

REVIEW ARTICLE

[View Article Online](#)
[View Journal](#) | [View Issue](#)

Excited-state proton coupled charge transfer modulated by molecular structure and media polarization

Alexander P. Demchenko,^{*a} Kuo-Chun Tang^b and Pi-Tai Chou^{*b}Cite this: *Chem. Soc. Rev.*, 2013, **42**, 1379

Received 31st May 2012

DOI: 10.1039/c2cs35195a

www.rsc.org/csr

Charge and proton transfer reactions in the excited states of organic dyes can be coupled in many different ways. Despite the complementarity of charges, they can occur on different time scales and in different directions of the molecular framework. In certain cases, excited-state equilibrium can be established between the charge-transfer and proton-transfer species. The interplay of these reactions can be modulated and even reversed by variations in dye molecular structures and changes of the surrounding media. With knowledge of the mechanisms of these processes, desired rates and directions can be achieved, and thus the multiple emission spectral features can be harnessed. These features have found versatile applications in a number of cutting-edge technological areas, particularly in fluorescence sensing and imaging.

^a Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, 9 Leontovicha street, Kiev 01030, Ukraine. E-mail: alexdem@ukr.net; Fax: +380 44 279 6365; Tel: +380 44 234 1106

^b Department of Chemistry, 1 Roosevelt Rd. Sec. 4, Taipei 106, Taiwan, R.O.C. E-mail: chop@ntu.edu.tw; Fax: +886 2 2369 5208; Tel: +886 2 3366 3894



Alexander P. Demchenko

Nanobiotechnology at the same institute. His research interests involve biophysics, photophysics and spectroscopy of proteins and biomembranes and the development of new fluorescence-based methods. He is the author of popular textbooks "Ultraviolet spectroscopy of proteins" (Springer 1986) and "Introduction to fluorescence sensing" (Springer 2009) and of about 150 research papers and reviews in rated journals.

Alexander (Oleksandr) P. Demchenko graduated from the Department of Physics of Kiev State Schevchenko University in 1967 and got his PhD in Biochemistry in 1972. He got Habilitation (Dr of Science) degree in Biophysics in 1982 and organized the Department of Biophysics at the Palladin Institute of Biochemistry at the National Academy of Sciences of Ukraine. Presently he is the head of laboratory of

1. Introduction

Proton transfer (PT), one of the most fundamental processes involved in chemical and biochemical reactions, deserves the attention of vast numbers of scientists, leading to thousands



Kuo-Chun Tang

Pi-Tai Chou's group at the National Taiwan University (2010–present) and his research interests and expertise are in the field of ultrafast spectroscopy on the excited-state dynamics.

Kuo-Chun Tang received his PhD degree from National Tsing Hua University, Hsinchu, Taiwan, in 2003, and pursued postdoctoral research at Department of Chemistry and the FOCUS (Frontiers In Optical Coherent and Ultrafast Science) center of the University of Michigan, Ann Arbor (2006–2010), after the discharge of his obligated military service (2004–2005). He is currently a postdoctoral research fellow working in Prof.

upon thousands of publications and numerous books on related topics.^{1–11} Various types of proton transfer reactions, depending on, *e.g.*, the reaction in the ground or excited states, whether they are adiabatic or non-adiabatic, and strong *versus* weak hydrogen bonding, with their driving forces based on acidity (proton donor) and basicity (proton acceptor), have been classified and identified.^{1,12–17} From the molecular point of view, proton transfer can be generally categorized into *intermolecular* and *intramolecular* cases. The former, which are frequently encountered in biological systems, involve transfer of protons from the donor to the acceptor and commonly require uniquely arranged proton relay systems.¹⁸ The host/guest type of proton transfer, which takes place upon formation of a dimer or complex between proton donating and accepting molecules, is also ascribed to this category.^{19–26} The latter cases involve proton transfer within the same molecular framework. Systems invoking excited state intramolecular proton transfer (ESIPT) are the focus of this review. ESIPT occurs in a unimolecular basis, which avoids bimolecular complexity, most intermolecular dynamics, and most entropic effects, allowing the observation of fluorescence response from both reactant and product forms as well as transitions between them with high resolution in the time domain, down to femtoseconds.¹ As a result, ESIPT can serve as a model to mimic, *e.g.*, the ground-state catalytic and biocatalytic reactions and allow better understanding of them.^{27–32} No less important are their realized and prospective technological applications.^{11,33,34}

ESIPT and the subsequent reverse proton transfer in the ground state achieve a fully reversible reaction cycle. Such cycles have been ubiquitously observed in aromatic molecules possessing proton donor and acceptor groups bridged with a hydrogen bond. Hydroxyl or amino groups generally serve as



Pi-Tai Chou

Pi-Tai Chou received his PhD degree from Florida State University, Tallahassee, in 1985, and is currently a Chair Professor of Chemistry Department and Director of Center for Emerging Material and Advanced Devices in National Taiwan University (NTU). Prior to joining NTU, he was a DOE postdoctoral fellow at University of California, Berkeley (1985–1987), an assistant professor at University of South Carolina, Columbia (1987–1994), and a professor of National Chung Cheng University (1994–2000). His research interests are in the area of ultrafast phenomena of excited-state proton/charge and energy transfer reaction; syntheses, photophysics, and applications of materials suited for OLED, solar energy cells, and nanotechnology. He is a Fellow of Royal Society of Chemistry (FRSC) and has published more than 350 SCI papers with current h-index of 50.

(1987–1994), and a professor of National Chung Cheng University (1994–2000). His research interests are in the area of ultrafast phenomena of excited-state proton/charge and energy transfer reaction; syntheses, photophysics, and applications of materials suited for OLED, solar energy cells, and nanotechnology. He is a Fellow of Royal Society of Chemistry (FRSC) and has published more than 350 SCI papers with current h-index of 50.

the proton donors, and carbonyl oxygen or azo nitrogen as the proton acceptors. Upon electronic excitation, the redistribution of electronic charge makes the proton donor more acidic and the acceptor more basic. Thermodynamically, the enhancement in the basicity/acidity factor, which is several orders of magnitude, makes ESIPT energetically favourable, provides the strong driving force for its occurrence, and explains why the same reaction does not occur in the ground state.

The redistribution of electron density does not only provide the driving force of ESIPT. The charge relocation can be reinforced by the integration of weak intermolecular interactions (such as solute/solvent dipole–dipole interactions), which dramatically influence both reaction kinetics and thermodynamics. Electronically excited to the Franck–Condon state, a molecule with an enhanced distribution of electronic charge and thus a large change of dipole moment strongly polarizes the surrounding medium. This leads to a dielectrically stabilized charge-transfer (CT*) excited state. The reaction leading to this state can be referred to as the excited state intramolecular charge transfer (ESICT) state. Such stabilization can also occur from the product side if the ESIPT reaction generates a substantial charge-transfer character.

The prerequisite of ESIPT lies in the formation of an intramolecular hydrogen bond. Recent advances have shown that the hydrogen bond plays an important role in the excited-state behaviour of molecular dyes. For instance, several theoretical approaches have explored the site-specific intermolecular excited-state hydrogen bonding (H-bonding) property^{35–38} and found interesting correlations for fluorescence quenching, the dynamics of intermolecular charge transfer, and the spectral shifts *versus* the intermolecular H-bonding strength in the excited state.^{35,37–40} Also, using coumarin 102 and phenol as a H-bonding prototype, the results show that intermolecular H-bonding is significantly strengthened in the excited state but dissociated within the first ~200 femtoseconds,⁴¹ implicitly implying that the H-bonding strength may affect the proton transfer dynamics. One would thus expect that the H-bonding properties, such as strength, distance and orientation, should affect ESIPT, ESICT, and consequently the ESIPT–ESICT coupled reaction described below (*vide infra*). Unfortunately, systematic studies of the relevant correlations,^{35–41} and especially experimental work, are sparse, and hence the correlations await future resolution.

The proton is a positively charged particle and, in solution, its transfer leads to a new redistribution of electron density in the molecule. This redistribution influences its interactions with the surroundings. When the ESIPT reaction is observed in condensed dielectric media, such as liquid solvents, polymer matrices, and aqueous-based biological systems, the dielectric polarization of the environment can have a substantial impact on photophysical events, stabilizing the charge-separated states. Therefore, the ESIPT process can couple with ESICT reaction both kinetically and energetically.

This coupling depends strongly on the molecular structure. In a number of ESIPT systems, due to the small changes of dipole moment before and after proton transfer reaction, the interaction with the surrounding medium and correspondent

perturbation is negligible. In this case, ESIPT is so fast, *i.e.*, having no or a small energy barrier, that the rate is modulated by certain vibrations associated with hydrogen bonds.^{42,43} In this case, the role of ESICT cannot be noticed. There are systems, however, in which the interplay between ESICT and ESIPT is apparent, rendering drastically different photophysical phenomena such as dual emissions simultaneously associated with both ESIPT and ESICT. For an oversimplified approach, two possibilities can be promptly realized here. First, when ESICT takes place upon Franck–Condon excitation, *i.e.*, N → N* (N: normal ground populated state, N*: electronically excited state), N* may thus have large charge separation. The subsequent N* → T* ESIPT (T* stands for excited tautomer), leading to strong redistribution of the electronic density, may be subject to drastic change of the dipole moment. For the other possibility, we consider that the ESICT state is not formed upon excitation, whereas ESIPT is accompanied by significant charge separation. Thus, the (T*) form attains the property of the ESICT state and then relaxes to the (N) state *via* the (T) state, closing a reaction cycle. In both cases, the medium plays a very important role in harnessing the reaction and hence the associated spectroscopy and dynamics.

These interesting cases, and in-depth examinations of the underlying mechanisms, will be covered in different sections of this Review. Here we emphasize the most important general features of ESIPT in condensed dielectric media.

(1) ESIPT reaction exhibits the most significant Stokes shift (defined as the difference between absorption and emission peak frequency) that can be obtained in any intramolecular photophysical reaction. This shift can be as large as 6000–12 000 cm⁻¹. In other words, this anomalously large separation lies in the fact that the absorption and emission originate from two proton-transfer isomers. Such a shift in terms of wavelength provides a large spectral window convenient for spectroscopic measurements, since it allows the scattering of excitation light to be excluded. Also, it lowers the probability of homo-FRET (fluorescence resonance energy transfer between the same molecules) and discriminates the autofluorescence background from the informative signal in sensing and imaging.

(2) Such a strong decrease in the energy of emission quanta and a fast rate of the basic process still allow, in many cases, observation of ESIPT emission and the emission from initially excited normal forms. Well separated spectrally, the normal (N*) and tautomer (T*) forms can be seen simultaneously in steady-state spectra and can also be easily analyzed by time-resolved techniques. In these cases, both kinetic control and thermodynamic control can be in the background of the simultaneous existence of two forms in emission. In the latter case, as elaborated in the later section, the initially excited state generally demonstrates the properties of the ESICT state. Essentially, the energy of the ESICT state can be close to that of the ESIPT state. Due to the often small barrier separating them, the interplay of two emissions from a single molecule can thus be modulated.

(3) Chemical modifications produced distantly from the site of ESIPT reaction, but changing the electronic distribution within the molecule, provide dramatic effects by switching

the emission between N* and T* forms in broad ranges. One can even design a white-light emitter by properly selecting the positions and intensities of two fluorescence bands.³⁴

(4) Since the ESICT states are involved in the reactant or product side of ESIPT reaction, the interplay between two emissions well separated on the frequency/wavelength domain can be modulated in a very distinct way, not only by chemical modifications but also by the change in its surrounding environment. This extends dramatically the possibilities in the design of wavelength-ratiometric fluorescence sensors and probes.

The present Review will focus on the above issues. Also, amid our delineation, depending on the occurrence of ESICT before, concurrently with, or after ESIPT, those dye structures experimentally designed will be categorized into class I and II types. The structures of these dyes have distinctive features; the proton acceptor (donor) and the electron acceptor (donor) site are designed to be overlapped for class I (class II) molecules. The distinct differences between I and II in terms of their associated reaction thermodynamics and dynamics harnessed by the media polarization will be reviewed and discussed. We will then show that proper knowledge of the properties of ESICT coupled ESIPT systems opens up unlimited perspectives in research and application.

2. Theory of proton transfer reactions

Describing the coupling of electron and proton motions in dielectric environments has been a challenging task, and this coupling has recently been intensively investigated in the case of ESICT–ESIPT reactions. On the one hand, the quantum mechanics of electrons are well developed.^{44,45} On the other hand, the proton is much heavier than the electron (~1836 times), so it can display both classical and quantum-mechanical features. There are at least two lines of experiments demonstrating the quantum-mechanical nature of protons in ESIPT reactions.

(1) For most ESIPT systems with strong intramolecular H-bonds, the basic ESIPT process is nearly barrierless and proceeds within the femtosecond time domain. Such a fast rate is retained for matrix-isolated molecules at temperatures close to absolute zero.⁴⁶ Cryogenic experiments with the organic dye 3-hydroxyflavone (3HF) have clearly shown that the intrinsic ESIPT reaction in matrix-isolation conditions is barrierless, and that intermolecular interactions and/or perturbation introduce the activation barrier.^{47,48}

(2) The ESIPT rate depends critically on the distance between the proton donor and acceptor; *i.e.*, on the length of H-bond separating them. This dependence should be much sharper than that for an electron, since a proton is much heavier and thus has a much narrower wave function. This distance can vary on the sub-angstrom scale when systems with H-bonds closing 5-member, 6-member and 7-member rings are compared based on their ESIPT ability.⁴⁹

Several attempts to provide basic descriptions of ESIPT reaction grounded on formalism have been developed for corresponding reaction of electrons. In this way, both proton

tunnelling and solvent reorganization can be considered by categorizing the process into two regimes: nonadiabatic and adiabatic pathways.^{50,51}

In nonadiabatic limit, the reactant and product states interact very weakly, so the coupling of the matrix elements containing the two states may be rather small. In this case, the dominant factor that determines the reaction rate is the vibrational mode connecting two states, and the effect of the environment of the reaction site is introduced as the solvent reorganization energy. In adiabatic limits, in contrast, the interaction between two states is strong, and the presence of a nonzero matrix element allows observation of a “zone” of crossing their potential surfaces.^{44,45} The rate of such reaction is coupled with the solvent fluctuation frequency in the reactant environment, which allows reaching this zone and channelling in the direction toward the product. In intramolecular electron transfer reactions with π -electronic coupling between donor and acceptor occurring in dielectric media, the adiabatic limit demonstrates a closer approximation to reality,⁴⁴ so it may be expected that it will be a similar case for ESICT reactions.

When applied to proton transfer reactions, this theory meets several lines of criticism. In ESIPT reactions, the proton transfer coordinate is not always (and probably never is) the reaction coordinate. The stretching and bending vibrations of the -OH or amino group, or even of the whole molecular skeleton, can be determinants of this process. The ultrafast reaction time scale may correspond to the period of certain low-frequency, large-amplitude motions incorporating the change in nuclear distance associated with the hydrogen bond. Accordingly, attempts to improve this theory incorporated a low frequency vibrational motion between proton donor and acceptor, the so-called Q modes, for example,⁵¹ but such attempts still did not lead to quantitative description of these reactions.

One key question to be addressed is how to deal with the motions of protons, which probably determine the whole picture. In order to resolve this challenging problem, we observe several recent developments in the relevant theory. These developments call into question the traditional definition of proton transfer as the movement of a hydrogen nucleus between a donor and an acceptor atom. It has been suggested that the process can be described in such a way that the proton does not move at all. The distribution of electronic density changes in such a way that the proton interaction with the donor becomes looser, and the proton becomes heavily attracted by the acceptor. In other words, in the strong intramolecular H-bonding system, a proton transfer reaction can be defined as the movement of electronic charge density from the donor-hydrogen covalent bond to the acceptor-hydrogen covalent bond.⁴⁸ This interpretation, in a certain sense, is reminiscent of some previous approaches based on the symmetry argument; *i.e.*, the distribution of the nodal plane between reaction and product, rather than the relocation of the proton, to describe ESIPT.^{52–54}

In this respect, we must recall the fact that an optical electronic excitation starting any photophysical event changes drastically the distribution of electronic density on a scale much faster than any nuclear motion. After this instantaneous change,

the whole system relaxes to its equilibrium state, including the electrons, protons and heavier nuclei. The electron–proton correlation in its dynamics, arising from their attractive electrostatic interaction, must be quite complicated. The proton is not a usual atom. Although much heavier than the electron, it is still much lighter than other nuclei. In view of the quantum mechanical behaviour of the proton, the traditional Born–Oppenheimer separation between electronic and nuclear motions, based on mass and timescale differences, may break down.⁵⁵

The above progress then addresses another question, whether we should consider an electron–proton coupled reaction in a concerted or in a sequential fashion. If the process is sequential, then the electron and proton motions can be described independently, and the overall reaction rate is determined by the slowest reaction. Conversely, if the transfers of proton and electron are coupled, then the process can be concerted; *i.e.*, it occurs in a single step without an intermediate. As stated elsewhere,⁴⁸ these reactions typically are vibronically nonadiabatic because the electron–proton quantum subsystem does not respond instantaneously to the solvent motions. In this regime, the reaction could be described in terms of nonadiabatic transitions between charge-localized diabatic electron–proton vibronic states. Within this regime, the proton transfer may be electronically adiabatic, wherein the electrons respond instantaneously to the proton motion, or electronically nonadiabatic, wherein the response of the electrons is slower than the proton motion. The theory of such concerted processes has started to be developed quite recently,^{48,56} and its validity has already been supported in experiments.⁵⁷

Concluding this section, we reemphasize here that despite the many important contributions to our understanding over the years, it cannot be claimed that a solid theoretical background describing ESICT coupled ESIPT reactions already exists. The more general description should incorporate electronic and protonic components, molecular vibrations, and solvation dynamics. This delineation requires convincing support from the experimental point of view. The design of molecules to conduct the relevant proton coupled charge transfer reaction will be reviewed in Section 4.

3. Solvation dynamics and its coupling with ESICT/ESIPT

3.1. General theory of solvation in energy domain

Many experiments have demonstrated that solvation dynamics is an important component of ESICT–ESIPT coupled reactions. The motions along “the solvation coordinate” play a pivotal role. The consequences of such motions modulate the energies of electronic excited states, especially those of the states with substantial differences from the ground state distribution of charges. In ESICT reactions, such motions, as an integral solvent response in fluorescence emission, are easily observed by three complementary experimental methods: solvent-dependent shifts of steady-state spectra,^{58,59} red-edge effects,⁶⁰

and time-resolved fluorescence.^{61,62} Theory on these reactions is also well developed on the classical and quantum mechanical levels.⁶¹ Recent developments in molecular dynamics (MD) simulation⁶³ and hybrid methods involving quantum mechanics^{64,65} and MD (so-called QM/MM techniques) allow our understanding of these processes to be extended with atomic and molecular-scale resolution.

A number of models describing solvation dynamics use quasi-continuous approximation.^{64,68} This allows simplification of the solvent sub-system, assigning to it the properties of an infinite (usually isotropic) medium characterized by the same averaged parameters as the bulk solvent. Within a quasi-continuous approximation, the solvation is displayed in terms of refractive index n , responsible for its electronic component, and dielectric constant ϵ , describing the re-arrangement of nuclei and molecular framework. These two functions compose a physical definition of polarity that combines two basic effects, *i.e.*, the electronic polarizability and nuclear polarizability, to electrostatically stabilize a given molecule in a particular environment. The electronic polarizability of the medium provides ultrafast response and can be described as the function of the square of the refraction index, n^2 . The nuclear polarizability, expressed as a function of dielectric constant, ϵ , describes the presence of molecular dipoles interacting with the probing dye and their much slower motion in the electric field created

by this dye. In most applications, the continuum may be thought of as a population-averaged or time-averaged solvent environment displayed under the condition of Boltzmann equilibrium.

Most classical and quantum mechanical treatments of solvation dynamics are based on the Onsager cavity model (see Fig. 1a), which allows description of this process as occurring in a self-consistent reaction field. This field is created by the dye located in the cavity center, the dipole moment of which can be changed upon electronic excitation, and by the medium surrounding this cavity. The medium responds in a self-consistent manner to the changes of the central dipole. When the effects of electronic polarizability are small (*e.g.*, in the case of fluorescence spectra in highly polar liquids), the Onsager polarity function can be expressed as

$$f(\epsilon) = 2(\epsilon - 1)/(2\epsilon + 1) \quad (1)$$

which can be used as a simplified estimate of polarity. In addition, the effects of generation of induced dipoles require a more complicated description in both ϵ and n^2 terms. The interaction giving rise to electronic and nuclear polarizability in the fluorophore environment is considered “universal”, in order to distinguish it from “specific” interactions arising from, *e.g.*, the intermolecular charge-transfer (CT) complexes and H-bonds.^{58,59}

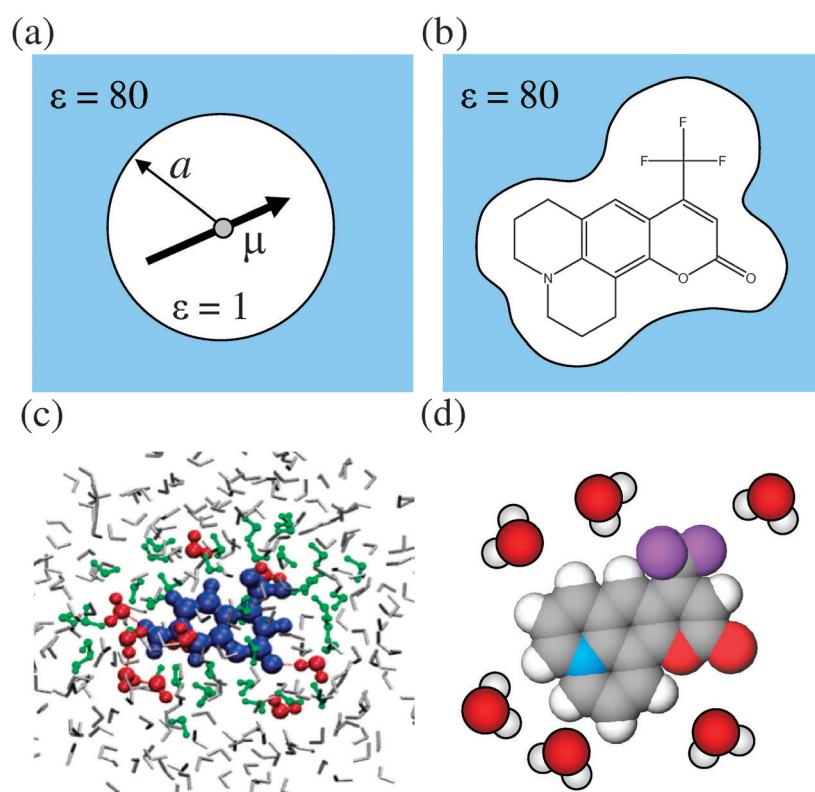


Fig. 1 Schematic view of various solvation models.⁶⁶ (a) Simple Debye–Onsager model. (b) Implicit solvation used in polarized continuum model.^{64,65} (c) Hierarchical solvation in quantum dynamics,⁶⁷ where different solvation shells are shown in different colors. (d) Explicit solvation. The coumarin 153 probe in water is used as an example. (With kind permission from Springer Science + Business Media: *Advanced Fluorescence Reporters in Chemistry and Biology III: Applications in Sensing and Imaging*, Part I General Aspects, 2011, P. 10, A. P. Demchenko and S.O. Yesylevskyy, Fig. 2.)

Thus, the solute dipole polarizes the medium, and the medium provides an integrated effect on it, creating the Onsager reactive field. The reactive field strength is proportional to the solute dipole moment in both ground and excited states. The energy shift on transition from a vacuum ($\hbar\nu_0$) to a dielectric ($\hbar\nu$) environment is proportional to the product of the reactive field vector R and $\Delta\mu = \mu_e - \mu_g$, the change of dipole moment on electronic excitation:

$$\hbar\Delta\nu = \hbar\nu_0 - \hbar\nu = \Delta\mu R/h \quad (2)$$

The simplest models consider the dye as a point dipole located in the center of the spherical cavity of the radius corresponding to the dye dimension (Onsager sphere radius a , see Fig. 1a). The frequently used Lippert–Mataga equation⁵⁸ is based on approximation, in which all polarization effects, except the generation of the reactive field, are neglected and the dipoles of the ground and the excited states (μ_g and μ_e) are oriented in the same direction. As the spectroscopic parameter, the model uses the Stokes shift, which is the difference between the positions of dye absorption and emission maxima in terms of the wave-number scale (in cm^{-1}). It describes how the general solvent effects expressed as the function of n^2 and ε can produce the relative shifts between absorption and fluorescence emission spectra:

$$\bar{v}_A - \bar{v}_{F^-} = \frac{2}{hc} \left(\frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1} \right) \frac{(\mu_e - \mu_g)^2}{a^3} + \text{const} \quad (3)$$

Here c is the speed of light, and h is Planck's constant. In eqn (3), the difference between the two terms inside the large parentheses is defined as $\Delta f(\varepsilon, n)$; *i.e.*,

$$\Delta f = \frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1} \quad (4)$$

and is commonly called the orientational polarizability. It is conceivable that n^2 contribution accounts for the ability of the electrons belonging to polarizable groups in the environment to be polarized in order to stabilize the dipole moment. Since such polarization is instantaneous and occurs during the absorption process, the consequence is that the absorption spectrum is shifted toward lower energies, decreasing the Stokes shift. In contrast, the dielectric constant ε term accounts for the relaxation process, which involves the rotational or translational motions of groups of atoms or whole molecules. This process evolves in time and results in the shifts to lower energies of the fluorescence spectrum, *i.e.*, in increase in the Stokes shift. The constant term in eqn (3) accounts for small additional spectral shifts due to excitation and emission to higher vibrational levels of the excited state and ground state correspondingly. The Δf values calibrated in different aprotic solvents can be used for determining $\mu_e - \mu_g$ values, which can serve as the empirical scale to assess the strength of ESICT.^{69,70}

There have been a number of attempts to improve the Lippert–Mataga equation.⁷¹ Bakhshiev considered the induced polarization terms and the non-parallel orientation of ground-state, μ_g , and excited-state, μ_e , dipole moments.⁷² Somewhat different Δf values are used for calibration when the induced

dipoles are taken into account, resulting in more complex functions of ε and n . In these treatments, all solute–solvent interactions are considered as “universal”, and the involvement of specific interactions (such as H-bonds) can be detected as the deviations from linearity in the Stokes shift *vs.* Δf plots (the Lippert plots).^{58,59}

Dealing with simple parameters obtained from experiments, this very simplified description does not provide sufficient details for understanding the mechanism of the photophysical process or the environment dynamics at the molecular level. In this respect, molecular dynamic (MD) simulations that allow computation of the forces between all atoms in the system and equilibration of the structure in a chosen thermodynamic ensemble^{73,74} have a much broader applicability. Nevertheless, strong limitations are also imposed. Based on the application of classical mechanics, they do not allow description of the electron motions. In addition, all-atomic description of the solvent (see Fig. 1d) yields excessive information that is hard to analyze. Coarse-grained models that involve coupling of several atoms or molecules into one unit⁷⁵ simplify the calculations and their analysis but still do not allow study of the reactions, in which the redistributions of the electronic density inside the molecules are essential, particularly for the excited-state reactions. In response to these demands, the development of hybrid simulation techniques, which combine molecular dynamics with quantum mechanics,^{76,77} has been boosted in recent years. Such combined techniques allow the computation of the electronic properties of small critical subsystems (such as organic dye molecules together with their binding sites) in realistic dynamic environments, which is described in terms of classical mechanics. The underlying quantum subsystems can provide the trajectories of individual atoms together with their electronic densities.

It must be emphasized again that atomistic simulations of the compounds in the excited electronic states are still rather challenging. Excitation is an electronic process that can be described adequately only by the methods of quantum mechanics, for which the computation in condensed medium is extremely intensive and even formidable. Therefore, a reasonable compromise is needed between the accuracy of the quantum description and the computational speed of the classical one. Comparative analysis of contemporary approaches has been reviewed recently,⁷⁸ and here we provide only short remarks.

First, a benefit could arise from the fact that the lifetimes of the excited states are commonly longer than the relaxation times in fluid environments.⁶³ Then the quantum mechanics (QM) calculations provide charge distributions in the ground and excited states, and the classical MD could be used, since the charge distribution in the electronically excited molecule could be approximated adequately by the point charges on individual atoms. When the excited state is short-lived and the perturbations caused by the excitation of charges of surrounding molecules are the only factor to be considered, the hybrid quantum mechanics/molecular mechanics (QM/MM) methods could be accessible. These advances reduce the computational burden of the quantum dynamics while keeping the accurate

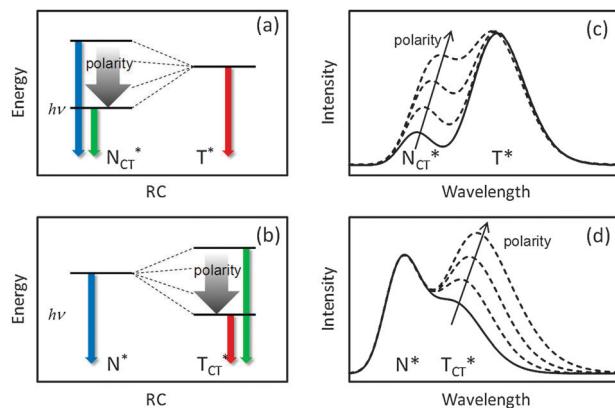


Fig. 2 Two limiting cases of coupled ESICT-ESIPT reactions, (a) ESICT first and (b) ESIPT first, of the ESICT/ESIPT dynamics and their corresponding steady-state spectra (c) and (d) respectively. Dashed lines in (c) and (d) denote the expected spectral change subject to solvent polarity change. For clarity, the intensity of emission with charge transfer character is intentionally set to increase as the solvent polarity increases. Note: for the convenience of demonstrating the dual emission, it is assumed that both N_{CT}^* and T_{CT}^* can be either thermally or kinetically populated (also see Fig. 3).

quantum description of the excited states. In the hybrid techniques, a small part of the system in the vicinity of the excited molecule can be described on the quantum level, while the rest of the system is purely classical (Fig. 2c).

Different versions of combination of QM and MM have been suggested using the approach depicted in Fig. 1. Recently, the influence of the polar water environment on the excited state of coumarin 151 dye was successfully modelled *via* treatment of solute at a simplified semi-classical level,⁷⁹ while the approach to the polarity effects in quantum calculations is based on the continuous implicit solvent model with the volume polarization.⁸⁰ Another direction of improvement of implicit solvent models is to take the time-dependent solvation effects into account. It was shown that introduction of the time-dependent continuous polarization into the quantum dynamics allows extremely good reproduction of the experimental time-dependent Stokes shift in coumarin 153 and the charge transfer in *N,N*-dimethylaniline.⁶⁵

As pointed out at the end of Section 2, the excited-state electronic charge transfer and the proton transfer reactions can be either concerted or sequential. This causes difficulties in describing the solvation dynamics effects. Two collective solvent coordinates may need to be introduced to describe the changes in equilibrium solvent polarization associated with electron and proton movements.⁸¹ Depending on the relative timescales of charge transfer and of solvent relaxation, the dynamics of these collective solvent coordinates may be coupled to the whole process. Thus, the equilibrium solvent configuration can be considered similar for the electronic ground state and the reaction (proton transfer) product state, but distinctly different for the photoexcited and relaxed (on the solvation time scale) ESICT state. The proton may not be transferred immediately upon photoexcitation, but transferred with the electronic density in the concerted step. In this case,

the proton potential is similar for the electronic ground state and the photoexcited donor state (*i.e.*, when the proton is bonded to the same atom), while the proton potential is distinctly different for the acceptor state (*i.e.*, the proton is bonded to a different atom). In such studies, the transferring proton was treated quantum mechanically, and the solvent was either represented by a dielectric continuum model⁸² or treated by molecular dynamics simulations with explicit solvent molecules.⁸³

3.2. Solvation dynamics and ESICT in time domain

Any reaction involving translocation of charge in dielectric medium is associated with response in that medium, which, in addition to ultrafast response in electronic polarization, develops as a slower rotational and translational motion of molecules or the groups of the associated atoms. After optical excitation, the surrounding solvent molecules appear in a nonequilibrium configuration because the polarization effect being created will still correspond to the equilibrium configuration for the ground state, rather than for the excited state. The strong distribution of electronic charge and hence the substantial change of the dipole moment after excitation polarize the surrounding medium, generating its relaxational mobility. Accordingly, the surrounding solvent molecules rearrange and reorient themselves to stabilize the new charge distribution in the excited state. Finally, a local equilibrium is reached, from which the fluorescence emission occurs. The time dependence of this rearrangement is characterized by “solvation relaxation time”, which is reflected in the continuous temporal red shift of the emission spectrum.⁶¹

To characterize this motion, the solvation time correlation function (STCF) was introduced and is defined as

$$S_{\text{obs}}(t) = \frac{v(t) - v(\infty)}{v(0) - v(\infty)} \quad (5)$$

where $v(t)$ denotes the time-dependent emission frequency (in cm^{-1}) of the solute chromophore. This function is so normalized that it decays from unity at $t = 0$ to zero at $t \rightarrow \infty$. Since the emission frequency scale is proportional to the scale of energy, eqn (5) describes the time evolution of solvation energy. The temporal characteristics of solvation then can be followed by monitoring this function *via* recording a series of time-resolved spectra, *i.e.*, a spectral temporal evolution.⁸⁴

If the ESICT states are formed, then large deviation from the ground-state equilibrium is expected. Therefore, these states can be recognized not only by a strong Stokes shift and the high polarity response of this shift, but also by time-dependent solvent response correlated with the rate of dielectric relaxations in the solvent. In addition, when the rate of solvent response is comparable to or slower than the rate of fluorescence emission, the Red-Edge effects in excited-state reactions can be observed in a steady-state manner^{85,86} and in time-resolved spectra,⁶² the extent of which correlate with the density in population of the surrounding dipoles. These features allow recognition of molecules exhibiting ESICT.

Thus, imagine that we observe in fluorescence spectra the two bands as a result of proton coupled charge transfer reaction.

One of them belongs to the ESICT state, with a strongly separated electronic charge, and the other is the state in which the charge separation is compensated by the relocation of the proton. To recognize the ESICT state, we can use the criteria elaborated above. The correspondent spectral band should exhibit a position strongly dependent on solvent polarity in a steady state manner (see Fig. 2),⁸⁷ the red-edge effect in a rigid environment,⁶⁰ and a shift in the spectrum in the time domain on the scale of solvent relaxations.⁸⁸ Such solvation dynamics effects in the N^* state were observed in 3-hydroxyflavone derivatives^{87–89} and in T^* state for 5-methoxysalicylic acid in the presence of diethyl ether acting as the H-bond acceptor.⁹⁰

3.3. Interplay of ESICT and ESIPT in steady-state emission

The fluorescence emissions from ESICT and ESIPT states can be recognized by the different positions of their spectra and also, in some cases, by their different polarizations and lifetimes. In order to explain the frequent appearance of two bands in steady-state emission simultaneously, it is necessary to accept that the N^* state responding to the fluorescence is not the initially excited Franck–Condon (F–C*) state, but rather a state accessed after some steps of relaxation to a local energy minimum. Formally, we have to consider the presence of at least two minima on the excited-state reaction coordinate and two possible mechanisms of transitions between them.⁹¹

(i) **Kinetics.** In this case (Fig. 3a), owing to a large energy gap between the N^* and T^* states, the equilibrium in the ESIPT reaction is shifted towards the T^* form, which makes the reaction practically irreversible. However, the rate of this reaction can be slow owing to some energy barrier, most plausibly the solvent polarity induced barrier (or perturbation by external H-bond) separating the two states along the solvation coordinate. In this case, during the reaction, the lifetime of the N^* form may be long enough to emit light, and this emission is termed the N^* band fluorescence. In more general expression, this case may also include those of intermolecular H-bonding perturbations, as observed for parent 3-hydroxyflavone¹² and 3-hydroxyquinolones.⁹² Then the factors that induce the barrier for ESIPT reaction (such as temperature and viscosity) along certain conformational coordinates can modulate the intermolecular interactions with H-bond donors (including water), generating a fluorescence signal from the N^* state.

(ii) **Thermodynamics.** In this case (Fig. 3b), the ESIPT reaction can be very fast. However, the presence of the reverse reaction on a similar timescale, which is faster than the fluorescence decay of both N^* and T^* forms, enables equilibrium to be established between these forms. This leads to the appearance of two emission bands that can be of comparable intensities. In this case, the distribution of intensities between two bands is determined by the Boltzmann distribution of excited-state species between these forms.⁹³ As we will see in the later sections, many designed 3-hydroxychromone derivatives are ascribed to this case, in which the stabilization of the N^* state occurs due to its transformation into the ESICT state by introduction of electron-donating substituents. The rapidly established equilibrium between two excited-state populations

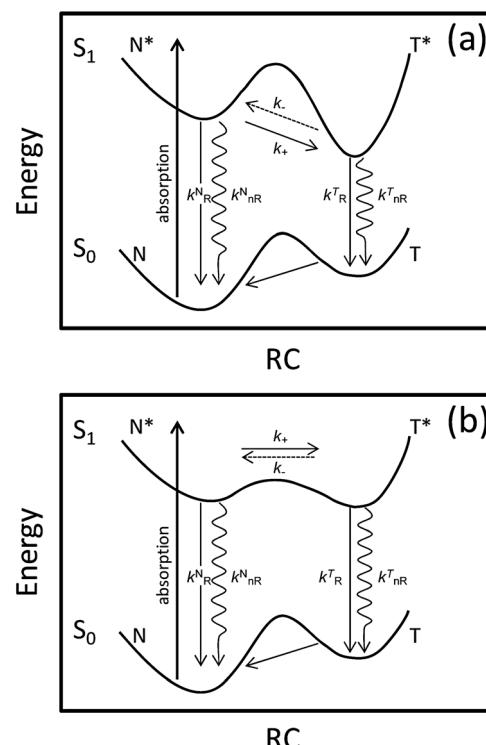


Fig. 3 Schematic representations of two mechanisms of ESIPT reaction, for which the two bands of similar intensities can be observed in fluorescence emission spectra. (a) Slow irreversible reaction is the case when $k_- \rightarrow 0$ or $k_+ \gg k_-$. Due to the appreciable barrier (see text), the reaction occurs on a similar time scale of emission, so the emission from the initially excited state N^* can be observed. (b) In the case of fast reversible reaction, the origin of observation of emission from the N^* state is the fast rates of both forward and reverse reactions (expressed by k_+ and k_-), so the distribution of intensity between two bands corresponds to the distribution of excited-state species at equilibrium. Note that the reaction coordinate RC is oversimplified, which may represent the sum of multiple dimensional pathways, including the solvent coordinate. (Reprinted from Chemical Physics, Vol. 342, V. I. Tomin et al., Dynamic quenching as a simple test for the mechanism of excited-state reaction, P. 127, Copyright © 2007 with permission from Elsevier, ref. 91.)

allows variation of the relative intensities of correspondent fluorescence bands as a function of polarity⁹⁴ and local electric field,⁹⁵ but as a result, they are insensitive to modulations of lifetime, e.g. by variations of temperature⁹⁶ or by dynamic quenchers.⁹¹

The difference between these cases may be recognized in a time-resolved manner (Fig. 4a and b). In the case of the kinetic mechanism, the precursor (N^*) and successor (T^*) type of relationship is obvious; that is, the decay rate of N^* band emission matches the rise time of T^* emission. In contrast, in the case of rapid establishment of thermodynamic equilibrium, the decay curves are two-component curves for both emissions. Shorter components (decaying for the N^* band and rising for the T^* band) reflect the rate of ESIPT reaction, whereas the long components are essentially equal and demonstrate depopulation of both states coupled by dynamic equilibrium. Kinetic and thermodynamic regimes of ESIPT reaction can also be distinguished by applying two recently suggested

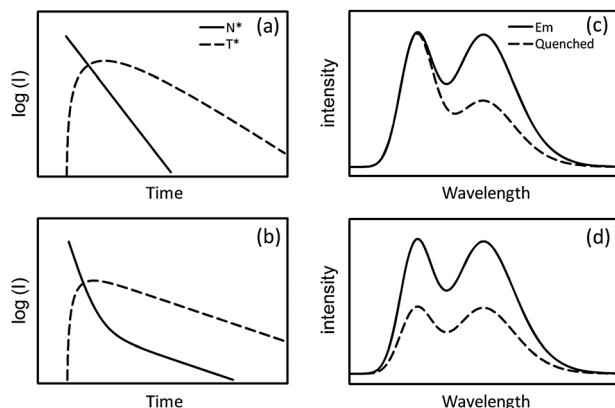


Fig. 4 Manifestation of (a) kinetic and (b) thermodynamic regimes in spectrally resolved fluorescence emission decays and in quenching-resolved steady-state spectra (c) and (d), respectively.

methods that use only steady-state measurements (Fig. 4c and d). They are based on thermal quenching⁹⁶ or the application of an efficient collisional quencher.⁹¹ Accordingly, one can demonstrate that the dynamic quenching decreases the intensities of both bands proportionally in the case of a thermodynamic regime, whereas in the case of a kinetic regime, the quenching of T* emission should be much stronger (Fig. 4).

The involvement of ESICT can change dramatically the regime of ESIPT reaction. Thus, for parent 3-hydroxyflavone, in which the excited-state charge separation is small, a kinetic regime is observed, and the presence of the N* band in emission in protic solvents can be explained by perturbation of this state *via* specific intermolecular H-bonding.⁹⁷ A kinetic barrier may appear, owing to the change in this dye of the relative configuration of the phenyl and chromone rings, which may be coupled with the solvent-induced barrier along the solvation coordinate.⁹⁸ Thermodynamic control of ESIPT reaction is frequently observed in the cases of a strong CT character of the N* state and an appreciable solvent-induced barrier separating the N* state from the T* state. For 3-hydroxyflavone derivatives, this is characteristic for molecules with dialkylamino substitutions in the phenyl ring.⁸⁸ Owing to its low oxidation potential, this dialkylamino group serves as a strong electron donor, which allows the creation of a large dipole moment and contributes to the strong CT character of the N* excited state. The ESICT state can be further stabilized in more polar solvents,⁸⁷ slowing down the ESIPT rate (*vide infra*) and essentially establishing a strong correlation between N* and T* relative emission intensities and polarity.

3.4 Time-resolved measurement and theoretical approaches

The associated ESICT/ESIPT mechanism can be further probed by time-resolved spectroscopy. Since the rates of ESICT, ESIPT, and the accompanying solvation dynamics (in low viscous solvents) are mostly on the subpicosecond-picoseconds time scale, the corresponding measurement could not be successful without exploiting ultrafast spectroscopic techniques.^{28,39,42,99–106} Among these methods, the femtosecond fluorescence up-conversion technique, which directly monitors the fluorescence rise/decay

dynamics, is the most-utilized method, owing to its very high detection sensitivity, dynamic range, and fine temporal resolution, which is as good as ~25 fs.^{28,101,103} The other customary technique for studying ESIPT/ESICT dynamics is femtosecond transient absorption, which, to a certain extent, provides complementary data to the fluorescence up-conversion method.^{103,99–101} This technique is particularly useful when the resulting fluorescence is subject to long radiative lifetime (*i.e.*, the small transition dipole), non-fluorescent intermediate, or even a dark state, such that the up-conversion method cannot be well operative due to the small integrated signal.^{107–110} Ultrafast vibrational spectroscopy is another powerful tool, since it directly probes the changes of molecular structure in terms of bonding character, which is reflected in the vibrational frequency changes of local modes, and can thus be corresponded well to the theoretical calculations if possible.^{42,103}

In view of theoretical calculations, DFT (density functional theory) based computational approaches are so far the methods most applied for analyzing the ESIPT and ESICT reactions, owing to the computational efficiency and the satisfactory results in a qualitative fashion from a level of approximation.¹¹¹ The TDDFT (time-dependent DFT) gives the vertical transition energies and oscillator strength of the excited states and is often used for comparison to the steady-state absorption and emission spectroscopy. However, a main problem of over-stabilization to the charge transfer state has been addressed and should be considered with the attenuation by asymptotically corrected functionals.^{112–114} Alternatively, the combination of HF (Hartree–Fock)/CIS (configuration interaction singles) for the stationary point of the excited state and the TDDFT provides a better precision level to compare the experimental data.¹¹⁵ To probe the reaction kinetics, molecular dynamics (MD) simulations with, *e.g.*, EVB (empirical valence bond) potentials obtained from DFT/CIS/TDDFT are commonly employed so that the results can be correlated with the ESIPT rate, IVR (intramolecular vibrational redistribution), temporal spectral shifting, solvation, *etc.*^{106,116,117} It should be noted, however, that Lischka, Barbatti, and co-workers very recently pointed out that excited-state calculations are still quite challenging due to the significant shift in electron density and the resulting irregular shapes of potential energy surfaces caused by the excited state dynamics after photoexcitation.¹¹¹ Therefore, it is not surprising that most of the theoretical approaches to date for the proton transfer coupled reaction are in the electronic ground state. Computation methods developed for the ESICT/ESIPT coupled reaction, especially when the ESICT and ESIPT are occurring from different sites of the molecular entity, are still in the initial stage.

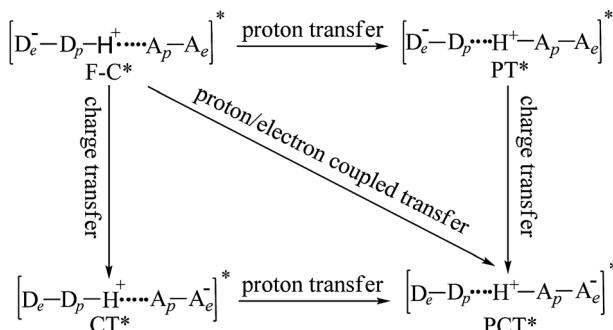
4. Conceptual ESIPT–ESICT coupled systems and their expected spectroscopic manifestations

In this section, we define various proton transfers, electronic charge transfers, and their coupled states in a more detailed

manner. This definition allows us to use the interplay between N^* and T^* emissions to determine the polarity of the medium.

Early theoretical advancements by Hynes and co-workers^{51,118} have incorporated both solvent reorganization and proton tunnelling and developed a framework similar to that of electron transfer reaction. As described above (Section 2), depending on the degree of coupling between the reactant and product potential energy surfaces, the overall proton transfer process can thus be categorized into two regimes, similar to the classification in electron transfer reaction, namely nonadiabatic⁴ and adiabatic⁵ limits. This formulation, which considers only pure proton migration, may not be sufficient to describe the situation when the charge transfer and proton transfer occur in different functional groups within the same molecular framework. In other words, an electron and a proton, in reality, may not transfer in a direct coupled manner from the same donor to the same acceptor.

For the more general expression, Hammes-Schiffer and co-workers have extended Hynes' work and established a model consisting of one set of four states relevant to different status of charge transfer and proton transfer.¹¹⁹ For the convenience of the later discussion, Scheme 1 shows a slightly modified reaction pathway based on Hammes-Schiffer's work, in which the electronically excited state is emphasized. In addition, the interrelation among the four states is linked by arrows to specify each reaction pattern. Here, we simply neglect the possible equilibrium established between two species, which will be elaborated in the later sections. Accordingly, the system could be described by one set of four diabatic states: the Franck-Condon excited state ($F-C^*$), charge transfer state (CT^*), proton transfer state (PT^*), and proton-coupled electronic charge transfer state (PCT^*). The functional groups D and A in each state symbolize donor and acceptor for charge (D_e and A_e) and proton (D_p and A_p) transfer. It should be noted that the $F-C^*$ state is not strictly restricted to the promptly excited level, but rather a state accessed after some steps of internal conversion and/or vibrational relaxation. On several occasions, hereafter, $F-C^*$ denotes the excited normal species (N^*) to have linkage with the notation used in the early sections.



Scheme 1 A schematic illustration of the proton-coupled electronic charge transfer reaction in the excited state (marked *). The functional groups in each state are represented as follows: D and A denote donor and acceptor for charge (D_e and A_e) and proton (D_p and A_p) transfer, while H^+ specifies the proton and the dotted lines representing hydrogen bonds.

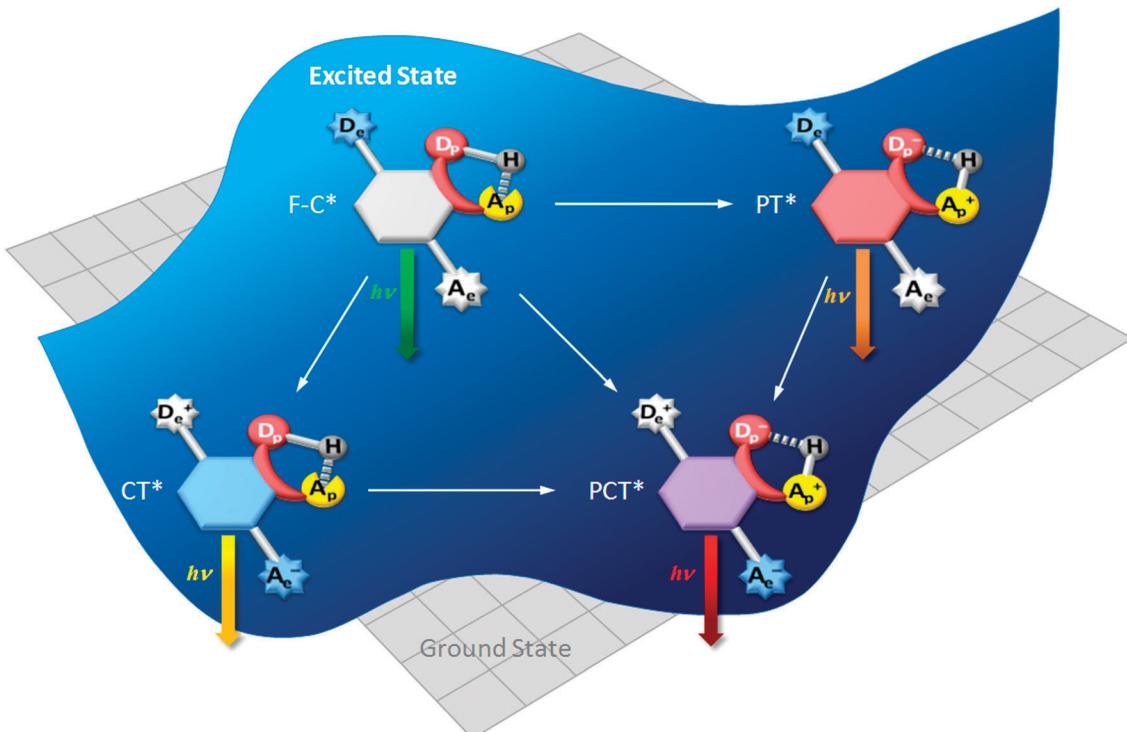
To give the reader a feel for the viewpoint of chemistry, Scheme 2 graphically imprints Scheme 1, utilizing the molecular illustration. As shown in Scheme 2, the requirement of photo-excitation unavoidably leads to the design of a core moiety composed of a $\pi\pi^*$ chromophore, which is represented by a benzene-like structure attached by four reaction sites, *i.e.*, proton and electron donor/acceptor.

Starting from the $F-C^*$ state, whether the proton/electron coupled reaction is sequential or concerted depends on the relative free energies of these four diabatic states, as well as on couplings between them. For example, when the PT^* and CT^* states are relatively higher in energy than that of $F-C^*$, the concerted mechanism is favoured. To fit the proposed reaction pathways shown in Scheme 1 (or 2), theoretically, it is convenient to treat the charge transfer (CT^*) as electronically nonadiabatic, and to treat the PT^* as electronically adiabatic. In this approach, the solvent environment is described by a dielectric continuum or explicitly by molecular mechanics, and the dynamical effect of proton donor-acceptor vibrational motion is also considered. The resulting rate constant expression is analogous to that derived by Hynes and co-workers, but the couplings, reorganization energy, and reaction energy are defined in terms of two solvent coordinates describing the solvent motions equilibrating two states.

Experimentally, efforts have been made to design and synthesize suitable molecules to serve as the model proton/electron coupled systems fitting the above criteria. One obstacle lies in the unavailability of molecules where the proton and electron donor/acceptor are fully separated into four independent sites. Instead, summarizing the literature reports, the ESIPT-ESICT coupled systems being designed and synthesized can be categorized into two classes:

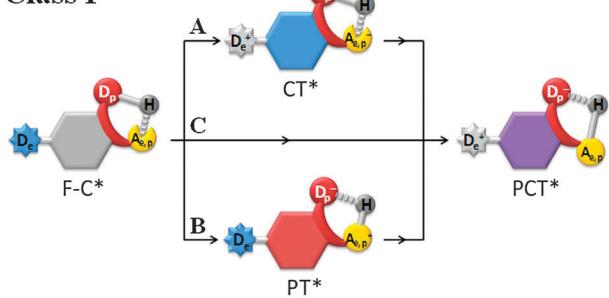
- Class **I** molecules, in which the proton acceptor and electron acceptor happen to be in the same group, but the electron donor and proton donor sites are different.
- Class **II** molecules, in which the proton donor and electron donor are indistinguishable, but the proton acceptor and electron acceptor sites are different.

This reality leads us to draw ESICT-ESIPT reaction models for class **I** and **II** molecules, depicted in Scheme 3. In most cases, D and A in the charge transfer process are separated by the aromatic moiety (represented schematically by a benzene-like structure; see also Scheme 2), and their relative positions are suited for either proton or charge transfer reactions. While the proximity (*-ortho*) in position between D_p and A_p is necessary, owing to the formation of the hydrogen bond, D_e and A_e can be far separated and undergo ESICT *via*, *e.g.*, polarization in a π -electron delocalized system. D_p provides a hydroxyl or amino hydrogen to form an intramolecular hydrogen bond with A_p .^{88,99,121–124} Due to the synthetic feasibility and decent proton acidity, the $-OH$ group seems to be ubiquitously applied as a proton donor. Chemically, the electron donating strength of $-OH$ may be weak. However, upon proton transfer, its conjugated base, *i.e.*, the oxide ion $-O^-$, drastically increases the electron donating ability. In the case of class **II**, the $-OH$ group is intentionally designed to be π -conjugated with the electron

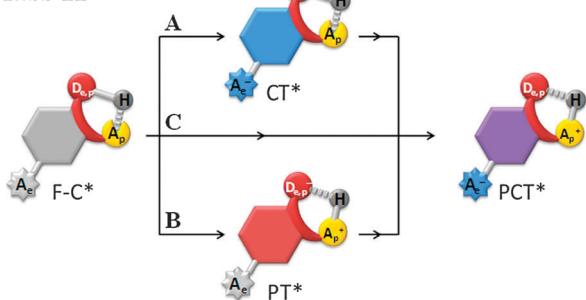


Scheme 2 A graphic illustration of the proton-coupled electronic charge transfer reaction in the excited state. Notation similar to those in Scheme 1 are used here. (Modified with permission from ref. 120. Copyright © 2008 American Chemical Society.)

Class I



Class II



Scheme 3 Generalized ESICT-ESIPT systems synthetically available up to this stage. Note that the notation used is slightly different from that in Scheme 1. Instead of using the symbol H^+ for the proton in Scheme 1, the hydrogen atom is used in Scheme 3. Also, the electron donor is uncharged to keep the system in a neutral form. $D_{e,p}$ and $A_{e,p}$ denote the donor and acceptor for both charge and proton transfer.

acceptor A_e , such that it may act as both proton and electron acceptor $D_{e,p}$ amid ESIPt.

For both class **I** and **II** types of molecules, depending on the reaction time domain, studies of ESICT vs. ESIPT can be classified into three pathways. When the rate of ESICT is faster than that of ESIPT, following $F-C^* \rightarrow CT^*$ charge transfer, the $CT^* \rightarrow PCT^*$ proton transfer reaction takes place (path A). Alternatively, when ESIPT takes place prior to ESICT, the overall reaction may be described as an $F-C^* \rightarrow PT^*$ proton transfer, followed by a $PT^* \rightarrow PCT^*$ charge transfer process (path B). Evidently, when the PT^* and CT^* states are relatively higher in energy than that of $F-C^*$, simultaneous proton coupled charge transfer reaction may take place in a concerted manner (path C).

To which category the proton and/or charge transfer reaction is ascribed strongly depends on the coupling between the reactant and product electronic states. For the case of proton transfer, its rate strongly depends on the distance between proton donor and acceptor, *i.e.*, the hydrogen bond length. The larger electronic coupling not only reduces the barrier height but also narrows the width. As mentioned earlier, compared to nonadiabatic electron transfer, the tunnelling probability in nonadiabatic proton transfer should be much more sensitive to the interatomic separation, simply because the proton is much heavier than the electron. Special attention is paid to pathway B, when ESIPT is followed by instantaneous ESICT. Kinetically, the reaction dynamics will behave more like the proton/charge transfer coupled reaction described by Hynes and co-workers in their theoretical work.^{51,118} This issue will be manifested in class **II**, in which the charge transfer will not take place due to

the weak $-\text{OH}$ electron donating strength until it undergoes ESIPT, forming the oxide ion $-\text{O}^-$. An exemplary case will be illustrated in Section 6.

In accordance with the above conceptual design and synthetic feasibility, a number of potential ESICT–ESIPT coupled systems have been synthesized and investigated. Prototypes include:

- Class **I** molecules such as *N,N*-dialkylamino-3-hydroxy-flavones,^{88,99,121–125} 2-hydroxy-4-(di-*p*-tolyl-amino)benzaldehyde¹²⁶ and 2-(2'-hydroxy-4'-diethylaminophenyl)benzothiazole,^{102,127} in which the *N,N*-dialkyl group is strategically designed to act as an electron donor, while the carbonyl oxygen or the nitrogen group within the parent ESPT moiety serves as an electron acceptor.

- Class **II** molecules such as 2-((2-hydroxyphenyl)benzo-[*d*]oxazol-6-yl)methylene-malononitrile (**diCN-HBO**)¹²⁸ and its analogues, in which either a mono-cyano or a di-cyano functional group is attached to act as the electron accepting group, while $-\text{OH}$ and its conjugated base act as the proton donating and electron donating groups, respectively. They are located at the same site and are denoted as $\text{D}_{\text{e,p}}$ for simplification (see class **II** in Scheme 3).

In our previous review article,⁸⁹ we have decently explained that the associated ESICT–ESIPT reactions are functions of crucial parameters such as reaction thermodynamics, the dipole moment difference between normal and tautomer forms, and the solvent polarity, the topics of which are keen on the fundamental approaches, especially in view of the dynamics of solvent relaxation. In the current Review, as shown in this and earlier sections, we are focusing more on the molecular design and generalization of the experimental results that can be fit to the theoretical framework without heavy involvement in the kinetic derivation. This tactic is intended to bring these classes of ESICT–ESIPT coupled molecules into a broad spectrum of interest in the field of materials science for their prosperous applications in bio-medicine and optoelectronics. On this basis, in the following section, the significance and influence of each case will be reviewed according to the strategic design of each designated molecular structure. Accordingly, representative molecules for classes **I** and **II** will be elaborated in Sections 5 and 6, respectively. Discussion on the sameness and difference in spectroscopy and dynamics, especially on the solvent effect, will be presented at the end of Section 6.

5. ESICT–ESIPT switching in class I molecules

To probe and to signify the solvation effects on ESICT–ESIPT coupled reactions, it is important to find prototype dyes in which strong redistributions of electronic density occur in the excited state, thus causing large changes in dipole moment. It is also desirable to incorporate both ESIPT and ESICT properties to achieve different modes of their coupling. For this reason, a facile synthetic approach is to exploit the already existing ESIPT molecules and carry out further chemical derivation to incorporate ESICT. In this respect, the well known ESIPT-molecule 3-hydroxyflavone (**3HF**) has played a special role as

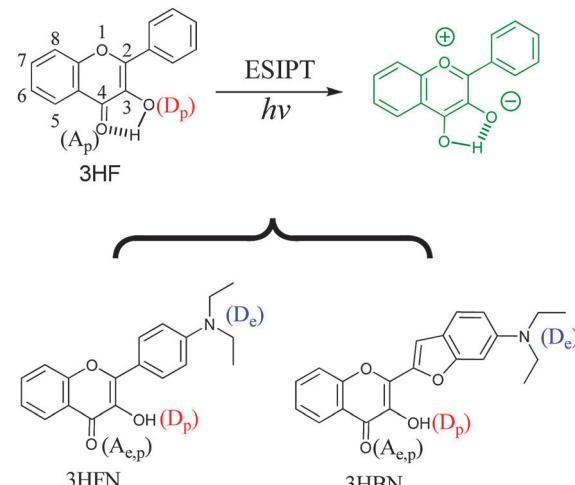


Fig. 5 Structures and atom numbering of **3HF** and its representative class **I** derivatives **3HFN** and **3HBN**. The parent molecule without substituent at position 2 is 3-hydroxychromone (**3HC**). Note that the pyrylium structure of the proton transfer tautomer is adopted from Sengupta and Kasha's seminal work,¹²⁹ which represents the popular structure existing in the flower pigments.

the model object, from which the designated molecules **3HFN** and **3HBN** (see Fig. 5) have been synthesized to demonstrate their prominent ESICT/ESIPT properties. As depicted in Fig. 5, **3HFN** and **3HBN** represent prototypes of class **I** molecules, in which the carbonyl group serves as both electron and proton accepting site, while the hydroxyl and dialkylamino groups act as proton and electron donor, respectively, which are far separated within the molecular framework.

5.1. Photophysics of 3-hydroxyflavones

Strong interest in photo-transformations of **3HF** and its derivatives lasted for many years after Michal Kasha proved the ESIPT origin of **3HF** emission (see Fig. 5),¹²⁹ establishing the four-level model for its reaction dynamics.^{12,130,131} There are many advantages to using this molecule as the ESIPT model. It is well soluble and highly emissive in many different solvents, and it has a rigid skeleton that does not allow it to isomerise in the ground or excited state (in contrast to other ESIPT dyes, in which the N^* emission appears due to the existence of ground state conformational isomers or a conformational isomerisation in the N^* state associated with a reorganization of the hydrogen bonds involved in proton transfer). In contrast to ESIPT in systems with symmetric proton transfer (e.g., tropolone and 9-hydroxyphenalenone), this reaction in **3HF** occurs between structurally and energetically asymmetric states and generates in the excited state a species with a changed electronic and nuclear configuration, the tautomer (T^*) form. The latter is isomeric to the initially excited normal (N^*) form and demonstrates a fluorescence spectrum dramatically shifted to lower energies (longer wavelengths), up to $5000\text{--}6000\text{ cm}^{-1}$. In nonpolar solvents such as cyclohexane, **3HF** exhibits the $\text{S}_0 \rightarrow \text{S}_1 (\pi \rightarrow \pi^*)$ transition maximized at 340 and 354 nm, while the fluorescence of **3HF** shows an anomalously large Stokes-shifted band maximized at $\sim 526\text{ nm}$ ($\Phi = 0.36$, $\tau_f = 3\text{ ns}$).¹³¹

Methylation of **3HF** at position 3 changes dramatically the fluorescence properties: 3-methoxyflavone demonstrates normal Stokes-shifted fluorescence maximized at ~ 360 nm in cyclohexane. It is thus clear that ESIPT takes place from the 3-hydroxyl proton to the carbonyl oxygen atom, giving rise to the proton-transfer tautomer emission. In a dry, extensively purified nonpolar solvent, such as cyclohexane, the time-resolved measurement renders an ultrafast time of ESIPT for **3HF** ($\tau_{\text{pt}} < 240$ fs),^{100,132} and ESIPT is essentially barrierless and perhaps triggered by the low-frequency skeletal motions associated with the hydrogen bond.¹³³ The effect of perturbations by intermolecular interactions or substituents in the **3HF** structure on the ESIPT rate is clearly observed in the studies in cryogenic Spolski matrices, where basic reaction is ultrafast and activationless.^{47,134}

Introducing an electron-donor substituent (dialkylamino group) into the *para* position of the phenyl ring dramatically changes the properties of **3HF**. The *N,N*-dialkylamino group is very strongly electron donating and, when conjugated with aromatic systems, it possesses an intramolecular charge transfer character. The presence of an electron donating group results in a large dipole moment inducing the difference between the normal (N^*) and the tautomer (T^*) forms in the excited-state. As a result, the proton transfer rate is significantly affected by the solvent polarization. Strong solvent-dependent variation of the position and intensity of the normal emission band is a clear indication of the ESICT state.^{87,135} Significant redistribution of the electron density from the electron donor to the 4-carbonyl acceptor was also shown by QM calculations,¹³⁶ and this was supported by direct measurement of dipole moments by electro-optical absorption measurements.¹³⁷ A variety of substitutions that change the length of a π -electronic system or modulate the excited-state charge distribution have been synthesized since then. Here we analyze the solvent-dependent behavior of classical and most typical ones, dialkylamino substituted **3HF**, among which the 4'-diethylamino-3-hydroxyflavone (**3HFN**) is the prototype (Fig. 5).

Experiments with femtosecond time resolution have shown that, upon photoexcitation, ultrafast ESICT takes place, generating the CT^* state on this time scale.¹²⁴ Subsequently, the solvent relaxation process from CT^* to CT_{eq}^* (subscript eq: equilibrium) and ESIPT occur competitively (see Fig. 6). After reaching the solvent equilibrium, proton transfer reaction from CT_{eq}^* to PCT^* takes place. As a result, in polar aprotic solvents, dual emission is observed: the shorter wavelength emission band (CT_{eq}^*) exhibits a strong positive solvatochromic shift from 460 nm in benzene to 520 nm in acetonitrile, while the longer wavelength emission band (PCT^*) exhibits almost solvent polarity-independent emission at 570 nm (Fig. 7).

These results, in combination with *ab initio* calculations,¹²⁴ led Chou *et al.* to unveil the importance of the relationship of the dipolar vectors among various states, and hence the corresponding solvation energetics in the overall ESIPT reaction. It is also concluded, as pointed out at the end of Section 3.1 for class I molecules, that a similar dipolar character is normally found between ground-state normal (N) and excited proton-coupled electronic charge transfer tautomer (PCT^*) species, whereas due to

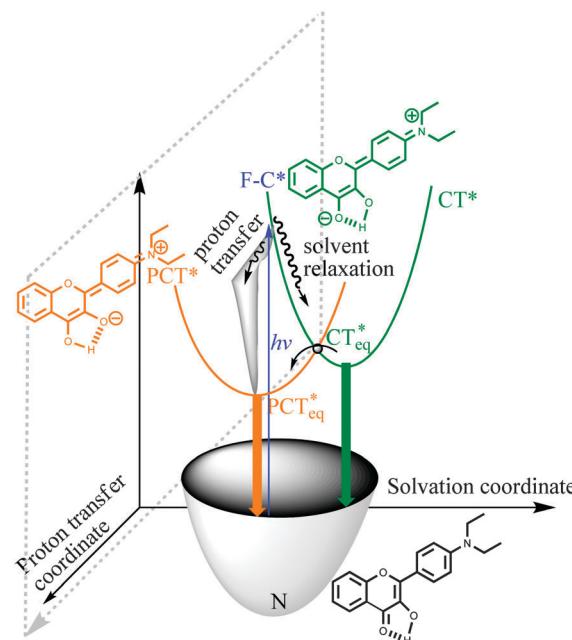


Fig. 6 Relaxation processes for case (A) ESICT/ESIPT system using **3HFN** as an example. For notation see Scheme 3. CT_{eq}^* and PCT_{eq}^* denote the polarization equilibrium of CT^* and PCT^* , respectively. Due to the complex coupling between CT^* and PCT^* , the drawing of the 3D potential energy surface is only for the ground-state N species. This is to show the origin of dual emission. Also note that $F-C^*$ may indicate a state being populated after ultrafast intramolecular vibrational redistribution (IVR) and internal conversion (IC).

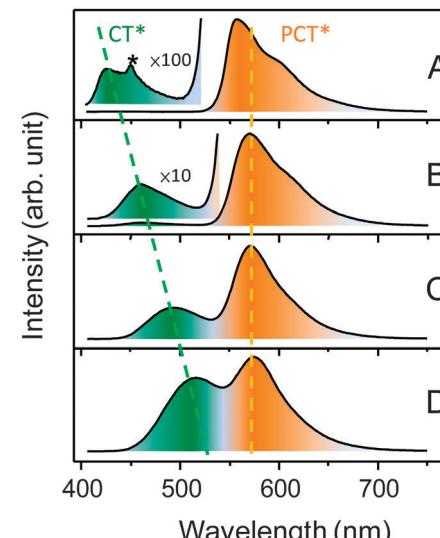


Fig. 7 Steady-state fluorescence spectra of **3HFN** in (A) cyclohexane, (B) benzene, (C) dichloromethane, and (D) acetonitrile at 298 K. The sign * shown in (A) denotes a Raman signal, which becomes obvious after magnifying the spectrum by ~ 100 folds. (Modified with permission from ref. 124. Copyright © 2005 American Chemical Society.)

the excited-state intramolecular charge transfer, the CT^* state possesses a large dipolar change with respect to N and PCT^* . ESIPT is thus energetically favourable even in the Franck-Condon excited state $F-C^*$, and its rate is competitive with

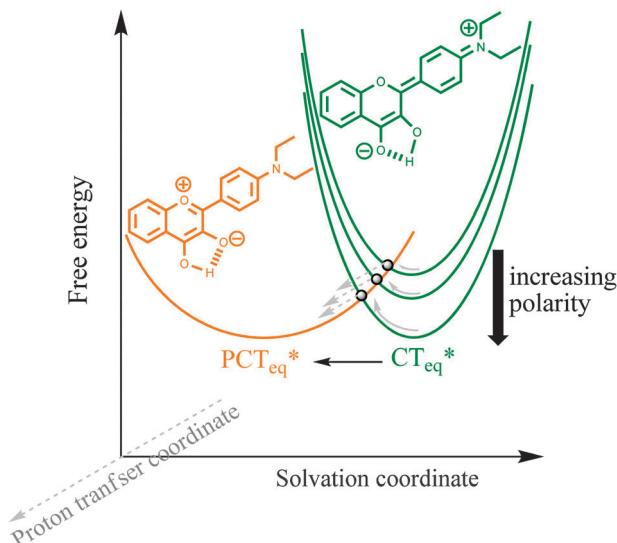


Fig. 8 The induced barrier for ESIPT as a function of solvent polarity using **3HF** as a model. Note that this figure is focused on the event after solvent relaxation to the CT^* state. The CT_{eq}^* state then undergoes $\text{CT}_{\text{eq}}^* \rightarrow \text{PCT}_{\text{eq}}^*$ proton transfer. The barrier is therefore increased as the solvent polarity increases. Note that as in Fig. 6, the solvent parabolic potential well for PCT^* is similar (in terms of position and curvature) to that of the ground-state N, which is not shown here to avoid complexity.

respect to the solvation relaxation process (see reaction along the proton transfer coordinate in Fig. 6). After reaching the solvation equilibrium of the CT^* state, the $\text{CT}_{\text{eq}}^* \rightarrow \text{PCT}_{\text{eq}}^*$ ESIPT then takes place. Due to the difference in equilibrium solvent polarization between CT_{eq}^* and PCT_{eq}^* , the energy of CT^* in the solvent parabolic potential well has to be brought to resonance with PCT^* (*i.e.*, the crossing point indicated by ● in Fig. 6) to execute ESIPT.

Interestingly, since the CT^* structure possesses a larger dipole moment (in terms of magnitude) than that of PCT^* , solvent polarity stabilizes the CT^* structure more than PCT^* . For the convenience of discussion, knowing that CT^* possesses a larger dipole moment, we draw only the solvent polarity dependent CT^* energy level and simply assume a negligible solvent stabilization effect on PCT^* . Fig. 8 then clearly reveals that the increase of solvent polarity results in the decrease of the CT_{eq}^* energy and hence the increase of the $\text{CT}_{\text{eq}}^* \rightarrow \text{PCT}_{\text{eq}}^*$ barrier, slowing down the rate of ESIPT. In a strong polar solvent such as CH_3CN , owing to substantially large stabilization, thermal equilibrium can be established even between the CT_{eq}^* and PCT_{eq}^* states. In this case, both forward and reversed ESIPT dynamics are significantly influenced by a solvent-induced barrier. This viewpoint has been supported in a number of reports based on steady-state,^{87,93,135} picosecond,⁸⁸ and femtosecond dynamic approaches.^{124,138}

5.2. Other class I systems exhibiting ESICT–ESIPT transformation

5.2.1 Benzazoles. Another system that has greatly contributed to ESIPT research is the benzazole type of dyes, such as

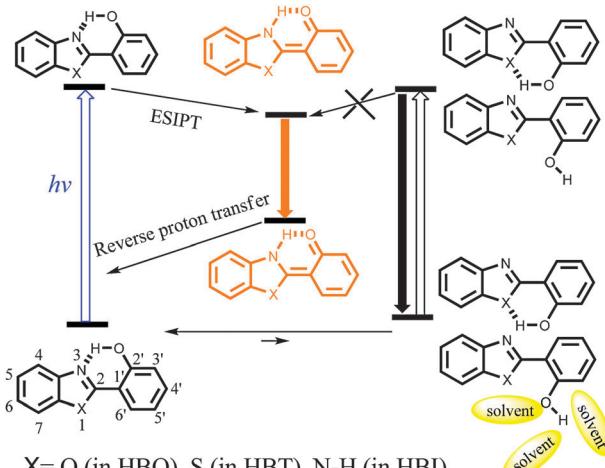


Fig. 9 The modified ESIPT mechanism of benzazole dyes.¹⁴² Only *enol-cis* conformer with an intact intramolecular H-bond can exhibit this reaction resulting in strongly Stokes-shifted fluorescence emission. A number of non-productive conformers that can be stabilized by X atom or by intermolecular H-bonding can be observed. They produce normal emission at shorter wavelengths.

2-(2'-hydroxyphenyl)benzimidazole (**HBI**), 2-(2'-hydroxyphenyl)-benzoxazole (**HBO**), and 2-(2'-hydroxyphenyl)benzothiazole (**HBT**). These dyes exhibit ESIPT that depends on the relative configuration of molecular fragments.^{139,140} These dyes can be denoted as the HBX compound series, where –X– in the benzazole ring stands for –NH–, –O– and –S– (Fig. 9). In their ground states in nonpolar and aprotic environments, their *enol-cis* form is the most stable conformer that upon excitation undergoes ESIPT to form the keto tautomer T^* . This transition gives rise to a strongly (by more than 100 nm) Stokes-shifted emission.¹⁴¹ Other conformers that are more stable in more polar and protic environments (for example, an *enol-anti* close or open rotamer) do not undergo ESIPT because of the significant distance between donor and acceptor sites and the lack of an intramolecular H-bond connecting them. These conformers are responsible for the fluorescence band of normal emission, N^* , observed at shorter wavelengths.

Upon introducing electron-donating substituents in the 4'-position of the N atom in the benzene ring (see Fig. 10),¹⁴² strong changes of properties along the ESICT–ESIPT coordinate can be achieved. For the 4'-diethylalkyl amino analogue of HBO, namely **HBON**, the ground-state “gating”, *i.e.*, the existence of open or closed *anti-enol* isomers in parent molecules (see Fig. 9), is negligible. This is rationalized by the increase of basicity in the benzazole nitrogen due to the electron donation from the 4'-diethylamino group *via* π -conjugation. As depicted in Fig. 10, this series of ESICT–ESIPT molecules are ascribed to the class **I** type, since the electron and proton acceptors are in the same benzazole nitrogen site. Therefore, similar behaviour to that observed for **3HF** derivatives (see Section 5.1) is expected; that is, upon exciting **HBON**, an ultrafast electronic charge transfer takes place from the diethylamino group to the benzazole nitrogen, forming the CT^* state.

After solvent relaxation, the $\text{CT}_{\text{eq}}^* \rightarrow \text{PCT}_{\text{eq}}^*$ proton transfer process is subject to the solvent-polarity induced barrier. Due to

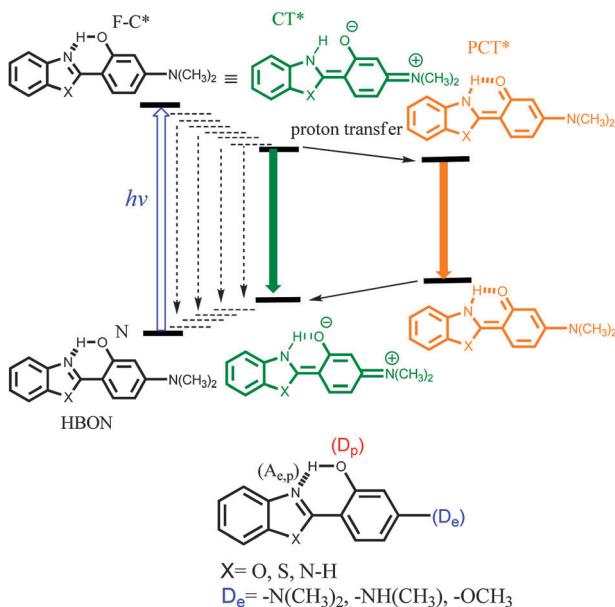


Fig. 10 Structures and ESICT-ESIPT mechanism (class I type) of benzazole dyes substituted by an electron donor at the 4'-position of the parent molecules (see Fig. 9). The equivalence sign drawn between $\text{F}-\text{C}^*$ and CT^* simply indicates the type of ultrafast optical electronic charge transfer process. The dotted arrow lines indicate the solvent relaxation.

the greater dipole moment and hence larger solvent stabilization of the CT_{eq}^* state (*cf.* PCT_{eq}^*), the rate of $\text{CT}_{\text{eq}}^* \rightarrow \text{PCT}_{\text{eq}}^*$ decreases upon increasing the solvent polarity. Qualitatively, this is also reflected from the steady-state fluorescence, showing dual emission, in which nonpolar solvent facilitates ESIPT and renders dominant PCT^* emission, while the CT^* emission is favourable in polar solvents.¹⁴² Likewise, the normal emission intensity consecutively decreases in the order of the decreasing electron-donating ability. Also, variations in the location of amino substituents¹⁴³ or fusion with a naphthalene group¹⁴⁴ modulates strongly the relative intensities of the CT^* and PCT^* bands in this family of fluorescence emitters.

5.2.2 *p*-N,N-Ditolyaminosalicylaldehydes¹²⁶. From the viewpoint of molecular structure, the simplest model to represent the class I system should be *p*-N,N-ditolyaminosalicylaldehydes (SAN), depicted in Fig. 11.

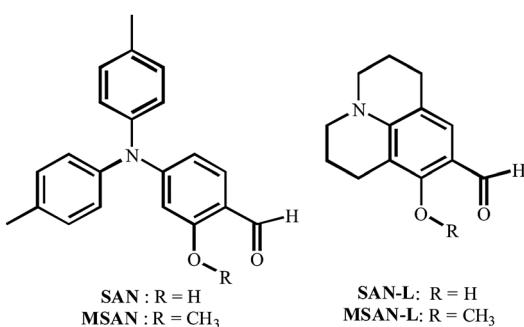


Fig. 11 Molecular structure of SAN and its analogue SAN-L with nitrogen lone pair electrons being locked. Also shown are their methylated derivatives.

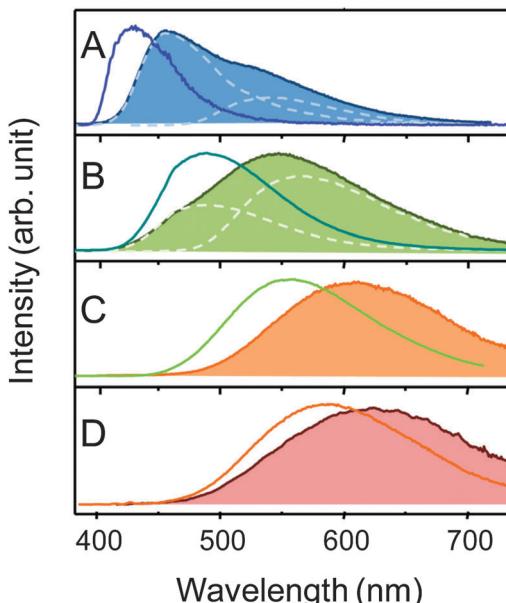


Fig. 12 Static fluorescence spectra of MSAN (solid lines) and SAN (filled spaces) in (A) cyclohexane, (B) benzene, (C) dichloromethane, and (D) acetonitrile at 298 K. Dashed curves express the spectra fitted by two Gaussian shapes for SAN in cyclohexane and benzene. The existence of both CT^* and PCT^* emissions in nonpolar solvent cyclohexane is thus apparent. (Modified with permission from ref. 126. Copyright © 2004 American Chemical Society.)

SAN presents a unique example among the class I molecules in that even in nonpolar solvent such as benzene (with quadrupole moment), following an ultrafast rate of ESICT, fast equilibrium takes place between CT_{eq}^* and PCT_{eq}^* , resulting in spectrally overlapped dual fluorescence maximized at 450 and 540 nm, respectively (see Fig. 12), as also evidenced by the identical population decay rate of 360 ps^{-1} for these two bands.¹²⁶ When the solvent polarity increases, the solvation (*e.g.* in CH_3CN) brings the CT^* state to even lower energy than that of the PCT^* state, so the $\text{PCT}_{\text{eq}}^* \rightarrow \text{CT}_{\text{eq}}^*$ reverse proton transfer becomes a prevailing process, resulting in the dominant CT^* emission. As shown in Fig. 12, this is clearly demonstrated by the nearly identical emission between SAN and MSAN in CH_3CN ; the latter is the methoxy derivative of SAN, so ESIPT is prohibited and only ESICT is operative.

Further structural verification of the charge transfer emission is given by SAN-L (see Fig. 11), in which the nitrogen lone pair electrons are locked, to a certain extent, in an unfavourable orientation such that the charge transfer efficiency may be largely inhibited (for example, see ref. 145 and 146). For a control, the methoxy derivative of SAN-L, *i.e.*, MSAN-L was prepared to represent the case for the lack of ESIPT. As shown in Fig. 13, the emission maximum of MSAN-L in various solvents is only slightly red-shifted from 375 nm in cyclohexane to 400 nm in CH_3CN . By contrast, in MSAN, the red shift of the emission maximum was much larger in different solvents (from 420 nm in cyclohexane to 590 nm in CH_3CN ; see Fig. 12).

The result firmly supports the proposed charge transfer mechanism incorporating diarylamino nitrogen in MSAN and

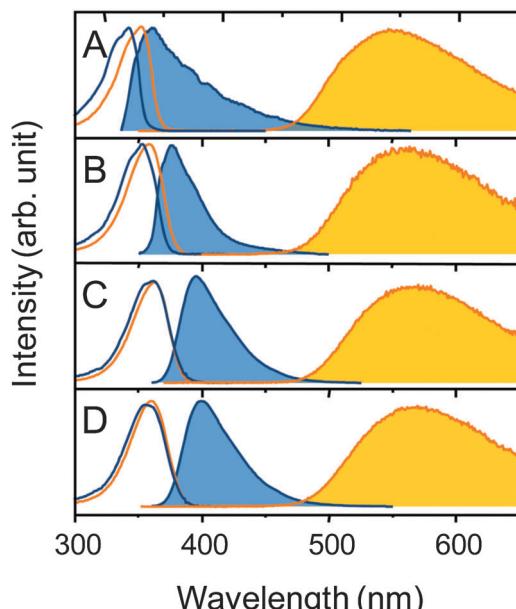


Fig. 13 Static absorption (solid lines) and fluorescence (filled spaces) spectra of SAN-L (orange) and MSAN-L (blue) in (A) cyclohexane, (B) benzene, (C) dichloromethane, and (D) acetonitrile at 298 K. (Modified with permission from ref. 126. Copyright © 2004 American Chemical Society.)

likewise SAN. Conversely, as shown in Fig. 13, SAN-L revealed a large Stokes-shifted, almost solvent-polarity independent \sim 530–540 nm emission band. Obviously, under the diminution of excited-state charge transfer, ESIPT is decoupled from the solvent perturbation. Accordingly, SAN-L is expected to exhibit photophysical properties similar to those of 2-hydroxybenzaldehyde (2HBA),^{147,148} *i.e.*, fast ESIPT reaction, resulting in a proton-transfer tautomer emission.

Important conclusions can thus be drawn from the above results of class I molecules presented. Upon excitation, an adiabatic type of ESICT, *i.e.*, an optical electron transfer, can be rationalized by a strong π -electron overlapping between donor and acceptor moieties, such that the electronic coupling matrix is much larger than that of the Marcus type of weak-coupling electron transfer process.^{149–151} For this case, promptly after ESICT, on the shortest time scale, the ESIPT reaction is seen as a barrierless process. The reaction barrier is low, since the initial surrounding solvent configuration is favourable to the excited-state proton transfer tautomer form (see Fig. 6). With time evolution, this reaction starts to compete with solvation dynamics, *i.e.*, the $\text{CT}^* \rightarrow \text{CT}_{\text{eq}}^*$ solvent relaxation process. The decay dynamics combining these two competitive pathways is complicated, and it is still pending resolution for differentiation. Upon reaching equilibrium polarization of the surrounding dipoles, since their equilibrium configurations become different between charge transfer species and proton-transfer tautomer forms, this creates the reaction barrier. The $\text{CT}_{\text{eq}}^* \rightarrow \text{PCT}_{\text{eq}}^*$ (and *vice versa*, $\text{PCT}_{\text{eq}}^* \rightarrow \text{CT}_{\text{eq}}^*$) proton transfer reaction then becomes a thermally activated process and proceeds more slowly, mainly due to the solvent polarity induced barrier, which channels into the overall reaction scheme.

The interplay of these processes allows realization of both kinetic and thermodynamic controls of the reaction rates depicted in Fig. 3.

6. ESIPT–ESICT switching in class II molecules

Up to this stage, most experimental model systems exhibiting the ESICT/ESIPT dynamics are ascribed to class I, in which ESICT takes place prior to ESIPT. Thus, upon Franck–Condon excitation, the associated reaction dynamics incorporate the competitive solvent relaxation and prompt the proton transfer process (see Fig. 6 and text in Section 5.1) before reaching the equilibrium polarization. As such, the study of early ESICT/ESIPT coupling reaction dynamics is commonly complicated and limited by the rate of solvent relaxation. In addition, it has been of great fundamental interest, as well as urgent, to seek an ideal system to probe ESICT/ESIPT coupling reactions that can be free from early solvent relaxation processes. In this context, class II molecules are an ideal case in point, as they are strategically designed so that they undergo ESIPT prior to the ESICT reaction. As depicted in Scheme 3, the $D_{e,p}$ group of class II prototypes is not a strong electron donating group in the excited state until it forms an anion *via* transferring a proton (H^+) to an acceptor (A_p). Since $D_{e,p}$ is π -conjugated with the electron acceptor A_e , a strong coupling matrix between D_e^- and A_e is then expected. Such an electronic charge transfer process should be adiabatic and its rate ultrafast. In other words, the occurrence of proton transfer is simultaneously coupled with the charge transfer process. We are thus essentially dealing with direct $\text{F}-\text{C}^* \rightarrow \text{PCT}^*$ ESIPT/ESICT coupling reaction, *i.e.*, pathway C in Scheme 3.

Bearing the above concept in mind, a series of class II molecules have been strategically designed by adding an electron accepting group that is π -conjugated with the hydroxyl ($D_{e,p}$) site of parent molecules such as HBO and HBT, among which the well known prototypes include 2-((2-(2-hydroxyphenyl)benzo-[d]oxazol-6-yl)methylene)-malononitrile (diCN-HBO), 2-((2-(2-hydroxyphenyl)benzo[d]thiazole-6-yl)methylene)-malononitrile (diCN-HBT),^{120,128} 2-((2-(2-hydroxyphenyl)benzo[d]oxazol-6-yl)methylene)-cyanoacetic acid (HBODC), and its ester form HBOCE¹⁵² (see Fig. 14).

From the molecular structure point of view, the lone pair of electrons of the benzazole nitrogen, which is perpendicular to the π -resonance system, does not participate in aromaticity. Therefore, its electron donating strength, compared with those of alkyl

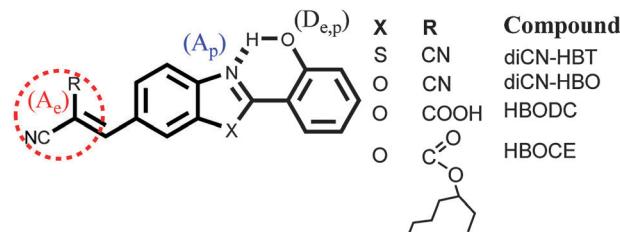


Fig. 14 Structures of prototypical class II molecules.^{120,128,152}

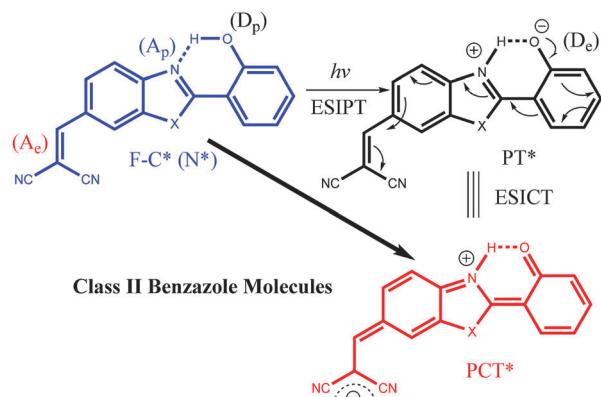


Fig. 15 The proposed ESIPT/ESICT coupled reaction for the class **II** benzazole molecules. (Modified with permission from ref. 89 and ref. 120. Copyright © 2010, 2008 American Chemical Society.)

and aryl amines in class **I**, is rather weak, and upon Franck-Condon excitation, the degree of charge transfer to the electron accepting cyano group (see (A_e) circled in red in Fig. 14) is negligible. On the other hand, as in their parent molecules, *i.e.*, those benzazoles with no A_e anchoring, ESIPT is expected to take place from the hydroxyl proton to the benzazole nitrogen, resulting in a proton transfer tautomer.¹⁵³ Once the proton is transferred, as shown in Fig. 15, the resulting oxide anion $-O^-$ becomes a powerful electron donating group, which executes charge transfer toward the electron accepting cyano-group *via* π -conjugation, *i.e.*, an adiabatic, ultrafast electron transfer process. As a result, for class **II** molecules, every proton nuclear motion should concomitantly accompany the ESICT process, such that the ESIPT reaction dynamics are directly coupled with the solvent polarization effects. The long-range solvent polarization interactions result in a solvent-induced barrier channelling into the overall proton transfer reaction dynamics.

Here, the CT* reaction *via* strong $-O^-$ electron donating strength is a revised model from the literature.^{120,128,152} Previous reports proposed that once the proton-transfer tautomer forms, the benzazole nitrogen becomes the secondary amino nitrogen and thus should act as a good electron donor. This mechanism requires the keto-formation and then the charge transfer from the oxide anion ($-O^-$) to the benzazole nitrogen, followed by another CT* to the cyano-electron accepting group. The overall process is essentially equivalent to the direct charge transfer from the hydroxyl group proposed in this Review.

As with class **I** molecules, mainly due to the solvent induced barrier, dual emission bands are also observed in class **II** systems, and their relative intensity as well as peak frequency are solvent polarity dependent. Despite this similarity, salient differences between these two classes can be perceived. First, different from the complicated relaxation dynamics in class **I** molecules, which involve competitive solvent relaxation and proton transfer in the early time domain (see Section 5), the relaxation dynamics for class **II** is straightforward, incorporating ESIPT (coupled simultaneously with ESICT), followed by the

solvent relaxation and then population decay. Secondly, from the angle of reaction view shown in Scheme 3, dual emission in class **I** comprises CT* and PCT* bands, whereas that of class **II** originates from F-C* and PCT* species, for which the PCT* possess a substantial charge transfer character. Accordingly, solvent stabilization is favourable for PCT* in class **II** in comparison with that for CT* in class **I** types. This makes the whole reaction irreversible (case (b) of Fig. 3)

We have seen in Fig. 7 and 12 that the short-wavelength CT* band in the class **I** system exhibits significant red shift upon an increase in the solvent polarity. In striking contrast, for the class **II** type, it is the long-wavelength proton transfer emission band that shows remarkable bathochromic spectral shift when the solvent polarity is raised. This viewpoint can be clearly demonstrated by the steady-state emission spectra of **diCN-HBO** in various solvents shown in Fig. 16. Evidently, despite the nearly solvent-polarity independent normal emission (N^* , or F-C* denoted in Scheme 3), the PCT* proton-transfer emission is largely subject to the solvent polarity, being shifted in peak wavelength from 540 nm (in cyclohexane) to 750 nm (in CH₃CN). The reader can make a fair comparison between Fig. 16 (class **II**) and Fig. 7 and 12 (class **I**) for clarity.

To provide more quantitative assessment, Fig. 17 shows the result of another class **II** molecule **HBOCE** (see also Fig. 14 for structure) reported by Park and co-worker,¹²⁸ in which both absorption and emission peak frequencies as a function of solvent polarity parameter (see eqn (3) and (4)) are plotted.

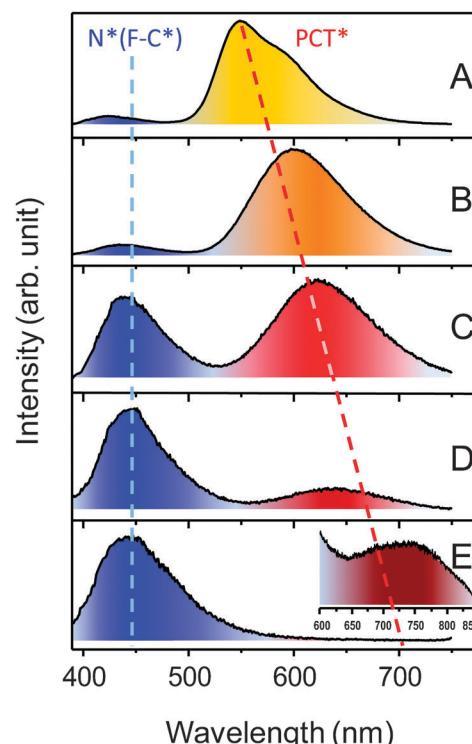


Fig. 16 Static absorption and fluorescence spectra of **diCN-HBO** at 298 K in (A) cyclohexane, (B) benzene, (C) chloroform, (D) dichloromethane, and (E) acetonitrile. Insert: obtained by a red-sensitive charge coupled detector. (Modified with permission from ref. 120. Copyright © 2008 American Chemical Society.)

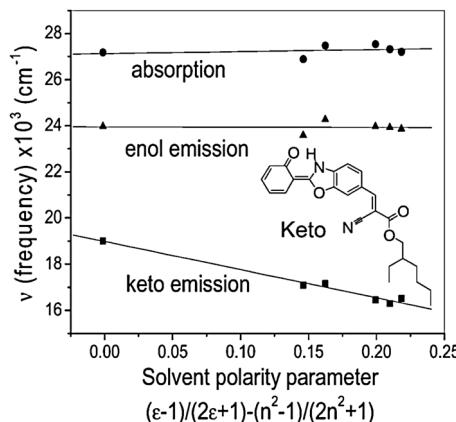


Fig. 17 Plot of absorption and emission maxima in enol (N and N^* , respectively) and emission of keto form (PCT*) of HBOCE as a function of solvent polarity parameter. (Reprinted with permission from ref. 128. Copyright © 2004 American Chemical Society.)

A strong solvatochromism in the PCT* (the *keto* form) emission band can be observed, whereas both N absorption and N^* emission bands are independent of solvent polarity.

Class **II** also exhibits drastically different solvent-polarity dependent reaction dynamics from that of class **I** system. As opposed to that of the class **I** molecules, which exhibit decreased rate of $CT_{eq}^* \rightarrow PCT_{eq}^*$ reaction (see Section 5), the rate of $F-C^*$ (or N^*) \rightarrow PCT* proton transfer reaction increases with the increase of solvent polarity. For example, in cyclohexane, the rate constant of ESPT of **diCN-HBO** was determined to be 1.1 ps. Upon an increase in solvent polarity, the ESPT rate constants were also determined to be 1.00 ± 0.13 ps in benzene, 0.60 ± 0.05 ps in CH_2Cl_2 , and 0.31 ± 0.03 ps in CH_3CN , respectively. These values reveal that increasing the solvent polarity tends to render an increase in the rate of ESIPT.¹²⁰ The overall reaction dynamics can thus be described by a mechanism incorporating both solvent polarization and proton-transfer reaction coordinates (Fig. 18).

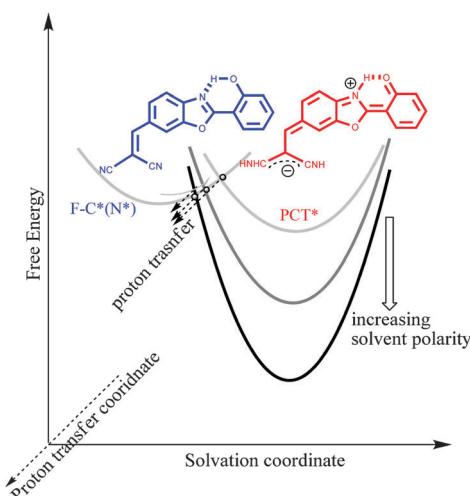


Fig. 18 The proposed ESIPT/ESICT reaction/relaxation dynamics using **diCN-HBO** as a model. (Modified with permission from ref. 120. Copyright © 2008 American Chemical Society.)

The proton transfer tautomer PCT*, possessing large degrees of charge transfer character, is obviously stabilized upon an increase in solvent polarity, while the $F-C^*$ state is not influenced, and thus the corresponding solvent-induced barrier is reduced.

It is noteworthy here that unlike the parent ESIPT molecule **HBO**, which executes ultrafast (~150 fs) ESIPT in nonpolar solvent such as cyclohexane,¹²¹ the ESIPT/ESICT coupled system **diCN-HBO** undergoes a finite time constant (1.1 ps) proton transfer in similar cyclohexane. This result can, on the one hand, be rationalized by the non-negligible quadrupole moment effect from cyclohexane,¹⁵⁴ which induces the small solvent-perturbed barrier. On the other hand, a dynamic polarization model in nonpolar solvents, which has been proposed by Hamaguchi and co-workers,¹⁵⁵ may explain the results. Because of the large dipolar change between $F-C^*$ and PCT* in class **II** systems, the induced-dipole/dipole interaction is considered to be non-negligible, inducing an appreciable barrier.

Regarding the class **II** molecules, straightforwardly, ESIPT may be viewed as being incorporated with solvent reorganization and proton tunnelling. The framework and associated mechanism should be similar to that of electron transfer reaction, which is also consistent with the theoretical advancements.^{2,6,48,51,81,83,118,119} On this basis, depending on the degrees of coupling between reactant and product potential energy surfaces, the overall proton transfer process can thus be categorized into two regimes similar to the classifications in electron transfer reaction, namely nonadiabatic (weak hydrogen bonding) and adiabatic (strong hydrogen bonding) limits.^{51,118} Since all currently designed class **II** systems possess strong hydrogen bonds, as indicated by the ultrafast ESIPT for their parent molecules (*vide infra*), the associated ESIPT/ESICT reaction rate constant k_{PCT} should be an adiabatic type, which can be expressed as

$$k_{PCT} = \frac{\omega_s}{2\pi} \exp\left(\frac{-\Delta G_{ad}^\neq}{RT}\right) \quad (6)$$

where ω_s stands for solvent fluctuation frequency in the reactant well, and ΔG_{ad}^\neq denotes the adiabatic reaction activation barrier, which is solvent polarity dependent. Support of this kinetic expression is given by the lack of deuterium isotope effect on k_{PCT} . In fact, similar kinetic expression should be applicable to the class **I** molecules in dealing with the $CT_{eq}^* \rightarrow PCT_{eq}^*$ proton transfer reaction after solvent relaxation.

Nevertheless, the above kinetic expression may impose a certain limitation due to the fact that proton transfer simultaneously involves bond breaking and making as well as strong electrostatic interaction with the surrounding solvent. Therefore, its motion may not be adequately described by the Marcus theory, which was originally derived for outer sphere electron-transfer reactions in solution.⁴⁴ Furthermore, electron transfer theory generally assumes that the electronic coupling between reactant and product states is small (*e.g.*, a kcal/mol or even less), whereas for the proton transfer, a typical electronic coupling value might be on the order of an electron volt. These, together with the very sensitive proton (*cf.* electron) tunnelling

to the reaction potential energy surface, make the ESIPT/ESICT reaction more complicated and deserve further in-depth understanding. It should be stressed, however, that in all known cases, solvation dynamics produces a stronger or weaker influence on the ESICT rate due to solvent interactions with the dye dipole moment that can be changed dramatically. The influence on the ESIPT rate is mechanistically different and can be revealed only in experiments with sub-ps time resolution in the systems, in which ESICT is suppressed. This is because ESIPT is fundamentally limited to short distances relative to electronic charge polarization.

7. Modulation of the ESICT–ESIPT process

From the chemistry point of view, chemical modification *via* ingenious design always serves as an essential and powerful stepping stone in gaining in-depth fundamental understanding and developing future application. In this section, we firstly demonstrate that various chemical approaches, depending on the π -conjugation length and electron donating strength, are capable of tuning the dual emission in terms of relative peak positions and ratiometric emission intensity (7.1). This concept is then exploited to render the proof of concept for the ESICT–ESIPT coupling reaction *via* fine-tuning the dipole functionality between reactant and product (7.2). Lastly, electrochromic modulation of ESICT–ESIPT equilibrium is introduced (7.3), aiming for its latent potential in applications described in Section 8.

7.1. Chemical modulation of ESICT–ESIPT

In theory, the degree of charge transfer should be sensitive to the strength of the electron donor and acceptor couple, and their electron coupling. For the designated ESICT–ESIPT systems elaborated above, the coupling is established *via* the π -conjugation. It is thus interesting to probe the dual emission properties in terms of relative peak positions and ratiometric emission intensity as functions of the π -conjugation length and electron donating/accepting strength. In the **3HF**s type of class I molecules, the 4-carbonyl serves as both proton and electron acceptor. With this and the 3-hydroxy group intact, a variety of modifications can be realized that allow distribution of π -electron density and attachment of electron-donor and electron acceptor groups. With the aim of fine-tuning the fluorescence emission properties and adapting the ESICT/ESIPT switching to a particular narrow range of solvent polarities and proticities, a large number of new compounds have been synthesized.

Stronger effects in switching can be achieved by variations of strength of electron-donor and electron-acceptor substituents, which can be provided at different sites of the parent 3-hydroxy-chromone (**HC**) molecule.^{156–158} For illustration of the dramatic effects of these substituents on ESICT/ESIPT equilibrium in compounds, in which the variations in electronic structures are relatively small, the spectra of two series of compounds derived from **3HF** and 2-benzofuryl-3**HC** (**3HB**), respectively, were compared in terms of fluorescence response (see Fig. 19 and 20).

Both series of these dyes are categorized as class I molecules, which all demonstrate dramatic variations of relative intensities

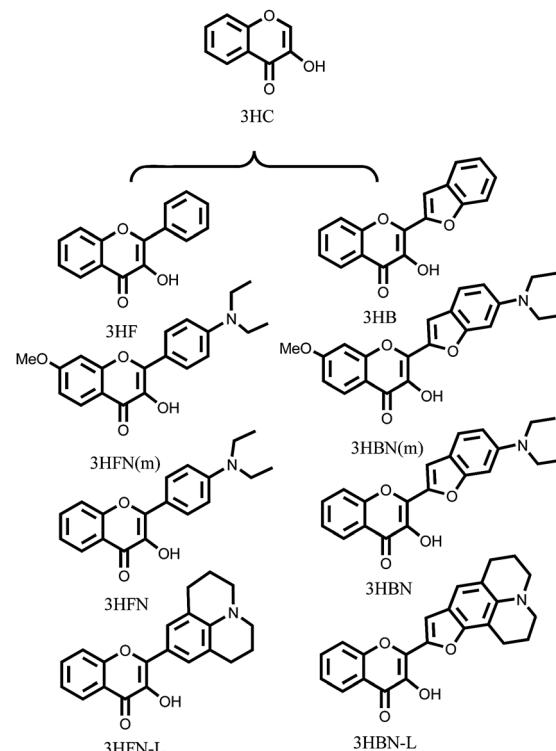


Fig. 19 Structures of two series of **3HC** dyes exhibiting dramatic variation of wavelength-ratiometric response to polarity of molecular environment. (Adapted from ref. 158 with permission from the Centre National de la Recherche Scientifique (CNRS) and The Royal Society of Chemistry.)

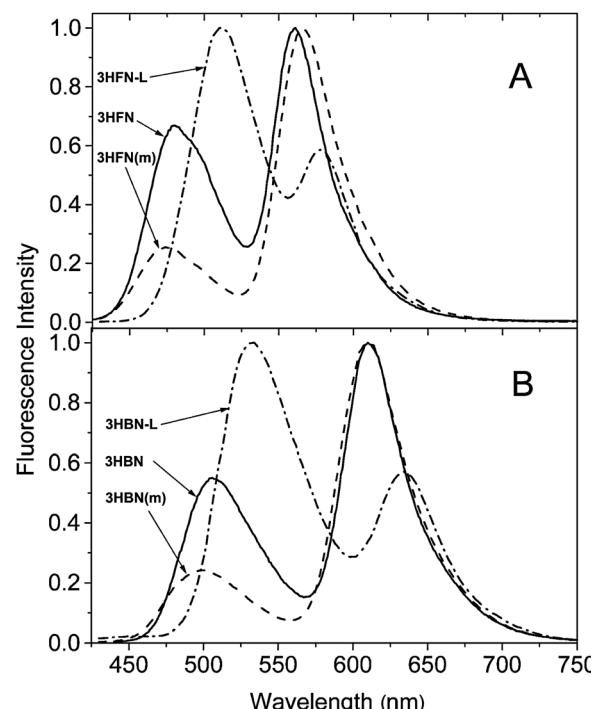


Fig. 20 Static Fluorescence spectra of the studied flavones **3HFN**, **3HFN-L** and **3HFN(m)** in chloroform (A) and benzofurylchromones **3HBN**, **3HBN-L** and **3HBN(m)** in toluene (B). Excitation wavelength of 420 nm. (Reproduced from ref. 158 with permission from the Centre National de la Recherche Scientifique (CNRS) and The Royal Society of Chemistry.)

due to the difference in dipole moment vector between CT* and PCT* as a function of D_e/A_e strength and the π -conjugation. The CT* peak wavelength also shows corresponding changes, whereas the shift of PCT* peak is relatively much smaller, a characteristic of class I molecules (*vide supra*). Lastly, it is noted that the nitrogen-locked forms **3HFN-L** and **3HBN-L** still exhibit substantial CT* emission, which is different from the lack of CT* emission in the salicylaldehyde type of molecule **SAN-L** (see Fig. 11 and 13). The result indicates that the restriction of nitrogen orientation to "stop" charge transfer, a theory based on twisted intramolecular charge transfer (TICT),^{145,146,159} may not be fully operative, a point which has caused a long-standing controversial debate.^{160,161}

In a continuous effort regarding the chemical modulation of the ESICT/ESIPT system, it is interesting to note that when an isothiocyanate group is attached at position 7 of **3HF**, this dye reacts with amino groups (e.g., in proteins). As an electron acceptor, this group is converted upon this interaction into an electron donor thiourea-based form. This transformation, reducing the dipole moment of 4'-diethylamino-**3HF**, shifts the reversible ESIPT reaction towards the ESIPT product with the two-band sensitivity in a new, shifted, polarity range.¹⁶² Presently, for any application in any condensed medium (including even supercritical fluids),¹⁶³ a **3HC** or **3HF** dye can be suggested for detection of fine changes in molecular properties with a strong variation of fluorescence emission color.

The ability of the **3HC** core to allow a broad variety of different substitutions was further explored in many different ways. In contrast to substitutions discussed above, adding a benzene ring at the 6,7 position (see Fig. 5 for numbering) does not change significantly the spectroscopic properties, and adding one to position 5,6 achieves a different effect; that is, the 4-carbonyl becomes protected from perturbations by intermolecular H-bonds, so the intensity ratio between two bands becomes fitted to the same polarity function for both aprotic and protic solvents.¹⁶⁴ In the meantime, attachment to the 6,7 position by fusing furan heterocycle increased dramatically the quantum yield and extended the separation between CT* and PCT* bands on a wavelength scale.¹⁶⁵

After establishing the important fact that the fluorescence spectra of 4'-dialkylamino substituted **3HFs** are strongly solvent-dependent, and thus quite different from those of the parent compound,^{87,135} a number of research publications aimed at investigating this issue in detail and to quantify the solvent response. In view of the absence of charge and relatively good solubility of the parent fluorophore, the whole broad range of solvent polarities (from hexane to dimethyl formamide and water) was open for research. It was observed that for each of these compounds, a full switching between CT* and PCT* emissions occurs within surprisingly narrow ranges of polarities, such that in order to describe them, the logarithmic scale for their intensity ratios as a function of solvent polarity has to be used.⁹³

In addition to reaction to solvent polarity, the fluorescence spectra of ESICT-ESIPT systems possessing intramolecular H-bonds respond to several more factors. The first one is the effect of high pressure, which suppresses the ESIPT reaction of **3HFN** in polymeric media.¹⁶⁶ Such a pressure tuning effect

requires the consideration of the additional factor of "rigidity" in the highly viscous media, complicating the overall process. Regarding chemical means, the ionization of a proton-donor -OH group occurs in **3HFs** in aqueous media at pH 8–9 and generates a new emission band.¹⁶⁷ This reaction can also occur in non-aqueous systems in the presence of strong proton acceptors, such as amines.¹⁶⁸ An intermolecular H-bond with the proton acceptor group is known to suppress the ESIPT reaction.¹⁶⁹ Generating a new band in emission, such a solvated form can respond to the presence of proton-donor groups in the medium.¹⁷⁰

7.2. Tuning ESICT-ESIPT *via* the dipolar functionality

Exploiting the powerful capability of modulating the photo-physical processes by chemical means, a particular emphasis in this section is the proof of concept regarding the involvement of a dipolar factor (among F-C*, CT* and PCT*) to harness ESICT-ESIPT thermodynamics and dynamics. This has been demonstrated by a comparative study of a series of **3HF** derivatives, namely **3HFN**, 7-N,N-diethylamino-3-hydroxyflavone (**3HFN(7)**), and 4'-N,N-dimethylamino-7-N,N-diethylamino-3-hydroxyflavone (**3HFN(4,7)**), depicted in Fig. 21A–C, respectively. These three models have been employed as a chemical approach to fine-tuning the ESICT coupled ESIPT reaction *via* the dipolar functionality of the molecular framework.¹²³ Accordingly, as shown

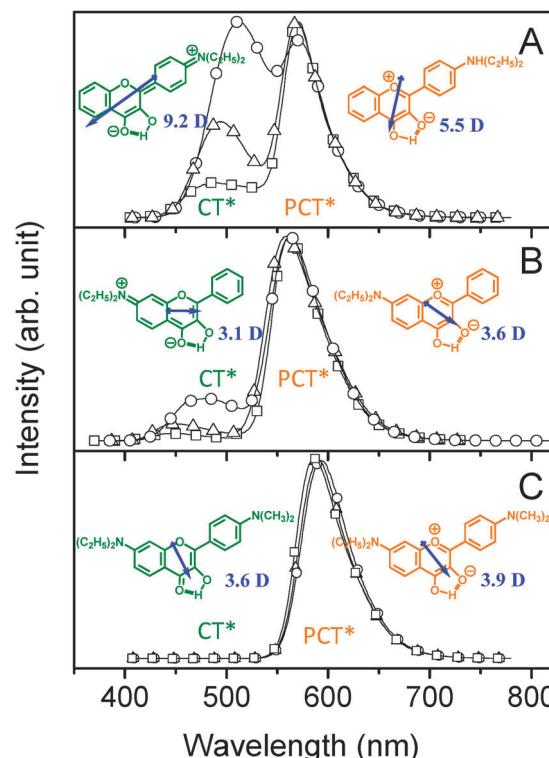


Fig. 21 The dipole orientation and emission spectra of (A) **3HFN**, (B) **3HFN(7)** and (C) **3HFN(4,7)** in ethyl acetate (□), CH₂Cl₂ (△) and CH₃CN (○). The excitation wavelength is 350 nm. The depicted vectors of the dipole moment for CT* state (left) and PCT* state (right) for (B) and (C) are taken from ref. 89, while that for (A) is from ref. 124. Note that the calculation of dipole vector is qualitatively good in orientation but not in magnitude, as indicated by its smaller value in CT* form for **3HFN(7)**.

in Fig. 21, both **3HFN** and **3HFN(7)** exhibit prominent dual emission due to the large differences in dipole vector (in terms of both magnitude and orientation) and hence different equilibrium solvent polarization between CT* and PCT*.

As a result, the solvent-polarity induced barrier is substantial and accounts for the observed dual (CT* and PCT*) emission and the associated slow relaxation dynamics.⁶² Conversely, for **3HFN(4,7)**, the interplay between two electron-donor entities, *i.e.*, 4'-diethyl amino and 7-diethyl amino groups, in the opposite direction with respect to the electron acceptor (4-carbonyl oxygen) causes the cancellation of CT*. The similar dipole moment vector between CT* and PCT* species (see Fig. 21C) leads to the decoupling of ESIPT from the solvent-polarity effect. Thus, the rate of ESIPT for **3HFN(4,7)** is ultrafast, resulting in a proton-transfer tautomer emission only. The results make further rational design of the ESICT-ESIPT coupled systems feasible simply by tuning the net dipolar effect. Accordingly, systematic investigation of the correlation in regard to the difference in dipolar vectors between ESICT and ESIPT *versus* solvent polarity-induced barriers becomes possible.²⁵ We will see the full expansion of chemical modification of ESICT-ESIPT systems in the application section.

7.3. Electrochromic modulation of ESICT-ESIPT equilibrium

An applied electric field modulates the energies of CT states. This phenomenon, known as electrochromism (or the Stark effect), is observed as the shifts of electronic (absorption and fluorescence) spectra as a function of strength and direction of this field.¹⁷¹ Influencing the energies of the ground and excited states, this effect depends on the value and orientation of the solute dipole moment. The concept of the Onsager reactive field (the field that modulates in a self-consistent way the ground-state and the excited-state energies) allows explanation of the strong connection between the effects of solvent polarity and of the molecular-scale electric field created by nearby charges and dipoles.¹⁷² Based on the Onsager cavity model (Fig. 2a), in a first approximation, the direction and magnitude of the shift can be expressed as:

$$\hbar\Delta\nu_{\text{obs}} = - (1/\varepsilon_{\text{ef}})|\Delta\vec{\mu}||\vec{F}|\cos\theta \quad (7)$$

Here \vec{F} is the electric field vector that averages all the fields acting on the solute dye, ε_{ef} is the effective dielectric constant, and $\Delta\vec{\mu}$ is the change of dipole moment associated with the spectroscopic transition. θ is an angle between \vec{F} and $\Delta\vec{\mu}$ vectors. It follows that in order to show maximal sensitivity to the field, the dye should exhibit substantial change of its dipole moment $\Delta\vec{\mu}$ upon electronic excitation. This implies a substantial unidirectional redistribution of the electronic charge density, which is a characteristic feature of ESICT systems. A concept of the modulation of ESICT/ESIPT reaction caused by an applied electric field is illustrated below (Fig. 22).

Working with **3HF** derivatives, Klymchenko *et al.*^{95,173} discovered a new phenomenon – electrochromic modulation of ESIPT reaction. They observed a strong electrochromic shift of the N* band due to its ESICT character. In contrast, the position of the PCT* band, which belongs to the state with a much

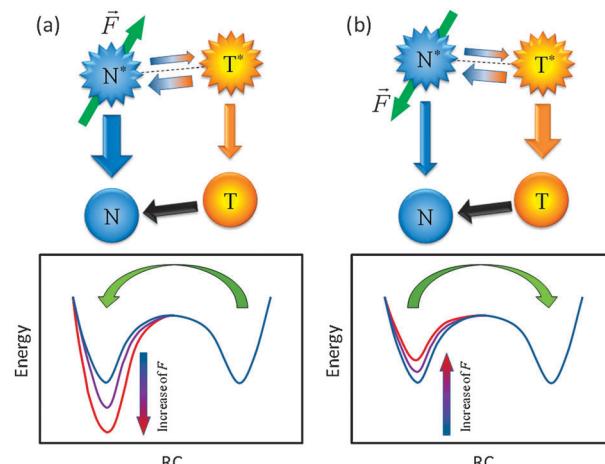


Fig. 22 The modulation of ESIPT reaction caused by an applied electric field. The shift of equilibrium between the two states with different sensitivity to perturbation by this field results in the redistribution of fluorescence intensity of N* and T* (*i.e.*, PCT*) forms. This is schematically shown by the arrows of different width for the opposite directions of the applied electric field F. Bottom panels show the variations of free energies of N* and T* forms for the electric fields of different directions and magnitudes. (Reprinted from Chemistry and Physics of Lipids, Vol. 160, A. P. Demchenko and S. O. Yesylevskyy, Nanoscopic description of biomembrane electrostatics: results of molecular dynamics simulations and fluorescence probing, P. 63, Copyright © 2009 with permission from Elsevier, ref. 78.)

smaller charge asymmetry, remains essentially unchanged, with no significant electric field sensitivity. Similar to the effects of polarity, the electric field-dependent switching between ESICT and ESIPT emissions was resolved. In this case also, the stronger interacting ESICT state (possessing decreased free energy) becomes more populated, just in accordance with the Boltzmann law. This leads to redistribution of the intensity between the two fluorescence bands, making the ratio of band intensities ($I_{\text{CT}}/I_{\text{PCT}}$) highly sensitive to the electric field. In isotropic media (such as dye solution in a neat solvent), this effect can be observed due to through-space interaction with nearby charges such that re-location of the charge to an opposite from the chromophore site produces the opposite effect (Fig. 23). It opens new possibilities for the studies of systems with structural and electrostatic anisotropy, such as biological membranes (see Section 8.3).

It was then demonstrated that this effect is really vectorial and depends strongly on the locations of nearby charges.⁹⁵ Being essential in the practical sense, the intensity ratio between ESICT and ESIPT bands demonstrates a strong amplification effect as compared to the commonly measured ratio obtained from the shift of a single excitation band. Since the two excited states coupled by ESICT-ESIPT reaction are neutral, this allows development of a series of dyes for probing electrostatics at interfaces, particularly in biological membranes (see Section 8). When they are used for labeling, the through-spacer dye attachments to $-\text{SH}$ and $-\text{NH}_2$ groups in proteins are clearly distinguished. The $-\text{NH}_2$ labelling creates a positive charge in proximity to the fluorophore, which results in a strong internal Stark effect.¹⁷³

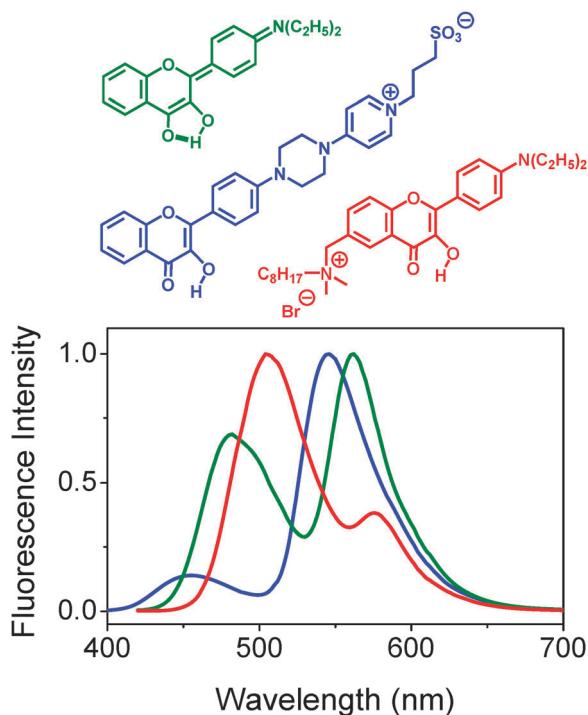


Fig. 23 Modelling the electrochromic shifts in ESICT-ESIPT equilibrium by covalent attachment of electronically uncoupled charged groups in neat solvents. The effect of through-spacer attachment of a positively charged group to 4'-dialkylamino group (blue) and at the opposite side – to chromone position 6 (red) on fluorescence spectrum of dialkylamino-**3HF** (green). The solvent is chloroform and the excitation is at 410 nm. (Data from ref. 95.)

8. Applications

Systems exhibiting the interplay of ESICT and ESIPT energies demonstrate the broadest possible variation of emission color achieved among the intramolecular processes. Moreover, such variation is gradual and controllable. It can be fine-tuned by both covalent modifications of the fluorescence emitter and by its non-covalent interactions with the surrounding medium. Due to these properties, such dyes have been applied in different areas. Some of them, such as novel UV photostabilizers, laser dyes, and electroluminescent emitters, are not based on intermolecular relaxations stabilizing the ESICT states, but rather benefit from extremely large Stokes' shifts and hence the absence of self-absorption and concentration quenching, from easy population inversion and four-level photocycle, or from extended width in the visible range of the two-band spectrum.³³ In addition, there is a range of applications in which the ESICT-ESIPT processes play the most essential role.

8.1. Polarity and its determination on molecular scale

Non-covalent intermolecular interactions that are in the background of the macroscopically defined term ‘polarity’ decrease preferentially the energies of dipolar ESICT states relative to the coupled ESIPT state, which commonly possesses smaller charge separation. This is reflected by long-wavelength shifts of the correspondent bands in correlation with macroscopic

polarity values.⁹³ Such dyes, being polarity-sensitive molecular sensors, demonstrate the variations of two-band relative intensities (F_{N^*}/F_{T^*}) by several orders of magnitude, even within the narrow polarity ranges, and can thus be considered as the most sensitive solvent polarity indicators ever suggested. This effect is most clearly seen when the ESICT-ESIPT emission occurs in a thermodynamic regime, such that Boltzmann distribution between the species populating these states occurs prior to emission. Such a dramatic change of color is clearly detected with even the simplest fluorescence technique, allowing comparison of intensities at two wavelengths.¹⁷⁴

Great versatility in polarity scaling can be achieved with **3HF** and **3HC** derivatives. Within the narrow range of polarities, they demonstrate switching between the two bands of relatively bright emission. With proper substituents, such switching can be adjusted for a particular range on the whole polarity scale (Fig. 24). On the side of hydrophobic environments are the dyes with the strongest excite-state dipole moments, which can be achieved, for instance, by attachment of a dimethylaminobenzofuryl group at position 2 of the chromone ring.^{94,175} Further increasing the dipole moment by introduction of an electron acceptor 7-(2-methoxycarbonylvinyl)-group in position 7 opposite to the electron donor group increases the relative N^* band intensity in hydrophobic solvents along with long-wavelength shifts of both absorption and emission spectra by 40–50 nm.¹⁷⁶

On the other side of achieving two-band switching in highly polar environments are the species with weak ESICT properties, such as 7-acetamido-**3HCs** substituted at position 2 with furanyl or benzofuranyl. Their small charge distributions are still subject to efficient CT* stabilization by polar solvent molecules, allowing observation of the two-band emission in water.¹⁶⁷

3HC dyes have proved to be potent H-bond sensors.¹⁶⁹ Recently, a series of new dyes with improved spectroscopic properties in water and other highly polar media were synthesized.^{177,178} Their 4'-methoxyphenyl-**3HC** fluorophore, similar to that of 2-furyl, presents an intermediate electron donor ability between phenyl and 4'-(dialkylamino)phenyl groups. These dyes display an enhanced sensitivity to intermolecular H-bonding perturbation of 4-carbonyl, which make them ideal molecular sensors of water in biological systems. Remarkably, the N^* band of these new dyes does not exhibit a strong shift as a function of polarity, which allows an excellent separation between N^* and T^* bands.

An opposite case is 5,6-benzo-derivative of **3HFN**, which is totally insensitive to hydrogen bonding but shows linear dependence of the $\log(N^*/T^*)$ ratio upon the function of dielectric constant ϵ for all sets of solvents, including protic ones. This unique probe opens the route to separate fixation of effects of hydrogen bonding and dipolarity and to determination of dipolarity level on the nanoscopic scale.¹⁶⁴

Thus, the principle of selecting the dyes for polarity sensing is based on the proper balance between ESICT and ESIPT energies. To observe the N^* emission band of comparable intensity and achieve ESICT-ESIPT switching in hydrophobic environments, a high ESICT dipole moment stabilizing this state is needed. In polar medium, in contrast, the ESICT state

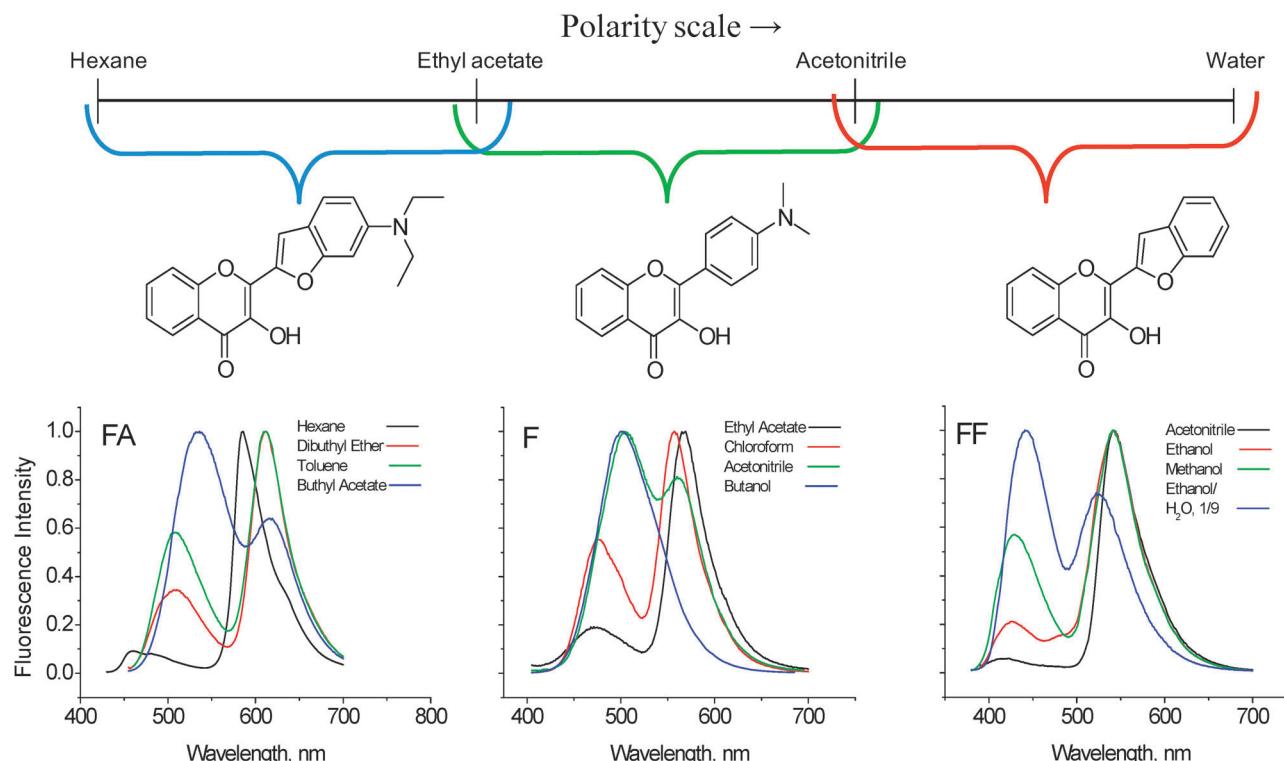


Fig. 24 Polarity scale marking the range of two-band ratiometric response of typical 3-hydroxychromone derivatives and their representative spectra in selected solvents.

should exhibit a much smaller charge separation, which in turn allows the required energy balance with the ESIPT state to be maintained. In this shaky balance, the sensitivity to H-bonding effects in terms of the band intensity switching is increased.

The most interesting applications of this simple technique for polarity sensing are observed in the study of systems of nanoscopic heterogeneity, such as direct¹⁷⁹ and reverse¹⁸⁰ micelles. The host-guest interactions in cyclodextrins have been studied¹⁸¹ based on dramatic differences in response between unbound dye and that located in a low-polar molecular cavity. The properties of thermosensitive polymers dependent on their composition¹⁸² and dynamic solvation in supercritical fluids¹⁶³ can also be investigated by this method. The intriguing properties of ionic liquids were characterized based on the dye two-color response in steady-state and time domains.^{183,184}

8.2. Probing the intermolecular interactions in biological systems

Proper fluorophore location allows study of the interactions with and between biological macromolecules,¹⁷⁴ and this opens many possibilities in sensing and imaging technologies. The binding sites of proteins can be labelled¹⁸⁵ to reflect their structural changes.^{186,187} An enzyme binding with its inhibitor can be observed in wavelength-ratiometric mode due to conformational change in the inhibitor influencing the solvent exposure of 3HCs label.¹⁸⁸ The strong ratiometric signal detected upon interaction of labelled antigenic peptide with

specific antibody^{189,190} suggests a novel approach for the development of biomolecular sensors and immunosensors.¹⁹¹

Many new possibilities appear from the studies of structures and interactions of nucleic acids. Using the labelled polycationic spermine as a probe, it is easy to distinguish its binding to single-stranded or double-stranded DNA by dramatic changes in the spectra.¹⁹² As shown in Fig. 25, using 2-furyl-3HC dye as a probe, a dramatic decrease of CT* band intensity is indicated upon incorporation of the label into a double-helical structure (*cf.* single-strand). It was also shown that the labelled

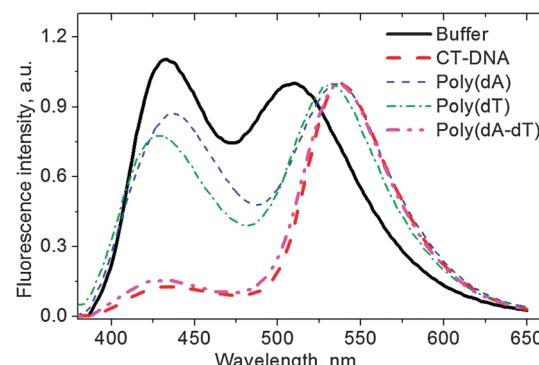


Fig. 25 Fluorescence spectra of spermine labeled with 2-furyl-3HC dye interacting with single-strand (blue) and double-strand (Red) DNA. Dramatic decrease of CT* band intensity indicates incorporation of the label into a double-helical structure. (Reprinted with permission from ref. 192. Copyright © 2008 American Chemical Society.)

peptide corresponding to the zinc finger domain of the HIV-1 nucleocapsid protein, upon interaction with target oligonucleotides, displays a strong sequence-dependent fluorescence response.¹⁹³

The efficiency of **3HF**s as intercalating dyes replacing the DNA abasic site for recognition of complementary nucleotide was demonstrated.¹⁹⁴ Good prospects in DNA and RNA sensing technologies promise oligonucleotides with brightly fluorescent and polarity-sensing **3HC**s covalently linked to replace the natural bases.¹⁹⁵ It was shown that they not only provide a bright signal on hybridization of DNA sequences but also strongly respond to the interaction of these sequences with proteins.¹⁹⁶ The possibility of replacing the natural bases in order to occupy the major and minor DNA grooves has also started to be explored.¹⁹⁷

Of great importance is the understanding of protein aggregation in the brain tissues, leading to neurodegenerative disorders, such as Parkinson's disease. The application of **3HF** dyes for probing the formed aggregates allowed observation of strong structural differences between the alpha-synuclein protein aggregates formed by wild-type protein and its mutants found in a human population.¹⁹⁸ The covalent attachment of dyes of the same type to different sites of this protein allowed continuous monitoring of protein aggregation and revealed its early and intermediate stages.¹⁹⁹ Moreover, this approach allowed study of the interaction of alpha-synuclein with biomembranes, since this process is thought to be crucial in the development of pathology.²⁰⁰

8.3. Probing the biological membranes

The strongest gradients of electric field, on the level of 10^8 V cm^{-1} , exist in the membranes of living cells, which suggests the application of electrochromic dyes.⁷⁸ Moreover, detection of the propagation of action potential in nerve cells requires sub-millisecond time resolution. This is achievable with the dyes, the electrochromic response of which is based on perturbation of ESICT-ESIPT equilibrium (see above). Since the electrostatic potential changes steeply and non-monotonously across the membrane on a sub-nanometer scale, the application of smart fluorescent dyes remains probably the only versatile experimental method to achieve this probing.⁷⁸ The **3HF** dyes are well suited to this approach. A series of dyes focused on this application were designed for insertion into biological membranes in different depths and orientations.^{201,202} Their location and sensitivity allowed probing of the contributions to electrostatic membrane potential – dipole^{203,204} and transmembrane potentials.¹⁵⁴

For dyes located at the charged and highly hydrated biomembrane surface, a combined response to hydration and to the surface potential is observed.²⁰⁵ These two types of response are mechanistically uncoupled, and they can be analyzed with simple software that involves both excitation and emission spectra.²⁰⁶ In the studies of membranes of intact living cells, the response of these dyes to their 'life stories' was so significant that it was possible to characterize the changes of the molecular order of biomembranes on depletion-addition of

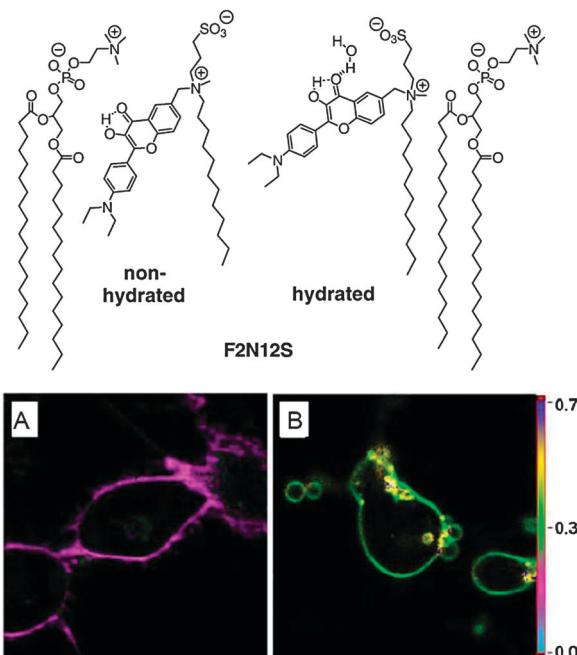


Fig. 26 Example of an application of functional **3HF** derivative F2N12S in cell microscopy. The mode of incorporation of these dyes in the phospholipid bilayer is shown above. Below are fluorescence ratiometric images of normal (A) and apoptotic (B) cells stained with F2N12S using two-photon excitation at 830 nm. The images are presented in artificial color based on intensity ratio at 520 and 580 nm. (Reprinted from Biochimica Et Biophysica Acta-Biomembranes, Vol. 1798, S. Oncul1, A. S. Klymchenko, O. A. Kucherak, A. P. Demchenko, S. Martin, M. Dontenwill, Y. Arntz, P. Didier, G. Duportail, Y. Mély, Liquid ordered phase in cell membranes evidenced by a hydration-sensitive probe: Effects of cholesterol depletion and apoptosis, P. 1441, Copyright © 2010 with permission from Elsevier, ref. 207.)

cholesterol²⁰⁷ and to detect early steps of apoptosis, *i.e.*, programmed cell death.²⁰⁸ (Fig. 26)

8.4. Molecular sensors

The possibility of realizing switchable dual emission is very attractive for different chemical sensing technologies.^{191,209} In contrast to the usually applied "ON-OFF" switching, based on quenching/enhancement of total intensity that is hard to quantify, the easily detectable two-channel "OR-OR" sensor response can be realized here.²¹⁰ The advantages are apparent; they provide independence on any instrumental factors and on concentration of fluorescence reporter, and thus allow the response to be displayed in quantitative terms. When the ESICT-ESIPT equilibrium is established much faster than the emission, the ratiometric response is robust against thermal and collisional quenching.^{91,96}

Metal cation sensing is probably the simplest task for this methodology. However, when bound to a CT* donor, such as azacrown substituent in **3HF**,^{211,212} an ejection of the ion occurs in the excited state. The situation is different when a cation is bound to a CT* acceptor. It enhances the electron withdrawing ability of this group along with inhibition of the ESIPT process such that the coordinated species emit ESICT fluorescence with the normal Stokes' shift.²¹³ In addition,

a different mechanism is explored for detection of biologically important Zn^{2+} ions; it is the disruption of the ESIPT process caused by ion binding at the site of this reaction²¹⁴ or re-formation of this site by the distantly bound ion.²¹⁵

Inhibition of ESIPT is also used in anion sensing.²¹⁶ Selectivity to anions here appears due to the dependence of the efficiency of ESIPT suppression upon the anion basicity and charge density. Still, the ability of analyte to modulate the ESICT-ESIPT equilibrium remains largely unexplored. From the basic considerations presented above, this mechanism must have much broader applicability, since it allows switching between two emissions by analyte binding at a distance from the ESIPT reaction site. This can be accomplished in different ways: by modulating the strengths of the CT* donor and acceptor substitutes, by changes in local polarity, and by providing electrochromic modulation of two-color response.

9. Conclusion and perspective

In this Review we attempt to demonstrate great potential of organic dyes exhibiting ESICT-ECIPT coupled transformations for basic science and for many different applications. Based on the structures and combination of the photophysical properties of these systems, we introduce a new classification of them, summarized in Table 1.

As class I, we distinguish the class of molecules in which the electron donor and proton donor occupy different positions but the same group serves as both proton acceptor and electron acceptor. Their ESICT transformation occurs before or simultaneously with the ESIPT reaction, so both kinetic and thermodynamic regimes for this reaction can be realized. Such molecules can exhibit steady-state dual emission with the position and relative intensity of the CT* band being solvent-dependent.

The other class (class II) of ESICT-ESIPT coupled systems are those in which the proton acceptor and electron acceptor are at different positions but the proton donor and electron donor are the same. Dual emission can also be observed in this case, but it is the long-wavelength proton-transfer tautomer band that exhibits the solvent-dependent variation of emission wavelengths. Dynamic solvation of the PCT*_{eq} state makes the overall reaction irreversible.

For both classes of molecules, a common feature lies in the strong intramolecular hydrogen bonding connecting the proton

donor and acceptor sites such that the intrinsic ESIPT is barrierless and the slowdown of the overall ESICT/ESIPT reaction is mainly due to a barrier induced by the stabilization of the charge transfer state by intermolecular interactions. Moreover, this charge transfer is through a π -conjugated chromophore and is deemed an optical electron transfer process, which instantaneously responds to the motion of proton. These are not the only possibilities, and other systems for study, analysis, and application can be rationally designed. We foresee that future expansion in the scope of the relevant research can focus on still obscure cases of weaker coupling between correspondent states of protons and electrons. Particularly, instead of D_e and A_e using the π -conjugation as the donor/acceptor bridge, they may be linked *via* σ -bonds or through-space interaction. The D_e/A_e electronic coupling constant will thus be drastically reduced and the charge transfer reaction will fall into the non-adiabatic regime with a slow reaction rate. ESIPT molecules possessing weak intramolecular hydrogen bonds have recently been reported.^{217–220} They are needed for better understanding of the cases in which the vibronic coupling along the proton transfer coordinate is weak, slowing down the proton transfer rate. The expected decrease in rates when these two processes are combined may represent a general case requiring two-dimensional solvation models to distinguish the solvent dependence of proton and electron motions. Doubtlessly, experimental progress requires supplementary support from theoretical approaches. The combination of both is believed to be a powerful way to develop in-depth insight into the ESIPT/ESIPT coupling reaction. The computation methods for the ESICT/ESIPT coupled reaction, though still in the early stage, are expected to develop quickly in the near future.

Also, we still need better understanding of cases in which the ESICT/ESIPT reactions are modulated by external H-bonding perturbations, particularly in water and other protic solvents. The role of water, and particularly the associated specific intermolecular hydrogen bonding effect, can thus be explored.^{6,48,81,119,221–224} In this approach, molecules such as 7-azaindole and its analogues,^{19–26,28–31,225–227} which, after charge transfer, undergo excited state proton transfer *via* the catalysis of water molecules, can be of use. Therefore, the associated CT* and PCT* emission may serve as a case in point to probe bulk and local microsolvation of water, respectively. The latter aspect is of great importance for understanding biocatalysis and biological transport phenomena.

Table 1 Characteristic features of two classes of ESICT-ESIPT systems

Features	Class I	Class II
Structures	Electron donors and proton donors are different and electron-proton acceptor is the same.	Electron-proton donor is the same and electron and proton acceptors are different.
Major sequence of photophysical events	$F - C^* \rightarrow CT_{eq}^* \rightarrow PCT^*$	$F - C^* \rightarrow PT^* \rightarrow PCT_{eq}^*$
Dipole moment	Large in CT* _{eq} state	Large in PCT* _{eq} state
Solvatochromism	Strong red shift and increased relative intensity with the increase of polarity of CT* _{eq} band.	Strong red shift and increased relative intensity with the increase of polarity of PCT* _{eq} band.
Emission decays ESIPT rate	Switchable between thermodynamic and kinetic regimes. Decrease with the increase of polarity.	Kinetic regime. Increase with the increase of polarity.

In this Review, we show that chemical modifications even at sites quite distant from those involved in ESIPT reaction provide practically unlimited possibilities for modulating the photophysical properties of ESICT–ESIPT systems and for optimising them for different applications. The attractions of these applications benefit from the ability to obtain sensing and imaging information based on medium-induced or target-induced switching between ESICT and ESIPT emission bands.

We consider that the ESICT–ESIPT interplay is the most general fundamental process characterizing the systems undergoing ESIPT reactions. Its exploration offers unlimited possibilities in applications. We thus hope that, from reading this chapter, the reader has gained fundamental knowledge of these processes and perspectives on their basic research and applications.

Acknowledgements

The authors are grateful to A. Burger, S. Yesylevskyy, V. Pivovarenko and V. Tomin for reading the manuscript and making valuable comments. Chou and Tang thank the National Science Council of Taiwan for financial support and the National Center for High-Performance Computing of Taiwan for computer time and facilities.

Notes and references

- 1 T. Elsässer and H. J. Bakker, *Ultrafast hydrogen bonding dynamics and proton transfer processes in the condensed phase*, Kluwer Academic Publishers, Dordrecht, Boston, 2002.
- 2 S. Hammes-Schiffer and N. Iordanova, *Biochim. Biophys. Acta, Bioenerg.*, 2004, **1655**, 29.
- 3 C. J. Chang, M. C. Y. Chang, N. H. Damrauer and D. G. Nocera, *Biochim. Biophys. Acta, Bioenerg.*, 2004, **1655**, 13.
- 4 M. H. V. Huynh and T. J. Meyer, *Chem. Rev.*, 2007, **107**, 5004.
- 5 T. J. Meyer, M. H. V. Huynh and H. H. Thorp, *Angew. Chem., Int. Ed.*, 2007, **46**, 5284.
- 6 S. Hammes-Schiffer, *Acc. Chem. Res.*, 2009, **42**, 1881.
- 7 C. J. Gagliardi, B. C. Westlake, C. A. Kent, J. J. Paul, J. M. Papanikolas and T. J. Meyer, *Coord. Chem. Rev.*, 2010, **254**, 2459.
- 8 S. Hammes-Schiffer, *Chem. Rev.*, 2010, **110**, 6937.
- 9 S. Hammes-Schiffer and A. A. Stuchebrukhov, *Chem. Rev.*, 2010, **110**, 6939.
- 10 G. J. Zhao and K. L. Han, *Acc. Chem. Res.*, 2012, **45**, 404.
- 11 J. Zhao, S. Ji, Y. Chen, H. Guo and P. Yang, *Phys. Chem. Chem. Phys.*, 2012, **14**, 8803.
- 12 M. Kasha, *J. Chem. Soc., Faraday Trans. 2*, 1986, **82**, 2379.
- 13 J. Waluk, *Conformational analysis of molecules in excited states*, Wiley-VCH, New York, Chichester, 2000.
- 14 P. T. Chou, *J. Chin. Chem. Soc.*, 2001, **48**, 651.
- 15 L. M. Tolbert and K. M. Solntsev, *Acc. Chem. Res.*, 2002, **35**, 19.
- 16 J. Waluk, *Acc. Chem. Res.*, 2003, **36**, 832.
- 17 T. E. Dermota, Q. Zhong and A. W. Castleman, *Chem. Rev.*, 2004, **104**, 1861.
- 18 C. A. Wright, *Biochim. Biophys. Acta, Bioenerg.*, 2006, **1757**, 886.
- 19 P. T. Chou, J. H. Liao, C. Y. Wei, C. Y. Yang, W. S. Yu and Y. H. Chou, *J. Am. Chem. Soc.*, 2000, **122**, 986.
- 20 P. T. Chou, M. L. Martinez, W. C. Cooper, D. Mcmorrow, S. T. Collins and M. Kasha, *J. Phys. Chem.*, 1992, **96**, 5203.
- 21 P. T. Chou, C. Y. Wei, C. P. Chang and M. S. Kuo, *J. Phys. Chem.*, 1995, **99**, 11994.
- 22 P. T. Chou, G. R. Wu, C. Y. Wei, M. Y. Shiao and Y. I. Liu, *J. Phys. Chem. A*, 2000, **104**, 8863.
- 23 P. T. Chou, W. S. Yu, Y. C. Chen, C. Y. Wei and S. S. Martinez, *J. Am. Chem. Soc.*, 1998, **120**, 12927.
- 24 P. T. Chou, W. S. Yu, C. Y. Wei, Y. M. Cheng and C. Y. Yang, *J. Am. Chem. Soc.*, 2001, **123**, 3599.
- 25 W. T. Hsieh, C. C. Hsieh, C. H. Lai, Y. M. Cheng, M. L. Ho, K. K. Wang, G. H. Lee and P. T. Chou, *ChemPhysChem*, 2008, **9**, 293.
- 26 W. P. Hu, J. L. Chen, C. C. Hsieh and P. T. Chou, *Chem. Phys. Lett.*, 2010, **485**, 226.
- 27 A. P. Demchenko, *Biochim. Biophys. Acta*, 1994, **1209**, 149.
- 28 M. Chachisvilis, T. Fiebig, A. Douhal and A. H. Zewail, *J. Phys. Chem. A*, 1998, **102**, 669.
- 29 A. Douhal, S. K. Kim and A. H. Zewail, *Nature*, 1995, **378**, 260.
- 30 O. H. Kwon and A. H. Zewail, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 8703.
- 31 H. Lim, S. Y. Park and D. J. Jang, *J. Phys. Chem. A*, 2010, **114**, 11432.
- 32 S. Y. Park, Y. S. Lee and D. J. Jang, *Phys. Chem. Chem. Phys.*, 2011, **13**, 3730.
- 33 J. E. Kwon and S. Y. Park, *Adv. Mater.*, 2011, **23**, 3615.
- 34 K. C. Tang, M. J. Chang, T. Y. Lin, H. A. Pan, T. C. Fang, K. Y. Chen, W. Y. Hung, Y. H. Hsu and P. T. Chou, *J. Am. Chem. Soc.*, 2011, **133**, 17738.
- 35 G. J. Zhao and K. L. Han, *ChemPhysChem*, 2008, **9**, 1842.
- 36 G. J. Zhao and K. L. Han, *Biophys. J.*, 2008, **94**, 38.
- 37 G. J. Zhao and K. L. Han, *J. Phys. Chem. A*, 2009, **113**, 14329.
- 38 G. J. Zhao, B. H. Northrop, K. L. Han and P. J. Stang, *J. Phys. Chem. A*, 2010, **114**, 9007.
- 39 G. J. Zhao, J. Y. Liu, L. C. Zhou and K. L. Han, *J. Phys. Chem. B*, 2007, **111**, 8940.
- 40 G. J. Zhao and K. L. Han, *J. Phys. Chem. A*, 2007, **111**, 9218.
- 41 G. J. Zhao and K. L. Han, *J. Phys. Chem. A*, 2007, **111**, 2469.
- 42 E. T. J. Nibbering and T. Elsaesser, *Chem. Rev.*, 2004, **104**, 1887.
- 43 K.-L. Han and G.-J. Zhao, *Hydrogen bonding and transfer in the excited state*, Wiley, Hoboken, N.J., 2011.
- 44 R. A. Marcus and N. Sutin, *Biochim. Biophys. Acta*, 1985, **811**, 265.
- 45 M. Maroncelli, J. Macinnis and G. R. Fleming, *Science*, 1989, **243**, 1674.
- 46 A. N. Bader, V. G. Pivovarenko, A. P. Demchenko, F. Ariese and C. Gooijer, *J. Phys. Chem. B*, 2004, **108**, 10589.
- 47 A. N. Bader, V. Pivovarenko, A. P. Demchenko, F. Ariese and C. Gooijer, *Spectrochim. Acta, Part A*, 2003, **59**, 1593.

- 48 S. Hammes-Schiffer, *J. Phys. Chem. Lett.*, 2011, **2**, 1410.
- 49 C.-C. Hsieh, M.-L. Ho and P.-T. Chou, *Organic dyes with excited-State transformations (electron, charge, and proton transfers)*, in *Advanced Fluorescence Reporters in Chemistry and Biology I. Fundamentals and molecular design*, ed. A. P. Demchenko, Springer, Springer Ser. Fluoresc., 2010, vol. 8, pp. 225–266.
- 50 D. Borgis and J. T. Hynes, *J. Chem. Phys.*, 1991, **94**, 3619.
- 51 P. M. Kiefer and J. T. Hynes, *J. Phys. Chem. A*, 2002, **106**, 1834.
- 52 S. Nagaoka, J. Kusunoki, T. Fujibuchi, S. Hatakenaka, K. Mukai and U. Nagashima, *J. Photochem. Photobiol. A*, 1999, **122**, 151.
- 53 S. Nagaoka, A. Nakamura and U. Nagashima, *J. Photochem. Photobiol. A*, 2002, **154**, 23.
- 54 S. Nagaoka, H. Teramae and U. Nagashima, *Bull. Chem. Soc. Jpn.*, 2009, **82**, 570.
- 55 C. Swalina, M. V. Pak, A. Chakraborty and S. Hammes-Schiffer, *J. Phys. Chem. A*, 2006, **110**, 9983.
- 56 Y. Georgievskii and A. A. Stuchebrukhov, *J. Chem. Phys.*, 2000, **113**, 10438.
- 57 B. C. Westlake, M. K. Brennaman, J. J. Concepcion, J. J. Paul, S. E. Bettis, S. D. Hampton, S. A. Miller, N. V. Lebedeva, M. D. E. Forbes, A. M. Moran, T. J. Meyer and J. M. Papanikolas, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 8554.
- 58 N. Mataga and T. Kubota, *Molecular Interactions and Electronic spectra*, Marcel Dekker, New York, 1970.
- 59 P. Suppan and N. Ghoneim, *Solvatochromism*, Royal Society of Chemistry, Cambridge, UK, 1997.
- 60 A. P. Demchenko, *Luminescence*, 2002, **17**, 19.
- 61 M. Maroncelli, *J. Mol. Liq.*, 1993, **57**, 1.
- 62 M. Vincent, J. Gallay and A. P. Demchenko, *J. Phys. Chem.*, 1995, **99**, 14931.
- 63 N. Nandi, K. Bhattacharyya and B. Bagchi, *Chem. Rev.*, 2000, **100**, 2013.
- 64 J. Tomasi, B. Mennucci and R. Cammi, *Chem. Rev.*, 2005, **105**, 2999.
- 65 B. Mennucci, *Theor. Chem. Acc.*, 2006, **116**, 31.
- 66 A. P. Demchenko and S. O. Yesylevskyy, *Interfacial Behavior of Fluorescent Dyes*, in *Advanced Fluorescence Reporters in Chemistry and Biology III*, ed. A. P. Demchenko, Springer, Berlin, Heidelberg, 2011, pp. 3–62.
- 67 J. Neugebauer, C. R. Jacob, T. A. Wesolowski and E. J. Baerends, *J. Phys. Chem. A*, 2005, **109**, 7805.
- 68 J. Tomasi and M. Persico, *Chem. Rev.*, 1994, **94**, 2027.
- 69 V. K. Sharma, S. P. D. R. C. Rastogi, S. K. Ghoshal and D. Mohan, *Spectrochim. Acta, Part A*, 2003, **59**, 1799.
- 70 M. Umadevi, P. Vanelle, T. Terme and V. Ramakrishnan, *J. Fluoresc.*, 2006, **16**, 569.
- 71 N. A. Nemkovich, A. N. Rubinov and V. I. Tomin, *Inhomogeneous broadening of electronic spectra of dye molecules in solutions*, in *Topics in fluorescence spectroscopy*, ed. J. R. Lakowicz, Plenum Press, New York, 1991, pp. 367–428.
- 72 N. G. Bakhshiev, *Spectroscopy of intermolecular interactions*, Nauka Leningrad, 1972.
- 73 H. J. C. Berendsen, *Science*, 1996, **271**, 954.
- 74 W. F. van Gunsteren, D. Bakowies, R. Baron, I. Chandrasekhar, I. Christen, X. Daura, P. Gee, D. P. Geerke, A. Glattli, P. H. Hunenberger, M. A. Kastenholz, C. Oostenbrink, M. Schenk, D. Trzesniak, N. F. A. van der Vegt and H. B. Yu, *Angew. Chem., Int. Ed.*, 2006, **45**, 4064.
- 75 S. O. Yesylevskyy, L. V. Schafer, D. Sengupta and S. J. Marrink, *PLoS Comput. Biol.*, 2010, **6**, e1000810.
- 76 U. C. Singh and P. A. Kollman, *J. Comput. Chem.*, 1986, **7**, 718.
- 77 H. Lin and D. G. Truhlar, *Theor. Chem. Acc.*, 2007, **117**, 185.
- 78 A. P. Demchenko and S. O. Yesylevskyy, *Chem. Phys. Lipids*, 2009, **160**, 63.
- 79 D. Kina, P. Arora, A. Nakayama, T. Noro, M. S. Gordon and T. Taketsugu, *Int. J. Quantum Chem.*, 2009, **109**, 2308.
- 80 D. M. Chipman, *J. Chem. Phys.*, 2009, **131**, 014103.
- 81 A. Hazra, A. V. Soudackov and S. Hammes-Schiffer, *J. Phys. Chem. B*, 2010, **114**, 12319.
- 82 R. Bianco, J. J. I. Timoneda and J. T. Hynes, *J. Phys. Chem.*, 1994, **98**, 12103.
- 83 E. Hatcher, A. Soudackov and S. Hammes-Schiffer, *J. Phys. Chem. B*, 2005, **109**, 18565.
- 84 B. Bagchi and B. Jana, *Chem. Soc. Rev.*, 2010, **39**, 1936.
- 85 A. P. Demchenko and A. I. Sytnik, *Proc. Natl. Acad. Sci. U. S. A.*, 1991, **88**, 9311.
- 86 A. P. Demchenko and A. I. Sytnik, *J. Phys. Chem.*, 1991, **95**, 10518.
- 87 P. T. Chou, M. L. Martinez and J. H. Clements, *J. Phys. Chem.*, 1993, **97**, 2618.
- 88 V. V. Shynkar, Y. Mely, G. Duportail, E. Piemont, A. S. Klymchenko and A. P. Demchenko, *J. Phys. Chem. A*, 2003, **107**, 9522.
- 89 C. C. Hsieh, C. M. Jiang and P. T. Chou, *Acc. Chem. Res.*, 2010, **43**, 1364.
- 90 M. Smoluch, H. Joshi, A. Gerssen, C. Gooijer and G. van der Zwan, *J. Phys. Chem. A*, 2005, **109**, 535.
- 91 V. I. Tomin, S. Oncul, G. Smolarczyk and A. P. Demchenko, *Chem. Phys.*, 2007, **342**, 126.
- 92 D. A. Yushchenko, M. D. Bilokin', O. V. Pyrovarenko, G. Duportail, Y. Mely and V. G. Pivovarenko, *Tetrahedron Lett.*, 2006, **47**, 905.
- 93 A. S. Klymchenko and A. P. Demchenko, *Phys. Chem. Chem. Phys.*, 2003, **5**, 461.
- 94 S. Ercelen, A. S. Klymchenko and A. P. Demchenko, *Anal. Chim. Acta*, 2002, **464**, 273.
- 95 A. S. Klymchenko and A. P. Demchenko, *J. Am. Chem. Soc.*, 2002, **124**, 12372.
- 96 S. Oncul and A. P. Demchenko, *Spectrochim. Acta, Part A*, 2006, **65**, 179.
- 97 A. J. G. Strandjord and P. F. Barbara, *J. Phys. Chem.*, 1985, **89**, 2355.
- 98 A. S. Klymchenko, V. G. Pivovarenko and A. P. Demchenko, *Spectrochim. Acta, Part A*, 2003, **59**, 787.
- 99 S. Ameer-Beg, S. M. Ormson, X. Poteau, R. G. Brown, P. Foggi, L. Bussotti and F. V. R. Neuwahl, *J. Phys. Chem. A*, 2004, **108**, 6938.

- 100 S. Ameer-Beg, S. M. Ormson, R. G. Brown, P. Matousek, M. Towrie, E. T. J. Nibbering, P. Foggi and F. V. R. Neuwahl, *J. Phys. Chem. A*, 2001, **105**, 3709.
- 101 P. T. Chou, Y. C. Chen, W. S. Yu, Y. H. Chou, C. Y. Wei and Y. M. Cheng, *J. Phys. Chem. A*, 2001, **105**, 1731.
- 102 W. Frey, F. Laermer and T. Elsaesser, *J. Phys. Chem.*, 1991, **95**, 10391.
- 103 C. C. Hsieh, P. T. Chou, C. W. Shih, W. T. Chuang, M. W. Chung, J. Lee and T. Joo, *J. Am. Chem. Soc.*, 2011, **133**, 2932.
- 104 H. Hosoi, H. Mizuno, A. Miyawaki and T. Tahara, *J. Phys. Chem. B*, 2006, **110**, 22853.
- 105 S. Takeuchi and T. Tahara, *J. Phys. Chem. A*, 1998, **102**, 7740.
- 106 C. Schriever, M. Barbatti, K. Stock, A. J. A. Aquino, D. Tunega, S. Lochbrunner, E. Riedle, R. de Vivie-Riedle and H. Lischka, *Chem. Phys.*, 2008, **347**, 446.
- 107 M. Glasbeek and H. Zhang, *Chem. Rev.*, 2004, **104**, 1929.
- 108 C. Z. Wan, T. Fiebig, O. Schiemann, J. K. Barton and A. H. Zewail, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 14052.
- 109 A. Sharonov, T. Gustavsson, V. Carre, E. Renault and D. Markovitsi, *Chem. Phys. Lett.*, 2003, **380**, 173.
- 110 K. C. Tang, A. Rury, M. B. Orozco, J. Egendorf, K. G. Spears and R. J. Sension, *J. Chem. Phys.*, 2011, **134**, 104503.
- 111 F. Plasser, M. Barbatti, A. J. A. Aquino and H. Lischka, *Theor. Chem. Acc.*, 2012, **131**, 1073.
- 112 M. J. G. Peach, T. Helgaker, P. Salek, T. W. Keal, O. B. Lutnaes, D. J. Tozer and N. C. Handy, *Phys. Chem. Chem. Phys.*, 2006, **8**, 558.
- 113 T. Yanai, D. P. Tew and N. C. Handy, *Chem. Phys. Lett.*, 2004, **393**, 51.
- 114 Y. Zhao and D. G. Truhlar, *Theor. Chem. Acc.*, 2008, **120**, 215.
- 115 K. C. Tang, C. L. Chen, H. H. Chuang, J. L. Chen, Y. J. Chen, Y. C. Lin, J. Y. Shen, W. P. Hu and P. T. Chou, *J. Phys. Chem. Lett.*, 2011, **2**, 3063.
- 116 O. Vendrell, M. Moreno, J. M. Lluch and S. Hammes-Schiffer, *J. Phys. Chem. B*, 2004, **108**, 6616.
- 117 M. A. Bellucci and D. F. Coker, *J. Chem. Phys.*, 2012, **136**, 194505.
- 118 P. M. Kiefer and J. T. Hynes, *J. Phys. Chem. A*, 2004, **108**, 11793.
- 119 S. Hammes-Schiffer and A. V. Soudackov, *J. Phys. Chem. B*, 2008, **112**, 14108.
- 120 C. C. Hsieh, Y. M. Cheng, C. J. Hsu, K. Y. Chen and P. T. Chou, *J. Phys. Chem. A*, 2008, **112**, 8323.
- 121 P. T. Chou, M. L. Martinez and J. H. Clements, *Chem. Phys. Lett.*, 1993, **204**, 395.
- 122 F. Parsapour and D. F. Kelley, *J. Phys. Chem.*, 1996, **100**, 2791.
- 123 P. T. Chou, C. H. Huang, S. C. Pu, Y. M. Cheng, Y. H. Liu, Y. Wang and C. T. Chen, *J. Phys. Chem. A*, 2004, **108**, 6452.
- 124 P. T. Chou, S. C. Pu, Y. M. Cheng, W. S. Yu, Y. C. Yu, F. T. Hung and W. P. Hu, *J. Phys. Chem. A*, 2005, **109**, 3777.
- 125 Y. M. Cheng, S. C. Pu, Y. C. Yu, P. T. Chou, C. H. Huang, C. T. Chen, T. H. Li and W. P. Hu, *J. Phys. Chem. A*, 2005, **109**, 11696.
- 126 P. T. Chou, W. S. Yu, Y. M. Cheng, S. C. Pu, Y. C. Yu, Y. C. Lin, C. H. Huang and C. T. Chen, *J. Phys. Chem. A*, 2004, **108**, 6487.
- 127 Y. M. Cheng, S. C. Pu, C. J. Hsu, C. H. Lai and P. T. Chou, *ChemPhysChem*, 2006, **7**, 1372.
- 128 J. Seo, S. Kim and S. Y. Park, *J. Am. Chem. Soc.*, 2004, **126**, 11154.
- 129 P. K. Sengupta and M. Kasha, *Chem. Phys. Lett.*, 1979, **68**, 382.
- 130 M. Kasha, *Acta Phys. Pol. A*, 1987, **71**, 717.
- 131 P. Chou, D. McMorrow, T. J. Aartsma and M. Kasha, *J. Phys. Chem.*, 1984, **88**, 4596.
- 132 B. J. Schwartz, L. A. Peteanu and C. B. Harris, *J. Phys. Chem.*, 1992, **96**, 3591.
- 133 C. Chudoba, E. Riedle, M. Pfeiffer and T. Elsaesser, *Chem. Phys. Lett.*, 1996, **263**, 622.
- 134 A. N. Bader, F. Ariese and C. Gooijer, *J. Phys. Chem. A*, 2002, **106**, 2844.
- 135 T. C. Swinney and D. F. Kelley, *J. Chem. Phys.*, 1993, **99**, 211.
- 136 S. O. Yesylevskyy, A. S. Klymchenko and A. P. Demchenko, *THEOCHEM*, 2005, **755**, 229.
- 137 N. A. Nemkovich, W. Baumann and V. G. Pivovarenko, *J. Photochem. Photobiol. A*, 2002, **153**, 19.
- 138 A. Douhal, M. Sanz, M. A. Carranza, J. A. Organero and L. Santos, *Chem. Phys. Lett.*, 2004, **394**, 54.
- 139 M. Mosquera, J. C. Penedo, M. C. R. Rodriguez and F. Rodriguez-Prieto, *J. Phys. Chem.*, 1996, **100**, 5398.
- 140 A. Suwattanamala and V. Ruangpornvisuti, *Struct. Chem.*, 2009, **20**, 619.
- 141 S. R. Vazquez, M. C. R. Rodriguez, M. Mosquera and F. Rodriguez-Prieto, *J. Phys. Chem. A*, 2007, **111**, 1814.
- 142 R. J. Wang, D. Liu, K. Xu and J. Y. Li, *J. Photochem. Photobiol. A*, 2009, **205**, 61.
- 143 F. S. Rodembusch, F. P. Leusin, L. F. Campo and V. Stefani, *J. Lumin.*, 2007, **126**, 728.
- 144 T. Iijima, A. Momotake, Y. Shinohara, T. Sato, Y. Nishimura and T. Arai, *J. Phys. Chem. A*, 2010, **114**, 1603.
- 145 P. C. M. Weisenborn, A. H. Huizer and C. A. G. O. Varma, *J. Chem. Soc., Faraday Trans. 2*, 1989, **85**, 1895.
- 146 B. Bliss, U. Lommatsch, C. Monte, W. Rettig and B. Brutschy, *Chem. Phys.*, 2000, **254**, 407.
- 147 S. Nagaoka, N. Hirota, M. Sumitani and K. Yoshihara, *J. Am. Chem. Soc.*, 1983, **105**, 4220.
- 148 F. Toribio, J. Catalan, F. Amat and A. U. Acuna, *J. Phys. Chem.*, 1983, **87**, 817.
- 149 M. J. Shephard, M. N. PaddonRow and K. D. Jordan, *Chem. Phys.*, 1993, **176**, 289.
- 150 M. N. PaddonRow and M. J. Shephard, *J. Am. Chem. Soc.*, 1997, **119**, 5355.
- 151 A. M. Napper, N. J. Head, A. M. Oliver, M. J. Shephard, M. N. Paddon-Row, I. Read and D. H. Waldeck, *J. Am. Chem. Soc.*, 2002, **124**, 10171.
- 152 C. H. Kim, J. Park, J. Seo, S. Y. Park and T. Joo, *J. Phys. Chem. A*, 2010, **114**, 5618.
- 153 H. Wang, H. Zhang, O. K. Abou-Zied, C. Yu, F. E. Romesberg and M. Glasbeek, *Chem. Phys. Lett.*, 2003, **367**, 599.

- 154 A. S. Klymchenko, H. Stoeckel, K. Takeda and Y. Mely, *J. Phys. Chem. B*, 2006, **110**, 13624.
- 155 K. Iwata, R. Ozawa and H. O. Hamaguchi, *J. Phys. Chem. A*, 2002, **106**, 3614.
- 156 A. S. Klymchenko, T. Ozturk, V. G. Pivovarenko and A. P. Demchenko, *Can. J. Chem.*, 2001, **79**, 358.
- 157 A. S. Klymchenko, T. Ozturk, V. G. Pivovarenko and A. P. Demchenko, *Tetrahedron Lett.*, 2001, **42**, 7967.
- 158 A. S. Klymchenko, V. G. Pivovarenko, T. Ozturk and A. P. Demchenko, *New J. Chem.*, 2003, **27**, 1336.
- 159 K. Rotkiewicz, K. H. Grellmann and Z. R. Grabowski, *Chem. Phys. Lett.*, 1973, **19**, 315.
- 160 W. Rettig and B. Zietz, *Chem. Phys. Lett.*, 2000, **317**, 187.
- 161 D. Rappoport and F. Furche, *J. Am. Chem. Soc.*, 2004, **126**, 1277.
- 162 A. S. Klymchenko, D. A. Yushchenko and Y. Mely, *J. Photochem. Photobiol. A*, 2007, **192**, 93.
- 163 M. Barroso, N. Chattopadhyay, A. S. Klymchenko, A. P. Demchenko, L. G. Arnaud and S. J. Formosinho, *J. Phys. Chem. A*, 2006, **110**, 13419.
- 164 A. S. Klymchenko, V. G. Pivovarenko and A. P. Demchenko, *J. Phys. Chem. A*, 2003, **107**, 4211.
- 165 A. S. Klymchenko, T. Ozturk and A. P. Demchenko, *Tetrahedron Lett.*, 2002, **43**, 7079.
- 166 A. Zhu, B. Wang, J. O. White and H. G. Drickamer, *J. Phys. Chem. B*, 2004, **108**, 891.
- 167 A. S. Klymchenko and A. P. Demchenko, *New J. Chem.*, 2004, **28**, 687.
- 168 A. P. Demchenko, A. S. Klymchenko, V. G. Pivovarenko, S. Ercelen, G. Duportail and Y. Mely, *J. Fluoresc.*, 2003, **13**, 291.
- 169 V. V. Shynkar, A. S. Klymchenko, E. Piemont, A. P. Demchenko and Y. Mely, *J. Phys. Chem. A*, 2004, **108**, 8151.
- 170 A. P. Demchenko, *J. Fluoresc.*, 2010, **20**, 1099.
- 171 G. U. Bublitz and S. G. Boxer, *Annu. Rev. Phys. Chem.*, 1997, **48**, 213.
- 172 W. Liptay, *Angew. Chem., Int. Ed. Engl.*, 1969, **8**, 177.
- 173 A. S. Klymchenko, S. V. Avilov and A. P. Demchenko, *Anal. Biochem.*, 2004, **329**, 43.
- 174 A. P. Demchenko, *FEBS Lett.*, 2006, **580**, 2951.
- 175 A. P. Demchenko, S. Ercelen, A. D. Roshal and A. S. Klymchenko, *Pol. J. Chem.*, 2002, **76**, 1287.
- 176 A. S. Klymchenko and Y. Mely, *Tetrahedron Lett.*, 2004, **45**, 8391.
- 177 O. M. Zamotaiev, V. Y. Postupalenko, V. V. Shvadchak, V. G. Pivovarenko, A. S. Klymchenko and Y. Mely, *Bioconjugate Chem.*, 2011, **22**, 101.
- 178 V. G. Pivovarenko, O. M. Zamotaiev, V. V. Shvadchak, V. Y. Postupalenko, A. S. Klymchenko and Y. Mely, *J. Phys. Chem. A*, 2012, **116**, 3103.
- 179 T. Ozturk, A. S. Klymchenko, A. Capan, S. Oncul, S. Cikrikci, S. Taskiran, B. Tasan, F. B. Kaynak, S. Ozbey and A. P. Demchenko, *Tetrahedron*, 2007, **63**, 10290.
- 180 A. S. Klymchenko and A. P. Demchenko, *Langmuir*, 2002, **18**, 5637.
- 181 J. A. Organero, L. Tormo, M. Sanz, A. Roshal and A. Douhal, *J. Photochem. Photobiol. A*, 2007, **188**, 74.
- 182 B. Szczupak, A. G. Ryder, D. M. Togashi, A. S. Klymchenko, Y. A. Rochev, A. Gorelov and T. J. Glynn, *J. Fluoresc.*, 2010, **20**, 719.
- 183 M. Fukuda, M. Terazima and Y. Kimura, *Chem. Phys. Lett.*, 2008, **463**, 364.
- 184 Y. Kimura, M. Fukuda, K. Suda and M. Terazima, *J. Phys. Chem. B*, 2010, **114**, 11847.
- 185 S. Ercelen, A. S. Klymchenko and A. P. Demchenko, *FEBS Lett.*, 2003, **538**, 25.
- 186 S. V. Avilov, N. A. Aleksandrova and A. P. Demchenko, *Protein Pept. Lett.*, 2004, **11**, 41.
- 187 S. V. Avilov, C. Bode, F. G. Tolgyesi, A. S. Klymchenko, J. Fidy and A. P. Demchenko, *Int. J. Biol. Macromol.*, 2005, **36**, 290.
- 188 C. Boudier, A. S. Klymchenko, Y. Mely and A. Follenius-Wund, *Photochem. Photobiol. Sci.*, 2009, **8**, 814.
- 189 K. Enander, L. Choulier, A. L. Olsson, D. A. Yushchenko, D. Kanmert, A. S. Klymchenko, A. P. Demchenko, Y. Mely and D. Altschuh, *Bioconjugate Chem.*, 2008, **19**, 1864.
- 190 L. Choulier, V. V. Shvadchak, A. Naidoo, A. S. Klymchenko, Y. Mely and D. Altschuh, *Anal. Biochem.*, 2010, **401**, 188.
- 191 D. Altschuh, S. Oncul and A. P. Demchenko, *J. Mol. Recognit.*, 2006, **19**, 459.
- 192 A. S. Klymchenko, V. V. Shvadchak, D. A. Yushchenko, N. Jain and Y. Mely, *J. Phys. Chem. B*, 2008, **112**, 12050.
- 193 V. V. Shvadchak, A. S. Klymchenko, H. de Rocquigny and Y. Mely, *Nucleic Acids Res.*, 2009, **37**, e25.
- 194 S. J. Xu, Y. Shao, K. Ma, Q. H. Cui, G. Y. Liu, F. Wu and M. J. Li, *Analyst*, 2011, **136**, 4480.
- 195 M. Spadafora, V. Y. Postupalenko, V. V. Shvadchak, A. S. Klymchenko, Y. Mely, A. Burger and R. Benhida, *Tetrahedron*, 2009, **65**, 7809.
- 196 D. Dziuba, V. Y. Postupalenko, M. Spadafora, A. S. Klymchenko, V. Guérineau, Y. Mély, R. Benhida and A. Burger, *J. Am. Chem. Soc.*, 2012, **134**, 10209.
- 197 D. Dziuba, I. Karpenko, B. Michel, A. Klymchenko, Y. Mely, R. Benhida, A. P. Demchenko and A. Burger, 2012, (submitted for publication).
- 198 M. S. Celej, W. Caarls, A. P. Demchenko and T. M. Jovin, *Biochemistry*, 2009, **48**, 7465.
- 199 D. A. Yushchenko, J. A. Fauerbach, S. Thirunavukkuarasu, E. A. Jares-Erijman and T. M. Jovin, *J. Am. Chem. Soc.*, 2010, **132**, 7860.
- 200 V. V. Shvadchak, L. J. Falomir-Lockhart, D. A. Yushchenko and T. M. Jovin, *J. Biol. Chem.*, 2011, **286**, 13023.
- 201 A. S. Klymchenko, G. Duportail, T. Ozturk, V. G. Pivovarenko, Y. Mely and A. P. Demchenko, *Chem. Biol.*, 2002, **9**, 1199.
- 202 A. P. Demchenko, Y. Mely, G. Duportail and A. S. Klymchenko, *Biophys. J.*, 2009, **96**, 3461.
- 203 A. S. Klymchenko, G. Duportail, Y. Mely and A. P. Demchenko, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 11219.

- 204 V. V. Shynkar, A. S. Klymchenko, G. Duportail, A. P. Demchenko and Y. Mely, *Biochim. Biophys. Acta, Biomembr.*, 2005, **1712**, 128.
- 205 A. S. Klymchenko, G. Duportail, A. P. Demchenko and Y. Mely, *Biophys. J.*, 2004, **86**, 31a.
- 206 W. Caarls, M. Soledad Celej, A. P. Demchenko and T. M. Jovin, *J. Fluoresc.*, 2010, **20**, 181.
- 207 S. Oncul, A. S. Klymchenko, O. A. Kucherak, A. P. Demchenko, S. Martin, M. Dontenwill, Y. Arntz, P. Didier, G. Duportail and Y. Mely, *Biochim. Biophys. Acta, Biomembr.*, 2010, **1798**, 1436.
- 208 V. V. Shynkar, A. S. Klymchenko, C. Kunzelmann, G. Duportail, C. D. Muller, A. P. Demchenko, J. M. Freyssinet and Y. Mely, *J. Am. Chem. Soc.*, 2007, **129**, 2187.
- 209 A. P. Demchenko, *Trends Biotechnol.*, 2005, **23**, 456.
- 210 A. P. Demchenko, *Lab Chip*, 2005, **5**, 1210.
- 211 A. D. Roshal, A. V. Grigorovich, A. O. Doroshenko, V. G. Pivovarenko and A. P. Demchenko, *J. Phys. Chem. A*, 1998, **102**, 5907.
- 212 A. D. Roshal, A. V. Grigorovich, A. O. Doroshenko, V. G. Pivovarenko and A. P. Demchenko, *J. Photochem. Photobiol. A*, 1999, **127**, 89.
- 213 A. Helala, M. H. Rashid, C.-H. Choi and H.-S. Kim, *Tetrahedron*, 2012, **68**, 647.
- 214 C. C. Yang, Y. Tian, C. Y. Chen, A. K. Y. Jen and W. C. Chen, *Macromol. Rapid Commun.*, 2007, **28**, 894.
- 215 Y. Q. Xu and Y. Pang, *Chem. Commun.*, 2010, **46**, 4070.
- 216 Y. K. Wu, X. J. Peng, J. L. Fan, S. Gao, M. Z. Tian, J. Z. Zhao and S. Sun, *J. Org. Chem.*, 2007, **72**, 62.
- 217 H. C. Chou, C. H. Hsu, Y. M. Cheng, C. C. Cheng, H. W. Liu, S. C. Pu and P. T. Chou, *J. Am. Chem. Soc.*, 2004, **126**, 1650.
- 218 M. Kijak, Y. Nosenko, A. Singh, R. P. Thummel and J. Waluk, *J. Am. Chem. Soc.*, 2007, **129**, 2738.
- 219 T. Y. Lin, K. C. Tang, S. H. Yang, J. Y. Shen, Y. M. Cheng, H. A. Pan, Y. Chi and P. T. Chou, *J. Phys. Chem. A*, 2012, **116**, 4438.
- 220 M. W. Chung, J. L. Liao, K. C. Tang, C. C. Hsieh, T. Y. Lin, C. Liu, G. H. Lee, Y. Chi and P. T. Chou, *Phys. Chem. Chem. Phys.*, 2012, **14**, 9006.
- 221 B. T. Kang, K. C. Ko, S. Y. Park, D. J. Jang and J. Y. Lee, *Phys. Chem. Chem. Phys.*, 2011, **13**, 6332.
- 222 A. Sirjoos Singh and S. Hammes-Schiffer, *J. Chem. Theory Comput.*, 2011, **7**, 2831.
- 223 A. Sirjoos Singh and S. Hammes-Schiffer, *J. Phys. Chem. A*, 2011, **115**, 2367.
- 224 C. Venkataraman, A. V. Soudackov and S. Hammes-Schiffer, *J. Chem. Phys.*, 2009, **131**, 154502.
- 225 J. Catalan, J. C. del Valle and M. Kasha, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 8338.
- 226 J. Catalan and M. Kasha, *J. Phys. Chem. A*, 2000, **104**, 10812.
- 227 J. Catalan, P. Perez, J. C. Del Valle, J. L. G. De Paz and M. Kasha, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 419.