Mol. Cryst. Liq. Cryst., Vol. 507, pp. 18–25, 2009

ISSN: 1542-1406 print/1563-5287 online
DOI: 10.1080/15421400903048024

Taylor & Francis
Taylor & Francis Group

Biomolecular Science of Liposome-Nanoparticle Constructs

Yan Yu[1], Stephen M. Anthony[2], Sung Chul Bae[1], Erik Luijten[1,3], and Steve Granick[1,2,3]
[1]Department of Materials Science and Engineering, University of Illinois, Urbana, IL, USA
[2]Department of Chemistry, University of Illinois, Urbana, IL, USA
[3]Department of Physics, University of Illinois, Urbana, IL, USA

Phospholipid-nanoparticle constructs, formed by allowing nanoparticles to adsorb to the outer leaflet of liposomes, are found to be stabilized against fusion with one another. Here, through single-particle tracking by epifluorescence microscopy, we explore their use as novel colloidal particles – flexible and hollow colloidal particles that contrast strikingly with colloids of the conventional type. At the single-liposome level, the distribution of diffusion coefficients is quantified. Biomolecular function is addressed through experiments in which we explore the access of receptor to liposome-immobilized ligand, finding that receptor binding persists over a range of nanoparticle surface coverage where liposome fusion and large-scale aggregation is prevented. This opens the door to designing newer and more flexible types of tailor-made materials with desirable functionality.

Keywords: biofunctionalization; colloids; diffusion; liposomes; nanoparticles

Phospholipid liposomes, submicron-sized artificially-constructed capsules of phospholipid bilayers, present an increasingly important platform for areas as diverse as biotechnology, nanomedicine, and analytical chemistry. They are tremendously biofunctionalizable; antibodies, protein receptors and other biosensor molecules can attach to them [1,2]. They comprise compartments that can be used to encapsulate and store various cargoes, such as enzymes, proteins,

This work was supported by the U.S. Department of Energy, Division of Materials Science, under Award No. DEFG02-02ER46019. SMA acknowledges the NSF for financial support in the form of a graduate research fellowship.

Address correspondence to Steve Granick, Departments of Materials Science and Engineering, Chemistry, and Physics, University of Illinois, Urbana, IL, USA. E-mail: sgranick@uiuc.edu

DNA and various drug molecules [3–5]. Their small and controllable size, diameter from tens to thousands of nm, signifies that individual liposomes comprise nanocontainers with volumes from zeptoliters (10^{-21} L) to femtoliters (10^{-15} L). When biomolecules or other chemical reactants are loaded into this biocompatible container, cellular processes and chemical reactions including protein expression, mRNA transcription and enzyme-catalyzed reactions can be performed inside [6–8]. To release the final products, one can either change the temperature to below the bilayer main phase transition temperature, beyond which lipid packing defects create transient pores in the membrane, or use strong electric pulses to break it apart.

In this paper, we are interested in the functions of phospholipid liposomes as novel colloidal particles – soft and flexible particles. Recent experiments show that submicron-sized phospholipid vesicles fail to fuse with one another when coated with adsorbed nanoparticles at surface coverages on the order of 25%. The idea of how to accomplish this is summarized in Figure 1. This route to stabilization is appealing because the low occupied surface area enables these liposomes to retain the potential to react functionally with their environment. An original study [9] addressed only the influence of anionic nanoparticles whose charge density was high. Moreover, those studies of shelf life, at dense concentrations were only qualitative, leaving open the question of the relative efficacy of nanoparticles of different electric charge, as well as the question whether liposomes retain fluidity, using this stabilization route, at dense concentrations. Later, the efficacy of cationic and anionic nanoparticles for the stabilization of DLPC, 1,2-dilauroyl-sn-glycero-3-phosphocholine was compared, and it was concluded that cationic nanoparticles stabilize these zwitterionic liposomes better than anionic ones [10]. This was rationalized by considering that because the phospholipid zwitterionic headgroup terminates with positive charge, lipids beneath an adsorbed nanoparticle bind more weakly when the nanoparticle charge is cationic.

In the present study, we prepared large unilamellar lipid vesicles (liposomes) with a maximum diameter of 200 nm in deionized water (Millipore) using the well-known extrusion method, employing procedures described in detail elsewhere [9]. Liposomes of DLPC were mixed by low-power sonication with these nanoparticles, diameter 20 nm. The molar ratio of 100:1, approximately 1:1 by weight, corresponds to the upper limit of $\approx$25% surface coverage if all nanoparticles adsorb. To image the liposomes, typically $\approx$500 DMPE-RhB probes per liposome were doped into a small fraction of the liposomes, leaving the rest unlabeled and free of fluorescence. To prepare concentrated suspensions, first the liposomes were prepared at 1 vol%, then were

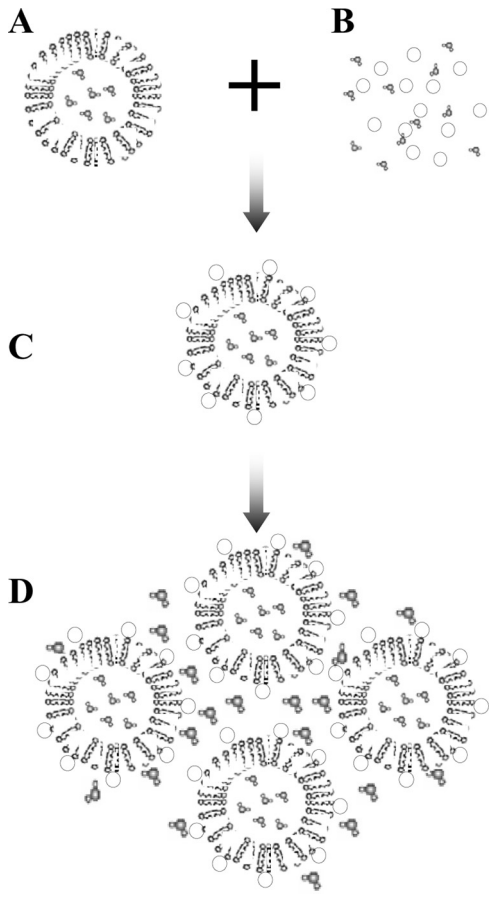

FIGURE 1 Schematic illustration of the strategy to produce nanoparticle-stabilized liposomes and their dense suspensions. Particles with diameter in the range 100–1000 nm can be concentrated reversibly up to volume fractions as high as 60% and remain stable for several months at least. The enabling idea is that if nanoparticles adsorb to the outer surface of a phospholipid liposome, this liposome is stabilized against fusion with other liposomes. Inspired by the phenomenon of particle-stabilized emulsions we find that individual phospholipid vesicles can be stabilized against fusion by adding nanoparticles that adsorb to the vesicle outer surface. (A) Liposomes are made using the extrusion method. (B) Nanoparticles (silica, polystyrene, or other material) with a diameter of <100 nm are prepared. (C) Nanoparticle-stabilized liposomes are formed by mixing A and B by sonication. (D) To condense the dilute liposome suspension C, pure nitrogen gas was blown gently over the suspension until reaching the desired volume fraction. Adapted from Ref. [9].

concentrated by bubbling nitrogen gas gently over them. To calculate the volume fraction, calculations included not only liposomes but also nanoparticles, and were determined from the difference between initial and final suspension volumes. An essential point is that liposomes prepared using this method are polydisperse; we estimated the ratio of standard deviation to liposome diameter as 0.34 [10].

This strategy of fluorescence labeling allowed single-liposome detection of fluidity in a homebuilt epifluorescence setup. Usually, the total observation time at a given focus spot was 50 sec, yielding 1000 time steps of 50 ms length. The diffusion of liposomes was tracked, using a modified implementation of standard single particle tracking algorithms described previously, with spatial resolution of 50 nm [11]. From the liposome trajectories, the mean square displacements $<\Delta x(t)^2> = <(x(\tau + t) - x(\tau))^2>$ were individually computed for all trajectories that lasted longer than 100 time steps. Figure 2A illustrates the mean square displacements for three individual stabilized liposomes at $\Phi = 0.50$. The displacements are in units of the liposome hydrodynamic radius. The log-log plot of individual $<\Delta x(t)^2>$ versus time all yielded slopes $\sim$1, which is expected for Fickian diffusion. Figure 2B shows a histogram of the diffusion coefficients $D = <\Delta x(t)^2>/4t$ calculated from individual mean square displacements. The distribution in D is attributed to polydispersity of the elementary liposomes.

But what happens when liposomes are concentrated to even higher concentrations? There is an analogy to concentrated hard-sphere colloidal systems near the glass transition, where it is known that dynamical heterogeneity occurs when particles in different regions show diverse mobility, both temporally and spatially, which is believed to be related to inhomogeneous structural relaxation [12]. A large variety of soft glassy materials exhibit fast dynamics related with the elasticity. At the same time, most of the systems also exhibit slow dynamics, often associated with dynamical heterogeneity and aging [13,14]. The origin of such dynamical heterogeneity is still poorly understood. Analyzing mean square displacements of individual liposomes for this system comprised of soft, flexible particles, we find heterogeneity in the diffusion for volume fractions up to 0.79. Two populations of liposomes with distinct power laws, $<\Delta x(t)> \sim 4Dt^{\alpha}$, where D is the diffusion coefficient of liposomes, can be identified. Examples of trajectories are displayed in Figure 3. Mean-squared displacements, plotted against time on log-log scales, are displayed in Figure 4. Conventional hard colloids lack the flexibility and charge elements of these liposome systems, so to observe distinctly new patterns of translational dynamics is exciting. This holds the potential to open new vistas of scientific investigation.

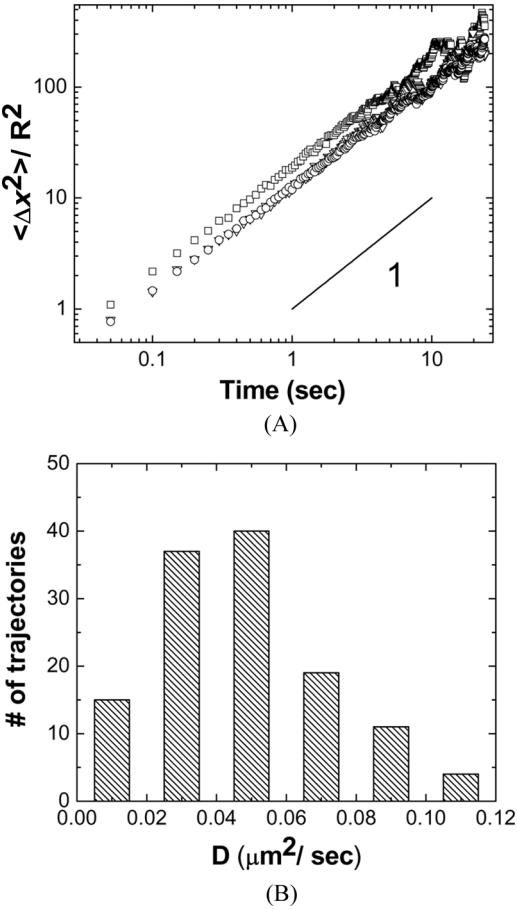

FIGURE 2 Diffusion of stabilized liposome at 50% volume fraction revealed by single liposome tracking. (A) Mean square displacements $<\Delta x^2>$ in units of liposome hydrodynamic radius are plotted against time on log-log scale for three individual liposomes. The line, a guide to the eye, has a slope of unity. (B) From the analysis of ≈130 trajectories, the distribution of diffusion coefficient, determined from data of the kind illustrated in Figure 2A, is plotted. In dilute solution, fluorescence correlation spectroscopy showed $D \sim 0.8\,\mu m^2/sec$, faster by more than one order of magnitude. Adapted from ref. 10.

Turning to biofunctionalization, it is interesting to note that the design of function in phospholipid vesicles is complicated by competing needs. On the one hand, their stability against fusion with one another is augmented by coating them with a protective layer such as PEG, polyethylene glycol. But the capability of vesicles to react chemically

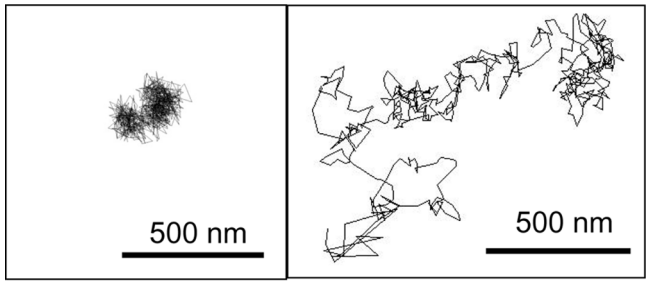

FIGURE 3 Typical trajectories for ~50 secs for $\Phi = 0.77$. (A) Sub-diffusive population, which corresponds to $<\Delta x^2(t)> \sim t^{1/3}$. (B) Diffusive population, which corresponds to $<\Delta x^2(t)> \sim t$.

with their environment requires that reactants have access to the vesicle surface. It is desirable to understand how protein-membrane binding is modulated by the repulsion that prevents deleterious aggregation or fusion of neighboring liposomes. A potential advantage of the nanoparticle-stabilization strategy outlined in Figure 1 is that the strength of the repulsion between liposomes is easily tuned by varying the surface coverage of adsorbed nanoparticles. In the case that nanoparticles adsorb sparsely, we have confirmed earlier experiments of others showing that streptavidin proteins not only bind to

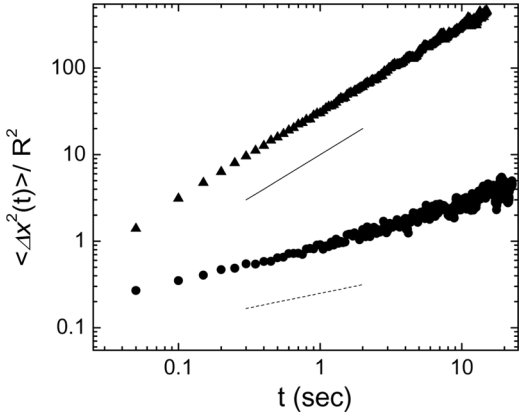

FIGURE 4 Typical assemble-average mean square displacement $<\Delta x^2(t)>$, scaled to the square of the hydrodynamic radius $R = 100$ nm, of two populations for $\Phi = 0.77$. The solid straight line has a slope of 1 in log-log plot. The dotted straight line has a slope of 1/3.

liposomes containing embedded biotin ligands but also can bridge between liposomes to form liposome networks or clusters [15]. Going beyond this, in the case of higher levels of nanoparticle surface coverage, large-scale aggregation between liposomes fails to occur but ligand-receptor binding remains effective [16]. This shows that at low surface coverage, $<50\%$, the outer surfaces of nanoparticle-stabilized liposomes remain biofunctionalizable. This opens another door to using liposomes in emerging biotechnological fields in which it is desirable to possess stable liposome suspensions while retaining the capacity for proteins embedded within the liposome to sense their environment without loss of bioactivity.

These results demonstrate the appealing potential of liposome-nanoparticle constructs to not only shed light on topical scientific questions but also to contribute to new technological possibilities in the biomolecualar arena. The capability demonstrated here, as a characterization tool, to follow the dynamics of individual liposomes within *concentrated* stabilized liposome suspensions offers a platform to reveal the dynamics of these flexibly-shaped objects in a variety of other situations. At least three factors may contribute to the distinct differences from conventional solid colloidal particles: the flexible lipid bilayer membrane, the polydispersity of the liposomes and the strong electrostatic repulsion between cationic nanoparticles.

REFERENCES

[1] Vamvakaki, V., Fournier, D., & Chaniotakis, N. A. (2005). *Biosensor & Bioelectronics*, *21*, 384.
[2] Oja, C., Tardi, P., Schutze-Redelmeier, M. P., & Cullis, P. R. (2000). *Biochim. Biophys. Acta.*, *1468*, 31.
[3] Sharma, A. & Sharma, U. S. (1997). *Inter. J. Pharma.*, *154*, 123–140.
[4] Dos Santos, N., Waterhouse, D., Masin, D., Tardi, P. G., Karlsson, G., Edwards, K., & Bally, M. B. (2005). *J. Controlled Release*, *105*, 89.
[5] Pantos, A., Tsiourvas, D., Paleos, C. M., & Nounesis, G. (2005). *Langmuir*, *21*, 6696.
[6] Yoshimoto, M., Wang, S. Q., Fukunaga, K., Treyer, M., Walde, P., Kuboi, R., & Nakao, K. (2004). *Biotech. Bioeng.*, *85*, 222.
[7] Rhoades, E., Gussakovsky, E., & Haran, G. (2003). *Proc. Nat. Acad. Sci. USA*, *100*, 3197.
[8] Chen, I. A., Salehi-Ashtiani, K., & Szostak, J. W. (2005). *J. Am. Chem. Soc.*, *127*, 13213.
[9] Zhang, L. & Granick, S. (2006). *Nano Lett.*, *6*, 694.
[10] Yu, Y., Anthony, S. M., Zhang, L., Bae, S. C., & Granick, S. (2007). *J. Phys. Chem. C*, *111*, 2833.
[11] Anthony, S. M., Zhang, L., & Granick, S. (2006). *Langmuir*, *22*, 5266.
[12] Weeks, E., Crocker, J. C., Levitt, A. C., Schofield, A., & Weitz, D. A. (2000). *Science*, *287*, 627.

[13] Cipelletti, L., Ramos, L., Manley, S., Pitard, E., Weitz, D. A., Pashkovski, E. E., & Johansson, M. (2003). *Faraday Discuss*, *123*, 237.
[14] Ramos, L. & Cipelletti, L. (2001). *Phys. Rev. Lett.*, *87*, 245503.
[15] Zhang, L., Dammann, K., Bae, S. C., & Granick, S. (2007). *Soft Matter*, *3*, 551.
[16] Chiruyolu, S., Walker, S., Israelachvili, J., Schimitt, F.-J., Leckband, D., & Zasadzinski, J. A. (1994). *Science*, *264*, 1753.