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Azide-Reactive Liposome for Chemoselective and Biocompatible Liposomal Surface Functionalization and Glyco-Liposomal Microarray Fabrication

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Abstract

A chemically selective liposomal surface functionalization and liposomal microarray fabrication using azide-reactive liposome are described. First, liposome carrying PEG-triphenylphosphine was prepared for Staudinger ligation with azide-containing biotin, which was conducted in PBS buffer (pH 7.4) at room temperature without catalyst. Then, immobilization and microarray fabrication of the biotinylated liposome onto streptavidin-modified glass slide *via* specific streptavidin/biotin interaction were investigated by comparing with direct-formed biotin-liposome, which was prepared by conventional liposome formulation of lipid-biotin with all other lipid components. Next, covalent microarray fabrication of liposome carrying triphenylphosphine onto an azide-modified glass slide and its further glyco-modification with azide-containing carbohydrate were demonstrated for glyco-liposomal microarray fabrication *via* Staudinger ligation. Fluorescence imaging confirmed the successful immobilization and protein binding of the intact immobilized liposomes and arrayed glyco-liposomes. The azide-reactive liposome provides a facile strategy for a membrane-mimetic glycoarray fabrication, which may find important biological and biomedical applications such as studying carbohydrate-protein interaction and toxin and antibody screening.

Keywords

Liposome; biotinylation; immobilization; Staudinger ligation; glycoarray

1. INTRODUCTION

Immobilization of liposome onto a solid surface has shown a great potential in biological and biomedical research and applications.¹ This discipline has been inspired by that liposome structurally retains the properties inherent in natural lipid membranes, and functionally can serve as model of biomembrane and also can encapsulate both hydrophobic and hydrophilic compounds such as drug and gene for delivery applications.² For example, immobilized liposomes have been investigated as model systems presenting lipid membranes for bioseparation,³ biosensor,⁴ and nanobioreactor⁵ applications. Recently, immobilized liposomes onto a biomedical device have been considered as a potential local drug delivery system, which release drug immediately to the environment surrounding the device, and reduce the toxic effects on other organisms and thus enhance the therapeutic effect of the drug.⁶ In addition, liposome microarray has been explored recently for applications in membrane biophysics, biotechnology, and colloid and interface science.⁷

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Surface-immobilized liposomes can be fabricated through either non-covalent such as bio-affinity interaction or covalent bond formation by synthesizing anchor group modified liposomes. Conventionally, the anchor group modified liposomes are prepared by direct liposome formation method, in which the anchor lipid is synthesized first and followed by formulation of the liposome with all other lipid components. In this direct liposome formation method, however, some anchor-lipid conjugates may have limited solubility and stability in solvent, or are incompatible with various stages of preparation, or even may have difficulty to form liposome due to the loss of its amphiphilic property. It is also well-known that the shape of the self-assembled liposomes may be influenced by the nominal geometric parameters of its molecule such as polar head surface, tail volume and chain length.⁸ Alternatively, anchor group modified liposomes can be synthesized by post chemical modification of reactive preformed liposomes.⁹ Variable successes using amide¹⁰ or thiol-maleimide coupling¹¹ as well as by imine¹² or hydrazone linkage¹³ have been reported. However, non-chemoselective, harsh reaction conditions and low efficiency of most these methods limited their practical applications.

Azide-based ligation reactions have been extensively explored for highly selective and biocompatible bioconjugation,^{14–16} polymer and materials science,^{17,18} and drug discovery.^{19,20} Specifically, the azide is a versatile bioorthogonal chemical reporter. Its small size and stability in physiological settings have enabled azide-functionalized metabolic precursors to hijack the biosynthetic pathways for numerous biomolecules, including glycans,²¹ proteins,^{15,22} lipids,²³ and nucleic acid-derived cofactors,²⁴ and therefore can afford a variety of azide-containing biomolecules for biomedical applications. Three reactions have been reported for tagging azide-labeled biomolecules. The two involve the reaction of azide with alkyne to give triazole, a process that is typically very slow under ambient conditions. The Cu(I)-catalyzed azide-alkyne cycloaddition also known as “click chemistry”, accelerates the reaction.^{25,26} However, the toxic copper catalyst may residue inside of the liposomes and thus cause problem in clinical application. Recently, the strain-promoted [3 + 2] cycloaddition removes the requirement for cytotoxic copper by employing cyclooctynes that are activated by ring strain.^{27,28} Nevertheless, two triazole regioisomers forms during the conjugation, which affords complicated products without controlling.³⁰ Another one, the Staudinger ligation capitalizes on the selective reactivity of triphenylphosphine and azide to form an amide bond.^{14,30,31} Most recently, we have demonstrated that Staudinger ligation of triphenylphosphine-carrying liposome with azide-containing biomolecules as a chemoselective liposome surface functionalization approach.³² The high specificity, high yield, biocompatible and the lack of residual copper reaction condition natures of the Staudinger ligation approach make it an attractive alternative to all currently used protocols for liposome surface functionalization. Herein, we investigated expanded application of this azide-reactive liposome for efficient and chemically selective liposome immobilization and microarray fabrication applications (Figure 1). First, biotinylation of a liposome carrying triphenylphosphine with azide-modified biotin and its immobilization onto a streptavidin coated glass slide were investigated. Next, microarray formation of biotinylated liposome onto a streptavidin coated glass slide was investigated. Finally, covalent liposomal microarray formation was investigated by printing the liposome carrying triphenylphosphine onto an azide-modified glass slide and followed by further glyco-modification with azide-containing carbohydrate so as to afford a membrane-mimetic glycoarray. This glyco-liposomal microarray will find important biological and biomedical applications such as studying carbohydrate-protein interaction and toxin and antibody screening and so on.

2. MATERIALS AND METHODS

2.1 Materials

1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[amino(polyethylene glycol) 2000] (ammonium salt) (DSPE-PEG₂₀₀₀), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[biotinyl(polyethylene glycol)-2000] (ammonium salt) (DSPE-PEG₂₀₀₀-biotin), 1,2-dipalmitoyl-*sn*-glycerol-3-phosphoethanolamine-*N*-(7-nitro-2-1,3-benzoxadiazol-4-yl) (DPPE-NBD) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Cholesterol, dicyclohexylcarbodiimide (DCC), diphenylphosphino-4-methoxycarbonylbenzoic acid, hexaethylene glycol, toluene sulfonyl chloride, imidazole, sodium azide, *N*-hydroxysuccinimidobiotin, *N,N*-dimethylformamide were purchased from Sigma (USA). All other solvents and reagents were purchased from commercial sources and were used as received, unless otherwise noted. Deionized water was used as a solvent in all experiments.

2.2. Synthesis of anchor lipid DSPE-PEG₂₀₀₀-triphosphine (1)

DSPE-PEG₂₀₀₀-NH₂ (100 mg, 35.8 μmol) was dissolved in 20 mL of CH₂Cl₂, and 0.2 mL of triethylamine was added. After stirring for 30 min at room temperature, a solution of succinimidyl 3-diphenylphosphino-4-methoxycarbonylbenzoate (33 mg, 71.6 μmol) in 50 mL of CH₂Cl₂ was added. The reaction mixture was stirred at room temperature for 24 h and then concentrated under vacuum to give a residue, which was purified by silica gel chromatography with chloroform/methanol (4:1, v/v) to afford product **1** (32 mg, 28.5 %). ¹H NMR (CDCl₃, 300 MHz) δ: 8.06 (m, 1H), 7.79 (m, 1H), 7.44 (m, 1H), 7.66 (m, 2H), 7.52-7.42 (m, 2H), 7.28-7.34 (m, 8H), 6.64 (m, 1H), 5.19 (s, 1H), 4.34-4.20 (m, 3H), 3.95-3.80 (m, 3H), 3.80-3.50 (br. s, 44H, O-CH₂-CH₂-O), 3.40-3.20 (m, 3H), 2.28 (br. s, 4H), 1.52 (br. s, 4H), 1.36-1.20 (s, 32H), 0.89 (t, *J*, 6.9, 6H), ³¹P NMR (CDCl₃, 121 MHz) δ: -2.7.

2.3. Synthesis of azidoethyl-tetra(ethylene glycol) ethylamino biotin (2)

Triethylamine (0.03 mL, 0.02 mmol) was added to a solution of amino-11-azido-3, 6, 9-trioxanundecane²⁰ (54 mg, 0.176 mmol) in DMF (3.5 mL). After the solution was stirred for 30 min, a solution of *N*-hydroxysuccinimidobiotin (50 mg, 146 μmol) was added. The reaction mixture was stirred for 12 h at room temperature and then concentrated under vacuum to give a residue, which was purified by silica gel column chromatography using acetone:hexane (4:1, v/v) as eluent to afford **2** (41 mg, 44%). ¹H NMR (CD₃OD, 300 MHz) δ: 6.75 (br. s, 1H), 6.75 (br. s, 1H), 6.52 (br. s, 1H), 5.88 (br. s, 1H), 4.51 (m, 1 H, -CH-1-Biotin), 4.32 (m, 1 H, -CH-4-Biotin), 3.70-3.63 (m, 16 H, -O-(CH₂CH₂O)₄-PEG), 3.56 (m, 2 H, -O-CH₂CH₂-N₃), 3.39 (m 4 H, -CH₂-NH and -O-CH₂CH₂-N₃), 3.24 (m 1 H, -CH-3-Biotin), 2.92 (dd, 1 H, *J* = 4.8, 12.8 Hz, -CH-2a-Biotin), 2.71 (m 1 H, -CH-2b-Biotin), 2.23 (t, 1 H, *J* = 7.6 Hz, -CH₂CO-Biotin), 1.76-1.63 (m, 4 H, -(CH₂)₂-Biotin), 1.50-1.40 (m, 2 H, -(CH₂)₂-Biotin).

2.4. Preparation of triphenylphosphine-liposome

DSPC and cholesterol at 2:1 mol ratio were used as the major components of all liposome. For liposome biotinylation, 0.5 mol % of anchor lipid DSPE-PEG₂₀₀₀-triphosphine was doped. To visualize the liposome immobilized onto the solid surface, all kinds of liposome were incorporated with DPPE-NBD (0.5 mg, 0.6 mol%). In detail, the mixture of lipids was first dissolved in chloroform. The solvent was gently removed on an evaporator under reduced pressure to form a thin lipid film on the flask wall and kept in a vacuum chamber overnight. Then, the lipid film was swelled in the dark with 2.5 mL PBS buffer (pH 7.4), followed 10 freeze-thaw cycles of quenching in liquid N₂ and then immersed in a 65 °C

water-bath to form multilamellar vesicle suspension. Finally, the crude lipid suspension was extruded through polycarbonate membranes (pore size 600, 200, and 100 nm, gradually) at a 65 °C to afford small unilamellar vesicles.

2.5 Preparation of biotin-liposome via Staudinger ligation

To 2.5 mL of triphosphine-liposome in PBS (pH 7.4) above, 1 mL of biotin-PEG₆-azide (30 mg, 56 μmol) in PBS (pH 7.4) was added; then the reaction mixture was incubated at room temperature for 6 h in an argon atmosphere. The unreacted biotin-PEG₆-azide was removed by gel filtration (1.5 × 20 cm Column of Sephadex G-50). The size of liposomes during the Staudinger ligation was monitored over time by using 90Plus particle analyzer.

2.6 Preparation of biotin-liposome via direct liposome formation

DSPC (43.2 mg, 54.7 μmol), cholesterol (10.6 mg, 27.4 μmol), DSPE-PEG2000-Biotin (2.5 mg, 0.83 μmol) (2:1:1% molar ratio) in 3 mL of chloroform. The solvent was gently removed on an evaporator under reduced pressure to form a thin lipid film on the flask wall and kept in a vacuum chamber overnight. Then, the lipid film was swelled in the dark with 2.5 mL PBS buffer (pH 7.4), followed 10 freeze-thaw cycles of quenching in liquid N₂ and then immersed in a 65 °C water-bath to form multilamellar vesicle suspension. Finally, the crude lipid suspension was extruded through polycarbonate membranes (pore size 600, 200, and 100 nm, gradually) at a 65 °C to afford small unilamellar vesicles.

2.7. Immobilization of biotin-liposome onto streptavidin-glass slide

Immobilization of the biotinylated liposomes above was performed by incubating with streptavidin-glass slide (Xenopore Corp) in a 5 mg/mL of biotin-liposome suspension (total lipid concentration) at room temperature for 4 h, followed by removing the un-immobilized liposomes in the surface of glass slide. The glass slide was washed by rinsing with PBS buffer for 1 h and then replacing with new buffer solution, and repeated three times. Finally, fluorescence scanning image detecting incorporated DPPE-NBD (0.6 mmol%) in the liposome was obtained by Typhoon 9410 Variable Mode Imager (Amersham Biosciences, USA).

2.8. Liposome array based on biotin/streptavidin interaction

The direct biotinylated and post biotinylated liposomes doping with DSPE-Rodamine (1 mol %, Avanti Polar Lipid, Inc) in the liposome lipid bilayer and encapsulating 5,6-carboxyfluorescein (5,6-CF, Sigma) (50 mM) into the liposome were diluted to desired lipid concentration (2.0 mg/mL, total lipid concentration) by PBS (pH 7.4) buffer, then was printed onto streptavidin functionalized glass slides (Xenopore Corp) by using LabNext XactII™ Compact Microarrayer (Lab Next, USA), and followed by incubating the slide for 2.0 hrs at room temperature, respectively. Next, the liposome-immobilized glass slide was washed by rinsing with PBS (pH 7.4) buffer for 2.0 hrs and repeated three times to remove unbound liposomes completely.

2.9. Liposome array based on Staudinger immobilization of liposome

The azide-reactive liposome doping with DSPE-Rodamine (1 mol%, Avanti Polar Lipid, Inc) in the liposome lipid bilayer and encapsulating 5,6-carboxyfluorescein (5,6-CF, Sigma) (50 mM) into the liposome was diluted to desired lipid concentration (2.0 mg/mL, total lipid concentration) by PBS (pH 7.4) buffer, then was printed onto azide-PEG6-functionalized glass slide by using LabNext XactII™ Compact Microarrayer (Lab Next, USA), and followed by incubating the slide for 2.0 hrs at room temperature. Next, the liposome-immobilized glass slide was washed by rinsing with PBS (pH 7.4) buffer for 2.0 hrs and repeated three times to remove unbound liposomes completely.

2.10. Staudinger glyco-functionalization of immobilized liposome

The glass slide with immobilized liposome carrying triphenylphosphine was incubated with 2-azideethyl-lactoside in PBS buffer (pH 7.4, 40 mg/mL) at room temperature for 2.0 hrs, followed by removing the glass slide from the reaction solution. Next, the glass slide was then washed by rinsing with PBS (pH 7.4) buffer for 2.0 hrs and repeated three times.

2.11. Specific lectin binding onto lactosylated immobilized liposome

The glass slide with lactosylated immobilized liposome was incubated with lectin (*Arachis hypogae*, FITC-labeled, Sigma) in PBS (pH 7.4) buffer solution (50 µg/mL) at room temperature for 2.0 hrs, followed by removing the glass slide from the reaction solution. Next, the glass slide was washed by rinsing with PBS (pH 7.4) buffer for 2.0 hrs and repeated three times. Finally, fluorescence scanning image detecting FITC-labeled lectin bound onto the lactosylated liposome was obtained by Typhoon 9410 Variable Mode Imager (Amersham Biosciences, USA).

2.11. Instrumental analysis

Dynamic Light Scattering was measured with 90plus particle size analyzer (Brookhaven Ins. Co., USA). Atomic force microscopes were carried out using PicoPlus 3000 (Molecular Imaging, USA). Liposome microarray was conducted with LabNext XactII™ Compact Microarrayer (Lab Next, USA) Fluorescence imaging was obtained by Typhoon 9410 Variable Mode Imager (Amersham Biosciences, USA).

3. RESULTS AND DISCUSSION

3.1. Chemically selective liposome biotinylation and its microarray formation

Streptavidin/biotin-based liposome immobilization has been widely used by synthesizing biotinylated liposome.^{5,33,34} Conventionally, the biotin anchor group modified liposome is synthesized by direct liposome formation method, in which the biotin-lipid is mixed with all other lipid components so as to afford a liposome with biotin oriented both outside and enclosed aqueous compartment. In the present study, we explored azide-reactive pre-prepared liposome carrying PEG-triphenylphosphine for chemically selective liposome surface biotinylation through Staudinger ligation. First, the anchor lipid DSPE-PEG₂₀₀₀-triphenylphosphine **1** was synthesized by amidation of commercially available DSPE-PEG₂₀₀₀-NH₂ with 3-diphenylphosphino-4-methoxycarbonylbenzoic acid NHS active ester synthesized as described in our previous study.¹⁵ Next, small unilamellar vesicles composed of phospholipids (DSPC) and cholesterol (2:1 mol ratio) and 1.0 mol % of the anchor biotin-lipid **1** were prepared by rapid extrusion through polycarbonate membrane with pore size of 600, 200, and 100 nm diameter, sequentially at 65 °C. This produced predominately small unilamellar vesicles showed an average mean diameter of 120 ± 12 nm as judged by dynamic light scattering (DLS) (Figure 2A). Finally, conjugation of azide-PEG₆-biotin (**2**) to the preformed liposomes was performed in PBS buffer (pH 7.4) at room temperature in an argon atmosphere for 6 hrs (Scheme 1). The azide-PEG₆-biotin (**2**) was synthesized by amidation of amino-11-azido-3,6,9, trioxaundecane¹⁶ with commercially available biotin NHS ester (Sigma). DLS technique was used to verify the integrity of the vesicles during and after the coupling reaction. As result shown in Figure 2B, there is no significant size change of the vesicles observed after biotinylation reaction. This result indicated that the reaction condition above does not alter the integrity of the liposomes and thus are harmless for liposome surface modification.

Next, streptavidin binding assay was examined to determine the success of the biotinylation and whether the grafted biotin residues are easily accessible at the surface of liposomes. It is well known that one streptavidin molecule is able to bind four biotin molecules and the

presence of streptavidin could induce aggregation of surface biotinylated liposome. The biotin/streptavidin combination measurement was performed by incubating streptavidin with the biotinylated liposome in PBS buffer (pH 7.4) at room temperature. After 2 hrs, streptavidin-induced aggregation of the biotinylated liposomes was confirmed by DLS (Figure 3A), while there was no aggregation observed for the liposomes without biotinylation (Figure 3B). Furthermore, the presence of free biotin (5.0 mM) prevented aggregates formation (not shown), confirming that the aggregation was due to the specific recognition of the biotin residues on the surface of liposome by streptavidin. These results indicated that the liposome surface has been biotinylated successfully and grafted biotin on the liposome surface was easily accessible. Similar result was obtained with the direct-formed biotin-liposome as positive control (Figure 3C). The cluster size of direct formed biotin-liposome (Figure 3C) is different from that of the post biotinylated liposome (Figure 3A). This might be due to different biotin density on the two kinds of liposomes.

Immobilization of the biotinylated liposome was performed by incubating with streptavidin-coated glass slide (Xenopore Corp) in PBS buffer (pH 7.4) at room temperature for 2 hrs followed by washing with PBS buffer (pH 7.4) three times. To confirm the immobilized liposome on the glass slide surface, fluorescence imaging study was examined with post biotinylated liposome and direct biotinylated liposome, respectively. DSPE-NTB (1 mol%, Avanti Polar Lipid, Inc) was doped in both liposomes as component for detecting by a microplate reader. As shown in Figure 4, both post biotinylated liposome (Figure 4A) and direct biotinylated liposome (Figure 4B) yielded a uniform fluorescence image, while there was no apparent fluorescence image observed for the control non-biotinylated liposome (Figure 4C). Taken together, these results indicated that biotinylated liposome could be immobilized onto streptavidin-coated glass slide through specific biotin/streptavidin interaction.

Liposome microarrays are versatile tools in biomedical research, as they can be used for applications in membrane biophysics, biotechnology, and colloid and interface science.⁷ Most liposome microarrays were fabricated through bio-affinity between anchoring group on the liposome surface and the counterpart group on the solid surface, such as biotin/streptavidin⁷ and DNA hybridization.^{35–37} In the present study, both post biotinylated liposome and direct biotinylated liposome were applied for liposome microarray fabrication. Briefly, both post biotinylated liposome and direct biotinylated liposome in PBS buffer (pH 7.4) (2 mg/mL of total lipid concentration) were printed onto streptavidin-coated glass slides (Xenopore Corp) followed by incubating for 2 hrs at room temperature, and then washing with PBS buffer (pH 7.4) three times to remove the unbound liposomes. In order to confirm the intact liposome immobilized on the glass slide surface, fluorescence imaging study was conducted by doping DSPE-Rodamine (1 mol%, Avanti Polar Lipid, Inc) in the liposome lipid bilayer so as to label lipid membrane and by encapsulating 5,6-carboxyfluorescein (5,6-CF, Sigma) (50 mM) into the liposome so as to image the inner compartment of the liposome. As results, either detecting 5,6-CF (Figure 5A1, 5B1) or Rhodamine (Figure 5A'1, 5B'1) yielded fluorescence image of the arrayed liposomes for both post biotinylated liposome and direct biotinylated liposome, while there was no apparent fluorescence image observed for liposomes without anchor biotin (Figure 5A2, 5A'2 and 5B2, 5B'2). These results indicated that the arrayed intact liposomes were achieved through specific biotin/streptavidin interaction.

3.3. Covalent liposomal microarray fabrication

In the present study, liposome microarray based on covalent immobilization was performed by printing preformed liposomes carrying PEG-triphenylphosphine in PBS buffer (pH 7.4) (2 mg/mL of total lipid concentration) onto azide-PEG-glass slide (Xenopore Corp) followed by incubating for 2 hrs at room temperature, and then washing with PBS buffer

(pH 7.4) three times to remove the unbound liposomes. In order to confirm the intact liposome immobilized on the glass slide surface, fluorescence imaging study was conducted by doping DSPE-Rhodamine (1 mol%, Avanti Polar Lipid, Inc) in the liposome lipid bilayer so as to label lipid membrane and by encapsulating 5,6-carboxyfluorescein (5,6-CF, Sigma) (50 mM) into the liposome so as to image the inner compartment of the liposome. As results, either detecting 5,6-CF (Figure 6A1) or Rhodamine (Figure 6A'1) yielded fluorescence image of the immobilized liposome for azide-PEG glass slide treated with liposomes carrying anchor group triphenylphosphine, while there was no apparent fluorescence image observed for azide-PEG glass slide treated with liposomes without anchor group triphenylphosphine (Figure 6B and 6B'). Furthermore, washing the liposome arrays with Triton X 100-containing PBS (pH 7.4, 1%) were conducted to confirm the immobilized intact liposome. As results, both liposome encapsulated 5,6-CF and doped Rhodamine-PE were not detectable any more due to the rupture of the immobilized liposome (Figure 6A2, 6A'2). Taken together, these results indicated that the arrayed intact liposomes were achieved through Staudinger ligation.

Next, glyco-modification was performed by conjugating of the immobilized liposomes carrying triphenylphosphine left over on the liposome exterior surface with 2-azideethyl-lactoside³⁸ in PBS buffer (pH 7.4) at room temperature under an argon atmosphere for 2 hrs. Specific lectin binding assay was investigated to confirm the success of glycosylation and whether the grafted lactose residues are easily accessible at the surface of the immobilized liposomes. The binding assay was conducted by incubating lactosylated arrayed liposomes in the solution of β -galactose binding lectin (*Arachis hypogae*, 120 kDa, FITC-Labeled, Sigma) in PBS (pH 7.4) buffer at room temperature for 2 hrs, followed by washing with PBS (pH 7.4) buffer three times. As shown in Figure 6, the specific binding of fluorescent labeled lectin was observed on the lactosylated liposome array spots (Figure 7B), while there was no apparent fluorescence image observed for liposomes with anchor group triphenylphosphine unmodified (Figure 7A) and glycosylated liposome treated with lactose pre-incubated lectin (Figure 7C). These results indicated the successful glycosylated liposomal array fabrication and its specific lectin binding. Continued study for glyco-liposomal array with different glycans and even different lipid components for specific detecting targets are under investigation.

4. CONCLUSION

An azide-reactive liposome has been developed for efficiently and chemically selective liposome surface modification and glyco-liposomal microarray fabrication applications. Specifically, microarray of liposome carrying triphenylphosphine onto azide-modified glass slide and further glyco-modification with azide-containing carbohydrate were demonstrated *via* Staudinger ligation. The high selective and biocompatible reaction condition natures make the reported method an attractive alternative to all currently used protocols for liposome surface functionalization and liposome microarray fabrication applications. Notably, there is no concern of catalyst left in the resultant liposome, which is common problem in other liposome modification methods since there is no catalyst used in modification and immobilization reaction. In the same chemistry, any biomolecules containing azide can be introduced onto arrayed liposome surface and thus will provide a variety membrane-mimetic array for both bioanalytical and diagnostic applications.

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Figure 1. Schematics of chemical selective and biocompatible liposome surface functionalization and immobilization and its further glyco-functionalization *via* Staudinger ligation.

Figure 2. DLS monitoring of size change of liposome before biotinylation (**A**) and after biotinylation (**B**) reaction.

Figure 3. DLS monitoring of Streptavidin binding assays of biotinylated liposomes: post biotinylated liposomes (**A**), plain liposomes without biotin (**B**) and direct biotinylated liposomes (**C**).

Figure 4.

Fluorescence image of immobilized liposomes onto streptavidin-coated glass slides: post biotinylated liposomes with DPPE-NBD (0.6 mmol%) incorporated (**A**), direct biotinylated liposomes with DPPE-NBD (0.6 mmol%) incorporated (**B**) and plain liposomes with DPPE-NBD (0.6 mmol%) incorporated but without biotin (**C**).

Figure 5.

Fluorescence images of biotinylated liposome arrays: selectively exciting 5,6-CF encapsulated in the direct biotinylated liposome (**A1**), post biotinylated liposome (**B1**) and Rhodamine-PE embedded in the direct biotinylated liposome membrane (**A'1**), post biotinylated liposome (**B'1**), and control liposomes without anchor biotin (**A2** and **A'2**, **B2** and **B'2**). – Bar size: 500 μm

Figure 6. Fluorescence images of covalent immobilized liposome arrays: selectively exciting 5,6-CF encapsulated in the liposome (**A1**) and Rhodamine-PE embedded in the liposome membrane (**A'1**) and followed by washing with PBS containing Triton X-100 (pH7.4, 1%) (**A2** and **A'2**), and control liposomes without anchor lipid DSPE-triphenylphosphine (**B** and **B'**). – Bar size: 500 μ m

Figure 7. Fluorescence image of lectin (FITC-labeled *Arachis hypogaea*) binding onto the lactosylated liposome arrays: liposomes with anchor group triphenylphosphine (**A**), lactosylated liposome (**B**), and lactosylated liposome treated with lactose pre-incubated lectin (**C**). – Bar size: 500 μm

Scheme 1.
Liposome biotinylation *via* Staudinger ligation