Anellated Hemicyanine Dyes with Large Symmetrical Solvatochromism of Absorption and Fluorescence

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A novel class of amphiphilic hemicyanine dyes is described where electron-pushing aniline and electron-pulling pyridinium are joined by anellated benzene rings. Enhancing the solvent polarity, the absorption band of these ANNINE dyes is shifted to the blue and the fluorescence band is shifted to the red at an invariant 00 energy. The increasing Stokes shift spans the whole visible spectrum. The divergent symmetrical solvatochromism of the positively charged chromophores is parametrized by a monopole−dipole model using a Born−Onsager−Marcus approach. From the intramolecular charge shift that is induced by electronic excitation, the linear Stark effect that is expected when the ANNINE dyes are used as voltage-sensitive probes in a biomembrane is estimated.

Introduction

Amphiphilic hemicyanine dyes are frequently used as fluorescent probes of voltage transients in neuron membranes.[1−3] Most popular are the styryl-type hemicyanines where aniline as an electron donor is joined to pyridinium as an electron acceptor by one, two, or three conjugated CC double bonds.[4−7] In bulk polar solvents, these dyes exhibit a symmetrically divergent solvatochromism with a blue shift of absorption and a red shift of emission that indicate a displacement of electrical charge from the pyridinium toward the aniline moiety.[8] It was postulated that such a charge displacement by electronic excitation gives rise to a molecular Stark effect for dyes in biomembranes with an identical shift in excitation and emission spectra and that the concomitant change of fluorescence intensity may be applied to the sensitive optical recording of neuronal excitation.[9] However, detailed spectrally resolved measurements of voltage sensitivity revealed that the fluorescence of styryl dyes in neuronal membranes is modulated in a rather complicated manner with different shifts of excitation and emission spectra and with changes of spectral shape and fluorescence quantum yield.[10−13]

The photophysical dynamics of styryl-type hemicyanines is rather involved because of their flexible carbon chain between the electron-pushing and electron-pulling moieties. In the electronically excited state, the molecules may undergo photoisomerism around the CC double bonds and photorotamerism around the CC single bonds.[14] To restrict the photoresponse of the dyes, the free CC double bonds were eliminated by including them in aromatic ring systems. Aniline and aminonaphthalene as electron donors were joined by a free CC single bond to pyridinium and isoquinolinium as electron acceptors.[15,16] These biaryl-type hemicyanines also exhibited a symmetrically divergent solvatochromism, which indicated an even stronger intramolecular displacement of charge than in the homologous styryl dyes.[8] But the rotamerism around the central CC single bond of the biaryls still leads to a twisted internal charge transfer (TICT) in the excited state, a process that could affect voltage sensitivity. To eliminate photorotamerism, the CC single bond of the simplest biaryl hemicyanine—aminophenylpyridinium—was bridged, and the TICT process was blocked.[17,18] On the basis of these results, it seemed possible to develop hemicyanine dyes for optical recording in neuron membranes that are free of photoisomerism and photorotamerism.

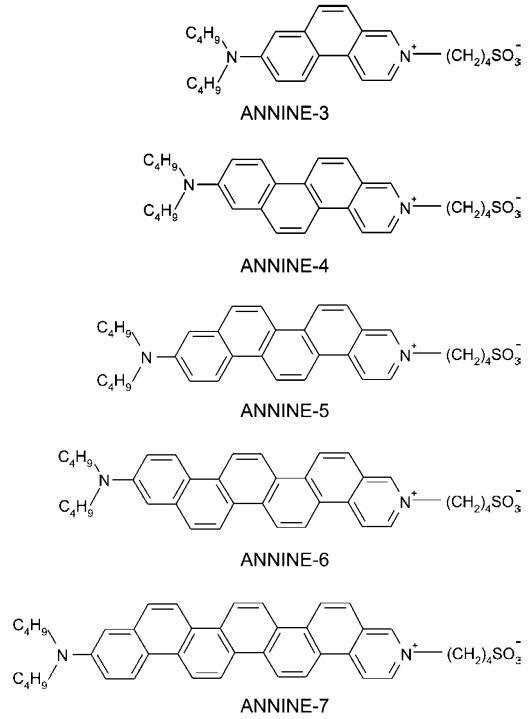

Figure 1. Hemicyanine dyes with three to seven anellated benzene rings (ANNINE-3 to ANNINE-7).

In the present paper, we describe a series of amphiphilic hemicyanines without free CC double bonds and CC single bonds. They consist of three to seven anellated benzene rings with integrated aniline and pyridinium as electron-pushing and electron-pulling moieties as shown in Figure 1. We describe

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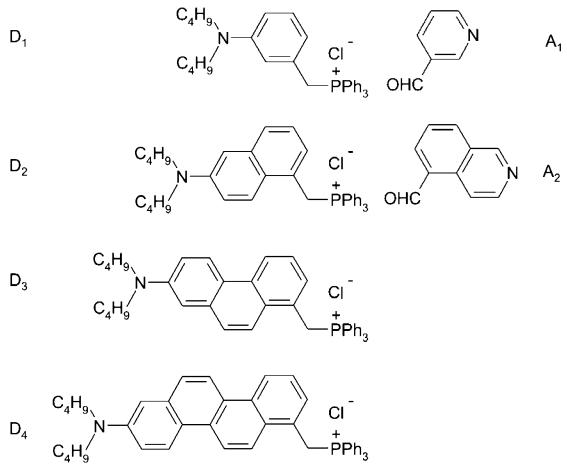

Figure 2. Scheme of the synthesis of the ANNINE dyes. The donor moieties D1−D4 and the acceptor moieties A1 and A2 are synthesized separately. The chromphores are formed in a Wittig reaction and by subsequent ring closure in a photochemical reaction. Finally, butylsulfonate groups are attached.

the synthesis of these ANellated hemicyaNINE dyes and characterize their spectra in a series of bulk solvents with increasing polarity. We evaluate their solvatochromism in terms of the classical concepts of Born, Onsager, and Marcus.[32−39] On that basis, we estimate the molecular Stark effect that is expected for the dyes bound to a neuron membrane. The voltage sensitivity of the ANNINE dyes in neurons is described in a subsequent paper.[19]

Materials and Methods

Synthesis. The ANNINE dyes were assembled by combining four different donor moieties (D_1-D_4) with two different acceptor moieties (A_1-A_2) as illustrated in Figure 2. Details of the synthesis are described in the Appendix.

Triphenylphosphonium salt D_1 was obtained in four steps from 3-aminobenzoic acid via 3-*N*,*N*-dibutylaminobutyl benzoate (1-iodobutane and K_2CO_3),[20] 3-*N*,*N*-dibutylaminobenzyl alcohol ($LiAlH_4$), 3-*N*,*N*-butylaminobenzyl chloride (with PCl_5), and subsequent treatment with PPh_3. This method is superior to the synthesis using 3-aminobenzaldehyde dimethyl acetal as a starting material.[18] For the synthesis of D_2, we brominated 2-nitronaphthalene; the resulting 1-bromo-6-nitronaphthalene[21,22] was reduced to 6-amino-1-bromonaphthalene ($SnCl_2$, HCl). Alklyation with 1-iodobutane leads to 1-bromo-6-*N*,*N*-dibutylaminonaphthalene.[20] Via 6-*N*,*N*-dibutylamino-1-cyanonaphthalene[23] (CuCN) and 6-*N*,*N*-dibutylaminonaphthalene-1-carboxylic acid (NaOH), we obtained by reduction ($LiAlH_4$) 6-*N*,*N*-dibutylamino-1-hydroxymethylnaphthalene.[24] (A direct synthesis starting from 1-bromo-6-*N*,*N*-dibutylaminonaphthalene via the lithiated intermediate and subsequent reaction with formaldehyde was less effective.) After the bromination (PBr_3)[24] of the alcohol, treatment with PPh_3 resulted in D_2.

To obtain D_3, we joined D_1 to 2-methoxymethylbenzaldehyde[25] by a Wittig reaction. Photocyclization of the alkenes *E*/*Z*-2-(3-*N*,*N*-dibutylaminostyryl)benzylmethyl ether produced 7-*N*,*N*-dibutylamino-1-methoxymethylphenanthrene. By ether cleavage, we obtained 7-*N*,*N*-dibutylamino-1-bromomethylphenanthrene, and with triphenylphosphine, product D_3. Starting from D_2, the same reaction sequence was performed to obtain D_4.

Acceptor moiety A_1 was commercially available. A_2 was obtained from 5-bromoisoquinoline[26] (BuLi then DMF).[27,28] Proper donor and acceptor moieties were chosen for Wittig reactions and subsequent photocyclization[29] to build up the scaffold of the ANNINES. For example, D_3 and A_2 were used for the formation of the six anellated rings of ANNINE-6. The last synthetic step was the reaction with 1,4-butane sultone. The structures of all dyes and intermediates were confirmed by mass spectrometry and ^{1}H NMR.

Solutions. We studied the absorption and fluorescence spectra in 17 solvents of increasing polarity: chloroform, decanol, benzyl alcohol, dichloromethane, octanol, cyclohexanol, hexanol, pentanol, cyclopentanol, butanol, propanol, ethylene glycol, dimethylformamide, acetone, ethanol, acetonitrile, and methanol. Solvents of the highest available purity were used (Merck, Darmstadt, Germany; Sigma, Taufkirchen, Germany). Stock solutions were made from methanol and chloroform in a volume ratio of 1:2 at a concentration of 1 mM for ANNINE-3, ANNINE-4, and ANNINE-5 and, because of lower solubility, 200 μM for ANNINE-6 and ANNINE-7. These stock solutions were diluted by the different solvents to a final concentration of 5 μM, with the exception of ANNINE-7 in pentanol, butanol, acetone, and acetonitrile where a concentration of 1 μM was used.

Spectra. Absorption spectra were measured with a Varian Cary 3E spectrometer (Varian, Mulgrave, Victoria, Australia) at room temperature. Emission spectra were measured with an SLM Aminco 8100 fluorescence spectrometer that was adapted to the long-wavelength region with a Spectra Pro-275 monochromator from Acton Research (Acton, MA) containing appropriate gratings for the wavelength region up to 1200 nm and an avalanche photodiode (APD) as a detector (Polytec, Waldbronn, Germany). The spectrometer was calibrated with a 45-W quartz−halogen tungsten coiled filament lamp (OL 245M, Optronic Laboratories, Orlando, FL) as a standard of spectral irradiance.[30] Calibration data beyond 1100 nm were specially provided by the manufacturer. The calibration of the fluorescence spectrometer required an Ulbricht sphere in the sample housing. Because no spectral data above 780 nm for the sphere were obtained from the manufacturer, the Ulbricht sphere was calibrated for the long-wavelength region with the standard lamp and the Spectra Pro-275 monochromator. First, we obtained the sensitivity of the emission monochromator together with the APD detector by positioning the lamp in front of the monochromator. Then we mounted the Ulbricht sphere to the entrance slit of the Spectra Pro-275 monochromator and the calibration lamp to the entrance hole of the Ulbricht sphere. Again, lamp spectra were measured. From these two measurements, the scattering efficiency of the Ulbicht sphere was obtained for the long-wavelength regime. These data were used to extend the spectral data obtained from the manufacturer to the whole spectral range. Finally, we connected the Spectra Pro-275 monochromator, the Ulbricht sphere, and the calibration lamp to the fluorescence spectrometer. From the scattering efficiency of the Ulbicht sphere and the spectrum of the calibration lamp, the sensitivity of the spectrometer was obtained. Calibration and measurement of the spectra were performed under magic angle conditions.[30] For ANNINE-3, the excitation bandwidth was 8 nm, and the emission bandwidth was 18 nm. For ANNINE-4, ANNINE-5, ANNINE-6, and ANNINE-7, we used an excitation bandwidth of 16 nm and an emission bandwidth of 18 nm.

Evaluation of Maxima. The maxima of the absorption spectra were automatically calculated by the Varian Cary software. To evaluate the fluorescence maxima, we fitted a log-normal curve to each spectrum[31] according to eq 1 with an amplitude $F_{\bar{\nu}}^{\max}$, a spectral maximum $\bar{\nu}^{\max}$, a spectral width W,

and a spectral asymmetry b, excluding the contribution of the S_0/S_2 transition.

$$F_{\bar{\nu}}(\bar{\nu}) = F_{\bar{\nu}}^{\max} \exp\left\{ - \frac{\ln^2[1 + \{2b(\bar{\nu} - \bar{\nu}^{\max})\}/\{W\}]}{\{b^2\}/\{\ln 2\}} \right\} \quad (1)$$

The maximum was taken from the fit. Only the spectral region around the maximum was used for solvents, where the second-order Rayleigh peak appeared on the flank of the spectrum.

Results

Solvatochromism. Wavenumbers $\bar{\nu}_{abs}$ and $\bar{\nu}_{em}$ of the maxima of absorption and emission, respectively, of the five hemicyanine dyes ANNINE-3 to ANNINE-7 in 17 solvents are plotted in Figure 3. The solvents are characterized by the polarity function $F_1(\epsilon,n)$ defined by eq 2[32] that depends on the static relative dielectric constant ϵ and the refractive index n of the solvent and on an intramolecular dielectric constant ϵ_i.

$$F_1(\epsilon,n) = \frac{1}{\epsilon_i}\left(\frac{\epsilon_i - n^2}{\epsilon_i + 2n^2} - \frac{\epsilon_i - \epsilon}{\epsilon_i + 2\epsilon} \right) \quad (2)$$

The choice of this particular polarity function will become clear in the discussion. The values of $F_1(\epsilon,n)$ with $\epsilon_i = 2$ are the following: chloroform 0.112, decanol 0.162, benzyl alcohol 0.170, dichloromethane 0.172, octanol 0.178, cyclohexanol 0.192, hexanol 0.197, pentanol 0.201, cyclopentanol 0.202, butanol 0.214, propanol 0.222, ethylene glycol 0.227, dimethylformamide 0.227, acetone 0.229, ethanol 0.234, acetonitrile 0.248, and methanol 0.250.

With all dyes, there is a common trend for absorption and fluorescence: increasing polarity shifts the absorption spectra to the blue and the emission spectra to the red. For each dye, this divergent solvatochromism is rather symmetrical over the whole range of accessible polarity (i.e., it seems that the solvatochromism can be assigned to an increasing Stokes shift $\bar{\nu}_{abs} - \bar{\nu}_{em}$ with an invariant average $(\bar{\nu}_{abs} + \bar{\nu}_{em})/2$). It should be noted that with ANNINE-7 there were serious problems with the solubility. We suspect that molecular aggregates may exist in some solvents.

Discussion

Charged Chromophores. The classical theory of solvatochromism by Lippert[33] and Mataga[34] refers to electrically neutral molecules where electronic excitation changes the dipole moment. A divergent, symmetrical shift of excitation to the blue and of emission to the red is predicted when the dipole moment is inverted, as considered by Liptay.[35] However, for the ANNINE molecules, we expect an increase in the dipole moment by excitation due to a displacement of positive charge from pyridinium toward aniline with increasing distance from the negative sulfonate group. Considering a similar discrepancy for hemicyanines of the styryl and biaryl classes, it was suggested that the solvatochromism of the neutral hemicyanines must be assigned to the positively charged chromophore alone, neglecting the sulfonate appendix.[8,15] A consistent treatment can be obtained with a monopole−dipole model[8] using a framework described by Brunschwig, Ehrenson, and Sutin[32] that relies on the concepts of Born,[36] Onsager,[37] and Marcus.[38,39]

Born−Onsager−Marcus Approach. Wavenumbers $\bar{\nu}_{ex}$ and $\bar{\nu}_{em}$ for the Franck−Condon transitions of excitation and emission are given by the excitation energy of the vibrationally relaxed molecule in the vacuum $hc\bar{\nu}_{00}$ (h is Planck's constant, c is light velocity) by the difference $w_E - w_G$ of the solvation energies for the relaxed excited state and ground state, and by the addition and subtraction of the intramolecular reorganization energy λ_{in} and the reorganization energy of solvation λ_{solv}, respectively. The latter is estimated with the help of a virtual molecular state E−G defined by the difference in the charge distributions of the excited state and the ground state.[38,39] We obtain eqs 3 and 4 with $\lambda_{solv} = w_{E-G}(n^2) - w_{E-G}(\epsilon)$, the work required to transfer the virtual molecule from a relaxed solvent with relative permittivity ϵ to a nonrelaxed solvent with optical permittivity n^2.

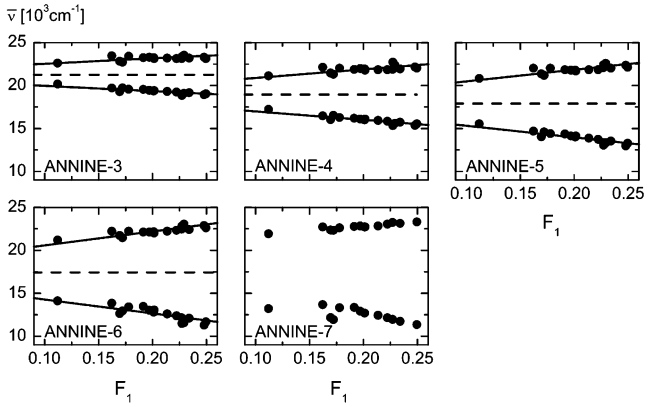

Figure 3. Spectral data of ANNINE dyes in polar solvents. The maxima of the absorption spectra (wavenumber $\bar{\nu}_{ex}$, upper dots) and the fluorescence spectra (wavenumber $\bar{\nu}_{em}$, lower dots) are plotted versus the polarity function F_1 of 17 solvents. The straight lines are obtained from the fits of $(\bar{\nu}_{ex} + \bar{\nu}_{em})/2$ and $(\bar{\nu}_{ex} - \bar{\nu}_{em})/2$ in Figure 4 using a monopole−dipole model. The dashed lines are the averages of the fitted relations for the excitation and emission wavenumbers.

$$hc\bar{\nu}_{ex} = hc\bar{\nu}_{00} + [w_E - w_G] + \lambda_{in} + [w_{E-G}(n^2) - w_{E-G}(\epsilon)] \quad (3)$$

$$hc\bar{\nu}_{em} = hc\bar{\nu}_{00} + [w_E - w_G] - \lambda_{in} - [w_{E-G}(n^2) - w_{E-G}(\epsilon)] \quad (4)$$

To compute the work of solvation w_j of the different species j, we use a monopole−dipole model with point charges q_j and point dipoles μ_j in the center of a sphere with radius a and internal permittivity ϵ_i.[8,32] With the vacuum permittivity ϵ_0 and the relative permittivity of the solvent $\epsilon_{solv} = \epsilon$ or $\epsilon_{solv} = n^2$, we obtain eq 5.[36,37]

$$w_j(\epsilon_{solv}) = \frac{q_j^2}{8\pi\epsilon_0 a}\left(\frac{1}{\epsilon_{solv}} - 1 \right) + \frac{\mu_j^2}{4\pi\epsilon_0 a^3}\frac{1}{\epsilon_i}\left(\frac{\epsilon_i - \epsilon_{solv}}{\epsilon_i + 2\epsilon_{solv}} - \frac{\epsilon_i - 1}{\epsilon_i + 2} \right) \quad (5)$$

When electronic excitation displaces an elementary charge e_0 from a position $\delta_{EG}/2$ to a position $-\delta_{EG}/2$ off center, the charge distribution in the ground state can be described by $q_G = e_0$ and $\mu_G = e_0\delta_{EG}/2$, in the excited state, by $q_E = e_0$ and $\mu_E = -e_0\delta_{EG}/2$, and in the virtual state, by $q_{E-G} = 0$ and $\mu_{E-G} = -e_0\delta_{EG}$. The charge and squared dipole moment are identical in the excited and ground states. Considering the uncharged virtual state where $\mu_{E-G}^2 = e_0^2\delta_{EG}^2$, we obtain from eqs 3 and 4 for the sum and difference of the wavenumbers of

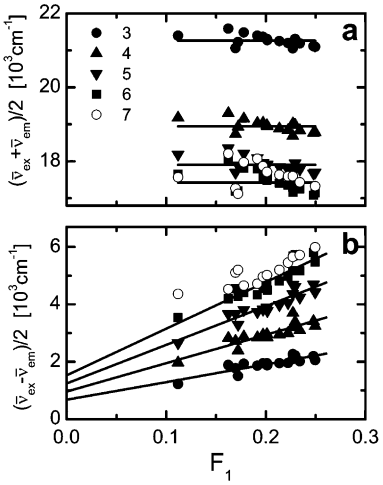

Figure 4. Spectral features of the ANNINE dyes in polar solvents. (a) Average $(\bar{\nu}_{ex} + \bar{\nu}_{em})/2$ of the spectral maxima of absorption and fluorescence versus polarity function F_1. The data for ANNINE-3 to ANNINE-6 are fitted with a constant wavenumber $\bar{\nu}_{00}$ for the 00 energy in vacuum. (b) Half of the Stokes shift $(\bar{\nu}_{ex} - \bar{\nu}_{em})/2$ versus polarity function F_1. The data for ANNINE-3 to ANNINE-6 are fitted by linear relations with slopes $\Delta\bar{\nu}_{solv}$ that characterize the solvatochromic sensitivity.

excitation and emission eqs 6 and 7 with $\bar{\nu}_{in} = \lambda_{in}/hc$ and with the polarity function $F_1(\epsilon,n)$ defined by eq 2.

$$\frac{\bar{\nu}_{ex} + \bar{\nu}_{em}}{2} = \bar{\nu}_{00} \tag{6}$$

$$\frac{\bar{\nu}_{ex} - \bar{\nu}_{em}}{2} = \bar{\nu}_{in} + \Delta\bar{\nu}_{solv}F_1(\epsilon,n) \qquad \Delta\bar{\nu}_{solv} = \frac{e_0^2}{4\pi\epsilon_0 hc}\frac{\delta_{EG}^2}{a^3} \tag{7}$$

The average wavenumber of absorption and emission is independent of solvent polarity and reflects the excitation energy of the relaxed molecule in the vacuum. The difference in absorption and emission wavenumbers is a linear function of the polarity function $F_1(\epsilon,n)$. Increasing polarity shifts the wavenumbers $\bar{\nu}_{ex}$ and $\bar{\nu}_{em}$ of the absorption and emission, respectively, in opposite directions with slopes $\pm\Delta\bar{\nu}_{solv}$.

Solvatochromism. From the experimental wavenumbers of absorption and emission in Figure 3, we evaluate the averages $(\bar{\nu}_{ex} + \bar{\nu}_{em})/2$ and differences $(\bar{\nu}_{ex} - \bar{\nu}_{em})/2$ and plot them versus $F_1(\epsilon,n)$. As shown in Figure 4a and b, the values of $(\bar{\nu}_{ex} + \bar{\nu}_{em})/2$ vary only by a few percent over the whole range of polarity, whereas the values of $(\bar{\nu}_{ex} - \bar{\nu}_{em})/2$ increase rather linearly. Of course, the invariance and linear increase are not perfect because the solvation by molecules of very different chemical structures cannot be completely described by a bulk dielectric constant and a bulk refractive index.

We fit the data of $(\bar{\nu}_{ex} + \bar{\nu}_{em})/2$ for ANNINE-3 to ANNINE-6 in Figure 4a by a constant 00 energy $\bar{\nu}_{00}$ according to eq 6 and the data of $(\bar{\nu}_{ex} - \bar{\nu}_{em})/2$ by straight lines according to eq 7. The 00 energies $\bar{\nu}_{00}$ decrease as summarized in Table 1. The slopes $\Delta\bar{\nu}_{solv}$ increase with the size of the chromophores as summarized in Table 1 and plotted in Figure 5. For the data of ANNINE-7, a meaningful fit is not possible.

The solvatochromic sensitivities $\Delta\bar{\nu}_{solv}$ of ANNINE 4, 5, and 6 are distinctly higher than those of the homologous styryl dyes RH364, RH-160, and RH-237 as shown in Figure 5.[8] That difference indicates a larger intramolecular charge displacement δ_{EG} in the ANNINE dyes according to eq 7. However, a value of the charge displacement could be assigned only if we knew the effective radius a of the chromophores.

TABLE 1: Solvatochromic Parameters of ANNINE-3 to ANNINE-6[a]

	$\bar{\nu}_{00}$ [cm^{-1}]	$\bar{\nu}_{in}$ [cm^{-1}]	$\Delta\bar{\nu}_{solv}$ [cm^{-1}]
ANNINE-3	21 261	672	6179
ANNINE-4	18 946	956	9916
ANNINE-5	17 905	1239	13 494
ANNINE-6	17 427	1523	16 276

[a] Wavenumber $\bar{\nu}_{00}$ of the 00 energy in vacuum, wavenumber $\bar{\nu}_{in}$ of the intramolecular reorganization energy, and solvatochromic sensitivity $\Delta\bar{\nu}_{solv}$.

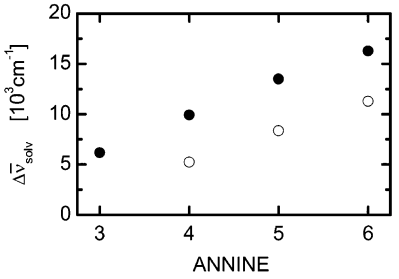

Figure 5. Solvatochromic sensitivity $\Delta\bar{\nu}_{solv}$ of ANNINE-3 to ANNINE-6 (●) and of the styryl hemicyanines RH-364, RH-160, and RH-237 (○) that are homologous to ANNINE-4 to ANNINE-6.

Electrochromism. When a dye with an intramolecular charge displacement δ_{EG} is placed in an external electric field, its absorption and emission bands are shifted by a linear molecular Stark effect. Amphiphilic hemicyanine dyes can be bound to the outer surface of cell membranes such that their intramolecular displacement of charge has a component $\delta_{EG}\cos\vartheta$ along the membrane normal. The change in the electric field is defined by the change in the intracellular voltage ΔV_M and the membrane thickness d_M. For charge displacement against the electric field, the spectral shift is given by eq 8.

$$\Delta\bar{\nu}_{electro} = \frac{\Delta V_M}{d_M}\frac{e_0}{hc}\delta_{EG}\cos\vartheta \tag{8}$$

By eliminating the charge displacement with eq 7, we obtain a general relation of electrochromic and solvatochromic sensitivity according to eq 9.

$$\Delta\bar{\nu}_{electro} = \frac{\Delta V_M}{d_M}\sqrt{\frac{4\pi\epsilon_0}{hc}\Delta\bar{\nu}_{solv}}\,a^{3/2}\cos\vartheta \tag{9}$$

However, there are two problems with a quantitative prediction of electrochromism from solvatochromism: (i) the uncertainty about the effective molecular radius a in bulk solvents and (ii) the uncertainty about the orientation $\cos\vartheta$ in the membrane. For illustration, we plot the expected electrochromic shift $\Delta\bar{\nu}_{electro}$ in a membrane of thickness $d_M = 4$ nm and a voltage change $\Delta V_M = 100$ mV versus the solvatochromic sensitivity $\Delta\bar{\nu}_{solv}$ in Figure 6 for three different radii $a = 5$, 7, and 9 Å assuming a perfect orientation of $\cos\vartheta = 1$.

The experimental solvatochromic sensitivities $\Delta\bar{\nu}_{solv}$ of the ANNINE dyes are marked by vertical lines in Figure 6. The experimental electrochromic shifts of ANNINE-5 and ANNINE-6 in a neuron membrane are indicated by dots.[19] The electric responses in the neuron are in a range predicted by solvatochromism, yet the increase in electrochromism from ANNINE-5 to ANNINE-6 is stronger than expected for constant orientation $\cos\vartheta$ and constant effective radius a. That tendency may be due to increasing orientation and increasing effective radius with the size of the chromophore.

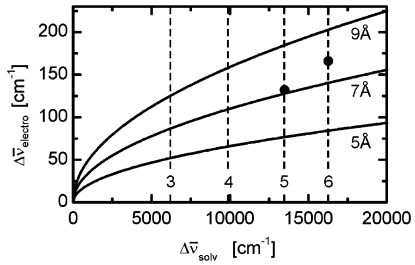

Figure 6. Electrochromism and solvatochromism. The electrochromic shift $\Delta\bar{\nu}_{electro}$ expected for a voltage change of $\Delta V_M = 100$ mV across a membrane of thickness $d_M = 4$ nm is plotted versus the solvatochromic sensitivity $\Delta\bar{\nu}_{solv}$ in polar solvents. The intramolecular displacement of electric charge is assumed to be in the direction of the membrane normal with $\cos\vartheta = 1$ against the direction of the electric field. Three different effective molecular radii $a = 5$, 7, and 9 Å are considered. The experimental solvatochromic sensitivities of ANNINE-3 to ANNINE-6 are indicated by four vertical lines (dashed). Dots mark the experimental electrochromism of ANNINE-5 and ANNINE-6 in a neuron membrane.

Conclusions

Hemicyanine dyes with anellated benzene rings exhibit no photorotamerism and no photoisomerism around CC double bonds and CC single bonds. Their solvatochromism in bulk solvents indicates an intramolecular displacement of electric charge that is distinctly larger than in homologous styryl-type hemicyanines. They are expected to be simple and the most efficient probes for voltage transients in biological membranes on the basis of the linear Stark effect. In addition, the ANNINE dyes may be used as simple probes of polarity and local electric fields in colloids and polymers. Also, strong nonlinear optical effects are expected.

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Appendix

Synthesis of the ANNINE Dyes. 3-*N*,*N*-Dibutylaminobutyl Benzoate (1).[20] 1-Iodobutane (114.3 mL, 1 mol) was added to a mixture of 3-aminobenzoic acid (Fluka, Switzerland, 34.3 g, 250 mmol) in 250 mL of DMF and K_2CO_3 (103.5 g, 750 mmol). The mixture was stirred at 100 °C overnight and distributed between EtOAc and water. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. Pure **1** (64.9 g, 85% yield, colorless oil) was obtained by distillation (bp 153 °C, 0.01 mbar).

3-*N*,*N*-Dibutylaminobenzyl Alcohol (2). To a magnetically stirred mixture of $LiAlH_4$ (5.3 g, 140 mmol) in 200 mL of anhydrous diethyl ether was added a solution of **1** (74.1 g, 240 mmol) in 200 mL of anhydrous diethyl ether dropwise. The mixture was stirred overnight and then quenched by the addition of ice-cold water and triturated with 40% NaOH. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. Distillation provided **2** (oil, bp 105−110 °C, 0.01 mbar, 91% yield).

3-*N*,*N*-Dibutylaminobenzyl Chloride (3). PCl_5 (46.0 g, 220 mmol) was added in portions to **2** (52.3 g, 220 mmol). The black, viscous mixture was stirred at 100 °C for 1 h and then at room temperature overnight. The reaction mixture was quenched with ice and diluted ammonia and extracted with ether. The extract was washed with brine and dried (Na_2SO_4), and the solvent was evaporated. Chromatography (SiO_2, EtOAc/heptane 18:5) provided pure **3** (oil, bp 95 °C, 0.01 mbar, yield 79%).

3-*N*,*N*-Dibutylaminobenzyltriphenylphosphonium Chloride (4). A solution of **3** (19.4 g, 152 mmol) and PPh_3 (40 g, 152 mmol) in 120 mL of toluene was refluxed for 16 h at 110 °C. Upon cooling, the white precipitate was collected, washed with Et_2O, and dried for 24 h at 50 °C in vacuo to yield pure **4** (mp 165 °C, yield 63%).

1-Bromo-6-*N*,*N*-dibutylaminonaphthalene (5). The synthesis was performed similarly to that previously described for 6-bromo-2-*N*,*N*-dibutylaminonaphthalene.[20] A mixture of 6-amino-1-bromonaphthalene[21] (29.15 g, 131.2 mmol obtained from 2-nitronaphthalene (Lancaster, U.K.) via 1-bromo-6-nitronaphthalene[21,22] and subsequent reduction), 1-iodobutane (52.5 mL, 492.2 mmol), and anhydrous K_2CO_3 (36.0 g, 260 mmol) was heated in 250 mL of dry DMF to 130 °C and held for 48 h. Upon cooling, water was added, and the mixture was extracted with $CHCl_3$ several times. The organic extracts were dried (Na_2SO_4), and the solvent were evaporated. By chromatography (SiO_2, EtOAc/heptane 3:7), pure **5** (brown oil, yield 77%) was obtained.

6-*N*,*N*-Dibutylamino-1-cyanonaphthalene (6).[23] A mixture of **5** (3.3 g, 10 mmol) and CuCN (1.07 g, 12 mmol) in 10 mL of dry pyridine was refluxed at 220 °C for 72 h. Upon cooling and evaporation of the solvent, compound **6** (yellow solid, mp 33 °C, yield 78%) was isolated by chromatography (SiO_2, EtOAc/heptane 3:7).

6-*N*,*N*-Dibutylaminonaphthalene-1-carboxylic Acid (7). A solution of **6** (1.4 g, 5 mmol) and NaOH (0.3 g, 7.5 mmol) in 20 mL of 1-pentanole was stirred at 170 °C for 72 h. Then the solvent was evaporated, and the residue was distributed between EtOAc and water and acidified with 1 N HCl to pH 6. The organic layer was separated, and the water was extracted twice with EtOAc. The combined organic layers were dried (Na_2SO_4) and evaporated to give **7** (yellow solid, mp 78 °C, quant.).

6-*N*,*N*-Dibutylamino-1-hydroxymethylnaphthalene (8).[24] To a stirred and ice-cooled mixture of $LiAlH_4$ (0.6 g, 15.8 mmol) and dry diethyl ether (50 mL), a solution of **7** (5.6 g, 18.7 mmol) in 70 mL of dry diethyl ether was added dropwise; then the cooling was removed, and the mixture was stirred overnight. After the addition of water, the resulting precipitate was removed by filtration; then the organic layer was washed with brine and dried (Na_2SO_4), and the solvent was evaporated. The resulting compound (**8**, oil, yield 90%) still containing minor impurities was used for the next step. The direct synthesis of **8** starting from **5** via the lithiated intermediate and subsequent reaction with formaldehyde is also possible but less effective.

1-Bromomethyl-6-*N*,*N*-dibutylaminonaphthalene (9).[24] To a solution of the alcohol (**8**, 14.5 g, 51 mmol) in 60 mL of toluene, PBr_3 (2.4 mL, 26 mmol) was added with vigorous stirring. After the reaction mixture was stirred overnight, ice-cold water and then 10% $NaHCO_3$ were added. The product was extracted with EtOAc, and the organic layer was washed with brine and dried (Na_2SO_4) and the solvent was evaporated. After purification by column chromatography (SiO_2, EtOAc/heptane 1:4) 13.2 g of **9** (yellow oil, which solidifies in the freezer, yield 75%) was obtained.

6-*N*,*N*-Dibutylaminonaphthyl-(1)-methyltriphenylphosphonium Bromide (10). A solution of **9** (12.7 g, 36.6 mmol) and PPh_3 (9.6 g, 36.6 mmol) in 40 mL was refluxed for 7 h. Upon cooling, 450 mL of diethyl ether was added, and the precipitate was washed with diethyl ether twice to give pure **10** (22 g, mp 185−188 °C, yield 99%).

2-Methoxymethylbenzaldehyde (11). 1,2-Bis(hydroxymethyl)-benzene (Lancaster, U.K.) was reacted to give 2-methyl-methoxybenzyl alcohol (1 equiv NaH in THF, then MeI, oil, yield 50%); subsequent oxidation (MnO_2 in $CHCl_3$) resulted in **11** (oil, bp 52 °C at 0.012 mbar, yield 82%).[25]

7-*N*,*N*-Dibutylaminophenanthryl-(1)-methyltriphenylphosphonium Bromide (12) and 8-*N*,*N*-Dibutyl-aminochrysenyl-(1)-methyltriphenylphosphonium Bromide (13). Phosphonium salt **4** (20 mmol) or **10** (20 mmol) with 20 mmol of sodium methoxide were dissolved in dry methanol and stirred at 60 °C for 3 h. Then, 20 mmol of aldehyde **11** in MeOH was added dropwise, and the solution was allowed to reflux overnight. After the evaporation of the solvent, the crude product was purified by chromatography (silica gel; EtOAc/heptane 1:5); the obtained alkenes *E*/*Z*-2-(3-*N*,*N*-dibutylaminostyryl)benzylmethyl ether **14a** (yield 50%) or *E*/*Z*-2-(6-*N*,*N*-dibutylamino-1-naphthyl)-vinylbenzylmethyl ether **15a** (yield 61%) were cyclized as described below (20 h, solvent CH_2Cl_2) to 7-*N*,*N*-dibutylamino-1-methoxymethylphenanthrene **14** (yield 40%, oil) and 2-*N*,*N*-dibutylamino-7-methoxymethylchrysene **15** (yield 50%, mp 215 °C), respectively. Ether **14** or **15** (10 mmol) was stirred with 20 mL of 62% HBr at 60 °C for 10 min. After neutralization ($KHCO_3$), the crude products were extracted with $CHCl_3$ and purified by chromatography (silica gel; EtOAc/heptane 1:5) to yield 7-*N*,*N*-dibutylamino-1-bromomethylphenanthrene **16** (yield 53%, oil) and 2-*N*,*N*-dibutylamino-7-bromomethylchrysene **17** (yield 50%, mp 186 °C (dec)), respectively. Treating **16** and **17** with triphenylphosphine yielded phosphonium salts **12** (yield 81%, mp 213−214 °C) and **13** (yield 93%, mp 206 °C), respectively.

5-Formylisoquinoline (**18**) was obtained from 5-bromoisoquinoline[26] by analogy to 4-formylisoquinoline[27] (mp 116 °C,[28] yield 55%).

***E*/*Z*-3-(3-*N*,*N*-Dibutylaminostyryl)pyridine (19).** Phosphonium salt **4** (3.5 mmol) was dissolved in dry MeOH; 190 mg (3.5 mmol) of NaOMe was added, and the mixture was allowed to reflux for 3 h. Then 3.5 mmol of 3-formylpyridine (Fluka) dissolved in MeOH was added, and the solution was refluxed overnight. The E/Z mixture was isolated by flash chromatography (silica gel, EtOAc/heptane 1:1). Yield 28%, yellow oil; EIMS *m*/*z*: 308 (M^+), 265 ($M^+ - C_3H_7$, $C_{21}H_{28}N_2$ requires 308.2).

***E*/*Z*-3-(6-*N*,*N*-Dibutylamino-1-naphthyl)vinylpyridine (20).** The same procedure as that described for **19** was used but with phosphonium salt **10**. Yield 85%, yellow oil; EIMS *m*/*z:* 358 (M^+), 315 ($M^+ - C_3H_7$, $C_{25}H_{30}N_2$ requires 358.2).

***E*-5-(6-*N*,*N*-Dibutylamino-1-naphthyl)vinylisoquinoline (21).** The same procedure as that described for **19** was used but with phosphonium salt **10** and aldehyde **18.** Yield 73%, yellow solid, mp 128−130 °C (EtOAc); EIMS *m*/*z*: 408 (M^+), 365 ($M^+ - C_3H_7$, $C_{29}H_{28}N_2$ requires 408.3).

***E*/*Z*-5-(7-*N*,*N*-Dibutylamino-1-phenanthryl)vinylisoquinoline (22).** The same procedure as that described for **19** was used but with phosphonium salt **12** and aldehyde **18**. Yield 82%, yellow solid, EIMS *m*/*z*: 458 (M^+), 415 ($M^+ - C_3H_7$, $C_{33}H_{34}N_2$ requires 458.3).

***E*/*Z*-5-(8-*N*,*N*-Dibutylamino-1-chrysenyl)vinylisoquinoline (23).** The same procedure as that described for **19** was used but with phosphonium salt **13** and aldehyde **18**. Yield 80%, yellow solid; FABMS *m*/*z*: 509.3 ($M + 1$, $C_{37}H_{36}N_2$ requires 508.3).

Cyclization of Alkenes 19−23.[29] A 5×10^{-3} M solution of the corresponding *E*- or *E*/*Z*-alkene was irradiated (45 min−40 h, duran glass, room temperature, and CH_2Cl_2 unless stated otherwise) by a Hg high-pressure lamp (TQ 150, Heraeus-Noblelight, Germany). After the evaporation of the solvent, cyclization products **24**−**26** were isolated by flash chromatography (EtOAc/heptane 1:1); crude **27** and **28** were obtained by precipitation from $CHCl_3$/MeOH and used for the final step without further purification.

7-*N*,*N*-Dibutylamino-2-azaphenanthrene (24): Yield 48%, 45 min of irradiation (quartz glass, −30 °C, 2-methyltetrahydrofuran), yellow oil; FABMS *m*/*z*: 307.2 ($M + 1$, $C_{21}H_{26}N_2$ requires 306.2).

8-*N*,*N*-Dibutylamino-2-azachrysene (25): Yield 22%, 45 min of irradiation (quartz glass, 0 °C, cyclohexane), pale-yellow solid, mp 213 °C (EtOAc); EIMS *m*/*z*: 356 (M^+), 313 ($M^+ - C_3H_7$, $C_{25}H_{28}N_2$ requires 356.2).

10-*N*,*N*-Dibutylamino-3-azapicene (26): Yield 25%, 40 h of irradiation (cyclohexane), pale-yellow solid (EtOAc), still containing minor impurities; FABMS *m*/*z*: 407.2 ($M + 1$, $C_{29}H_{30}N_2$ requires 406.2).

11-*N*,*N*-Dibutylaminobenzo[*m*]-3-azapicene (27): Yield 40% (crude product), 15 h of irradiation; EIMS *m*/*z:* 456 (M^+), 413 ($M^+ - C_3H_7$, $C_{33}H_{32}N_2$ requires 456.3).

12-*N*,*N*-Dibutylaminonaphtho[5,6-*m*]-3-azapicene (28): Yield 46% (crude product), 15 h of irradiation; EIMS *m*/*z*: 506 (M^+), 463 ($M^+ - C_3H_7$, $C_{37}H_{34}N_2$ requires 506.3).

ANNINES 3−7. A mixture of 2 mmol of **24**−**28** and 5.4 g (40 mmol) of 1,4-butane sultone was stirred at 120 °C for 4 h.[20] The resulting precipitate was collected, washed with ether, purified by flash chromatography (silica gel, MeOH or CH_2Cl_2/MeOH/H_2O 50:20:4 and Sephadex LH 20 (Pharmacia), MeOH), and recrystallized.

ANNINE-3: Yield 24%, yellow solid, mp > 300 °C (MeOH/heptane); FABMS *m*/*z*: 443.2 ($M + 1$, $C_{25}H_{34}N_2O_3S$ requires 442.2).

ANNINE-4: Yield 35%, yellow solid, mp > 300 °C (MeOH); FABMS *m*/*z*: 493.5 ($M + 1$, $C_{29}H_{36}N_2O_3S$ requires 492.2).

ANNINE-5: Yield 40%, red-orange solid, mp > 300 °C (MeOH); FABMS *m*/*z*: 543.3 ($M + 1$, $C_{33}H_{38}N_2O_3S$ requires 542.3).

ANNINE-6: Yield 21%, red-orange solid, mp > 300 °C (MeOH); FABMS *m*/*z*: 593.4 ($M + 1$, $C_{37}H_{40}N_2O_3S$ requires 592.3).

ANNINE-7: Yield 10%, red solid, mp > 300 °C (MeOH); FABMS *m*/*z*: 643.5 ($M + 1$, $C_{41}H_{42}N_2O_3S$ requires 642.3).

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