pH-Dependent Assembly of DNA–Gold Nanoparticles Based on the i-Motif: A Switchable Device with the Potential of a Nanomachine

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The pH-dependent self-assembling of gold nanoparticles is described. Oligonucleotides containing four or six consecutive dC residues are immobilized on 15-nm gold nanoparticles. Their assembly is based on the formation of a DNA i-motif as determined by the color change from red to blue between pH 5.5 and 6.5. The process occurs within a narrow pH range and is reversible. The i-motif is formed by the antiparallel intercalation of two parallel duplexes provided by two different gold nanoparticles. This assembly process can be utilized to generate novel systems for colorimetric sensing, applications in medical imaging and therapy, and for the construction of a proton-driven nanomachine.

Introduction. – Already in 1676, *J. Kunckel* concluded that in aqueous gold solutions, gold must be present in such a degree of communition that is not visible to the human eye [1]. A solution of deep red colloidal gold was prepared from chloroaurate ($AuCl_4^-$) by *M. Faraday* in 1857 [1]. Gold nanoparticles are one of the chemically most stable metal species allowing surface modification. Recent advances have led to the development of functionalized nanoparticles being covalently linked to biological molecules such as nucleic acids, peptides, and proteins [2–4]. One of the most successful approaches is the DNA–gold-nanoparticle system which has been used to construct a variety of highly ordered nanoassemblies [3][5][6]. The DNA–gold-conjugate concept is based on the combination of the favorable properties of the gold nanoparticles and the DNA molecules. DNA represents a powerful molecular recognition system leading to self-assembly. Its stiff structure and easy access by automated DNA synthesis make it ideal for the construction of nanodevices [7]. The DNA–gold system is used in the bottom-up strategy of nanotechnology. This assembly is not limited to single-stranded or duplex DNA, also higher-ordered DNA structures such as triplexes, quadruplexes, and pentaplexes are readily formed depending on particular sequence motifs [8].

Repetitive DNA sequences which are interspersed throughout the human genome are capable of folding into a variety of complex structures. Cytosine-rich regions such as the centromer and telomer domains as well as the insulin minisatellite are assumed to form a unique tetrameric structure which is designated as i-motif [9–13]. The i-motif consists of two sets of paired duplexes containing stretches of cytosine residues to form a quadruplex as shown in *Fig. 1*, *a*. The duplexes are stabilized by hemiprotonated noncanonical cytosine–cytosine base pairs in which a protonated dC^+ is situated oppo-

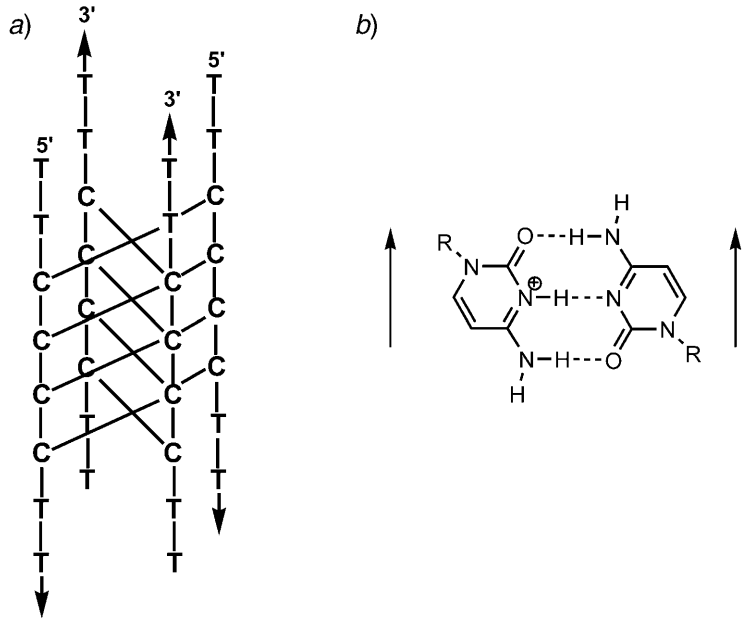

Fig. 1. a) *The i-motif assembly of* **1** *is stabilized by* b) *hemiprotonated* $dC \cdot dC^+$ *base pairs and intercalation*. T = dT and C = dC.

site to an unprotonated dC residue with parallel chain orientation of the phosphodiester backbone (*Fig. 1, b*). Two of these duplexes are associated in an antiparallel way by base-pair intercalation. The cytosine residues have a right-handed twist of 17–18°. The i-motif displays two wide and two narrow grooves with close sugar contacts. Crystal structures of the intercalated i-motif have been reported [14][15]. Consistent with the hemiprotonation of the cytosine residues, the i-motif assembly is formed under weak acidic conditions (pH 5.5) [16–18].

Recently, the synthesis and properties of multiple-stranded DNA–gold conjugates by using the ion-specific aggregation of the dG quartet hairpin 5′-d($G_4T_4G_4$)-3′ were reported by our laboratory [6]. These observations have been used later by others for the same purpose [19]. In continuation of our studies, we became interested in the construction of a DNA–gold nanoparticle system based on the pH-dependent four-strand assembly of the i-motif. Herein, we report for the first time on the pH-dependent self-assembling of DNA–gold conjugates to generate a switchable nanoscopic device. The self-assembling of the gold–i-motif construct has the potential of a proton-driven DNA nanomachine.

Results and Dicussion. – At first, four different oligonucleotides, namely 5′-d(TTC CCC TT)-3′ (**1**) and 5′-d(TTC CCC CCT T)-3′ (**2**) and their 5′-thiol-modified derivatives **3** and **4** (see *Table*) were prepared. For the synthesis of compounds **3** and **4** a 5′-[P^O-(6-mercaptohexyl)]-modified phosphoramidite was used (*Glen Research*, US). The oligonucleotide syntheses were performed in an automated DNA synthesizer by means of solid-phase synthesis. The purification followed the standard protocol [20],

and the oligomers were characterized by MALDI-TOF mass spectra. The calculated masses were in good agreement with the measured values (*Table*).

Table. *Molecular Masses of Oligonucleotides Determined by MALDI-TOF Mass Spectrometry*

	$[M+H]^+$ [Da]	
	calc.	found
5′-d(T-T-C-C-C-C-T-T)-3′ (**1**)	2312	2311
5′-d(T-T-C-C-C-C-C-C-T-T)-3′ (**2**)	2890	2891
5′-d{[Trityl-S-$(CH_2)_6$-O(PO_2H)O]T-T-C-C-C-C-T-T}-3′ (**3**)	2751	2750
5′-d{[Trityl-S-$(CH_2)_6$-O(PO_2H)O]T-T-C-C-C-C-C-C-T-T}-3′ (**4**)	3329	3328

The distinct characteristics of the i-motif can be monitored by circular-dichroism (CD) spectra. In the aggregated state, a positive band around 280 nm and a concomitant negative band around 260 nm are typical for the i-motif structure. They appear under slightly acidic conditions and change in alkaline medium [21][22]. The formation of the i-motif structure of the unmodified oligonucleotides **1** and **2** (data not shown) as well as of the modified derivatives **3** and **4** carrying a bulky protecting group was established by CD measurements (*Fig. 2*) [22]. A large positive lobe at 282 nm and a negative lobe at 256 nm (pH = 5.2) are indicative for the i-motif structure of **4** shown in *Fig. 2*,*a*. The CD spectra change by increasing the temperature (*Fig. 2*,*a*) or by shifting the pH towards alkaline medium due to the disassembly of the i-motif (*Fig. 2*,*b*).

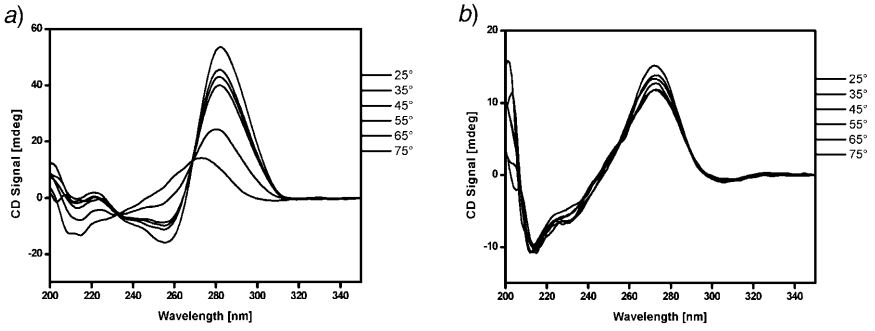

Fig. 2. *CD Spectra of* a) *the i-motif construct 5′-d{[trityl-S-$(CH_2)_6$-O(PO_2H)O]T-T-C-C-C-C-C-C-T-T}-3′* (**4**) *measured in 10 mM sodium phosphate buffer containing 0.3 M NaCl at pH 5.5 and* b) *the single-stranded species at pH 8.0*

To test the viability of our concept, gold-nanoparticles were functionalized with the cytosine-rich oligonucleotides **3** and **4**. The solutions of the 15-nm gold nanoparticles were prepared from a $HAuCl_4$ solution by citrate reduction as it was originally reported by *Turkevitch* and later described by *Letsinger* and *Mirkin* [23][24]. Prior to functionalization, the gold-nanoparticle solution was brought up from pH 5.5 to pH 9.5 avoiding i-motif formation of the nonderivatized oligonucleotides. The UV/VIS spectrum of the alkaline solution of the unmodified nanoparticles shows the characteristic plasmon resonance at 520 nm appearing at the same wavelength as observed for the acidic solution.

In the next step, the functionalization of the gold-nanoparticles with the oligonucleotides **3** or **4** was performed. The gold-nanoparticle samples **5** and **6** were prepared by mixing the alkaline gold-nanoparticle solution (6.4 ml) with the oligonucleotide solutions (3.5 ml) obtained after trityl-group removal and purification [24]. The coupling reaction was performed at slightly elevated temperature. The resulting gold–DNA conjugates **5** and **6** show the expected plasmon resonance at 525 nm under alkaline conditions (*Fig. 3*, *b*) indicating a nonaggregated state. A plurality of oligonucleotides are bound to each individual gold nanoparticle. The DNA–gold-nanoparticle solutions are stable in sodium phosphate buffer containing 0.1 or 0.3M NaCl at pH 8. No detectable aggregation was observed after 1–2 months as evidenced by UV/VIS spectroscopy.

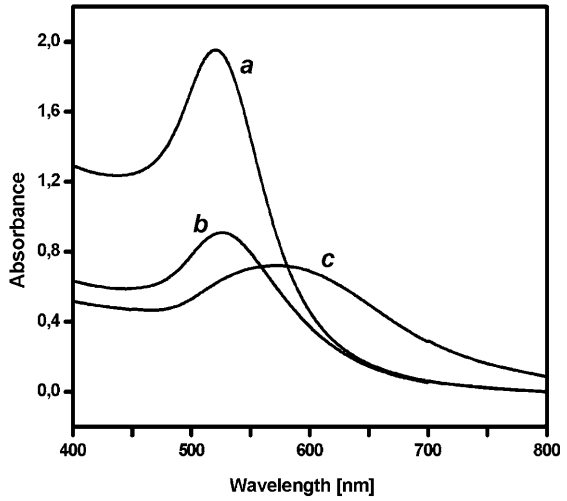

Fig. 3. *UV/VIS Spectra of* a) *the alkaline solution* (pH 9.5) *of 15-nm-diameter gold nanoparticles. Gold nanoparticle conjugate* **6** b) *at pH 8.0 and* c) *at pH 5.0 after i-motif formation measured in 10 mM phosphate buffer containing 0.3M NaCl.*

The response to an external stimulus is a basic requirement of a switchable nanoscaled device. As dC-rich DNA forms an i-motif under acidic condition (pH 5.5), it was expected that the same occurs on the nanoparticle surface with an on-state below pH 5.5 and off-state at higher pH values. If this process takes place between oligonucleotides bound to nanoparticles, it leads to the self-assembly of the DNA–gold conjugate **5** or **6**. Consequently, the pH-dependent assembly of the nanoparticles was examined by acid or base addition to the solution. The reaction was followed spectrophotometrically in 1-cm quartz cuvettes. As shown in *Fig. 4*, *a* the color of the DNA–gold-nanoparticle solution changed from deep red to blue between pH 6.5 and 5.5. The color change occurs within less than a second, is fully reversible, and can be repeated by multiple working cycles as evidenced by the experiment shown in *Fig. 4*, *b*. Multiple cyclic additions of HCl and NaOH to the functionalized gold-nanoparticle solution (700 µl; 10 mM phosphate buffer with 0.1M NaCl) results in changes of the UV absorbance measured at 610 nm. This confirms the nanoparticle assembly induced by

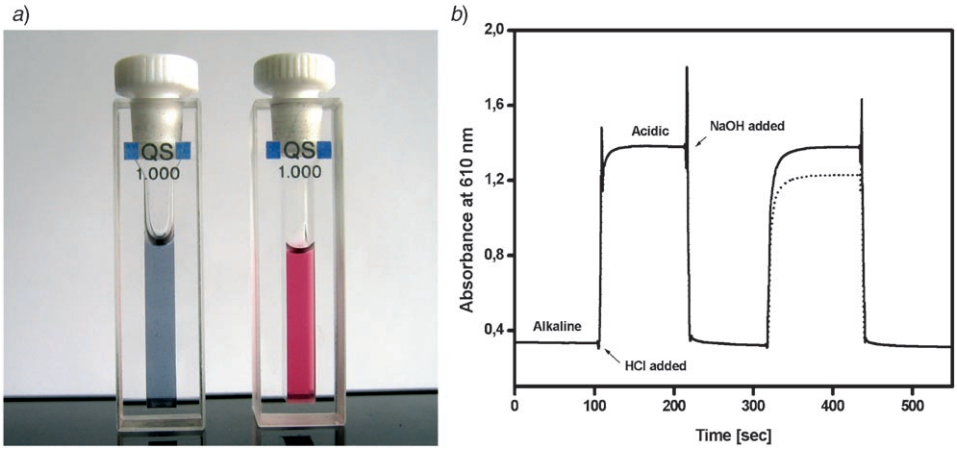

Fig. 4. a) *Color change of the solution of the DNA–gold conjugate* **5** (derived from **3**) *in 10 mM phosphate buffer containing 0.1M NaCl at pH 5.5* (left) *and pH 6.5* (right). b) *Multiple cycling of the color change of the DNA–gold conjugate* **5** *in 10 mM phosphate buffer with 0.1M NaCl.* The cyclic absorption changes were induced by repetitive addition of 1M HCl or 1M NaOH. The absorbance was corrected by a factor resulting from dilution with acid and base (… → —).

the i-motif. Only a prolonged storage at an acidic pH value led to precipitation of the gold nanoparticles forming a black precipitate at the bottom of the cuvette while the solution became clear. However, even the black precipitated turned back into solution by addition of NaOH, thereby forming a deep red, fully disperse and clear solution. Additional evidence for the i-motif assembly is given by the fact that the unmodified gold solution as well as gold-nanoparticle solutions carrying nonhybridized single-stranded 12-mer oligonucleotides stay red under these conditions.

The formation of the i-motif assembly requires oligonucleotide chains containing at least three consecutive cytosine residues. In principle, every single chain participitating in the i-motif assembly might be linked to an individual nanoparticle. Thus, the assembly has to accommodate four bulky gold nanoparticles at the terminus of the quadruplex structure. As the length of the oligonucleotide chains used in this study is shorter than 5 nm and the size of the nanoparticle is *ca.* 15 nm, it is difficult to imagine that such a crowded species is formed during the aggregation. It is much more likely that already two oligonucleotide chains are provided with one individual gold-nanoparticle thereby forming an i-motif containing only two gold species as it is demonstrated in *Fig. 5*. The i-motif formation requires two parallel and two antiparallel oligonucleotide chains, therefore the gold nanoparticles are located at opposite ends of the tetraplex.

Conclusions and Outlook. – Gold nanoparticles functionalized with cytosine-rich oligonucleotides are able to form the i-motif structures leading to a pH-sensitive nanoscopic device. The i-motif structure acts as a pH-dependent switch causing a reversible assembly of the gold nanoparticles at acidic pH and a disassembling into a disperse nanoparticle solution under alkaline conditions. According to the steric requirements, it is likely that one individual nanoparticle provides two dC-rich oligonucleotide chains

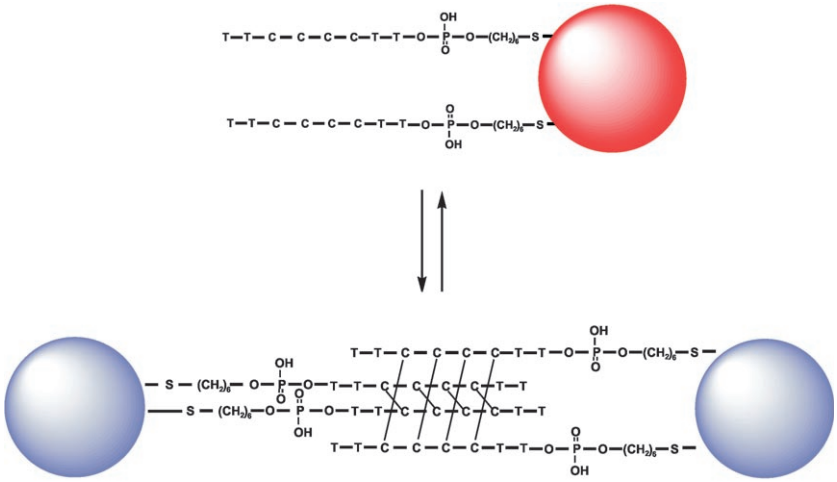

Fig. 5. *Schematic representation of the assembly of one unit of compound* **5** (derived from **3**). The number of oligonucleotides attached to an individual gold nanoparticle is arbitrary. The structure is held together by hemiprotonated dC-pairs. T = dT and C = dC.

and the tetrameric i-motif assembly is formed by two of those units. The described system comprises the option to be used as a colorimetric sensor. The acid-induced gold assembly makes the system also applicable for tumor-cell diagnostics and therapy, as these cells often grow in an acidic environment [25]. In a recent approach, the i-motif structure has been used to design a molecular nanomachine that is driven by pH changes using a quenched and a nonquenched state of a dye induced by the addition of a single-stranded dG-rich oligonucleotide [26]. Our system already represents a proton fuelled-nanomachine which requires only an i-motif–gold conjugate, acid, and base but no other additional molecule. Further studies on the properties of i-motif-derivatized gold nanoparticles containing modified cytosine residues and their assembling are under current investigations.

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Experimental Part

General. $HAuCl_4 \cdot 3\ H_2O$ and trisodium citrate were purchased from *Aldrich* (*Sigma-Aldrich Chemie GmbH*, Deisenhofen, Germany). The 5′-mercapto modifier 6-(triphenylmethyl)-S-$(CH_2)_6$OH *O*-2-cyanoethyl diisopropylphosphoramidite was obtained from *Glen Research* (Virginia, USA). UV/VIS Spectra: *U-3200* spectrophotometer (*Hitachi*, Tokyo, Japan); λ_{max} (ε) in nm. CD Spectra: *Jasco-600* (*Jasco*, Japan) spectropolarimeter with thermostatically (*Lauda-RCS-6* bath) controlled 1-cm quartz cuvette; accumulations of three scans.

Synthesis, Purification, and Characterization of the Oligonucleotides **1**–**4**. The oligonucleotides were synthesized in an automated DNA synthesizer (*ABI 392*, *Applied Biosystems*, Weiterstadt, Germany) at a 1-μmol scale according to the standard protocol for 3′-(2-cyanoethyl phosphoramidites) employing the 5′-*O*-[(tritylthio)hexyl] modifier for the oligonucleotides **3** and **4** [27]. Deprotection and purification of the unmodified oligonucleotides **1** and **2** was performed as described earlier [20]. The modified oligonucleo-

tides **3** and **4** were deprotected with 25% aq. NH_3 soln. (60°, 16 h). The trityl-protected oligonucleotides were purified by reversed-phase HPLC in the trityl-on modus. The molecular masses of oligonucleotides **1**–**4** were determined by MALDI-TOF with a *Biflex-III* instrument (*Bruker Saxonia*, Leipzig, Germany) and 3-hydroxypicolinic acid (3-HPA) as a matrix (*Table*).

The trityl protecting groups of **3** and **4** were removed immediately before modification with the gold nanoparticles. The trityl protecting group was cleaved by treatment of the dry oligonucleotide samples with 150 µl of a 50 mM $AgNO_3$ soln. A milky suspension was formed which was allowed to stand for 20 min at r.t. Then, 200 µl of a 10 µg/ml soln. of dithiothreitol (5 min) were added. A yellow precipitate was formed which was removed by centrifugation (30 min, 14000 rpm) [24]. Aliquots of the samples were purified on a *NAP-10* column (*Sephadex G-25* medium, DNA grade; *Amersham Bioscience AB*, S-Uppsala; equilibrated with 15 ml of nanopure H_2O). The effluents from 0 to 2.5 ml were collected, and the volume was adjusted to 3.5 ml with nanopure H_2O.

Oligonucleotide-Modified Gold Nanoparticles: {[5′-d{[6-(Mercapto-κS)-(CH$_2$)$_6$-O(PO$_2$H)O]T-T-C-C-C-C-T-T}-3′}(1−)}gold (**5**) *and {[5′-d{[6-(Mercapto-κS)-(CH$_2$)$_6$-O(PO$_2$H)O]T-T-C-C-C-C-C-C-T-T}-3′}(1−)}gold* (**6**). The soln. of the 15-nm gold nanoparticles was prepared as reported by *Turkevitch* and later described by *Letsinger* and *Mirkin* [23][24]. Prior to modification, the gold-nanoparticle soln. was brought to pH 9.5. The oligonucleotide-modified gold nanoparticles were synthesized by treating the alkaline gold-nanoparticle soln. (6.4 ml) with 5′-(mercaptohexyl)-modified oligonucleotide soln. (3.5 ml). The mixture was allowed to stand for 20 h at 40° followed by the addition of 4.8 ml of 0.1M NaCl in 10 mM phosphate buffer soln. (pH 7). The mixture was kept for further 2 days at 40°. The sample was centrifuged using screw-cap micro tubes for 30 min at 14000 rpm. The clear supernatant was removed, and the red oily precipitate was washed twice with 8.4 ml of 0.1M NaCl in 10 mM phosphate buffer soln. (pH 7) and redispersed in 9.6 ml of 0.1M NaCl or 0.3M NaCl in 10 mM phosphate buffer soln. (pH 8.5).

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