Wrap to sort

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Modelling the structure and behaviour of vesicles in cells requires liposomes with precise sizes, but producing liposomes with a narrow size distribution is challenging. An approach has now been developed to accurately size-sort liposomes in a scalable way by coating them with customized structures based on DNA nanotechnology.

The self-assembly of biological molecules constitutes a remarkable toolkit for scientists to engineer a myriad of functional materials. An example is found in liposomes — artificial vesicles formed by lipid self-assembly — with contributions as drug carriers, biomimetic reactors or cell membrane models, among others. The versatility of their chemical composition, number of bilayers and size, which can range from several micrometres down to a few tens of nanometres, provide a large variety of chemical and physical features that can be tailored to target specific applications. For instance, these parameters can influence the interactions of liposomes with cells and cargo loading, thus directly impacting their drug delivery capabilities. Likewise, accurate control of these features is desirable for establishing robust model systems to investigate the function of membrane-interacting proteins. There are already several methods to size-regulate small liposomes, such as extrusion, sonication or microfluidic-based systems (with different levels of complexity). Nevertheless, narrowing the size distribution of liposomes with a diversity of lipid-type formulations that incorporate functional elements, such as membrane proteins, in a scalable fashion remains challenging. Now, two studies use DNA nanotechnology to address this issue.

Writing in Nature Chemistry, Hongzhou Gu, Chenxiang Lin and collaborators describe an approach to sort sub-150-nm liposomes of different compositions into populations of well-defined size with a high degree of homogeneity1. Their method uses isopycnic centrifugation, which separates liposomes based on their buoyancy. Liposomes that differ in size but have the same bilayer and internal contents do not considerably vary in their buoyant density, so Lin and co-workers proposed amplifying this difference by creating a surface coating with a dense material, DNA. This way, smaller DNA-coated liposomes acquire more density than larger ones, enabling high-resolved size-sorting upon isopycnic centrifugation (Fig. 1). Rather than using individual DNA strands, the team exploited DNA self-assembly to build larger DNA-based nanostructures (DNs), customized with a hydrophobic cholesterol moiety for lipid bilayer anchoring, which guarantees high surface coverage.

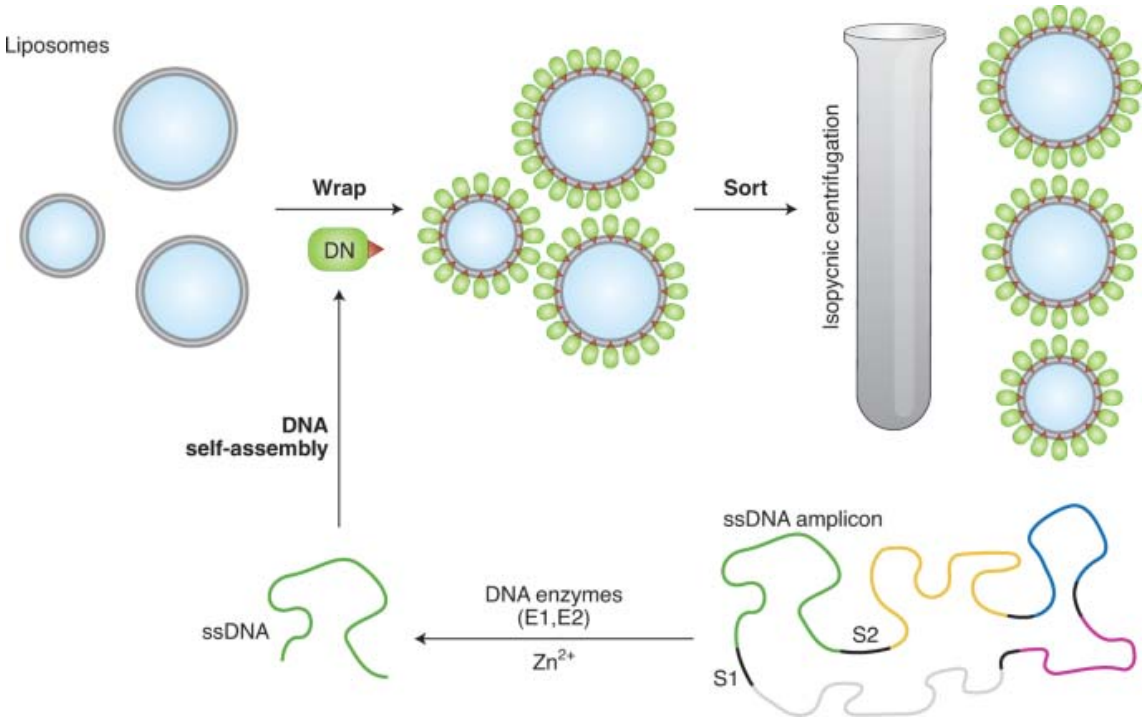

Fig. 1: Scheme showing the liposome size-sorting approach.
Liposomes are wrapped with DNA nanostructures (DNs) and sorted by isopycnic centrifugation. DNs are shown in green. The red triangle represents the cholesterol moiety that anchors the structure to the lipid bilayer. The single-stranded DNA (ssDNA) amplicon contains sequences to encode several DNs in tandem (each represented by a different colour) and DNA-enzyme-sensitive sequences (S), which are shown in black. Selective cleavage by DNA enzymes E1 and E2 (operating on S1 and S2) in the presence of Zn2+ leads to an ssDNA that self-folds into the DN required for liposome coating.

Gu, Lin and colleagues used electron microscopy to verify the narrow size distributions of the different gradient centrifugation fractions. They loaded the sorted liposomes with deoxyribozymes (DNA oligonucleotides able to catalyse chemical reactions) that self-cleave in the presence of Zn2+ — the liposomes remained intact in a Zn2+ solution until detergent was applied, demonstrating membrane integrity and leakage-resistance. An interesting aspect is that the DNs coating gives prolonged storage life to the sorted liposomes, yet enzyme-mediated DNA digestion can be used to remove the coverage and recover naked liposomes. The fine size distribution, and hence well-defined curvature exhibited by each fractionated liposome population, enabled the researchers to apply this system to gain new insights and validate previous predictions on the membrane curvature dependency function of two proteins: ATG3, an enzyme involved in autophagy, acting at the periphery of the autophagosome membrane, and SNAREs, which play a key role in many membrane fusion processes within cells.

It should be mentioned that the use of DNs to adapt the size of liposomes has been explored before. Indeed, previous reports by Lin's group and co-workers showed designer DNs that template the assembly of liposomes2. However, each liposome size required a matching de novo fabricated DN partner, which is certainly detrimental for cost-effective scalability. Conversely, the present procedure only necessitates one DN type, affording larger production at lower material cost (a tenfold reduction compared to the previous method, as estimated by the team)1. Still, time-consuming electron microscope analysis on each sorted fraction is needed to determine the liposome size.

DNs used by Gu and Lin's team, with either a three-point star or a six-helix-bundle rod shape, are simple constructs composed of a few strands. Sorting the larger (>100 nm) liposomes was better achieved by using the rod-type DN, which exhibits a higher molecular weight. Due to the programmability uniquely provided by DNA nanotechnology, heavier DNs can be customized as desired to adapt this sorting strategy to the separation of even larger liposomes. Nevertheless, the fabrication of DNs for sorting large quantities of liposomes significantly enhances material costs.

Writing in Chem, Hongzhou Gu and collaborators present successful size-dependent liposome sorting with DNs prepared by a method — with lower production costs — that relies on the folding of a single DNA strand3. The fabrication of DNs using a single strand confers some advantages over those produced from multiple strands, such as the possibility of amplification with high fidelity using biotechnological procedures, and is not affected by an imbalanced concentration of strands4. Gu's team generated artificial 'genes' that contain precisely selected sequence fragments to encode either a single or several DNs in a tandem arrangement. Rather than using protein enzymes to facilitate the release of the DN-encoding fragments4,5,6, Gu's team used a DNA-based approach. By introducing distinct deoxyribozyme-sensitive sequences flanking the DN-encoding fragments, the researchers achieved the selective scission of the desired DN in the presence of the deoxyribozyme and Zn2+ (Fig. 1). In vitro or in vivo (using phagemid vectors in bacteria5,6,7) amplification methods were used to augment the mass production of the designed artificial 'genes'. The in vivo strategy is especially appealing to scale future industrial fermenter-based manufacture production up to a gram-scale of DNA material in a cost-affordable fashion3,7.

An interesting aspect of this work is that the DNs folded with a single strand showed superior resistance to nuclease degradation than constructs with a similar surface-area/mass ratio but assembled with multiple strands. Additionally, Gu and colleagues demonstrate size-dependent internalization of the DN-coated liposomes in two types of cancer cell lines, while the uptake exhibited by their naked counterparts was negligible. This feature expands the usefulness provided by the DN-coating strategy from merely assisting size-sorting of liposomes to rendering hybrid nanomaterials for potential biological applications with precise size control and an enhanced biostability. It is foreseeable that future investigations will shed light on the potential effect exerted by the type of DN coverage on the liposome internalization pathways and their intracellular fate. A plethora of therapeutical applications can also be conceived by equipping the DN coating with biologically active sequences, such as cell-type targeting (RNA/DNA) aptamers, gene regulators, and/or immunostimulatory oligonucleotides.

The outstanding design versatility of DNs (yielded by DNA nanotechnology), together with the fine control on liposome size provided by Gu and Lin's approach and the favourable cost-effective production of DNs presented by Gu, paves the way for establishing precise synthetic combinations that are tuned to address current and upcoming challenges in the biomedical realm.