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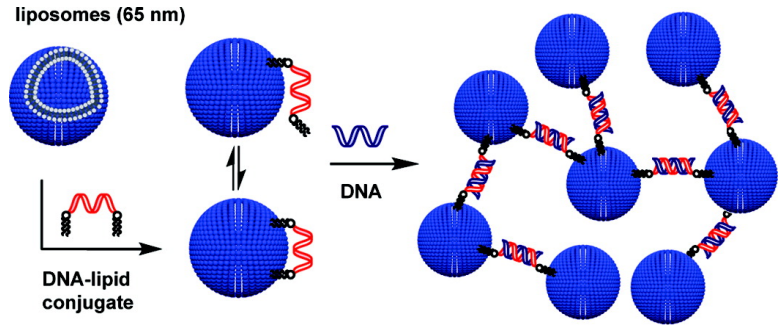

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DNA-Controlled Assembly of Soft Nanoparticles

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DNA-controlled assembly of DNA-encoded nanoparticles has been reported for a number of different solid nanoparticles, including applications in biodiagnostics.[1] Immobilization of DNA (encoding) on solid nanoparticles requires surface chemistry, which is well established for gold surfaces but often tedious and not generally applicable for many other inorganic surface materials. Noncovalent attachment of DNA to soft nanoparticles, like liposomes, is an attractive technology as no surface chemistry is needed. Successful applications of noncovalent DNA encoding of liposomes, for example, liposome tethering to surfaces toward liposome arrays[2,3] and liposome fusion, have been reported.[2e]

Here we report a DNA-controlled assembly of liposomes (POPC, 65 nm, 62 nM probe DNA concentration in 100 mM NaCl, 10 mM HEPES buffer, pH 7.0) in solution and on solid supported membranes; this process displays remarkably sharp thermal transitions from an assembled to a disassembled state, allowing application of DNA-controlled liposome assembly for the detection of polynucleotides (e.g., DNA) with single mismatch discrimination power. The method is based on a single DNA strand (contains two lipid membrane anchors), which is able to noncovalently attach to a liposome surface. This design enables detection of biological polynucleotide targets as the complimentary strand can be unmodified DNA or RNA strands.

The key concept is shown schematically in Figure 1 illustrating how the membrane anchors (a total of four palmityl chains) are attached to each end of the probe DNA conjugate.[4] Three additional T bases are attached on each end to avoid self-aggregation of the respective DNA conjugates. We have shown that such DNA−lipid conjugates form very stable duplexes with cDNA.[4] The conformationally flexible ssDNA probe is presumably anchored reversibly into the liposomes since permanently double anchored DNA strands would not allow efficient hybridization to a complementary target sequence. After hybridization of the DNA probe to a complementary target DNA, both duplex ends are not able to be simultaneously anchored into the same liposome, as this would require bending of the rigid dsDNA. Therefore, one of the membrane anchors is released into solution, and subsequent interliposomal membrane anchoring does occur and is highly favored. The exclusive interliposomal anchoring of the DNA duplex leads to liposome cross-linking and liposome assembly. The process continues until target or probe DNA strands have been consumed since liposomes are present in excess. This process is reported using a 17-mer DNA, but it works equally well with 24-mer and 27-mer dsDNA (not shown). The required duplex rigidity is not compromised by single mismatches, deletions or insertions. Cooperative effects like DNA melting and the entropically favored disassembly of liposome aggregates are presumably responsible for the sharp thermal transitions observed.

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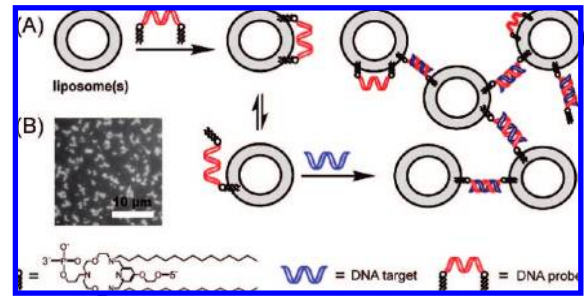

Figure 1. (A) Schematic representation of liposome aggregation upon duplex formation between a lipophilic probe DNA (red) and an unmodified target DNA (blue). Liposomes and DNA strands are not drawn to scale. Inset: (B) Fluorescence microscopy image showing liposome assembly and increased aggregate size (MICA solid supported POPC membrane,[5] 62 nM DNA concentration, pH 7.0, HEPES buffer).

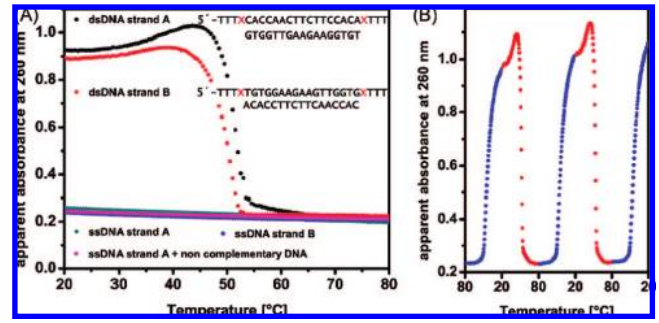

Figure 2. UV monitored thermal denaturation data for ssDNA probe strands (green and blue), with noncDNA (light red) and the corresponding duplexes with cDNA (black and red) in the presence of liposomes (A). Reversibility of the assembly process is shown by multiple heating−cooling cycles (blue cooling/red heating). X = lipid anchor (Figure 1).

The increase in particle size upon liposome assembly has been measured by dynamic light scattering. Higher DNA concentrations (>1 μM) finally result in visible precipitation of large liposome aggregates. The precipitated liposomes can be redissolved by heating above the thermal denaturation temperature (T_m) of the respective DNA duplex. The liposomes do not release their content or fuse during this process as shown by a calcium-arsenazo III assay and light scattering (see Supporting Information for details). Notably, the liposome assembly is a fast process (<5 min).

The process of liposome assembly and disassembly can be monitored by ultraviolet spectroscopy at 260 nm but also at higher wavelengths, up to 420 nm, although with decreasing signal intensity. Repeated T_m measurements show that liposome assembly is fully reversible when cycling the temperature between 20 and 80 °C (Figure 2B).

The changes in absorbance are caused by light scattering of liposome aggregates rather than changes in absorbance of the nucleobases during thermal denaturation. The T_m curves in Figure

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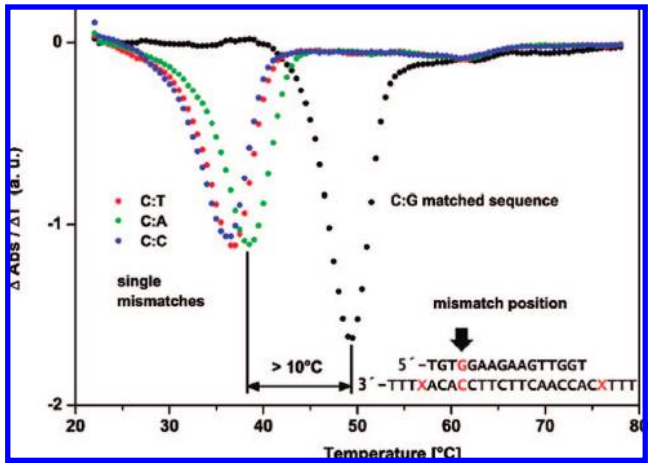

Figure 3. First derivative of the UV T_m data of a 17-mer DNA probe and the corresponding single mismatches (17-mer DNA target).

2A are therefore reversed and of much higher intensity (2 orders of magnitude) compared to conventional T_m curves of DNA duplexes (strong absorption of liposomes aggregates below T_m and weak absorption of free liposomes above T_m). The remarkably sharp thermal transitions (disassembly within 2−3 °C, Figure 2A), compared to duplex DNA in the absence of liposomes (typical thermal denaturation within 15−25 °C), is an important advantage over fluorophore-based detection systems. Thermal melting profiles of this type have only been reported for a Au-nanoparticle system.[1] Digestion of the dsDNA links in liposome aggregates with DNase I resulted in complete disassembly, which verified dsDNA as a linker between individual liposomes. DNA−lipid conjugates with only a single palmitoyl substituent at each end of the DNA strand, incorporated in the same sequence context, cannot mediate liposome assembly (see Supporting Information).

The narrow temperature range for the disassembly process and nonoverlapping thermal transitions between complementary and single mismatched sequences (Figure 3) can be utilized to turn the liposome assembly process into a powerful detection system for single nucleotide polymorphisms (SNPs). The ΔT_m values for single mismatch discrimination, in the presence and absence of liposomes, closely resemble experimental data for unmodified DNA, which allows traditional probe sequence design.

DNA controlled tethering of liposomes to solid supported membranes has been demonstrated using terminal cholesterol[2] or dialkyllipid[3] functionalized DNA, based on designs involving at least two modified DNA strands. However, none of the previously reported systems has been applied to detection of polynucleotides. The lipid-modified DNA conjugates described here can also be used to tether liposomes to a solid supported membrane (on mica, POPC, Figure 4A−B).[5] Thermal denaturation of the liposome aggregates by heating to temperatures above the T_m of the corresponding DNA duplex (T_m = 46 °C) leads to complete release of the attached liposomes into solution and can be visualized directly by fluorescence (Figure 4C). The liposomes reattach to the solid supported membrane upon cooling below the T_m, which indicates that the probe DNA, despite an ~620 times excess of total POPC bilayer surface in solution, does not strongly partition into free liposomes (0.5 mM POPC) in solution (Figure 4D). This finding is attributed to the membrane anchor structure with two palmityl groups[4]

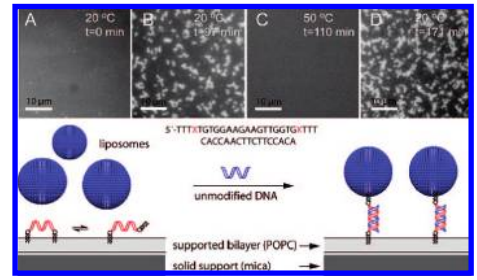

Figure 4. Schematic representation of DNA mediated liposome anchoring on a solid supported membrane (A to B). Reversible attachment upon thermal denaturation (C and D, see Supporting Information for details).

anchoring the DNA strand more strongly to the solid supported membrane, which we speculate consists of a more ordered and densely packed POPC bilayer compared to the POPC bilayer (fluid L_α state) of the free liposomes.[5] Individual attached liposomes, as well as clusters, are resolved in the images (Figure 4) and diffuse laterally as expected from the fluidity of the underlying supported membrane (POPC).[3b]

The DNA-controlled assembly of liposomes reported here is general and efficient for liposomes of sizes 50−200 nm in solution and on supported membranes. In contrast to solid nanoparticles, the lipophilic DNA probe strand is inserted noncovalently, which avoids development of tedious conjugation chemistry and enables a rapid assembly process. The method presented here is the first application of liposome assembly to the detection of polynucleotides with single mismatch discrimination power in solution. The reversible assembly of liposomes is accompanied by remarkably sharp and highly reproducible thermal transitions between assembled and disassembled states. This versatile assembly strategy can be generally applied to tethering and encoding of liposomes for detection of polynucleotides and for other assembly processes, for example, immobilization of proteoliposomes.

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Supporting Information Available: Materials and experimental methods for UV T_m studies, liposome preparation and experiments on supported membranes. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Mirkin, C. A.; Letsinger, R. L.; Mucic, R. C.; Storhoff, J. J. *Nature* **1996**, *382*, 607. (b) Rosi, N. L.; Mirkin, C. A. *Chem. Rev.* **2005**, *105*, 1547. (c) Taton, T. A.; Mirkin, C. A.; Letsinger, R. L. *Science* **2000**, *289*, 1757.
(2) (a) Svedhem, S.; Pfeiffer, I.; Larsson, C.; Wingren, C.; Borrebaeck, C.; Höök, F. *ChemBioChem* **2003**, *4*, 339. (b) Pfeiffer, I.; Höök, F. *J. Am. Chem. Soc.* **2004**, *126*, 10224. (c) Maru, N.; Shoda, K.; Sugawara, T. *Nucleic Acids Symp. Ser.* **2004**, *48*, 95. (d) Zhang, G.; Forooqui, F.; Kinstler, O.; Letsinger, R. L. *Tetrahedron Lett.* **1996**, *37*, 6243. (e) Stengel, G.; Zahn, R.; Höök, F. *J. Am. Chem. Soc.* **2007**, *129*, 9584.
(3) (a) Yoshina-Ishii, C.; Boxer, S. G. *J. Am. Chem. Soc.* **2003**, *125*, 3696. (b) Yoshina-Ishii, C.; Miller, G. P.; Kraft, M. L.; Kool, E. T.; Boxer, S. G. *J. Am. Chem. Soc.* **2005**, *127*, 1356. (c) Graneli, A.; Yeykal, C. C.; Prasad, T. K.; Greene, E. C. *Langmuir* **2006**, *22*, 292–299. (d) Chandra, R. A.; Douglas, E. S.; Mathies, R. A.; Bertozzi, C. R.; Francis, M. B. *Angew. Chem., Int. Ed.* **2006**, *45*, 896–901.
(4) (a) Rohr, K.; Vogel, S. *ChemBioChem* **2006**, *7*, 463. (b) Jakobsen, U.; Rohr, K.; Madsen, R. K.; Vogel, S. *Nucleosides, Nucleotides Nucleic Acids* **2007**, *26*, 1221.
(5) Simonsen, A. C.; Bagatolli, L. A. *Langmuir* **2004**, *20*, 9720.

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