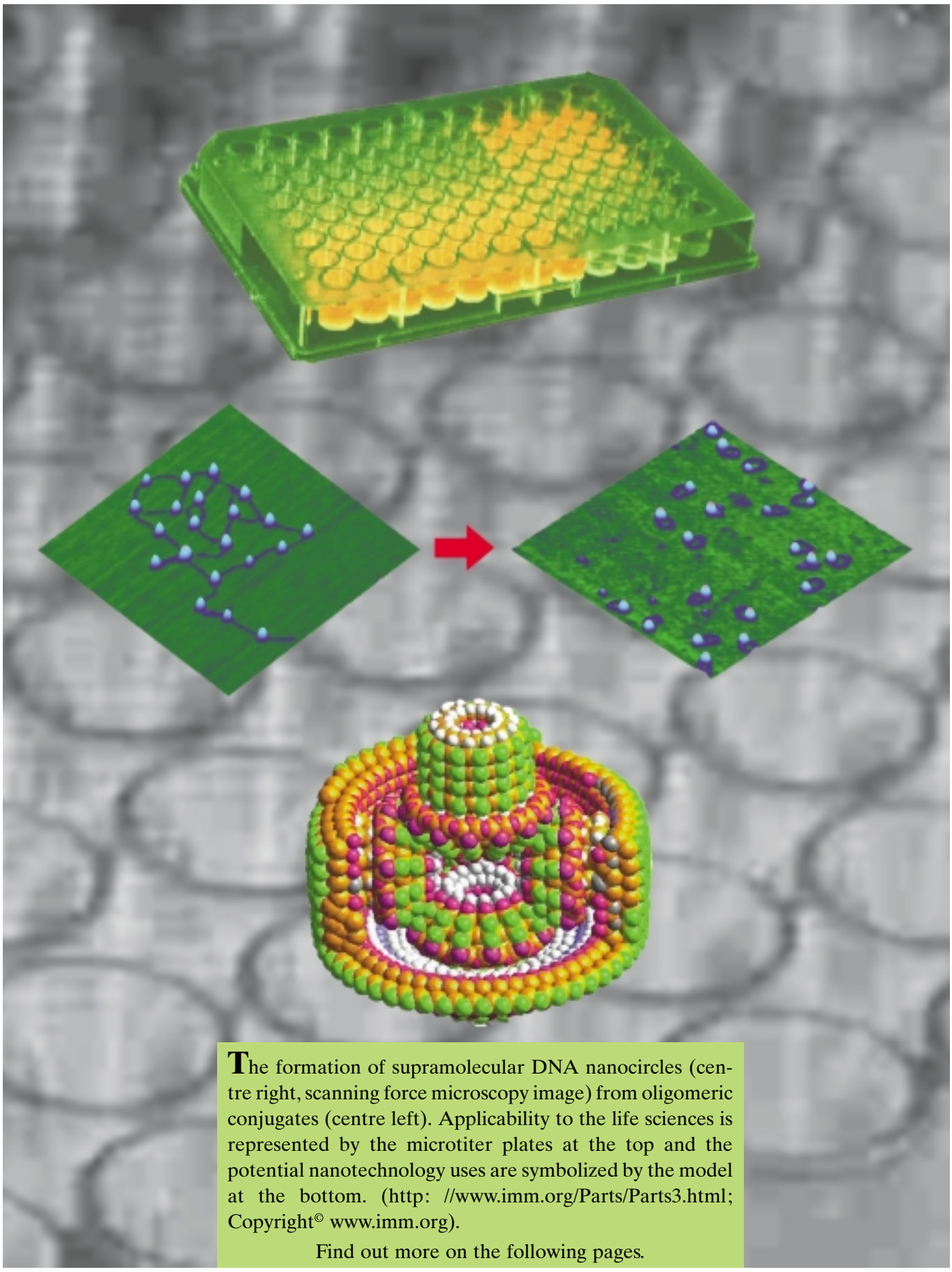

The formation of supramolecular DNA nanocircles (centre right, scanning force microscopy image) from oligomeric conjugates (centre left). Applicability to the life sciences is represented by the microtiter plates at the top and the potential nanotechnology uses are symbolized by the model at the bottom. (http: //www.imm.org/Parts/Parts3.html; Copyright© www.imm.org).

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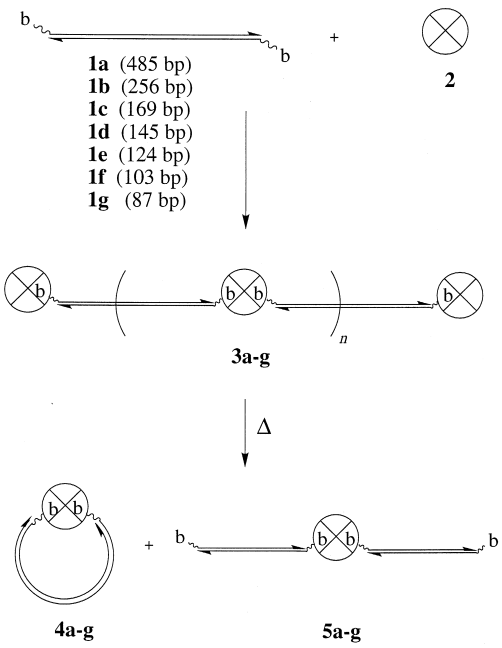
Supramolecular Nanocircles Consisting of Streptavidin and DNA**

Christof M. Niemeyer,* Michael Adler, Song Gao, and Lifeng Chi

The self-assembly of small molecular building blocks programmed to form larger nanometer-sized, structural and functional elements is an important goal of molecular nanotechnology.[1] Due to its unique and simply predictable recognition capabilities, high physicochemical stability, mechanical rigidity, and high precision processing ability, DNA is a promising construction material for the fabrication of nanostructured systems.[2, 3] As an example, the work of Seeman et al. indicates that DNA can be used to synthesize nanostructured scaffolding,[2b,c] surface architecture,[2d,e] and, even, nanomechanical devices.[2f] Moreover, the highly specific hybridization of complementary nucleic acids can be used to selectively position macromolecules, such as proteins[4] and metal nanoclusters.[4b, 5]

We have recently reported on the synthesis of nanostructured oligomeric conjugates **3**, which can be obtained by the self-assembly of bisbiotinylated DNA **1** and the biotin-binding protein streptavidin (STV, **2**).[6] Scanning force microscopy (SFM) analysis of the oligomers **3** revealed that the STV preferentially functions as a bi- or trivalent linker molecule between adjacent double-stranded DNA (dsDNA) fragments, despite its binding capacity for four molecules of biotin. Thus, linear and two-dimensionally branched supramolecular chains are formed (Scheme 1). Due to the remaining biotin-binding capacity, the DNA–STV oligomers **3** are powerful reagents for immuno-polymerase chain reactions (immuno-PCR),[7] a highly sensitive method for the trace analysis of proteins and other antigens. We here report on the synthesis of novel supramolecular DNA nanocircles **4** which can conveniently be generated by thermal treatment of the oligomers **3**.

During the exploitation of the supramolecular DNA–STV oligomers **3** we intended to investigate their thermal denaturation response. For this purpose, oligomers were prepared by coupling of STV (**2**) with varying molar equivalents of a 256 base pair (bp) dsDNA fragment **1b**. In the case of a molar excess of STV the typical fingerprint-like band pattern was visible by gel-electrophoretic analysis representing the population of the supramolecular oligomers **3**. In contrast, an excess of dsDNA led to the formation of the typical blurry band of the high molecular weight oligomers in addition to



Scheme 1. Synthesis of supramolecular DNA–streptavidin nanocircles **4**. Oligomeric DNA–STV conjugates **3a–g** are prepared from 5′,5′-bisbiotinylated double-stranded DNA (dsDNA) of various lengths **1a–g** and streptavidin (STV, **2**). Note that the schematic drawing of **3** is simplified, since branch points occur due to the presence of tri- and tetravalently conjugated STV (see Figure 2d). The oligomeric conjugates **3** are heated for two minutes at 95 °C and subsequently cooled down rapidly in an ice bath. As a consequence, supramolecular DNA nanocircles **4a–g** and linear DNA_2–STV conjugates **5a–g** are formed as the chief products. For simplification, complementary DNA strands are drawn as parallel lines. The 3′-ends are indicated by the arrow heads. It is unclear whether the two STV-bound dsDNA fragments are conjugated to adjacent or opposite subunits of the tetrameric STV. However, the model of Sinha and Chignell suggests that binding to opposite subunits should lead to more stable complexes.[19]

distinct bands indicating the presence of free dsDNA and DNA–STV conjugates of varying stoichiometry, such as DNA_2–STV (Figure 1, lanes 1–4).[6] The various preparations of **3b** were denatured by heating to 95 °C for two minutes, and subsequently, the samples were rapidly cooled in the ice bath. This treatment led to a significant simplification of the electrophoretic band patterns and the products were formed to a varying extent (Figure 1, lanes 5–8). The chief products are the $dsDNA_2$–STV conjugate **5b** (position d in Figure 1) and the nanocircle **4b** (position b).[8] A comparison of lanes 4 and 8 indicates that free **1b** is reformed only when an excess of dsDNA was present initially (position a). Quantification of the bands by densitometry revealed that the formation of **4b** proceeded most efficiently (60% yield) in the case of an initial 1:2 coupling ratio of DNA:STV.

Further studies showed a strong dependency of the product formation on the educt concentration. Increasing the concentration of **3** from 0.2 to 0.4 μM led to a decrease in the relative amount of **4** to about 45%. An increase in the concentration of **3** to 1 μM further decreased the product yield to about 20% of **4**, and, instead, high molecular weight oligomers were formed. The cooling rate subsequent to thermal denaturation of **3** also influenced the formation yields of the nanocircles. For example, the slower cooling rate of 0.5 °C min^{-1} led to the

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[**] This work was supported by the Deutsche Forschungsgemeinschaft (SPP 1072), the Fonds der Chemischen Industrie, and the Tönjes-Vagt Stiftung. We thank Prof. D. Blohm and Prof. H. Fuchs for generous support.

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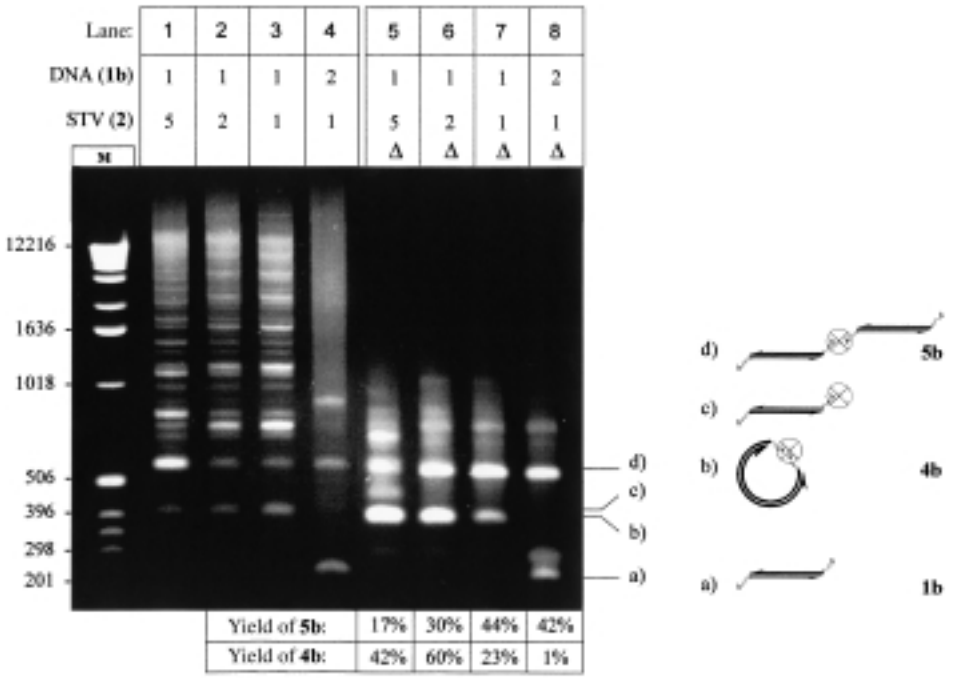

Figure 1. Electrophoretic analysis of the synthesis of nanocircle **4b**. A nondenaturing 1.5% agarose gel stained by ethidiumbromide is shown. Lanes 1–4 contain the oligomeric DNA–STV conjugates **3b**, prepared from varying coupling stoichiometries of STV and dsDNA **1b**. Thermal denaturation and rapid cooling in an ice bath leads to the transformation of the various preparations of **3b** to the low molecular weight products. The main products are the linear conjugate DNA_2–STV **5b** and the nanocircle **4b** (lanes 5–8). The dependency of the product formation on the initial coupling stoichiometry of DNA:STV is demonstrated by the percentage yields indicated below lanes 5–8. The numbers were obtained by densitometrical quantification of the band intensities. Lane M contains a DNA molecular weight marker; the lengths are given in base pairs.

predominant formation of highly oligomerized DNA–STV products, and the yield of **4** was decreased to about 35%. The quantitative oligomerization, indicated by the appearance of an immobile band in the gel electrophoretic analysis, was obtained by the slow cooling of a 1 μM solution of **3**. The subsequent denaturation and rapid cooling of this oligomeric network, led to the reformation of products **4** and **5**, respectively. These results, which concern the dependency of the product formation on the temperature and concentration, were in agreement with earlier reports on the self-assembly of oligovalent building blocks.[9, 10]

To further investigate the phenomenon of the circle formation, we modified the length of the dsDNA fragments in **3**, which led to the formation of nanocircles **4** of miscellaneous size (**4a**–**g**). A comparison of the rates of product formation (Table 1) indicated that the yield of **4** decreased with a reduced length of the dsDNA. Instead, higher molecular weight products and free dsDNA were formed. This result should be due to the greater rigidity of short dsDNA fragments which counteracts intramolecular cyclization.[11]

To characterize **4**, the cyclic structure was established by means of a Ferguson plot.[12] In this analysis, the electrophoretic mobility of linear and cyclic DNA–STV species is plotted against the density of the gel matrix. The plot of the linear supramolecules revealed an identical slope of the species **5a**–**c**, while the slopes of the nanocircles **4a**–**c** grew with increasing length of the dsDNA. This observation allows one to estimate that the friction constant of nanocircles **4a** and **4b** is greater than the analogous linear conjugates **5a** and **5b**, while **4c** has a smaller friction coefficient than **5c**.[13] Moreover, a comparison of the Ferguson plots of, for example, **5b** and a linear 500 bp dsDNA fragment indicates that the coupling of the STV molecule leads to a decreased friction constant. Probably, this is a consequence of the enhanced flexibility of the two DNA moieties linked by the central STV molecule in **5** compared to the rigid DNA double helix.

Samples of the nanocircles **4** were purified by electrophoresis and analyzed by SFM (Figure 2). The SFM visualization allowed clear verification of the supramolecular cyclic structure and the equimolar coupling ratio of the nanocircles for DNA sizes down to the 103 bp dsDNA of circle **4f**. Statistical analysis of the SFM images indicates that more than 90% of the supramolecules reveal the characteristic cyclic structure. The ring diameters measured by SFM correlate well with the theoretical values. However, the

Table 1. Yields and dimensions of the nanocircles **4**

	a	**b**	**c**	**d**	**e**	**f**	**g**
dsDNA length [bp]	485	256	169	145	124	103	87
amount of **4** prior to denaturation [%][a]	30	10	10	4	2	< 1	< 1
amount of **4** after denaturation [%][a]	77	60	62	49	50	52	28
amount of **5** after denaturation [%][a]	20	30	25	12	13	11	17
diameter of **4** [nm][b]	61	29	22	17	15	15	–
deviation [%][b]	21	14	9	6	4	2	–
calculated diameter [nm][c]	54.6	29.9	20.4	17.8	15.5	13.2	11.5

[a] Determined by densitometrical analysis of the gel electrophoretic band intensities; the average deviation is about ±5%. [b] Determined from the scanning force microscopy images. The number of individual structures measured was more than 50. The data indicate the average arithmetical values and the percentage deviation of the average ring diameters. [c] Calculated from the length of the dsDNA fragments with assumption of a B-DNA conformation. The diameter of the STV was estimated to be 4.6 nm[20] and the length of the two alkyl spacers between the DNA and the STV was estimated as 2 nm.

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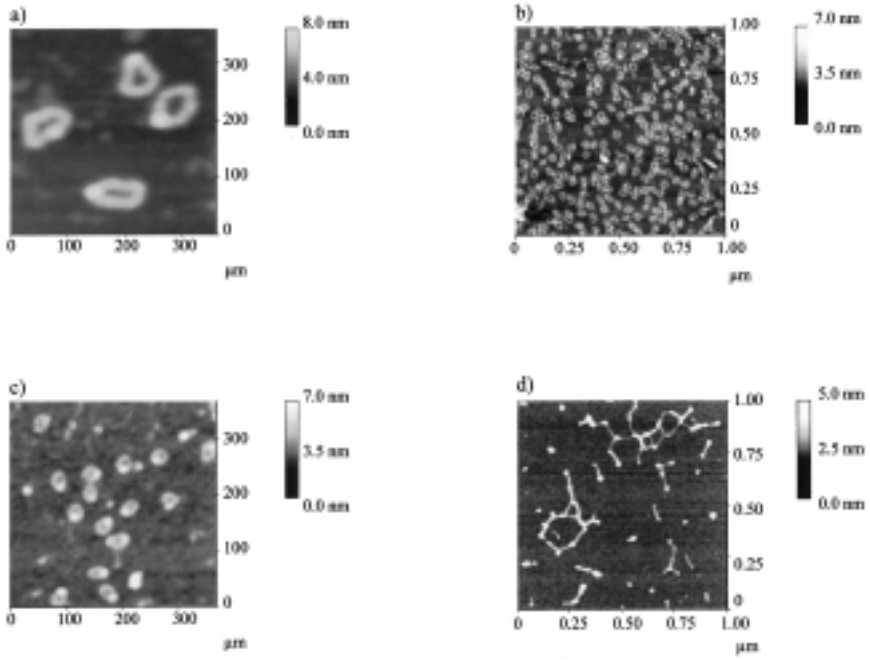

Figure 2. Scanning force microscopy (SFM) images of the DNA nanocircles **4a** (a), **4b** (b), **4c** (c), and the oligomeric DNA–STV conjugate **3b** (d).

average deviation of the various diameters which were determined decreases with reduced ring size (Table 1). This is most likely a consequence of the increasing ring tension within the small circles. As a consequence, the structure of **4c** is more similar to an ideal circle than that of **4a** (Figure 2).

The reasons for the dependency of product distribution on the initial coupling stoichiometry, as well as the mechanism of nanocircle formation, are as yet vague. However, it is known that the denaturation temperature of ligand-saturated STV is 112 °C.[14] Thus, it seems probable that during thermal denaturation a quasi transition state species exists that consists of STV conjugated with two biotinylated single-stranded DNA molecules; these DNA molecules can intramolecularly hybridize, provided that they are complementary to each other. From the statistical point of view, however, only 50 % of the bivalently conjugated STV molecules in the quasi transition state carry two complementary single-stranded DNA moieties. Thus, the product yields of **4** that are up to 77 %, suggest that other mechanisms might contribute to the nanocircle formation. Denaturation of **3** in the presence of free D-biotin leads to the displacement of the dsDNA from the STV. This indicates that the interaction between the biotinylated dsDNA and STV becomes labile at elevated temperatures, which suggests that ligand exchange reactions might also contribute to the formation of **4**.[15]

We have reported here on the simple and efficient synthesis of novel supramolecular DNA nanocircles **4**, obtainable by thermal denaturation and rapid cooling of the oligomeric DNA–STV networks **3**. The generation of DNA circles and their potential use as building blocks for the fabrication of nanostructured elements has previously been reported.[10, 16, 17] This work extends the preceeding approaches since the endogenous STV molecule within the nanocircles can conveniently be used as an anchor group for further functionalization.[4b, 18] Work regarding the modification and lateral organization of the nanocircles is under way.

Experimental Section

A solution of STV (24 µL, 1.0 µM) in Tris buffer (10 mM, pH 7.3) containing ethylenediaminetetraacetate (EDTA, 2 mM) was mixed with a solution of the bisbiotinylated dsDNA **1c** (6.8 µL, 1.75 µM) in the same buffer. The final concentration of dsDNA was 0.39 pmol µL^{-1}. The solution was incubated for 15 min at room temperature on a vertical shaker and, subsequently, diluted with the same Tris buffer to a final concentration of 0.2 µM of DNA. The oligomeric DNA–STV conjugate was denatured for 2 min at 95 °C and the solution was cooled to 0 °C in the ice bath. Analysis and purification of the products was achieved by nondenaturing gel electrophoresis on an agarose gel. The agarose concentration varied according to the length of the dsDNA fragment: 1 % (DNA > 400 bp), 1.5 % (400–150 bp), or 2.2 % (150–80 bp). The gel is stained with ethidiumbromide and the main product bands are cut out. The products are eluted from the gel slices by centrifugation for 10 min at 5000 rpm using a Micropure 0.22 µm filtration device (Millipore). Immobilization of **4** on mica and SFM analysis are carried out as previously described.[6]

Received: December 22, 1999 [Z 14450]

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single-stranded DNA. Moreover, conjugates containing STV and one or more non-complementary single-stranded DNA molecules were determined to migrate as constituents of the diffuse background band. The formation of the single-stranded species occurs due to the rapid cooling rates inhibiting quantitative intermolecular rehybridization.

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Dynamic Observations of the Hydrolysis of a DPPC Monolayer at the Air/Water Interface Catalyzed by Phospholipase A_2**

Junbai Li,* Zhijian Chen, Xiaoli Wang, Gerald Brezesinski, and Helmuth Möhwald*

Many chemical reactions, especially those in the crystalline state, depend on the local environment of the reactants. Hence in order to study their mechanism one has to investigate these reactions as a function of local order, and in addition, the change of order should be measured as the reaction proceeds. For this purpose amphiphile monolayers are highly suited, because their order can be varied and characterized, and the chemical reaction can be followed by newly developed spectroscopic and microscopic techniques. In addition the reactants can be exchanged with the adjacent phase in a controlled way.

One biologically and technologically important system is the phospholipase A_2 (PLA_2) that catalyzes the hydrolysis of a phospholipid monolayer at an interface. It is known that this reaction depends upon the state of the monolayer. It is now possible to study the molecular arrangement within monolayers, even in individual domains, with microscopic resolution. For this investigation the main methods used are surface X-ray diffraction, FT-IR spectroscopy, and Brewster angle microscopy (BAM). These techniques are used here to follow the enzyme reaction in detail. The correlation of changes in the molecular arrangement with catalytic activity should provide an understanding of reaction mechanisms.

PLA_2 is a calcium-dependent enzyme abundant in living organisms. The enzymatic reaction with the membrane surface consists of a molecular recognition process and a cleavage reaction.[1] PLA_2 stereoselectively hydrolyzes the *sn*-2 ester linkage of L-phospholipids to release fatty acids and lysophospholipids (Figure 1a). The activity of PLA_2 at the interface of aggregated substrates, such as phospholipid monolayers, is 10000-fold greater than with the corresponding monomeric substrate.[2] PLA_2 has an α-helix-enriched conformation in aqueous solution (Figure 1b). During an enzymatic reaction a change in this conformation may occur as soon as the enzyme reaches the interface.

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[**] This work was supported by the research contract between the German Max-Planck-Society and the Chinese Academy of Sciences as well as the National Natural Science Foundation of China (NNSF). J.L. thanks the president fund of the Chinese Academy of Science and the National Personal Department of China. DPPC = L-dipalmitolphosphatidylcholine.

  1433-7851/00/3917-3059 $ 17.50+.50/0