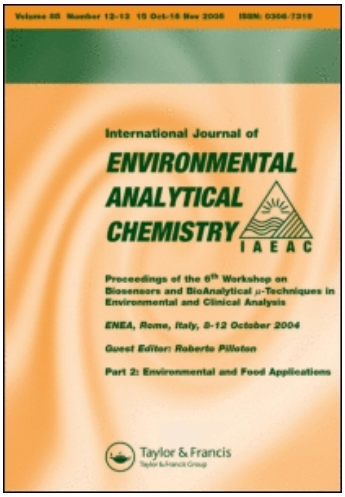

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Self-assembled bioconjugates for biochip technologies

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Self-assembled bioconjugates for biochip technologies

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This article gives an overview on current research in the area of self-assembled bioconjugates comprised of DNA-, protein- and nanoparticle building blocks. In particular, the highly specific molecular recognition capabilities of short DNA oligomers are utilized to organize inorganic nanoparticles and proteins, thereby allowing for the fabrication of functional biomolecular hybrid devices. These devices are employed, for instance, to generate programmable biomaterials and probes for bioanalytical methods, such as immunological assays and microarray technologies.

Keywords: Bioconjugates; DNA; Proteins; Microarrays; Self-assembly

1. Introduction

Current developments in microarray technologies, concerning miniaturized, high throughput analyses in genome- and proteom research, biomedical diagnostics, drug screening and other applications, essentially depend on efficient chemical means for the conjugation of bioactive molecular components [1]. To this end, our research concerns the development and utilization of biologically programmed self-assembly processes. This so-called 'bottom-up' biomimetic assembly of programmed molecular building blocks provides a novel strategy for the generation of nanometer-scaled functional devices and materials [2]. Due to their size and evolutionary optimized recognition capabilities, biomolecules, such as DNA and proteins, are currently investigated as building blocks for the self-assembly of nanostructured architecture [3, 4].

2. Highly sensitive detection of proteins by immuno-PCR

We have developed novel classes of semisynthetic DNA–protein conjugates, self-assembled oligomeric networks consisting of streptavidin (STV) and double-stranded

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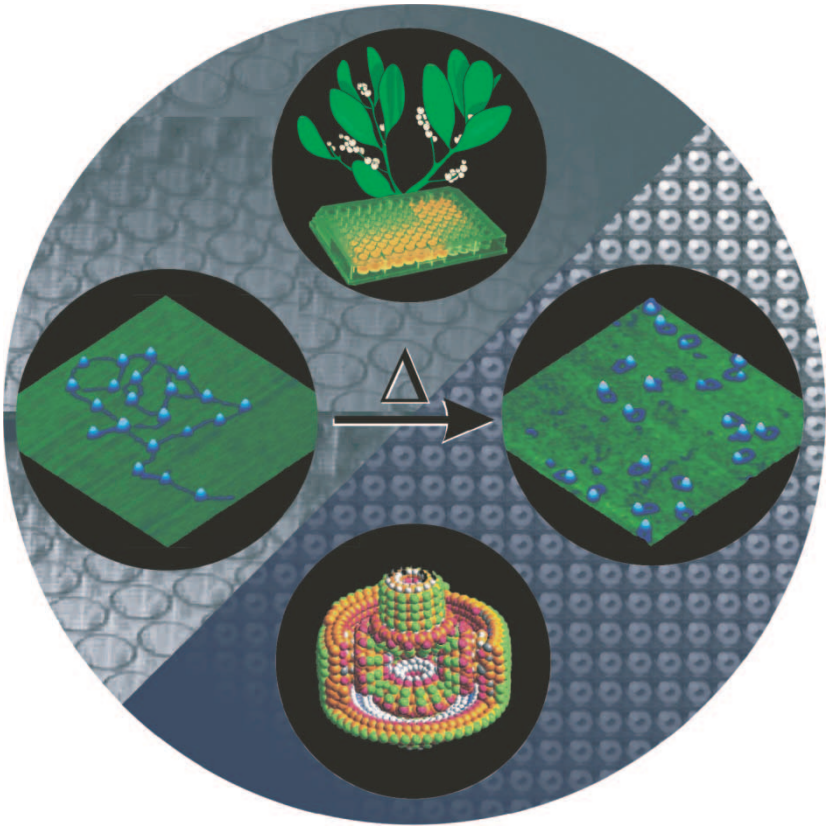

Figure 1. Scanning force microscopy images of nanostructured DNA-protein conjugates as examples of self-assembled biomaterials at the crossroads of life-sciences and biomolecular nanotechnology.

DNA [5] that can be converted to well-defined supramolecular nanocircles [6] (figure 1). The DNA–STV conjugates are applicable as modular building blocks for the generation of novel immunological reagents for the ultra-sensitive trace analysis of proteins and other antigens by means of the immuno-polymerase chain reaction (IPCR) methodology [5, 7, 8]. IPCR combines the specificity of an antibody-based immunoassay with the exponential amplification power of PCR, thereby leading to a 1000-fold enhanced sensitivity, as compared to standard enzyme-linked-immunosorbent assay (ELISA) techniques. With its broad scope of applications ranging from the detection of proteins [9] to small-molecules [10], IPCR is a prime example of how biomolecular nanostructure can add new performance to well-established immunoassay methodology of biomedical diagnostics [11].

Other applications of the self-assembled DNA–STV conjugates concern the rising field of nanobiotechnology. For instance, the conjugates are used as model systems for ion-switchable nanoparticle networks [12], nanometer-scaled 'soft materials' calibration standards for scanning probe microscopy [13, 14], or as programmed building blocks for the rational construction of complex biomolecular architecture [15] which might be used as templates for the growth of nanometer-scaled inorganic devices [2, 16].

3. Biochip technologies: nanostructures and self-assembly enhance the performance of conventional approaches

Our work in the area of microarrays aims at the development of immobilization methods which allow for the functionalization of microstructured surfaces with nucleic acids, proteins, as well as small-molecule analytes. This type of bioconjugation can be achieved using chemically activated substrates, prepared, for instance, by amino silylation of glass or metal oxides, and subsequent transformation with homo- and heterobifunctional crosslinking reagents. We have recently employed polyamidoamino dendrimers, containing a large number of primary amino groups in their outer sphere, as an intermediate layer between the bioactive component and the solid substrate [17, 18]. This approach not only leads to an increase in signal intensity and, thus, sensitivity in analytical assays, but also yields highly homogeneous biochips with an outstanding physico-chemical stability, thus enabling efficient preparation of protein and small-molecule biochips [19]. A recent comparative study of antibody microarrays revealed that our dendrimer-based surfaces offer highest signal intensities and the best limits-of-detection amongst other commercially available products [20].

Other developments of our group concern covalent conjugates of single-stranded DNA oligomers and STV [21], which can be utilized as biomolecular adapters for the immobilization of biotinylated macromolecules at solid substrates via nucleic acid hybridization (figure 2). This 'DNA-directed immobilization' proceeds with high immobilization efficiencies and allows for reversible and site-selective functionalization of solid substrates with proteins [21–23], metal and semiconductor nanoparticles [24], and small-molecule compounds [25]. The refinement of this technique for commercial products, i.e., reagent kits that allow the user to prepare highly-active protein biochips without the need for expensive spotting instrumentation, includes the bioinformatic design of oligomer sequences [26] and the adaptation of this technique to real-life sample materials, such as blood serum [27, 28].

The DNA-directed immobilization (DDI) method has implications on various fields of technology: (a) DDI of proteins and small molecules can be used for the self-organized fabrication of biochips to be employed as a platform in biosensing [29, 30], proteomics [31] and lab-on-a-chip devices [32]; (b) DDI of gold nanoparticles enables powerful strategies for microarray-based high sensitivity nucleic acid analyses [33, 34];

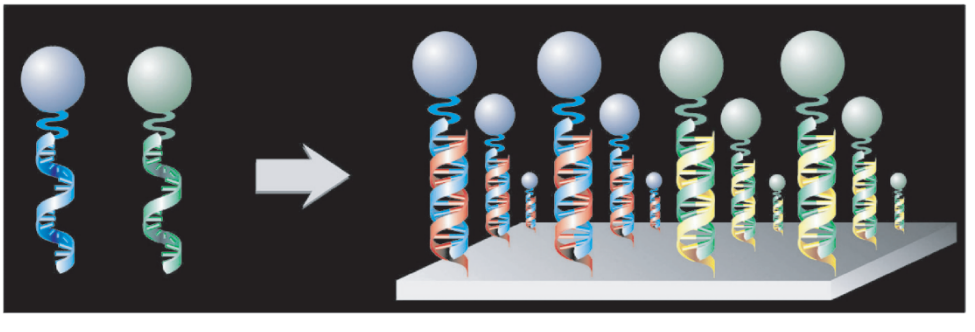

Figure 2. DNA-directed immobilization of macromolecular and colloidal components on solid substrates.

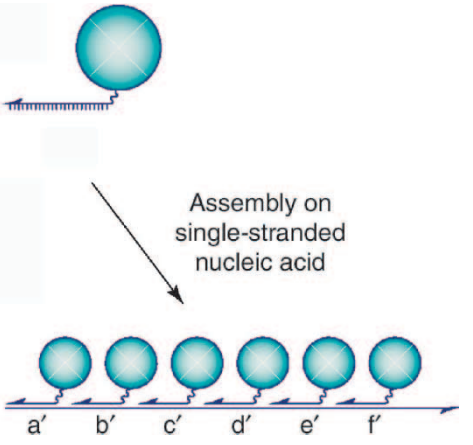

Figure 3. DNA-directed nanoscale assembly of macromolecular and colloidal components.

(c) DDI of metal and semiconductor nanoparticles opens up novel ways for the self-organized formation of complex heterostructures and functional materials [35, 36].

4. Novel biolabels by means of nanoscale assembly

Covalent DNA–STV conjugates are also convenient for constructions at the nanometer-scale. For instance, they have been used for the DNA-directed functionalization of gold nanoparticles with immunoglobulins [37]. The resulting hybrid bioconjugates, which combine the high specificity recognition capabilities of immunoglobulins and the extraordinary stability of DNA-functionalized colloidal gold, are applicable for the biochip-based detection of antigens.

Moreover, due to the high specificity of Watson–Crick base pairing, the covalent DNA-STV conjugates allow for selective positioning of biotin-derivatized molecular components along a single-stranded nucleic acid carrier molecule (figure 3) [21, 38]. Examples include the spatially controlled assembly of enzymes to form artificial multienzyme complexes with enhanced biocatalytic activity [39], the fabrication of optically active nanoscale assembly from fluorescent proteins [40], and functional biometallic nanoarrays from antibodies [41] and gold nanoparticles [42], applicable as tools in bioanalytics and materials science.

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