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Supporting Information

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Cooperative Self-Assembly of Adenosine and Uridine Nucleotides on 2-D Synthetic Template

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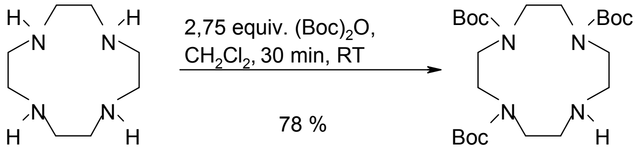
1. Synthesis of an amphiphilic cyclen-derivative:

A bis-cyclen building block was substituted with a long alkyl chain.

In the first step of the synthesis three of the four secondary amines of the cyclen (A0) are protected with a tert-butyloxycarbonyl group, using a literature known method.[1] The use of the protected azamacrocycle (1,4,7-tris-tert-butyloxycarbonyl-1,4,7,10-tetraazacyclododecane A1) in the synthesis has two advantages over the unprotected cyclen.

Firstly, the protecting group reduces the polar character of the tetraamines, thus making the preparative handling of these compounds more practical. Secondly this protection strategy prevents multi-N-substitution.

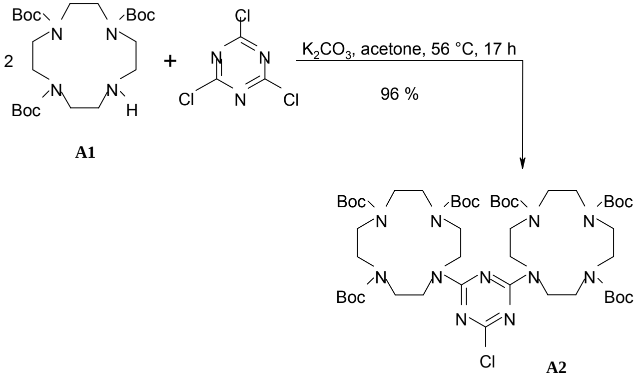


A0

A1

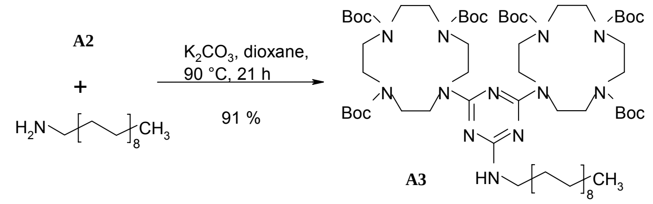
Scheme 1: Synthesis of 1,4,7-tris-tert-butyloxycarbonyl-1,4,7,10-tetraazacyclododecane (A1).

In the second step of the synthesis two protected azamacrocycles are bridged by an aryl-spacer. For this purpose the useful aryl-spacer trichlorotriazine was used. Due to its electron deficient character the trichlorotriazine is strongly favoured for nucleophilic aromatic substitution. Scheme 2 shows how two equivalents of 1,4,7-tris-tert-butyloxycarbonyl-1,4,7,10-tetraazacyclododecane react with trichlorotriazine to form the aryl-bridged bis-product A2 in 96 % yield.



Scheme 2: Synthesis of the aryl-bridged bis-cyclen A2.

In the next step of the synthesis the remaining chlorine atom on the triazine is substituted with octadecylamine. Through this a C-18 building block is produced which gives the target structure adequate hydrophobic properties.

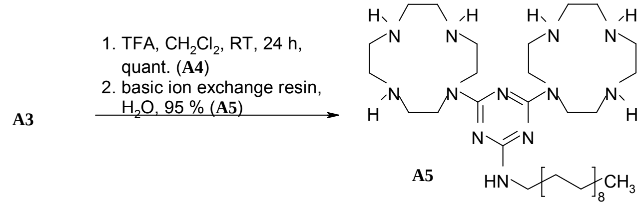


Scheme 3: Synthesis of A3.

For the synthesis of A3 it is necessary to conduct the reaction with dioxane as the solvent. When acetone is used as the solvent, the yields are low (~ 30 %) even if the reaction time is increased dramatically. This is due to the low boiling point of acetone. When dioxane is used as the solvent the product can be isolated in yields of 91 %.

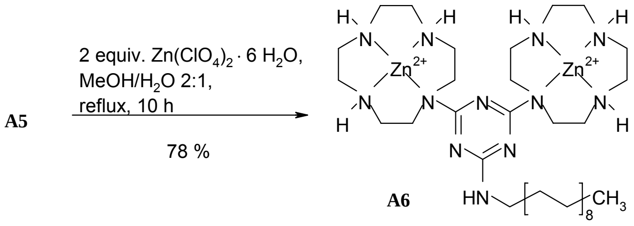
The boc-protected cyclen derivative is deprotected with 14 equivalents of trifluoroacetic acid per boc group to give the corresponding ammonium salt (A4) in quantitative yields. The exact protonation level of this compound was not determined.

The trifluoroacetate salt is converted into the free amine by passing over a strongly basic ion exchange resin. The free amine A5 is obtained in 95 % yield.



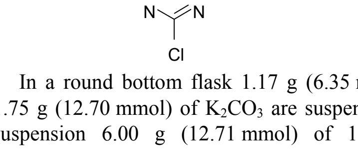
Scheme 4: Deprotection of A3 and conversion to amine A5.

Finally the complexation of the amine with two equivalents of $Zn(ClO_4)_2 \cdot 6\ H_2O$ yield the bis-zinc(II) complex in 78 % yield.



Scheme 5: Synthesis of the bis-zinc(II) complex A6.

~~4-Chloro-2,6-bis-(1,4,7,10-tetraazacyclododecane-4,7,10-tricarboxylic acid-tri-tert-butylester-1-yl)-[1,3,5]-triazine (A2):~~

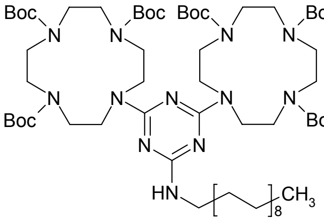


In a round bottom flask 1.17 g (6.35 mmol) of trichlorotriazine and 1.75 g (12.70 mmol) of K_2CO_3 are suspended in 40 ml acetone. To this suspension 6.00 g (12.71 mmol) of 1,4,7-tris-tert-butyloxycarbonyl-

1,4,7,10-tetraazacyclododecane in 30 ml of acetone are slowly added via a syringe. The reaction mixture is heated under reflux for 17 h. The solvent is removed under reduced pressure and the raw product chromatographed on silica gel (EE/PE 30:70) to yield A2 (6.54 g, 6.19 mmol, 98 %), as a colourless solid (R_f = 0.44, EE/PE 50:50).

Mp.: 124 °C; IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 2976, 2925, 1697, 1565, 1365, 1167, 779 UV/Vis (CH_3CN): □$_{max}$ [nm] (lg □) = 237 (4.579), 268 (3.698); ^{1}H NMR (250 MHz, $CDCl_3$): δ [ppm] = 1.42 (s, 18 H, CH_3-Boc), 1.47 (s, 36 H, CH_3-Boc), 3.23-3.67 (m, 32 H, CH_2-cyclen); ^{13}C NMR (63 MHz, $CDCl_3$): δ [ppm] = 28.4, 28.5 (+, CH_3-Boc), 49.9, 50.5, 50.9 (-, CH_2-cyclen), 80.0, 80.2 (C_{quart}, C-Boc), 156.4, 157.0 (C_{quart}, C=O Boc), 165.1 (C_{quart}, triazine-C_{Aryl}-N), 169.0 (C_{quart}, triazine-C_{Aryl}-Cl); MS (ESI, CH_2Cl_2/MeOH + 1 % AcOH): m/z (%) = 1057 (100) [MH]$^+$, 957 (14) [MH - Boc]$^+$; Elemental analysis calculated for $C_{49}H_{86}N_{11}O_{12}Cl_1$: C 55.69, H 8.20, N 14.58; found C 55.91, H 8.37, N 14.68.

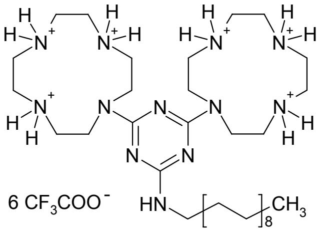
2,6-Bis-(1,4,7,10-tetraazacyclododecane-4,7,10-tricarboxylic acid-tri-tert-butylester-1-yl)-4-octadecylamino-[1,3,5]-triazine (A3):



1.20 g (1.14 mmol) of the starting material A2 and 0.79 g (1.70 mmol) of octadecylamine is dissolved in 20 ml of dioxane and 0.47 g (3.42 mmol) of K_2CO_3 added to this solution. The reaction mixture is heated to 90 °C for 21 h, the solvent removed in vaccuo and the residue chromatographed on silica gel (EE/PE 50:50) to yield A3 (1.33 g, 1.03 mmol, 91 %) as a colourless solid (R_f = 0.32, EE/PE 50:50).

Mp.: 84 °C; IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 2974, 2926, 2855, 1695, 1541, 1249, 1167, 972, 777; UV/Vis (CH_3CN): □$_{max}$ [nm] (lg □) = 227 (4.651); ^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] = 0.88 (t, 3 H, ^{3}J = 6.7 Hz, CH_3), 1.21-1.35 (m, 32 H, CH_2-alkyl chain), 1.44 (s, 18 H, CH_3-Boc), 1.46 (s, 36 H, CH_3-Boc), 3.34-3.70 (m, 34 H, CH_2-cyclen and CH_2-alkyl chain □-position to amine), 4.69 (bs, 1 H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ [ppm] = 14.1 (+, CH_3), 22.6, 27.0, 29.3, 29.5, 29.6, 29.7, 30.1, 31.9 (-, CH_2-alkyl chain), 28.4, 28.5 (+, CH_3-Boc), 40.6 (-, CH_2-alkyl chain □-position to amine), 50.3 (-, CH_2-cyclen), 79.7 (C_{quart}, C-Boc), 156.3 (C_{quart}, C=O Boc), 165.8 (C_{quart}, triazine); MS (ESI, CH_2Cl_2/MeOH + 10 % NH_4Ac): m/z (%) = 1290 (100) [MH]$^+$; Elemental analysis calculated for $C_{67}H_{124}N_{12}O_{12}$: C 62.39, H 9.69, N 13.03; found C 62.20, H 9.41, N 12.95.

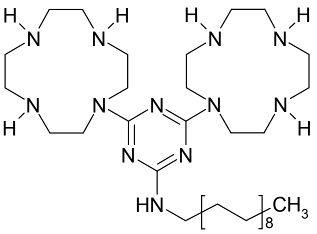
[4,6-Bis-(1-aza-4,7,10-azonia-cyclododec-1-yl)-[1,3,5]-triazin-2-yl]-octadecylamine-hexakis-trifluoroacetate (A4):



Compound A3 (1.14 g, 0.89 mmol) is dissolved in 30 ml CH_2Cl_2. To this solution 5.7 ml (8.49 g, 74.5 mmol) of TFA are added. The reaction mixture is stirred for 24 h at room temperature, the solvent removed in vaccuo and product A4 (1.29 g, 0.89 mmol, quantitative) isolated as a colourless oil.

IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 2952, 2853, 1667, 1201, 1136, 901, 844; UV/Vis (CH_3CN): □$_{max}$ [nm] (lg □) = 223 (3.419); ^{1}H NMR (400 MHz, CD_3CN): δ [ppm] = 0.88 (t, 3 H, ^{3}J = 6.8 Hz, CH_3, 1.21-1.31 (m, 30 H, CH_2-alkyl chain), 1.54 (m, 2 H, CH_2-alkyl chain □-position to amine), 2.96-3.32 (m, 32 H, CH_2-cyclen), 3.39 (t, 2 H, ^{3}J = 7.0 Hz, CH_2-alkyl chain □-position to amine, 7.56, 9.63 (bs, 11 H, $^+NH_2$); ^{13}C NMR (100 MHz, CD_3CN): δ [ppm] = 14.4 (+, CH_3), 23.4, 27.5, 30.0, 30.1, 30.3, 30.4, 32.7 (-, CH_2-alkyl chain), 29.5 (-, CH_2-alkyl chain □-position to amine), 42.1 (-, CH_2-alkyl chain □-position to amine), 44.6, 45.7, 46.9, 48.3 (CH_2-cyclen), 117.4 (C_{quart}, q, $^1J_{C,F}$ = 290 Hz, CF_3), 157.4 (C_{quart}, triazine), 161.0 (C_{quart}, q, $^2J_{C,F}$ = 36.6 Hz, C=O trifluoroacetate); MS (ESI, MeOH + 10 % NH_4Ac): m/z (%) = 345 (100) [MH_2]$^{2+}$, 690 (60) [MH]$^+$.

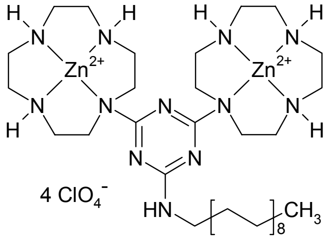
[4,6-Bis-(1,4,7,10-tetraazacyclododec-1-yl)-[1,3,5]-triazin-2-yl]-octadecylamine (A5):



A strong basic ion exchange resin is swollen for 15 min in water/MeOH and washed neutral with water. A column is charged with resin (20 ml, 18.0 mmol hydroxy equivalents at a given capacity of 0.9 mmol/ml). The ammonium salt A4 (1.29 g, 0.89 mmol) is dissolved in water (50 ml) and MeOH (5 ml) and is given onto the column and eluated with the same amount of solvent mixture. The eluate is lyophilised to yield 0.58 mg (0.85 mmol, 95 %) of free base A5, as colourless oil.

IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 3441, 2924, 2853, 1553, 1495, 1362, 811; UV/Vis (CH_3CN): □$_{max}$ [nm] (lg □) = 228 (3.273); ^{1}H NMR (400 MHz, MeOH-d4): δ [ppm] = 0.90 (t, 3 H, ^{3}J = 6.7 Hz, CH_3), 1.29-1.34 (m, 30 H, CH_2-alkyl chain), 1.55 (m, 2 H, CH_2-alkyl chain □-position to amine), 2.64-2.74 (m, 16 H, CH_2-cyclen), 2.91 (bs, 8 H, CH_2-cyclen), 3.30-3.32 (m, 2 H, CH_2-alkyl chain □-position to amine and MeOH), 3.73-3.75 (m, 8 H, CH_2-cyclen); ^{13}C NMR (100 MHz, MeOH-d4): δ [ppm] = 14.5 (+, CH_3), 23.8, 28.2, 30.6, 30.9, 31.3, 33.2 (-, CH_2-alkyl chain), 30.7 (-, CH_2-alkyl chain □-position to amine), 41.6 (-, CH_2-alkyl chain □-position to amine), 45.0, 45.7, 47.8, 48.4 (-, CH_2-cyclen), 167.2 (C_{quart}, triazine-alkyl chain), 168.2 (C_{quart}, triazine-cyclen); MS (ESI, MeOH + 10 % NH_4Ac): m/z (%) = 345 (65) [MH_2]$^{2+}$, 690 (100) [MH]$^+$; HRMS ($C_{37}H_{76}N_{12}$): calculated 689.6394 [MH]$^+$, found 689.6387 [MH]$^+$ ± 1.8 ppm.

[4,6-Bis-(1,4,7,10-tetraazacyclododec-1-yl)-[1,3,5]-triazin-2-yl]-octadecylamine-zinc(II)-di-perchlorate (A6):



400 mg (0.58 mmol) A5 are dissolved in 30 ml MeOH. To this solution 432 mg (1.16 mmol) zinc(II)-perchlorate in 10 ml MeOH are slowly added. The reaction mixture is stirred for 16 h at room temperature and then heated to reflux for a further 10 h. The solvent is removed in vaccuo and the residue then dissolved in 15 ml MeOH/Water 2:1. 10 ml of petroleum ether are added to precipitate the product A6. The solid is filtered off and dried in vaccuo. Compound A6 (461 mg, 0.45 mmol, 78 %) is isolated as colourless solid.

Mp.: 212 °C; IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 3438, 3278, 2923, 2853, 1566, 1144, 1089, 813, 627; UV/Vis (CH_3CN): □$_{max}$ [nm] (lg □) = 228 (4.244); ^{1}H NMR (300 MHz, CD_3CN): δ [ppm] = 0.84 (t, 3H, ^{3}J = 6.5 Hz, CH_3), 1.22-1.36 (m, 30 H, CH_2-alkyl chain), 1.51-1.54 (m, 2 H, CH_2-alkyl chain □-position to amine), 2.68-3.63 (m, 34 H, CH_2-cyclen and CH_2-alkyl chain □-position to amine), 4.19-4.28 (m, 6 H, NH), 6.28 (bs, 1 H, NH); ^{13}C NMR (75 MHz, CD_3CN): δ [ppm] = 14.5 (+, CH_3), 23.4, 27.8, 30.1, 30.3, 30.4, 32.7 (-, CH_2-alkyl chain), 41.7 (-, CH_2-alkyl chain □-position to amine), 44.5, 45.7, 46.3, 47.3 (-, CH_2-cyclen), 166.8, 170.8 (C_{quart}, triazine); MS (ESI, H_2O/CH_3CN): m/z (%) = 292 (75) [M^{4+} + CH_3COO^-]$^{3+}$, 306 (30) [M^{4+} + ClO_4^-]$^{3+}$, 468 (100) [K^{4+} + ClO_4^- + OH^-]$^{2+}$, 1035 (15) [K^{4+} + 2 ClO_4^- + OH^-]$^+$.

References

[1] S. Brandes, C. Gros, F. Denat, P. Pullumbi, R. Guilard,

Bull.Soc.Chim.Fr. 1996, 133, 65-73.

2. Langmuir Monolayer and LB Film Formation.

a) monolayer formation

A home-made, fully automated Langmuir surface balance with Wilhelmy glass plate was used for monolayer studies. Teflon barriers and a trough of surface area 300 cm^2 were sequentially rinsed with a hot mixture of chromic and sulfuric acids, and then with acetone and with pure water. Monolayers were formed by spreading 60 μL of freshly prepared Zn(II)-BC solution (2.4×10^{-5} M) in chloroform on the surface of deionized water or dilute aqueous solution of sodium hydroxide of pH 6.80±0.05; 7.85±0.05; 8.52±0.05. Spreading was done using an automatic micropipette (Gilson) delivering 5 μl drops onto a subphase surface in a chessboard-like pattern to distribute the monolayer uniformly. Subphase pH was controlled in situ during the experiments in order to keep its deviations within the range of ±0.05. The pH meter was calibrated to read hydrogen ion concentration directly. The solvent was allowed to evaporate for 15 min prior to the monolayer compression at the rate of 4.8 Å^2·molecule^{-1}·min^{-1}.

b) surface pressure vs area isotherms of Zn(II)-BC monolayers

As it is illustrated by Fig.1, Zn(II)-BC forms expanded monolayers of low collapse pressure on pure water (curve 1).

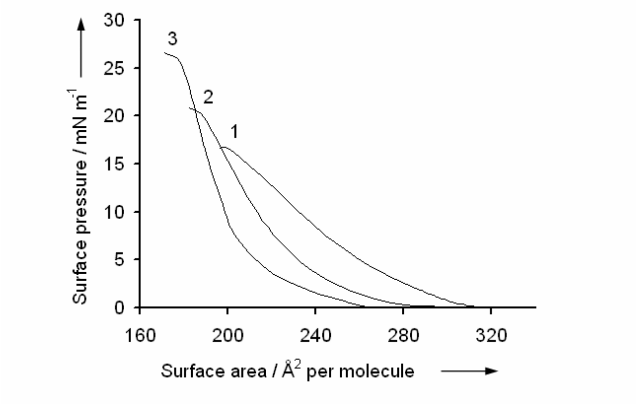


Figure 1. Surface pressure vs area isotherms for Zn(II)-BC on deionized water at pH 6.80 (1) and on basic subphases with pH 7.85 (2) and 8.52 (3).

The quality of a monolayer as a precursor for further fabrication of composed film on solid platform is, however, improved trough the increasing of subphase pH (curves 2 and 3).

b) SAM-LB film formation

The vertical "down-stroke" transfer of Zn(II)-BC monolayers onto thiolated SPR plates and CVA electrodes from a subphase with pH 8.52 was carried out at surface pressure 17±0.1 mN·m^{-1} (area per Zn(II)-BC molecule 187±1 Å^2) with a constant speed of 0.5 mm·min^{-1} and with a transfer ratio 0.82. "Down-stroke" transferring mode implies slow passage of hydrophobized support through the monolayer in the direction from the air into subphase solution. After transfer completion, the precursor monolayer was thoroughly removed from the subphase's surface and the modified support was drawn out from the trough keeping the backside of platform up and parallel to the plane of air/water interface ("face-down passage"). The hydrophobic sublayers of both loosely packed thiol monolayer and comparatively expanded Langmuir monolayer overlap in a course of transfer thus forming interdigitated-layered structure. We note especially, that surfaces covered with densely-packed SAMs or other hydrophobic materials are inappropriate for the modification with Zn(II)-BC monolayers because of the poor adhesion of LB monolayer to the bottom surface.

3) Staged cyclic voltammetry of SAM-Zn(II)-BC surface

Electrochemical, home-made instrument consisted of an electronic potentiostate IPC-compact, an electronic frequency response analyzer counter FRA-2 and MATLAB compatible software. All measurements were carried out in the three-electrode system. An Ag/AgCl double-junction reference electrode was used, the external chamber was filled with 0.5 M KCl solution containing 1 mM of both $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$.

Cylindrically-shaped platinum cellular counter-electrode had a surface area ~100 times greater than that of the working electrode. Gold working electrodes on silicon support were fabricated by first etching the electrode's shape into silicon oxide and then chemical depositing of a nickel adhesion layer (10 nm thick) and a 500 nm gold layer into the grooves until the top layer of the deposited metals coincided with the flat portion of the silicon wafer. This procedure allowed elimination of edge effects occurring on commonly used, thermally evaporated electrodes, and increased stability and reliability of the electrodes. Working round-shaped area of the electrode was of 0.08 cm^2. LB films on the electrodes were formed as described above.

The electrochemical response was measured by cyclic voltammetry with a scanning rate of 0.1 V·sec^{-1} in the voltage range from +0.5 V to –0.2 V.

Relative surface coverage δ was estimated as the extent of Red/Ox inhibition $\delta = (Q_{gold} - Q_{film})/Q_{gold}$, where Q_{gold} is an integrated charge for the reaction on bare gold, Q_{film} – that for electrode covered with SAM or SAM-Zn(II)-BC; Q_{gold}, Q_{SAM} and $Q_{SAM\text{-}Zn(II)\text{-}BC}$ were obtained through the integration of corresponding CVA curves in the range from 0.1 to 0.45 V.

According to CVA data; δ_{SAM} ranges up to 32% and $\delta_{SAM\text{-}Zn(II)\text{-}BC}$ – to 70%. All electrodes covered with SAM-Zn(II)-BC were stable in Red/Ox solution within no less than 5 hours.

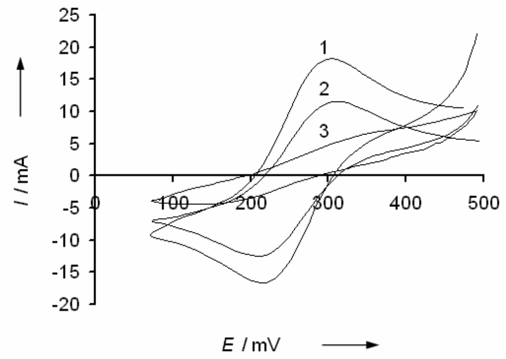


Fig. 2 CVA curves obtained for bare gold (1); gold surface thiolated in 1 mM octanethiol in absolute ethanol for 2 min (2); electrode covered with SAM-Zn(II)-BC (3). Red/Ox 0.5 M KCl/1 mM $[Fe(CN)_6]^{2-}$/1 mM $[Fe(CN)_6]^{3-}$.

4) Additional SPR data

The SPR plates covered with bare SAM of alkanethiol were measured in solutions of both types of nucleotides (5'-AXP and 5'-UXP); no increase in SPR signal that might be attributed even to non-specific adsorption of substance to SAM was registered.

According to our observations, there was no desorption of SAM-Zn(II)-BC film substance in pure water within at least 5 hours of SPR monitoring of platform placed to the water-filled SPR cell.

a) Selectivity of recognition of purine and pyrimidine bases by SAM-Zn(II)-BC surface

The binding of organic bases– nucleotide constituents – by immobilized Zn(II)-BC follows the recognition pattern similar to that observed for Zn(II)-cyclen derivatives in solution. The sensing platform responded to the base containing imide group (uracil, Fig.3, curve 1) and was inactive with respect to pyrimidine one (adenine, curve 2).

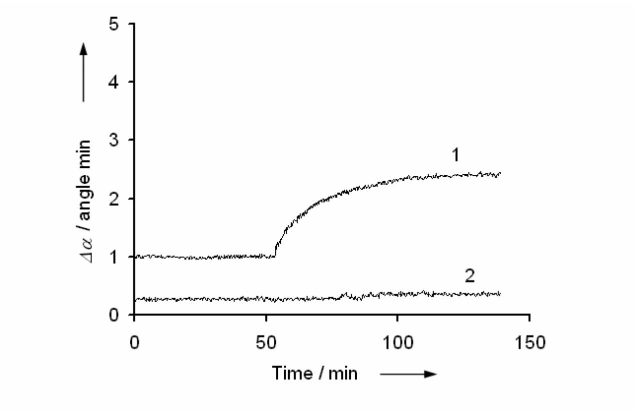


Fig.3 SPR sensogram for the adsorption of 0.1 mM uracil (curve 1) and adenine (curve 2) on SAM-Zn(II)-BC platform preliminary equilibrated in pure water; pH 7.8. Curve 2 is shifted down along y-axis by 0.7 angle minutes for clarity; both solutions were measured on different sides of the same SPR slide covered with sensing film.

b) Binding of inorganic phosphates at SAM-Zn(II)-BC film.

Fig. 4 illustrates the SPR monitored kinetics of the adsorption of dibasic and monobasic inorganic phosphates at SAM-Zn(II)-BC sensing platform (Fig.4, curve 1 and 2, correspondingly). These interactions were highly specific -- we did not observe the anion binding upon introduction of 1 mM NaH_2PO_4 into the analyzed solution. In contrast, the SPR signals of great intensity were measured for sensing surfaces exposed to the solution of 0.1 mM Na_2HPO_4 at pH 7.5.

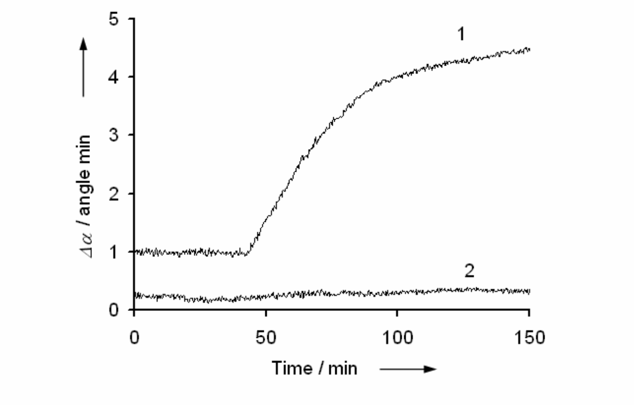


Fig.4 SPR-sensogram for the adsorption of HPO_4^{2-} on SAM-supported LB monolayer of Zn(II)-BC in aqueous solutions of 0.1 mM Na_2HPO_4 with pH 7.5 (curve 1). The SPR-curve obtained for the same sensing film in the solution of 1 mM NaH_2PO_4 with pH 5.5 (curve 2). Curve 2 is shifted down along y-axis by 0.7 angle minutes for clarity; both solutions were measured on different sides of the same SPR slide covered with sensing film.

c) Concentration effect on kinetics of initial binding of 5'-ATP to SAM-Zn(II)-BC.

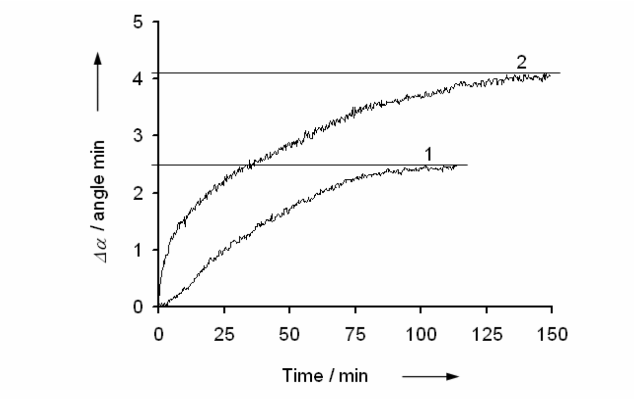


Fig.5 SPR sensograms for the adsorption of 0.02 mM (1) and 0.04 mM 5′-ATP on Zn(II)-BC sensing platform; pH 7.5.

d) Stepwise assembly of 5'-ATP/5'-UMP binary combination at SAM-Zn(II)-BC template

As mentioned in the main text of the paper, the initial assembly of UMP at immobilized Zn(II)-BC inhibits further adsorption of ATP. The inversion of the binding order – the adsorption of ATP followed by that of UMP - results in kinetic curve similar to that observed for other binary combinations.

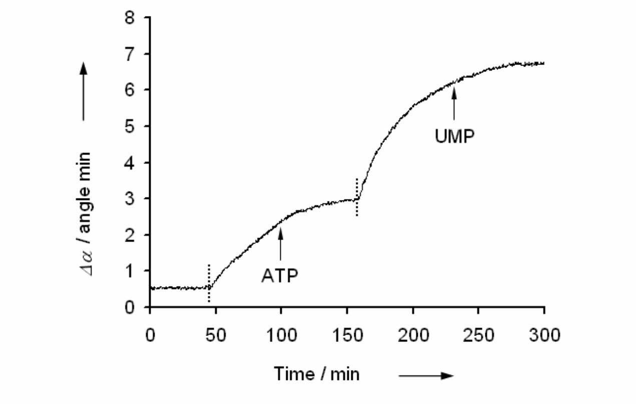


Fig. 6 SPR sensogram for stepwise adsorption of 0.02 mM 5′-ATP and 0.02 mM 5′-UMP at SAM-Zn(II)-BC sensing film; pH 7.5.

e) Uninterrupted one-step binding of 5'-ATP/5'-UTP equimolar mixture at SAM-Zn(II)-BC surface

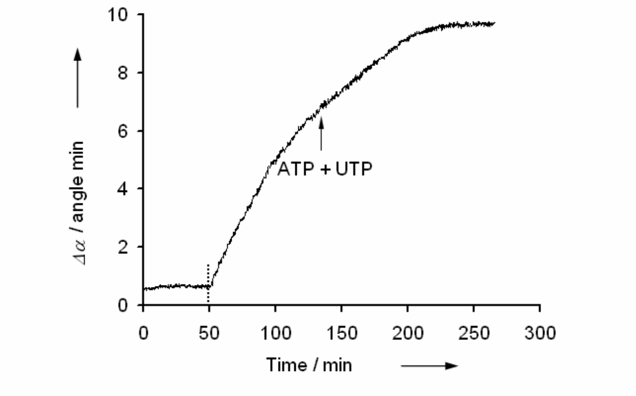


Fig. 7. SPR sensogram for uninterrupted one-step adsorption of equimolar mixture of 5′-ATP and 5′-UTP (0.02 mM of each nucleotide) at SAM-Zn(II)-BC sensing film; pH 7.5.

Table 1. Summarized data on averaged maximal SPR responses $\Delta\alpha_i$ measured at different stages of stepwise self-assembly of various nucleotides from their 0.02 mM aqueous solutions on SAM-Zn(II)-BC surface at pH 7.5.

Nucleotide	$\Delta\alpha_i$, angle minutes		
	$\Delta\alpha_1$[a]	$\Delta\alpha_2$[a]	$\Delta\alpha_3$[a]
5′-AMP	1.7	3.7	1.4
5′-ADP	2. 3	4.8	2.0
5′-ATP	2.7	5.4	2.4
5′-UMP	2.2	3.6	1.2[b]
5′-UDP	2.0	4.2	1.8
5′-UTP	2.5	5.3	2.2

[a] $\Delta\alpha_1$ corresponds to initial (first-step) adsorption, $\Delta\alpha_2$ – to secondary one, $\Delta\alpha_3$ is a maximal signal measured at third stage completing self-assembly.

[b] Third-stage response to UMP was measured after prior stepwise adsorption of UDP/ADP at sensing film.

Table 2. Maximal SPR responses $\Delta\alpha_{\text{one-step}}$ measured in a course of uninterrupted self-assembly of 5′-AXP/5′-UXP from their equimolar binary mixtures containing 0.02 mM of each nucleotide at SAM-Zn(II)-BC surface; pH 7.5. Maximal experimental SPR responses $\Delta\alpha_{\text{multi-step}}$ estimated from some stepwise kinetic curves are given for comparison.

Mixture composition	$\Delta\alpha_i$, angle minutes	
	$\Delta\alpha_{\text{one-step}}$	$\Delta\alpha_{\text{multi-step}}$
5′-ADP+ 5′-UDP	8.1	8.5[a]
5′-ATP+ 5′-UTP	9.0	10.4[b]
5′-ATP+ 5′-UDP	8.7	9.1[a]

[a] Measured for stepwise adsorption started from 5′-UDP

[b] Measured for stepwise adsorption started from 5′-UTP