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Hydrogen-Bond-Guided Self-Assembly of Nucleotides on a Receptor-Array Surface

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Abstract: The hydrogen-bond-guided self-assembly of 5′-ribonucleotides bearing adenine(A), cytosine (C), uracil (U), or guanine (G) bases from aqueous solution on a lipid-like surface decorated with synthetic bis(Zn^{II}–cyclen) (cyclen = 1,4,7,10-tetraazacyclodododecane) metal–complex receptor sites is described. The process was studied by using surface plasmon resonance spectroscopy. The data show that the mechanism of nucleotide binding to the 2D template is influenced by the chemistry of the bases and the pH value of the solution. In a neutral solution of pH 7.5, the process is cooperative and selective with respect to Watson–Crick pairs (A–U and C–G), which form stable double planes in accordance with the Chargaff rule. In a more acidic solution at pH 6.0, the interactions between complementary partners become non-cooperative and the surface also stabilizes mismatched and wobble pairs due to the pH-induced changes in the receptor coordination state. The results suggest that hydrogen bonding plays a key role in the self-assembly of complementary nucleotides at the lipid-like interface, and the cooperative character of the process stems from the ideal matching of the orientation and chemistry of all the interacting components with respect to each other in neutral solution.

Keywords: hydrogen bonds · interfaces · macrocycles · nucleotides · self-assembly

Introduction

The role of hydrogen bonds and base pairing in the stabilization of natural and synthetic supramolecular structures is a subject that remains of considerable interest.[1,2] For biologically relevant processes, studies have shown that the role of hydrogen bonding in the formation and stabilization of defined aggregates has been largely overestimated.[3] Kool and co-workers reported that hydrogen bonds are not necessary for selective pairing between natural DNA bases and nonpolar base shape mimics and for efficient and selective replication by polymerases.[4] For synthetic supramolecular systems, it is generally accepted that noncovalent synthesis through multiple hydrogen bonding and base pairing in aqueous media is severely limited because of the competitive action of water molecules. The majority of hydrogen-bonded synthetic assemblies are produced in apolar solvents,[5] and only a few examples of hydrogen-bonded complexes that are stable in water have been reported.[6] Most of these assemblies were synthesized in a hydrophobic core of water-soluble polymers or micelles.[7]

Hydrogen-bonding and electrostatic interactions are, however, drastically enforced at the lipid/water interface because of a dramatic decrease in the polarity and the dielectric constant in the critical near-surface zone relative to parameters in bulk water.[8,9] This effect results in the experimentally observed enhancement of binding affinities (up to ca. 7 orders of magnitude) of nucleobases and similar structures, which are capable of selective hydrogen bonding, in molecular recognition or self-assembly at floating monolayers, membrane surfaces, and so forth.[9] The difference in the energy of the hydrogen bonds in bulk water and at the air/water interface has also been corroborated by computational modeling and calculations.[10]

Overall, hydrogen bonding in the vicinity of water/lipid interfaces may energetically compete with other noncova-

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lent forces, such as base stacking,[11] although hydrogen bonds are still considered as important contributors to the specificity of the resulting structure rather than as stabilizing interactions.

The contribution of hydrogen bonding to the stability and specificity of structures formed on lipid-like surfaces contacted by water is essential for the rational design of sensing surfaces when using selective base pairing for molecular recognition. Cooperative interactions lead to self-assembled monolayers with attached oligo- and polynucleotides, and such sensing systems are already used in genomic research, drug discovery, and so forth.[12] However, for nucleobases and "small" nucleotides, such as adenosine triphosphate (ATP), nicotinamide adenine dinucleotide (NADH), flavin adenine dinucleotide (FAD), and others, which are of key importance for many metabolic pathways, the desired selectivity of recognition has been achieved only in structurally labile Langmuir monolayers of laterally ordered complementary amphiphiles.[8] The applicability of floating films as sensing elements is, nevertheless, practically limited. At the same time, self-assembled monolayer (SAM)-coated solids that present similar planar assemblies of laterally aligned nucleobases demonstrated poor selectivity and low efficiency of the binding that results from steric hindrance and interactions of the template functionalities within tightly packed SAMs.[13]

The use of synthetic receptor templates that can binding nucleotide terminal phosphate groups can help to overcome these limitations through a guided bottom-up self-assembly of nucleotide bilayers by the stepwise coordination of nucleotides to the receptor surface and base pairing between complementary partners.[14a]

We recently reported an experimental observation of the self-assembly of adenosine and urdine 5′-mono-, di-, and triphosphates (hereafter, AXP and UXP; X = mono (M), di (D), or tri (T)) into double planes (i.e., planar bilayers) on a novel supramolecular template in aqueous solution at pH 7.5.[14a,b] The template consists of amphiphilic bis(Zn^{II}–cyclen) (cyclen = 1,4,7,10-tetraazacyclodododecane) complexes (Zn^{II}–BC) immobilized on a thiolated gold surface.[14a] The affinity of the zinc complexes to the phosphate anions and their spatial orientation at the surface leads to a guided cooperative self-assembly of ATP–UTP nucleotide layers. The data suggested that the attachment of either nucleotide to the Zn^{II}–BC receptor enhanced further binding of the complementary partner. These stepwise cooperative interactions resulted in the Chargaff ratio of nucleotides adsorbed on the surface, that is, the amount of the bound uracil (U) nucleotide was equal to that of the adenine (A) nucleotide and vice versa.

These observations led to several important questions: What is the chemical origin of the cooperative character of self-assembly? What a role does hydrogen bonding play in the stabilization of the nucleotide double planes on a surface? What is the selectivity of self-assembly with respect to other combination of nucleotides, including mismatching ones?

Herein, we report our investigations on the molecular recognition of complementary and mismatched nucleotides presenting A, guanine (G), U, and cytosine (C) bases on Zn^{II}–BC templates. The reported results suggest that the mechanism of self-assembly depends on the coordination state of the receptor and varies with the pH value of the media. The cooperative process, which was observed in neutral solution, showed selectivity with respect to Watson–Crick (A–U and G–C) pairs. This selectivity implies that Watson–Crick interactions provide better stability of nonpolymerized nucleotide pairs when compared to noncomplementary combinations at the lipid/water interface. The observed effects may contribute to a better understanding of the role of hydrogen bonding and base pairing in the similar noncovalent self-assembly of natural and synthetic structures.

Results and Discussion

First, the binding of another Watson–Crick pair (C–G) on a receptor array was studied. The stepwise adsorption of 5′-CTP/5′-GTP on the Zn^{II}–BC template was monitored by using surface plasmon resonance (SPR) spectroscopy at pH 7.5 (Figure 1) and showed a close resemblance to that

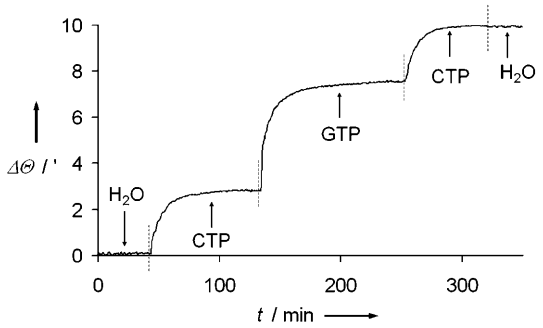


Figure 1. SPR sensograms for the stepwise adsorption of a 5′-CTP/5′-GTP binary combination on a SAM-Zn^{II}–BC surface. The experiment started by probing with CTP (0.02 mmol) followed by subsequent addition of GTP (0.02 mmol) and CTP (0.02 mmol) at pH 7.5.

previously reported for complementary AXP/UXP pairs.[14b] The nucleotide CTP (0.02 mmol) was first introduced into the SPR cell. The CTP terminal phosphate group reversibly coordinates to one metal complex of the macrocyclic host Zn^{II}–BC . It was previously assumed that the adsorbed nucleotide sterically shields the opposite Zn^{II}–cyclen moiety from the interaction with another CTP molecule. This initial binding of CTP translates into the SPR maximal signal $\Delta\Theta_1$. The second step of the kinetic curve corresponds to the adsorption of GTP (0.02 mmol), which was added to the SPR cell after the completion of the initial adsorption of CTP. This secondary adsorption of a complementary nucleotide was faster than the initial one. The SPR maximal signal $\Delta\Theta_2$ was twice as large as the value of $\Delta\Theta_1$. This result implies the recognition of two GTP molecules per one already-ad-

sorbed CTP nucleotide. In accordance with the principal mechanism, which we suggested previously,[14b] the observed cooperative effect arises as a consequence of multipoint intermolecular interactions between complementary nucleotides and the macrocyclic host (Figure 2a). The already-

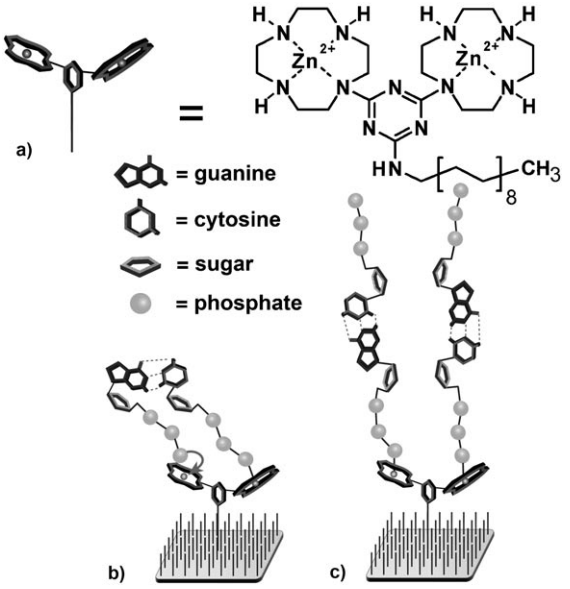


Figure 2. a) Chemical structure of amphiphilic bis-(Zn^{II}–cyclen) (Zn^{II}–BC). b,c) Illustration of the possible mechanism of cooperative self-assembly of complementary nucleotides on a single Zn^{II}–BC molecule immobilized on a thiolated gold center. b) Intermediate cooperative structure that involves the already bound CTP and the incoming GTP. The complementary base pairing orientates the GTP terminal phosphate group to the free "head" of Zn^{II}–BC. c) The proposed structure of the resulting "double plane", which harbors equal amounts of C and G bases within the nucleotide bilayer.

bound CTP recognizes the incoming GTP through Watson–Crick base pairing. Base coupling gives a spatial arrangement of interacting species that leads to the coordination of the GTP terminal phosphate group to the unoccupied Zn^{II}–cyclen unit of Zn^{II}–BC (Figure 2b). These intermediate interactions are stabilized through base stacking. Hydrogen bonds between the adsorbed CTP and GTP molecules dissociate, and the disengaged bases become available for the interaction with their complementary partners from solution. The immobilized CTP binds a second GTP molecule, thereby forming the incomplete top layer of nucleotides. The formation of a double plane (schematically depicted in Figure 2c) is completed through the third-stage adsorption of 0.02 mmol CTP, which translates into an SPR maximal signal $\Delta\Theta_3$. This value was very close to the $\Delta\Theta_1$ value, thus indicating that the resulting bilayer comprises equal amounts of C and G nucleotides (Figure 2c). A similar kinetic pattern was observed for the reversed order of nucleotide adsorption (i.e., initial addition of GTP followed by CTP and finally adsorption of GTP; the data are available in the Supporting Information).

The cooperative character of the C–G double-layer formation manifests itself in complex kinetics of the process that corroborates the proposed mechanism. Figure 3a–c rep-

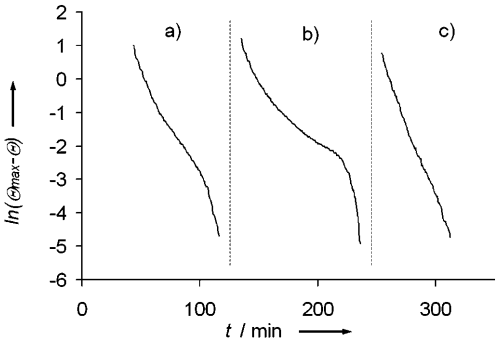


Figure 3. Plots of $\ln(\Theta_{max}-\Theta)$, where Θ_{max} is the maximal SPR signal, as a function of time for the stepwise adsorption of CTP (a) followed by subsequent addition of GTP (b) and CTP (c) (for the corresponding portions in SPR sensogram, see Figure 1). The nonlinearity of plots (a) and (b) indicates the cooperative character of nucleotide binding to the template surface.

resents the dependencies of $\ln(\Theta_{max}-\Theta)$ versus time for each step of the nucleotide adsorption on the template. As indicated by the graphs, the first two steps of nucleotide binding to a surface do not fit to a pseudo-first-order process, which can be expected for a simple Langmuir adsorption model [Eq. (1)].

$$(\Theta_{max}-\Theta)/(\Theta_{max}-\Theta_0) = e^{-kt} \quad (1)$$

Instead, for the initial CTP binding (Figure 3a), the linear first-order portion of the curve is very short and is followed by the nonlinear and slightly sigmoidal portion. The curve in Figure 3b, which corresponds to the secondary adsorption of GTP, is pronouncedly sigmoidal and has no linear portions at all. Such kinetic behavior is consistent with cooperative substrate binding. Only third-step recognition of CTP can be approximated as a linear function that fits pseudo-first-order kinetics of adsorption (Figure 3c).

It is noteworthy, however, that the assisted character of the initial binding was observed only for 5′-CXP nucleotides. For the first-step adsorption of U, G, and A nucleotides, the kinetics are different to that of 5′-CXP nucleotides, as exemplified by the curve obtained for the initial UTP binding (Figure 4). This binding yields a comparatively long straight line of a pseudo-first order followed by a hyperbolic transition to the zero-order portion that is typical for heterogeneous reactions. The binding of U, G, and A nucleotides can be therefore described as adsorption followed by the formation of a coordination bond between Zn^{2+}–cyclen and the nucleotide phosphate group. For these nucleotides, the value of the pseudo-first-order rate constants k_1 determined from linear portions of the kinetic curves are $90–102\times10^{-4}\ s^{-1}$, whereas the value of k_1 estimated for the initial adsorption of CTP is significantly greater (ca. $188\times10^{-4}\ s^{-1}$). The origin

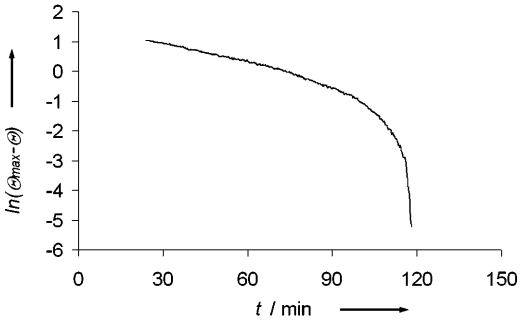


Figure 4. Plot of $\ln(\Theta_{max}-\Theta)$ as a function of time for the first-step adsorption of UTP on a SAM/Zn^{II}–BC surface at pH 7.5. The corresponding SPR sensogram is available in the Supporting Information.

of this difference in the first-step kinetics of CXP and other nucleotides is currently unclear, though it may be related in part to the chemistry of cytidine, which is discussed below.

With these findings, the cooperative character of the nucleotide interactions and the stability of the resulting "double planes" on the Zn^{II}–BC surfaces at pH 7.5 are proven for complementary pairs presenting the A–U and G–C bases, respectively. The six-phase Fresnel simulations to the experimental SPR curves give an average geometrical thickness of double layers of 3.4 nm ($n=1.36$; see the Experimental Section), which is consistent with the molecular geometry of the nucleotides used. Data from atomic force microscopy (AFM; see the Supporting Information) give a smaller value of approximately 2 nm, which is in better agreement with a model of a noncondensed thin film with an expectedly tilted arrangement of ligands.

However, the kinetics of the adsorption of naturally mismatched or wobble pairs (i.e., C–A, U–G, G–A, and U–C, respectively) was different from that observed for complementary partners under similar conditions. Figure 5a illustrates the adsorption of 0.02 mmol ATP on the Zn^{II}–BC surface with already immobilized CTP at pH 7.5. The CTP-covered surface responded to the purine nucleotide; however, the second-step maximal increase in the SPR signal $\Delta\Theta_2$ was much lower than the value of $\Delta\Theta_1$ measured during a course of the initial recognition of CTP. Moreover, the adsorbed ATP was easily removed from the surface by rinsing the SPR cell with water. The SPR signal decreased during the desorption of ATP and finally reached the value of $\Delta\Theta_1$. The interactions of naturally mismatched nucleotides are therefore weak, non-cooperative and nonspecific at the solution/template interface. The adsorption of ATP on the template-bound CTP layer results in the formation of an unstable system, whereas the layers assembled from complementary partners are stable in water. For the opposite order of nucleotide addition, we observed poor secondary adsorption of CTP on a layer of immobilized ATP followed by the release of CTP from the surface upon a flow of water (Figure 5b).

Similar behavior was found for all the studied mismatched pairs irrespective of the order of nucleotide addition (data are provided in the Supporting Information). The template-

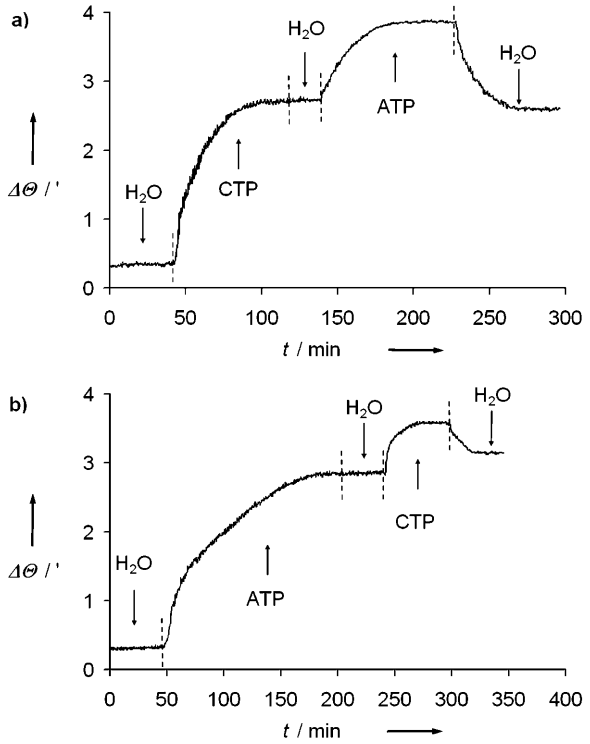
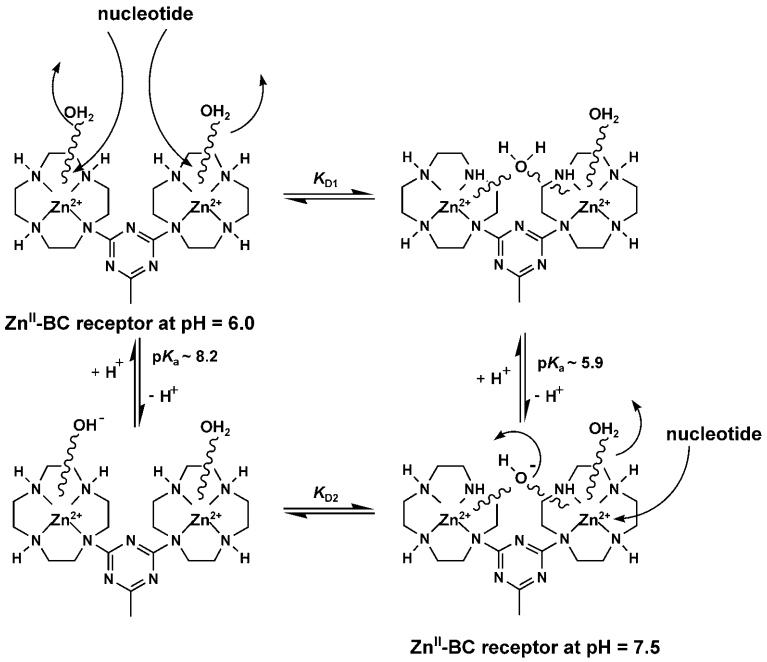


Figure 5. SPR sensograms for the stepwise adsorption of a 5′-CTP/5′-ATP mismatch combination on a SAM/Zn^{II}–BC surface. a) The experiment started by probing with CTP (0.02 mmol) followed by subsequent addition of ATP (0.02 mmol). b) The "vice versa" subsequent binding of ATP (0.02 mmol) and CTP (0.02 mmol) at pH 7.5.

bound nucleotides remained active for ordered self-assembly with complementary nucleotides after the desorption of mismatched partners: The Zn^{II}–BC surface with bound UTP showed cooperative binding of ATP after removal of 0.02 mmol CTP, which was added beforehand.

These observations suggest that the self-assembly mechanism of nucleotides on Zn^{II}–BC surface templates at pH 7.5 is highly susceptible to the mismatching between nucleobases and relies on their natural ability to form complementary hydrogen-bonded pairs. Such dependence of self-assembly on the nucleobase matching supports our previous hypotheses that hydrogen bonding plays the key role in the formation of nucleotide "double planes" on a Zn^{II}–BC template at a given pH value. These comparatively weak interactions are responsible for the base selectivity of the process. A key step of the self-assembly is the hydrogen-bond guided placement of a complementary nucleotide at the second receptor binding site next to the initially bound nucleotide. Potentiometric titrations have shown the presence of an acidic water molecule ($pK_a \sim 5.9$) that bridges the two metal centers in bis-cyclen in solution.[14c,d] The dinuclear surface receptor is occupied at pH 7.5 by one hydroxide and one water ligand (Scheme 1). The initial nucleotide coordination displaces the water molecule, but the second binding site remains occupied by the hydroxide ion. Its replacement requires the hydrogen-bond-guided intramolecular attack of the pyrophosphate unit of a complementary nucleotide, as discussed



Scheme 1. Possible mechanism of nucleotide binding by Zn^{II}–BC at pH 6.0 and 7.5; for details, see reference [14c].

above. There are two major forces that might stabilize the intermediate state required for such attack: hydrogen bonding and base stacking. Base stacking, though somewhat sterically dependent, is much less selective than strongly directional hydrogen bonding. At the same time, base stacking is considered to be a main stabilizing force in DNA and related structures in polar media. Moreover, recent studies showed that base stacking of nonpolar nucleobases (i.e., those unable to form hydrogen bonds) at the end of a DNA helix was more stabilizing than the pairing of natural bases.[3a,b] In our case, however, even the pairing between strongly stacking purine nucleotides cannot stabilize the forming nucleotide layer, which is readily destroyed with water. We hypothesize that the comparatively small differences in the energy of interactions between the complementary and mismatched or wobble bases in solution might increase in the sterically confined environment at the interface and that this increase favors the more significant hydrogen-bonding stabilization of the evolutionary optimized base pairs.

The key role of hydrogen bonding in the stabilization of the base-pair intermediates was directly proven by an A–U combination, in which the A nucleotide lacks the hydrogen-bonding ability due to chlorine substitution of the amine group of the purine base. Figure 6a shows the two-step adsorption of 5′-UDP and 5′-AMP;[15] consequently, the secondary binding of the A nucleotides was cooperative and very similar to that previously described for this complementary combination. However, the second-stage binding of chlorine-substituted 5′-(Cl)AMP to the UDP-covered template showed a close similarity to that observed for mispairs (Figure 6b). The adsorption was weak and non-cooperative and moreover the adsorbed 5′-(Cl)AMP was easily removed from the surface by rinsing with water. The ability to form complementary hydrogen bonds is therefore essential for the stability of the forming bilayer rather than base-stacking energetics and structural similarity.

The specificity of the self-assembly of complementary nucleotides into double planes stems therefore from the ideal matching of the receptor structure, its coordination state at a given pH value, the orientation and chemistry of all the interacting components with respect to each other, and the strength of the hydrogen-bonding interaction for a given nucleotide combination.

These inferences are supported by several observations that we made related to the adsorption of the nucleotide on the bis-cyclic template at pH 6.0. Both binding sites of the zinc complex at pH 6 are presumably occupied by water molecules (Scheme 1); therefore, both sites are identical with respect to the coordination of the nucleotides to a single Zn^{II}–BC receptor unit. The interaction of Zn^{II}–BC with nucleotides in more acidic solutions

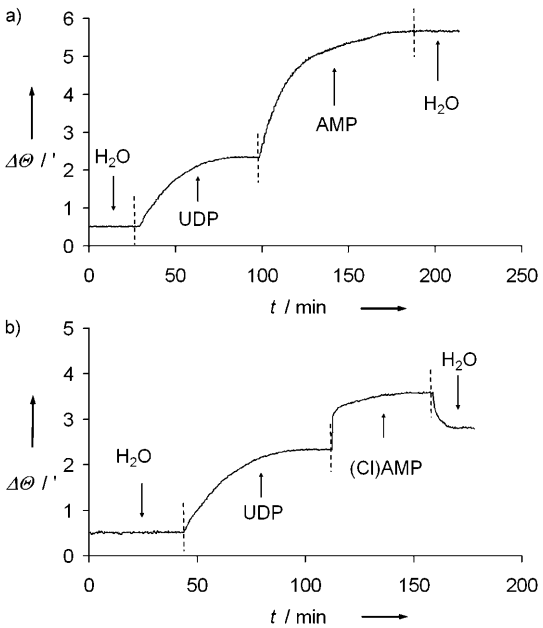


Figure 6. a) SPR sensogram for the two-step adsorption of 5′-UDP/5′-AMP adsorption on a SAM/Zn^{II}–BC surface at pH 7.5. b) The SPR curve for the adsorption of 5′-UDP followed by the addition of 5′-(Cl)AMP, which lacks the ability to form hydrogen bonds (pH 7.5).

actually became different from that observed at physiological pH (except for CTP; see below). The initial adsorption of either GTP, ATP, or UTP (0.02 mmol) on the template resulted in a maximal increase in the SPR signals $\Delta\Theta_1$, which had values that were almost double the values measured for these nucleotides at pH 7.5 (Figure 7a–c). These observations suggest that the Zn^{II}–BC molecule binds two identical nucleotides, either G, A, or U bases at pH 6.0, whereas under more basic conditions only one nucleotide molecule was allowed to coordinate to a single bis-cyclic receptor. Therefore, the effect of the pH value on the initial assembly process originates from the Zn^{II}–BC receptor sites.

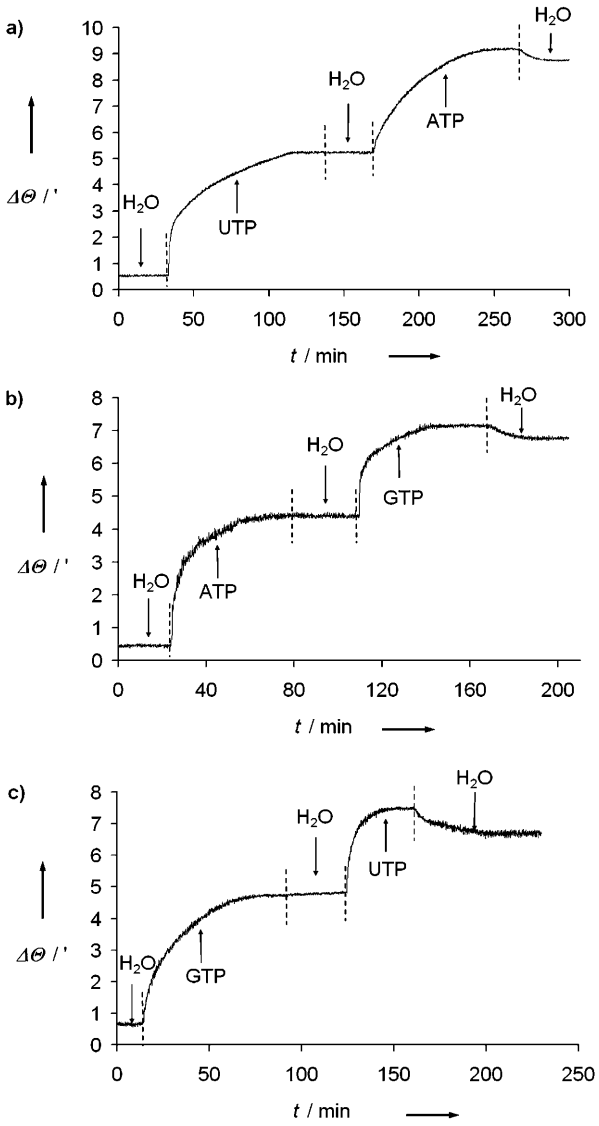


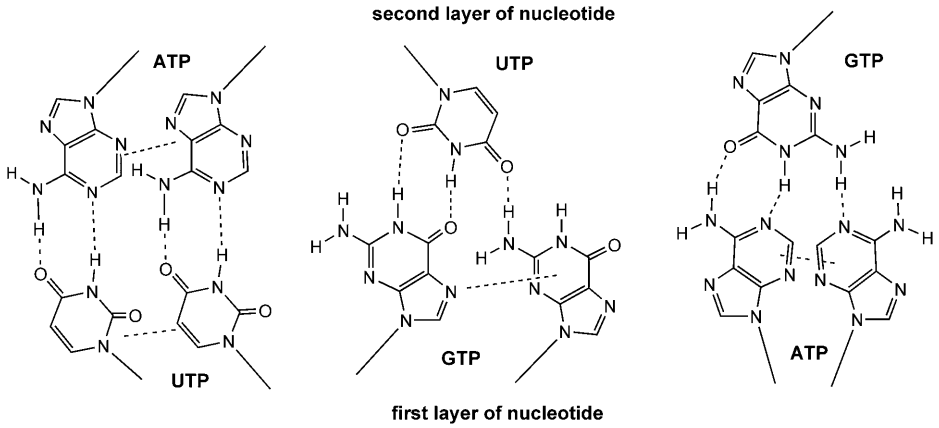
Figure 7. SPR sensograms for the stepwise adsorption of various combinations of 5′-ATP, 5′-UTP, and 5′-GTP on a Zn^{II}–BC surface equilibrated at pH 6.0. a) The adsorption of UTP (0.02 mmol) followed by the subsequent addition of complementary ATP (0.02 mmol). Stepwise probing of Zn^{II}–BC template with mismatched combinations: b) ATP (0.02 mmol) adsorption followed by the addition of GTP (0.02 mmol) and c) subsequent binding of similar GTP and UTP probes (0.02 mmol) at pH 6.0.

The secondary recognition of nucleotides by the already adsorbed monolayers became nonselective and non-cooperative in the acidic solution. The ATP-, GTP-, and UTP-covered Zn^{II}–BC surfaces at pH 6.0 could respond both to incoming complementary partners and the molecules presenting mismatching nucleobases (A or U for template-bound GTP; C or G for ATP; and C or G for UTP, respectively). The kinetics of complementary binding (Figure 7a) was very similar to that of mismatched pairing (i.e., pairing of the incoming nucleotide with those presented by the homogeneous bottom layers) described in Figure 7b.

The secondary adsorption of mismatches translated into a maximal increase of the SPR signal $\Delta\Theta_2$, which was about one half of the value of $\Delta\Theta_1$ measured during the course of the initial adsorption. That is, one molecule from the solution is recognized by two identical nucleotides already bound to Zn^{II}–BC. For the ATP–UTP pair, the values of $\Delta\Theta_1$ measured for the initial adsorption of either ATP or UTP were very close to $\Delta\Theta_2$ obtained for the second-stage recognition of UTP and ATP, respectively, thus suggesting a 1:1 interaction between the complementary partners.

All the complementary systems studied herein were comparatively stable in water within no less than three hours. The stability of noncomplementary bilayers varied depending on the chemistry of the nucleobases involved in the mispairing. The mismatched top layers adsorbed on the ATP- or UTP-covered Zn^{II}–BC surface were stable in water, whereas the substantial desorption of the mismatched nucleotides from the layer of template-bound GTP was observed upon a flow of water (Figure 7c). The composition of the top layer, which formed through secondary adsorption, might be explained through the donor–acceptor patterns of nucleotide interactions: the nucleotides coordinated as the initial layer on the Zn^{II}–BC binding sites present their hydrogen-bonding donor and acceptor sites toward the substrate. This template for potential hydrogen bonding guides the next layer of interaction with the added nucleotide. Although double UTP still allows for the interaction with two ATP molecules, ATP and GTP provide complementary binding sites for only one molecule of GTP or UTP, respectively (Scheme 2).

However, a decrease in the pH value of the measured solution did not influence the affinity of the Zn^{II}–BC surface for the cytidine phosphate groups. The magnitude of the template response $\Delta\Theta_1$ to 0.02 mmol CTP registered at pH 6.0 remained equal to that obtained at pH 7.5. Moreover, the secondary adsorption of GTP nucleotides on the CTP-covered Zn^{II}–BC surface equilibrated at pH 6.0 exhibited a cooperative character similar to that observed in neutral solution (Figure 8a). Most interestingly, the templates with an already immobilized CTP did not stabilize the pairs with mismatching nucleotides (Figure 8b). The stability of the kinetic pattern of CTP–template interactions with respect to the media acidity might originate from the chemistry of the cytidine nucleobase. The cytidine heterocyclic system is the most basic of all the nucleotides as the ring nitrogen atom has a pK_a value of about 4.2 (increasing in nu-



Scheme 2. Possible hydrogen-bonding patterns in the nucleotide bilayers on the Zn^{II}–BC surface.

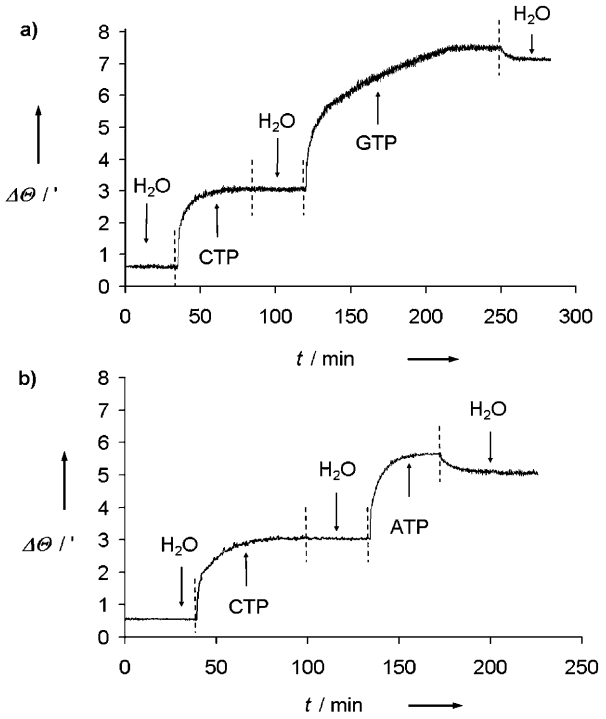


Figure 8. SPR sensograms for the stepwise adsorption of complementary and mismatched nucleotide combinations involving 5′-CTP on the Zn^{II}–BC template at pH 6.0. a) The assembly of 5′-CTP (0.02 mmol) followed by probing with 5′-GTP (0.02 mmol). b) Initial adsorption of 5′-CTP (0.02 mmol) followed by subsequent addition of 5′-ATP (0.02 mmol).

cleotides to $pK_a = 4.5$). This basicity may be sufficient to lead to protonation of CTP when in proximity to the lipid–water interface. A protonated CTP nucleotide only coordinates with one nucleotide to the Zn^{II}–BC binding site due to electrostatic repulsion between the bases. The assembly of added GTP proceeds through the well-known GC^+ Hoogsteen base pairing in analogy to the assembly process at pH 7.5.

When considered along with our previous observations on the nucleotide self-assembly on Zn^{II}–cyclen templates, the reported results allow some interesting conclusions regarding the nature and the mechanism of this phenomenon to be drawn. First, the solid surface decorated with a Zn^{II}–cyclen motif can induce selective self-assembly by coordination through the nucleotide phosphate group, thereby promoting the nucleobase interactions. Second, the molecular structure of the receptor and the experimental conditions can be varied to a certain degree and still allow the nucleotide adsorption on the surface, whereas the kinetic pattern of the process and the proposed composition of the resulting nucleotide layers changes. At the same acidity of the solution (pH 7.5–7.6), the self-assembly depends on the receptor structure. The monocyclic template gives double planes through simple 1:1 interactions of complementary partners, each of these planes presents one type of base depending on the order of nucleotide addition (e.g., A on the bottom as the initially adsorbed layer and U in the top layer[14a]). The ditopic structure of bis(Zn^{II}–cyclen) only favors the cooperative self-assembly of complementary nucleotides (A–U and G–C pairs). For the same receptor structure (biscyclic host), the resulting self-assembled layer structure depends on the acidity of the medium. Although the self-assembly is very specific and cooperative at pH 7.5, it becomes non-cooperative and tolerant to base mismatching under more acidic conditions. Finally, there is a certain "window of conditions" that only allows for the formation of complementary layers in accordance with the Chargaff rule (i.e., each plane that consists of the equal amount of purine and pyrimidine nucleotides represents the replica of another layer).

It is noteworthy that the stability of nonpolymerized nucleotide bilayers, when formed cooperatively, shows sensitivity to the nature of the base pairs and that Watson–Crick interactions result in the most stable system, similar to the polymer double-helix of DNA.

Most importantly, this cooperative self-assembly does not require drastic or unusual conditions to be applied: the essential components are a lipid-like interface decorated with linked (ditopic) zinc–cyclen complexes and small (naturally complementary) ribonucleotides in an aqueous solution at physiological pH.

We believe that this cooperative and selective self-assembly, which is realized through the cascade of coordinative binding and base pairing, provides a novel approach for the modification of surfaces with various combinations of nucleotides in a controllable fashion.

Experimental Section

Materials: The synthetic procedure for 4,6-[bis-(1,4,7,10-tetraazacyclododec-1-yl)]-1,3,5-triazin-2-yloctadecylamine/zinc(II) diperchlorate (Zn^{II}–BC; Figure 2a) has been previously described.[14b,c] $CHCl_3$ (Merck) was used as the solvent. Hydrochloric acid and sodium hydroxide were obtained from Fluka. All the nucleotides (adenosine 5′-monophosphate; adenosine 5′-diphosphate disodium salt hydrate; adenosine 5′-triphosphate disodium salt hydrate; uridine 5′-monophosphate disodium salt hydrate; uridine 5′-diphosphate disodium salt hydrate; uridine 5′-triphosphate trisodium salt hydrate; guanosine 5′-triphosphate sodium salt hydrate, guanosine 5′-diphosphate disodium salt, guanosine 5′-monophosphate disodium salt hydrate; cytidine 5′-triphosphate disodium salt hydrate, cytidine 5′-diphosphate sodium salt hydrate, cytidine 5′-monophosphate) were of analytical-reagent grade and obtained from Acros Organics (Belgium). The nucleotides were dissolved in water deionized to 16 MΩ·cm resistivity (initial pH 6.62 ± 0.05) and preliminary adjusted to pH 7.50 ± 0.05 or 6.00 ± 0.05 by the addition of a small amount of aqueous sodium hydroxide; these solutions were used for SPR measurements immediately after their preparation. Aqueous solution of 6-chloropurineriboside-5′-monophosphate (5′-(Cl)AMP) at pH 7.5 was purchased from Jena Bioscience (Germany).

Fabrication of SAM/LB films: A KSV Minitrough (KSV Instrument Ltd, Helsinki, Finland) equipped with a Wilhelmy plate was used for the preparation of Langmuir–Blodgett (LB) monolayers. TF-1 glass supports covered with a Cr adhesion sublayer (5 nm) and a polycrystalline Au layer (50 nm) supplied by Analytical MicroSystem (Germany) were modified by immersion into a solution of octanethiol in absolute ethanol (1 mM) for 2 min. The monolayers of Zn^{II}–BC were spread onto the basic subphase at pH 8.52 ± 0.05, compressed to 17.0 ± 0.1 mN m^{-1} (area per molecule $= 187 \pm 1$ Å^2), and transferred onto supports with a transfer ratio of 0.82. All SAM-supported films were transferred in a down-stroke fashion at a constant speed of 0.5 mm min^{-1}. Down-stroke transferring is a slow passage of a hydrophobized support through the interface in the direction from the air into the subphase solution. After the completion of the transfer, the monolayer was thoroughly removed from the subphase and the modified support with its thiolated side facing down into the subphase was drawn out from the trough.

SPR measurements (SPR): The SPR Kretschmann-type spectrometer Biosuplar-2 (Analytical MicroSystem; light-emitting diode light source, $\lambda = 670$ nm) equipped with peristaltic pump (flow rate $= 0.3$ mL min^{-1}) was used for kinetic monitoring. Freshly prepared solutions were added in portions (15 mL) to the pump vessel when the previous portion was nearly all used. An SPR cell was rinsed with pure water for 15–20 min prior to the addition of each next probe. The experimental SPR spectra of nucleotide adsorption on a Zn^{II}–BC surface were fitted to the theoretical curves based on six-phase Fresnel calculations that used the Neelder-Mid algorithm of minimization.[16] Although the exact value of the refractive index of nucleotide double layers on the Zn^{II}–BC template is unknown, there is a certain similarity of our system to hydrated thin films of short-chain oligonucleotides, for which such data are available. For data analysis, the refractive index n for a noncondensed film of nucleotide tetramers was assumed to be around $n = 1.36$, according to the reported dependence of refractive index on the degree of hydration of oligonucleotides.[17]

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[15] The available 6-chloropurine monophosphate was used to confirm the essential role of hydrogen bonds in the self-assembly because the number of phosphate groups in the added nucleotide does not influence the mechanism of secondary binding, even for a NDP–NMP combination (N = nucleobase; Figure 6a). The chemistry of the nucleotide, which forms the initially adsorbed layer, is nevertheless important for such a study: when monophosphates are adsorbed on the already bound complementary triphosphates, we observed a decrease in the rate and efficiency of secondary adsorption, most likely, because of steric factors (unpublished); on the other hand, UMP interacts with Zn^{II}–BC through simultaneous divalent coordination of phosphate and imide groups to both macrocycles of the single receptor, thereby making the surface inactive with respect to secondary binding of the incoming partner (see ref. [14b]). For these reasons, UDP was used to form the initially adsorbed layer in these experiments.

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