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Ultrathin film sensory system based on resonance energy transfer between the monolayers consisting of non-covalently linked fluorophores

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An ultrathin film, wherein a resonance energy transfer (FRET) from the energy donor monolayer to the analyte sensitive acceptor monolayer is controlled by definable cation binding, was produced, thus proving the feasible concept of non-covalently linked FRET couple based sensors.
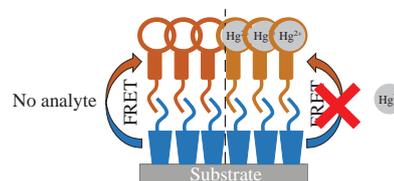
Sensory systems employing fluorescent properties of the analyte receptor molecules remain one of the most promising fields of study in modern supramolecular chemistry.[1–9] A large subset of such systems relies on the phenomenon of so-called Förster resonance energy transfer (FRET) for the enhancement of fluorescent signal or the shift of operational wavelength.[9–16] Implementation of this requires two chromophore molecules located in a close proximity and characterized by the spectral overlap of the energy donor emission spectrum and the energy acceptor absorption spectrum. These two chromophores, pre-selected by their photophysical properties, are usually covalently linked to each other *via* a non-conjugated spacer group.[15] It is necessary to note that the FRET efficiency in highly organized planar systems consisting of ultrathin films of donor and acceptor chromophoric components can be significantly higher than that in bulk unorganized systems of the same composition.[17–21] Moreover, the necessity of covalent binding between the components of donor–acceptor couple can be avoided by the formation of such planar films, wherein the donor and acceptor are not chemically linked, but still remain in close proximity. However, to the best of our knowledge, such bilayer planar architecture, wherein FRET occurs between the two fluorophore layers not bound covalently, was never employed for the fabrication of sensory devices. Thus, this work was aimed at the investigation of principal possibility of employing the above described approach.
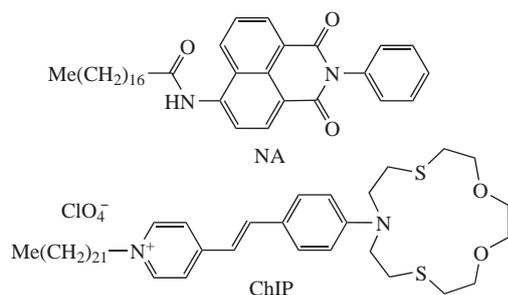
We evaluated the FRET process occurring in the planar supramolecular system comprised of an energy donor monolayer (naphthalimide derivative, NA) deposited onto a quartz substrate using Langmuir–Blodgett technique, and an energy acceptor monolayer (functionalized hemicyanine chromoionophore, ChIP) deposited on top of a donor monolayer using Langmuir–Schaefer procedure.[†] Due to the presence of ionophoric dithia-aza-crown ether moiety conjugated with the chromophoric part of the ChIP molecule, the latter also acts as the receptor element of the system, capable of binding mercury cations followed by a change of its optical properties.[22,23] These compounds were chosen due to their relatively high photostability, fluorescence quantum yield,[15,24–27] and compatibility with the Langmuir monolayers method.[22,23,25,28] Most importantly, however, the choice was made owing to the fact that NA emission spectrum overlaps free-form ChIP (ChIP molecule which is not yet bound to the analyte) absorbance band, thus allowing FRET to occur between these chromophores, while the shift of the ChIP absorbance band upon mercury cation binding[22] drastically reduces this overlap, thus decreasing the efficiency of FRET process (Figure 1).[‡] This allowed us to employ the change of FRET efficiency and, consequently, the quenching/dequenching of NA fluorescence as the response to the binding of mercury cations by ChIP.

At the first stage of this work, the individual Langmuir–Blodgett films (LBF) of NA and ChIP on quartz substrates were produced in order to objectively estimate the efficiency of the FRET process occurring in the studied system. Spectral properties

NA

ChIP

[†] The alkylated dithiaaza-crown-hemicyanine dye (ChIP), 4-{(*E*-2-[4-(1,4-dioxa-7,13-dithia-10-azacyclopentadec-10-yl)phenyl]ethenyl}-1-docosylpyridinium perchlorate[23] and modified naphthalimide (NA), *N*-(1,3-dioxo-2-phenyl-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-6-yl)stearamide[18] were prepared according to the known procedures.

[‡] UV-VIS absorbance spectra of solutions and solid films on quartz substrates were recorded using a Shimadzu UV 2450 PC spectrophotometer. Fluorescence spectra of the studied systems were recorded on a Shimadzu RF-5301 PC spectrofluorimeter.
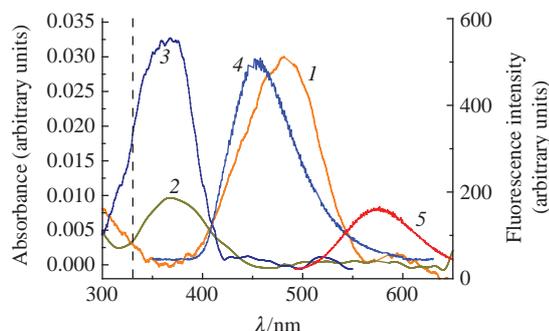
Figure 1 UV-VIS absorbance spectra of the ultrathin films of (*1*) free-form ChIP, (*2*) [ChIP·Hg^{2+}] complex and (*3*) NA; (*4*) fluorescence spectrum of NA film upon excitation at $\lambda_{ex} = 330$ nm (denoted by a dashed line) and (*5*) typical fluorescence emission spectrum of a ChIP film upon excitation at $\lambda_{ex} = 480$ nm.

of the ChIP film were studied before and after dipping into 1 mM aqueous solution of mercury perchlorate Hg(ClO$_4$)$_2$, so as to obtain spectral data on both free form of the molecule and its mercury complex ([ChIP·Hg^{2+}]).§ Perchlorate salt was chosen since ChIP molecule also contains the perchlorate anion, which simplifies the analyte reception process. After the dipping, the film was allowed to dry for 10 min, and then the fluorescence spectra were recorded. Figure 1 shows electronic absorption spectra of all mentioned films and the fluorescence spectrum of NA film. It is notable that spectral overlap between NA emission and unbound ChIP absorbance peaks (see Figure 1, curves *4* and *1*) is significantly larger than that in case of NA and [ChIP·Hg^{2+}] (see Figure 1, curves *4* and *2*) due to both large hypsochromic spectral shift upon complex formation, and significant decrease in the molar extinction.

For NA monolayer and all the systems studied further, the excitation wavelength of 330 nm was chosen (denoted by a dashed line in Figure 1). Such light wavelength provides a significant excitation of NA (Figure 2, curve *3*) and, at the same time, leads to no observable emission of the free-form ChIP monolayer (see Figure 2, curve *1*) and only insignificant fluorescent signal from the [ChIP·Hg^{2+}] complex (see Figure 2, curve *2*).

The next step of this work was to assemble a bilayer film containing both NA and ChIP on a quartz substrate. The first layer of NA was transferred from the aqueous subphase using Langmuir–Blodgett (*i.e.* vertical deposition) procedure¶ to obtain a film, in which the hydrophobic alkyl chains of the molecule are oriented outwards. The second layer of ChIP, preorganized

§ Ultrapure water (18 MΩ cm) deionized with a millipore Milli-Q water purification system was used as a subphase in Langmuir monolayer studies, if not noted otherwise, and for the preparation of solutions of barium and mercury perchlorates (acquired from Sigma-Aldrich and used as received). The studied hemicyanine and naphthalimide dyes were solubilized at a concentration in the range of 1.0–1.5×10^{-4} M in CHCl$_3$ for monolayer formation (all solvents were HPLC grade, Sigma-Aldrich). Mercury perchlorate for the sensory studies was purchased from Sigma-Aldrich and used as received.
¶ Langmuir–Blodgett device KSV Minitrough (Finland) with surface area of 273.0 cm^2, the trough made of Teflon, which provides chemical resistance and prevents subphase leakage, and barriers made of hydrophilic polymer polyacetal, was used for Langmuir monolayers formation. Compression isotherms were recorded using automated Langmuir balance and platinum Wilhelmy plate. Before experiments, Langmuir–Blodgett trough was washed with chloroform, acetone and distilled water, and polyacetal barriers – with ethanol and distilled water. The monolayers were formed by spreading the chloroform solutions onto the air/water interface using a chromatographic syringe. Then the system was left undisturbed for 15 min in order to fully evaporate the solvent from the interface. After that, monolayer compression at the rate of 5 mm min^{-1} commenced.
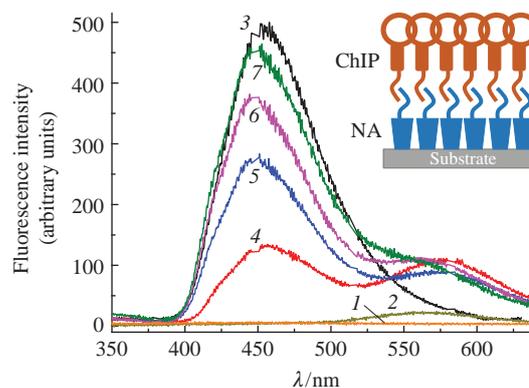
Figure 2 Fluorescence spectra of single layer LBFs of (*1*) free-form ChIP, (*2*) [ChIP·Hg^{2+}] complex, (*3*) NA, (*4*) LBF consisting of a layer of NA and a layer of ChIP. Spectra (*5*), (*6*), and (*7*) correspond to the bilayer donor–acceptor film after its immersion into 1 mM solution of Hg(ClO$_4$)$_2$ for 20, 80, and 540 s, respectively. $\lambda_{ex} = 330$ nm (for all the spectra). Inset shows a schematic structure of the studied bilayers.

for maximum sensory response,[22] was deposited onto the NA film by Langmuir–Schaefer technique (*i.e.* horizontal deposition).†† This approach provides such bilayer organization, where hydrophobic parts of both molecules would be in contact and the ionophoric crown-ether group of ChIP would be on the external part of the bilayer, thus quite available for the interaction with analyte (see Figure 2, inset). This configuration maintains the distance between the donor and acceptor fluorophore moieties of about 5 nm that is close to the optimal value for the maximal efficiency of FRET in planar systems.[18,19]

Fluorescence emission spectrum observed for this system (see Figure 2, curve *4*) upon photoexcitation of NA ($\lambda_{ex} = 330$ nm) drastically differs from that of individual components (curves *1* and *3*): NA emission is quenched by ~3.5 times, and ChIP fluorescence band at *ca*. 580 nm appears. This indicates an efficient realization of resonance energy transfer from the NA donor monolayer to the ChIP acceptor monolayer. For a rough estimation of the efficiency of this process, it is suitable to introduce a relative efficency coefficient $K = I_{452\ nm}/I_{580\ nm}$, where $I_{452\ nm}$ and $I_{580\ nm}$ are the corresponding emission intensities of the system upon excitation at $\lambda_{ex} = 330$ nm.

For an the initial NA/ChIP bilayers, wherein the two distinct bands corresponding to both fluorophores were observed, the value of K is about 1.18±0.06 indicating quite efficient FRET. If one denote as K_0 this value of K for the bilayer film not yet exposed to a mercury salt solution, then the analyte binding can be characterized by the value of the parameter $P = K/K_0 - 1$ (which for the initial film would amount to 0).

To investigate the possibility of obtaining the on/off signal for the mercury cations, the studied bilayer was immersed into 1 mM aqueous Hg(ClO$_4$)$_2$ solution for various amount of time, and its fluorescence spectra were measured afterwards. As expected, the spectrum of system brought into contact with the analyte solution for 20 s (see Figure 2, curve *5*) shows significant dequenching of the NA fluorescence since formation of the [ChIP·Hg^{2+}] dramatically reduces the overlap between the NA

†† Bilayer films were produced by Langmuir–Blodgett–Schaefer technique, which comprised two stages of monolayer transfer from air/water interface onto quartz substrates. At the first stage, vertical transfer of NA monolayer was carried out according to Langmuir–Blodgett technique at constant surface pressure of 20 mN m^{-1}, which provides orientation of hydrophobic parts of the molecules outwards from the substrate. Then, according to Langmuir–Schaefer method, a second layer of ChIP was transferred onto horizontally oriented substrate with single-layer film, resulting in contact of hydrophobic parts of the substances between two layers, thus forming a bilayer.

emission and hemicyanine absorption. Value of P increases to 1.56±0.11, indicating the decrease of FRET efficiency as well. Further exposition of the system to the mercury perchlorate solution for 80 s in total (curve *6*) leads to increase in P value to 2.26±0.12. At last, after 540 s (curve *7*), NA emission intensity almost reaches the level observed for individual NA film, and P significantly increases to 3.46±0.12. It should be noted, that, in case of the studied bilayer, K cannot reach infinitude or even the value observed for individual NA monolayer, due to the presence of residual [ChIP·Hg^{2+}] fluorescence upon excitation at $\lambda_{ex} = 330$ nm (see Figure 2, curve *2*) and miniscule overlap between NA emission and [ChIP·Hg^{2+}] absorption spectra (see Figure 1, curves *4* and *2*, respectively). The change in the value of P from 0 for the initial film up to 3.46±0.12 for the film close to saturation with analyte cations indicates the possibility of further employing the studied system as a basis for sensory devices.

Note that the control experiments, wherein both individual films of ChIP and ChIP:NA bilayer were immersed into deionized water did not show any signs of the films deterioration or change in their photophysical properties.

In conclusion, we have for the first time developed the proof-of-concept FRET-based sensory ultrathin film system, wherein planar energy donor and energy acceptor layers are not chemically bound to each other. It has been demonstrated that this approach is as feasible as the case of covalently linked FRET couples. Its implementation opens up possibilities for the design of novel sensor devices that do not require the tedious and complicated synthesis of fluorophore couples. Moreover, as was shown earlier for such systems, the FRET efficiency can be fine-tuned by introduction of spacer interlayers,[18] further improving the proposed multilayer design.

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