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ARTICLE

General Strategy for Tuning the Stokes Shifts of Near Infrared Cyanine Dyes

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We report a significant Stokes shift enhancement in near-infrared fluorescing cyanines as a result of C4'-substiution with cyclic or acyclic amines. Based on a combined experimental and density functional study, a simple strategy for optimizing the Stokes shift is proposed. By tuning the relative energies of cyanine-like and bis-dipolar conformers, differing in the rotational angle of the amine substituent, it is possible to develop molecules that undergo conformational change upon excitation, resulting in a predictable Stokes shift.

Introduction

Fluorescent dyes have been instrumental in cellular biology, biomedical drugs design, advanced materials engineering, energy capture and conversion, and many other fields.[1] Amongst many fluorophores for optical imaging, near-infrared (NIR) dyes, and in particular cyanine dyes, enjoy significant advantages due to their brightness and their narrow emission profile in a region that is devoid of many interferences.[2] However, most cyanine dyes exhibit small Stokes shifts, which lead to significant background and light scattering interferences in optical imaging. Although there are occasional reports of cyanine dyes with larger Stokes shift, there is a lack for a general strategy for tuning the Stokes shift in a rational manner with commercially available fluorophores. Accordingly, the development of a Stokes shift tuning strategy for cyanine dyes would be of great importance for bioimaging, new probe design, and stimulated emission depletion microscopy techniques.

Heptamethine cyanine dyes with a rigid central chlorocyclohexenyl ring have found broad application in optical imaging and drug delivery, as many commercially available examples have a chloride on the C4' carbon that can be easily substituted. Pioneering work to increase the Stokes shifts of cyanine dyes have explored primary amine substitutions, geometrically twisted structures, and novel donor groups, achieving nearly 160 nm Stokes shift by various modalities.[1i, 3] Nonetheless, these methods have not offered a reliable strategy for tuning or rational means of predicting the Stokes shift. We had synthesized a number of amine substituted heptamethine cyanine dyes for their evaluation as light harvesting agents in solar concentrators.[4] A variety of cyclic and acyclic amine substituents were screened in the prior study. Herein, we have expanded the structural variations of the substituents and the cyanine backbone to understand the underlying principles that affect the spectroscopic characteristics, and in particular, the Stokes shift. In doing so, we have identified a simple strategy to tune the Stokes shift of heptamethine cyanine dyes by tuning the relative energies of conformers of the dye differentiated by rotation about the central amine group. Our study sheds light onto the fundamental factors that govern Stoke shift changes in cyanine dyes, and could pave the way for further optimizations of this family of dyes, as they have gained utility and prominence in the materials energy, imaging, and medical fields.

Results and Discussion

The standard heptamethine cyanine dye **1** was chosen to investigate the effects of different nitrogen substituents on the observed Stokes shift. Two categories of cyanine dyes (substituted with acyclic and cyclic amines) were synthesized via addition–elimination, promoted with diisopropylethylamine (DIPEA) and excess amine in acetonitrile. Acyclic amine substituted cyanine dyes include dimethyl (**2**), methyl ethyl (**3**) and diethyl groups (**6**), whereas cyclic rings include methyl aziridine (**4**), azetidine (**5**), pyrrolidine (**7**) and piperidine (**8**). Attempts to synthesize the azepane substituted cyanine failed as the product was not stable and could not be isolated in its

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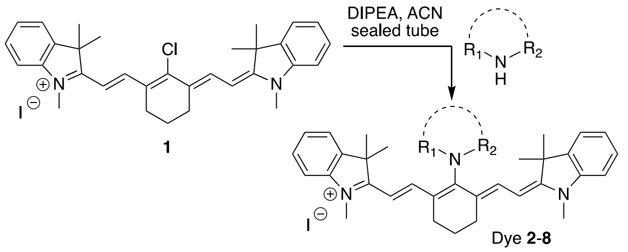
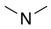
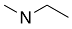
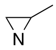
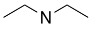
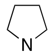
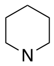
pure form. Table 1 summarizes spectroscopic properties for the latter dyes. Cyanine dye **1** exhibits an absorption maximum in the NIR region (λ_{max} = 785 nm), emission maximum (λ_{em} = 806 nm), with a small Stokes shift (21 nm) in DCM. To our surprise, while dyes **2** and **4** share a similar structure, they are quite different spectroscopically (Figure 1). Dye **2** absorbs at 664 nm and emits at 777 nm in DCM, leading to a 113 nm Stokes shift. In contrast, aziridinyl substrate dye **4** only has a 24 nm Stokes shift. Interestingly, the quantum yield is not improved by using aziridinyl or azetidinyl rings (25% for dye **4** and 26% for dye **5**).[5]

Table 1. Synthesis of the cyanine dyes and spectroscopic data. λ_{max}, absorption maximum; λ_{em}, emission maximum; *SS*, Stokes shift; φ, quantum yield. All spectroscopic data were measured in DCM.



Dye	NR$_2$	λ_{max} (nm)[a]	λ_{em} (nm)[a]	*SS* (nm/eV)	*FWHM (nm)*	φ[b]
1	---	785	806	21/59.0		15
2		664	777	113/9.5	117	28
3		684	778	94/13.2	110	29
4		742	766	24/51.7	94	25
5		615	704	89/13.9	80	26
6		699	778	79/15.7	98	31
7		604	784	180/6.9	85	33
8		668	785	117/10.6	133	23

[a] Absorptions and emissions were recorded in DCM at rt; [b] Absolute quantum yields were measured by Hamamatsu Quantaurus fluorimeter in DCM at rt.

On the other hand, the opposite trend is observed for dyes **6** and **7** (Figure 1). Diethylamine substrate **6** yields a moderate 79 nm Stokes shift (absorption at 699 nm and emission at 778 nm), while the cyclic pyrrolidine substituted cyanine **7** leads to an even larger Stokes shift (180 nm). Notably, their quantum yields are similar (31% for dye **6** and 33% for dye **7**). These observations indicate that the Stokes shift of cyanine dyes can be easily tuned by their amine substituents ranging from 24 nm up to 180 nm. Inspection of this first set of data indicates some trends. Cyclic and acyclic amines appear to behave differently; while the smaller acyclic amines lead to the larger Stokes shifts, the smaller cyclic amines yield the smaller Stokes shift. Another notable observation is that most of these dyes (except dye **5**) share similar emission wavelength (around 770 nm). The change in Stokes shift is dictated mainly by the blueshift of the absorption. Thus, we speculated that these dyes may have different ground state geometries.

To examine the transferability of the trend described above, similar amine substitutions were made on indocyanine green (ICG) and benzothiazole based cyanine dyes. Photophysical characteristics of these dyes are listed in Table 2. As compared to cyanine dyes **2**-**8** in Table 1, ICG type dyes **9**-**15** with a more conjugated aryl ring are red shifted by around 40 nm, while maintaining the same Stokes shift trend. The largest Stokes shift comes from dye **14** with the pyrrolidine ring substitution (187 nm Stokes shift) and lowest is the aziridinyl functionalized dye **10** (56 nm Stokes shift). Most of the dyes listed in Table 2 have a slightly lower quantum yield than their analog in Table 1, with only the azetidine substituted dye **10** with a slightly higher quantum yield (30%). We also chose 3 representative substituents: dimethyl, diethyl and pyrrolidinyl groups to study the effects on benzothiazole based cyanine dyes. Gratifyingly, the same trend was also observed in these dyes featuring a large Stokes shift with pyrrolidinyl (171 nm for dye **18**) and a moderate Stokes shift with diethyl (69 nm for dye **17**).

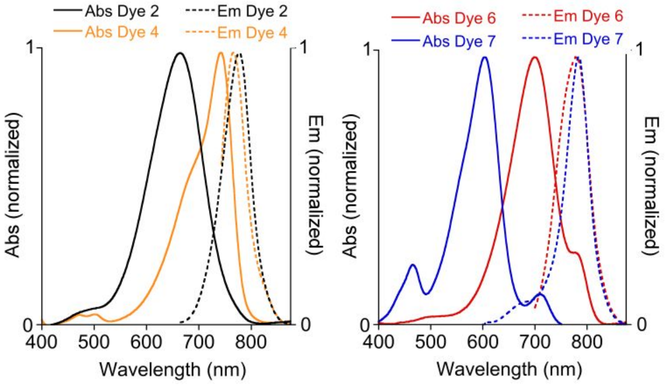


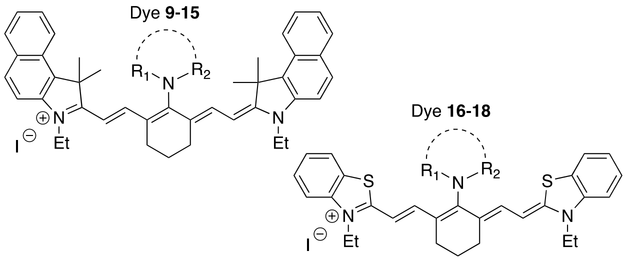
Figure 1. Normalized absorption and fluorescence emission spectra (measured in DCM) for dyes **2** and **4** (left), and dyes **6** and **7** (right).

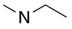
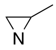
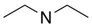
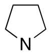
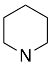
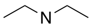
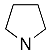
In order to investigate the mechanism underlying the observed change in the Stokes shift we carried a computational study of dyes **2**-**8.** Calculating the spectroscopic properties of cyanine dyes remains a challenging task.[6] For example, commonly used time-dependent density functional theory (TD-DFT) functionals cannot provide accurate transition energies for cyanine dyes, overestimating excitation energies by 0.3 -1.0 eV. Nonetheless, TD-DFT is established to provide an accurate description of the shape of the excited potential energy surfaces of these molecules.[6b, 7] Thus, one can expect TD-DFT to provide an accurate description of the Stokes shift and the associated displacement coordinate, even when it does not provide quantitative predictions of the excitations energies themselves.

Before turning our attention to the excited state, we examined the ground state conformer. Geometry optimizations at the CAM-B3LYP/6-31G* level[8] (performed with the TeraChem software package[9]) show the *s-trans* conformer to have a lower energy than the *s-cis* structure (differentiate by flipping of the iodolium ring) for all dyes in the series **1** to **8**. Interestingly, all seven of the amine-substituted dyes **2** to **8** have two distinct *s-trans* ground state minimum structures that differ in the rotation angle of the amine group. In one minimum, the amine is rotated to be roughly in plane with the heptamethine chain, which we refer to as the parallel conformer. In the other structure, referred to as the perpendicular conformer, the amine is rotated approximately 90° with respect to the heptamethine chain. Structures for all optimized systems can be found in Supporting Information.

The existence of two conformers is closely related to a concept that is known to determine the spectroscopic properties of substituted cyanine dyes: the distinction between bis-dipolar and cyanine-like electronic structures (Figure 2). A bis-dipolar system is characterized by alternating double and single bonds, as may occur when the amine nitrogen conjugates to the π system of the chain (Figure 2, left). In a cyanine-like system, bond orders of all carbon-carbon bonds in the heptamethine chain are roughly 1.5 (Figure 2, right). The excitation energy typically increases with increasing bis-dipolar character. The reader is referred to the elegant work by Guennic, Andraud, Maury and coworkers for a complete description of this concept.[10] It is important to note that all perpendicular structures will exhibit cyanine-like character, because twisting about the amine bond breaks conjugation with the chain. Parallel structures, however, may exhibit a continuum of electronic character, from more bis-dipolar to more cyanine-like character, depending on the degree of π electron donation from the amine to the chain (see dashed box, Figure 2).

Table 2. Spectroscopic data of two other type cyanine dyes. λ_{max}, absorption maximum; λ_{em}, emission maximum; *SS*, Stokes shift; φ, quantum yield. All spectroscopic data were measured in DCM.



Dye	NR_2	λ_{max} (nm)[a]	λ_{em} (nm)[a]	*SS* (nm/eV)	*FWHM* (*nm*)	φ[b]
9		705	818	113/11.0	124	24
10		724	816	92/13.5	116	26
11		746	802	56/22.1	128	24
12		649	737	88/14.1	84	30
13		739	819	80/15.5	107	24
14		636	823	187/6.6	85	25
15		708	824	116/10.7	143	21
16		703	801	98/12.7	123	26
17		733	802	69/18.0	129	27
18		636	807	171/7.3	105	35

[a] Absorptions and emissions were recorded in DCM at rt; [b] Absolute quantum yields were measured by Hamamatsu Quantaurus fluorimeter in DCM at rt.

Interestingly, several of the amine-substituted dyes (**2**-**3**, **6**, and **8**) favor the perpendicular conformation, while the minority (**4** and **5**) favor the parallel conformation. Only those dyes with relatively narrow amine substituents (**4** and **5**) favor the parallel conformation. Analysis of the C-C distance between the carbon atoms in the amine substituent and those in the polymethine chain suggests that steric interaction between these groups determines the ground state conformation. The parallel conformers of **2**-**3**, **6**, and **8** all have C-C contacts between the amine substituent and the polymethine chain in the range 2.91-2.93 Å. These close contacts suggest that the parallel conformers of these dyes are sterically destabilitzed. On the other hand, the parallel conformers of **4** and **5** have no contacts shorter than 3.00 Å, and therefore are not sterically destabilized. For **7**, the calculations predict the difference in energy to be <1.5 kcal/mol, which is effectively equal, given the margin of error for this DFT approach. In this case, the closest C-C contact between the amine substituent and the polymethine chain is 2.94 Å, which is intermediate between the two extremes. For the molecules with a clear lowest energy conformer (**2**-**6** and **8**), computing a Stokes shift is straightforward. In each of these cases, a two excited state minima (parallel and perpendicular) were optimized at the TD-CAM-B3LYP/6-31G* level, and in all cases the energetically favored conformer was the same as in the ground state.

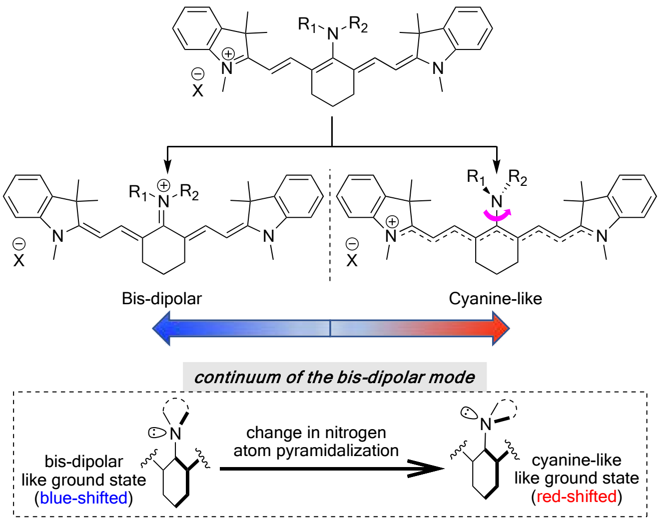


Figure 2. Generalized cyanine-like and bis-dipolar structures of cyanine dyes.

Figure 3 is a scatter plot with the computed excitation energies on the x-axis and the experimentally measured spectral maxima on the y-axis. Blue marks indicate the experimental absorption maxima as a function of the computed vertical excitation energies from the ground state minimum structure. Red marks indicate the experimental emission maxima as a function of the computed vertical excitation energy at the excited state minimum structure. Though DFT consistently overestimates the experimental energies by 0.6-0.7 eV, a compelling pseudo linear relationship between experiment and theory is observed for molecules **2**-**4**, **6**, and **8**. This suggests that the basic physics underlying the Stokes shift is well described. As will be discussed below, molecules **5** and **7** do not follow the trends due to other factors. The experimental excitation energies correlate strongly with displacement along a bond length alternation coordinate (BLA) as determined from the calculated structures, supporting the assignment of variances in the excitation energies to a shift between cyanine-like and bis-dipolar electronic character (see Figure S1).

Applying the same analysis to **7**, the molecule with the largest Stokes shift, does not result in good agreement with experiment. The fit is poor regardless of whether the perpendicular ($\mathbf{7}_{\perp}$) or parallel ($\mathbf{7}_{\parallel}$) conformer is chosen. In the parallel case, the absorption point falls on the line, but the emission point does not. On the other hand, in the parallel case, the emission point falls on the line, but the absorption point does not. This suggests the intriguing possibility that the molecule converts from the parallel to the perpendicular conformation upon excitation. A shift from a strongly bis-dipolar character in the ground state to more cyanine-like character in the excited state is indicated by a reduction in BLA from 0.089 Å (the largest of all dyes studied here) to 0.034 Å. Such a reorganization mechanism has previously been recognized,[3a, 11] but here we demonstrate the delicate energy balance that determines whether such rotation occurs or not.

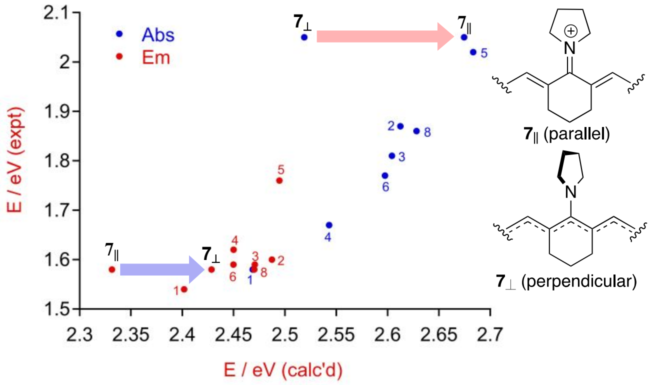


Figure 3. Scatter plot of the experimental spectral energy maxima as a function of the DFT-computed theoretical excitation energies. Absorption and emission energies are shown by blue and red dots, respectively. The lowest energy conformer is chosen for **1-6** and **8**, while for **7**, both the parallel ($\mathbf{7}_{\parallel}$) and perpendicular ($\mathbf{7}_{\perp}$) conformers are shown (see Figure 2 for full structures).

As noted above, all perpendicular structures exhibit cyanine-like electronic structure. This includes both ground and excited states structures for **2**-**3**, **6**, and **8** as well as the excited state structure for **7**. To determine the nature of the electronic structure of the parallel structures (both ground and excited states structures of **4** and **5**, and the ground state structure of **7**), we must consider additional coordinates. Table 3 presents the computed BLA displacement and pyramidalization angle about the amine nitrogen atom of these five structures. The computed ground state pyramidalization angles increase as the amine ring become more constrained, with dyes **7**, **5**, and **4** having angles of 2.1, 7.2, and 43.4°, respectively. This change in hybridization from a nearly planar sp^2 conformation in **7** to a more sp^3 conformation in **4** results in decreased coupling of the amine lone pair to the π system of the heptamethine chain (see dashed box, Figure 2). The reduction in the bis-dipolar character is evidenced by a decrease in the computed BLA (respectively 0.069, 0.057, and 0.036 Å) and increasing experimental λ_{max} (respectively 604, 615, and 742 nm). A similar trend is seen in the excited state structures, with **5** being the dye with more bis-dipolar character upon excitation (BLA of 0.044 Å) and a correspondingly high emission energy (λ_{em} = 704 nm).

With the latter experimental and computational results, a physical description of the trends observed in Table 1 can be summarized as follows. Dyes with small amine substitutions orient such that the nitrogen atom lone pair may participate in conjugation (bis-dipolar), thus leading to the observed blue-shift in absorption. Note, size in this context, is the width of the substituent, not necessarily the smallest overall moiety. Therefore, the width of the pyrrolidine substituent is less than dimethylamine (dye **7** vs **2**). As the lateral size of the substituent increases, the degree of blue-shift is reduced as the bis-dipolar orientation is more difficult to achieve. The observed trend in Stokes shift (**8** > **2** > **3** > **6**) follows the size description with the anticipated increasing difficulty in adopting the necessary orientation to have the nitrogen atom lone pair in conjugation with the polyene.

Table 3. Important geometric parameters that determine the electronic character (bis-dipolar vs. cyanine-like) of the five structures in the parallel conformation.[a]

Dye	State[b]	BLA (Å)	Pyr. Ang. (°)
4	GS	0.036	43.3
	ES	0.023	49.4
5	GS	0.057	7.2
	ES	0.044	15.9
7	GS[c]	0.069	2.1

[a] Geometric parameters are computed at the TD-CAM-B3LYP/6-31G* level; [b] Ground and excited state structures are abbreviated GS and ES, respectively; [c] Only the GS structure is presented for **7**, because the ES minimum energy structure has a perpendicular conformation. A table containing these parameters for all structures can be found in Supporting Information, along with explicit definitions of the BLA and Pyr. Ang. coordinates.

Dyes **4** and **5** (as well as the analogous **11** and **12**), however, defy the latter description**;** though they are both smaller than **7**, they do not have higher-energy absorption. Participation of the aziridine nitrogen atom in the bis-dipolar electronic structure would require it to adopt the more strained sp^2 hybridization, which is energetically disfavored.[12] Thus, dye **4** has the lowest Stokes shift as it exists in a more cyanine-like conformation in both the ground and excited state, even though it adopts the 'bis-dipolar' rotation about the C-N bond. Based on the absorption and emission spectra of dye **5** (both blue-shifted), the azetidine substituent present a unique case. It is small, and thus can adopt the bis-dipolar conformation, and in contrast to the aziridine, rehybridization of its nitrogen atom is not as energetically costly. In fact, iminium formation of azetidines are well documented.[13] Yet, it resists conformational change to the structure that favors cyanine-like character in the excited state, presumably because rehybridization of the nitrogen atom is costlier in the small ring, and therefore emits from the bis-dipolar conformation. Dye **7** finds itself with the correct balance of size, and conformational flexibility to easily traverse from a more bis-dipolar conformer to a more cyanine-like conformations upon excitation, leading to the observed large Stokes shift.

Conclusions

In summary, we demonstrate a simple strategy to selectively engineer Stokes shifts for cyanine dyes, which are of importance in biomedical engineering and imaging. Stokes shifts of different cyanine dyes can be easily tuned via nitrogen substituents (from 24 nm up to 180 nm). Rotation of the amine substituent upon excitation is responsible for the largest Stokes shifts, and an enhanced Stokes shift is observed when the dye may convert from the bis-dipolar form to the cyanine-like form upon excitation. Tuning of the energies of bis-dipolar and cyanine-like conformers into near degeneracy provides a novel strategy for designing cyanine dyes with enhanced Stokes shifts. Some of the cyanine dyes described here have already been tested in luminescent solar concentrator systems with good results. The method presented here can be further explored in advanced material engineering and new probes for imaging.

Conflicts of interest

J.Z., C.Y, B.L., R.L., and B.B. have filed a patent based on some of the work in this manuscript. All other authors declare no competing financial interest.

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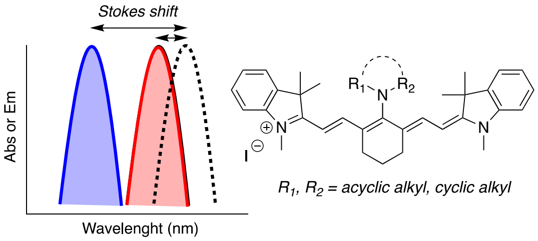

A series of amine substituted cyanine dyes were synthesized. Their spectral characteristics are highly dependent on the structure of the substituted amine, with a Stokes shifts that range from 24 -180 nm. Computational analysis is used to inform on the ground and excited state structures.