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The iron complex of dimethyl chlorin e_6–thioctic acid conjugate and its monolayers on the water and gold surfaces

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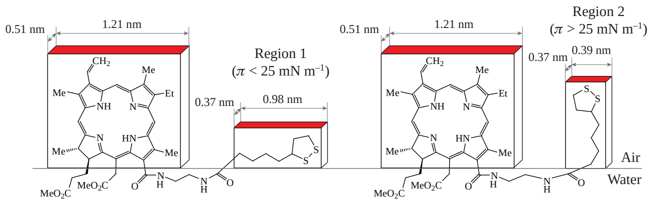
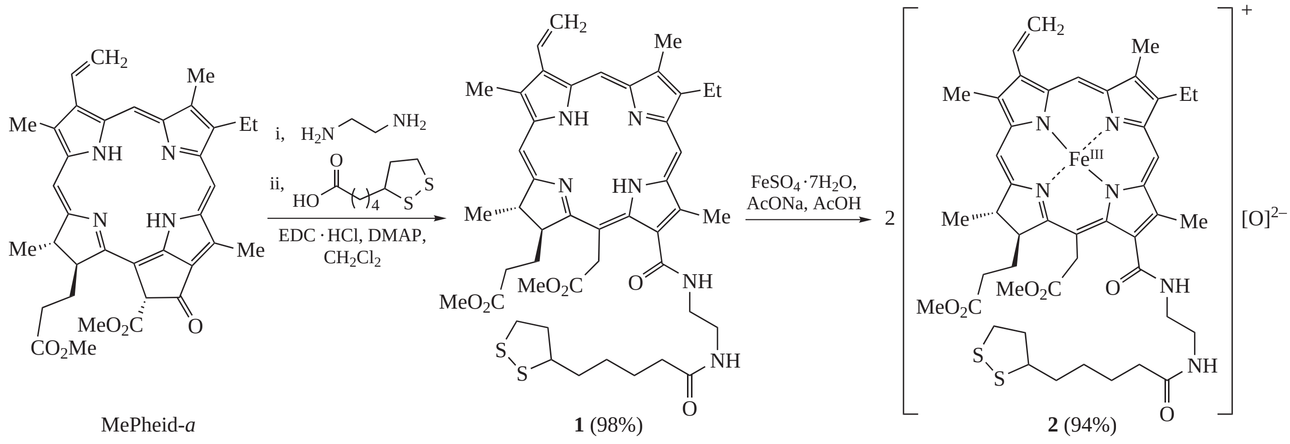
A μ-oxo-Fe^{3+} complex of chlorin e_6–thioctic acid conjugate was synthesized and explored as a new sensing material for nitric oxide. This conjugate can be used as a model for NO-binding potential biosensors.



Nitric oxide is an important endogenic molecule, which plays a crucial role in physiological processes such as nerve signal transmission, gene transcription and translation and determination of endothelial relaxation called endothelium-derived relaxing factor (EDRF).[1,2] The concentration of NO correlates with cardiovascular and immune system performance and ensures antibacterial, cytotoxic, anti-inflammatory and *in vivo* antioxidant actions. The human health and longevity depend on the level and activity of nitric oxide in the body.[3–6]

The primary screening of NO-releasing agents can be carried out using an indicator reaction of nitrogen monoxide with cytochrome *c*.[7–9] A methodology based on the preparation of Langmuir–Blodgett (LB) or Langmuir–Schaefer (LS) metal–porphyrin monolayer films for the detection of NO should be noted.[7–9] Another method supposes the application of self-assembled supramolecular systems such as metal complexes with polydentate macrocyclic ligands bearing alkanethiol fragments or precursors, providing good affinity to the surface of devices.[10–12] Natural and synthetic porphyrins, for example, cytochromes, are commonly used for the construction of NO-binding systems based on the above methods.[7–9,13–18]

Here, we report the synthesis of the iron complexes of dimethyl chlorin e_6 conjugates with thioctic (α-lipoic) acid, the preparation of their monolayers on gold and quartz surfaces and the ability of these monolayers to bind nitric oxide. The conjugate moiety responsible for homogeneous grafting to a gold surface was thioctic acid. This acid containing a terminal 1,2-dithiolan-3-yl moiety and a carboxyl group connected through a short alkyl linker is a cofactor of at least five enzyme systems. The disulfide moiety in the five-membered ring splits upon interactions with gold, providing bidentate covalent bonding to the surface and a better anchoring in comparison with related thiols.[19] This unique property of thioctic acid is intensively used to create self-assembled monolayers on gold surfaces for molecular electronics,[20] biosensor



Scheme 1

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constructions[21] and iron-containing alligator-type single-molecule magnet monolayers.[22]

The iron-containing dimethyl chlorin e_6 complex is proposed as a cytochrome mimetic. Chlorin e_6 belongs to natural metabolites of chlorophyll *a*[23], which are widely used as effective photosensitizers in photodynamic therapy.[24] These derivatives can be easily prepared from methylpheophorbide *a* (MePheid-*a*) using a semi-synthetic methodology.[25] Indeed, the five-membered exocycle in MePheid-*a*[26] readily undergoes ring opening in the presence of an excess of ethylenediamine to form the corresponding amide, which is used in the next step after extraction (Scheme 1). The primary amino group was then acylated with thioctic acid in the presence of *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC·HCl) and DMAP in CH_2Cl_2 to form amide **1** in 98% yield.† Compound **1** was treated with iron(II) sulfate in acetic acid in contact with air to afford μ-oxo-Fe^{III} complex **2** in 94% yield.

The π–A isotherm method was used for the analysis of monolayers of conjugates **1** and **2** on the aqueous subphase. The monolayers were characterized by the limiting area (area per molecule in close-packed monolayers) A_0 of conjugates, which was determined in the intersection point of the linear part of the π–A isotherm and the horizontal axis. Surface compression modulus C_s^{-1} of 2D-films was calculated as $C_s^{-1} = -A_0(\mathrm{d}\pi/\mathrm{d}A)_{p,T,n_i}$ (in mN m^{-1}). Figure 1 shows the 2D-phase transitions characterized by a plateau (see also Figure S1, Online Supplementary Materials). It may be proposed that liquid-expanded films (C_s^{-1} = 95 and 106 mN m^{-1}) transformed to liquid-condensed (C_s^{-1} = 176 and 295 mN m^{-1}) ones (Table S1).

The twofold increase of the limiting area (from 1.02 to 2.02 nm^2) can be related to μ-oxo-Fe^{III} complex **2** in monolayers

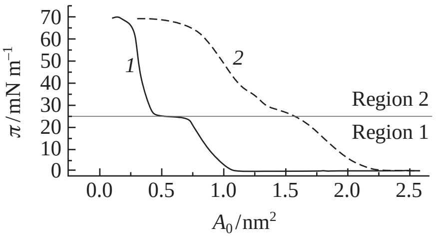

Figure 1 π–A isotherms of conjugates (*1*) **1** and (*2*) **2** on the water subphase.

† *Synthesis of compound* **1**. Ethylenediamine (0.15 g) was added to a solution of MePheid-*a* (0.05 g, 83 μmol, 1 equiv.) in dichloromethane with stirring. The mixture was stirred until the full consumption of MePheid-*a* (TLC control), then diluted with CH_2Cl_2 (100 ml) and extracted with water (3 × 30 ml). The organic phase was dried with Na_2SO_4, and the solvent was removed under reduced pressure. The residue was transferred to a Schlenk flask, and (±)-α-lipoic acid (0.051 g, 248 μmol, 3 equiv.), EDC·HCl (0.047 g, 245 μmol, 3 equiv.), DMAP (0.01 g, 83 μmol, 1 equiv.) and CH_2Cl_2 (1.25 ml) were added. The mixture was stirred under argon at 0 °C for 2 h and at room temperature for 12 h, then diluted with CH_2Cl_2 (100 ml) and washed with water (3 × 30 ml). The organic phase was dried with Na_2SO_4, and the product was isolated by column chromatography (1.5% Et_3N : 2.5% MeOH : 96% $CHCl_3$) to afford 0.069 g (81 μmol, 98%) of compound **1** as black solid. For its ^{1}H and ^{13}C NMR spectra, see Online Supplementary Materials.

Synthesis of compound **2**. Salt $FeSO_4 \cdot 7H_2O$ (0.331 g, 1.19 mmol, 7 equiv.) and AcONa·$3H_2O$ (0.70 g, 5.1 mmol, 30 equiv.) were added to a solution of compound **1** (0.145 g, 170 μmol, 1 equiv.) in acetic acid (0.17 ml) with stirring. The mixture was stirred under reflux for 20 min. Then, the solvent was removed under reduced pressure. The compound was isolated by column chromatography (5% MeOH : 95% $CHCl_3$) to afford 0.146 g (79.6 μmol, 94%) of product **2** as deep-green solid. MS (MALDI), *m/z*: 907.8 [M]$^+$ (calc. for $C_{46}H_{56}FeN_6O_6S_2^+$, *m/z*: 908.3), 923.9 [M + O]$^+$ (calc. for $C_{46}H_{56}FeN_6O_7S_2^+$, *m/z*: 924.2), 939.6 [M + 2O]$^+$ (calc. for $C_{46}H_{56}FeN_6O_8S_2^+$, *m/z*: 940.2).

(region 1), as predicted by quantum calculations carried out on the B3LYP/6-31g basis using the Gaussian 03W program.[27] The 2D-phase transitions may lead to film structure changes up to bilayers for conjugate **1** in region 2. This assumption is confirmed by a decrease of the limiting area from 1.02 (region 1) to 0.44 nm^2 (region 2), which is about a half of the calculated values (0.76 nm^2, Table S1).

The B3LYP/6-31g calculations of geometrical parameters of conjugates **1** and **2** allowed us to assume the orientation of conjugates at the air–water interface (Figure S1). The coincidence of the calculated and observed limiting areas A_0 in regions 1 and 2 of the compression isotherms of a monolayer of **2** indirectly confirms the correctness of the proposed orientation (Figure 1). Under compression of conjugate **2**, molecules in the monolayer are closely packed and do not tend to associate in contrast to conjugate **1** aggregated at π > 25 mN m^{-1} (Figures 1 and S1).

The formation of the monolayer films of **2** on the gold surface was investigated using atomic-force microscopy (AFM). The scanning was performed in the semi-contact mode. The gold surface had a grain structure with an average grain size of 60–100 nm and an approximate surface relief height of 12 nm [Figures S2(*a*) and S3(*a*)]. The AFM visualization of the first transferred monolayer of **2** from deionized water to the gold surface indicated the formation of a continuous surface film [Figure S2(*b*)] with a grain size of ~200 nm and a surface relief height of ~8 nm.

A similar situation was observed when two or three monolayers of **2** were transferred onto the gold surface [Figure S2(*c*)]. These facts are consistent with the results obtained by wetting methods (Figure S4). The contact angle θ_a measured by water drop on the initial gold surface (θ_a = 72°) was changed up to θ_a = 78° when the first LS transfer of monolayer that may be considered as a self-assembled monolayer (SAM). A drastic change of the contact angle when two or more layers were transferred on the gold surface may be owing to the film surface structure changes caused by the loss of regular orientation of molecules in the first layer.

The UV-VIS spectrum of conjugate **2** containing Fe^{3+} in a chloroform–methanol solution has three absorption maxima at 684, 584 and 391 nm (Soret band), which were shifted in comparison with the bands in the spectrum of **1** at 661, 500 and 401 nm, respectively (Figure S5). The broadening and the bathochromic shifts of Q-bands in the spectra (500 → 585 nm and 661 → 684 nm) indicate[28] that the porphyrin core in μ-oxo-dimer **2** is more oxidized as compared with that in conjugate **1**. It is known[28] that the reduction of μ-oxo-dimeric porphyrin units in $[(TPP)Fe]_2O$ occurs in one or two steps with the half-wave potentials $E_{1/2}$ = –1.09 and –1.60 V, respectively. Nitric oxide can act simultaneously as an oxidizing and reducing agent for both porphyrins and iron ions. Therefore, in order to estimate qualitatively the reducing ability of μ-oxo-dimer **2**, we used hydrazine sulfate ($E_{1/2}$ = 0.35–0.65 V),[29] which can reduce only Fe^{3+} without affecting the porphyrin fragments. Indeed, the UV-VIS spectra of reaction mixtures of conjugate **2** with hydrazine sulfate demonstrate the narrowing and threefold increase of Soret band absorption and the appearance of a new clearly defined Q-band at 602 nm (Figure 2), which can be explained by redox transformations Fe^{3+}/Fe^{2+} and the inclusion of hydrazine into the coordination sphere of iron.

To construct an optical sensor model for nitric oxide detection, the monolayers of compound **2** were transferred onto quartz. As the result, the bathochromic shift of Soret band absorption and only a band at 681 nm were observed in the spectrum (Figure 3). The increase of the number of transferred layers from 1 to 20 lead to a linear increase in optical density at 396 and 681 nm. This dependence approves the formation of regular layers on the quartz.

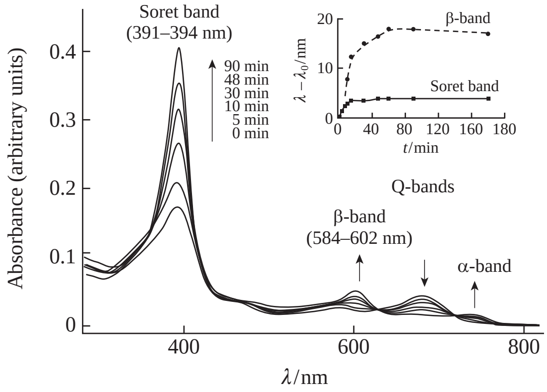

Figure 2 UV-VIS spectra of conjugate **2** and hydrazine sulfate in methanol. Insert: the red shifts of Soret and Q-bands *vs.* time.

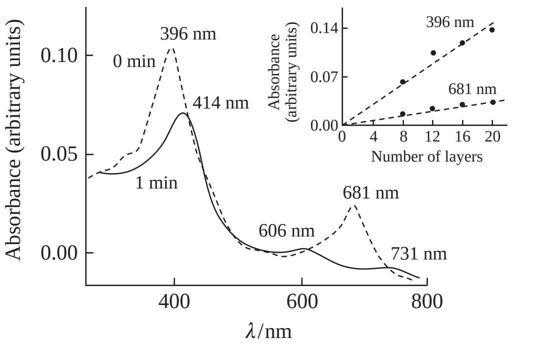

Figure 3 UV-VIS spectra of 12 monolayers of conjugate **2** transferred onto quartz under the action of NO (1.2 mM). Insert: the plot of $A = f(n)$, where n is the number of transferred layers.

When the monolayers of **2** on quartz were treated with gaseous NO in an oxygen-free glove-box at room temperature for 1 min, the visible spectrum of conjugate **2** was changed (Figure 3): the band at 681 nm was shifted to 606 nm and the Soret band at 395 nm was shifted to 414 nm; this can be due to the nitrosilation reaction of the iron atom in conjugate **2**. This data support the idea of the potential application of **2** monolayers on quartz for the construction of an NO-optical sensor with the limit of NO detection in a concentration range of 0.1–10 mM.

In conclusion, a μ-oxo-Fe^{3+} complex of chlorin e_6–α-lipoic acid conjugate **2** was explored as a new sensing material for nitric oxide using the Q and Soret absorption bands of UV-VIS spectra as an optical response. Conjugate **2** forms a stable monolayer at a water surface, and it can be transferred onto gold or quartz surfaces by the LS method. Sulfur atoms in conjugate **2** are involved in a self-assembly process on the gold surface to significantly increase the stability of transferred monolayers such as SAMs. The conjugate can be used for the model of NO binding, which is of interest for potential applications in biosensors and new medicines.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2017.11.024.

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