Supporting Information for

Design Amphiphilic Janus Nanoparticles with Tunable Lipid Raft Affinity via Molecular Dynamics Simulation

Xiaoqian Lin[1,2], Xubo Lin[1,*]

1. Institute of Single Cell Engineering, Key Laboratory of Ministry of Education for Biomechanics and Mechanobiology, Beijing Advanced Innovation Center for Biomedical Engineering, School of Biological Science and Medical Engineering, Beihang University, Beijing 100191, China.
2. Shen Yuan Honors College, Beihang University, Beijing 100191, China.

*Correspondence to linxbseu@buaa.edu.cn (XL)

1. Interactions between Different Coarse-grained (CG) Beads in Martini Force Field.

In the manuscript, we have shown a series of quantitative analyses to reveal the roles of ligand amphiphilicity in Janus NPs' preferred localization in the phase-separated lipid membranes. As for the exact molecular mechanism for different membrane partitioning thermodynamics, it can be ascribed to the non-bonded interactions between amphiphilic Janus NPs and lipids/waters. Janus NPs used here are hydrophilic (P1, P5) and hydrophobic (C1, C5). Hence, the non-bonded interactions are mainly Lennard-Jones (LJ) interactions. The interaction differences among C1, C4, C5, P1, P4 and P5 are shown in **Table S1**. These interaction differences can induce different membrane portioning thermodynamics of ligand-modified Janus NPs.

Table S1. The level of interactions between different coarse-grained (CG) beads in Martini force field.

	C1-C1	C1-C5	C1-P1	C1-P5	C1-C4	C1-P4	C5-C4	C5-P4	P1-C4	P1-P4	P5-C4	P5-P4	P4-P4
ε（kJ/mol）	3.5	3.1	2.7	2.0	3.1	2.0	3.5	3.1	3.5	4.5	2.7	5.6	5.0
σ (nm)	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47

*Water molecules use P4 type beads.

2. Effects of Ligand Amphiphilicity on the Partitioning Dynamics of Janus NPs in Phase-separated Lipid Membranes.

As shown in **Fig. S1**, more hydrophobic Janus NPs (C1 type NP) tend to reside in the raft domain, while less hydrophobic Janus NPs (C5 type NP) preferentially exist in the non-raft domain. What's more, the contact ratio of the Janus NPs to the DPPC/DUPC/CHOL was obtained (**Fig. S2**), which show that the affinity of C1-P5 Janus NP to the DPPC is greater than that of C1-P1 Janus NP, which is consistent with the result of our manuscript.

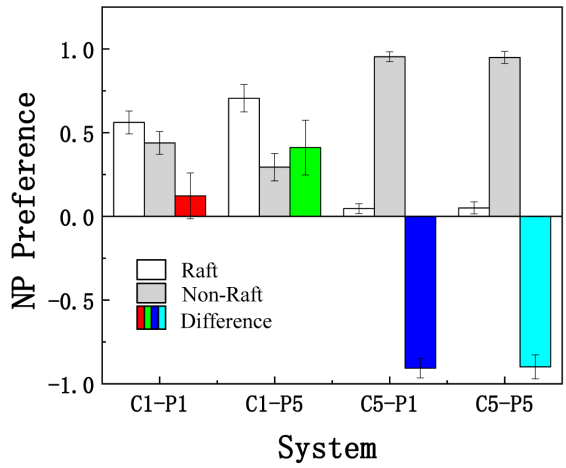

Figure S1. Percentage contact of membrane-bound Janus NPs with raft domain, non-raft domain, and their differences.

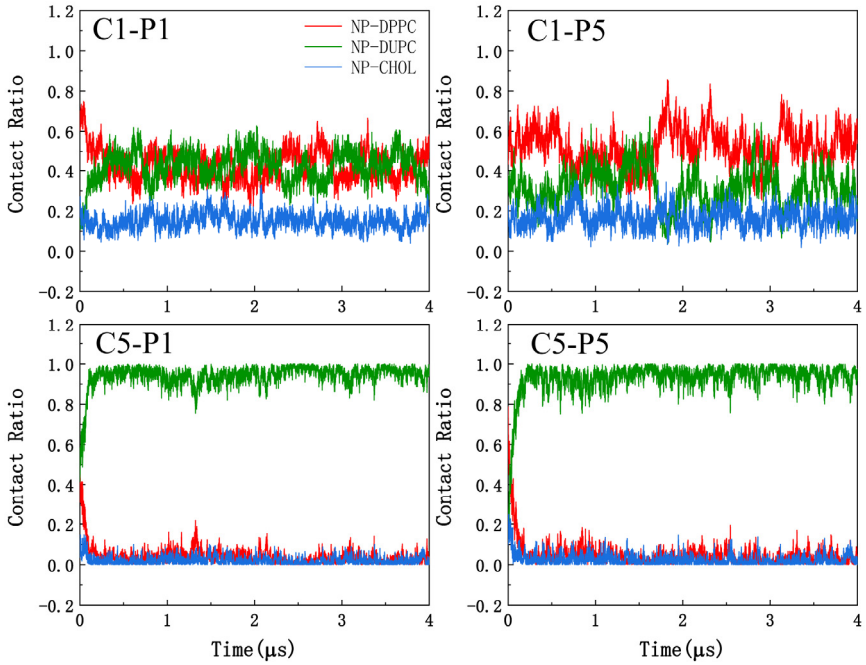

Figure S2. Time evolution of contact ratio of NP with DPPC/DUPC/CHOL.

2. Insertion of Amphiphilic Janus NPs Shows Little Effects on the Lipid Raft Dynamics.

As shown in **Fig. S3**, the phase separation processes of DPPC/DUPC/CHOL bilayers are not significantly affected by amphiphilic Janus NPs. We analyzed lipid chain order parameters (**Fig. S4a**) and cholesterol preferences (**Fig. S4b**) in these systems. There are minor differences among membrane systems with or without membrane-bound Janus NPs. Besides, these Janus NPs also have little effects on the diffusion coefficients of either saturated (DPPC) or unsaturated lipids (DUPC) as shown in **Fig. S5** and **Table S2**.

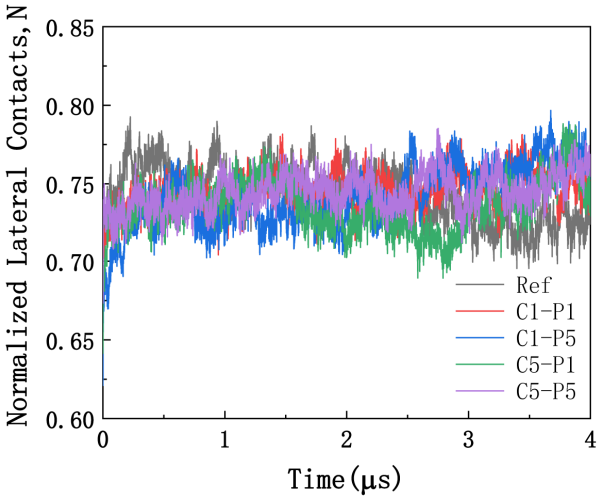

Figure S3. Time evolution of normalized lateral contact N of unsaturated lipids for lipid membrane systems with or without membrane-bound Janus NPs.

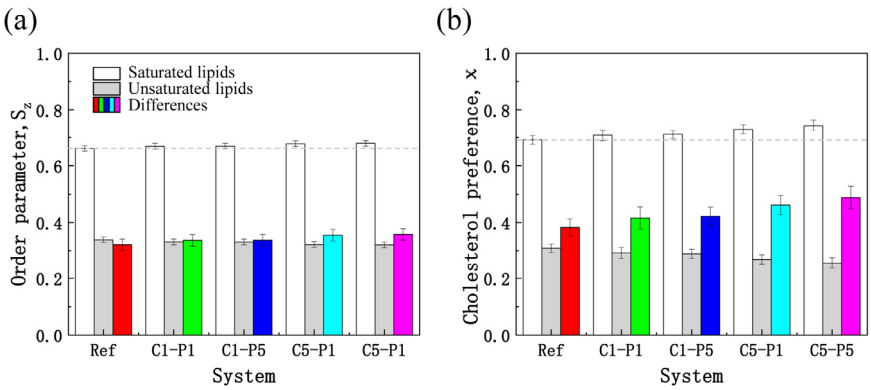

Figure S4. Insertion of NPs shows little effects on the lipid raft dynamics. (a) Lipid chain order parameter and **(b)** Percentage contact with cholesterol, χ of saturated lipids, unsaturated lipids, and their differences for lipid membrane systems with or without membrane-bound Janus NPs.

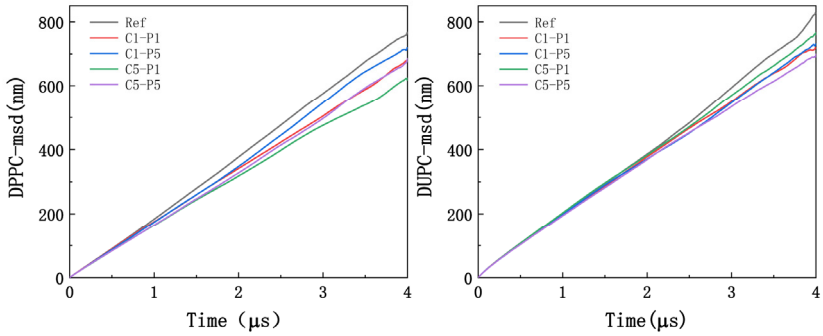

Figure S5. Time evolution of mean square displacement of saturated lipids and unsaturated lipids for JNP-embedded lipid membrane systems and the NP-free membrane system (Ref).

Table S2. The diffusion coefficients of saturated lipids (DPPC) and unsaturated lipids (DUPC).

System	Diffusion Coefficient	
	DPPC (10^{-5} cm^2/s)	**DUPC (10^{-5} cm^2/s)**
Ref	0.0485±0.0020	0.0496±0.0067
C1-P1	0.0418±0.0005	0.0447±0.0025
C1-P5	0.0459±0.0048	0.0446±0.0004
C5-P1	0.0384±0.0026	0.0464±0.0006
C5-P5	0.0464±0.0006	0.0428±0.0043

3. Local Disturbance of Membrane-bound Janus NPs on the Phase-separated Lipid Membranes.

In order to further validate the results shown in **Fig. 4**, the same analysis was performed for systems with C1-P5 NP (**Fig. S6**), C5-P1 NP (**Fig. S7**) and C5-P5 (**Fig. S8**).

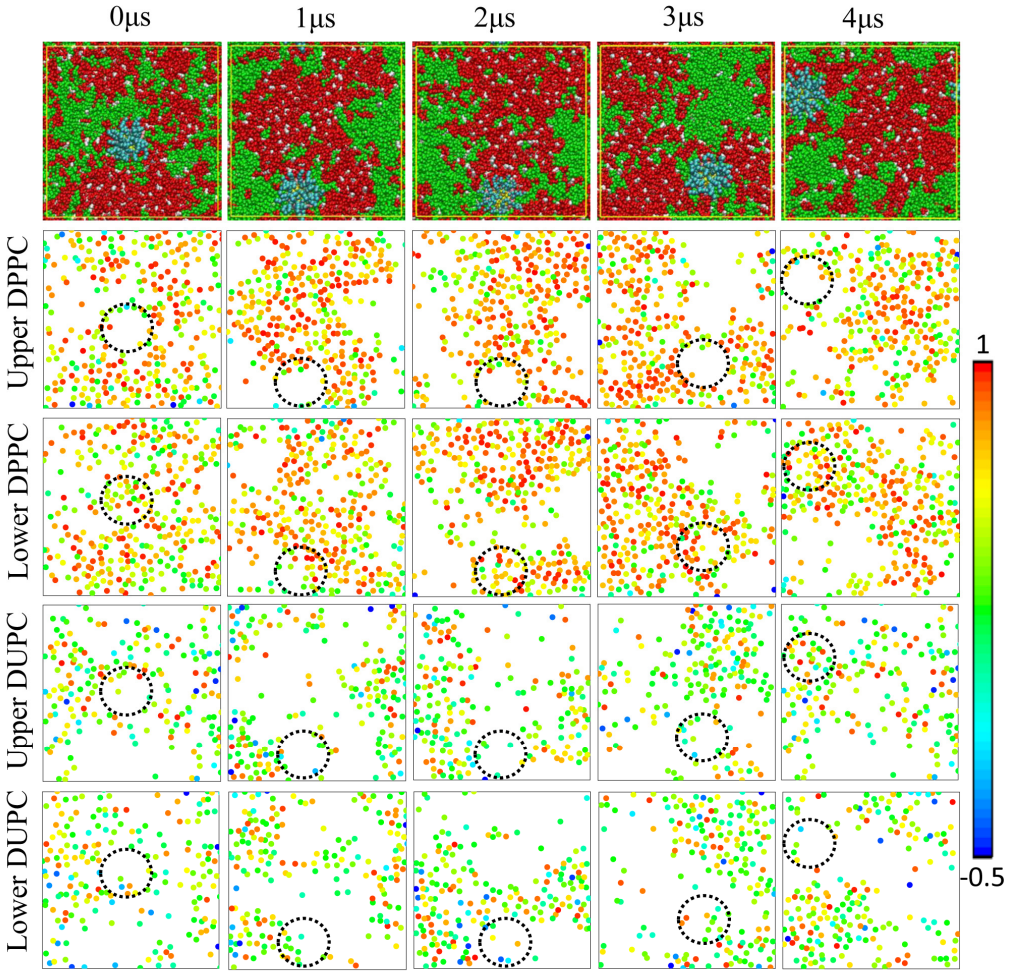

Figure S6. Time evolution of system snapshots and lipid order parameters for lipid membrane system with membrane-bound C1-P5 Janus NP. For snapshots, Janus NP core (C5) is colored in yellow, hydrophobic ligand (C1) in pink, hydrophilic ligand (P5) in blue. DPPC is colored in red, DUPC in green, CHOL in white. For lipid order map, each point represents one DPPC/DUPC molecule, and its color shows the averaged chain order parameters. The dashed black circle indicates the localization of the Janus NP.

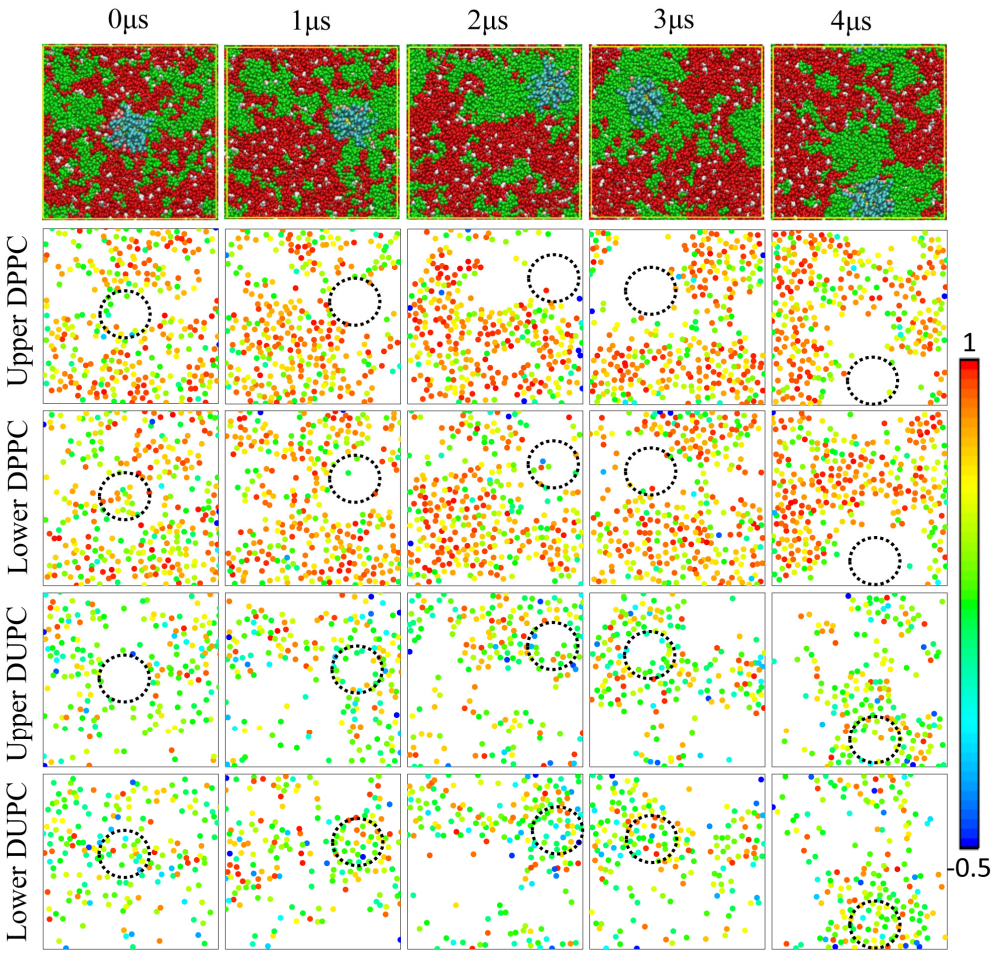

Figure S7. Time evolution of system snapshots and lipid order parameters for lipid membrane system with membrane-bound C5-P1 Janus NP. For snapshots, the coloring style is the same as in **Fig. S6**. For the lipid chain order map, each point represents one DPPC/DUPC molecule, and its color shows the averaged chain order parameters. The dashed black circle indicates the localization of the Janus NP.

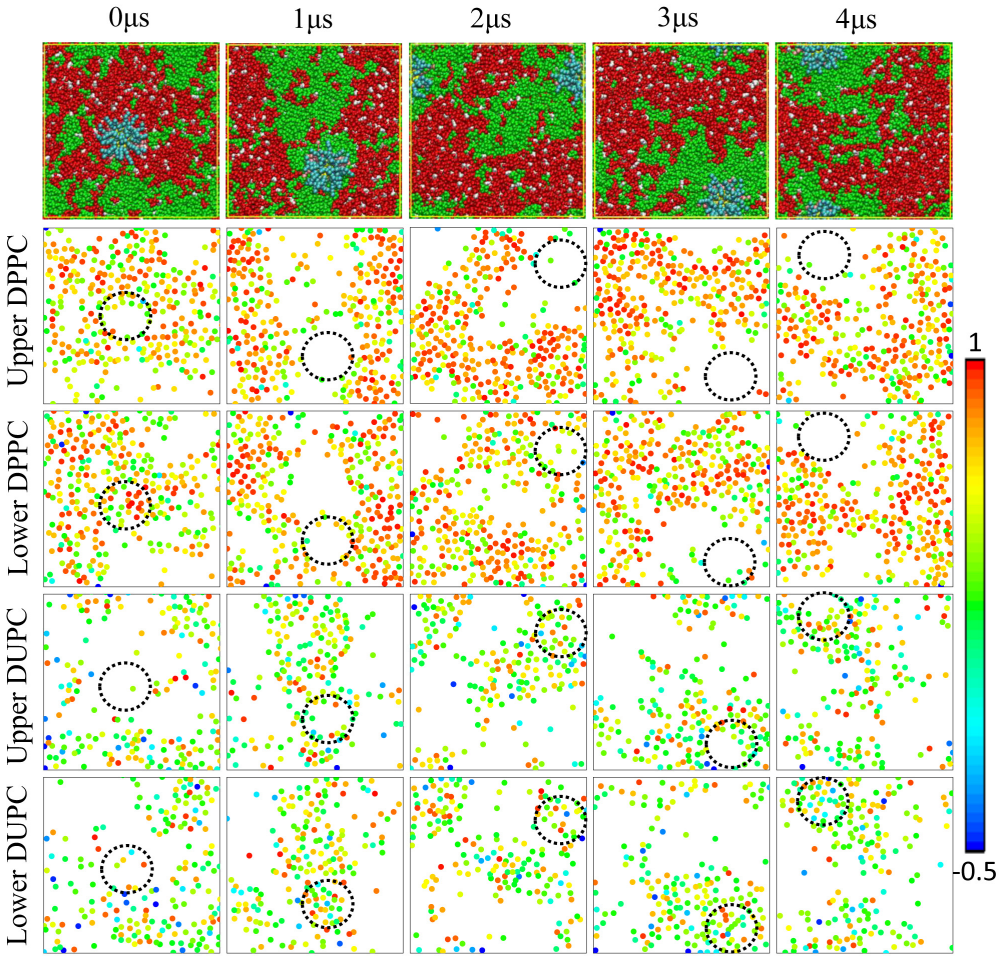

Figure S8. Time evolution of system snapshots and lipid order parameters for lipid membrane system with membrane-bound C5-P5 Janus NP. For snapshots, the coloring style is the same as in **Fig. S6**. For the lipid chain order map, each point represents one DPPC/DUPC molecule, and its color shows the averaged chain order parameters. The dashed black circle indicates the localization of the Janus NP.

4. Effects of Ligand Properties on the Lateral Membrane Partitioning Thermodynamics of Ligand-modified Janus NPs.

The analysis data of another independent copy for **Fig. 2** is provided as follows, which shows consistent results as **Fig. 2**.

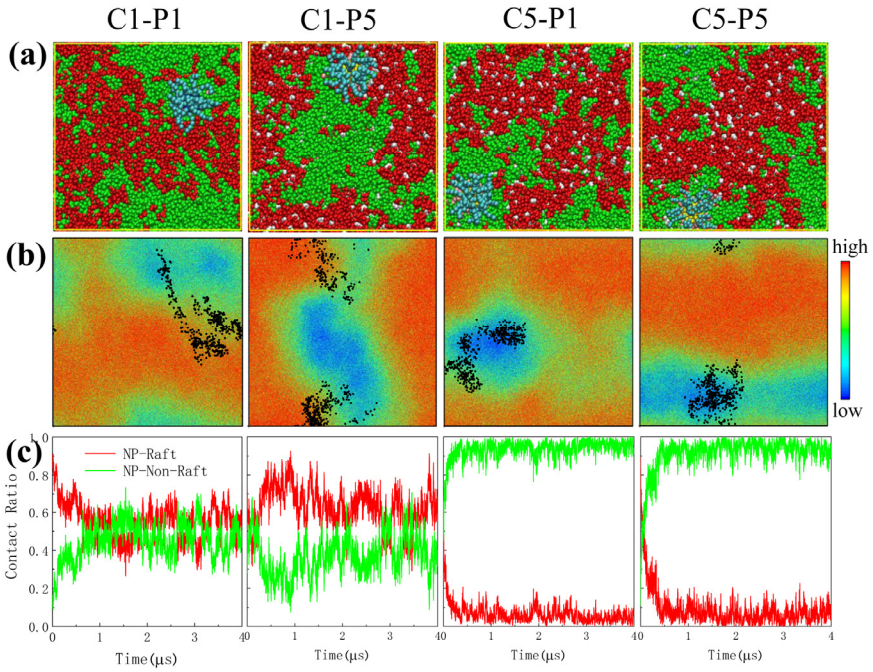

Figure S9. **Effects of ligand properties on the membrane partitioning dynamics of ligand-modified NPs.** **(a)** Top-view system snapshots of the last frame of each 4 μs trajectory. **(b)** 2D number-density maps of DPPC molecules and the instantaneous location of NP (black points) derived from analysis over the last 500 ns trajectory. **(c)** Time evolution of contact number of NP with Raft/Non-Raft domain. The coloring style is the same as in **Fig. S6**.

5. Convergence of the Potential of Mean Force (PMF).

PMF profiles were calculated using the weighted histogram analysis method over the last 400 ns of each 500 ns trajectory. The partial overlapping of the neighboring umbrella sampling windows reflects an adequate sampling for PMF calculation (**Fig. S10**).

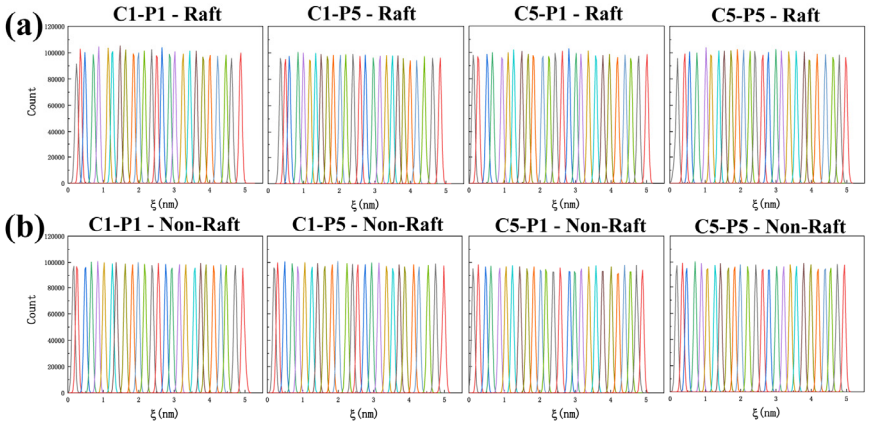

Figure. S10. The histogram of 26 umbrella windows based on the last 400 ns trajectories.

6. Effects of NP Initial Location on Its Membrane Partitioning

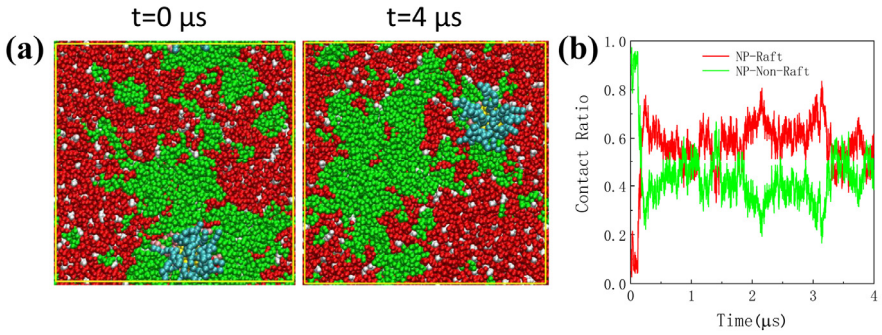

Figure S11. Effects of NP initial location on the membrane partitioning of C1-P1 Janus NP. (a) Top-view system snapshots of the first and the last frame of 4 μs trajectory. The coloring style is the same as in **Fig. 1**. **(b)** Time evolution of contact number of NP with Raft/Non-Raft domain.

6. Detailed Procedures of Voronoi Tessellation Analysis.

The Voronoi tessellation analysis were performed in MATLAB. First, x/y components of DPPC/DUPC center-of-mass coordinates and x/y box dimensions are obtained. Then, the boundary problem was solved by introducing its nearby 8 periodic copies. Last, MATLAB tools such as “voronoin”, “polyarea” and “patch” was used to obtained the Voronoi diagram shown in the manuscript (Detailed commands are shown as follows).

```
%Voronoi Tessellation Analysis
A=20;% Input the size of the box.
x=[];% Input x coordinates of per lipid COM.
y=[];% Input y coordinates of per lipid COM.
x1=x-A;% Periodic boundary.
x2=x+A;
y1=y-A;
y2=y+A;
x0=[x1 x x2 x1 x x2 x1 x x2];
y0=[y1 y1 y1 y y y y2 y2 y2];
[v,c]=voronoin([x0;y0]');
n=length(c);
B=zeros(n,1);
for i = 1:length(c)
C=polyarea(v(c{i},1),v(c{i},2));% Calculate the area value of per lipid.
B(i)=C;
patch(v(c{i},1),v(c{i},2),B(i));% Color according to area value.
```

```
set(0, 'defaultfigurecolor','w');
axis([0 A 0 A]),axis square;
```