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Site-specific Deposition of Colloidal Pd Nanoparticles on Self-assembled Microtubules from Biolipid

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Lipid microtubules with wound ribbon features were fabricated by self-assembling method, and the deposition patterns of colloidal Pd particles on tubular template were investigated. The result indicates that colloidal Pd nanoparticles are preferentially decorated on the helical markings in the interior and on the exterior of preformed tubule and to the edge of loosely helical ribbons to obtain helical deposition features. The multi-bilayer microstructure of tubules can be marked by fine Pd nanoparticles deposited at the edge of helical ribbon. There are the site-specific interactions between lipid tubular template and colloidal Pd particles at the helical edge. A new route was illustrated that colloidal Pd particles firstly attach at the edge of thin flat membranes, and then thin membranes roll up and reassemble into tubule together with particles to form helical deposition patterns. The site-specific deposition of Pd is unbeneficial to obtain the homogeneous metal film on tubules, but it can be utilized to reveal the different chemical nature of lipid molecular assembly.

Keywords moleculear self-assembly, lipid microtubule, Pd nanoparticle, site-specific deposition

Introduction

Biologically molecular self-assembly and template site-specific chemistry play an important role in biomineralization and new material fabrication.[1-5] Recently, it was reported that scientists fabricated different metallic nanostructures (highly oriented Pd nanocluster or nanowires) on biomolecular template via the chemical deposition of nanoscale palladium.[6-9] These nanostructures will have special electrical, magnetic, and quantum effect and have potential application in microelectronics, and these templates include different biological molecules, such as DNA, S-layer protein (surface layers), protein cages, and bacterial rhapidosomes.[6-12] Lipid biological membrane is one of typically molecular assemblies. Lipid molecules due to their amphiphile natures can self-organize to a variety of thermodynamically stable microstructures, for example, micelle, vesicle, helical ribbon and tubular microstructures.[13] Among them, an important research field is biolipid tubular structures, such as those formed from synthetic diacetylenic glycerophosphatidylcholine.[14-17] Lipid tubules have been utilized as a template to fabricate two kinds of materials. One kind is to coat the tubule with thin metal films.[18,19] The other is to fabricate organic-inorganic composites with high aspect ratio by depositing different nanoparticles, such as silica, gold, alumina.[20-23] Metallized lipid microtubules can be utilized to develop a new electroactive composite and a new vehicle in controlled release of marine antifouling agent to prevent ship from biofouling on ship hull.[13] Metal Pd is an important catalyst to initiate electroless metallization. However, to date, few researches on the detailed study of the deposition of colloidal Pd on the lipid tubules have been reported.

Colloidal Pd solution, which has been a catalyst in industry, plays an important role in electroless metallization on different substrates.[24,25] Colloidal solution of Pd in excess stannous chloride serves Pd particles to adhere to substrate surface without selectivity. However in our experiment, colloidal Pd particles are primarily decorated on the helical markings of lipid tubules on the exterior tubule and in the interior to form helical features, exhibiting a kind of site-specific deposition characteristics.

Hence, the detailed deposition features of colloidal Pd nanoparticles on lipid self-assembled tubular template are investigated. We suggest a new route to form site-specific deposition, not on the preformed tubules, but from the reassembly of lipid thin membrane with Pd nanoparticles deposited at the edge. To date, this would be the first time to illustrate this process. The site-specific deposition of Pd can mark the multi-bilayer structures of lipid tubules, and initiate inhomogeneous deposition of Ni on template. Meanwhile, the underlying mechanism of site-specific deposition is also dis-

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Experimental

1,2-Bis-(tricosa-10,12-diynoyl)-*sn*-glycero-3-phosphatidylcholine ($DC_{8,9}PC$, Figure l), was obtained from Avanti Polar Lipid Co., tested by thin layer chromatography and used after Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) analysis. Sodium tetrachloride palladate and dimethyl amine borane (DMAB) were purchased from Fluka Co., and used directly. Palladium chloride and stannous chloride for colloidal solution were analytically pure reagents. Double distilled water was used in all experiments.

Fabrication of lipid microtubules

The self-assembled lipid microtubules were fabricated through thermal cycling of lipid dispersions.[26] The procedure is as follows: adding $DC_{8,9}PC$ 10 mg into 10 mL of mixture solvent (ethanol∶water, 7∶3, *V*∶*V*), raising the temperature to 60 ℃, and cooling the solution to room temperature at a rate of 1 ℃/h. A white colloidal substance was precipitated from the solution at 32 to 34 ℃, which showed a kind of cylindrical shape under an optical microscope, and a tubular microstructure with helical ribbon wound observed under a SEM and TEM.

Deposition of colloidal Pd nanoparticles and Ni particles

Two kinds of colloidal Pd solutions were utilized to deposit on the lipid template. The first colloidal Pd solution was prepared in excess stannous chloride solution in conventional ways.[27] The colloidal Pd solution was treated about 4 h by ultrasonication method in the process of solution age in order to obtain finer Pd particles. An equal volume of colloidal Pd solution was added into preformed lipid tubule dispersion after dialysis to remove excess ethanol in system. Then large amount of water was added repeatedly to wash off excess colloidal solution after staying overnight, till the solution mixture reached almost neutrality. After that, solution mixture was treated by dilute NaOH solution (0.1 mol/L) about 5 min to release the activity of Pd. Because colloidal Pd particles enveloped by excess stannous chloride contain a large amount of flocculent substance, it is difficult to distinguish individual particles on the template. In order to get clearer image of site-specific patterns, the lipid tubule suspension treated after deposition of Pd was electrolessly plated by a 10% dilute Ni plating in less than 5 min, and the plating reaction was quenched by large amount of water. The sample was washed several times to remove remaining salts in solution. The Ni plating bath was prepared as described in the reported method.[19]

A second colloidal Pd solution was prepared from sodium tetrachloride palladate in excess sodium chloride solution.[28] Pd ion complex with chloride ions showed a kind of negative charge in solution. An equal volume of colloidal Pd solution was added into the preformed lipid tubule dispersion after dialysis to remove excess ethanol in system. After staying for 24 h, the mixture was dialyzed to remove excess Pd complex ion. A dilute DMAB solution (0.2%) was added into the above mixture, and the lipid suspension became gray with gas (H_2) bubbling out of the solution. Several minutes later, the solution was centrifuged and washed several times to do further observation.

Microscopic examination

An optical observation of lipid suspension was performed on glass slide by using an Olympus BH-2 biological microscope. The suspension sample was pipetted onto a formvar film-coated copper grid, washed gently by water, and air-dried for microscopic examination. Scanning electron microscopy (SEM) was performed by using a JEOL JSM-5610LV microscope (Japan), and the samples were coated with a thin layer of Pt to increase conductivity by using a JEOL JFC-1600 auto fine coater before examination. Electron dispersion X-ray spectroscopy (EDS) for composition analysis was performed by using EDAX-falcon system. The transmission electron microscopy (TEM) was performed by using a JEOL JSM-100 microscope (Japan), with an accelerative voltage of 60 kV, and a Philip CM200 microscope (Holland), with an accelerative voltage of 200 kV. Three dimensional topological image was observed by using a SPA 400 instrument (SEIKO, Japan) under a dynamic force mode (DFM), with a scanning tip of silicon nitride, a force constant of 17.5 N/m, and a scanning field of 20 μm×20 μm. High-resolution transmission electron microscopy (HRTEM) (JEM2010, Japan) was used to examine whether there were any lattice fringes of Pd nanoparticles on the tubules with an accelerative voltage of 200 kV.

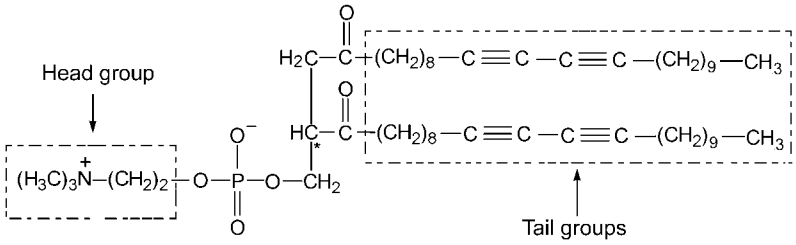

Figure 1 Chemical structure of lipid molecules $DC_{8,9}PC$ (* means chiral center in the molecule).

Results and discussion

Microscopic examination indicates that the lipid tubules were fabricated with high yields in lipid dispersion by self-assembling method. Under an optical microscope, the prepared microtubules stack each other disorderly just like a bird-nest or soda straw (Figure 2a). However, they show hollow structures and clear helical wrapping patterns under an electron microscopy (Figures 2b and 2c). Tubules are formed by helically wound ribbons and exhibit ribbon-wrapping features. An average diameter of microtubules ranges from 0.4 μm to 1.0 μm, and lengths from tens to hundreds micrometers. There also are few helical ribbons in loose way in solution (Figure 2d). The results are consistent with those in literatures.[14-17]

The formation process of microtubules can be described in Figure 3. First of all, the long diacetylenic hydrophobic groups (tail-groups, seen in Figure 1) point inside of lipid bilayer, and the short choline hydrophilic group (head-groups) in molecule directs outside in lipid bilayer to obtain lipid membrane (Figure 3a). The driving force to form a thin membrane comes from molecular amphiphilic nature. As to the formation mechanism of microtubules, most researches and experiments indicate that the diacetylenic group introduces a kink into the acyl chain, which imposes a steric hindrance to the molecules packed parallel to each other (Figure 3b); the nonparallel packing of the molecules can impart either a counterclockwise or clockwise twist to the lipid bilayer they form (Figure 3b). The chirality of lipid molecules causes one orientation to be energetically preferable, eventually driving the lipid membrane to roll into stable tubules.[29] However, lipid molecules with no diacetylene group finally form vesicles.[13]

The deposition of colloidal Pd nanoparticles on lipid tubules was investigated (Figure 4), and the experimental results indicate that the colloidal Pd particles are primarily deposited on the helical markings of tubules. The helical line deposition patterns along the edge of helical ribbon are presented (Figure 4a and 4b). The deposition of colloidal Pd happens not only on the exterior of tubules but also in the interior. In the interior wall, three Pd nanoparticle lines parallel with each other and form helical lines along the helical markings (Figure 4b). The outside rim of the tubules appears smooth and the turning points of helical lines appear in the interior of membrane, which strongly suggests that Pd helical lines are deposited inside the tubules. When the tu bular wall is very thin, such as 2-3 lipid bilayers, the clear images of helical deposition both in the interior and on the exterior can be observed. In most cases, deposition features in the interior and on the exterior of tubule were obtained simultaneously. Meanwhile, the same deposition pattern happens at the edge of loosely helical ribbon (Figure 4c). Because lipid tubules and helical ribbon

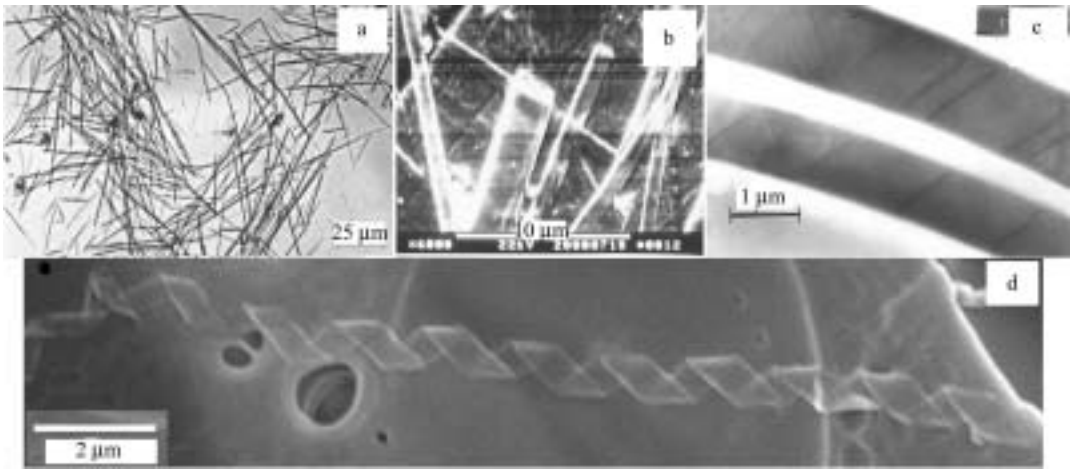

Figure 2 Microscopic observation of lipid microtubules (a) Optical observation, scale bar 25 μm; (b) SEM observation, scale bar 10 μm; (c) TEM observation; (d) Loosely helical ribbon.

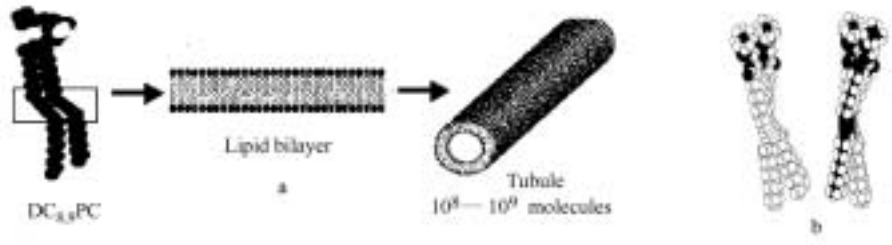

Figure 3 (a) Formation process of microtubule from lipid molecule via lipid bilayer. The black frame stands for a kink introduced by rigid diacetylene groups. (b) nonparallel packing of neighboring molecules. (a) and (b) are cited in Refs. 13 and 29.

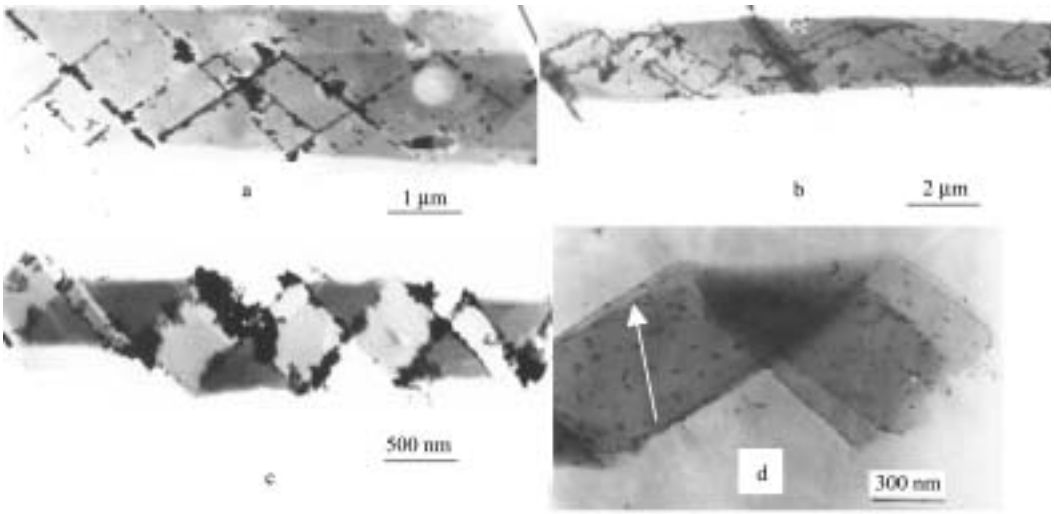

Figure 4 Site-specific deposition features of colloidal Pd particles at the helical edge of lipid tubules. (a) Tubule with Pd/Ni at the edge of helical markings; (b) Parallel helical deposition lines in the interior tubules; (c) Helical ribbon with the colloidal Pd/Ni at the edge; (d) Multi-layer deposition of Pd nanoparticles due to the lipid multi-bilayer features at the edge of helical ribbon (the arrow points).

have high aspect ratio, it is difficult to completely remove the outer colloidal layers of Pd particles in short treatment time with alkaline solution. Therefore, there are some black flocculent substances in the interior tubules (Figures 4a—4c). The samples in Figure 4a—4c were treated with Ni plating in several minutes to obtain better images of Pd particles, and the deposition patterns on the exterior of tubules in Figures 4a and 4c are better and clearer than those in Figure 4b. That is because plating bath is difficult to diffuse into the interior tubule in short treating time. In Figure 4d, the lipid tubules were treated by the colloidal Pd solution with excess NaCl, and then, reduced by DMAB. Pd nanoparticles at the edge of helical ribbon can exhibit multi-layer pattern due to the finer size, because the lipid membrane shows the multi-bilayer structure at the helical edge (The arrows direct in Figure 4d), and there are about 4 lipid bilayers.

Many people ever reported the depositions of different nanoparticles (silica, gold, alumina, platinum) on lipid tubules, but they did not obtain the multi-layer deposition.[20-23] The main reason is because the nanoparticles they used are larger than the thickness of one lipid bilayer. The theoretical thickness of one lipid bilayer is 6.6 nm,[15] however, the size of Pd nanoparticles at the edge in Figure 4d is about 2—4 nm under high power TEM, far less than the thickness of one bilyer. Hence, the deposition of very fine Pd nanoparticles can be a marker to mark the number of lipid membrane.

In the past, people overstressed the nonspecific deposition of colloidal Pd particles in excess stannous chloride solution on substrate, but neglected its selectivity or specificity.[24,25,27] In the paper, the deposition features of Pd nanoparticles indicate that there is a kind of site-specific deposition between tubular template and Pd particles. Lvov[23] think that these helical markings in the lipid tubules are weak lines, and there is at least one bilayer difference at the helical edge of ribbon causing more polarization to accumulate impurities and particles. In both colloidal Pd solutions in our paper, the Pd complex ions all show negative charge,[27,28] however, the choline headgroup with three methyl groups combined with nitrogen atom has positive charge (Figure 1), and has a large steric hindrance. Hence, it could be presumed that the arrangement of choline headgroups at the flat surface of lipid membrane would be in a closer and tighter state to keep the tubular microstructure perfect resulting in more steric hindrance that is unbeneficial to the interaction between the choline groups and Pd complex ions. On the contrary, the helical wrapping of lipid ribbon and membrane causes clear dislocation between lipid bilyers at the edge (the arrowhead in Figure 4d), and it would cause more choline groups to expose in a free environment, in which the headgroup would become much more relaxed or polarity, and the steric hindrance would be reduced. Eventually it is beneficial to strengthen interaction between Pd ions and choline groups through neutralizing the excess polarization at the helical edge of membrane, and to reduce the free energy in the whole system. Here, the helical dislocation could be utilized to describe this effect. According to the above analysis, we are trying to design a molecule to bind with choline group to reveal the different chemical natures between the flat surface and helical edge in lipid membrane.

There are two lipid membranes with colloidal Pd particles deposited at the edges that are being wrapped into two tubules, which have the same spiral markings of Pd deposition (two arrowheads in Figure 5). The upper tubule is being formed from a wide flat membrane with two lipid bilayers, and the lower little tubule is from a narrow ribbon with single bilayer, meanwhile, there is one tubule occasionally loaded on the lipid membrane. The result indicates that lipid thin membranes, even together with Pd particles deposited at the edge, still have an intrinsic driving force to roll up into

tubules. It also confirms the theory that tubular microstructure is the most stable structure in solutions.[29] The lipid ribbon or thin membrane is a kind of intermediate, which can reassemble into lipid tubules. The driving force should come from the molecular chirality and diacetylenic group. From the result, it is also suggested that there is a new route to form the spiral deposition pattern of Pd nanoparticles on tubules. Colloidal Pd particles firstly attach at the edge of thin flat membranes, and then, thin membranes roll up and wrap into tubules together with particles to form the site-specific deposition patterns. In general, it has ever been regarded that helical tubules are firstly formed, and then, some nanoparticles deposit primarily on preformed tubular template.

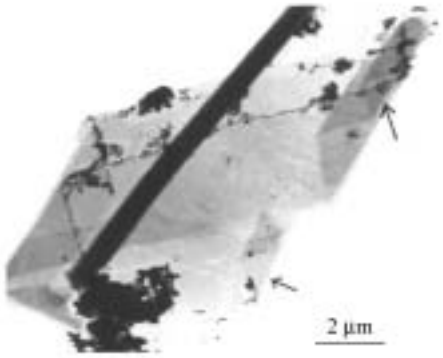

Figure 5 Thin membranes with Pd at the edge are being wrapped up into tubules to form helical Pd deposition pattern. Colloidal Pd in excess $SnCl_2$ solution treated the lipid samples.

The clear deposition features of colloidal Pd nanopaticles are also shown in the AFM image (the arrows in Figure 6). The fine Pd particles deposit mainly at the helical edge, and the helical ribbon shows multi-bilayer patterns, consistent with the result in Figure 4d. The tubules become flat in the dried state. The clear lattice fringes of Pd particles [Pd (200)] on lipid tubules were obtained under a HRTEM (Figure 7), and the results

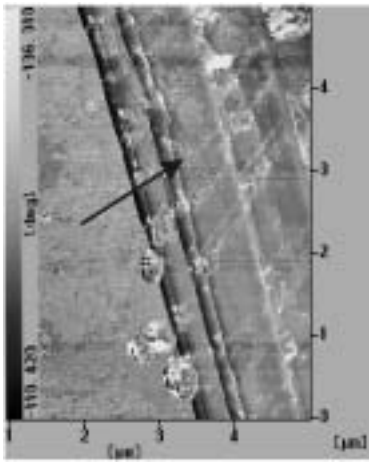

Figure 6 Phase images of Pd particles deposited at the helical edge of lipid tubules observed under AFM. The arrow points the edge of helical ribbon with a multi-bilayer feature.

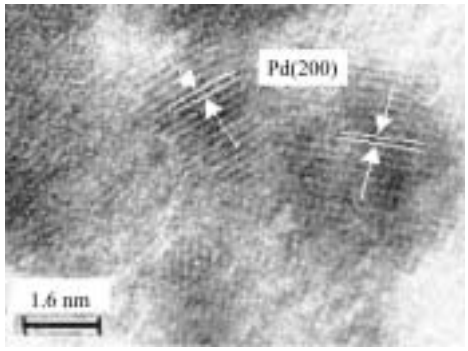

Figure 7 HRTEM image of Pd particles on lipid tubule.

also indicate that the orientation of lattice fringes of different Pd particles is stochastic. Hence, it would be difficult to utilize the lattice fringes of Pd to judge the molecular arrangement on lipid template.

The electroless plating of Ni on template indicates that the deposition of metal Ni happens mainly at the edge of helical tubules and loosely ribbon, which is catalysed by the site-specific deposition of Pd (Figure 8a). EDS for composition analysis confirms that there are two strong Ni peaks and a weak Pd peak, and a strong Sn peak comes from colloidal catalyst solution (Figure 8b). Therefore, the site-specific deposition of colloidal Pd is unbeneficial to form homogeneously thin metal film on template. Further research can be done to modify the surface of tubules to obtain uniform metal coat to develop new materials.

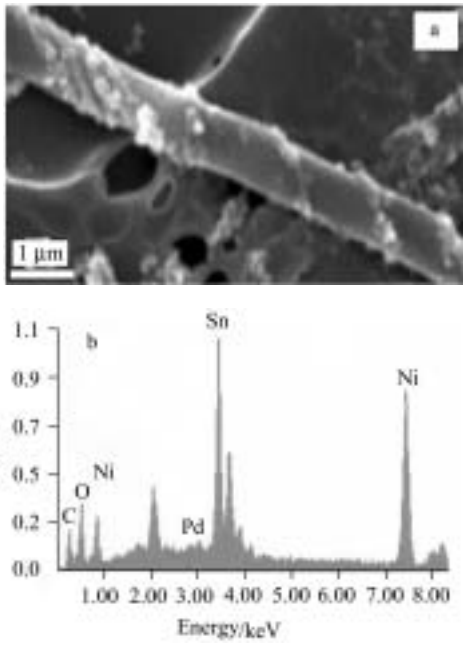

Figure 8 Deposition patterns of Ni catalyzed by colloidal Pd deposited at the edge of helical ribbon (a), and the EDS for composition analysis under SEM (b).

Conclusion

The lipid microtubules with multi-bilayer structure are formed by molecular self-assembly from a kind of

biolipid. Colloidal Pd nanoparticles can selectively deposit at the helical edge of lipid tubules and ribbon in the interior and on the exterior. The helical dislocation at the edge of lipid ribbon can cause much more activity for lipid headgroups to absorb colloidal Pd nanoparticles. A new reassembly process is shown that colloidal Pd firstly settles on the lipid thin membrane, then, lipid thin membrane with colloidal Pd at the edge rolls into tubules with spiral markings of Pd. Catalyzed by Pd nanoparticles, metal Ni has the same deposition pattern on tubules via electroless plating. The research will be significant for us to obtain more information on the assembly nature of lipid molecules in the living system. Meanwhile, metallization on biological template can help us to provide these templates with many new properties and to translate this biomolecular knowledge into material science.

References

1 Mirkin, C. A.; Letsinger, R. L.; Mucic, B. C.; Storhoff, J. J. *Nature* **1996**, *382*, 607.
2 Service, R. F. *Science* **1997**, *277*, 1036.
3 Niemyer, C. M. *Curr. Opinion in Chem. Biol.* **2000**, *4*, 609.
4 Nedelec, F. J. T.; Surrey, A. C.; Leibler, M. S. *Nature* **1997**, *389*, 305.
5 Whitesides, G. M.; Mathias, J. P.; Seto, C. T. *Science* **1991**, *254*, 1312.
6 Behrens, S.; Kahn, K.; Habicht, W.; Bohm, K.-J.; Kosner, H.; Dinjus, E.; Unger, E. *Adv. Mater.* **2002**, *14*, 1621.
7 Richter, J.; Kirsch, R.; Mertig, M.; Pompe, W.; Plaschke, J.; Schackert, H. K. *Adv. Mater.* **2000**, *12*, 507.
8 Pompe, W.; Mertig, M.; Kirsch, R.; Wahl, R.; Ciacchi, L. C.; Richter, J.; Seidel, R.; Vinzeberg, H. *Z. Metallkd.* **1999**, *90*, 1085.
9 Mertig, M.; Kirsch, R.; Pompe, W.; Engelhardt, H. *Euro. Phys. J. D* **1999**, 45.
10 Wong, K.; Mann, S. *Adv. Mater.* **1996**, *8*, 928.
11 Pazirandeh, M.; Baral, S.; Campbell, J. R. *Biomimetics* **1992**, *1*, 41.
12 Mann, S. *Biomimetic Material Chemistry*, VCH Publishers, Inc. S. Mann, School of Chemistry, University of Bath, Bath, BA27AY United Kingdom, **1996**, pp. 1—38.
13 (a) Schnur, J. M. *Science* **1993**, *282*,1669.
(b) Yager, P.; Schoen, P. E. *Mol. Cryst. Liq. Cryst.* **1984**, *106*, 371.
14 Schnur, J. M.; Price, R. R.; Schoen, P. E.; Yager, P. *Thin Solid Films* **1987**, *152*, 181.
15 Spector, M. S.; Selinger, J. V.; Singh, A.; Rodriguez, J. M.; Price, R. R.; Schnur, J. M. *Langmuir* **1998**, *14*, 3493.
16 Spector, M. S.; Price, R. R.; Schnur, J. M. *Adv. Mater.* **1999**, *11*, 337.
17 Schnur, J. M.; Shashidhar, R. *Adv. Mater*. **1994**, *6*, 971.
18 Schnur; J. M.; Schoen, P. E.; Yager, P.; Calvert, J. M.; George, J. H.; Price, R. R. *US 4911981*, **1990** [*Chem. Abstr.* **1990**, *113*, 89592s].
19 Markowitz, M.; Baral, S.; Brandow, S.; Singh, A. *Thin Solid Films* **1993**, *224*, 242.
20 Chappell, J. S.; Yager, P. *J. Mater. Sci. Lett.* **1992**, *11*, 633.
21 Burkett, S. L.; Mann, S. *Chem. Commun.* **1996**, 321.
22 Letellier, D.; Sandre, O.; Menger, C.; Cabuil, V.; Lavergne, M. *Mater. Sci. Eng., C* **1997**, *5*, 153.
23 Lvov, Y. M.; Price, R. R.; Selinger, J. V.; Singh, A.; Spector, M. S.; Schnur, J. M. *Langmuir* **2000**, *16*, 5932.
24 Shipley, Jr. C. R. *Plating Surf. Finish.* **1984**, *71*, 92.
25 Osaka, T.; Nagasaka, H. *J. Electrochem. Soc., Electrochem. Sci. Tech.* **1980**, *127*, 2343.
26 Schoen, P. E.; Yager, P.; Schnur, J. M. *US 4990291*, **1991** [*Chem. Abstr.* **1991**, *115*, 142267j].
27 Shipley, Jr. C. R. *US 3011920*, **1961** [*Chem. Abstr.* **1962**, *56*, 5731a].
28 Brandon, S. L.; Calvert, J. M. *J. Electrochem. Soc.* **1997**, *144*, 3425.
29 Selinger, J. V.; Specter, M. S.; Schnur, J. M. *J. Phys. Chem., B* **2001**, *105*, 7157.

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