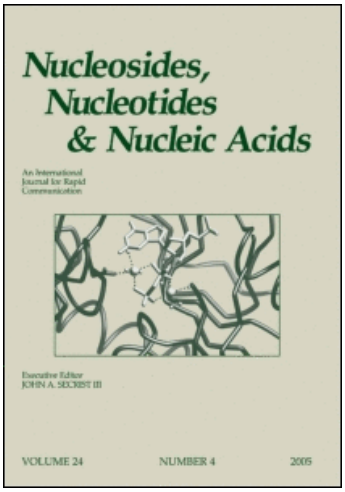

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SELFORGANIZATION OF NUCLEIC ACIDS VISUALIZED BY SCANNING FORCE MICROSCOPY

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ABSTRACT: SFM-investigations visualize domains and microdomains of selforganizational DNA- and RNA-adlayer patterns on graphite substrates and contribute by this to general approaches of elucidating biomesogen pre-life states complexity.

1. INTRODUCTION: Developmental pathways of chemistry from molecules to macromolecules and of physics from phase to microphase centre in the complexity of supramolecular biomesogen organizations[1-3] (FIG. 1). Adequate experimental elucidations and theoretical descriptions suffer from the deficiencies of methodological arsenals, providing broad spectra of investigation facilities for solid and liquid (solution)systems, but being devoid of a suitable instrumentary to cope with the complexity of highly condensed lower-order mesophase systems[3]. Among the recent achievements to overcome these disadvantages, molecular resolving microscopies[4-6] will offer intriguing insights into preintelligent organizations, from which living systems might have origined. While so far scanning tunneling microscopy and scanning force microscopy (STM and SFM) investigations of nucleoprotein components have mainly been devoted to characteristics of molecular individuals[4-9], we have tried to approach their selforganizational facilities.[3a]

2. MATERIALS AND METHODS: *2.1. Polynucleotides:* SFM-, SPR- as well as corresponding Tm-characterizations have been obtained as follows: high-molecular polydisperse chicken-erythrocyte DNA (1) (REANAL), 35μg/ml, 1mM NaCl in water, pH 7.0; polyuridylic polyadenylic acid: $(U)_n \cdot (A)_n$-duplex (2) (SERVA, K-salt, S_{20w} 5.7 and 8.8, respectively), 0.1mM solutions in water, PBS: 0.15m NaCl, 0.01m Na-phosphate, pH 7.4, hybridized according to [10,11]. 36-mer DNAs (3 and 4) (FIG. 2): oligodeoxyribonucleotide-duplexes were synthesized from commercial phosphoamidites (inclusive biotin-phosphoramidite - MWG) with a 380B-Synthesizer (Applied Biosystems), purified by common procedures, controlled by ion-exchange-chromatography, subsequently hybridized and characterized by Tm-profiles.[10,11] Polarizing microscopy- and SPR-probes were prepared according to SFM-samples, except for polynucleotide concentrations: chicken-DNA: 180mg/ml; $(U)_n \cdot (A)_n$: 100mg/ml. 36-mer 3: 10μg/ml. *2.2. Polarizing Microscopy:* The liquid-crystalline textures of DNAs and RNAs (1-4) (FIG. 3) have been detected by means of a polarizing microscope, type Leitz LaborLux 12S, equipped with a Hitachi Video Color Camera KP-551. In all cases samples were placed on a slide under a partially sealed coverslip, so that slow evaporation took place. Samples were observed through crossed polarizers and photographed with magnification 320x. *2.3. Surface Plasmon Resonance (SPR):* SPR-results (FIG. 4) have been obtained with the help of a BIAcore (Pharmacia Biosensor, Uppsala, Sweden) and sensor

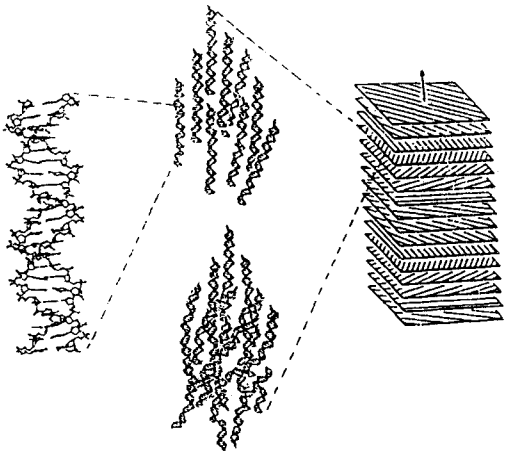

FIG. 1: Mesophase domains and microdomains between single-macromolecule and classical liquid-crystalline phase

5'---NCGCATATATGCGCGCATATATGCGCGCATATATGCG--- 3' 3
3'-----GCGTATATACGCGCGTATATACGCGCGTATATACGCN- 5' 4: Des-5'-N

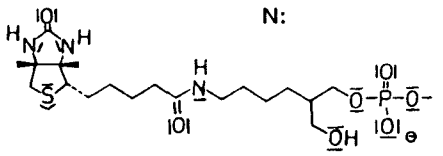

FIG. 2: (Biotinylated) self-complementary 36-mer duplexes 3 and 4

chips SA5 (research-grade, type 0420, 0802 and 0602), precoated with approximately 4ng/mm^2 of streptavidin covalently linked to a carboxymethylated dextran-layer.[12] *2.4. Scanning Force Microscopy (SFM):* SFM-images[4-6] were collected at room temperature in air using commercially available Nanoscope-III-instruments; for triple-layer-imaging (3) on chip gold-surface (FIG. 4), Si-tips (5-10nm radius range) have been used in tapping mode (2Hz). Adlayer(1,2)-imaging (FIG. 5) has been performed with Si_3N_4-tips (4-40nm radius range) in contact force mode (30.5, 10.2, 3.1Hz). For sample preparation, 20µl of each nucleic acid complex were allowed to roll uniformly across the substrate surface of freshly cleaved highly oriented pyrolytic graphite (HOPG). Hereby evaporation took place. The nucleic acid images were obtained at 15-20nN net repulsive, collecting the scans from left to right with 512x512 pixel information density. *2.5. Molecular Modelling:* molecular modelling of the streptavidin-DNA(3)-patterns by SYBYL (Vers. 6.0, TRIPOS Acc. Inc. St. Louis MO 63114) according to the corresponding X-ray-investigations.[13,14] Bond-lengths as well as bond- and torsion-angles optimized by TRIPOS-forcefield.

3. RESULTS AND DISCUSSION: Solid phase physics unravelled statics and, to a certain degree, foreseeable dynamics of life's informational component. Liquid-phase solution experiments elucidated

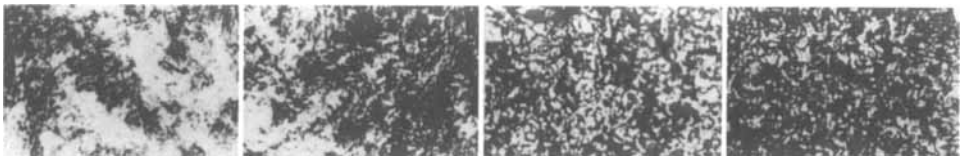

FIG. 3: Mesophase textures (left to right): poly- and oligo(deoxy)nucleotides 1-4

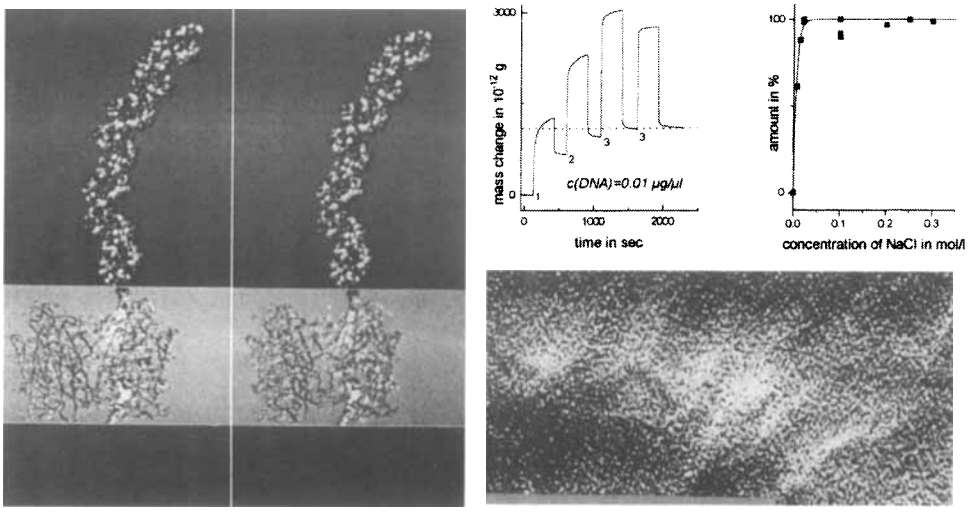

FIG. 4: Dextran-streptavidin-oligodeoxynucleotide(3)-triple-layer elucidations (left to right and top to bottom): triple-layer abstraction with detailed modelling of nucleic-acid and streptavidin components (possible further biotin-binding between streptavidin layer and matrix-support indicated by biotin-spacer, distal biotin-spacer of 3 omitted for clarity); SPR-monitoring of biotin-mediated docking of 3 to the streptavidin matrix; SFM-imaging of nucleic acid-streptavidin-dextran-triple-layer system on gold support (bar-scale: 50nm)

its single-molecule operation modes. Late, selforganizational facilities redirected the extrema-views to the broad landscape of diverse mesophase areas. Volume-phase investigations (FIG. 3) yielded more indirect insights into the three-dimensional statics and dynamics of large (idealized) molecular ensembles[3,15]. Langmuir-Blodgett(LB)-approaches[2,3a,16-18] reach, within two-dimensional lateral mesophase extensions, vertically the basic operation units of biomesogenic logic-blocks.[2,3] In both cases, near-field microscopies seemed promising to direct our insights to nano- and subnano-scales.

The here presented texture observations are indicative of the surprising aptness of broad ranges of nucleic-acid species to obtain even the idealized states of classical liquid-crystalline mesophases, irrespectively whether polydisperse high-molecular DNAs and RNAs, or shorter definite stretches of

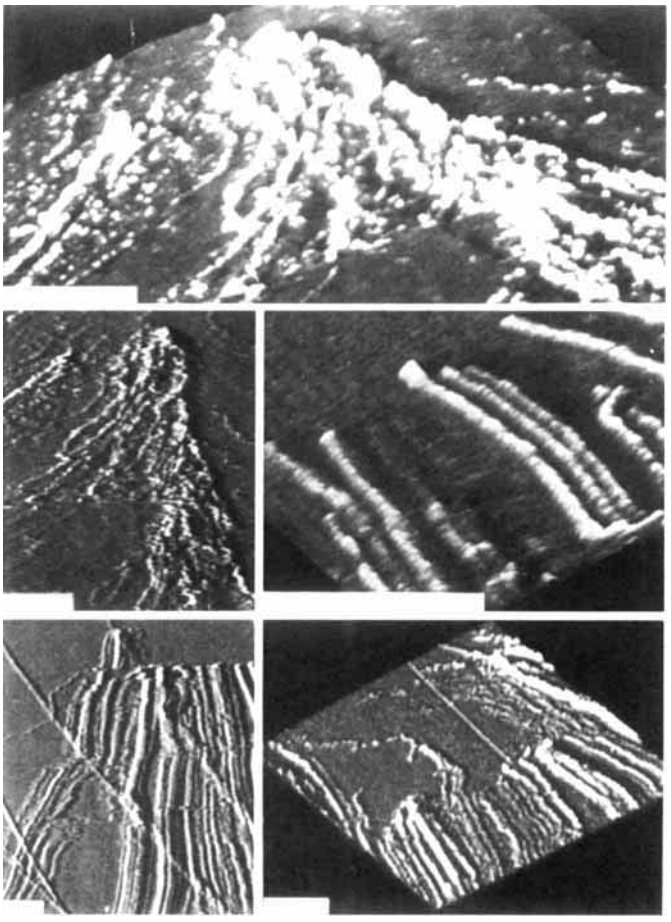

FIG. 5: SFM-visualizations of nucleic acids HOPG-adlayer domains and microdomains (left to right and top to bottom): polydisperse chicken DNA 1 in 3d- and 2d- overall-views (scale: 1μm/dark bars) - as well as in single-molecule-3d-imaging (scale: 0.25μm/bright bar); $(U)_n \cdot (A)_n$ (2) in 2d- and 3d-imaging (scale: 0.25μm/bright bars)

oligodeoxynucleotides - even with the phase-restrictions of larger biotin-spacer arrangements - represent the different objects of interest.[3,15] Contrary to the easiness of adopting mesophases, however, and indicating the requirements of evolutionary diversities in contrast to man-made artificials, the adjustments of definite molecular patterns to unspecific texture expressions remain an open problem.[3,15]

Approaches to apply near-field microscopies to the visualization of these evolutionary species[3a,19] and their models[20], as well as to artificially organized representatives in LB-arrangements[2,3a,17], reflect at first in FIG. 4, notwithstanding promising SPR-monitorings, with the SFM-indications of rather disordered nucleic-acid strand networks the difficulties in building up

complicated multilayer-systems, even with the help of biotin-forced layer adjustments. While here more work, especially in the integrations of different methodologies, will be needed[3,17], our first approaches to SFM-investigations of nucleic-acid domain and microdomain selforganizations in adlayers to solid supports unravelled intriguing pictures of first nano- and subnano-insights into supramolecular biomesogen-arrangements within selforganizational patterns[3a] (FIG. 5). It had been the employments of meanwhile in the elucidations of single-molecule details disdained methodologies, that turned out unexpectedly successful in this case. While graphite, after first euphoria, has been accused to mimic within its surface-steps, clefts and breaks nucleic-acid design, it provided with its lower adhesion forces to nucleic acids in highly concentrated solutions a rather ideal support to tolerate nucleic acid-selforganization within the slowly evaporating adlayer. Even the more obtuse pyramidal tips in the convenient contact force mode proved favourable in comparison to more acute abrasive versions in the sensitive tapping mode. FIG. 5 with its imaging of DNA- and RNA-domains and microdomains of selforganizational adlayer patterns sets a starting point, from which static and even futural dynamic investigations of selforganizational phenomena of nucleo-protein system components may origin and contribute to a general dual structure-phase view of biomesogen order-disorder patterns between chaotic origin and complexity tensions.

4. CONCLUSIONS: Near-field microscopies proved highly efficient in the elucidation of single molecule details. Contrary to recent progress in the imaging of mesophase areas of low-molecular-weight artificials, investigations in the field of biomesogen species[1-3,19] and their models[20] are still in their infancies.[1-3,19,20] SFM-visualizations of nucleic-acid adlayer domains and microdomains, preferently on graphite substrates, offer first insights into the operation modes of their selforganizational patterns. Further progress in statics and dynamics might use native milieus in liquid-cells and will profite from continuing improvements in the rapidly developing equipment.

The imaging of both biomesogen subjects an their relatedness in phase cooperativities and complexities contributes in all the insufficiencies of first approaches to the impressive coherencies of duality inherencies.

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