Proton Translocation in Monomolecular Langmuir−Blodgett Films Including 2-Naphthol and 1,4-Anthraquinone Derivatives

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Received: August 6, 2004; In Final Form: February 6, 2005

The scope of the present work is the investigation of proton transport through monomolecular Langmuir−Blodgett (LB) films. The films were formed from amphiphilic molecules: 2-naphtholo-6-sulfonamide of dodecylamine (N) and 1,4-anthraquinono-2 sulfonamide of dodecylamine (A). The 2-naphthol derivative can act as a proton donor due to excited state proton transfer (ESPT) and the 1,4-anthraquinone group can play the role of proton acceptor because of protonation of the reduced form if it is present. Absorption and emission spectra of LB films containing N and A were registered and separated into component bands. Individual absorption and emission peaks observed were assigned to given forms of chromophores. The behavior of different component bands reflects the state of anthraquinone dependent on proton concentration. A correlation of rate and efficiency of ESPT, with changes of the spectra of A, may be expected to yield information concerning the transport of protons from N to A. The influence of the donor−acceptor distance, sample humidity, film arrangement and the presence of protonophores (Gramicidin A) on proton transfer is studied. Our results indicate that the proton can be transported through the film but its concentration vanishes at the distance greater than 30 Å. The efficiency of proton transfer depends strongly on water content, film structure and the presence of ion channels.

Introduction

In the past decades of the former century great progress was made in exploration of the details of the role of proton transport in energy transforming membranes of living cells. However, some important problems such as interrelation between electron and proton movement and their interaction with the hydrophobic medium still escape our full understanding. This problem is also essential in construction of photovoltaic cells and signal transducing elements.

The aim of the present work is the study of the proton and electron movement in a simple model resembling biological energy transforming systems. We used Langmuir−Blodgett (LB) films formed from amphifilic molecules oriented perpendicularly to the layer surface, showing structural analogy to biological membranes. The advantage of our model is a possibility of direct measurement of the electronic spectra as well as electric currents and potentials within the film and to control the distance between proton donor and acceptor.

Several models of the photosynthetic apparatus, with electron donating dyes included in liposomes and other assemblies analogous to cell membranes were constructed and investigated. Light induced charge separation, by electron and/or proton transport was obtained for these systems.[1−3] To our knowledge no attempt has been made of using monomolecular LB films as a model of proton transporting medium though deprotontion of naphthol derivatives in LB films has been studied.[4]

We deposited on quartz substrates parallel layers containing 2-naphtholo-6-sulfonamide of dodecylamine (N) and 1,4-anthraquinono-2-sulfonamide of dodecylamine (A). N plays a role of a proton donor due to excited state proton transfer (ESPT)[5,6] that was observed in LB films containing only N.[7] A is used as a trap for protons and electrons, thus simulating proton and electron carriers in biological systems. We suppose that if the proton released from N in ESPT reaches A in another molecular layer, it will influence equilibria of its redox and prototropic states, inducing definite changes in the electronic spectra. By studying these effects, it should be possible to extract information concerning the protonation and reduction of A.

Following flowcharts of the processes of electron and proton transport in LB films may be taken into account: (i) charge recombination by returning of H^+ to the naphtholate after some time delay comparable to excite state lifetime, (ii) charge separation by proton transport to another molecular layer, leading to measurable electric effects, (iii) concomitant proton and electron transfer, which may give no electric response but can result in a decrease of the concentration of the naphtholate form of N, and (iv) the product of ESPT transported not as H^+ but as a hydrogen atom,[8] which would lead to similar effects as (iii).

From the investigation of electronic spectra and measurements of electric effects, it should be possible to conclude which of the above-mentioned variants of the fate of the proton and electron is realized. By a study of the dependence of the spectral effects observed, on the separation of N and A, water content, addition of acids and bases and of protonophores (gramicidin A) it is possible to draw conclusions concerning the mechanism of proton transfer in LB films.

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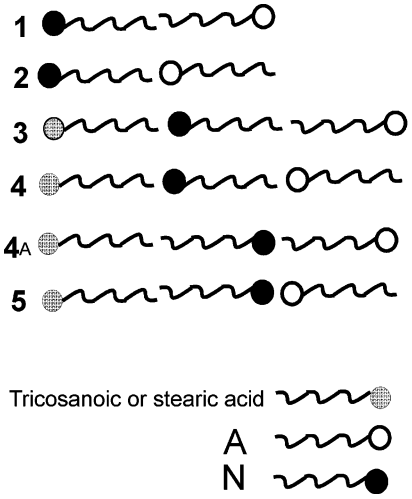
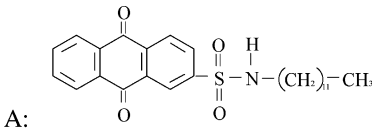
10.1021/jp046464r CCC: $30.25
Published on Web 03/11/2005





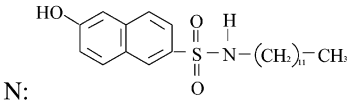


Figure 1. Film layouts used in the experiments.

I. Materials and Methods

I.1. Synthesis of 2-Naphtholo-6 sulfonamide of Dodecylamine (N) and 1,4-Anthraquinono-2-sulfonamide of Dodecylamine (A). N was synthesized as described in ref 9. A was obtained analogously from 1,4-anthraquinone-2-sulfonic acid (AQS) whose synthesis was performed according to reference.[10] Thus AQS was treated by a small molar excess of thionyl chloride in dimethylformamide.[11] and the resulting sulfonyl chloride (AQS−Cl) was reacted with dodecylamine in methylene chloride in the presence of triethylamine. The product was dissolved in $CHCl_3$, the solution was filtered, and insoluble residue was discarded. The solvent was removed by evaporation, and A was crystallized from methanol. (R_f $CHCl_3$:C_5H_{12} 9:1 = 0.168 Anal. Calcd: 68.6% C, 7.2% H, 3.1% N, 7.0% S. Found: 68.7% C, 7.0% H, 3.0% N, 7.3% S). Mp = 152−155 °C. Gramicidin A from Bacillus braevis was purchased from Fluka and kept at 4 °C. Other reagents were of analytical grade and were used without further purification.

I.2. Preparation of LB Films. The films were deposited by means of a KSV 5000 (Finland) trough, on quartz plates (fluorescence), or gold electrodes (electrometric measurements). The procedure of the deposition of LB films was described in more detail in ref 7. The film layouts used are shown in Figure 1. Each layout[1−5] was used in four variants: (a) N and A without additions, (b) N and A with addition of gramicidin (G) to the layer of N, A, or both (N+G:A, N:A+G, N+G:A+G), (c) N and A diluted (at 0.05−0.2 molar fraction) with stearic acid (S) or octadecylamine (ODA), and (d) both N and A diluted with S and with addition of G (molar fraction 0.01−0.02) (N+S: A+S+G, N+S+G:A+S+G). The identities of the film layouts in Figure 1 are verified by the deposition procedure itself as well as by the shape of the transfer isotherms (1S−2S, Supporting Information), measurements of the transfer ratio (07−1.9 in most cases), and measurements of the surface pressure during transfer, which was 30−40 mN/m unless stated otherwise.

I.3. Spectrophotometric and Spectrofluorometric Measurements. The samples of LB films containing N and A were excited at 320 nm, and the emission spectra in the range 350−600 nm were registered by means of the spectrofluorometer SF1 (Optel, Poland). The fluorescence spectra were corrected with respect to detector response. For more details of spectroscopic measurements, see refs 7 and 12. The deconvolution of the luminescence spectra was performed analogously as for samples containing only N,[7] but taking into account the possibility of occurring of three additional bands from A, besides two peaks of N, the number of component bands in the program for bands separation was five. The emission intensity of a given form was estimated in the band maximum or/and by integration of the component band.

The fluorescence quantum yield of our chromophores was determined from[13,14]

$$\phi_x = (F_x A_{st}/F_{st} A_x)\phi_{st} \quad (1)$$

where F_x is the corrected fluorescence intensity at band maximum, A_x is the absorbance of the sample at the exciting wavelength, and F_{st} and A_{st} are analogous values for the standard. As a standard the protonated form of N included in LB films was used ($\phi_{st} = 0.1$[7]). For excluding or promoting ESPT (see ref 7) the composition of the atmosphere surrounding the sample during the measurements was modified by addition 0.05 cm^3 of various solvents (e.g., glacial acetic acid, or H_2O) on the bottom of the closed cuvette.

The efficiency (E_T) of intermolecular energy transfer from the naphthol derivative to various species of A was determined by two independent procedures: one of these methods is based on formula 2 (see refs 13, 14).

$$E_T = (R_0)^6/(R_{NA}{}^6 + R_0{}^6) \quad (2)$$

where R_{NA} is the distance between the centers of N and A chromophores and R_0 is the distance where deactivation of the energy donor (N) by means of energy transfer (ET) is of equal probability as that of all other excited state deactivation processes in N. [$R_0 = (8.8 \times 10^{-25}\kappa^2 n^{-4}\phi_D{}^0 J_{AD})^{1/6}$ [cm], where κ is the donor−acceptor orientation factor, $\phi_D{}^0$ is the donor fluorescence quantum yield of N in the absence of energy transfer, and n is the refractive index of the medium. $J_{AD} = \int \epsilon_{A,\lambda}\lambda^4 F_{D,\lambda}\, d\lambda / \int F_{D,\lambda}\, d\lambda$, where $\epsilon_{A,\lambda}$ is acceptor extinction coefficient at wavelength λ. $F_{D,\lambda}$ is fluorescence intensity of the donor at λ. R_{NA} may be calculated for given layouts of layers using the values of bond lengths and bond angles in N and A molecules in extended conformations. In the calculation of R_0 and J_{DA} the donor acceptor orientation factor was assumed to be $\kappa = 1$, which seems to be the most appropriate for transition moments of N and A in a LB film, practically frozen within the excited state lifetime. For the value of $\epsilon_{A,\lambda}$ the absorption coefficient of the oxidized form (Figure 2) or of various reduced forms, taken from transient absorption spectra given in the literature,[16−18] was used. Thus a set of eight R_0 values for various

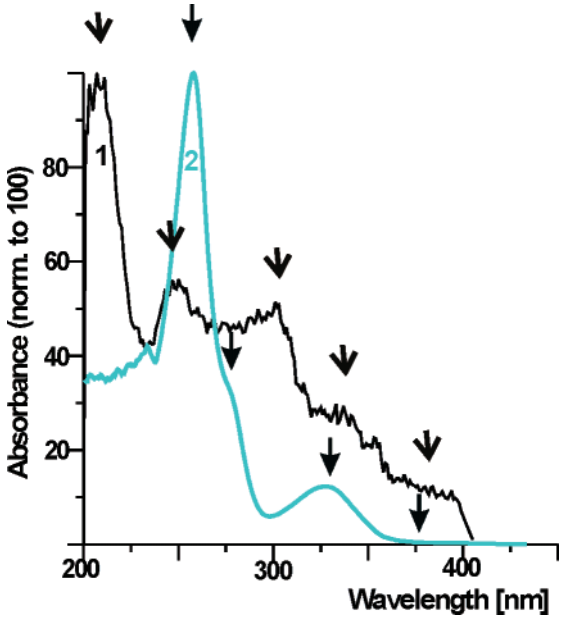


Figure 2. Absorption spectrum of A (normalized to 100): (1) in LB film (three layers thick); (2) in chloroform. Peaks are marked by arrows.

combinations of donor (N, two forms) and acceptor (A, four forms) was obtained (see S3). These values ranged from 9 to 12 Å and were treated as limiting values of R_0 in further calculation. In determination of R_{NA}, bond lengths and angles given in ref 20 were used. It was assumed thereby that (1) the molecules N and A are in the maximally extended conformation of the fluorophores and (2) N and A in LB films have the sulfonamide group exposed to the outer face of the layer and the aromatic rings directed toward the interior of the layer. (See also 4S, Supporting Information).]

Another procedure[13] for determination of E_T consists of using formula 3 based on the reduction of the fluorescence intensity of the energy donor due to ET.

$$E_T = 1 - \phi_D/\phi_D^0 \quad (3)$$

where ϕ_D^0, ϕ_D is the fluorescence quantum yield of N in the absence and in the presence of ET, respectively.

The luminescence decays were measured by means of the spectrofluorometer produced by Edinburgh Analytical Instruments (GB) type FL 900 by exciting the samples at 337 nm and analyzing at 360, 420, 580, and 620 nm. These wavelength values were thought to be optimal for exciting luminescence because of the characteristics of the flashlamp filled with N_2 and due to spectral properties of various forms of N and A that may be present in our samples (see Figure 2 and 5S).

I.4. Electrometric Measurements. Electrometric measurements were performed similarly as described in ref 15. Thus the dependence of the electric current (dc) on field strength (0−200 V/cm) applied parallel to the film surface was registered. The separation of electrodes was 0.2 mm. The current−voltage plot was taken during illuminating the samples by the light of 311 nm (HBO). For more details of the experimental setup see 6S.

II. Results

II.1. Absorption and Fluorescence Spectra of LB Films Containing Pure Naphthol (N) and Anthraquinone (A) Derivatives. II.1.1. Absorption. The absorption spectrum of N in LB films has been published previously,[7] and it is similar

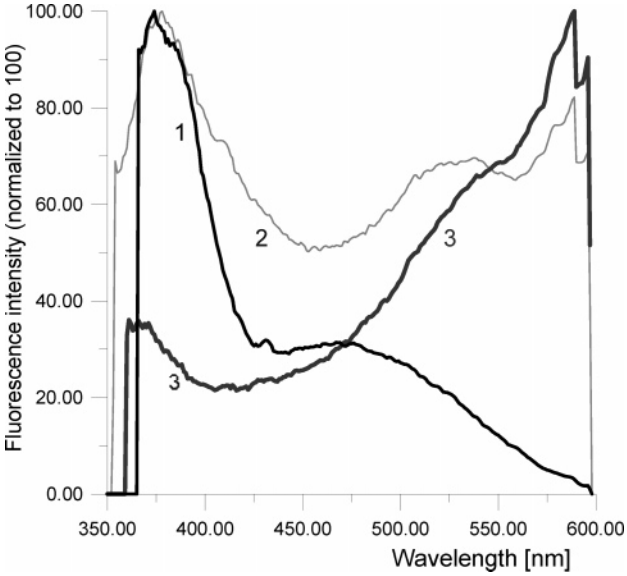


Figure 3. Fluorescence spectra of the systems of three layers (from left to right) T, N, A of following layouts.

1) T N No.3 in Fig.1

2) T N N No.4 in Fig.1

3) T N A No.5 in Fig.1

to the spectrum in chloroform solution. In Figure 2 the absorption spectrum of A in LB films (curve 1) is compared to that in solution (curve 2).

In the spectrum of A in chloroform (curve 2) four bands may be discerned at decreasing wavelength (λ) values of the maxima: (a) at 374 nm, the band scarcely visible in Figure 2 but evident in the spectrum of higher concentrated solution, (b) at 328 nm, (c) a shoulder at 274 nm, and (d) a band at 258 nm. Additionally, a fifth band at $\lambda < 200$ nm must also be present because it is evident in Figure 2 that the absorbance does not fall down at this spectral range. This band probably corresponds to the highest energy transition in the spectrum of A in LB film (curve 1) where five peaks are observed with maxima at (a) 380 nm, (b) 333 nm, (c) 301 nm, (d) 250 nm, and (e) 207 nm. Absorption peaks in LB films are red shifted 5−8 nm with respect to correspondent bands in solution with the exception of band (c), which appears in a LB film at a wavelength value 27 nm higher than that in the solution spectrum and band (d), which is blue shifted. The observed spectral shift, analogous to that in the case of LB films containing pure N, is probably caused by interchromophoric interaction and exciton effects. This suggests that analogous to the LB films containing only N, chromophoric molecules aggregate, forming domains of different spectroscopic properties, which leads to considerable reduction of the fluorescence quantum yield of the chromophores.

II.1.2. Luminescence. The fluorescence spectra of LB films containing parallel N and A (both undiluted) layers of various film layouts are shown in Figure 3.

The emission of pure N in LB films has been described,[7] and typically it is composed of two bands with maxima near 375 and 440 nm. The shape of the curves (1−3) in Figure 3, differing from the fluorescence of N, suggests that the spectra are composed of several peaks. Pure A in LB film, excited at 320 nm, practically shows no signals in our measuring spectral range (330−600 nm). AQS in water solution treated with a reducing agent, under N_2, shows luminescence with a maximum

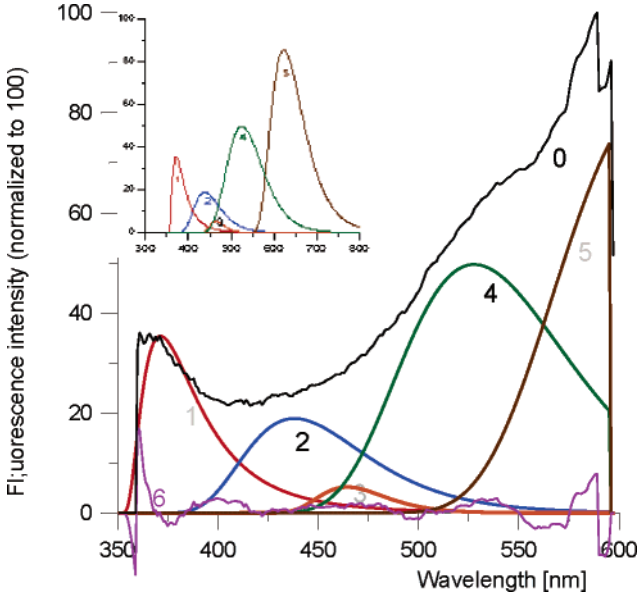


Figure 4. Deconvolution of spectrum 3 in Figure 3: (0) experimental spectrum; (1) N-protonated (ROH*); (2) N-ionized (RO^-*) form; (3)−(5) bands of A; (6) error distribution. Fit parameters: $\chi^2 = 0.4$; band maximum positions L1 = 377 nm, L2 = 448 nm, L3 = 469 nm, L4 = 536 nm, and L5 = 636 nm. Band heights [relative units]: F1 = 35, F2 = 18, F3 = 5, F4 = 48, and F5 = 81. Inset: reconstructed spectrum for 350−800 nm.

at about 500 nm rapidly disappearing by exposure to air. The assignment of the bands at 500−600 nm to A is in accord with the published[16−18] data on emission spectra of various reduced and protonated forms of AQS and its derivatives in the spectral range 500−900 nm. This makes us believe that the emission at 500−600 nm, observed from LB samples, is due to some protonated and reduced forms of A, absent normally in the films containing only A.

II.1.3. Components of the Fluorescence Spectra of LB Films Containing Pure N and A and Their Assignment to Various Redox and Prototropic Species. The spectra presented in Figure 3 can be separated into five components by fitting log-normal function[19] to the experimental data as described in ref 7. This is shown in Figure 4 for one of the spectra of Figure 3 (curve 3).

The positions of band maxima (L) and band intensities (F) obtained from the fitting procedure are given in the legend to Figure 4. Other emission spectra of the undiluted fluorophores N and A, included in LB films, were deconvoluted analogously, and the results are given in Table 1.

The positions of the highest energy maxima (L1, L2) in Table 1 and other parameters of the function fitted, are nearly the same as those of pure N in LB films. On the basis of the analogy of the fluorescence band parameters in Table 1 and ref 7, the peak at about 377 nm (Table 1, L1) may be assigned to the protonated form of 2-naphthol (ROH*) and that at 448 nm (L2) to the naphtholate (RO^-*) produced by ESPT. The emission band centered near 500 nm (Table 1), because of a similarity to that of reduced AQS in solution (results not shown), and on the grounds of literature data,[16] may be contributed to the fully reduced and doubly protonated form of antrahydroqinone ($AQSH_2$, 5S, Supporting Information). On the basis of transient absorption data given in refs 16−18 the band at 530−550 nm (Figure 4, Table 1) is probably due to semiquinone anion (AQSA). The lowest energy emission band centered at 600 nm may be contributed to semiquinone (AQSS).[17] In most cases the number of five bands in the deconvolution procedure seems sufficient because error distribution (cf. Figure 4) does not suggest the presence of additional peaks.

The results of Figure 3 and Table 1 show that the intensities (I3−I5) of the bands due to A increase considerably and the bands assigned to N decrease with reduction of the distance between 2-naphthol and anthraquinone groups in LB films. The effect indicated may be caused by protonation of A by H^+ produced in ESPT, enhanced by approaching the chromophores, leading to formation of emissive forms of A. Alternatively, this result can be explained by resonance energy transfer (ET) from 2-naphthol to the anthraquinone molecule in a reduced form. It is known that various reduced forms of AQS absorb at the spectral range between 370 and 700 nm,[16−19] which implies overlap of the fluorescence spectra of 2-naphthol and absorption of 1,4-anthraquinone, enabling ET according to Foerster's mechanism ref 13. Reduced and protonated forms of anthraquinone show the usual emission[16,17] in the indicated spectral range. Production of the reduced, forms of A, as energy acceptors, may be induced by protonation and/or by some other processes. To decide what is the influence of ESPT and protonation of A, on the concentration of the emissive forms of the chromophore, it is necessary to determine the efficiency of ET from N to A.

II.1.4. Estimation of the Influence of Energy Transfer and Proton Transfer between N and A on the Fluorescence Spectra of LB Films. Using bond lengths and angles in the aliphatic and aromatic moieties,[20] molecular dimensions of N and A were calculated, which allows estimation of the interchromophoric distance (R_{NA}) in our samples (see discussion after eq 2 and 3S and 4S, Supporting Information). On the basis of the literature data[7,16,17] concerning the spectral properties of N and A, R_0 values for this chromophore pair were calculated, which together with R_{NA} enables determination of the efficiency of energy transfer from N to A (E_T) from eq 2.

From the measurement of the fluorescence quantum yield of N in the presence and absence of ET, one can determine the E_T for given distances R_{NA} by means of eq 3 (4S, Supporting Information).

R_0 values needed for calculation of E_T by means eq 2 depend on the spectroscopic properties of energy donor and acceptor. Absorption and emission spectra differ for various forms of N (ROH*, RO^-*) and of A (AQS, $AQSH_2$, SAQSA, SAQS; see above). For various combinations of these forms, eight different values of R_0 were obtained (see 3S, Supporting Information) but the dependency of E_T on R_{NA} for the lowest value of R_0 (9 Å, curve 1 in Figure 5) and the highest one (12 Å, curve 2) do not differ greatly (Figure 5). The actual plot of $E_T = f(R_{NA})$ for our case, must lie somewhere between curves 1 and 2 (solid lines) in Figure 5.

E_T values determined using eq 3 (open circles) seem to fit well to a plot of E_T as a function of R_{NA} for any value of R_0 within the range 9−12 Å. On the other hand the contribution of A to the total luminescence (C_A, mean values from Table 1, filled circles and dashed line in Figure 5) seems to depend on R_{NA} in a quasilinear manner. These results suggest that another factor, besides ET, controls the observed enhancement of the emission of A due to reduction of R_{NA}. This effect may be due to protonation of A dependent on excited state proton transfer (ESPT) from N. In LB films probably a quasilinear proton gradient is formed with the H^+ concentration falling down to 0 at $R_{NA} > 30$ Å.

Some verification for this hypothesis may be provided by a study of the impact of acetic acid on the fluorescence of LB films.

TABLE 1: Fluorescence Band Maxima (*L*, nm) and Integrated Band Intensities (I, Relative Units) for LB Films of Various Distances between N and A (R_{NA})[a]

	Film layout R_{NA} [Å]	L1	L2	L3	L4	L5	I1[3]	I2	I3	I4	I5	C_A[2]	χ^2
1	T:N:A 6.2	377	448	469	536	636	1505	1530	224	5379	8712[3]	0.82	0.4
2	T:N:A 12.4	371	425.	509	600	610	3202	7280	2396	7412	1342	0.51	0.9
3	T:N: A 23.7	382	464	514	550	600	10778	3207	2271	0	0	0.14	1.1
4	T:N:A 6.2	378	460	-	551	630	2320	1709	-	11366	20	0.74	0.9
5	T: N: A 6.2	386	430	499	544	618	4091	296	1506	1119	3066	0.55	1.4
6	T:N:A 12.4	380	450	500	530	615	5361	3338	2529	2547	1524	0.43	1.5

[a] See discussion after eq 2 for the method of calculation of R_{NA}. [b] $C_A = (I3 + I4 + I5)/\Sigma(I)$. [c] Integration range of all bands was from 350 to 900 nm. Because our measuring spectral range is 300−600 nm, some bands were reconstructed in the full wavelength range by using deconvolution parameters (see inset to Figure 4).

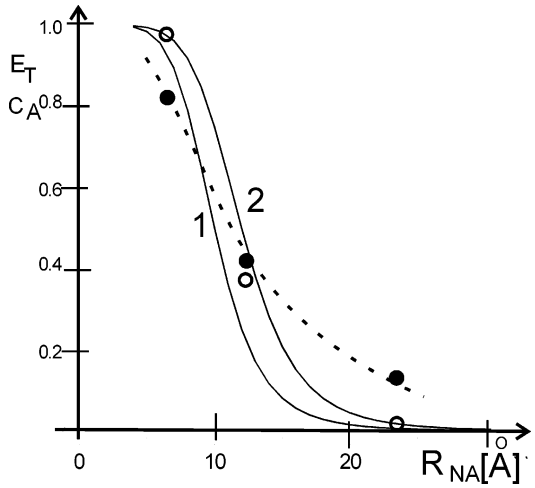


Figure 5. Dependence of energy transfer efficiency (E_T) and of A emission on R_{NA}. (E_T: solid lines, calculated by assuming $R_0 = 9$ Å (1) or 12 Å (2)). The luminescence of A (dashed line) is expressed as the contribution of A to the overall emission spectrum of a sample. C_A = IA/IF, where IA = $\int$F3 dλ + $\int$F4 dλ + $\int$F5 dλ and IF = $\int$F1 dλ + $\int$F2 dλ + $\int$F3 dλ + $\int$F4 dλ + $\int$F4 dλ + $\int$F5 dλ.

II.1.5. Influence of Acetic Acid on the Emission of LB Films Containing N and A. It has been shown[7] that acetic acid vapor prevents ESPT in 2-naphthol derivative included in LB films. Therefore, if the observed enhancement of A emission, in the presence of N, is caused by the reaction of A with protons released from N, this effect should disappear in the presence of acetic acid vapor.

In accord with this prediction for the sample of film layout no. 3 (R_{NA} = 23.7 Å) by admission of acetic acid vapor, the fluorescence signal over 500 nm disappears (cf. Figure 6, curve 1, and Figure 3, curve 1). However, for lower interchromophoric distances, considerable emission at 500−600 nm is observed (Figure 6, curves 2−3), even in acetic acid vapor. Each of the spectra in Figure 6 were separated into five component bands by fitting the log-normal function to our experimental data. The fit parameters were used to reconstruct component bands in all

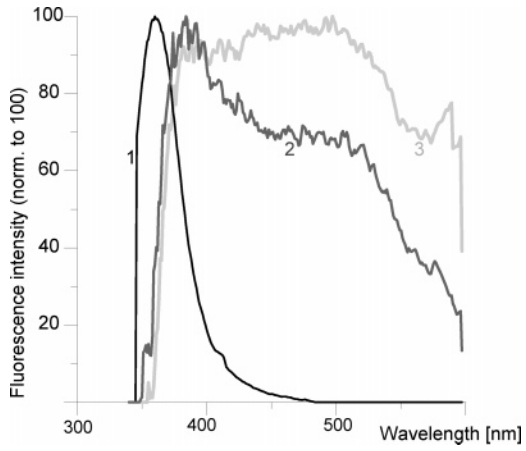


Figure 6. Influence of acetic acid vapor on the emission spectra of LB films containing N and A layers of the layout.

1) T N A No.3 in Fig.1

2) T N A No.4 in Fig.1

3) T N A No.5 in Fig.1

TABLE 2: Comparison of the Contribution of A to the Overall Emission of LB Films in Neutral Atmosphere (C_{NE}) and in Acetic Acid Vapor (C_{AA})

film layout	R_{NA}, Å	C_{NE}	C_{AA}	$C_{NE} - C_{AA}$
no. 3	23.7	0.139	0	0.139
no. 4	12.4	0.363	0.242	0.121
no. 5	6.2	0.816	0.599	0.217

the spectra in the range 340−900 nm (for reconstruction of the spectra in neutral atmosphere: see Figure 4, inset).

Integrated luminescence intensities (I_F) of the emission bands, in a neutral atmosphere, were compared (Table 2) with those in acetic acid vapor. It can be supposed that the contribution of A (C_A) to the luminescence of LB film in acetic acid vapor is proportional to the pure effect of energy transfer (ET) from the protonated (ROH*) form of N to A, because ESPT is inhibited

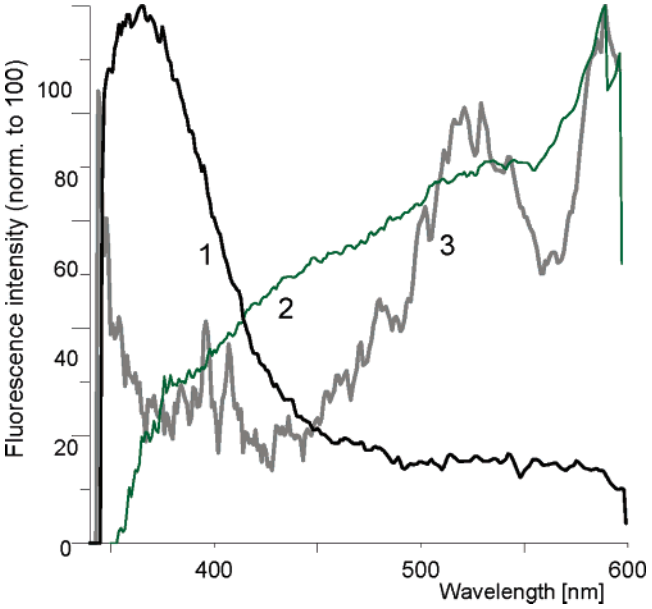


Figure 7. Influence of water on the fluorescence spectrum of a LB film of layout no. 5 in Figure 1 (R_{NA} = 6.2 Å): (1) after drying in a desiccator 48 h; (2) after drying in a desiccator 6 h; (3) in atmosphere saturated with water vapor.

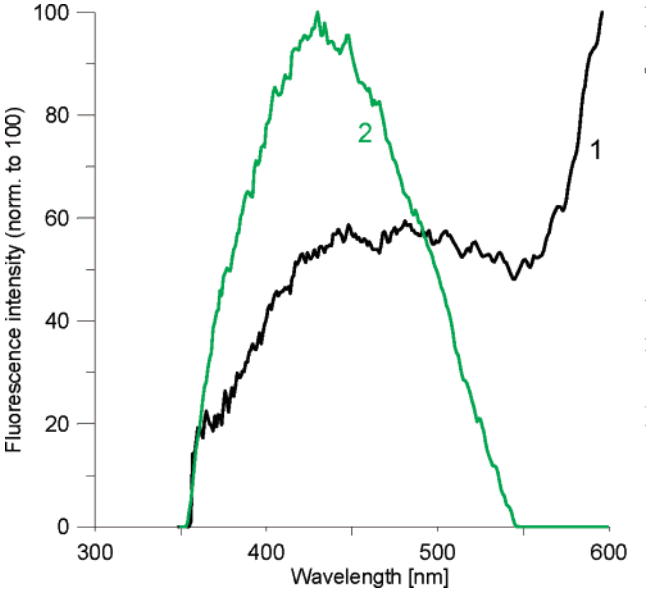


Figure 8. Influence of octadecylamine (ODA) on the efficiency of protonation and reduction of A: (1) film layout T:N+S:A+ODA; (2) film layout N+S:A+S. Addition of ODA to the layer of N has practically no influence on the spectrum. The molar fraction of N was 0.11, and that of A was 0.19.

at these conditions. The C_A value, in the neutral atmosphere, is considered to be proportional to the combined effects of proton transfer by ESPT and ET. The difference between C_A in neutral atmosphere (C_{NE}) and in acetic acid (C_{AA}) may be treated as a measure of the effect of ESPT dependent on R_{NA}. The values of C_{NE}, C_{AA}, and their difference are shown in Table 2.

Direct comparison of the signal intensity in neutral and acidic atmosphere might be spurious because interaction of acetic acid with the film leads to de-aggregation of fluorophores, resulting in a great (up to an order of magnitudes) increase of emission intensity.[7] The results of Table 2 prove that the contribution of A to the emission spectrum is always lower in acetic acid than in neutral atmosphere, and this confirms our view that the impact of the distance between N and A molecules in LB film, on the emission intensity of A, is partly (up to 60%) due to energy transfer and partly to proton migration from N to A. The dependence of $C_{NE} - C_{AA}$ on R_{NA} may be considered as an approximate gauge of proton gradient ($-dH^+/dR$) in LB film. This value appears to be the most negative at low R (near 6 Å) reach plateau at 7−24 Å and disappears at $R > 30$ Å (see also Figure 5). This picture differs from that in Figure 5. It must be noticed, however, that energy transfer (ET) contributes the values of C_A in Figure 5 and $C_{NE} - C_{AA}$ (Table 2) for film layout nos. 4 and 5 are probably underestimated with respect to C_A in Table 1 because of competition between ET and ESPT in neutral atmosphere disappearing in acid vapor.

II.2. Influence of Water on the Fluorescence of N and A in LB Films. The fluorescence spectra of LB film containing pure N and A, of layout no. 5 (Figure 1, R_{NA} = 6.2 Å), at various ambient humidities are shown in Figure 7. Preliminary NMR measurements indicate that water content in our samples (without drying) is considerable (7S, Supporting Information). A comparison of curve 1 to curve 3 of Figure 3 shows that by drying the LB sample, of the same film layout, over 48 h, the emission intensity of A at 500−600 nm dramatically decreases. This is easily understandible as it is known that protonation and reduction of A, which are necessary for its ability to show emission, are dependent on the presence of water.[16,17] An inspection of curve 2 of Figure 7 shows that drying of a LB film for only 6 h has a much lower impact on the luminescence spectrum. This picture may be explained by taking into account the literature data of the impact of water on the concentration of protonated and triplet states of anthraquinone-2-sulfonate (AQS)[17] in acetonitrile solution.

It follows from these results that protonation of AQS is enhanced initially by water addition, reaches a maximum, and decreases at a higher water content. It is possible, therefore, that in our experiments presented in curves 2−3 of Figure 7 the dependency of protonation of A on water content in our system approaches a plateau region. Relative growth of the band of the ionic form (RO^-*) of N, in the dried sample, is in contrast to the behavior of the fluorescence of LB films containing pure N, where opposite effect was observed.[7]

II.3. Luminescence Spectra of LB Films Containing A and/or N Mixed with Stearic Acid or Octadecylamine. If LB film is deposited from a mixture of N and A with stearic acid (SA), a completely different fluorescence pattern than that described above, is obtained (spectra not shown). Even at the lowest interchromophoric distance (R_{NA} = 6.2 Å) practically only one component, with the maximum at 473 nm, of low intensity, possibly due to A, may be distinguished (Figure 8and 8S, Supporting Information), and the luminescence spectrum is similar to the spectrum of the LB film containing N but without A (see ref 7). This may be explained by hindering the proton movement (and of ET) in the direction transverse to the molecular axes of N and A in the LB film, which would be required for protonation of A by H^+ released from N. However, if at the same film, layout S in the layer of A is replaced by octadecylamine (ODA, Figure 8 and 9S, Supporting Information), an increase of the signal at 500−600 nm, probably due to emission from A is observed.

We suppose that S probably plays a role of some trap for protons and ODA may function as electron donor enhancing reduction and in its consequence protonation of A. An addition of ODA to the layer of A can induce also some changes in the film structure promoting ESPT and/or ET. Addition of ODA to the layer of N gives practically no effect.

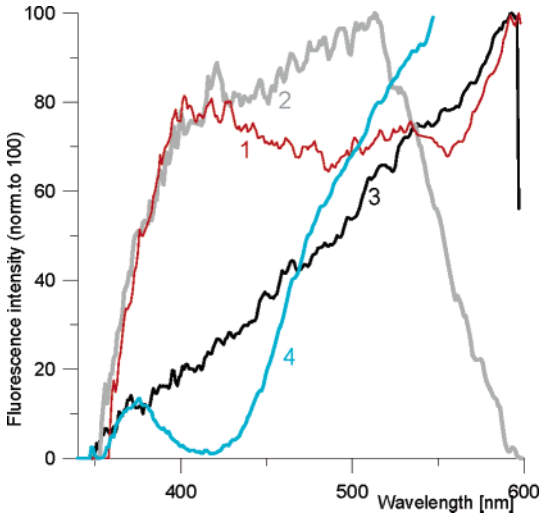


Figure 9. Luminescence spectra of a LB film containing N and A and gramicidin (G, molar fraction 0.01). In (1)−(3) the humidity of the atmosphere was 55%. (1) Film layout no. 1 (Figure 1), $R_{NA} = 23.7$ Å, S:N:A+G. (2) Film layout no. 4, $R_{NA} = 12.4$ Å, S:N:A+G. (3) Film layout no. 5, $R_{NA} = 6.2$ Å, N+G:A+G. (4) Film layout no. 5, $R_{NA} = 6.2$ Å, N+G:A+G, in H_2O vapor 100% saturation.

II.4. Influence of Gramicidin A on the Emission Spectra of LB Films Composed of N and A. Emission spectra of N and A, included in LB film, by addition of gramicidin to the layer of A are presented in Figure 9. Gramicidin A is a peptide antibiotic of amino acid sequence:

OHC-L-Val-Gly-L-LAla-D-Leu-L-Ala-D-Val-L-Val-D-
Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-
$NHCH_2CH_2OH$

It is known[21] that gramicidin transports protons through cell membranes by forming channels, of diameter 3 Å and length 30 Å, composed of two molecules oriented head to head. The rate of proton transport through the channel, usually very high (10^9 s^{-1}), depends strongly on membrane thickness and structure.[21] If protonation of A by ESPT takes place at all it must be promoted by gramicidin.

Confronting curve 1 in Figure 9 with curve 1 of Figure 3, concerning the sample with the same interchromophoric distance ($R_{NA} = 23.7$ Å), instantly shows that gramicidin (G) causes great enhancement of the emission component of A as compared to the sample without G. The same conclusion follows from the results of Table 3, where it is evident that in the presence of G, the sum of intensities of the emission bands due to A (I3−I5), increases as compared to analogous data (Table 1) for LB films of the same layout but without G.

An increase of the signal from A, in the spectra, induced by the presence of the protonophore in LB film, is analogous to the changes observed by reduction of R_{NA} (section 1.3). Both these effects should be attributed to the enhancement of the proton transport through the LB film from N to A. It should be noted, however, that the increase of the emission from A connected to a reduction of the distance to N, visible in Figure 9 and Table 3, in contrast to the analogous effect for samples without G, where strong contribution of ET must be allowed for—is probably due mainly to protonation of A by ESPT and not to ET, because it disappears in acetic acid: the spectrum in neutral atmosphere like that shown in Figure 9 (curve 3) is converted in acetic acid vapor to the form analogous to curve 1 in Figure 6 (see 10S, Supporting Information).

The lack of ET in the samples containing G may be due to successful competition of proton transfer with ET in the presence of G or to some influence of G on the film arrangement that is probably suggested by transfer isotherms (see 1S and 2S, Supporting Information) where it can be noticed that the surface pressure during transfer was lower and area per molecule is higher in the presence of the peptide than that without its addition.

The enhancement of the emission from the reduced forms of A, due to the protonophore, in laboratory atmosphere, seems to be greater for $R_{NA} = 23.7$ and 6.2 Å and less marked, or null for intermediate N−A distance. Greater influence of G at R_{NA} 23.7 than that at R_{NA} 12.4, may be explained by the length of the channel formed by the gramicidin dimer ($R = 30$ Å), which supplies protons in a more remote site than that where A is situated. In an atmosphere saturated with H_2O (Table 3), the impact of N on the emission from A rises with a decrease of R_{NA} analogously as for samples without G.

The promotion of the emission of the bands of A, by ESPT, due to addition of G, is also observed in LB films with the chromophores diluted by stearic acid (results not shown) where no considerable signal at wavelength values 500−600 nm is observed without G (8S, Supporting Information).

A remarkable feature of the luminescence spectra of LB films containing N, A, and G is also the inversion of the intensity ratio of the bands due to protonated (ROH*) and deprotonated (RO^-*) forms of N. Although for the samples without G (Table 1) in nearly all cases the intensity of RO^-* (I2) is higher than that of the ROH* form, on the contrary, in the spectra of LB films of a similar layout, but containing G, the ROH* band (I1, Table 3) dominates, and in 100% humidity by $R_{NA} = 6.2$ Å, the RO^-* band disappears. This observation indicates to a possibility of electron abstraction from RO^-* with oxidation of N to a nonfluorescent, quinoid form. This finding suggest that the deprotonated naphthol group can be a source of electrons for reduction of A in LB films investigated.

II.4.1. The Fluorescence of Tryptophan Residues of Gramicidin. The emission spectra in Figure 9 were taken at exciting light wavelength 320 nm, and therefore, the fluorescence of tryptophan residues in G is not observed, because of its lack of absorption ability at this spectral range. The spectrum of LB film composed of N, A, and G excited at 295 nm, where tryptophan is also excited, is shown in Figure 10 (unmarked curve).

The results of the resolution of this spectrum into component bands (curves 1−5) and error distribution (curve 6) are also shown. It can be supposed that if G is transporting protons, the peptide dynamics will be changed, leading to a spectral shift in luminescence. In coincidence with this prediction the emission band of tryptophan in G (Figure 10, curve 1) is red shifted with respect to that of LB film containing only G (band maximum at 330 nm, results not shown). Analogously, the fluorescence peak of N in the films containing G in most cases is red shifted with respect to that of the samples without G (cf. Table 1 and Figure 10, lines 2 and 3).

II.5. Emission Decay of LB Films Containing N and A. The lifetime (τ) of the excited state and amplitude (α) of the exponential decay function, of LB films containing N and A, obtained by excitation at 337 nm, and observed at various spectral ranges, are presented in Table 4.

Two lifetimes, near 0.7 and 1 ns observed at 420 and 580 nm, respectively, for the LB film containing only N (no. 1), are similar to those published previously[7] for analogous samples. As expected, the same decay components appear in

TABLE 3: Integrated Band Intensities (I)[a] in LB Films Containing N and A in the Presence of Gramicidin in the Layer of A in Neutral Laboratory Atmosphere (N) and in H_2O Vapor (H)

No.	R_{NA} [Å], film layout	I1	I2	I3	I4	I5	C_A	χ^2
1	6..2 (N)	237	345	56	11691	10377	0.97	2.9
2	12.4 (N)	3582	5275	5485	2420	18	0.47	0.9
3	23.7(N)	3936	5077	3183	4005	6441	0.60	0.9
4	6.2 (H)	206	28	4302	4876	11312	0.99	1.8
5	12.4 (H)	2524	1188	2528	3007	6040	0.76	1.5
6	23.7 (H)	5052	1943	3462	1266	7	0.40	0.8

[a] The numbers (1−5) standing by integral band intensities (I) refer to five emission bands present in our spectra, separated by our bands separation procedure.

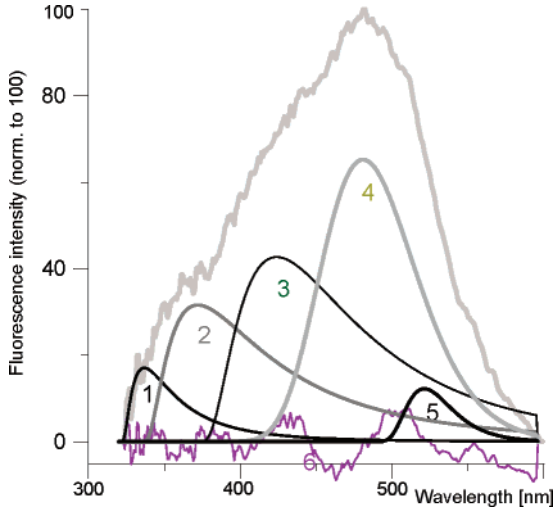


Figure 10. Emission spectrum of LB film containing N and A diluted with stearic acid (S) excited at 295 nm Film layout:

T:N+S+G:A+S+G, $\chi^2 = 0.663$, molar fraction N 0.098, A 0.19, G in N layer 0.024, G in A layer 0.022. F1 = 20, F2 = 37, F3 = 44, F4 = 64, F5 = 12, L1 = 339.7, L2 = 380, L3 = 438.5, L4 = 487, L5 = 526.5. The meaning of symbols is the same as in Figures 3 and 4.

the samples containing N and A. These lifetime values are probably due to 2 forms of N chromophore, quenched by exciton effects. The lifetime near 5 ns is analogous to that observed in solutions of 2-naphthol derivatives[9,12] and may be ascribed to some unquenched form of N that may be also present in an LB film.[7] The decay components near 7−8 ns on the grounds of analogy to solution spectra[12] may be ascribed to the unquenched naphtholate (RO^-*) but assignment to anthrahydroquinone ($AQSH_2$) must be taken into account because the RO^-* signal at 580, in the stationary spectra, is negligible.[12]

Very long-lived species, with lifetimes of 10−65 ns and of very low amplitudes (0.058), seem to be due to some triplet form of A. This long-lived component was found only by observation at 500−580 nm. Drying of a sample during 48 h gives great enhancement of long-lived components observed at 580 nm and invisible at 420 nm.

II.6. Electrometric Measurements. By applying voltage (0.2−40 V, dc) parallel to the surface of the film containing three layers of N and three layers of A, alternately deposited, in the presence of light (311 nm, Figure 10), electric current through the layers was observed. In the absence of light, practically no effect was noticed at the same conditions.

The current intensity (absolute value) varied considerably by changing the direction of the field applied (Figure 11). This effect is probably connected to a deviation of the molecular axes of N and A from the orientation perpendicular to the film surface and may be due to a superposition of an electric field created within the film by light with the applied voltage. A skew arrangement of molecules of such type was also found experimentally for some dyes in LB films.[22]

III. Discussion and Conclusions

The spectrofluorometric results presented above suggest that protons, dissociated from the excited N, in a LB film, may be transported to another molecular layer. Investigations of the emission decay and electric effects confirm this conclusion.

In the absence of gramicidin the protons released from N by ESPT are transferred through the film, probably by chains of hydrogen bonded water molecules[7,12] or another substance absorbed from the atmosphere. After some time delay, some H^+ ions reach another layer containing the anthraquinone derivative (A). They can approach excited A molecules and thus enhance their reduction leading to the appearance of the emitting species.

The sequence of reactions leading to the reduction of A can be represented by (a)−(c)[16,17] below:

$$AQS + e^- \rightarrow AQS^{\bullet -} \qquad \text{(a)}$$

$$AQS^{\bullet -} + H^+ \rightarrow AQSH^{\bullet} \qquad pK_a = 3.5^{17} \qquad \text{(b)}$$

$$AQSH^{\bullet} + H^+ + e^- \rightarrow AQSH_2 \qquad \text{(c)}$$

$$ROH^* \xrightarrow{\text{ESPT}} ROH^{-}* + H^+ \qquad \text{(d)}$$

Reaction (b) depends on pH. Therefore, production of $AQSH_2$ is expected to be enhanced by (d) even if the main source of $AQSH_2$ is disproportionation[16,17] of $AQSH^{\bullet}$ rather than (c). The source of electrons in (a) may be some reaction connected with photochemical production of $OH^{\bullet}$ radicals by the 1,4-anthraquinone group.[23,24]

We suppose that in a LB film, within the time interval exceeding the excited state lifetime of A, some quasi equilibrium

TABLE 4: Excited State Lifetimes of LB Films Containing N and A

Sample no., film layout and decay observation conditions	τ_1 [ns]	α_1	τ_2 [ns]	α_2	τ_3 [ns]	α_3	τ_4 [ns]	α_4	χ^2
1) S:N observed at 420 nm	0.721	0.843	5.32	0.157	-	-			1.02
1) S:N observed at 580 nm	1.064	0.827	8.18	0.172	-	-			1.07
2) S:N:A observed at 420 nm	0.793	0.942	4.97	0.058	-	-			1.07
2) S:N:A observed at 580 nm	0.058	0.081	0.840	0.866	4.97	0.053			1.01
3) S:N:A observed at 500 nm	1.08	51.5	7.28	0.067	25.1	0.159	65.8	0.226	35.0
3) S:N:A obs. at 580 nm. D[1)]	0.4	0.115	5.4	0.023	33.7	0.351	96.7	37.4	69.1
4) S:N:A observed at 420 nm	1.59	0.855	7.34	0.145	-	-			1.06
4) S:N:A observed at 580 nm	1.11	0.792	4.97	0.150	22.1	0.058			1.14

[a] The sample after drying in a desiccator 48 h.

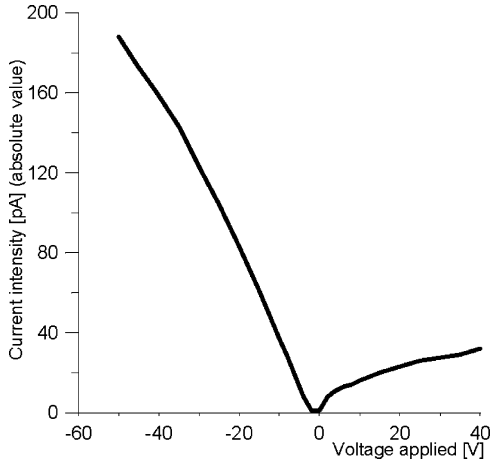


Figure 11. Current−voltage plot for LB film layout N+G:A+G:N+G:A+G:N+G:A+G (six layers) in head to tail arrangement

R_{NA} = 12.4 Å during illumination (311 nm): The separation of electrodes is 0.2 mm.

is established between processes such as ESPT in N, protonation of A, charge separation, and recombination, leading to reduction and protonation of A. Thereby, a nearly linear proton gradient, within the distance of about 30 Å across the layer, is formed.

Protonophores (section II.4), enhance greatly the efficiency of proton and electron transport in LB films. At these conditions the source of electron for the reduction of A may be charge abstraction from the excited naphtholate. Electrometric measurements suggest that even in this situation some charge separation may take place. The intensity of the luminescence of A, in the presence of the protonophore, does not depend linearly on R_{NA}; the dependence becomes a more complex one.

The proton movement in LB films in the direction perpendicular to the axes of the main structural elements is hindered (section II.3) but proton migration parallel to the molecular axes, both in and out of the layer, is equally probable. Because of the lack of directionality of charge transport in LB films, much lower efficiency of charge separation, than that in photosynthesis ($\Phi = 0.105$[25]), is to be expected.

Approximating the quantum efficiency of proton transport from N to A (Φ_{PT}) by Φ_A (formula 1) and using the fluorescence of N in a LB film as a standard ($\Phi_{St} = 0.1$), we obtain the expression for very rough estimation of $\Phi_A = (F_A/F_N)0.1$. Taking into account that without G only 12−20% (Table 2) of the value of C_A is connected with ESPT, one obtains for $R_{NA} = 6.1$ Å $\Phi_{PT} = 0.015$, for $R_{NA} = 12.3$ Å $\Phi_{PT} = 0.005$, and for $R_{NA} = 23.7$ Å $\Phi_{PT} = 0.001$.

It is evident that the efficiency of proton transport from N to A in LB films is rather low. However, some our results presented above (see Figures 8 and 9 and S6, Supporting Information) open the possibility of improving the efficiency by optimizing the film arrangement, composition, and sample humidity and by addition of charge transporting elements (as ion channels) and of suitable electron donors. Obviously, further work would be required.

Investigation of the model presented may help in understanding the coupling between proton and electron transport in biological membranes and other energy transforming devices.[1,2] The systems of the type presented above may also find an application in molecular switches[26] because of the various responses (spectral, electric) of the sample, dependent on parameters, that can be controlled, such as atmosphere composition and the distance between chromophores.

Abbreviations

N: 2-naphtholo-6-sulfonamide of dodecylamine
A: 1,4-anthraquinono-2-sulfonamide of dodecylamine
T: tricosanoic acid
S: stearic acid
G: gramicidin A
ESPT excited state proton transfer
ROH*: Excited molecule of N in the protonated form
RO^-*: Excited molecule of N in the ionized form
AQS: 1,4-anthraquinono2-sulfonic acid sodium salt
$AQSH_2$: antrahydroquinone
AQSS: semiquinone of AQS
AQSA: aninon of AQSS

Supporting Information Available: Transfer isotherms of substances deposited. Results of calculation of R_0 and J_{DA} for various combinations of donor (N) and acceptor (A) forms. Efficiencies of energy transfer (E_T) calculated from eq 3. Details of the system used for electrometric measurements. Free precession signal of the protons of water in the preparation of N. Luminescence spectrum of the LB film containing N and A diluted by stearic acid, deconvoluted into component bands. Luminescence spectrum of the LB film containing N, A diluted by ODA, deconvoluted into component bands. Luminescence spectra of LB film containing N and A and G in neutral atmosphere and in acetic acid. This material is available free of charge via the Internet at http://pubs.acs.org.

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