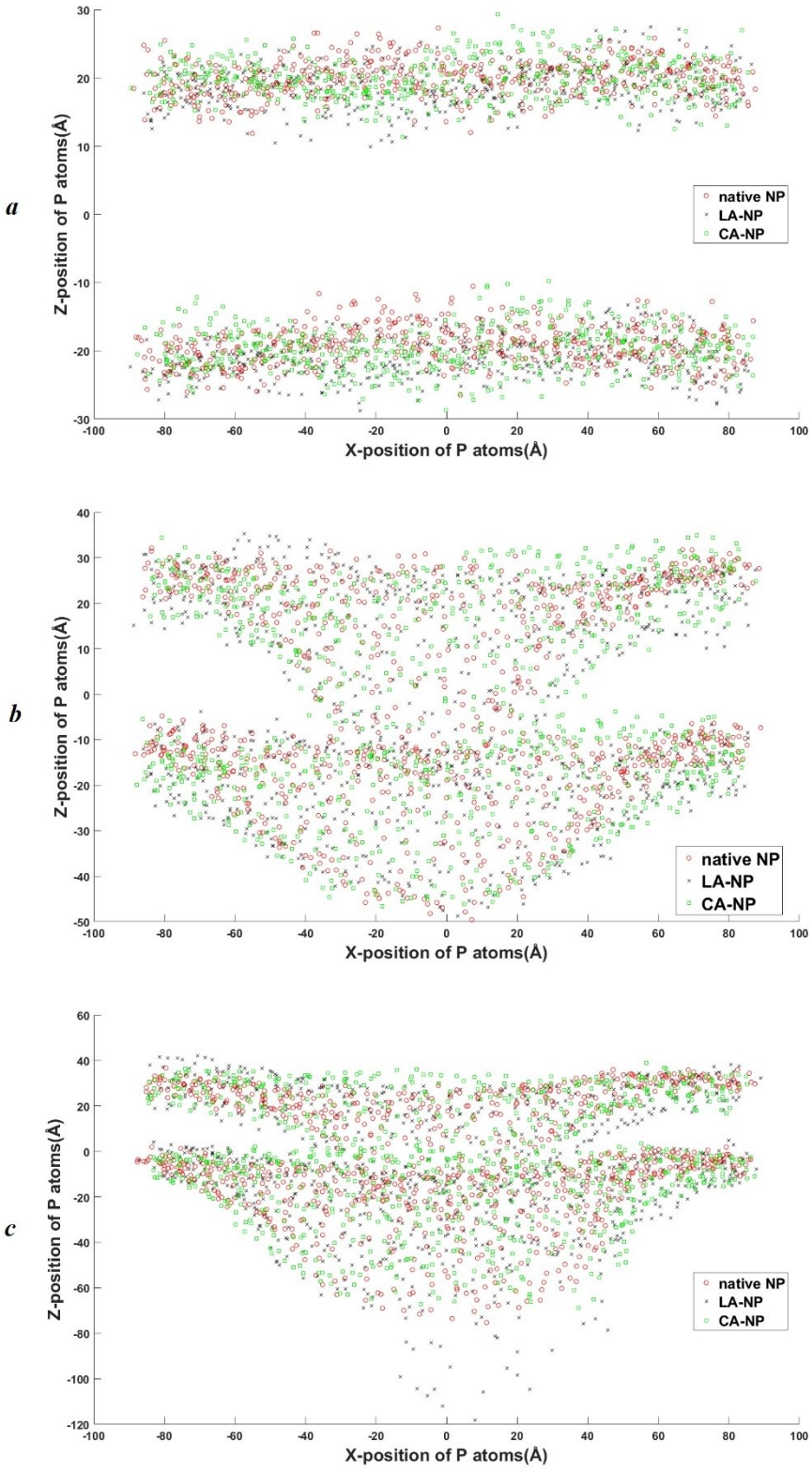

Figure S3. Position of phosphate (P) atoms of the membrane at selected time of the penetration process: (a) 0 ns, COM of NP at –80Å, (b) 30 ns, COM of NP at –4Å, and (c) 64 ns, COM of NP at 80Å.

S4: Relative Orientation of siRNAs

In Section 3.3 of the main text, the relative orientation of the 2 siRNAs was monitored by measuring the angle (θ) between two vectors each defined in one siRNA. This vector was defined by connecting two atoms at the opposite ends of the siRNA, which are C1' of the 18th residue in each strand. These vectors are schematically shown in Fig. S4. We then measured the angle θ between the two vectors as a function of COM position of the NP.

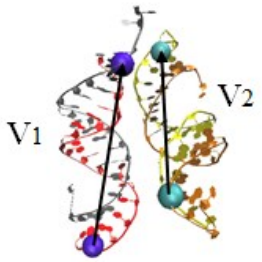

Figure S4: Schematic representative of defined vectors. Each color represent one strand of siRNA molecules. The spheres are $C_1^{'}$ atoms of the 18th residue in each strand.

S5: Angle between siRNAs of NPs and Unperturbed Membrane Surface

In Section 4.1 of the main text, the angle between each siRNA of the NPs and the unperturbed membrane surface is shown (Fig. 7) at four locations where snapshots in Fig. 5 are taken. Here, we show this angle during the entire penetration process (Fig. S5). For all the three systems, we have observed the rotation of each siRNA from its relatively upright initial configuration to a relatively parallel orientation during embedment, and returning to a relatively upright orientation again during detachment. The upright orientation is more evident in native NP and CA-NP than in LA-NP.

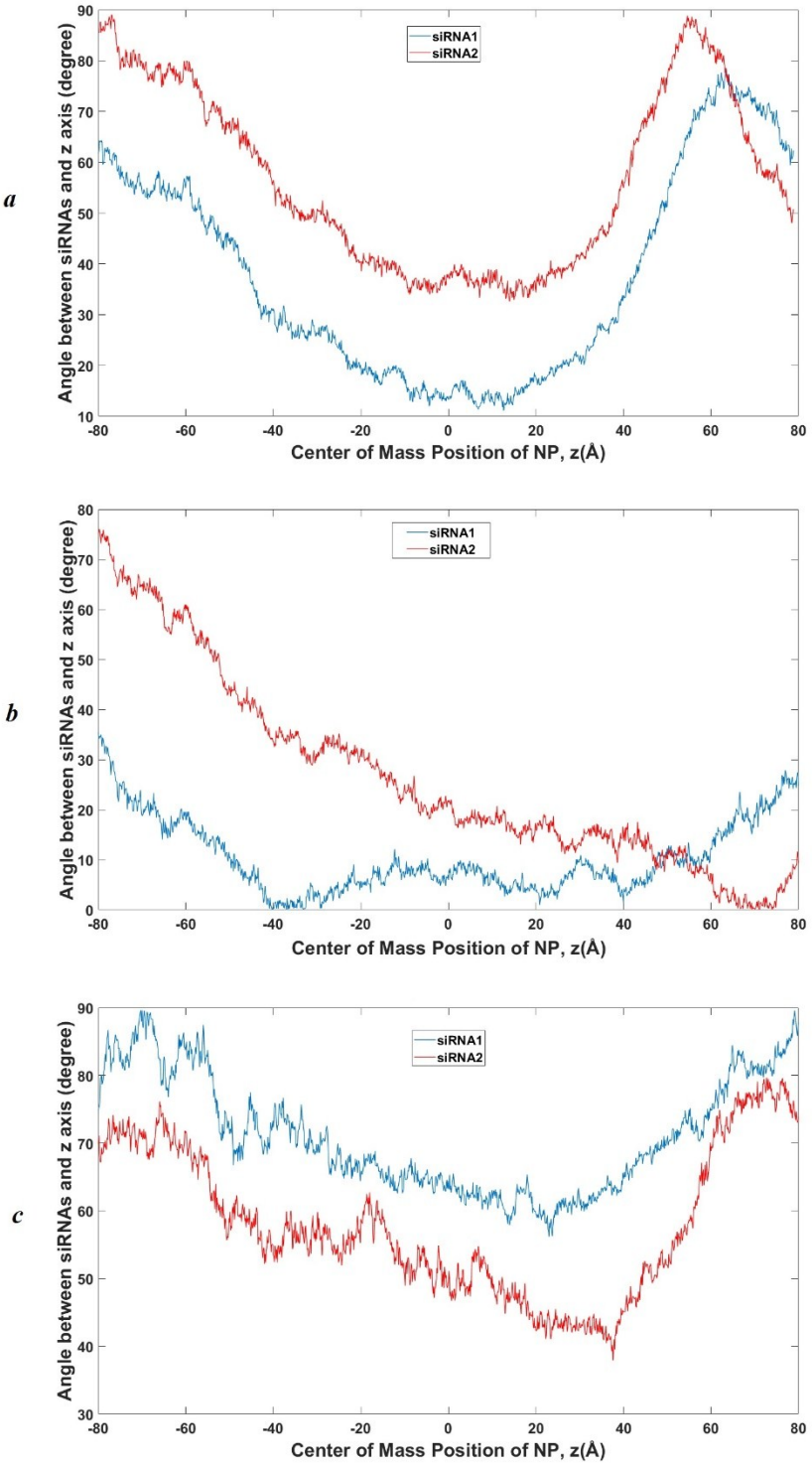

Figure S5. Angle between each siRNA and the unperturbed membrane surface during the penetration process for (a) native NP, (b) LA-NP and (c) CA-NP.

S6: Pore Resealing after Restraint Removal

A series of MD simulations were performed in absence of the pulling force on the NPs. In particular, for each NP, two configurations obtained from the SMD simulation were selected: (I) one corresponding to maximum compaction of the NP (minimum R_g) and (II) one from the end of the SMD simulation. Each configuration was used as the initial configuration for a MD simulation in which the restraint on the COM of NP was removed and the system was simulated for 20 ns. The time evolutions of COM position of the NP, R_g of the NP, COM distance between the two siRNA molecules and relative angle between the two siRNA molecules were monitored. Figure S6 shows the results for initial configuration (I), and Figure S7 shows the results for initial configuration (II).

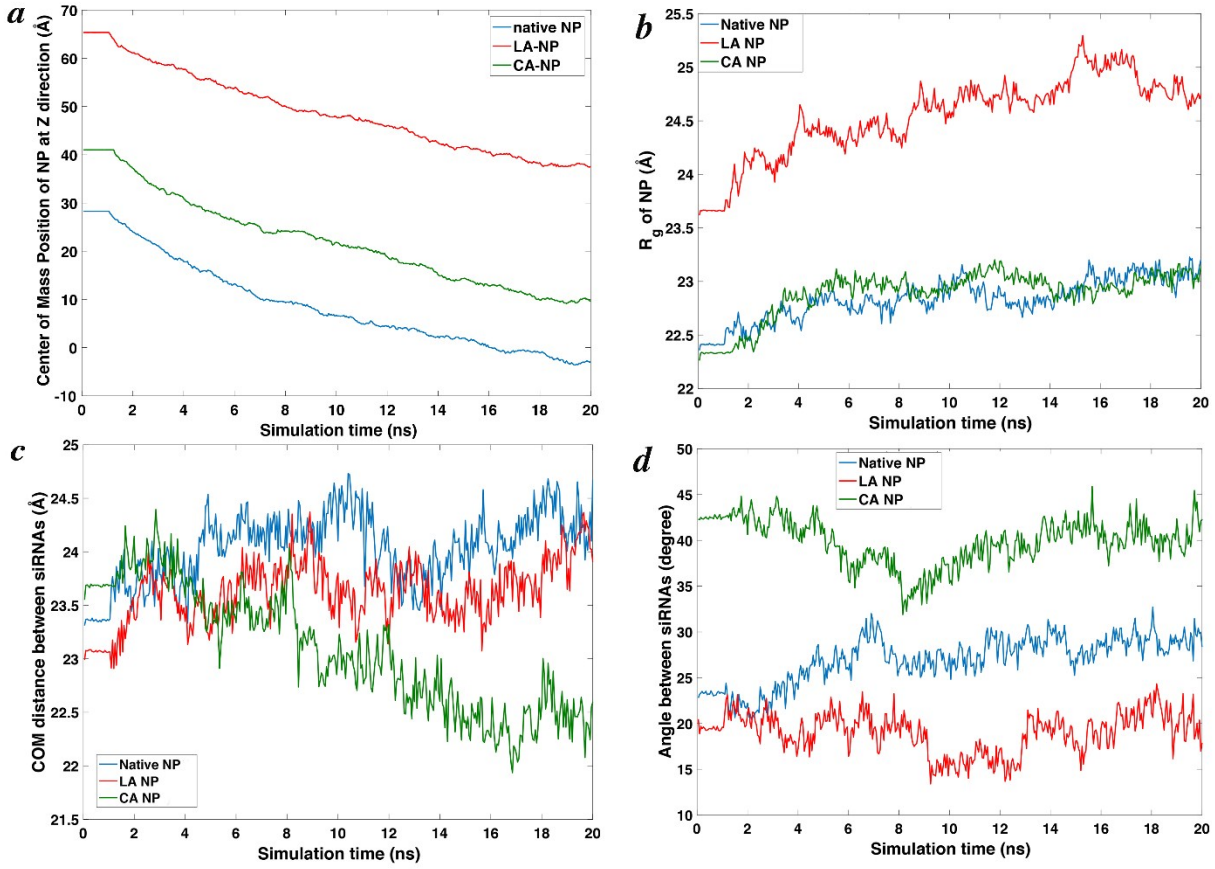

Figure S6 (*a*) COM position of NP, **(*b*)** Gyration radius of the NP, (***c***) COM distance between the two siRNA molecules, and (***d***) Relative angle between the two siRNA molecules, each as a function of simulation time during the standard MD simulation. The initial configurations for these simulation were selected from the SMD simulation at the maximum compaction of the NP.

For all the three systems, COM position of NP (Fig S6a) decreases with time, indicating that once the pulling force is removed the NP is repelled from the membrane and hence the induced pore is unstable. During the NP's separation from the membrane, R_g of each NP (Fig S6b) increases and the increase is more noticeable for LA-NP. For LA-NP and native NP, the COM distance between the siRNAs slightly rises (Fig.S6c), while it decreases for CA-NP. For all three systems, the relative angle between the siRNAs (Fig.S6d) shows an insignificant change. Similar repulsion of NP by the membrane is also observed in Fig S7 with initial configuration (II). CA-NP has a slight reduction in R_g, while the change is insignificant for LA-NP and Native NP. COM distance between the siRNAs is relatively stable for Native NP, while increases slightly for LA-NP and CA-NP. For all three NPs, the change in relative angle between the siRNAs (Fig.S6d) is insignificant.

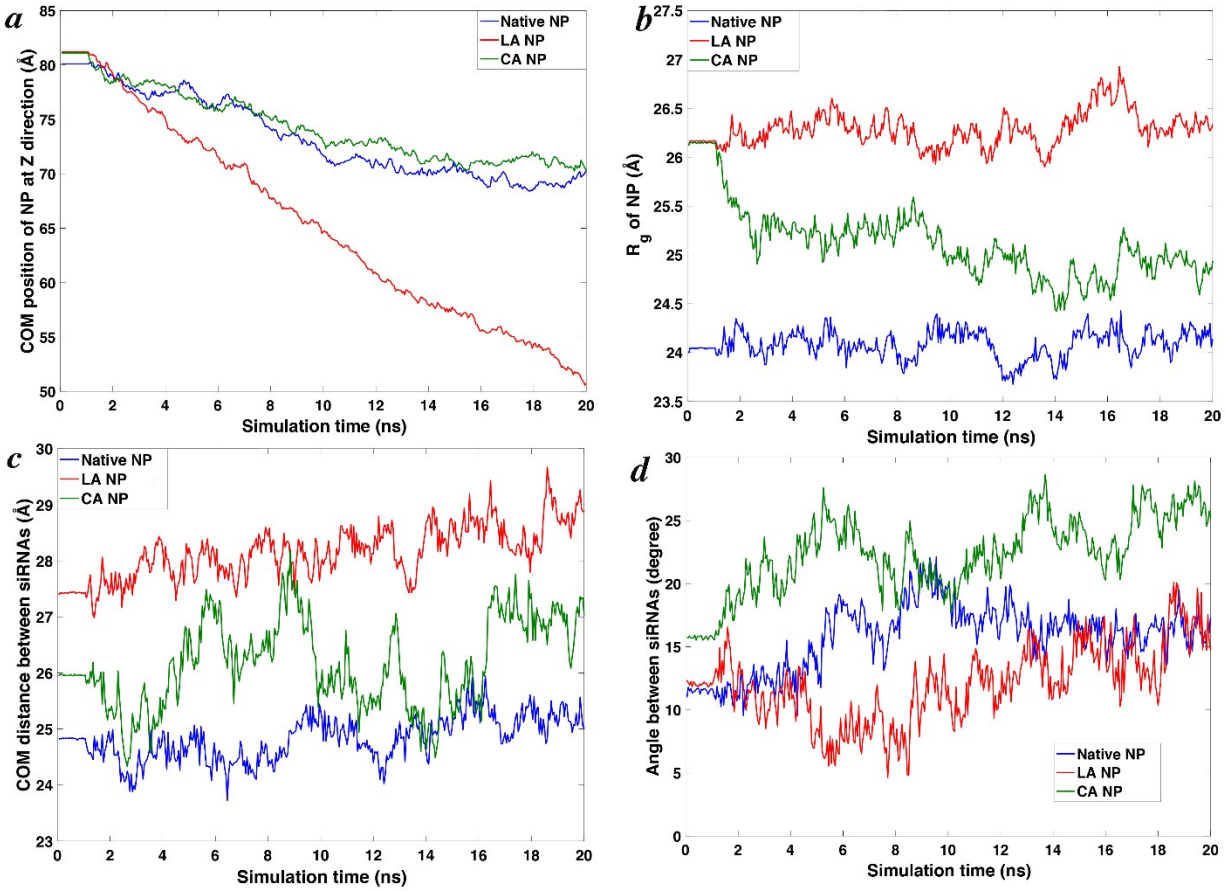

Figure S7 (***a***) COM position of NP, (***b***) Gyration radius of the NP, (***c***) COM distance between the two siRNA molecules, and (***d***) Relative angle between the two siRNA molecules, each as a function of simulation time during the standard MD simulation. The initial configurations for these simulations were selected from the end of the SMD simulation.

S7: Sensitivity of Result to Pulling Speed

To evaluate the sensitivity of our result to the pulling speed, we performed a series of SMD simulations on the CA-NP using two other speeds of 5 Å/ns and 10 Å/ns (usual pulling speed was 2.5 Å/ns). Fig. S8 displays the force profile, R_g, the siRNA separation distance and relative angle between siRNAs as functions of COM position of the CA-NP. The results show that while the magnitude of the force rises upon increase in the pulling speed, the qualitative behavior of the CA-NP is the same irrespective of the pulling speed. Importantly, the same behavior was observed at all pulling speeds for the gyration radius (compaction of CA-NP), siRNA separation distance, and change in the relative angle between the siRNAs (siRNA alignment).

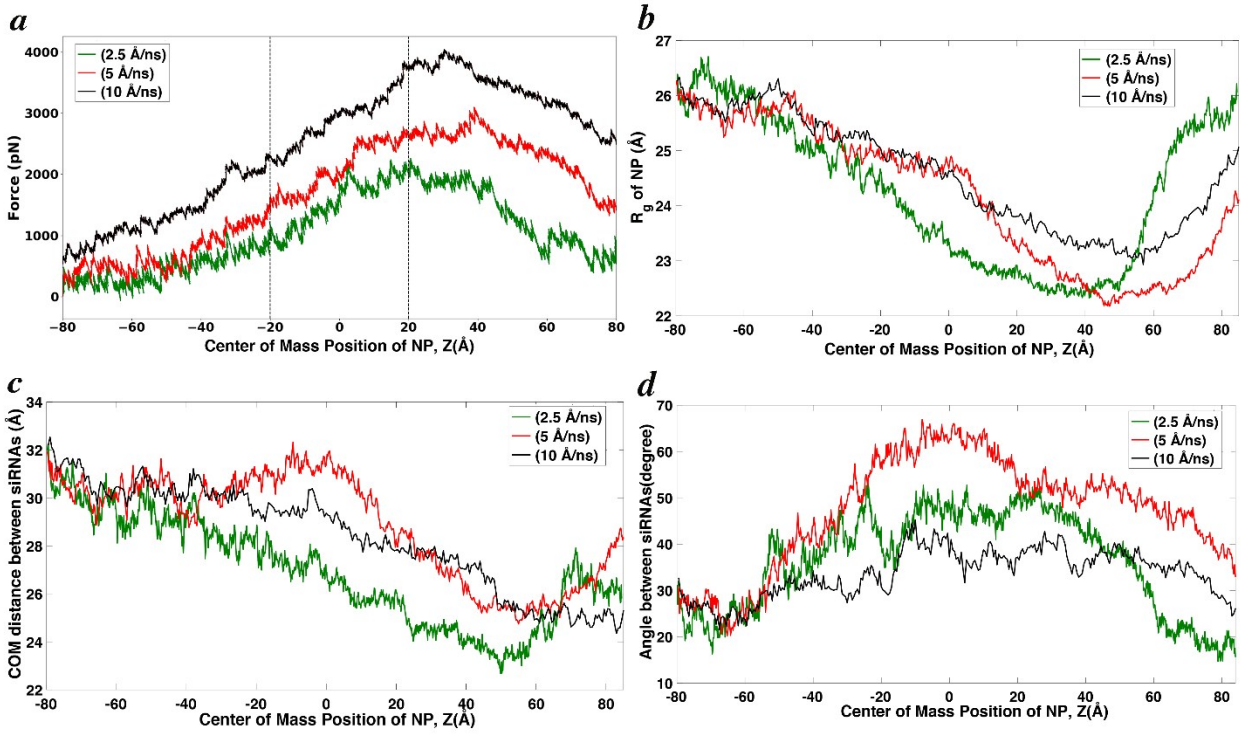

Figure S8 (***a***) Force profile (***b***) Gyration radius (***c***) COM distance between the two siRNA molecules, and (***d***) Relative angle between the two siRNA molecules, each as a function of the NP COM. All simulations are for the CA-NP.

Reference

1 C. Sun, T. Tang and H. Uludag, *Biomaterials*, 2013, **34**, 2822–2833.