Available online at www.sciencedirect.com
ScienceDirect
Mendeleev Commun., 2010, **20**, 329–331
Mendeleev Communications

New self-assembled monolayer coated cantilever for histidine-tag protein immobilization

Petr V. Gorelkin,*[a,b] Dmitry S. Mukhin,[b,c] Alexander G. Majouga,[a] Renata B. Romashkina,[a] Elena K. Beloglazkina,[a] Igor V. Yaminsky[a,b,c] and Nikolai V. Zyk[a]

[a] *Department of Chemistry, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation. Fax: +7 495 939 1009; e-mail: gorelkin@genebee.msu.ru*
[b] *Biosensor Academy, 119311 Moscow, Russian Federation. Fax: +7 495 939 1009; e-mail: muhinds@gmail.com*
[c] *Department of Physics, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation. Fax: +7 495 939 1009; e-mail: yaminsky@nanoscopy.org*

DOI: 10.1016/j.mencom.2010.11.009

A new way to histidine-tag protein immobilization with a new type of terpyridine ligand, 4′-(12-mercaptododecanyloxy)-[2,2′;6′,2″]terpyridine, on gold coated cantilever has been proposed.

The sensitive and selective detection of biological agents using protein specific binding depends on the conformation orientation of detected molecules on sensor surface during immobilization process. Various methods of controlling protein orientation at interface were developed since 1975[1] when the nitrilotriacetic acid/histidine-tag technology has been developed. This technology has become a powerful tool in bioscience for the single-step isolation and purification of protein and enzymes modified at the N- or C-terminus with histidine residues.[2] The histidine-tag (HT) technology is currently used for the prediction and control of protein orientation on a surface for biosensor applications.[3–5] This method of oriented surface protein immobilization can also be applied to multi-cantilever sensor development.

The nanomechanical cantilever systems have been used as a platform for the development of physical, chemical and biological sensors.[6–8] A unique microcantilever feature is that, when molecular adsorption is confined to one side of the microcantilever, it undergoes bending due to adsorption-induced stress.[9] In order to reach this feature, it is possible to use cantilevers one side of which is coated with gold modified by thiols.

Thiol-stabilized interfaces are applied in a range of experiments: from stabilization of gold nanoclusters in studies of their size effects on electronic, optical and chemical properties to the development of novel nanodevices (see, *e.g.*, refs. 10–12 and cited therein). The self-assembled monolayers (SAMs) formed by thiols or disulfides, which contain additional terminal (*i.e.*, solvent exposed) functional groups, are of special interest for these applications. In the cases when such terminal capping groups contain chelating moieties capable of complexing with transition metals, SAMs of these ligands produce metal complex on surfaces upon complexing with metals. These modified interfaces can be potentially used in catalysis or for modeling natural metal enzymes embedded in biomembranes.

Here, we propose a new method for oriented protein immobilization on gold cantilever surface. It is based on HT technology on copper metal complex formed on SAM of thiol, which contains terminal groups with chelating moieties.

We have synthesized a bifunctional thiol derivative containing a terminal terpyridine chelating group responsible for metal ion complexation. It is well known that 2,2′;6′,2″-terpyridine and its derivatives can effectively coordinate metal ions by forming complexes (2:1 and 1:1).[13,14]

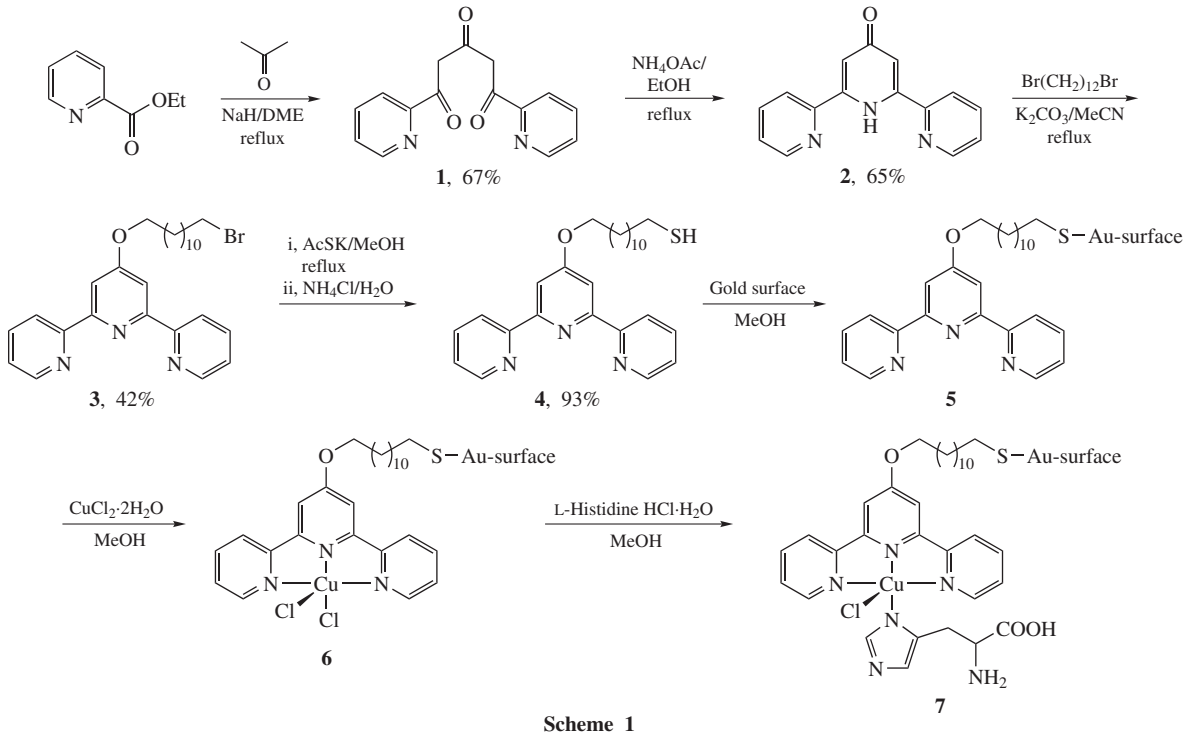
Terpyridine-functionalised thiol **4** was synthesized according to Scheme 1. Pyridone **2** obtained by a typical procedure† was alkylated by dibromoalkane in the presence of K_2CO_3, and the corresponding bromide was converted into desirable thiol by treatment with potassium thioacetate.‡

A Bioscan nanomechanical two-cantilever system (Biosensor Academy, Russia) and gold coated silicon cantilevers CSG 01 were used for cantilever surface stress measurements in the surface reaction (Scheme 1): chemisorption of 4′-(12-mercaptododecanyloxy)[2,2′;6′,2″]terpyridine (MDTP) **4** to furnish **5**,

† 1,5-Bis(2′-pyridyl)pentane-1,3,5-trione **1** and 2,6-bis(2′-pyridyl)-4-pyridone **2** were synthesized according to the previously reported protocol.[15]

‡ *Synthesis of 4′-(12-bromododecanyloxy)[2,2′;6′,2″]terpyridine* **3**. To a stirred solution of 2,6-bis(2′-pyridyl)-4-pyridone **2** (0.34 g, 1.4 mmol) in 20 ml of dry acetonitrile, potassium carbonate (0.37 g, 2.8 mmol) and 1,12-dibromododecane (0.89 g, 2.8 mmol) were added. The mixture was stirred under reflux for 24 h. The white precipitate was filtered off, and the solvent was removed under reduced pressure. The residue was purified by column chromatography (light petroleum–ethyl acetate, 4:1). Yield, 0.28 g (42%), white solid, mp 97–98 °C. ^{1}H NMR, δ: 8.66 (d, 2H, HC^6, HC$^{6''}$-Py, J 4.1 Hz), 8.62 (t, 2H, HC^3, HC$^{3''}$-Py, J 7.5 Hz), 8.04 (s, 2H, HC$^{3'}$, HC$^{5'}$-Py), 7.81 (t, 2H, HC^4, HC$^{4''}$-Py, J 7.8 Hz), 7.29 (dd, 2H, HC^5, HC$^{5''}$-Py, J_1 4.7 Hz, J_2 7.4 Hz), 4.26 (t, 2H, CH_2O, J 6.4 Hz), 3.37 (t, 2H, CH_2Br, J 6.8 Hz), 1.88 [m, 4H, $(CH_2)_2$], 1.56 (m, 2H, CH_2), 1.40 [m, 14H, $(CH_2)_7$]. ^{13}C NMR, δ: 167.0, 156.6, 156.2, 148.7, 136.1, 123.3, 121.0, 107.5, 67.8, 33.0, 32.8, 29.5, 29.4, 29.1, 28.8, 28.2, 26.1. Found (%): C, 65.56; H, 7.02; N, 8.31. Calc. for $C_{27}H_{34}N_3OBr$ (%): C, 65.32; H, 6.85; N, 8.47.

Synthesis of 4′-(12-mercaptododecanyloxy)[2,2′;6′,2″]terpyridine **4**. Potassium thioacetate (0.17 g, 1.5 mmol) was added to a stirred solution of 4′-(12-bromododecanyloxy)[2,2′;6′,2″]terpyridine **3** (0.25 g, 0.5 mmol) in methanol (15 ml). The mixture was stirred under reflux for 24 h under an argon atmosphere. After the reaction was complete, the saturated solution of NH_4Cl (30 ml) was added to the mixture. The formed precipitate was filtered off and dried *in vacuo*. Yield, 0.22 g (93%), pale pink solid, mp 83–84 °C. ^{1}H NMR, δ: 8.68 (d, 2H, HC^6, HC$^{6''}$-Py, J 4.0 Hz), 8.62 (d, 2H, HC^3, HC$^{3''}$-Py, J 8.1 Hz), 8.02 (s, 2H, HC$^{3'}$, HC$^{5'}$-Py), 7.83 (t, 2H, HC^4, HC$^{4''}$-Py, J 7.7 Hz), 7.31 (t, 2H, HC^5, HC$^{5''}$-Py, J 4.8 Hz), 4.25 (t, 2H, CH_2O, J 6.3 Hz), 2.66 (q, 2H, CH_2S, J 7.4 Hz), 1.88 [m, 4H, $(CH_2)_2$], 1.58 (m, 2H, CH_2), 1.41 [m, 14H, $(CH_2)_7$]. ^{13}C NMR, δ: 167.2, 156.8, 156.2, 148.9, 136.4, 123.5, 121.2, 107.4, 68.0, 39.1, 34.0, 29.5, 29.3, 29.1, 28.4, 26.0, 24.6. Found (%): C, 72.49; H, 7.85; N, 9.23; S, 6.95. Calc. for $C_{27}H_{35}N_3OS$ (%): C, 72.16; H, 7.79; N, 9.35; S, 7.13.



Scheme 1

copper chelate complex **6** formation, and replacement of chlorine by histidine to afford copper chelate complex **7**.§

Figure 1(*a*) shows measured signals obtained by monitoring cantilever deflections from two gold-coated cantilevers. Positive bending of cantilever (to modified with SAM side of cantilever) for 250 nm was obtained for 150 min of adsorption (the experiment was repeated six times, and the cantilever deflection deviation was ±20 nm).

A positive deflection ($\Delta z > 0$) corresponds to the upward bending of the cantilever due to lateral interactions between neighbour molecules in a layer. The main factor affecting the cantilever deflection is π–π interactions between neighbour molecules in a self-assembled monolayer of ligands because each molecule of MDTP contains three aromatic rings of pyridine (Figure 2). The π–π interactions provoke compressive forces in the layer, which make cantilever deflect in upward direction.

The deflection about –600 nm was obtained during copper metal complex formation (cantilever deflection deviation is ±60 nm). Time dependence of cantilever deflection during copper chelate complex formation is shown in Figure 1(*b*). A negative deflection ($\Delta z < 0$) corresponds to a downward bending of the cantilever (to the silicon surface) due to repulsive lateral forces, which appear between neighbour molecules.

The CuN_3Cl_2 polyhedra are essentially tetragonal pyramids, though the influence of the rigid tridentate terpyridine ligands to deviations from this geometry[15] takes place. Coordination of $CuCl_2·2H_2O$ on a gold surface modified by MDTP is attended by formation of chelate with the same structure. Repulsive interactions between neighbour molecules in a self-assembled layer, which influence cantilever deflection induced by conformation transformation in the layer of MDTP during chelating with

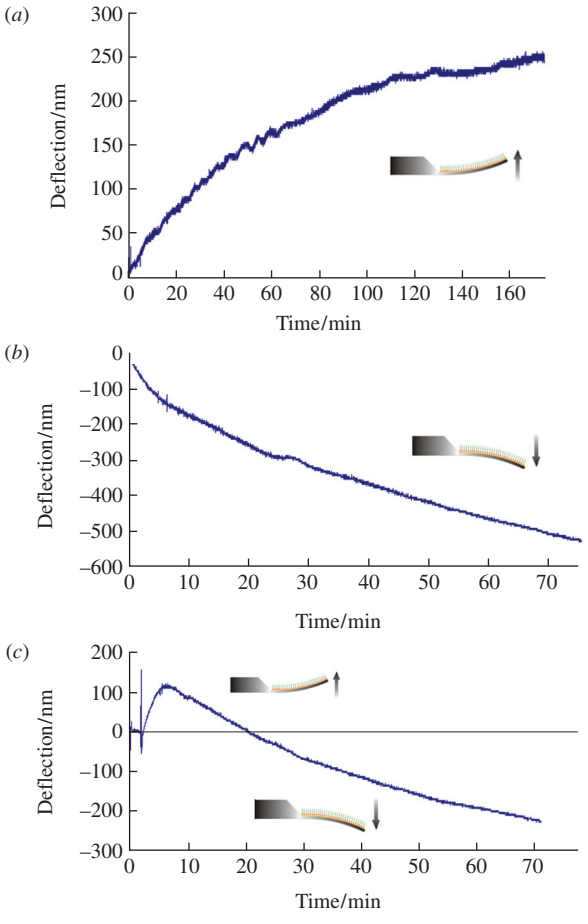


Figure 1 Time dependence of cantilever deflections during incubation in 10^{-3} M methanol solutions of (*a*) MDTP, (*b*) $CuCl_2·2H_2O$ and (*c*) histidine.

§ All experiments were carried out in methanol solution with gold coated silicon cantilever CSG01 (NT-MDT, Russia) size, 350×30×1 um, which was cleaned with a piranha solution ($H_2O_2·H_2SO_4$). Liquid cell of BioScan nanomechanical cantilever system (Biosensor Academy, Russia) was filled with methanol for signal stabilization. Incubation of the system in methanol was provided for ~1 h. The signal from two cantilevers has gone on plateau during this period of time. Then methanol was displaced by 10^{-3} M methanol solution of MDTP. When MDTP adsorption was finished liquid cell of BioScan was washed with pure methanol and then nanomechanical cantilever system was filled with MeOH for signal stabilization. Replacement of methanol by 10^{-3} M methanol solution of $CuCl_2·H_2O$ was provided before the second stage of measurements. After completion of the surface metal complex formation, the BioScan liquid system was washed with pure methanol. Displacement of water molecule in surface chelate complex by histidine was controlled at the last stage of the experiment.

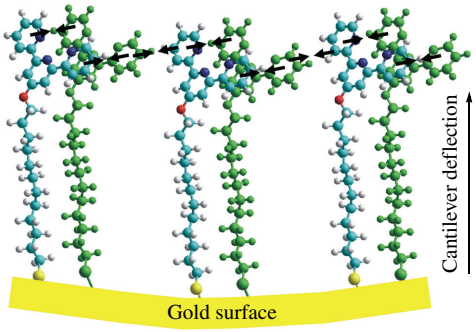


Figure 2 Model of neighbour molecules of MDTP immobilized on the gold surface of cantilever. Black arrows show the direction of π–π interactions.

$CuCl_2 \cdot 2H_2O$. Repulsions between electronegative chlorine atoms in neighbour molecules also affect surface tension formation.

We propose the model reaction for HT technology based on L-histidine adsorption on a modified surface of cantilever. Such a process of histidine immobilization on cantilever interface with MDTP modification is the same as for protein and enzymes modified at the N- or C-terminus with histidine residues. A positive deflection (~100±20 nm) was obtained for first several minutes of histidine adsorption [Figure 1(*c*)]; then, the negative deflection (~250±20 nm) took place after 10 min.

The proposed mechanism of chlorine substitution in metal complex is the following: first, histidine molecules diffuse to the surface modified with copper chelate complex and form the intermediate complex on the surface (positive deflection of the cantilever); then, the cleavage of bonds between chlorine and copper atoms occurs to produce new bonds between nitrogen atoms of histidine molecules and copper atoms (negative deflection of the cantilever). The same reaction was observed earlier for bromine substitution by imidazole in a similar terpyridine ligand.[16] For this reason, the obtained time dependence of cantilever deflection includes one extremum.

The results illustrate the potential of this technology for acting as a generic template for fabrication of oriented protein films on the surface. This type of protein immobilization provides new possibilities for biological sensing, diagnostic and other applications. The use of HT technology for protein oriented immobilization opens new horizons in nanomechanical cantilever biosensor chip creations.

This work was supported by the Russian Foundation for Basic Research (project nos. 03 10 00677, 10-02-06030-g and 10-04-01574-a), the Federal Agency of Science and Innovations of the Russian Ministry of Education and Science (project no. 02.512.11.2279), the Federal Agency of Education of the Russian Ministry of Education and Science (project no. P 255), NATO Science for Peace Program (project no. CBN.NR.NRSFP 983204) and the Korean Institute of Science and Technology (joint project KIST–MSU).

References

1 J. Porath, I. Carlsson, I. Olsson and G. Belfrage, *Nature*, 1975, **258**, 598.
2 K. Terpe, *Appl. Microbiol. Biotechnol.*, 2003, **60**, 523.
3 C. Mateo, G. Fernandez-Lorente, E. Cortes, J. L. Garcia, R. Fernandez-Lafuente and J. M. Guisan, *Biotechnol. Bioeng.*, 2001, **76**, 269.
4 G. S. J. Chaga, *Biochem. Biophys. Methods*, 2001, **49**, 313.
5 W. Jiang, B. Graham, L. Spicia and M. T. W. Hearn, *Anal. Biochem.*, 1998, **255**, 47.
6 J.-M. Antonietti, J. Gong, V. Habibpour, M. A. Röttgen, S. Abbet, C. J. Harding, M. Arenz, U. Heiz and Ch. Gerber, *Rev. Sci. Instrum.*, 2007, **78**, 054101.
7 G. Oliviero, P. Bergese, G. Canavese, M. Chiari, P. Colombi, M. Cretich, F. Damin, S. Fiorilli, S. L. Marasso, C. Ricciardi, P. Rivolo and L. E. Depero, *Anal. Chim. Acta*, 2008, **630**, 161.
8 K. Park, J. Jang, D. Irimia, J. Sturgis, J. Lee, J. P. Robinson, M. Toner and R. Bashi, *Lab Chip*, 2008, **8**, 1034.
9 I. Yaminsky, P. Gorelkin and G. Kiselev, *Jpn. J. Appl. Phys.*, 2006, **45**, 2316.
10 D. Lee, R. L. Donkers, G. Wang, A. S. Harper and R. W. Murray, *J. Am. Chem. Soc.*, 2004, **126**, 6193.
11 O. Lioubashevsky, V. I. Chegel, F. Patolsky, E. Katz and I. Willner, *J. Am. Chem. Soc.*, 2004, **126**, 7133.
12 Y. Negishi, Y. Takasugi, S. Sato, H. Yao, K. Kimura and T. Tsukuda, *J. Am. Chem. Soc.*, 2004, **126**, 6518.
13 E. C. Constable and M. D. Ward, *J. Chem. Soc., Dalton Trans.*, 1990, 1405.
14 T. Yutaka, I. Mori, M. Kurihara, N. Tamai and H. Nishihara, *Inorg. Chem.*, 2003, **42**, 6306.
15 W. Henke, S. Kremer and D. Reinen, *Inorg. Chem.*, 1983, **22**, 2858.
16 B. Verdejo, S. Blasco, E. Garcia-Espana, F. Lloret, P. Gavina, C. Soriano, S. Tatay, H. R. Jimenez, A. Domenech and J. Latorre, *Dalton Trans.*, 2007, 4726.

Received: 17th May 2010; Com. 10/3527