ELECTRONIC SUPPORTING INFORMATION

pH-Dependent Aggregation and pH-Independent Cell Membrane Adhesion of Monolayer-Protected Mixed Charged Gold Nanoparticles

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Table S1: Force field parameters according to Martini coarse-grained force field

non-bond		**bond**[1]			**angle**[2]		
building block	type	building block	R_0	k_{bond}		θ_0	k_{angle}
Au	C5	S-$(CH_2)_4$	0.445	1250	S-$(CH_2)_4$-$(CH_2)_4$	180	25
S	N0	$(CH_2)_4$-$(CH_2)_4$	0.470	1250	$(CH_2)_4$-$(CH_2)_4$-$(CH_2)_4$	180	25
$(CH_2)_4$	C1						
-NH_3^+	Qda						
-COOH	Qda						

[1] R_0 in nm, k_{bond} in $kJmol^{-1}nm^{-2}$. [2] θ_0 in degree, k_{angle} in $kJmol^{-1}rad^{-2}$.

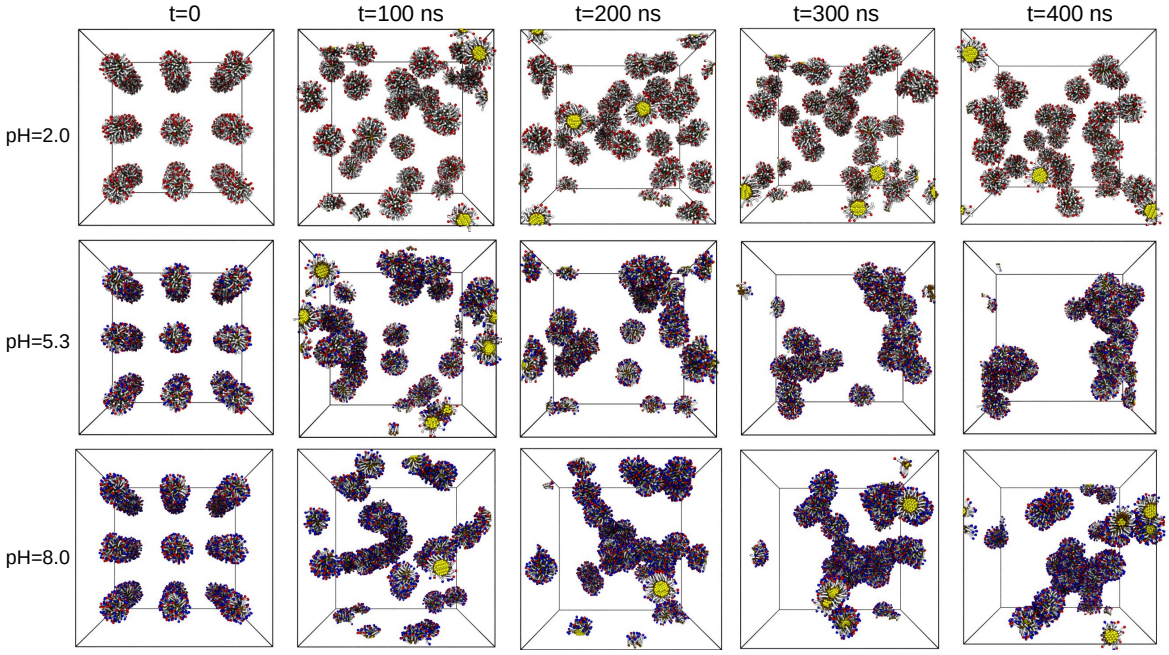

Figure S1: Evolution of configurations about 27 AuNPs in the simulation box at different pH values.

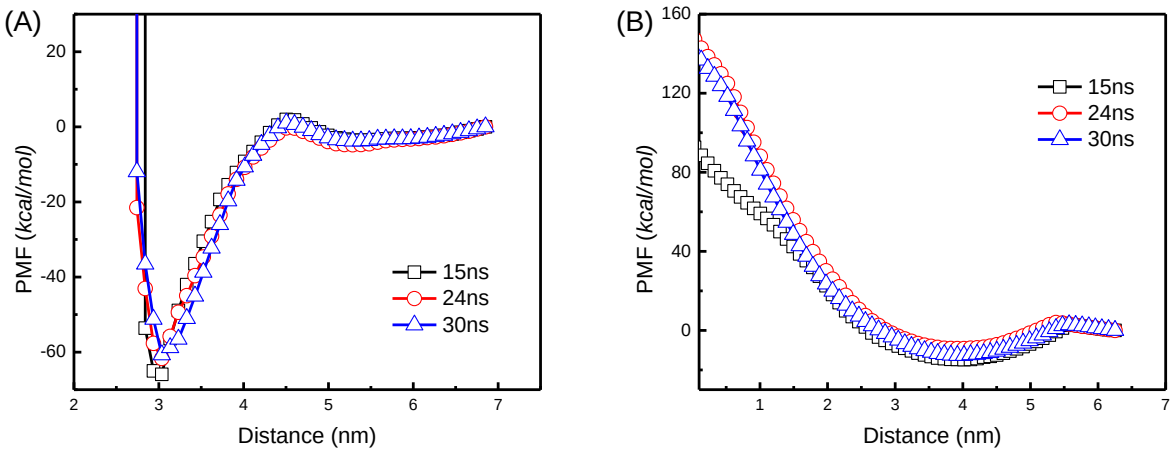

Figure S2: (A) The convergence of potential of mean force (PMF) calculation over the simulation time per window for two AuNPs at pH= 8.0. (B) The convergence of PMF calculation over the simulation time per window for AuNPs penetration at pH= 2.0.

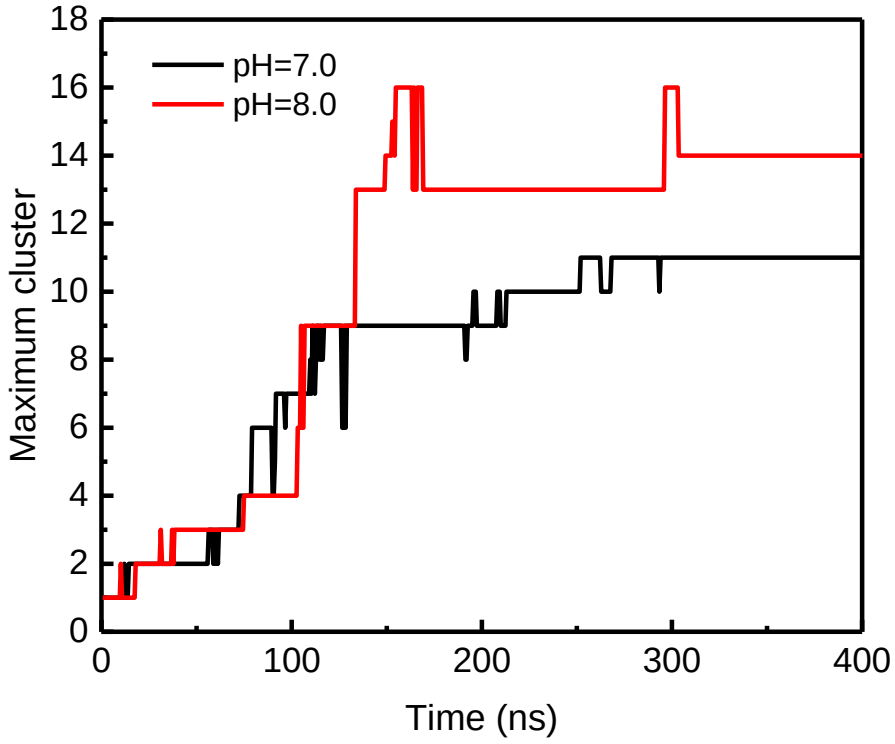

Figure S3: Evolution of maximum cluster for AuNPs at pH= 7.0 and pH= 8.0. As we can see, the maximum cluster of AuNPs barely increases after the time of 200 ns. Moreover, after 300 ns, the maximum cluster sizes do not change. It indicates that the AuNPs at pH= 7.0 and pH= 8.0 will not grow their size along with simulation time.

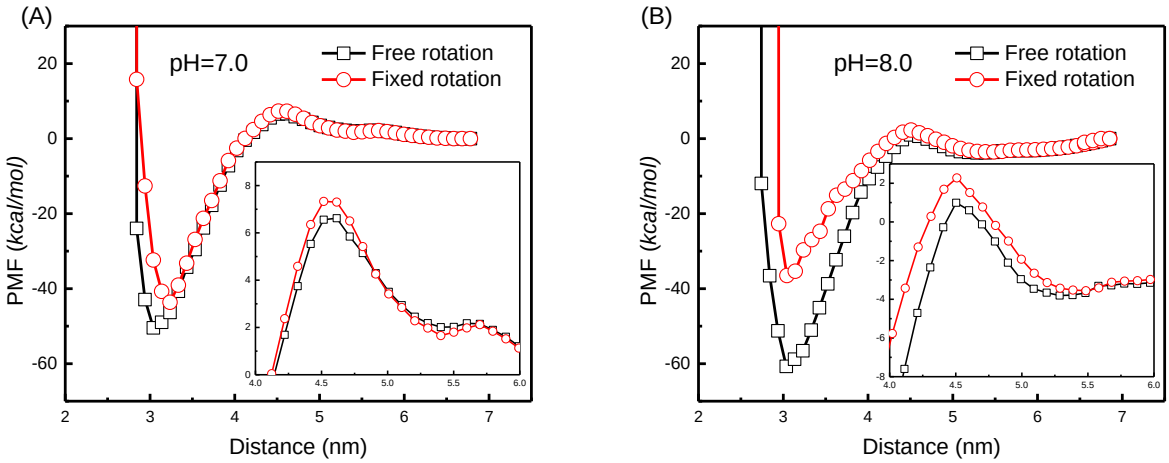

Figure S4: Comparisons of PMF profiles between AuNPs with free rotation and fixed rotation during the umbrella sampling process at (A) pH$=7.0$ and (B) pH$=8.0$. The insets are the enlarged figures at the distance between 4 nm and 6 nm. As we can see, the two PMF curves are slightly different. With the fixed rotation, both AuNPs at pH$=7.0$ and pH$=8.0$ have larger maximum and minimum values compared to the ones with free rotation. Furthermore, the ΔE_1 values with fixed rotation condition at pH$=7.0$ and pH$=8.0$ are 5.7 $kcal/mol$ and 5.8 $kcal/mol$, respectively. Both of them are larger than the ones of 4.6 $kcal/mol$ and 4.8 $kcal/mol$ at pH$=7.0$ and pH$=8.0$ with free rotation. Moreover, the ΔE_2 values with fixed rotation condition at pH$=7.0$ and pH$=8.0$ are 50.9 $kcal/mol$ and 38.7 $kcal/mol$, respectively, which are smaller than the ones with free rotation. All of these indicate that the orientation of AuNP will affect the PMF values during aggregation.

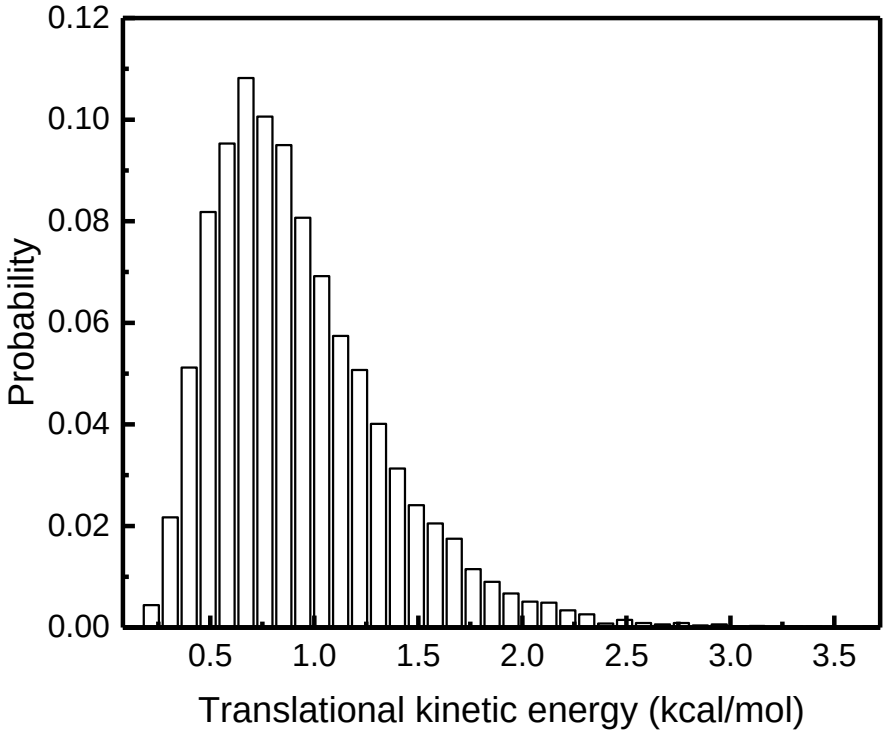

Figure S5: The translational kinetic energy distribution of a single AuNP under the thermal fluctuation during a simulation period of 300 ns. As we can see, the mean value of the translational kinetic energy is around 0.93 $kcal/mol$. The maximum translational kinetic energy during the testing time is 3.67 $kcal/mol$. This maximum value indicates that an energy barrier of 6.5 $kcal/mol$ is large enough to prevent the aggregation of AuNPs.

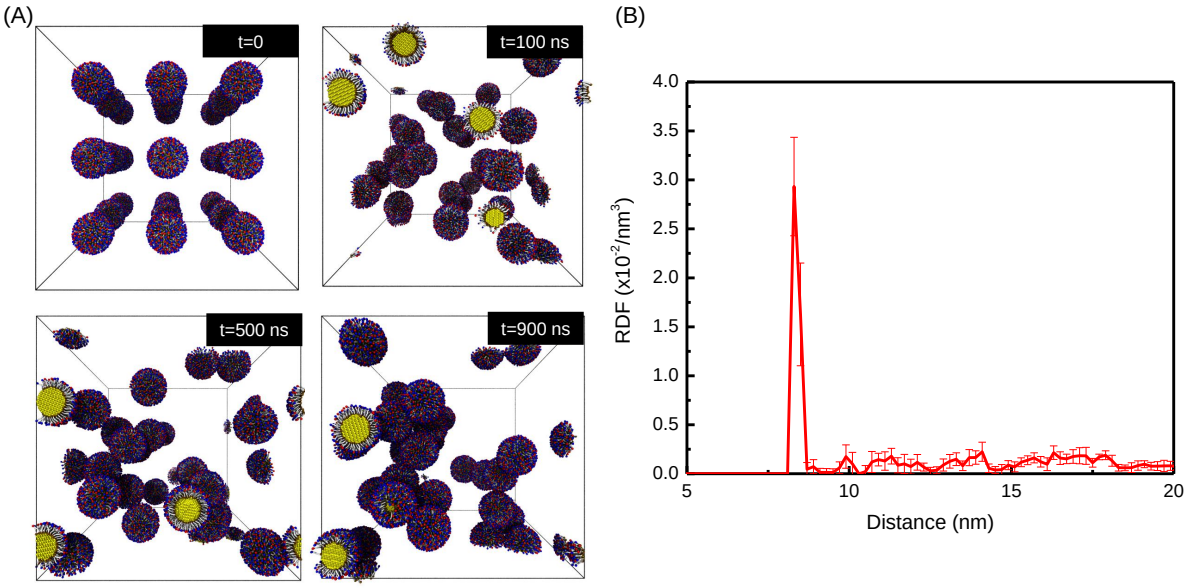

Figure S6: (A) Evolution of configurations about 27 AuNPs in the simulation box at pH$=8.0$. The Au core diameter here is 5.0 nm. The overall diameter of the monolayer-protected AuNP is around 8.2 nm. The simulation box size is $(46.5 \times 46.5 \times 46.5)$ nm^3 (B) Radial distribution function (RDF) of AuNPs at pH$=8.0$ with diameter 5.0 nm. Compared to the RDF values of AuNPs at pH$=8.0$ with core diameter 2.2 nm in the main text (Fig.3), we can observe two important things: (1) There is only one peak value in the RDF curve for larger size AuNPs; (2) This peak value is smaller than the one of smaller AuNPs at the same pH value. The cluster size of larger AuNPs is 1, if we take the cutoff distance as its diameter, indicating the non-aggregation behavior of these AuNPs.

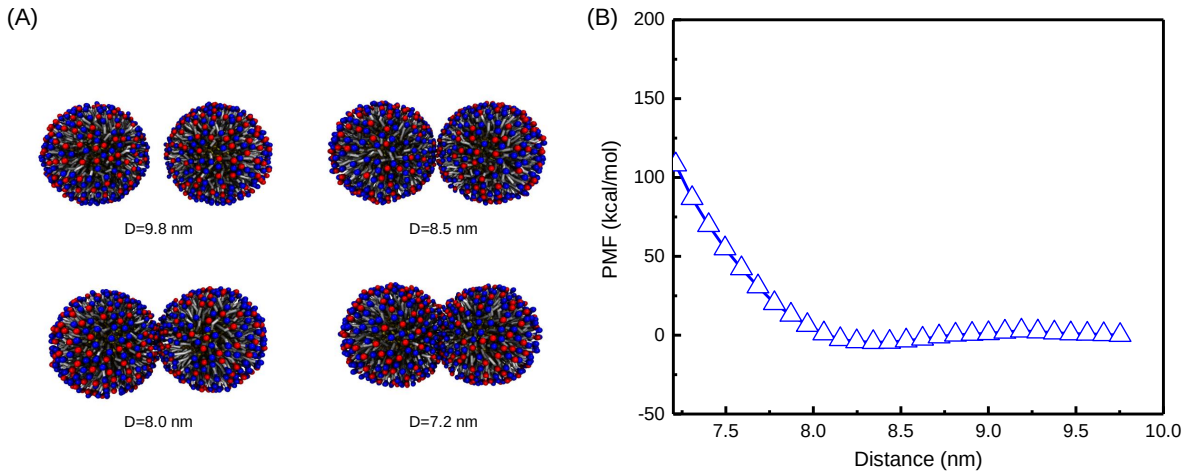

Figure S7: (A) Configurations of AuNPs at pH=8.0 at different COM distances during the free energy analysis process. The core diameter of AuNP is 5.0 nm. (B) The profile of PMF between two AuNPs against center-of-mass. Consistent with the RDF peak position in Fig.S6, there is a local minimum value in RDF curves around 8 nm. It is important to note that comparing with the PMF curves of small AuNPs in the main text, these PMF values here will not decrease when the hydrophobic alkane chain interact with each other ($D < 8.0$ nm). This is mainly caused by the decreasing curvature of large AuNPs, which contributes to a more tight packing state of ligands at the same grafting density. Due to the tightly packed ligands, the steric interaction between AuNPs will dramatically increase when they approaching each other. All of these results in Fig.S6 and Fig.S7 indicate that a larger size AuNP might have a better stability than the smaller one at high pH value.

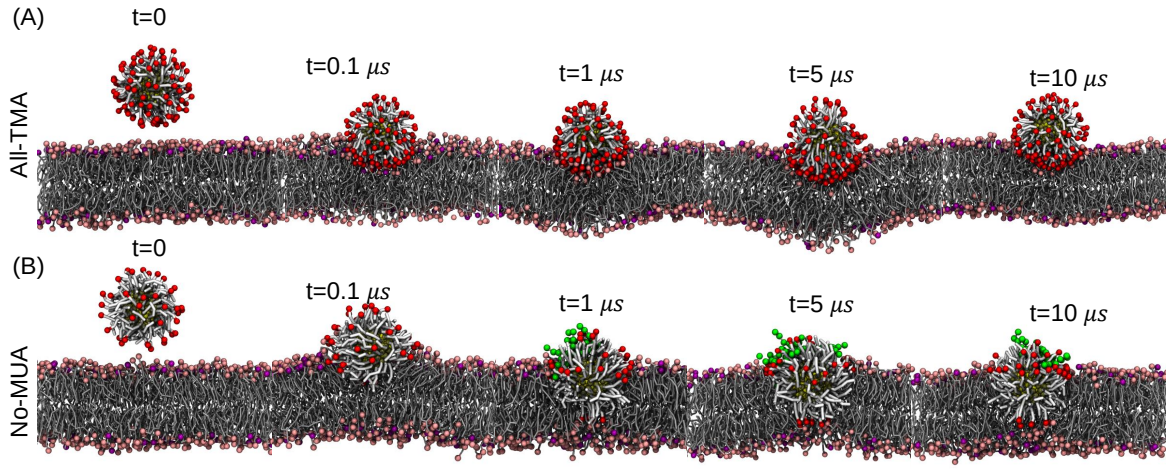

Figure S8: (A) Adhesion process of AuNP with only positive TMA ligands (all-TMA AuNP). (B) Penetration process of AuNP with positive TMA ligands and alkane chain ligands (no-MUA AuNP). Comparing with the pH-responsive AuNPs in the main text, the no-MUA AuNP replaces all the MUA ligands by the pure hydrophobic alkane chains. The setting of no-MUA AuNP is similar to the one in Ref [1]. As we can see, though the all-TMA AuNP has a larger positive surface charge than the pH-responsive AuNP at pH=2.0, it still can not penetrate into the membrane. However, if we change the MUA ligands on pH-responsive AuNPs to pure hydrophobic alkane chains as given for the no-MUA AuNP, the AuNP without the MUA can quickly penetrate into the membrane within 1 μs.

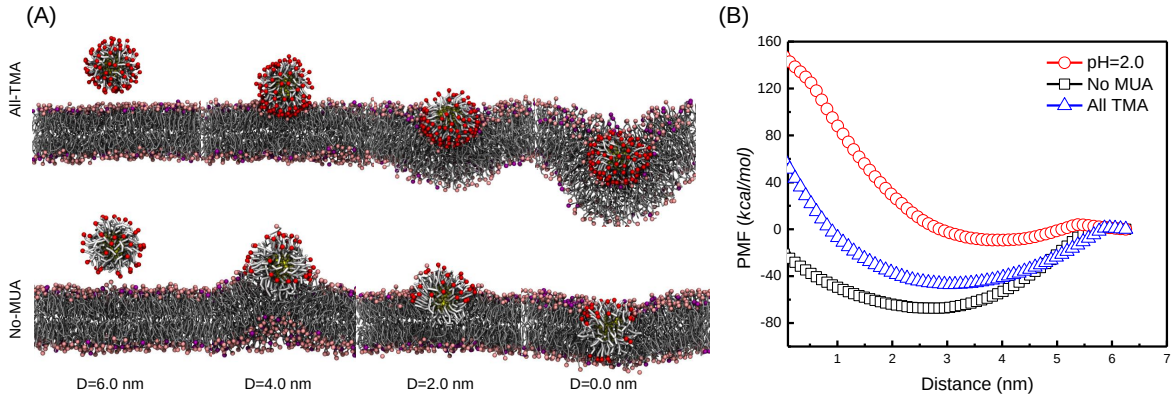

Figure S9: (A) Configurations of AuNPs (all-TMA and no-MUA) and lipid bilayer during the free energy analysis process. (B) Comparison of PMF profiles when directing an AuNP towards a lipid bilayer. As we can see for the all-TMA AuNP, it adheres on the lipid bilayer surface at $D = 4.0$ nm. When the distance further decreases, the membrane starts to bend at $D = 2.0$ nm. And the membrane wraps the majority part of AuNPs surface at $D = 0.0$ nm. During this entire process, the alkane chains on the AuNPs surface do not have the chance to interact with the hydrophobic lipid tail. In comparison, the alkane chains on no- MUA AuNP are easier to interact with the lipid tail. And the no-MUA AuNPs finally penetrates into the lipid bilayer. Comparing the PMF values, we can find that the PMF values of all-TMA AuNP is smaller than that of AuNPs at pH$= 2.0$, which is caused by its larger positive surface density. Additionally, the no-MUA AuNP has the smallest PMF values because of the hydrophobic interaction. Combining the results of pH-responsive AuNPs and the AuNPs in Fig.S8 and Fig.S9, we can conclude that the penetration behavior of mixed charged AuNPs is totally different from the ones with positive and pure hydrophobic ligands in Ref. [1].

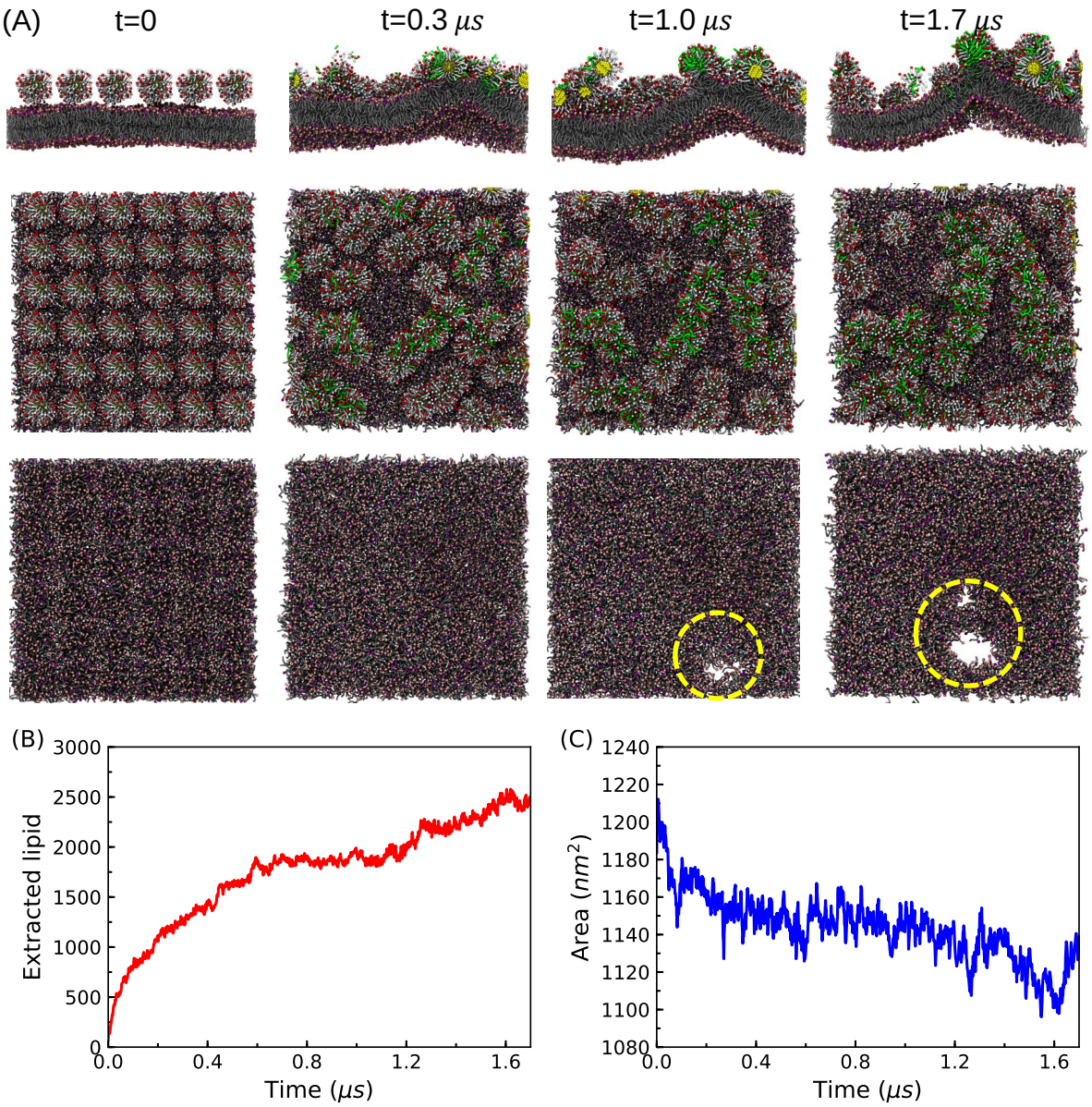

Figure S10: (A) Snapshots of interaction process between multiple AuNPs and lipid bilayer. The lipids extracted by AuNPs are highlighted in green. A pore opened in lipid bilayer is highlighted. (B) Number of extracted lipids over simulation time. (C) Evolution of the lipid bilayer area. The pH value in the system is 2.0. There are 36 NPs placed above the membrane at $t = 0$. And the membrane size at $t = 0$ is (35×35) nm^2. The extracted lipid number can increase to 2500 during the simulation time. Additionally, the membrane size also decreases with the amount around 100 nm^2.

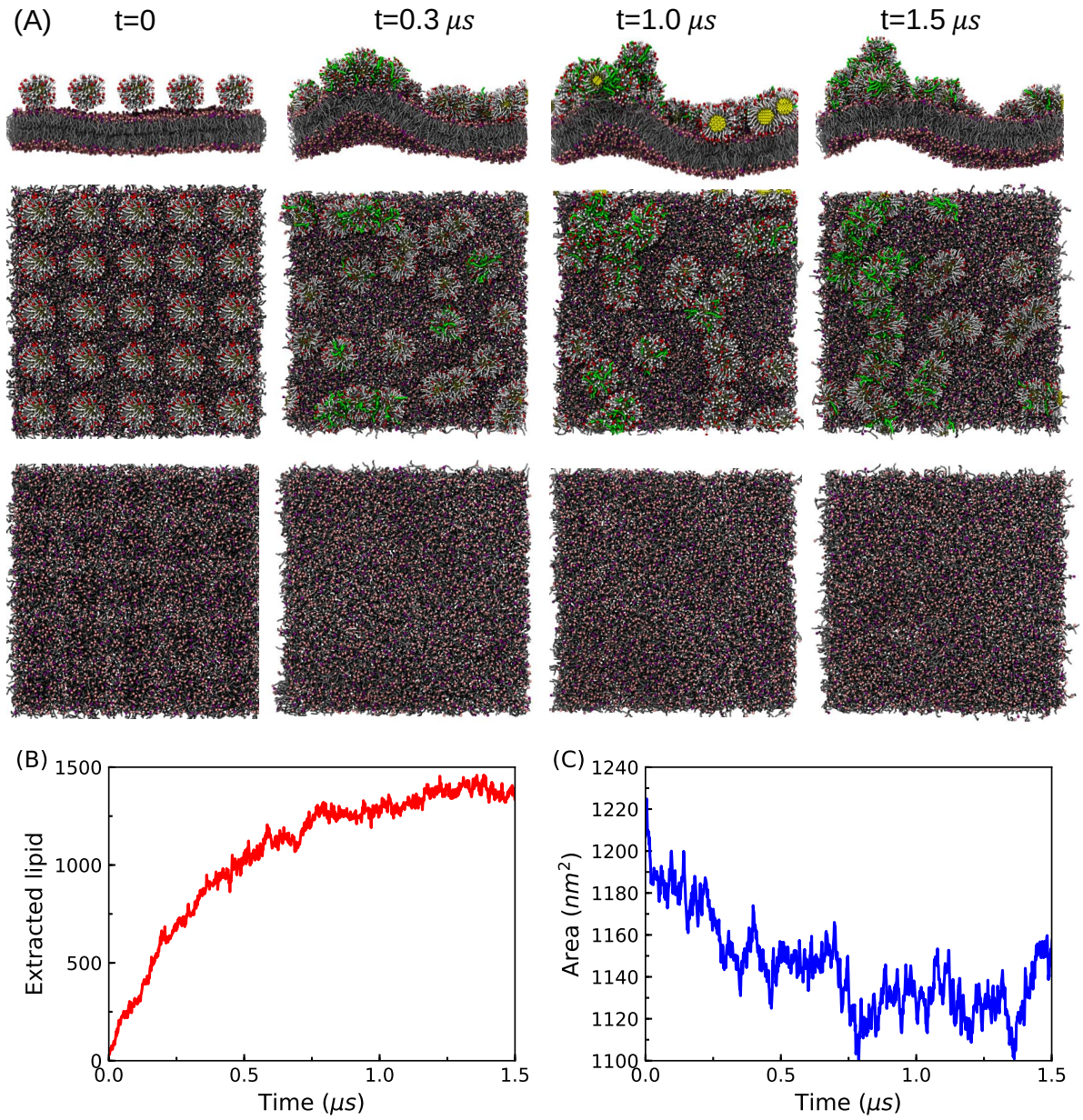

Figure S11: (A) Snapshots of interaction process between multiple AuNPs and lipid bilayer. The lipids extracted by AuNPs are highlighted in green. (B) Number of extracted lipids over simulation time. (C) Evolution of the lipid bilayer area. The pH value in the system is 2.0. There are 25 NPs placed above the membrane at $t = 0$. And the membrane size at $t = 0$ is (35×35) nm^2. The extracted lipid number can increase to 1500 during the simulation time. The membrane size also decreases with the amount around 80 nm^2. Comparing with the one in Fig.S10, an open pore doses not appear, which might be related to the reduced area density of AuNPs. The results in Fig.S10 and Fig.S11 suggest that the extraction of lipid and bilayer dehydration should be a general phenomena for pH-responsive AuNPs with mixed charges.

References

[1] Jiaqi Lin, Hongwu Zhang, Zhen Chen, and Yonggang Zheng. Penetration of lipid membranes by gold nanoparticles: insights into cellular uptake, cytotoxicity, and their relationship. *ACS Nano*, 4(9):5421–5429, 2010.