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Molecule Manipulation at Electrified Interfaces using Metal Nanogates

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ABSTRACT

Previously documented results on molecule manipulation using metal nanogates are comprehensively reviewed to conclude novel characteristics, which could be widely used for the developments of the systems at electrified interfaces. Novel systems to segregate molecules from self-spreading lipid bilayers in electrolyte solutions have been developed by using metal nanogate structures on solid surfaces. The spatial distribution of target molecules in the lipid bilayer changes depending on the molecule characteristics, such as charge, size, and flexibility. Energy dissipation during the spreading of the molecules on the surface contributes to the compression of the lipid bilayer at the nanogate, leading to the formation of a gradient of the electrochemical potential of the bilayer system including the target molecule in the vicinity of the nanogate (<100 nm from the gate). The gradient results in a segregation force being applied to target molecules in the order of 10^{-15} N per molecule. The segregation property can be tuned by changing the electrolytes, lipid molecules, temperature, and the width and surface hydrophobicity of the metal nanogate. Introduction of asymmetry to the nanogate leads to further effective diffusion control in a direction perpendicular to the spreading. It has been demonstrated that target molecules with a different number of glycolipid per protein in the lipid bilayer are effectively separated based on the Brownian ratchet mechanism.

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1. Introduction

Effective energy exchange between electrons, photons, and molecules often occurs at heterogeneous interfaces.¹ The origin of the energy conversion is the huge gradient of the electrochemical potential at the molecular scale (less than a few nanometers) reaching values around 10^7 V cm⁻¹. The gradient confined at interfaces promotes charge separation, catalytic reaction, and photon emission via the formation of the optimized structures of various molecules, such as solvents, ions, and reaction substrates. Although tremendous efforts have been made to control both the geometrical and electronic structures of the interfaces, there is still room for improvement of the precise control at the nanoscale. Developments in the technology for these controls are expected to contribute to the construction of effective electrochemical energy devices and/or smart materials, such as novel batteries, very efficient solar cells, and superior catalysts, which work based on the novel architecture.

Techniques have been developed for the structural control of electrochemical interfaces. The resulting structures of the systems are monitored by in situ spectroscopic characterization at the atomic and molecular scales. The information obtained from the observations gives the unique properties of the systems, showing novel functionalities to control the flow of electrons, photons, and molecules at electrodes. In this article, several important demonstrations about the control on flow of molecules have been shown comprehensively. Before going to details on molecule manipulation,

significant impacts on the control of photons and electrons are briefly summarized to clarify the importance on the structure control of metal nanostructure at electrified interfaces.

An example of novel structure-induced functionality on the flow of electrons is the quantized conductance of metal atomic contact investigated under electrochemical potential control.² It has been shown that monoatomic metal contact shows quantized conductance ($G_0 = 2e^2/h = 12.9$ K Ω^{-1}) limited by the density of the electronic states in free-electron metals.³ This conductance value of an atomic contact, however, is reduced by molecule adsorptions and structural changes in the contact. In the situation under electrochemical potential control, the changes in the values can be used to monitor the catalytic activity of the monoatomic contact. It has been found that the conductance histogram of Rh atomic contacts varies with the electrochemical potential.²⁴ When the electrochemical potential of the contact was kept at the potential where the bare Rh metal surface was exposed, the conductance histogram did not show any features. At the potential for under potential deposited hydrogen potential, the conductance histogram showed a feature around $2G_0$ that agreed with the conductance value of a clean Rh atomic contact, which was only observed in ultrahigh vacuum at low temperature. This result indicates that the monoatomic contact can be successfully stabilized by electrochemical potential control at room temperature in solution. Furthermore, the conductance histogram showed novel features around 0.5 and 1 G_0 at the hydrogen evolution potential. This behavior is attributed to stabilization of the metal monoatomic

contact because of atomic hydrogen adsorption.^{2c,2f,2o} The conductance behavior of the Rh atomic contact has been discussed by comparing previously reported results for Au, Ag, Cu, Pt, Pd, Ni, Co, and Fe.² The conductance behavior of the metal atomic contacts is related to the strength of the interaction between hydrogen and the metal surface. One of the most interesting points in these observations, even in Au and Cu^{2d,2i} which normally do not show catalytic activity of hydrogen adoption, is that the formation of the monoatomic contact results are comparable with other catalytic active metals, such as Rh, Pd, and Pt. These finding proved that the nanostructuring of metals at the atomic scale show novel catalytic activity of chemically inert noble metals.⁴ The results can be regarded as the example of nanostructure-induced functionality to control the flow of electrons at electrified interfaces.

Another example is the effect of nanostructuring of metals on optical properties at the interface. Nanostructuring of free-electron metals in the size range from a few to a hundred nanometers shows characteristic optical properties in the visible to infrared range because of plasmon excitation.⁵ Plasmon excitation is the collective excitation of free electrons in metals. Thus, such excitation produces highly localized electromagnetic fields at the interface between metals and outer substances. Various studies have focused on the application of the localized field as the perturbation for chemical reactions at electrified interfaces.^{6–8} Although the intrinsic cross-section of molecules for interaction with photons limits the probability of the electronic excitation, recent attempts to use localized plasmons for photoexcitation of molecules suggest the possibility of modifying the cross-section.^{9,10} By changing the structure of the metal at the nanometer scale, the localization, energy, phase, and wave vector of the electromagnetic field of localized plasmons can be controlled. Such highly localized fields are expected to change the photoexcitation process of the electrons in the electronic states, because highly localized polarization less than the size of molecules breaks the symmetry of wavefunctions. Surface-enhanced Raman scattering (SERS) is a promising tool for detecting the interactions between molecules and localized plasmons, especially for monitoring the plasmon-induced photoexcitation process.^{11–14} Based on detailed SERS measurements of an isolated single-walled carbon nanotube (SWNT) by introducing a metal nanodimer with controlled nanogap, SWNTs with certain chirality have been observed via the resonant excitation of normally forbidden transitions.¹⁵ The newly developed extended discrete dipole approximation method showed that the highly confined electromagnetic field at the nanogap (less than 2 nm) generates an intensity gradient of the field, resulting in the excitation of higher order polarization in the direction of the radial breathing mode in the SWNT. These results demonstrate the breakdown in the optical electronic transition selection rules of an isolated SWNT located in a nanogap using SERS measurements, because a very intense electromagnetic field with a high gradient appears in the nanogap induced by the surface plasmons of the metallic nanodimer.^{15,16} This breakdown in the selection rules means that there are increments in the optical excitation channels beyond the selection rules, indicating the possibility of improving the light harvesting capability necessary for photoenergy conversion and photochemical reactions in the future.¹⁶

These examples in two subjects for the flow of electrons and photons indicate that nanostructuring at interfaces can open up the possibility to change the framework of the electrical and optical limitations of materials defined by the bulk properties. Highly localized perturbation could contribute to activate normally hidden or extremely weak and slow chemical processes. These successful demonstrations prompt us to promote nanostructuring at electrified interfaces for novel functionalities for the control of molecules. In this article, previously reported results of molecule segregation in lipid bilayers through metal nanogates are reviewed comprehensively

to discuss the contribution of nanostructuring at electrified interfaces for novel electrochemical devices.

2. Manipulation of a Small Number of Molecules in Self-Spreading Lipid Bilayers through Metal Nanogates

2.1 Strategies of molecule manipulation using energy dissipation of self-spreading lipid bilayers compressed at metal nanogates

Precise motion control of molecules is an important technique for ultrasensitive recognition, manipulation, and fabrication of materials. Among the techniques for molecular manipulation, highly selective segregation of target molecules from an ultrasmall amount of sample is the main issue. Molecular separation systems based on this unique molecular motion have recently received considerable attention because of their high performance, novel separation mechanism, and the possibility of miniaturizing a separation device driven by ultrasmall energy.¹⁷ The Brownian ratchet,¹⁸ entropic trap,¹⁹ and Ogston sieve²⁰ are typical examples that have been proposed based on molecular manipulation in small spaces.

Molecular manipulation on a lipid bilayer is an effective approach to segregate, manipulate, and purify biomolecules in their native environment. However, most reported methods are based on electrophoresis,²¹ which imposes the crucial restriction that uncharged molecules cannot be manipulated in ultrasmall spaces. To overcome this limitation, self-spreading lipid bilayers have recently attracted interest because of their superior molecular transport ability (Fig. 1).²² The self-spreading phenomenon of the lipid bilayer is thermodynamically driven collective molecular flow and can be used as a low-energy consuming transport medium for any molecule, even uncharged molecules. We have found a molecular filtering phenomenon by passing a self-spreading lipid bilayer through a periodic array of metallic nanogates (Fig. 2).^{23–42} The formation of a highly localized compressed phase at the gates acts as the electrochemical potential barrier for the incorporated dye-labeled lipids. The potential barrier reduces the passing ability of dye lipids through the gate, leading to the emergence of the molecular filtering phenomenon.²³ Various types of metal nanostructures with controlled gate width have been developed to improve the ability of molecule segregations (Fig. 3).

Figure 1. The schematic illustration of self-spreading lipid bilayer.

Figure 2. (Color online) The schematic illustration of self-spreading lipid bilayer through metal nanogate for molecule segregation. Reprinted with permission from Ref. 23, Copyright 2005, American Chemical Society.

(A) nanogates prepared by nanosphere lithography **(B) nanogates prepared by EB lithography** **(C) asymmetric Ratchet gates**

Figure 3. (Color online) Structures of metal nanogates; controlled gate width prepared by nanosphere lithography (A), channels with distinct gate width prepared by electron beam lithography (B), asymmetric Ratchet gates (C). ((A) reprinted with permission from Ref 23, Copyright 2005, American Chemical Society. (B) reprinted with permission from Ref 27, Copyright 2009, American Chemical Society. (C) reprinted with permission from Ref. 40, Copyright 2014, American Chemical Society.)

2.2 Characteristics of molecule segregation at metal nanogates

A new methodology for nanoscopic molecular filtering was developed using a substrate with a periodic array of metallic nanogates with various widths between 75 and 500 nm has been developed. Distinct dye-labeled molecules doped in the self-spreading lipid bilayer were filtered after spreading less than a few tens of micrometers on the nanogate array. Quantitative analysis of the spreading dynamics suggests that the difference in the highly localized chemical potential barrier determines the ability of the doped dye-labeled molecules to penetrate the gate. Significant dependence of molecule effective size in the lipid bilayer was observed in the system (Fig. 4).²⁷

We also developed a quantitative analysis of the spreading energy carried by the bilayer in electrolyte solutions. Theoretical calcu-

lations based on extended Derjaguin–Landau–Verwey–Overbeek theory, considering the van der Waals, electrostatic, and hydration energies, supports the observed electrolyte concentration dependence on the spreading velocity, reflecting the dissipation energy of the system. The numerical analysis of the spreading velocities proves that the estimated dissipation energy gives information on the density of the bilayer as well as the electrostatic energy, which is the main factor that contributes to the spreading.^{24,25}

As well as the width of nanogates, the shape of the gate and the microscopic structure of the substrate surface are also important factors to determine the dissipation energy of the spreading bilayer through the gates. Further fine-tuning of the spreading energy was achieved by surface modification of the nanogate using thiol molecules for the formation of a self-assembled monolayer on the surface of the metal gate (Fig. 5). The molecular distribution and spreading dynamics of lipid bilayers can be tuned by the hydrophobicity of the nanogate surface. Interactions between lipids and the surfaces alter the localized compression of lipids at the gate, leading to the change in molecular filtering. The hydrophilic surface can tune the spreading velocity without changing the molecular distribution in the spreading bilayer, whereas the hydrophobic surface provides a molecular concentrating function to the nanogates. The results prove that a combination of unmodified, hydrophobic, and hydrophilic nanoarchitectures has a wide range of potential applications because it can be used to independently control the self-spreading dynamics and the molecular distribution.³²

2.3 Origin of molecule selectivity discussed based on single molecule observation

Further detailed study of the origin of the filtering processes was conducted based on the observations using the single molecule tracking method, which enables precise characterization of molecular diffusion dynamics. The presence of the chemical potential barrier for the molecule can be detected as a modulation in diffusion dynamics. Single molecular tracking was carried out for a lipid molecule in a self-spreading lipid bilayer on a glass substrate with and without Ag nanoarchitectures. Without Ag architectures, the mean square displacement (MSD) analyses showed that lipid molecules randomly diffuse within the self-spreading lipid bilayer with a diffusion coefficient comparable with the value for an artificial solid-supported lipid bilayer. However, in the presence of Ag architectures, dye molecules undergo hop diffusion between two neighboring compartments surrounded by the Ag architectures, which significantly decreases the MSD value. The smaller diffusion coefficients observed for the substrates with the Ag architectures are attributable to suppressed diffusion at the gap between the Ag architectures. The escape probability from the initial compartment to the neighboring compartment estimated from the MSD analysis agreed well with the values theoretically estimated from the compartment configuration, which proves that the Ag nanoarchitectures act as a diffusion barrier. These results provide a new mechanistic insight into how molecules feel the chemical potential barrier during the nanogate filtering process.³⁰

The numerical analyses of the inhibition of the diffusion provide additional quantitative information on the origin of the control of the molecular motion. Size-dependent changes in the MSD values and diffusion constants give the value of the average velocity of the molecules. For example, it has been found that the average velocities of molecules along the self-spreading direction decreased from the control value of 610 nm s^{-1} at the substrate without a metal gate to ca. 420, 470, and 260 nm s^{-1} at substrates with ca. 220, 130, and 80 nm gate widths. These decreases are attributable to the inhibition of the diffusion at the gate, as shown in the previous section. The observed decrease in the velocity was discussed quantitatively by estimating the additional force F applied to the molecular motion due to the spreading (Fig. 6). Assuming a rigid sphere model, the force

Figure 4. (Color online) Three-dimensional snapshots of fluorescence microscope images. Intensity of the fluorescence is shown as blue to red colors, indicating strength. Reprinted with permission from Ref. 27, Copyright 2009, American Chemical Society.

Figure 5. (Color online) (a) AFM images of NSL substrates. (b–f) Fluorescence microscopy images of a self-spreading lipid bilayer on various substrates. The brightness of each image was adjusted to give a clear image. Lower panels show enlarged images. The white arrows in (d) indicate the presence of a leading edge in front of the self-spreading lipid bilayer. Reproduced from Ref. 32 with permission from the PCCP Owner Societies.

on the diffusing molecule F was calculated to be 0.38 and 0.17 fN per molecule for the control and the 82 nm gate channel, respectively. Thus, the force acting on the molecule as the deceleration (F') on the 82 nm gate channel can be estimated as 0.21 fN per molecule. It should be noted that this value was found to be the same order as the electrostatic force (0.40 fN per molecule) required for generating a molecular concentration gradient in the homogeneous lipid membrane with applied field strength of $10^{-15} \text{ V cm}^{-1}$.²⁶ This indicates that molecular distribution control as effective as that of the electric-field dependent system can be achievable without any external field when a self-spreading lipid bilayer is used as the molecular transport medium. The estimated values of the force are average values. Further detailed analysis assuming inhomogeneity of the

compression of the lipid bilayer gives higher values, such as 4 fN per molecule at a distance from the gate $w = 60 \text{ nm}$, which is 10 times higher than the values in the electrophoretic system. We further estimated the force imposed on molecule near the nanogate, leading to the confirmation on the contribution of highly localized compression at the gates. The advantage of the system with metal nanogate using the self-spreading again becomes apparent from the fact that no external bias is imposed to generate relatively high perturbation applied on molecules.³¹

We have also proved that high precision measurements at the single molecule level give novel characteristics of lipid membranes that could not be clarified by other ordinary ensemble measurements. The molecular orientation of molecules was obtained through

Figure 6. (Color online) Trajectory of a single molecule through metal nanogate (upper); applied force F' to molecule locally imposed at single nanogate as a function of w . The inset shows the schematic illustration of nanogate region which induces filtering effect. Reproduced from Ref. 31 with permission of The Royal Society of Chemistry.

Figure 7. (Color online) Schematic presentation for the effect of cholesterol addition on the structure of the lipid bilayer. Defocus images of dye-labeled lipid molecules are also shown. Reproduced from Ref. 38 with permission from the PCCP Owner Societies.

defocused imaging under the observation of a single molecule diffusion (Fig. 7).^{38–40} The observation clarified the microscopic features, such as cholesterol-induced changes in the local packing structure. Diffusion analysis gave insight into the macroscopic aspects of the phase distribution in the heterogeneous bilayer system. Combining these two independent investigations, we revealed the effect of cholesterol addition to the lipid bilayer on the microscopic local packing and macroscopic phase structures. Our observations showed a phase transition from loosely to tightly packed phases upon the addition of cholesterol, which was not evident from previous domain shape observations. The results indicate that the orientation and diffusivity of molecules offer useful information regarding the phase distribution, which may be hindered

Figure 8. (Color online) The separation of different CTB-GM1 aggregates in the self-spreading lipid bilayer by the Brownian ratchet obstacles. Reprinted with permission from Ref. 40, Copyright 2012, American Chemical Society.

by the apparent phase structure in a heterogeneous lipid bilayer that contains cholesterol. The present single-molecule orientation observation technique is useful for detailed investigative studies of the microscopic structures of heterogeneous biological membranes.³⁸ This cholesterol-induced change in the micro domain structure is now used to increase the potential barrier because of the compression of the bilayer membrane at the nanogate. Improvement in the separation efficiency was achieved by the introduction of cholesterol into the spreading bilayer.⁴¹

2.4 Use of thermal fluctuation at ratchet structure for molecule segregation

As shown in previous sections, fabrication of the metal nanogate into the substrates gives the capability of molecule segregation at the spreading bilayers. The filtering efficiency is determined by the potential barrier formed at the nanogate because of compression of the spreading bilayer. Single molecule observation quantifies the biased motion of the molecule even under thermal fluctuation. Under these conditions, Brownian motion can be used to improve the separation efficiency of particles with random motion, if one can introduce asymmetric potential into the system works as Brownian Ratchet (Fig. 8). We have proposed a new approach for two-dimensional molecular separation based on the Brownian ratchet mechanism using a self-spreading lipid bilayer as both a molecular transport and separation medium.^{37,40} In this method, a two-dimensional ratchet substrate is introduced on the substrate. As the target molecule, Cholera toxin subunit B (CTB) binding to GM1 glycosphingolipid molecules in the spreading bilayer was selected, because multivalent CTB-GM1 binding properties are very important for diagnosis. Although the number of GM1 molecules binding to CTB has been proposed to be from one to five, direct observation of the binding states has not yet been achieved. We applied single molecule observation to characterize the diffusion of CTB molecules binding to GM1 in the spreading bilayer at the substrate with Brownian ratchet structure.^{34,40}

Single molecule tracking determines the diffusivity of CTB binding to GM1 molecules. Analysis of the diffusion coefficients of each CTB-GM1 complex showed the presence of at least three types of CTB-GM1 complexes with different diffusivities. The presence of three components also appeared in the control experiment using a glass substrate without metallic nano-obstacles. The diffusion coefficients on the glass substrate had slightly higher values than those on the ratchet substrate because of the absence of metallic obstacles acting as diffusion barrier. To consider the effect of nano-obstacles, we will discuss the diffusivity on the ratchet substrate. The diffusivity is dominated by the viscous dragging force, which is determined by the molecular size in the lipid bilayer. Because CTB has five binding sites to GM1, CTB can bind one to five GM1 molecules. The number of GM1 molecules in each CTB-GM1 complex determines the diffusivity; i.e., a multivalent CTB-GM1 complex with a larger number of GM1 molecules would

Figure 9. (Color online) (top) Schematic illustration of three different ratchet obstacles. (bottom) AFM images of each ratchet obstacles. (a) Normal ratchet obstacles. Rectangles with aspect ratio 1:4 are tilting 45° from the flow direction. (b) Horn-shaped ratchet obstacles. Additional triangle part for normal ratchet obstacles is shown in red. Narrowest gap width between obstacles is 100 nm. (c) Channel-shaped ratchet obstacles. Additional part for normal ratchet obstacles is also shown in red. Channel width is 175 nm. Reprinted with permission from Ref. 40, Copyright 2014, American Chemical Society.

exhibit slower diffusion. The observation clearly demonstrates that at least three types of CTB–GM1 complexes with different binding configurations were formed in the self-spreading bilayer.

The capability of the two-dimensional molecular separation is characterized by the perpendicular displacement reflecting the biased angle. A difference in the perpendicular displacement depending on the diffusivity of CTB molecules at the Brownian ratchet substrate was clearly observed. Numerical analysis was carried out to characterize the displacement according to the method as a simple geometric solution for the molecular transport mechanism perpendicular to the flow direction based on the two-dimensional Brownian ratchet mechanism. The analysis showed that the ability of the separation at the present system is quite high. A single molecule tracking observation proved that a carefully designed ratchet substrate produced the maximum ratchet separation angle. To clarify the origin of this extremely high separation efficiency, we developed a comprehensive model of the ratchet separation over a wide range of diffusivities, i.e., 3 orders of magnitude wider than those used in previous systems. We proposed a new geometrical model in the calculation; i.e., a higher-order lane shift was taken into account for molecules with high separation. Furthermore, the use of a self-spreading lipid bilayer induced a local velocity decrease at the gate region. This system enabled the separation of molecules even with the same separation by causing an additional ratchet shift that depended on the molecular structures, in this case the difference in the number of glycolipid per protein.

We have adopted a new strategy for enhanced Brownian ratchet using uniquely shaped ratchet obstacles. Using the commonly used Brownian ratchet obstacles, which are composed of simple rectangle-shaped obstacles (normal ratchet), the deformed obstacles with narrower gates (horn-shaped ratchet) and longer gates (channel-shaped ratchet) were used as the separation substrates (Fig. 9). Single molecule tracking observation gives the structure dependent values of the average separation angles at the respective substrates (Fig. 10). Although the average separation angle was 8.3° for the normal obstacles, the average separation angle of the horn-shaped ratchet obstacle increased to 12.6° . Furthermore, a much higher separation angle (21.0°) was obtained for the channel-shaped obstacles. This high separation angle resulted from the high

Figure 10. (Color online) Typical trajectories of bright objects observed in spreading lipid bilayer on ratchet substrate with three different ratchet obstacles. In each image, the lipid bilayer spread from the top to the bottom vertically. Obstacle shape: (a) normal, (b) horn-shaped and (c) channel-shaped. Different tracking time (t) and diffusion coefficient (D) were obtained in each trajectory: (a) $t = 1.06$ s, $D = 2.52 \mu\text{m}^2/\text{s}$; (b) $t = 1.37$ s, $D = 2.04 \mu\text{m}^2/\text{s}$; and (c) $t = 1.35$ s, $D = 2.47 \mu\text{m}^2/\text{s}$. (d) Average separation angle for each ratchet obstacles. Values of average separation angle are also shown on each bars. Error bars indicate standard error. Reprinted with permission from Ref 40, Copyright 2014, American Chemical Society.

perpendicular displacement under low parallel displacement, which is because of the geometrical effect of the obstacles and/or the nanogate effect discussed above.

To evaluate the characteristics of the observation, the random walk system in the Chemistry simulation program^{43,44} was used to simulate the motion of molecules at the respective substrates. The simulations were performed to investigate the diffusion behavior of a single particle in two-dimensional sieves with microscopic obstacles. The behavior of the diffusion was quantitatively evaluated by the average number of cavities the particle travels through, and by the MSD analysis. We constructed periodic arrays of impermeable parallelogram diffusion barriers, which serve as obstacles for the particle's diffusion process in the sieve, as with the experiments. Our simulations aimed to investigate the influence of the shape of the barriers on the diffusion behavior of the particle. The typical simulation trajectories for each ratchet obstacle are shown in Fig. 11. The average separation angles were calculated according to the same analysis of the experiment. Although the simulated average separation angle with the horn-shaped ratchet obstacle is slightly higher than that for the other two systems, no clear substrate dependence was found in the calculations.

This result indicates that the apparent effect of the separation angle depending on the substrate structure observed by experiment cannot be simply explain by the simple Brownian motion of molecules. Thus, we should consider additional effects for the present system. We have proven that the molecular filtering effect at the nanogate increases with decreasing gate size around the size region of interest. As a result, the gate effect could be apparent in the order channel-shaped > horn-shaped > normal. The present result that the horn-shaped geometry (gate width = 100 nm) caused a higher ratchet effect than the normal geometry (gate width = 250 nm) is reasonable, because the molecular filtering effect is known to be enhanced when using metallic channel-shaped width.⁴² In our ratchet experiment, the filtering effect should be more obvious on the channel-shaped ratchet obstacles than on the horn-shaped

Figure 11. (Color online) (a–c) Typical simulation trajectories of diffusion particles with three different ratchet obstacles within 5 s. (d) Average separation angles of three different ratchet obstacles based on the simulation results. Reprinted with permission from Ref 40, Copyright 2014, American Chemical Society.

ratchet obstacles. An increment in the retention time of molecules at the gates leads to the highest separation angle at channel-shaped obstacles. Based on these considerations, the importance of the geometrical effect becomes apparent under the comparable condition of effective molecular filtering. This result demonstrated the importance of the combination of the gate effect and the Brownian ratchet to achieve effective molecular separation using the self-spreading bilayer. We succeeded in the construction of novel molecular manipulation system by minute energy consuming external perturbation in ultrasmall spaces.⁴⁰

3. Conclusions

The present study proves that the two-dimensional arrays of metal nanostructures at electrified interfaces can be used as a tool for the effective segregation of target molecules in a single lipid bilayer. Optimization of the interactions between molecules, electrolyte ions, solvents, and substrate surfaces results in controlled self-spreading to provide sufficient energy for segregation from a zepto (10^{-21}) molar amount of lipid. Nanostructures constructed on the substrate contribute to make a gate effect, i.e., a gradient of electrochemical potential in a size scale less than 100 nm. Such perturbation leads to application of the segregation force to respective molecules in the order of 10^{-15} N, which is comparable with that of the usual molecule separation method electrophoresis. Thermal fluctuation in the system can be also used to improve the selectivity by introducing a newly designed Brownian ratchet structure at the substrate. Flow of the bilayer induced by the spreading constructs the asymmetric potential to separate molecules in the direction parallel to the spreading. As a result, improved Brownian ratchet segregation emerges in two dimensions by the combination of the self-spreading bilayer at the nanostructured substrate. The lipid membrane can be regarded as a model of a cell membrane, in which a variety of sophisticated functions, such as ion transport, molecule segregation, and stimulus-response of potential, develop. Effective processes in biological systems work well even under the condition with thermal noise at room temperature. A cell membrane is physically

compartmentalized with compartment sizes ranging from tens to thousands of nanometers. Unknown principles probably contribute to these sophisticated functionalities in the structures hierarchy. Artificial construction of nanostructures at electrified interfaces is still not enough to realize perfect control of molecule manipulation showing effective thermodynamics close to the Carnot limit. The findings from the systems comprehensively summarized in this article may provide an insight on the specially confined electrochemical potential, which is the source of energy conversions. The present findings are expected to contribute to the development of electrochemical devices via creation of novel functionalities from nanostructuring.

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