Alternating (Squaraine−Receptor) Sensory Polymers: Modular One-Pot Synthesis and Signal Transduction via Conformationally Controlled Exciton Interaction

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ABSTRACT: A novel class of nonconjugated polysquaraines consisting of isolated chromophores bridged by variable receptor linkers has been synthesized by a two-step one-pot procedure. Substantial reorganization of the polymer backbone conformation occurs upon external physical or chemical stimuli, leading to characteristic absorbance and emission signal responses both at long wavelengths. This novel approach to macromolecular sensor design in which signal transduction is based on backbone folding induced exciton interactions affords sensors with tunable selectivity.

Introduction

Natural evolution has brought forth unrivaled sensors displaying unprecedented specificity and incredible sensitivity that are essential to communication and information processing at the biological level. One approach in designing similar yet artificial high-performance sensors is based on macromolecular systems.[1,2] Apart from better processability, the exploitation of additive and possibly even positive cooperative effects in sensory polymers allows for an amplified response[3] to a recognition event and thus enhances sensitivity as compared to classical sensors based on small molecules.[1] In particular, conjugated polymers with receptors attached to the backbone have been developed for sensing purposes,[2] and the signal transduction process after binding of the analyte is typically based on either a twisting of the backbone leading to a change in the effective conjugation length[2] or the formation of local traps leading to quenching via electron transfer.[2,4] The corresponding signal response is generally based on a change of the polymer's optoelectronic properties and therefore analyzed by potentiometric, colorimetric, or fluorescence techniques. Alternative nonconjugated polymeric systems are based on traditional styrenic or acrylic polymers having analyte receptors grafted to the "spectator" backbone, but they usually lack enhanced sensitivity or straightforward monitoring of the binding event.[5] It should be noted that significant work has been carried out in the field of nonconjugated dimeric sensors.[1b] However, to our knowledge, most of the promising concepts established in this area have not been applied to the polymeric case yet.

Herein, we describe an alternative strategy for the design of sensory polymers in which the linear polymer backbone is consisting of isolated chromophores bridged by receptor units for analyte recognition (Figure 1); i.e., the receptors are an integral—and not appended—part of the structure. The transduction mechanism is based on a controlled change of the receptor conformation in response to external physical or chemical stimuli that is altering the interaction between adjacent chromophores and therefore affects their dimerization equi-
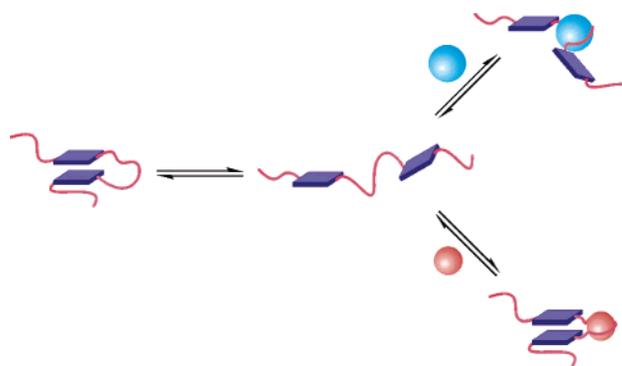

Figure 1. Illustration of the signal transduction process in polymers consisting of isolated chromophores linked by flexible receptors: The conformational equilibrium between folded H-dimer (left) and extended unimer (center) is affected by the analyte binding event (right) and can be conveniently monitored due to characteristic exciton interactions causing changes in absorption ratio and fluorescence intensity. Depending on the size of the cation to be detected and the receptor length, the H-dimer state is either selectively depopulated (top right) or populated (bottom right). For clarity, only part of the polymer structure is shown.

librium. Because of strong electronic interactions in the chromophore dimer, this information is translated to changes in both absorption ratio of isolated monomer (unimer) to H-dimer and fluorescence intensity.[6] Our system consists of alternating (squaraine−oligoethylene glycol) polymers, and its novel modular one-pot synthesis as well as spectroscopic behavior as a function of receptor structure, temperature, solvent composition, and cation content are discussed herein.

Squaraine chromophores were chosen as ideal candidates for signal transduction units in polymer backbones for several reasons. Squaraines[7] display narrow and intense absorption bands (extinction coefficients ϵ > 300 000 M^{-1} cm^{-1}) and fluorescence emission with high quantum yields (ϕ_f up to 0.9) both at long wavelengths (λ_{max}, λ_{em} > 600 nm),[8] rendering them attractive for biological/intracellular[9] probing and other applications as photoreceptors[10] relying on intrinsic brightness.[11] In addition, squaraine chromophores range among the smallest organic, charge neutral dye molecules facilitating synthesis and purification, and they display strong and characteristic exciton interaction[12]

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due to H-dimer formation.[13] This last property is exploited in our approach to signaling binding events. Squaraine dyes have been utilized for some chemosensor designs,[14] for instance by covalently linking them to crown ethers[14a,b] as well as open-chained aminoethylenes[14c] and ethylene glycols.[14d] In addition, one example using a conjugated poly(pyrrole-*alt*-squaraine) sensor has been reported.[14e] All these squaraine-based sensors rely on single signal changes in the absorption or emission spectra, necessitating exact knowledge of concentration. In contrast, the dual signal signature of the unimer/H-dimer equilibrium provides an internal calibration, thus rendering such ratiometric sensors potentially more accurate. The preparation of a polymer strand with isolated yet neighboring squaraine units displaying conformationally dependent exciton interactions should therefore inspire the design of a new class of sensory polymers.

Results and Discussion

Synthesis. The limited availability of functional, i.e., reactive, squaraine derivatives in combination with their intrinsically low solubility precludes the direct use of squaraine dyes for polymer synthesis. Therefore, a synthetic route, in which the squaraine units are formed during polymerization, was chosen. Traditional squaraine synthesis involves the condensation of two electron-rich aromatic moieties, usually *N*-alkylated aniline or pyrrole derivatives, with squaric acid in two consecutive steps.[7b,15] Phloroglucinol (1,3,5-trihydroxybenzene)-type derivatives were used as electron-rich components, providing additional advantages apart from high reaction yields: After condensation to yield the squaraine chromophore, the ortho hydroxyl groups of the dye form intramolecular hydrogen bonds with the oxygen atoms of the central four-membered squaryl ring. This leads to high squaraine rigidity and reduces the number of radiationless transitions available to the excited chromophore, thereby dramatically increasing the quantum yield ϕ_f. In the case of a monomeric squaraine dye, ϕ_f increases by a factor of 20 ($\phi_f = 0.04$ for the unsubstituted compound while $\phi_f = 0.86$ for the hydroxylated derivative).[10]

Monomer Synthesis. For later comparison studies, model monomer bis(4-dibutylamino-2,6-dihydroxyphenyl)squaraine (**4**) was chosen due to its similarity to the corresponding polymers and ready accessibility. The synthesis of this well-known dye was adopted from literature procedures,[16] i.e., condensation of dibutylamine with phloroglucinol via enamine formation followed by condensation with squaric acid under constant removal of generated water, yielding the desired model monomer **4**.

Polymer Synthesis. A key requirement for the sensory polymer synthesis is its flexibility with respect to a facile exchange of receptor moieties for tuning analyte binding selectivities and hence sensitivities toward selected cations. The following approach based on a novel modular two-step one-pot procedure allows for a convenient and rapid access to a whole range of polymer structures. The synthesis is based on three components: an α,ω-diamine as receptor and linker between the chromophores as well as phloroglucinol and squaric acid (1,2-dihydroxycyclobuten-3,4-dione) as condensation partners for generating the squaraine moiety. To attach the receptor unit to the activated aromatic system, a procedure based on the versatile conversion

of secondary α,ω-diamines **1** into bis(3,5-dihydroxyphenyl)-terminated tertiary α,ω-diamine building blocks **2**[17] using enamine formation with phloroglucinol was applied (Figure 2).[16] Without isolation, this linking unit **2** was reacted with equimolar amounts of squaric acid[14b,16] whereby the electron-rich aromatic termini undergo iterations of facile squaraine condensations, leading to formation of the desired polymers **3** via an $A_2 + B_2$ polycondensation process. Polycondensation was affected by azeotropic water removal using standard toluene/1-butanol solvent mixtures. Our nonoptimized reaction conditions afford oligomers displaying polydispersities as expected for a polycondensation process and chain lengths typical for polysquaraines. As the diamine linker, i.e., receptor unit, can easily be varied in this procedure, the rational design of sensory polymers becomes accessible by conveniently selecting a suitable receptor to match the desired analyte to be recognized. The following diamine linkers were used as starting materials: piperazine **1a**, (*N*-octyl)-terminated ethylene glycols **1b**−**d**, and diaza-crown ether **1e** (**1a** and **1e** are commercially available).

The syntheses of spacers **1b**−**d** are shown in Figure 3 whereby the *n*-octyl group was introduced to enhance solubility of the final polymers **3b**−**d**. For spacers **1b** and **1c**, the terminal amino groups of commercially available **5b** and **5c**, respectively, were reacted with octanoyl chloride, and the resulting diamides reduced to the desired diamines. Spacer **1d** was synthesized by a convergent procedure. 2-Aminoethanol (**9**) was reacted with octanoyl chloride, yielding the corresponding amide **10**, which was then coupled to both termini of bis(tosyl)-tetraethylene glycol (**8**). The resulting diamide **11** was finally reduced to afford diamine **1d**.

Polymer **3a** based on piperazine **1a** was synthesized in two steps, including isolation and purification of initial adduct **2a** of piperazine **1a** and phloroglucinol, followed by polycondensation with squaric acid, to test the validity of our two-step one-pot procedure. Although **3a** was poorly soluble in common solvents due to the lack of solubilizing side chains and intrinsic stiffness of the resulting polymer backbone, product morphology as well as the ^{1}H NMR and gel permeation chromatography (GPC) of the soluble fraction confirmed the successful polymerization and demonstrated the potential of the synthetic approach. Encouraged by these preliminary results, we prepared polymers with ethylene glycol derived spacers and solubilizing side chains. Although polysquaraine **3b** possesses long straight *n*-octyl side chains, the spacer itself seems to be too short to induce any decent flexibility in the polymer backbone since only pentamers ($M_n = 2500$ g/mol, polydispersity index (PDI) = 1.10) could be detected by GPC in the THF-soluble fraction. Matrix-assisted laser desorption ionization−time-of-flight mass spectrometry (MALDI−TOF MS) could not be used for determining degrees of polymerizations since it proved difficult to ionize the polysquaraines. As expected, polymers **3c** and **3d** displayed good solubilities due to the presence of solubilizing *n*-octyl side chains as well as long and flexible ethylene glycol linkers. Their masses were determined as M_n(GPC) = 6300 g/mol, PDI = 1.16 and M_n(GPC) = 9700 g/mol, PDI = 2.56, corresponding to a decamer and dodecamer, respectively. These degrees of polymerization are in the range of literature values reported for other (conjugated) polysquaraines.[19] The dimeric fraction of polymer **3c**, which was isolated by
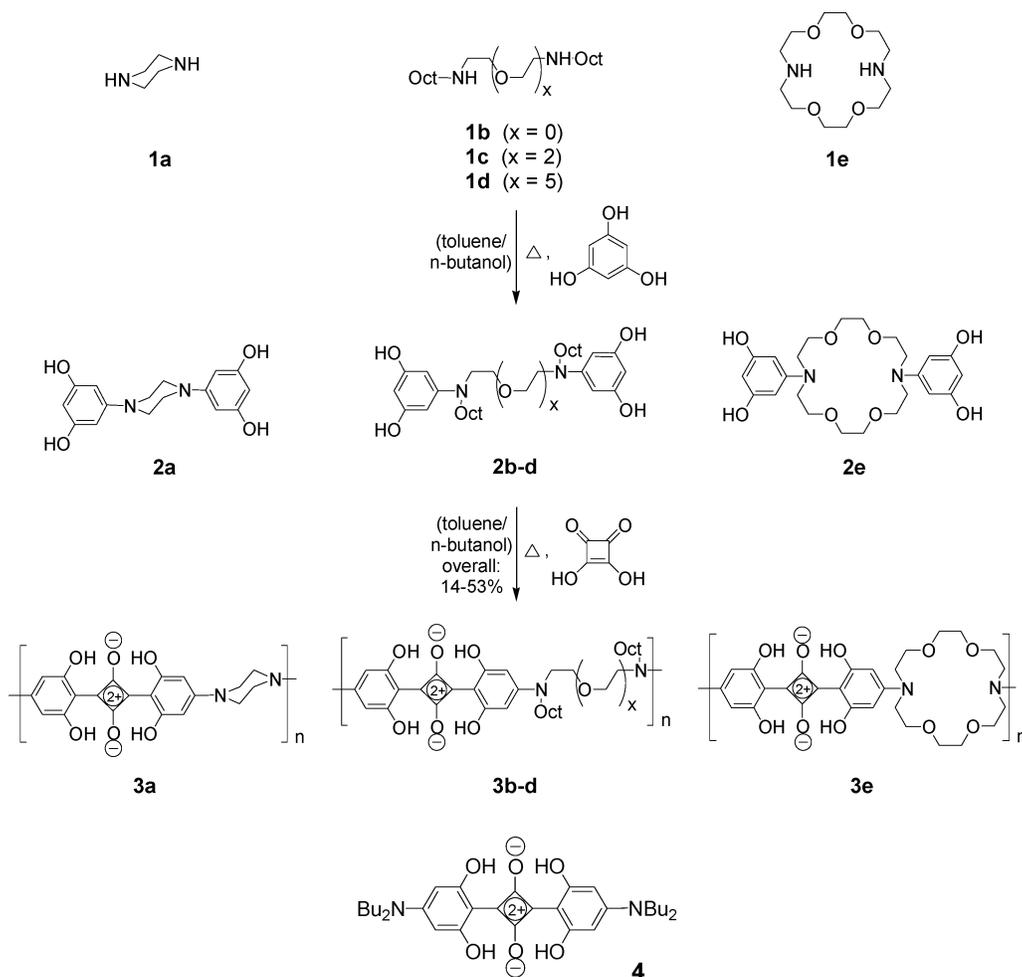
Figure 2. Modular one-pot procedure from terminal α,ω-diamines to polysquaraines (Oct = n-C_8H_{17}).
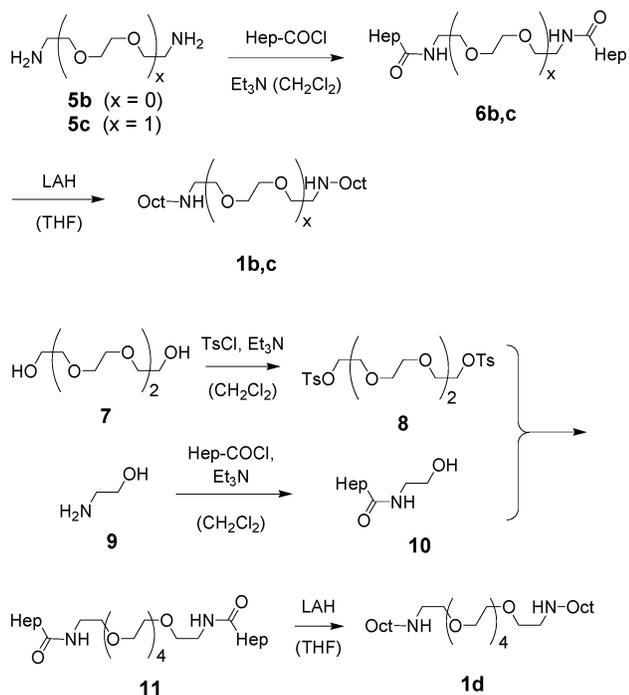

Figure 3. Synthesis of diamine receptors **1** (Hep = n-C_7H_{15}; Oct = n-C_8H_{17}).

preparative GPC, served as the dimer model **12** in subsequent comparison studies. Polymer **3e** having diaza-crown ether moieties and therefore lacking the

solubilizing groups yielded polymers of only lower molecular weight (tetramers, M_n(GPC) = 1800 g/mol, PDI = 1.10). These results suggest that the elementary step of condensing phloroglucinol-derived aromatic moieties with squaric acid is not yet efficient enough to produce much longer polymers. Without further optimization of the polycondensation conditions, the prepared polymers were investigated with regard to their spectroscopic behavior.

Spectroscopy. The UV/vis spectra of squaraines[8,20] and squaraine-based polymers[14e,21] exhibit absorption bands in the red region of the spectrum, corresponding to high molar extinction coefficients. Explicitly, the synthesized structures display absorption maxima between 642 and 653 nm—typical for squaraines—depending on used spacer and solvent, respectively (Figure 4, vide infra). While the single monomer squaraine **4** shows a narrow absorption band (λ_{max} = 640 nm, ϵ = 342 000 M^{-1} cm^{-1}, fwhm = 22 nm in cyclohexane), the dimeric and polymeric compounds exhibit a more or less pronounced, blue-shifted second absorption maximum between 597 and 609 nm caused by the exciton interaction of H-dimer-type aggregates, in which two squaraine chromophores are arranged parallel with respect to each other, permitting an electronic coupling of their excited states.[6] The intensity ratio of these two absorption bands related to unimer and H-dimer (I_U/I_D) enables a facile and rapid evaluation of the equilibrium between nonfolded (isolated) and folded (interacting) squaraine units and thus provides
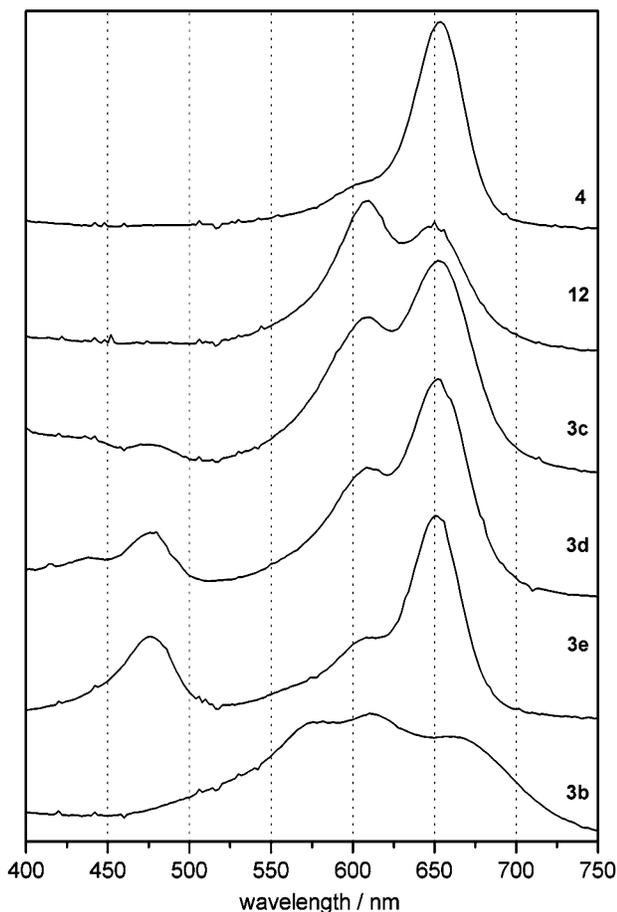
Figure 4. Absorption spectra of squaraine dye containing monomer **4**, dimer **12**, and polymers **3c**, **3d**, **3e**, and **3b** having different receptor-linking units (DMSO, 25 °C).

Figure 5. Absorption spectra of polymer **3c** with increasing temperature from 25 to 75 °C in DMSO. Inset shows absorbance ratio of unimer to H-dimer signal (I_U/I_D) as a function of temperature (line is included as a guide to the eye).

Figure 6. Absorption spectra of polymer **3d** with increasing temperature from 5 to 75 °C in acetonitrile.

insight into the conformation of the polymer backbone (vide supra). The almost negligible H-dimer absorption band in the spectra of monomer model **4** is due to intermolecular aggregation and can be further diminished by diluting the monomer solution. On the contrary, in dimer **12** and polymers **3** the local squaraine concentration is much higher; thus, the second absorption band associated with the H-dimer is much more prevalent and hence clearly visible. In a set of experiments varying the concentration, it could be demonstrated that the exciton formation is in fact due to intramolecular dimerization.[18]

Squaraines also display intense fluorescence emission. The model dye **4** emits at $\lambda_{em} = 646$ nm in cyclohexane, while the dimer and oligomer emission maxima are located between 644 and 673 nm, depending on polymer and solvent, respectively. Most significantly, only the nonaggregated chromophore units (unimers) emit, contrasting the totally quenched emission in the case of H-dimer formation.[13] Therefore, also the change in emission intensity can be exploited for evaluation of the backbone conformation. These spectroscopic signatures were used to investigate the conformational behavior of polymers **3** as a function of backbone structure, temperature, solvent composition, and presence of cations as discussed in the following sections.

Influence of Backbone Structure. The nature of the spacer between two adjacent squaraine units has a direct influence on the conformational equilibrium between unimers and H-dimers (Figure 4). A more flexible and longer spacer as present in polymers **3c** and
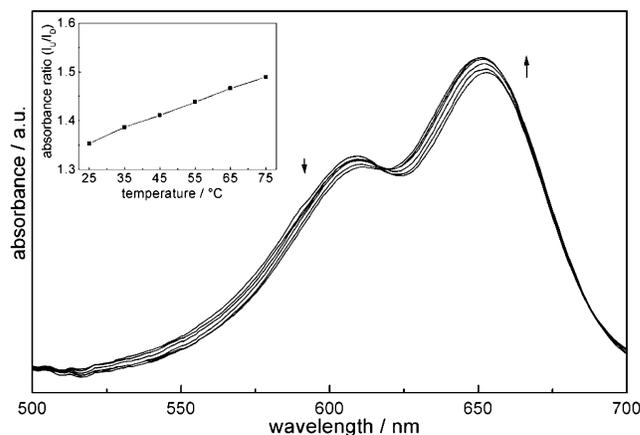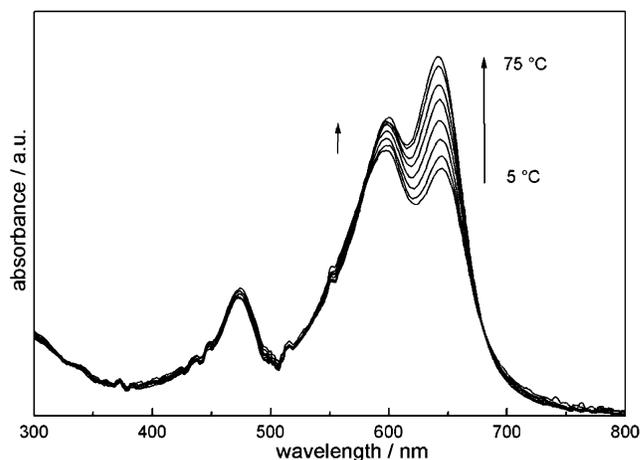
3d not only enhances solubility but more importantly facilitates chromophore dimerization, yielding a more intense exciton signal as compared to polymer **3e** with a somewhat rigid crown ether spacer. Polymers **3c**–**e** display an additional band around 480 nm assigned to higher aggregates[22] that is most pronounced in the case of polymer **3e**. Polymer **3b** possesses a broad undefined absorption region due to strong aggregation presumably caused by its inherent rigidity. The poor solubility of the polymers having more rigid spacers is clearly reflected in the lower degrees of polymerization as compared to other more flexible polymers that were synthesized under the same reaction conditions. Because of the poor solubility of polymers **3a**, **3b**, and **3e**, only polymers **3c** and **3d**, as well as monomer **4** and model dimer **12**, were investigated with respect to their conformational behavior in response to external stimuli as discussed below.

Influence of Temperature. In analogy to biological protein folding and DNA hybridization that show pronounced temperature dependence, the folding equilibrium between unimer and H-dimer can be directly influenced by temperature changes. Temperature studies were performed in DMSO (25–75 °C, Figure 5) and in acetonitrile (5–75 °C, Figure 6). In DMSO the absorbance ratio I_U/I_D remains constant for the monomer **4** and dimer model **12** (not shown), while in the case of polymers **3** it increases slightly with tempera-
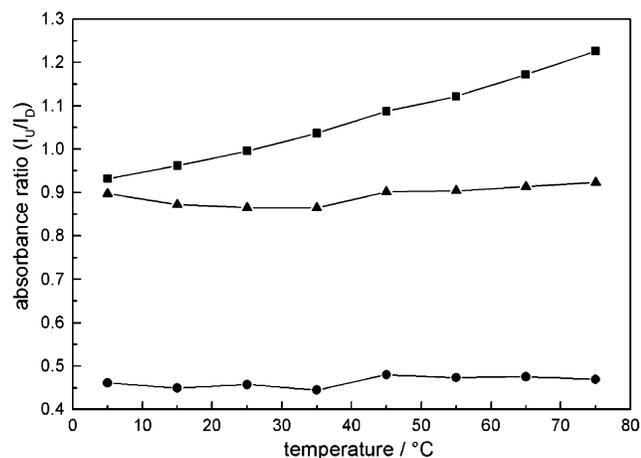
Figure 7. Absorbance ratio of unimer and H-dimer signal I_U/I_D in acetonitrile as a function of temperature: polymer **3d** (■), polymer **3c** (▲), dimer **12** (●). Lines are included as a guide to the eye.

Figure 8. Absorption spectra of polymer **3d** in different solvents (25 °C): cyclohexane (green), toluene (light blue), methylene chloride (black), acetonitrile (blue), and DMSO (red).

Figure 9. Absorption spectra of polymer **3d** in toluene/acetonitrile mixtures with increasing amount of acetonitrile from 0 to 100% (25 °C).

ture, revealing the exergonic nature of the chromophore dimerization process (Figure 5, inset).[23] An isosbestic point is clearly visible at 617 nm, pointing to a two-state unimer/H-dimer equilibrium without formation of intermediates.

In acetonitrile, the absorbance ratio I_U/I_D for the dimer model **12** and polymer **3c** vary with temperature only marginally (Figure 7). The signal change is strongest for polymer **3d** with the longest flexible linker pointing toward nonneighboring, nonlinear, potentially cooperative effects. This finding may be explained by the fact that, in a polymer backbone having a flexible linker, a chromophore unit can more easily find a partner for H-dimer formation since it can dimerize not only with its adjacent chromophore but also with units further apart. Clearly, in dilute solution the local chromophore concentration is much higher in the case of the polymer as compared to monomer **4** or dimer **12**.

Surprisingly, closer inspection of the absorption spectra in acetonitrile reveals the expected intensity increase of the unimer band, but at least to a certain extent also the H-dimer band is enhanced. This is in contradiction to the spectra recorded in DMSO (Figures 5 and 6). It should be noted that for polymer **3d** no thermal effect was observed for the absorption band below 500 nm assigned to higher aggregates (vide supra). For monomer model **4**, the unimer band diminishes even faster than the H-dimer, leading to a deviating intensity ratio behavior (not shown). This suggests that an additional temperature effect is superimposed on the conformational reorganization process. Consequently, another complementary method, i.e., solvent titration, was employed to study the polymer's conformational behavior.

Influence of Solvent Composition. Squaraines display strong solvatochromicity in both their absorption and emission spectra since solvent polarity has a strong influence on the equilibrium between unimer and H-dimer. Thus, the polarity of the solvating medium was systematically changed to investigate the conformational behavior of the polymer backbones. A series of UV/vis and fluorescence spectra were recorded in various solvents, showing varying degrees of H-dimer formation as well as higher aggregates at wavelengths below 500 nm, in particular for polymer **3d** (Figure 8). In general, dimerization and aggregation seems to be
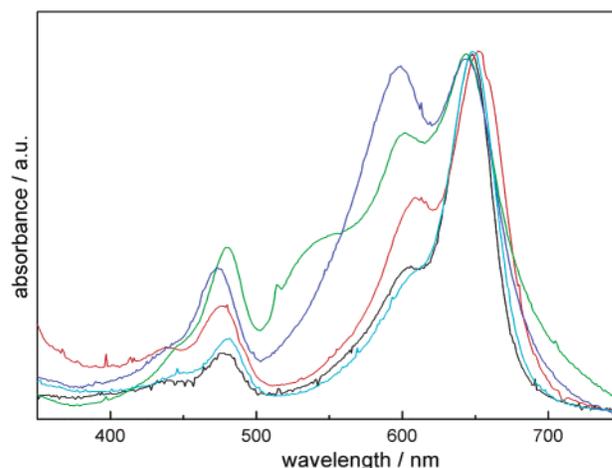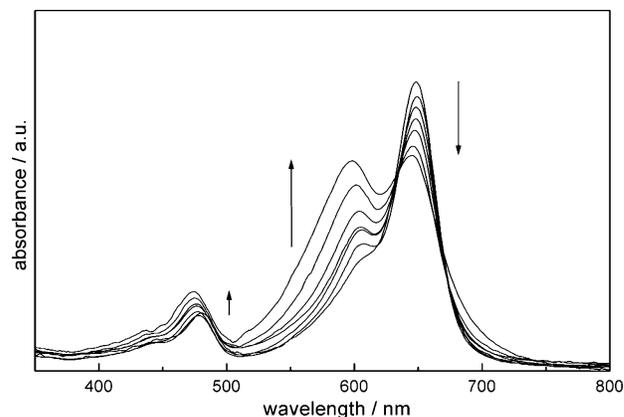
favored in both strongly polar and nonpolar solvents. In addition to the solvatochromicity associated with the unimer−dimer equilibrium, slight solvatochromic shifts of the respective absorption bands are also observed.

To cover a broad range of polarity, the amount of acetonitrile in toluene was increased stepwise from 0% to 100%, promoting the chromophore dimerization event (Figure 9). Similar to the temperature experiment, an isosbestic point is present at 634 nm. Also, the absorption at 480 nm assigned to higher aggregates increases with solvent polarity, but here, the lack of an isosbestic point suggests that this band is caused by undefined intermolecular aggregation.[22] As our interest focused on the discrete unimer and H-dimer states, no further analysis of this hypsochromic signal was done. The corresponding Taft parameters[24] describing solvent polarities for toluene ($\pi^* = 0.54$), acetonitrile ($\pi^* = 0.75$), and the linearly interpolated, intermediate values were used as a reference scale.

The effect of solvent polarity on the conformational equilibrium is negligible in the case of dimer model **12** (Figure 10). Instead, polymer **3d** with long spacers is well solvated and hence extended in nonpolar solvents displaying the highest absorbance ratio I_U/I_D among all compounds. Yet polymer **3d** also allows for an effortless formation of more thermodynamically stable backfolded H-dimers in polar solvents; hence, a strong decrease in
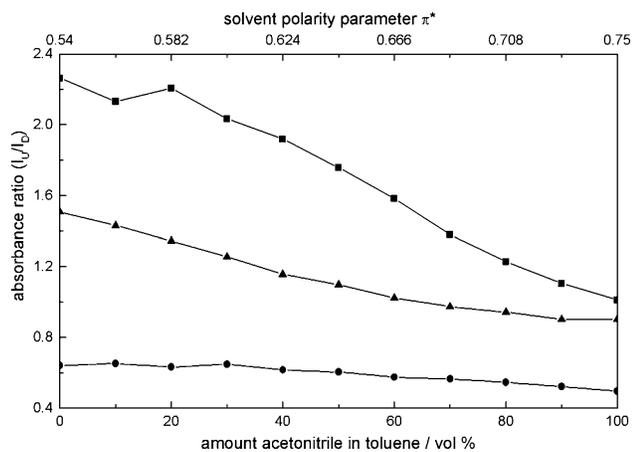
Figure 10. Absorbance ratio of unimer to H-dimer signal I_U/I_D as a function of solvent composition (25 °C). Solvent polarity parameter π^* is given according to ref 24: polymer **3d** (■), polymer **3c** (▲), and dimer **12** (●). Lines are included as a guide to the eye.

Figure 11. Relative change of absorbance ratio of unimer to H-dimer signal I_U/I_D after addition of cations (in DMSO or CH₃-CN, 25 °C): monomer **4** (■), dimer **12** (▼), polymer **3c** (▲), and polymer **3d** (●). If not stated otherwise, metal acetates were used as cation sources.

the absorbance ratio occurs, rendering a high overall folding degree. In contrast to temperature experiments, the intensity of the band below 500 nm assigned to higher aggregates increases with solvent polarity. Polymer **3c** displays a similar behavior for unimer and H-dimer bands, but less accentuated, as it possesses shorter linker units. It presumably attains a more folded conformation in nonpolar media than polymer **3d** as its shorter linkers provide less solubility, yet in polar solvents both polymers show nearly the same absorbance ratio I_U/I_D. This clearly demonstrates the importance of spacer length and flexibility and points toward nonlinear, potentially cooperative effects among polymers **3** when compared to model dimer **12**, but for a qualitative determination a series of oligomers of different lengths would be needed. Apart from physical stimuli, the local conformation of the polymer backbone should also be influenced by chemical stimuli in case the spacers between chromophore units are able to serve as receptor sites.

Cation Sensing. The linkers for bridging chromophore units were chosen in a way that they can recognize and bind cations through their electron-rich O and N heteroatoms. In the event of a favorable cation complexation, such a receptor supposedly wraps around the metal ion in a crescent fashion, leading to dramatic changes of its own conformation and hence of the relative orientation of the linked nearby chromophores. This alteration of the chromophores' orientation either promotes or prevents the dimerization of chromophore pairs, thus influencing the overall unimer to H-dimer equilibrium (Figure 1). Spectral changes expressed in absorbance ratio (I_U/I_D) and fluorescence intensity can be used to directly analyze this conformational reorganization, allowing for an exploitation of this phenomenon for analyte sensing applications.

The complexation of a cation to a linker relates to an equilibrium itself, and a successful binding event depends on several variables, mainly being geometric (cation size and solvation shell, linker length, and conformational flexibility) as well as electronic (cation Lewis acidity, nature and number of binding sites on linker, i.e., binding affinity) in character.[25] Oligoethylene glycols represent open-chain crown ether analogues and are well-known to discriminate and bind cations, despite their more or less flexible cavity geometry.[26] At
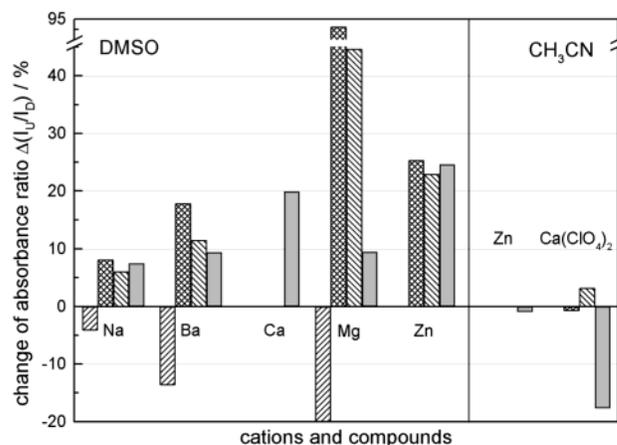
the same time, single squaraine dyes are known to associate with cations without the need for a linked, specific receptor, aggregating in the form of stacks with intercalated cations leading to excimer formation.[27] When compared to our polymer systems, the introduction of additional binding moieties (linkers) leads to a more complex system as the receptors and chromophores both compete for cation complexation. The optical responses to different cations based on changes in the absorbance ratio I_U/I_D are summarized in Figure 11. It should be noted that all observed $\Delta(I_U/I_D)$ values are positive, hence suggesting metal binding at the oligoethylene glycol linkers rather than the squaraine units (see negative $\Delta(I_U/I_D)$ for model monomer **4**[28]). Metal salt concentrations (micromolar range) and signal response intensities were similar to conditions described in the literature for single squaraine sensors.

Sodium and zinc acetates were chosen as cation sources as well as different earth alkaline acetates to study the complexation of a series of similar cations with increasing sizes, i.e., Mg^{2+} (72 pm), Ca^{2+} (100 pm), and Ba^{2+} (135 pm).[29] Sodium (102 pm) has the smallest influence on the conformational equilibrium/absorbance ratio for all tested compounds, i.e., monomer and dimer models **4** and **12** as well as polymers **3c** and **3d** as expected from the relatively weak binding constants of Na^+ to ethylene glycols.[30] Higher binding constants are realized with doubly charged cations. In general, dimer **12** tends to show higher sensitivity toward most cations in DMSO when compared to the polymers pointing toward negative nonlinear effects. This might be tentatively explained with the following simple model. In an isolated dimeric molecule of two chromophores connected by one linker one cation is sufficient to disrupt chromophore–chromophore interactions; i.e., saturation is reached at half of the equivalent of cations as compared to chromophores. In a polymer, more combinations for chromophore dimerizations are possible; thus, more cations are needed to reach saturation when compared to the dimeric compound.[31] In DMSO solutions, Mg^{2+} and Zn^{2+}, having a similar size around 73 pm, triggered the strongest responses for dimer **12** and polymer **3c** having the same triethylene glycol linker. The more intense signal response toward Mg^{2+} is explained by the stronger Lewis acidity of Mg^{2+} and thus higher binding affinity. For polymer **3d** having
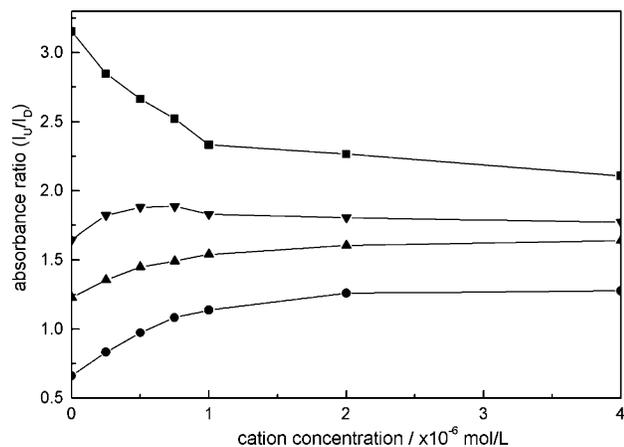
Figure 12. Absorbance ratio of unimer to H-dimer I_U/I_D as a function of Mg^{2+} cation concentration (in DMSO, 25 °C): monomer 10 (■), dimer **12** (●), polymer **3c** (▲), and polymer **3d** (▼).

Figure 13. Emission spectra of polymer **3c** (in DMSO, 25 °C): without cations (blue) and with Na^+ (light blue), Ba^{2+} (red), Mg^{2+} (green), and Zn^{2+} (black).

hexaethylene glycol receptors, Mg^{2+} is too small to efficiently prevent H-dimer stacking (vide infra) while Ba^{2+} exceeds the size range required for a stable analyte-to-linker binding. Here, Ca^{2+} is much more efficient, while Na^+ possesses an ion size in the same range, but a much lower binding affinity,[30] thus again leading to a weaker response.

As most of the compounds exhibited in DMSO the highest sensitivity toward magnesium, a series of titration experiments was performed under these conditions (Figure 12). All compounds showed typical saturation curves approximating receptor saturation at 1–3 equiv of cations per receptor, i.e., 2 for dimer **12**, 3 for polymer **3c**, 1 for polymer **3d** (Figure 12, upper axis), and 2 equiv of cations per chromophore for the model monomer **4**. The titration results for triethylene glycol-containing compounds **12** and **3c** illustrate the limitations in sensitivity of utilizing conformationally flexible open-chain receptors required for reversible folding instead of more rigid cyclic or bicyclic receptors such as crown ethers or cryptants, which display higher binding affinities (sensitivity) as well as selectivities.[26]

The solvent not only directly influences the unimer to H-dimer equilibrium as shown before but also influences the cation binding event itself. Strongly solvated ions expose considerably increased effective radii toward binding sites, thus affecting their complexation.[32] In DMSO medium, cation exposure apparently restrains chromophore dimerization, as the unimer to H-dimer absorbance ratio I_U/I_D changes in favor of the unimer for all compounds apart from model monomer **4** (Figure 11, left entries). As DMSO is known as a strongly complexing ligand for cations, these analyte–solvent species of bulkier hydrodynamic volume most likely prevent the complete wrapping and hence chromophore dimerization.[33]

In comparison, cation solvation in acetonitrile is far less pronounced thus facilitating cation–ligand complexation. Ethylene glycols can more easily replace solvent molecules as ligands from metal cations, therefore allowing for a complete wrapping of the receptor around the analyte[26,32] and hence leading to an increase of the H-dimer formation upon the binding event as evidenced for Zn^{2+} and Ca^{2+} (Figure 11, right entries). The ideal match of Ca^{2+} cations and hexaethylene glycols[34] could be impressively shown with polymer **3d** having such receptor moieties when compared to tri-
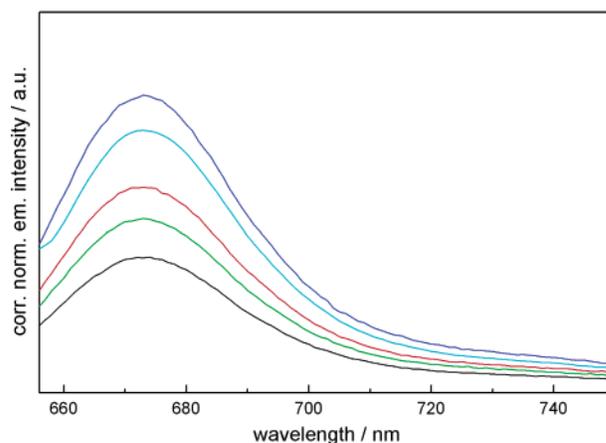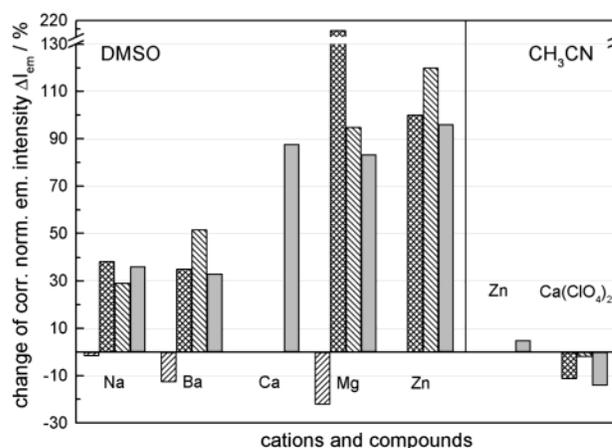

Figure 14. Relative change of emission intensity after addition of cations (in DMSO or CH_3CN, 25 °C): monomer **4** (///), dimer **12** (XXX), polymer **3c** (\\\), and polymer **3d** (■). If not stated otherwise, metal acetates were used as cation sources.

ethylene glycol-based polymer **3c**. The selectivity of sensing Ca^{2+} with hexaethylene glycol spacers was further improved by the restrictions of squaraine dimerization demands.[14d] For an efficient signal transduction the receptor has to encircle the cation in a way that both linked chromophores end up in a position where exciton formation is possible. For example, polymer **3d** with hexaethylene glycol receptors was saturated first with Mg^{2+} (Figure 12) but displayed by far the weakest response signal due to the undersized analyte. It should be noted that all discussed effects are undoubtedly due to the specific nature of the cations as shown by control experiments.[18]

Fluorescence can also be used as a response signal to the chemical stimulus; in fact, the average response is significantly more sensitive than absorption (Figure 13). It should be noted that a direct comparison of the population of folded vs nonfolded states is not possible due to the complete absence of fluorescence arising from H-dimers. In DMSO, monomer **4** was the only compound where the fluorescence actually diminishes after cation addition (Figure 14). In this case, positively charged species presumably promote aggregation of single squaraines,[27] thereby strongly quenching fluorescence. Signal responses for dimer **12** as well as polymers **3c** and **3d** increase upon cation addition as chromophore dimerization is decreased in agreement with UV/vis

absorption spectroscopy (vide supra). In contrast, the emission of **3d** upon addition of Ca^{2+} in acetonitrile is partly quenched as expected. In general, the emission experiments independently confirm the results obtained by UV/vis absorption, demonstrating the importance of receptor length for effective binding of the desired cation as well as the importance of binding affinities of cations in general.

Conclusion

A new approach to the design of sensory polymers has been demonstrated. Thereby, a set of polysquaraines having alternating receptor and squaraine chromophore units was synthesized. The novel modular and versatile two-step one-pot procedure presented herein allows for rapid and facile access to this new class of polymers. These nonconjugated polysquaraines undergo significant conformational changes that are controlled by physical and chemical stimuli such as temperature, solvent polarity, and addition of various cations. The binding of analytes induces changes in the polymer backbone conformation, leading to either preferential folding to, or unfolding from, chromophore H-dimers. Hence, the binding event is translated into a shift in the unimer to H-dimer equilibrium of the squaraine chromophores that can be conveniently visualized by UV/vis and fluorescence spectroscopy and thus successfully be exploited for cation sensing. In the event of analyte recognition, our polysquaraines respond with an unusual dual signal pattern allowing for internal self-calibration and display acceptable sensitivity and specificity, rendering them suitable for future sensory applications, in particular if receptors of higher selectivity and binding affinity would be incorporated into the polymers.

Supporting Information Available: Syntheses and characterizations of monomers, model compound, and polymers. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Czarnik, A. W., Ed. *Fluorescent Chemosensors for Ion and Molecular Recognition*; American Chemical Society: Washington, DC, 1993. (b) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515−1566 and references therein.

(2) For a review, see: McQuade, D. T.; Pullen, A. E.; Swager, T. M. *Chem. Rev.* **2000**, *100*, 2537−2574 and references therein.

(3) Swager, T. M. *Acc. Chem. Res.* **1998**, *31*, 201−207.

(4) See for example: Chen, L.; McBranch, D. W.; Wang, H.-L.; Helgeson, R.; Wudl, F.; Whitten, D. G. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 12287−12292.

(5) For an early example of cooperative cation binding, see: (a) Kopolow, S.; Hogen Esch, T. E.; Smid, J. *Macromolecules* **1973**, *6*, 133−142. An example involving energy migration and hence signal amplification in grafted, nonconjugated systems has very recently been reported in: (b) Broadwater,

(6) Kasha, M. *Radiat. Res.* **1963**, *20*, 55−70. Chromophores that can electronically interact with each other forming "pseudo-excimers" exist in two possible states: When isolated from each other they are referred to as unimers while aggregated dimeric complexes displaying pseudo-exciton interactions are labeled H-dimers. In contrast, the term monomer is used for defining single molecule compounds, i.e., nonpolymeric chemical structures.

(7) (a) Treibs, A.; Jacob, K. *Angew. Chem., Int. Ed. Engl.* **1965**, *4*, 694−695. For a review, see: (b) Schmidt, A. H. *Synthesis* **1980**, 961−994.

(8) For reviews, consult: (a) Das, S.; Thomas, K. G.; George, M. V. *Mol. Supramol. Photochem.* **1997**, *1*, 467−517. (b) Law, K.-Y. *Mol. Supramol. Photochem.* **1997**, *1*, 519−584. (c) Law, K.-Y. *Chem. Rev.* **1993**, *93*, 449−486.

(9) Terpetschnig, E.; Szmacinski, H.; Ozinskas, A.; Lakowicz, J. R. *Anal. Biochem.* **1994**, *217*, 197−204.

(10) Law, K.-Y. *J. Phys. Chem.* **1988**, *92*, 4226−4231.

(11) Brightness is defined as product of molar absorption coefficient ϵ and fluorescense quantum yield Φ_f. See: Minta, A.; Kao, J. P. Y.; Tsien, R. Y. *J. Biol. Chem.* **1989**, *264*, 8171−8178.

(12) "Exciton interaction" is a common term used in squaraine literature for describing the effect of electronic interactions between squaraine pairs, although it is strictly speaking a "pseudo-excimeric" interaction.

(13) For an example, see: Liang, K.; Farahat, M. S.; Perlstein, J.; Law, K.-Y.; Whitten, D. G. *J. Am. Chem. Soc.* **1997**, *119*, 830−831.

(14) (a) Das, S.; Thomas, K. G.; Thomas, K. J.; Kamat, P. V.; George, M. V. *J. Phys. Chem.* **1994**, *98*, 9291−9296. (b) Oguz, U.; Akkaya, E. U. *Tetrahedron Lett.* **1997**, *38*, 4509−4512. (c) Dilek, G.; Akkaya, E. U. *Tetrahedron Lett.* **2000**, *41*, 3721−3724. During the course of our investigations, a calcium sensor based on a bis(squaraine) molecule containing an ortho-hydroxyl groups that exhibits enhanced dimer aggregation upon binding has been reported: (d) Ajayaghosh, A.; Arunkumar, E.; Daub, J. *Angew. Chem., Int. Ed.* **2002**, *41*, 1766−1769. (e) Chenthamarakshan, C. R.; Ajayaghosh, A. *Tetrahedron Lett.* **1998**, *39*, 1795−1798.

(15) For a comprehensive discussion of the squaraine condensation mechanism, consult: Law, K.-Y.; Bailey, F. C. *Can. J. Chem.* **1986**, *64*, 2267−2273.

(16) Dirk, C. W.; Herndon, W. C.; Cervantes-Lee, F.; Selnau, H.; Martinez, S.; Kalamegham, P.; Tan, A.; Campos, G.; Velez, M.; Zyss, J.; Ledoux, I.; Cheng, L.-T. *J. Am. Chem. Soc.* **1995**, *117*, 2214−2225.

(17) Tertiary amines are necessary to prevent squaramide formation. For the only exception, see: Bello, K. A.; Corns, S. N.; Griffiths, J. *J. Chem. Soc., Chem. Commun.* **1993**, 452−454.

(18) See Supporting Information.

(19) See for example: (a) Havinga, E. E.; Pomp, A.; Ten Hoeve, W.; Wynberg, H. *Synth. Met.* **1995**, *69*, 581−582. For a review, see: (b) Ajayaghosh, A. *Chem. Soc. Rev.* **2003**, *32*, 181−191 and references therein.

(20) Law, K.-Y. *J. Photochem. Photobiol. A* **1994**, *84*, 123−132.

(21) Chenthamarakshan, C. R.; Eldo, J.; Ajayaghosh, A. *Macromolecules* **1999**, *32*, 251−257.

(22) See, for example: (a) Liang, K.; Law, K.-Y.; Whitten, D. G. *J. Phys. Chem.* **1994**, *98*, 13379−13384. (b) Chen, H.; Farahat, M. S.; Law, K.-Y.; Whitten, D. G. *J. Am. Chem. Soc.* **1996**, *118*, 2584−2594.

(23) The quantitative determination of the degree of chromophore dimerization within the polymer is not possible. There are two unknown variables, i.e., the molar extinction coefficient of the H-dimer and the equilibrium constant K_{eq}, which cannot be determined independently from each other because the pure H-dimer state is not accessible.

(24) Kamlet, M. J.; Abboud, J. L. M.; Abraham, M. H.; Taft, R. W. *J. Org. Chem.* **1983**, *48*, 2877−2887.

(25) Ouchi, M.; Inoue, Y.; Kanzaki, T.; Hakushi, T. *J. Org. Chem.* **1984**, *49*, 1408−1412.

(26) Vögtle, F.; Weber, E. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 753−777.

(27) Langley, G. J.; Hecquet, E.; Morris, I. P.; Hamilton, D. G. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 165−170.

(28) As the H-dimer bands in the monomer spectra are rather small, the absorbance ratios I_U/I_D for model monomer **4** might be associated with a larger error.

(29) In *Handbook of Chemistry and Physics*, 81st ed.; Lide, D. R., Ed.; CRC Press: Washington, DC, 2000; pp 12−14.

S. J.; Hickey, M. K.; McQuade, D. T. *J. Am. Chem. Soc.* **2003**, *125*, 11154−11155.

(30) In general, singly charged alkaline metal cations display smaller binding constants to oligoethylene glycol chains as compared with the doubly charged earth alkaline metal cations. See for example: Izatt, R. M.; Eatough, D. J.; Christensen, J. J. *Struct. Bonding (Berlin)* **1973**, *16*, 161−89.

(31) Actually, close to twice as many cations are needed to disrupt all possible chromophore dimerizations in a polymer; i.e., at a certain cation and the same overall chromophore concen-trations, the dimeric compound is exposed to a stimulus twice as strong as the polymers.

(32) Valeur, B.; Pouget, J.; Bourson, J.; Kaschke, M.; Ernsting, N. P. *J. Phys. Chem.* **1992**, *96*, 6545−6549.

(33) Honda, H.; Ono, K.; Murakami, K. *Macromolecules* **1990**, *23*, 515−520.

(34) Kawakami, J.; Komai, Y.; Ito, S. *Chem. Lett.* **1996**, 617−618.