



Cite this: *Chem. Commun.*, 2015, 51, 4815

Received 10th December 2014,
Accepted 9th February 2015

DOI: 10.1039/c4cc09857a

www.rsc.org/chemcomm

Decoloration rates of a photomerocyanine dye as a visual probe into hydrogen bonding interactions†

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We have developed a visual marker for the investigation of hydrogen bonding (HB) effects. The decoloration rate of a photochromic dye that incorporates a latent intra-molecular HB feature can be linked to the HB character of the media. Kinetic and thermodynamic parameters of this simple decoloration approach for HB sensing are investigated both experimentally and by high level theoretical studies. This principle has been applied for the detection of changes in the HB character of stationary and fluidic systems. A major finding is the observation of a shear-related perturbation of the balance between intra- and inter-molecular HB within a dynamic thin film.

Hydrogen bonds are central structural and reactivity elements in chemistry and biology,¹ and hydrogen bonding (HB) experimental and theoretical scales are essential for the manipulation and prediction of equilibrium properties.² For instance, HB plays a critical role in determining the conformation of naturally occurring complex macromolecules, such as proteins,³ and the manner in which other man-made systems such as polymers assemble,⁴ in both cases determining the functional properties of these systems. The ability to control HB *via* solution composition or physical parameters such as shear in a hydrodynamic system is therefore critical to the design of appropriate processing and assembly of structures containing such macromolecular systems.

The original and most widely accepted quantitative scale of HB acceptor (β , HBA) basicities and HB donor (α , HBD) acidities is that of Kamlet and Taft (K-T) which is based on a model solvatochromic experiment with a thoughtfully chosen dye.⁵ We report a new analytical tool for probing HB interactions, where rather than using precise

measurements of the energy of an ultraviolet electronic transition, as in the original K-T model, we instead employ a simpler visual marker involving HB effects on the decoloration rate of a photochromic dye.

Although key mechanistic questions still lack definitive answers,⁶ the reversible spiroopyran/merocyanine transformation (SP/MC, Fig. 1) of 6-nitro-substituted spiro[2H-1-benzopyran-2,2-indolines] (BIPS) has been intensively studied.⁷ This is in no small measure due to the broad range of physical and chemical stimuli that can tweak the SP/MC equilibrium.⁸ For instance, upon UV irradiation, very rapid^{7b} coloration is generally observed for solutions of 6-NO₂ BIPS (SP-1 in this work). Coloration accompanies the ring-opening of the colourless SP into the highly conjugated MC isomer, however, with only a few exceptions,⁹ the MC forms of 6-NO₂ BIPS derivatives are thermally unstable at room temperature; *i.e.* within seconds or minutes of photo-coloration the MC form reverts back to the closed-ring SP isomer. The MC/SP relaxation follows a first-order rate law, as a function of structure, solvent polarity and temperature,^{6-7,10} and kinetic data for the parent SP-1 molecule suggests a slower relaxation in polar solvents (Fig. S1, ESI†).⁶ In an attempt to expand the pool of analytical tools for probing HB effects we envisioned that the addition of a latent catechol (1,2-dihydroxybenzene) moiety, as in SP-2 (6-NO₂-8-OH BIPS, Fig. 1), would add an experimental

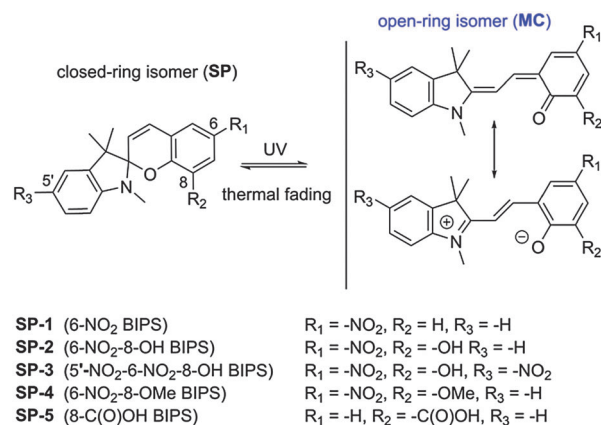


Fig. 1 Closed-ring spiro (SP) and open-ring merocyanine (MC) forms of photochromic molecules SP-1 to SP-5.

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c4cc09857a

handle to link the MC stability to the HB character of the media. The two phenolic groups of catechol are known to form a weak intra-molecular HB ($\sim 10\text{--}17\text{ kJ mol}^{-1}$)¹¹ and while this chemical feature is masked in the closed-ring isomer of **SP-2**, its open-ring MC form displays a catechol-like unit, hence it is capable of intra-molecular HB ($\sim 21\text{ kJ mol}^{-1}$,¹² *trans*-MC isomer, Tables S1–S3, ESI†). Guided by theoretical predictions (*vide infra*) we intended this intra-molecular HB scheme to preferentially stabilize MC over both SP and the putative transition states for the MC/SP relaxation, hence reducing the rate of the thermal reversion process and, most importantly, linking the HB character of the media to the decoloration rate.

The aim of this work was to experimentally investigate the balance of intra- vs. inter-molecular hydrogen bonding by simply monitoring a decoloration process. We here define the scope and limitations of this approach in stationary systems and illustrate an application in a thin film fluidic device. The latter is particularly relevant as (i) the concept of a shear-related perturbation of the HB environment has been tentatively suggested in recent computational studies,¹³ and (ii) the ability to probe shear-related changes to the HB balance may help to clarify an understanding of the thermodynamic stability self-organized systems, including the secondary structure of proteins in fluidic devices.¹⁴

In order to extract physically meaningful information from the decoloration rate of **SP-2** it was first necessary to ascertain (i) the presence and strength of the intramolecular HB, and (ii) under what conditions the HB acidity and basicity of the media is not outweighed by other solvents descriptors, most notably by the solvent polarity. With regards to the first point, a clear fingerprint of the existence of an intramolecular HB in **SP-2** comes from the observation of a red-shift in the infrared (IR) resonance for the hydroxyl stretch. The putative catechol-like hydroxyl absorption in the open-ring MC isomer is centred at 3541 cm^{-1} , while it is blue-shifted at 3608 cm^{-1} in the closed-ring form (Fig. S2 and Table S2, ESI†). These assignments of experimental IR frequencies and HB-related shifts were aided by computations performed at the M06-2X/6-31G(2df,p) level of theory (Fig. S3 and Tables S2 and S3, ESI†). Theoretical results, both in the gas-phase and when implicit solvent effects are considered, confirm an increase in the hydroxyl IR frequency from the hydrogen-bonded open-ring MC isomer to the closed-ring SP form (3467 cm^{-1} to 3585 cm^{-1} , gas-phase, Fig. S3, ESI†). This modelling prediction is in line with the data available for *o*-alkoxyphenols, where the reduction in frequency for hydroxyl stretches involved in hydrogen-bonding HB is around 100 cm^{-1} .¹⁵ Additional support for the IR assignments comes from the observation of a single hydroxyl absorption at 3548 cm^{-1} for **SP-3** (computed gas-phase value is 3595 cm^{-1} , Fig. S3, ESI†). **SP-3** (5'-NO₂-6-NO₂-8-OH BIPS) is designed as a model compound which would give the non-catechol (blue-shifted)^{15a} hydroxyl IR absorption. **SP-3** is a structural analogue of **SP-2** but with the key difference that the HB phenolate acceptor site is chemically masked in **SP-3**. This is because the 5'-nitro substitution on the indoline portion of **SP-3** destabilizes the MC form to the extent that, regardless of solvent polarity, no dark-equilibrium traces of the MC form can be detected (NMR and UV-visible data Fig. S4–S8, ESI†).¹⁶

Several published theoretical studies have focused on various aspects of the MC/SP conversion.¹⁷ For all compounds in this work (Table S4, ESI†) Gibbs free energies for the stationary points along the reaction profiles were determined by combining gas-phase

values, calculated with the composite G3MP2(CC) method,¹⁸ with free energies of solvation predicted using the SMD continuum solvation model¹⁹ (see Section S1.2, ESI†). Reaction profiles for **SP-2** are shown in Fig. S9 and S10 and in Tables S4–S7 (ESI†). Most importantly, both in the gas-phase and when considering solvents effects HB is predicted to preferentially stabilize MC over both the closed-ring SP isomer as well as over the putative transition states for the MC/SP relaxation. Hence, computational data suggest that the MC/SP reaction rate is reduced by roughly one order of magnitude by the HB; a crucial observation when accounting for environmental effects on the decoloration rate of **SP-2** (*vide infra*).

Experimental UV/vis data on the thermal decay of the photochemically ring-opened MC to the SP form were obtained by conventional methods and are summarized in Tables S9–S11 (ESI†). Upon removal of the UV stimulus from solutions of **SP-2** (Fig. S11, ESI†) the absorbance in the red portion of the visible spectra decreases and relaxes to its dark stationary value following first-order kinetics. We evaluated rate constants, k_{obs} , for the thermal fading in 29 solvents ranging from 2,2,2-trifluoroethanol to toluene (Table S9, ESI†). This has enabled correlations to be made between k_{obs} for the MC/SP back relaxation and a number of solvents parameters. Despite being aware of the limitations of using a single physical quantity in defining solvent properties we initially used the solvent permanent electrical dipole, μ , as our solvent descriptor. Given that a single macroscopic physical quantity cannot account for a range of solvent-solute interactions, the poor dependency of k_{obs} on μ (Fig. 2(a)) is perhaps not surprising. A sizable scatter is particularly evident at intermediate μ values (2.5–1.2 D), however, it is this subset of data that is of interest as the scatter in k_{obs} can be linked to the relative HB acidity to basicity character of the media. In other words, it is only when data for solvents at either ends of the μ scale are excluded, and μ only in the range 2.5 to 1.2 D is considered, that it becomes possible to dissect HB effects on k_{obs} from those mediated by other solvents descriptors. When this is done, experimental data then offer a correlation between the thermal fading rate of **SP-2** and the K–T solvent descriptors of HB (Fig. 2(b)). The direction of the displacement in k_{obs} is consistent with the chemistry involved (Fig. 2(b) inset) and its magnitude does indeed track the algebraic difference between the K–T empirical β and α values. Hence, the intra-molecular catechol-like HB scheme in the MC isomer brings about a reduction in the rate of the thermal reversion process and it is possible to predictably reduce this thermodynamic cost by altering the HB character of the media. Increasing the ability of the solvent to out-compete the intra-molecular HB increases in turn the rate of decoloration (Fig. 2(b)) and provides a simple visual sensor of the HB character of the media.

Our interpretation of the data is supported by a strong correlation between the rate of ring closure and calculated quantities. For example, computed Gibbs free energies of activation for the MC/SP relaxation of **SP-2** increase by *ca.* 5 kJ mol^{-1} due to HB formation (Table S6, ESI†), roughly translating into a factor of 8 reduction in the cyclization rate. As an example, when moving from a poor HB solvent such as 1,2-dichloroethane ($\alpha = \beta = 0$, (11)) to an excellent HB acceptor such as pyridine ($\alpha = 0$, $\beta = 0.64$, (14)) a 6 fold increase in k_{obs} is measured (Table S9, ESI†).

The impact of the intra- vs. inter-molecular HB balance on the thermal reversion rate of **SP-2** is further supported by control

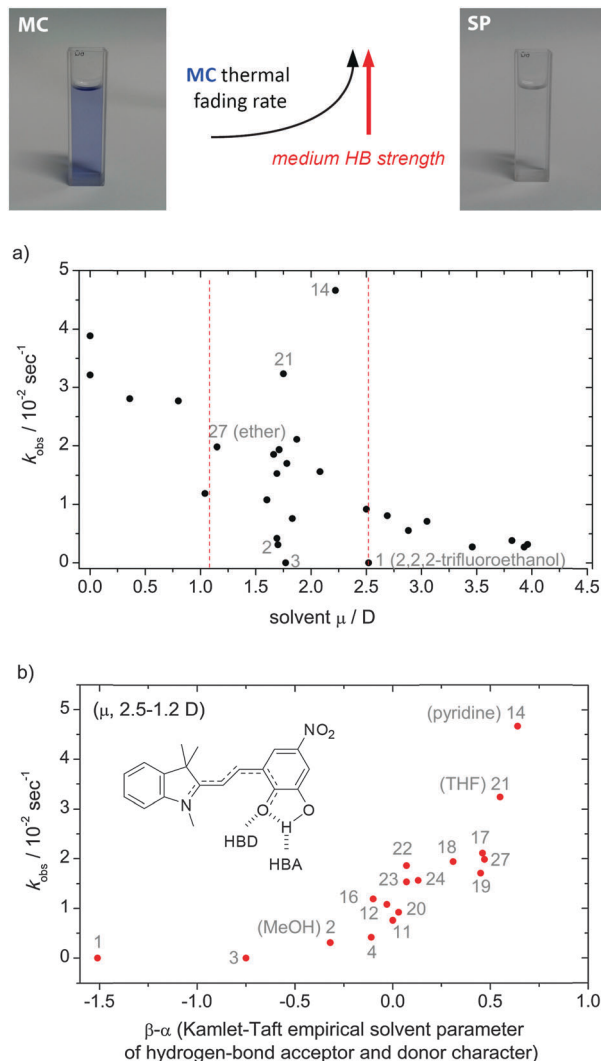


Fig. 2 Solvent effects on the thermal decoloration of **SP-2** (MC/SP relaxation). Plots of experimental k_{obs} vs. (a) solvent permanent electrical dipoles (μ), and (b) algebraic difference between the Kamlet and Taft empirical β and α values (HBA and HBD, respectively). Panel (b) includes only solvents systems of intermediate μ values (ca. 2.5–1.2 D). Solvents are numbered in order of increasing $E_{\text{T}}(30)$ value (Section S1.1.1, ESI[†]).

experiments where we deliberately removed hydrogen-atom donation in the open-ring isomer. Where HB is excluded by means of chemically masking the 8-hydroxyl moiety as a methyl ether (**SP-4**), any acceptable correlation is lost from plots of k_{obs} versus the K–T solvent descriptors $\beta - \alpha$ (Fig. S12 and Table S11, ESI[†]). Furthermore, activation barriers are increased in **SP-2** over **SP-4** (Fig. S13 and S14, ESI[†]), which is consistent with a slower fading when HB is present (Table S8, ESI[†]).

For this optical HB sensing approach to work there needs to be a fine balance between the intra- and inter-molecular HB strengths in the open-ring MC. For instance, attempts to increase the strength of intra-molecular HB beyond the ca. 21 kJ mol⁻¹ of **SP-2** (MC-TTC isomer, Table S1, ESI[†]), so as to access a broader range of β values (HBA), were unsuccessful. Data for control **SP-5** suggest that a HB of ca. 36 kJ mol⁻¹ (Table S1, ESI[†]) hampers the ability of the dye to probe the HB character of the media.

As shown in Fig. S15–S17 (ESI[†]), dark-equilibrated solutions of **SP-5** do not thermally fade, showing a MC-only population.^{9a}

As mentioned above, the relative importance of solvent polarity and HB character on the decoloration rate of **SP-2** shifts in favour of the former at extreme values of μ . Experimental k_{obs} values in solvent systems of μ values greater than 2.5 D (2,2,2-trifluoroethanol) or smaller than 1.2 D (diethyl ether) are outside the trend of Fig. 2(b). This aspect of the work will require a more detailed investigation, nevertheless, a plausible explanation lies in the high degree of charge separation of the **SP-2** transition states for the MC/SP relaxation. Computational data on implicit solvents effects over the two most likely reaction pathways strongly suggest a degree of charge separation for key transition states (Fig. S13 and S14, ESI[†]).²⁰ As for the parent compound **SP-1** (Fig. S1 and S14, ESI[†]),²¹ a transition state with zwitterionic character would account for very fast and very slow relaxations of **SP-2** in solvents at either end of the polarity scale (Fig. 2(a) and Fig. S18, ESI[†]), thereby masking HB effects on decoloration rates.

The ability of shearing fluidic forces to tweak nanoscale assembly processes is well detailed in the literature;²² some authors have tentatively ascribed this to fluidic effects on HB forces.²³ We asked the specific question of whether changes in the decoloration rate of **SP-2** could be used to probe perturbations of the HB environment under shearing forces in a fluidic device. We have subjected the **SP-2** photomerocyanine dye to a well-characterized fluidic processor, namely a vortex fluidic device (VFD²³). Here any change in the decoloration rate of **SP-2** arising from the shear present in the dynamic thin film may reflect perturbations to the HB environment. The VFD is a flow processor that is likely to become a more versatile alternative to conventional fluidics schemes, such as the spinning disk processor. In a VFD (Fig. 3) sub-mL samples can be maintained within the tube, hence removing the need for large volumes of liquid, while the shear in the film is controlled simply by the speed and the orientation of the tube with the presence of Stewartson/Ekman layers. Remarkably, kinetic data on the decoloration of **SP-2** solutions reveal a fluidic effect on the apparent HB balance for this particular system (Table S9, ESI[†]). For solvents with either very large or moderate K–T β values (DMSO and THF, Fig. 3(a) and (b), respectively) it becomes apparent that under shear the balance between intra- and inter-molecular HB tends to shift in favour of the former; *i.e.* the solvents “lose” some of their HBA character as experimental k_{obs} values drop to ca. 70% of their stationary value. On the other hand, we found no evidence of shear effects on the apparent HB balance with solvents of negligible HB character (DCM, Fig. 3(c)) or when HB is deliberately excluded from the chemical structure of the probe molecule (control **SP-4**, Fig. 3(d) and Table S11, ESI[†]). Although a quantitative analysis of this shear effect on HB will require collecting data over a broader range of shear regimes (Fig. S19, ESI[†]), we believe these initial observations may account for some of the fluidic effects reported in the protein literature (*vide infra*).

We have experimentally confirmed the usefulness of this simple visual sensor of the HB character of a medium. The concept of adding a latent intra-molecular HB feature to a spiropyran dye and the possibility of visually gauging changes to the thermodynamic cost of this feature is in principle applicable to other families of photochromic dyes, such as chromenes or spirooxazines. It is possible to anticipate broader applications of this technique, for instance changes

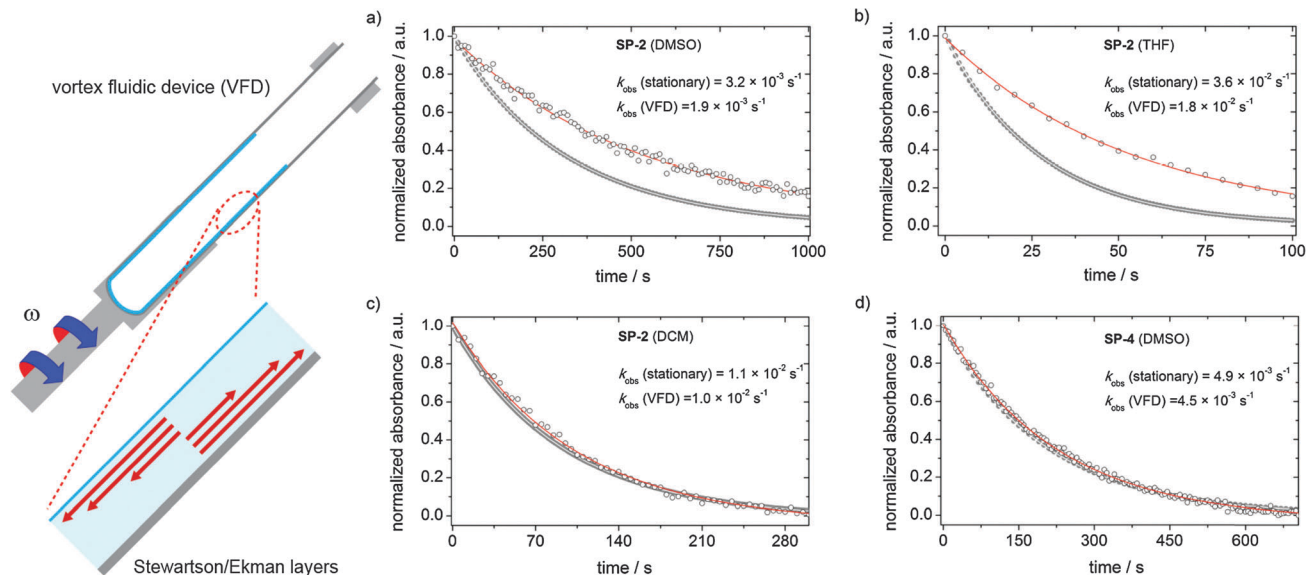


Fig. 3 Gauging shear-induced perturbations to the intra- vs. inter-molecular hydrogen-bonding balance. (a)–(c) Representative plots of absorbance data up to ca. 4 half-life times for the thermal decoloration of **SP-2** (MC/SP relaxation) under either stationary conditions (●) or under the effect of shear forces in a vortex fluidic device (VFD at 4000 rpm, tilt is 45°; (○)) highlighting the shear generating Stewartson/Ekman layers. Solid lines are best fits to the data (—). (d) Control trials by deliberately removing hydrogen-atom donation in the open-ring isomer (**SP-4**). Solvent system and refined k_{obs} values are indicated in each panel.

to the decoloration rates of **SP-2** could help elucidating controversial issues such as the nature of intermolecular interactions in ionic liquids.²⁴ We have also shown that in the specific case of **SP-2**, the intra-/inter-molecular HB balance is altered by a shear stress stimulus in a fluid. Although still debated,^{14a} but most certainly with broad reaching implications, the protein literature contains references to the possibility of shear-related protein denaturation.^{14b,c,25} Clear insights into the relationship between shearing forces and the thermodynamic stability of proteins are still lacking, but our findings for **SP-2** have implication for understanding protein folding under shear stress.

SC acknowledges the University of Wollongong for a Vice Chancellor Fellowship. CLR acknowledges financial support from the Government of South Australia. Funding from the ARC Centre of Excellence Scheme and resources provided through the National Computational Merit Allocation Scheme are also gratefully acknowledged.

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