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1,2,4,5-Tetrazines: An intriguing heterocycles family with outstanding characteristics in the field of luminescence and electrochemistry.

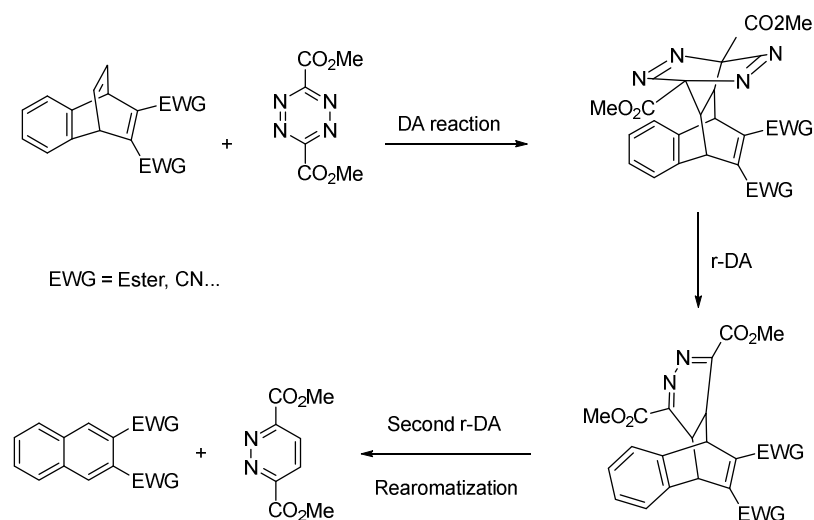
Fabien Miomandre¹ and Pierre Audebert^{*1,2}

1) PPSM – CNRS UMR 5831– ENS Paris-Saclay, 4 avenue des sciences 91190 Gif s. Yvette, France

2) Univ. Limoges, CNRS, XLIM, UMR 7252, F-87000 Limoges, France

Introduction.

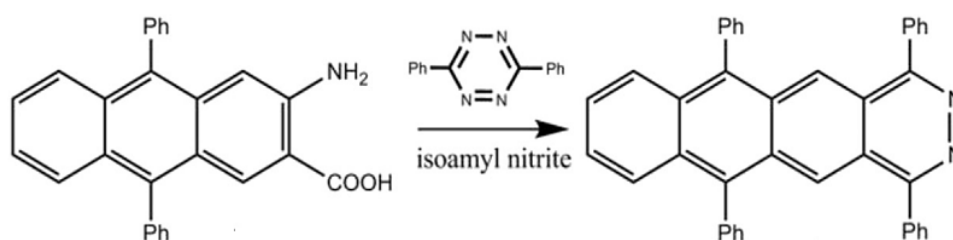
The 1,2,4,5-tetrazine, or s-tetrazine is a small, 6-membered aromatic heterocycle, the benzene-type (six-membered) heterocycle which contains the highest allowed proportion of nitrogen vs carbon in a single ring (indeed pentazine and hexazine are not stable). Despite they have been known for about 150 years, they suffered a relative lack of interest until the end of the last century, after which on the contrary, research works linked to this family of molecules finally skyrocketed during the last 20 years. Just to illustrate this, the total number of publications on tetrazines during the entire past century was 667, while it rose up to 492 during the first decade of our century only, to finally reach, during our (not completely achieved) present decade (11-20) already 1412 articles¹. While ancient reviews appeared in the past, recently the overall chemistry of tetrazines has been thoroughly reviewed by Saracoglu in 2007(1). This nice review, quoting 174 references, makes the point on more than 10 years of chemistry on tetrazines in general, reverently picking the most recent papers into a global amount of 1000 papers published since the first publication by Pinner in the 19th century(2). However, at the turn of this century, the situation totally changed, with the (almost simultaneous) occurrence of two new discoveries. The first was the (re)discovery of tetrazines optical properties (especially fluorescence and electrofluorochromism), initially driven by the work of our group, and that we already reviewed in 2010 (3). The second, and even larger, was the outstanding uprising of inverse Demand Diels-Alder (iDDA) reactions (or tetrazine click chemistry), in a special case, the 2-H-tetrazines with strained alkenes; the tetrazines owe this reactivity to their relatively low aromaticity energy ($E \approx 14-15$ kcal/mol (4), as compared to benzene, 33 kcal/mol). This reaction is an unusual, extremely fast, clean, and tolerant cycloaddition coupling. Much more than their synthetic usefulness, the reasons for this upraise was their widespread use in biochemistry and medicinal applications, and called in this context biorthogonal click chemistry (or “bioclick”).



¹ source Web Of Science

Scheme 1: Elaboration of 2,3-disubstituted naphthalenes through the intermediacy of one iDDA and two successive retro-DA reactions. (from ref. 4)

Although at the limit of the scope of this review, it is however worthwhile mentioning that modern iDDA chemistry of tetrazines is not limited to “bioclick”. A significant application of this chemistry is the synthesis of new 2,3-disubstituted naphthalenes and anthracenes that are difficult to obtain otherwise (Scheme 1). This has been notably reported by Saracoglu in his recent work(5). The synthesis of gem-diazatetracenes(6) and related compounds(7) is also a nice recent outcome of this reaction, these classes of compounds being very difficult to obtain otherwise (Scheme 2). This has been developed notably by Q. Zhang, who was able to generate these compounds through iDDA of the tetrazines with an in situ generated benzyne intermediate (Scheme 2)



Scheme 2: Synthesis of a diazatetracene derivative through cycloaddition on a benzyne intermediate (not represented, partly adapted from ref. 5)

However, beyond their use in sensing, and click chemistry, s-tetrazines have exceptional photophysical properties, not only by themselves, but also found in some of their derived products like some cycloadducts, which we intend to review in the present report.

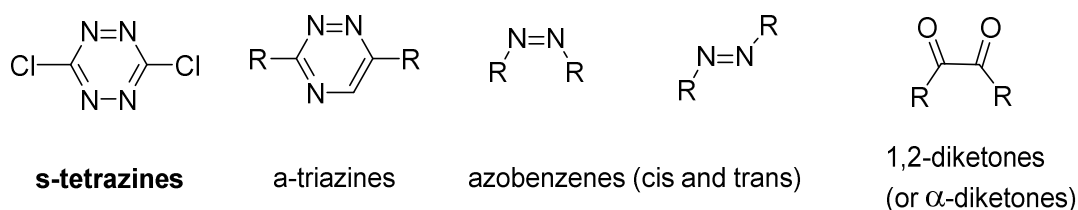
Actually, the tetrazines' photophysical properties can be classified in three main large categories.

- 1) A large subclass of tetrazines display intrinsic fluorescence, with average to high fluorescence yields (usually between 0.1 and 0.5 (8)(9)), which makes this family the smallest fluorophores in organic chemistry with unusual consequences and uncommon characteristics (e.g. very long luminescence lifetimes, usually in the 50 to 200 ns range).
- 2) These tetrazines all display reversible electrochemical reduction, which makes them choice compounds for the study of their electrofluorochromism (EF) and the development of electrofluorochromic devices.
- 3) Another subclass, the 3-H-tetrazines (or H-tetrazines), as aforementioned, easily undergo iDDA reactions, and in several cases, e.g. when the tetrazine is already conjugated, give birth to a class of conjugated pyridazines, another new family of fluorescent molecules.

Finally, these 3 fundamental characteristics of tetrazines, often associated to their very low molecular weight, have given birth to several unique applications in various areas, ranging from fingerprints revelations to low viscosity fluorescent liquids, owing to properties unreachable with more standard fluorophores. Meanwhile, the conversion of the second family of tetrazines into fluorescent pyridazines has led to a few recent applications in the domain of sensing and biosensing. Recording of these various applications will be the topic of the last 4th section of this review.

I – Tetrazines and tetrazines' derived materials optical properties.

As noticed from the very beginning, *s*-tetrazines are colored. This very unusual situation for a small ring is due to the existence of a slightly allowed $n\text{-}\pi^*$ transition, observed only in three other families of organic molecules, azobenzenes 1,2,4-triazines and 1,2-diketones (or α -diketones) (Scheme 3). At the exception of α -diketones (but their absorption coefficients are very low, around $15 \text{ L.Mol}^{-1}.\text{cm}^{-1}$), these molecules all have in common the presence of two neighboring nitrogen atoms, linked by a double bond. This specific situation allows some mixing of the n -orbitals of the nitrogen (resp. oxygen) atoms, causing a lowering of the $n\text{-}\pi^*$ separation, which reaches the visible range.



Scheme 3: The four only families of small molecules possessing a $n\text{-}\pi^$ transition in the visible domain.*

Actually, all the four families quoted above are fluorescent, but this fluorescence is not usable for the three families other than tetrazines. First, only locked azoic compounds are fluorescent(10, 11), all the other displaying photochromism instead; however, 2,3-diazabicyclo[2.2.2]oct-2-ene has a nice fluorescence with a very long lifetime up to the μs range (11), depending on the solvent, and the quenching of which was discussed(12). However, this compound has a low absorption coefficient ($\epsilon \approx 200$) and is too photounstable, which is a serious drawback to its use. While the fluorescence of α -triazines has only been noticed and never studied(13), it lies at the UV-visible limit of the spectrum, like α -diketones, whose (already mentioned) absorbance is in addition extremely weak (eg. diacetyl has the color of table oil, with $\epsilon \approx 15 \text{ L.M}^{-1}.\text{cm}^{-1}$; a value of $15.6 \text{ l.mol}^{-1}.\text{cm}^{-1}$ has been reported at 285 nm in cyclohexane (14))

Therefore, the tetrazines remain the only usable fluorophores to work on a $n\text{-}\pi^*$ transition. This gives them the unique features to be at the same time the smallest organic fluorophores (15), and to display the longest life-times (up to 180 ns) (16). Their photostability was never measured in details, but, at the exception of dialkyltetrazines, they display a fair photostability comparable to for example fluoresceine (17).

Indeed the photophysical properties of the generic H,H-*s*-tetrazine, and also dimethyl-*s*-tetrazine have been the subject of quite detailed studies, in specific conditions like gas phase spectroscopy (18), crystals (19), and spectrophysics (20, 21) of highly diluted compounds. Despite this former start, and while synthetic chemistry of tetrazines still continued to appear regularly, the interest of physical chemists in tetrazines dropped in the early 80's, at the exception of Neugebauer who prepared and studied the cation and anion radicals (22, 23) and published the photophysics (24) of various simple and more elaborated *s*-tetrazines (23). Actually the fluorescence of H,H-tetrazine and dimethyltetrazine was recognized long ago(25, 26), and some spectroscopic investigations followed up to the 90's for other tetrazines(27), even including the measurement of the triplet level

(28). However, these works were scattered, and strangely investigated the emission of weakly emissive tetrazines, either because of fast bleaching (case of H,H-tetrazine and dimethyltetrazine) or intrinsic low yields (aminotetrazines). Strangely enough the fluorescence of dichlorotetrazine had been noticed, but never studied, at the same period (24). It was only as late as in 2005 that within our group, we (serendipitously at the time) rediscovered tetrazines' fluorescence, along with the identification of the most fluorescent tetrazines, those bearing chlorine or alkoxy groups (15, 29-31) (Figure 1). We also demonstrated early on the possibility to produce active polymers including tetrazines in their chain (32), or substituted (33). All these findings up to 2009 have been summarized and detailed in our previous review (3). On the other hand, up to now, our group and others have continued to work on these exciting class of molecules, in several directions. Of course, because of the interest in many applications, fluorescence remained the central topic of most research works. However, new tetrazines were developed for engineering NLO properties, high oxidation potentials and/or new materials and devices.

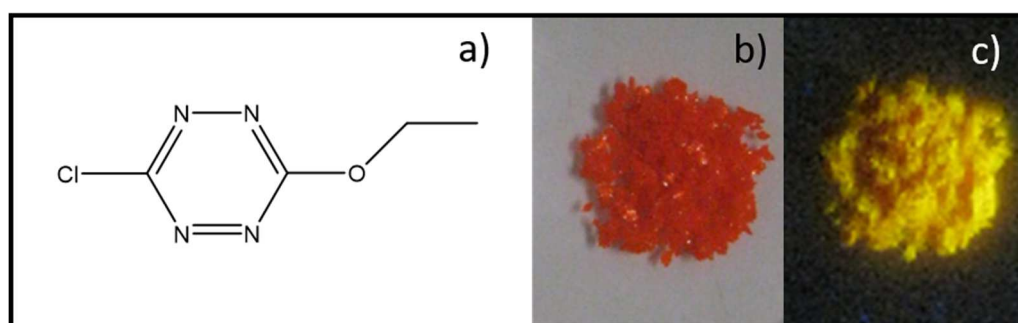
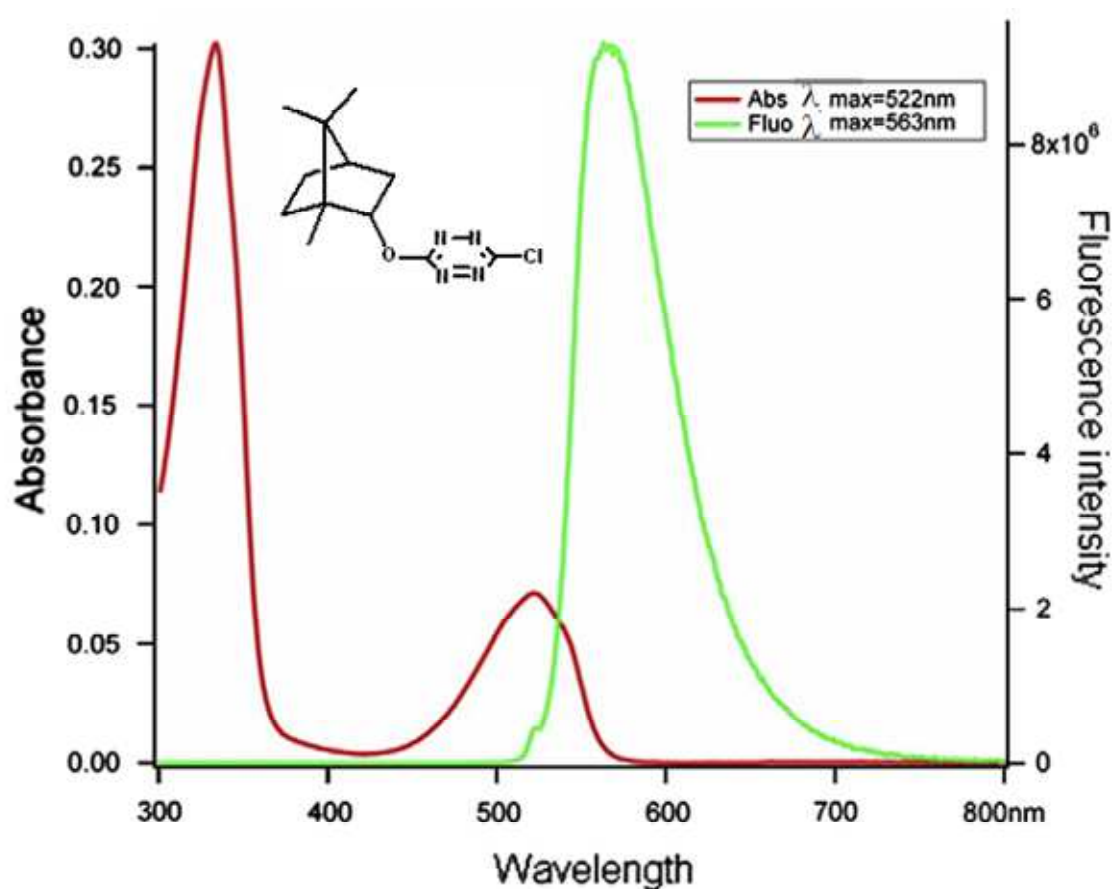


Fig. 1: Bottom: Formula of a famous luminescent tetrazine (used for Lumicyano®) typical of all chloroalkoxytetrazines, with pictures in white light and UV illumination. Top: The UV and fluorescence spectra of a typical chloroalkoxytetrazine (from ref. (34), Exc. $\lambda = 522 \text{ nm}$, solvent dichloromethane).

a) Theoretical insights on tetrazine orbitals and discussion on the fluorescence origin.

The orbitals of tetrazines are arranged as represented on Fig 2 below.

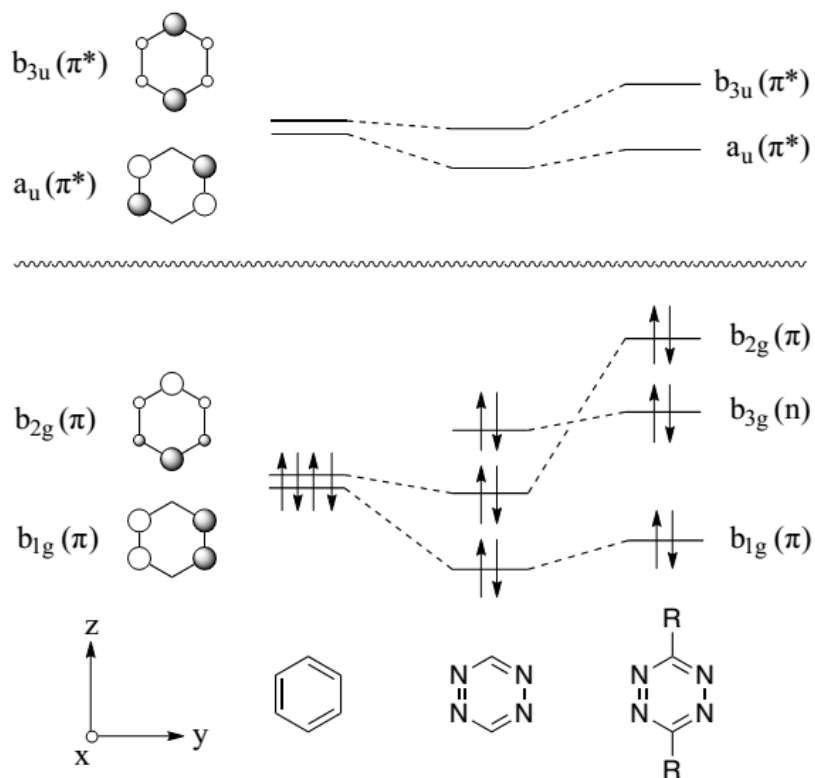


Fig. 2: Scheme of tetrazine orbitals, along with benzene for comparison. The n orbital is not represented but is located on the lone pairs of the nitrogen atoms, with mixing between the two neighboring nitrogen atoms. The drawing represents the orbital inversion which occurs when introducing donor substituents (reproduction from (35), itself adapted from J. Waluk(21)).

It is clear on the diagram, and from our former review, notwithstanding the precursor articles by F. Neugebauer and R. Gleiter, that the π orbitals of tetrazines are logically much more sensitive to substituents effects (especially π donors) than the n and the π^* (which interestingly vary only a little, and in opposite direction, which makes the emission color, when the tetrazine emits, almost constant). A more accurate investigation was published by us later on (16), showing that a very important parameter to be considered to observe high QY was the energy difference between the n and the π orbitals of the tetrazines. When the π orbital is the HOMO, no or very little fluorescence is observed (36). This is exemplified by our former calculations about 3-chloro-6-aziridinotetrazine, and 3-chloro-6-pyrrolidinotetrazine (Fig. 3) below. With the aziridine locked small ring, the nitrogen lone pair makes an angle which prohibits mixing with the tetrazine ring, while the freely moving pyrrolidine allows mixing. While the n and π^* orbital level (which are sensitive mainly to inductive effects) practically do not change, on the other hand the π orbital noticeably rises, becoming the HOMO, which in turn has the consequence to extinct the fluorescence.

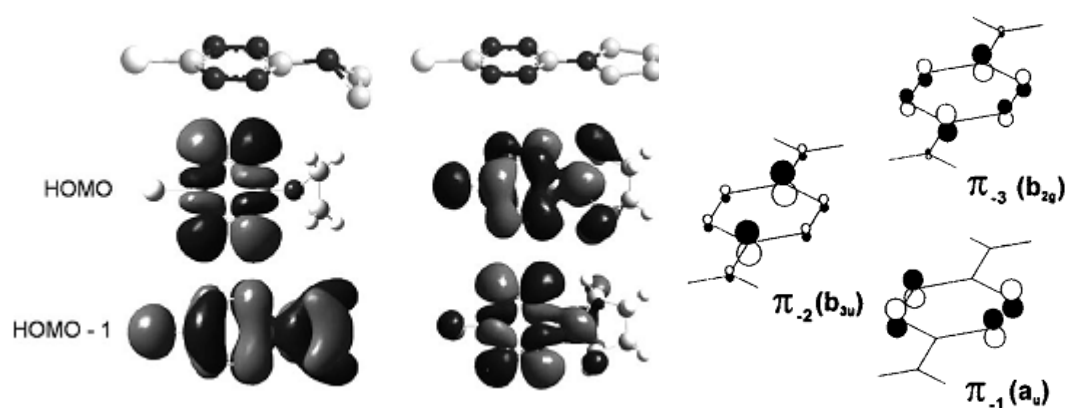


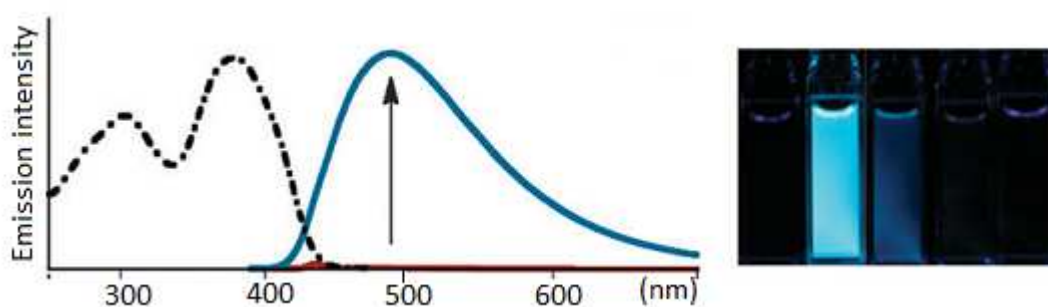
Fig. 3: Left: Structures, along with HOMO and HOMO-1 representations, for resp. 3-chloro-6-aziridinotetrazine (left), and 3-chloro-6-pyrrolidinotetrazine (right), showing the HOMO's orbitals inversion. The structures of the LUMOS's of these tetrazines were never fully calculated. In regard (right) are displayed the LUMO's of diaminotetrazine (adapted from ref. (21)), which do not differ much from those of 3-chloro-6-aziridinotetrazine and 3-chloro-6-pyrrolidinotetrazine (as already mentioned in the text, while occupied orbitals are very sensitive to substituent effect, vacant orbitals are weakly affected).

While our earlier works had demonstrated that utilizable fluorescence, with reasonable quantum yields (QY) could be obtained from chloroalkoxytetrazines, it remained like a “thumb rule” that electron-withdrawing enough substituents needed to be introduced on the ring to obtain

efficient emission. When the n orbital is the HOMO, noticeable fluorescence appears, to some extent the higher the gap, the higher the QY. However, other effects, possibly linked to the dipole moment, but also to the efficiency of non-radiative deactivation, themselves in turn linked to more hidden features, do interfere. For example, dichlorotetrazine, which has one of the largest n- π difference, has a modest QY of 11%, compared to the one of chloromethoxytetrazine, 33%. The reasons why some tetrazines displayed a high fluorescence yield are, surprisingly, still not completely elucidated. We prepared recently tetrazines from perfluorinated alcohols, a particularly strong electron-withdrawing substituent (37, 38). These tetrazines are fluorescent and exhibit occasionally high QY in the 40-44% range (38), but not all of them. Precise determination of the rate constants (radiative and non-radiative) have shown that for these tetrazines, the radiative deactivation was all the time of the same order, and that QY variations were mainly due to differences in non-radiative deactivation. The amount of fluorine atoms may play a protecting character, which lowers the non-radiative deactivation rate. Another more puzzling example of a high fluorescence yield is the case of butylbutoxytetrazine, which unexpectedly reached a 52% QY (39) (however this tetrazine, like many alkyltetrazines, has a quite moderate photostability).

Finally, it should be noted that very recently, A. Kedzia et al.(9) described new electron-deficient bisaryltetrazines, substituted by oxadiazoles or thiadiazoles, emitting in the violet to bluish-green region with very high QY's, sometimes up to almost unity. This very unusual behavior opens likely the track to new "super emitting" tetrazines with in addition increased emission frequencies.

On the other hand, and to conclude, *no one yet knows* why the n1- π -1* (this time calling precisely the orbitals with reference to Gleiter's work (24)) transition actually produces fluorescence, while the π - π * transition, when it has the lower energy (rarer cases, basically amino tetrazines), does not. I must however mention one single paper, by T. Kawai(40), which describes the *blue* fluorescence of a di(pyridylamino)tetrazine. However, this occurs *only* in water and *solely* in the presence of Zn²⁺ cations (Fig. 4). While the fact in itself is quite interesting, it is indeed quite puzzling! Not only the case is highly special, but the authors argue on an inversion between the π and the n orbitals, which not only their data do not confirm, but which also occurs usually in a few other tetrazines, without triggering on fluorescence. In addition, as previously stated, it is still unclear why the n- π * transition produces fluorescence, while the π - π * transition does (usually) not. Clearly more



delicate theoretical work is needed to raise the veil on this strange problem.

Fig. 4: Left: Absorption and fluorescence of di(pyridylamino)tetrazine in water, red curve, in the absence, and blue curve, in the presence of Zn²⁺ cations. Right, picture of the blue emission (second vial, the other vials contain the same tetrazine in other solvents, note the very faint emission in MeOH at the immediate right of the water solution): Adapted from ref. 29)

b) New “super emissive” fluorescent tetrazines.

Tetrazines are outstanding fluorophores, owing to their very long life-times, and small size, among other unique features. However, one drawback is their low absorption coefficients, compared to usual fluorophores like e.g. rhodamine (however, note that the absorption coefficients of tetrazines are far from ridiculous for a small ring; absorption coefficients lie in the $500\text{-}1000\text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ for the visible band, and $2000\text{-}3000\text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ for the UV band, to compare to the $200\text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ of benzene for the same atom number). Our group has engaged in a solution to overcome this situation, which is to link the emitting tetrazine to a suitable absorber, acting like an antenna. However, there are important restrictions to the choice of this antenna. Besides the obvious spectral matching, and a reasonably high absorption coefficient, an important restriction is that electron transfer should be blocked between the tetrazine and the antenna. It means that, nor the fundamental state, nor the excited state of the antenna should be an electron donor, in order to block electron transfer, both between the excited state of the antenna and the tetrazine, and the excited state of the tetrazine and the antenna. The choice has been made on an imide moiety, focusing more especially on naphthalimide ones, which gather all the desirable characteristics. Several functionalized tetrazines (Figure 2) have been prepared(41-43), and show more or less appreciable energy transfer. However, extensive studies have been performed essentially on a model molecule, composed of a chloroalkoxytetrazine, attached to a naphthalimide at the other end of the alkyl spacer, designed as NITZ (Figure 5), which definitely showed evidence for this occurrence (43).

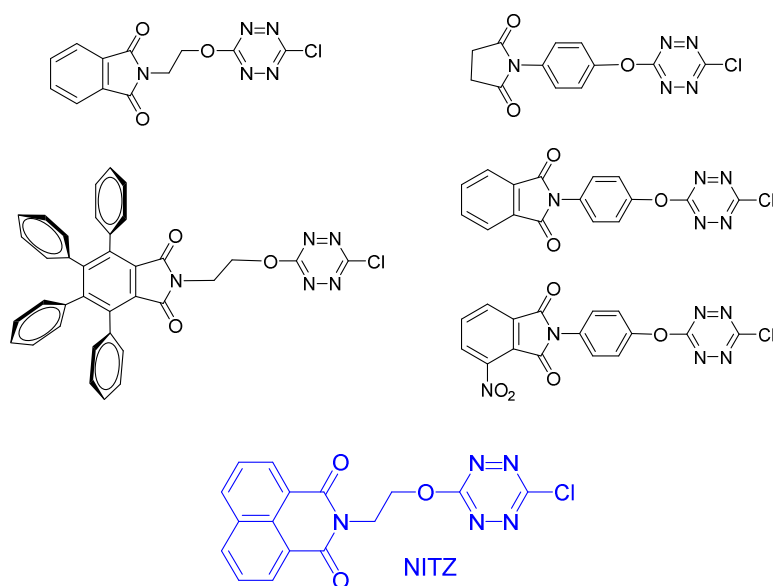
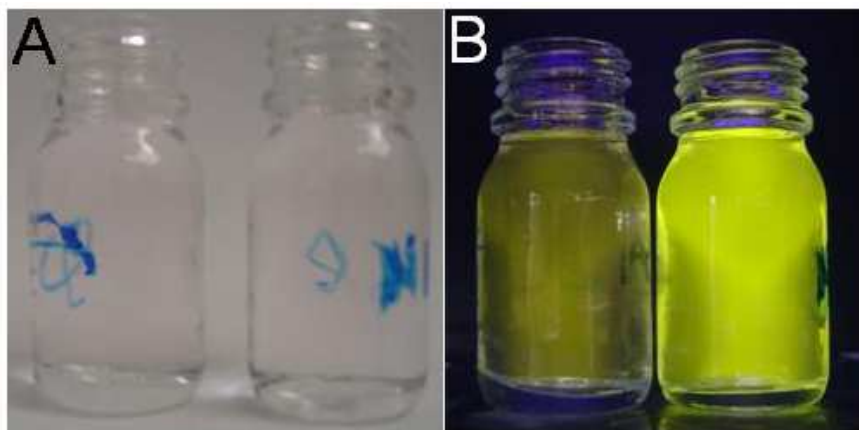


Fig. 5: Chart of some “antenna” activated tetrazines, showing especially the most efficient NITZ molecule, in blue at the bottom. (adapted from ref. (43))

The results show that a quasi-complete energy transfer occurs with this dyad, and that the brightness of the molecule (reminding: the brightness B of a molecule, is equal to the product of its absorption coefficient, ϵ , by its emission quantum yield Φ : $B = \epsilon \times \Phi$) was considerably increased. In our case, of course, the QY to be considered is the product of the tetrazine effective QY by the relative efficiency of the energy transfer, so $B = \epsilon \times \%_{\text{ET}} \times \Phi$. The tetrazine brilliance in the NITZ dyad was evaluated about 7.5 times higher when it is excited at 355 nm (selective of imide moiety) rather



than at 517 nm (selective of tetrazine moiety), as is evidenced in Figure 6, and the visual evaluation on the picture in the insert. The yield of the energy transfer, measured by two independent methods, was found to be approx. 90%. This very original result is solely due to the proximity of the two chromophores, since no change is observed in a fluorophore mixture.

Fig. 6: Picture of a $5 \cdot 10^{-6}$ solution of, left vial, chloromethoxytetrazine, and, right vial, NITZ. A) daylight illumination and B) Standard UV laboratory light (broad emission centered around 365 nm) adapted from ref. (43).

c) Photoredox catalysis and new high redox potential tetrazines.

As previously mentioned, tetrazines have relatively high redox reduction potentials, typically in the -0.5V-1.2V range, according to the substituents, to which they are very sensitive (in other words, the substituents have an important effect on the LUMO level). This characteristic makes tetrazines all the better oxidants in the excited state, compared to other organic photooxidants, since they all have more or less similar bandgaps. (The HOMO orbital, which has generally a n character, and is centered on the nitrogen lone pairs mixing, is indeed on the other hand weakly sensitive to substituents effects).

The use of tetrazines in organic photocatalysis was therefore a quite logical direction to undertake. However, it was only in 2013 that P. Biswas, for the first time, described the photochemically mediated conversion of 1,2-aryldiamines or 1,2-thioarylamines to resp. benzimidazoles or benzothiazoles (this multi-step condensation involves an oxidation step in the middle, mediated by dipyridyltetrazine) (44). The same group published two additional papers, on resp. the oxidation of thiols to disulfides(45), and alcohols to aldehydes(46), using the same tetrazine as the photocatalyst, as well as other papers where the tetrazine is supported onto various types of nanoparticles(47-49). However, in the case of NP's supported tetrazines, the mechanism is less straightforward, and reduction reactions can be observed, sometimes being the base for sensing (50) or biosensing (48). However, strangely enough, Biswas did not try to investigate changing the tetrazine to adjust the potential to the reaction envisaged. Actually, the pyridine substituents make the dipyridyltetrazine an obvious candidate for coordination on various supports, and its multigram scale synthesis is quite easy. However, its relatively low redox potential (around -0.9 V vs Ag/Ag⁺ (46)(51)) makes this tetrazine simply a honest candidate for photooxidation processes.

Recently, we were interested to investigate the efficiency of various tetrazines in our hands, with redox potentials in the excited state spanning a range between +1.31 V (bis-(1-adamantanymethoxy)-tetrazine) to +1.96 V (bis-(3,5-bistrifluoromethylphenoxy)-tetrazine). Those tetrazines (Fig. 7) were engaged in the photooxidation of thioaminals, followed by nucleophilic addition on the electrophile generated (likely the stabilized carbocation) by the photocatalytic reaction (52). The results show interestingly that the yields of the reaction are strongly influenced (among a couple of other parameters, like the fluorescence time) by the redox potential of the tetrazines, with yields ranging from 33% for the weakest oxidants, to 90% for the strongest ones. In a related approach, R. Tayebbe was able to show that supported tetrazines on magnesium oxide were able to photooxidize catalytically methylene blue and ciprofloxacin (53).

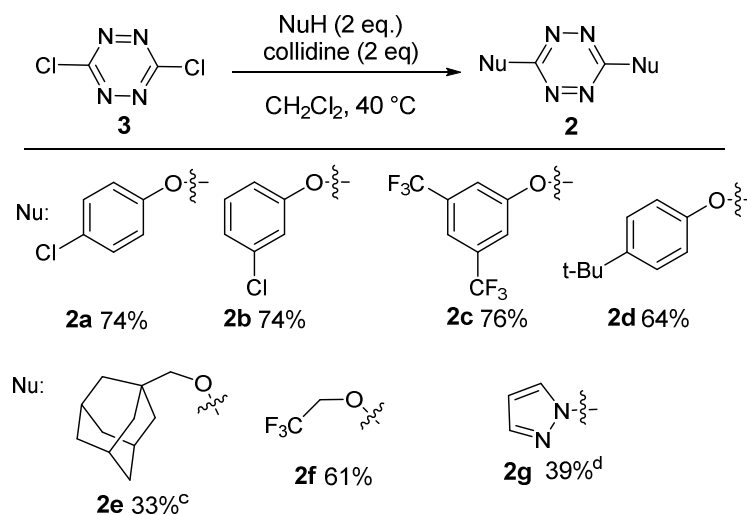


Fig. 7 : Chart of some catalytically active tetrazines (bottom) with their synthetic path (top); reproduced from ref. (52)

Sometimes the tetrazines are oxidizing enough, for the catalytic process to take place without light. An original example from G. Masson, in collaboration with us, is the oxidation of hydroxylamines into nitroso compounds, that can be further engaged into a Hetero-Diels cycloaddition (54). The first oxidation reaction is a slightly uphill reaction, but it is driven to completion by the consumption of the reactive N=O intermediate by the following cycloaddition.

Besides photocatalysis, other possible applications of tetrazines, like sensors, require the fabrication of tetrazines with the highest possible oxidation potential (or electron deficiency). While the correlation is far from straightforward (55), the iDDA reactivity is also linked to the electron deficiency (56). Therefore a few highly electron deficient tetrazines like bis-(trifluoromethyl)-tetrazine have been described in the literature (57), and tested in iDDA reactions a long time ago (58, 59); however, their redox potential was never measured, nor of course their photocatalytic properties. The synthesis of this compound is anyhow not straightforward, and other research directions may be envisaged. The choice of neutral electron-withdrawing substituents being limited (F, CF₃) and, as aforementioned, raising serious synthetic difficulties, we reasoned that cationic substituents could be a nice alternative, in this direction. We were able to prepare several tetrazines, substituted by onio-groups, mainly imidazolium and pyrazolium cations, by simple quaternization with methyl triflate of the easily accessible neutral precursors (Figure 8) (60). These very interesting molecules owned very high oxidation potential, and one of them, the 3,6-bis(2-methylimidazolium-1-yl)-s-tetrazine, with a calculated redox potential of 2.12 V (vs ferrocene) in the excited state, was found able to photooxidize even benzene, albeit sluggishly. However, one drawback was, for the

most electron deficient of them, their vulnerability to nucleophilic attack, and among them especially moisture, the electrophilicity unfortunately going along with the increase of the oxidant character.

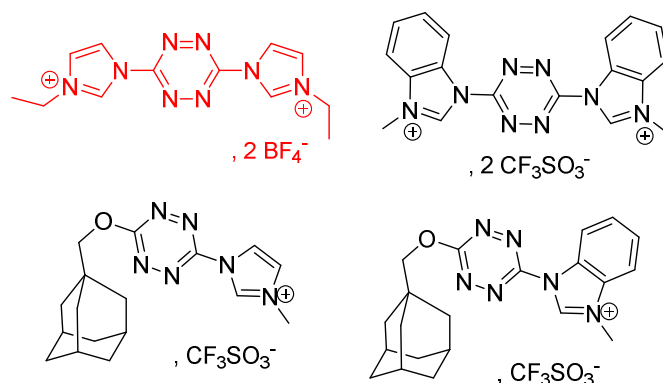


Fig. 8: Examples of cationic high potential tetrazines (adapted from ref. (60)); 3,6-bis(2-methylimidazolium-1-yl)-tetrazine (top left, red) has the highest potential (2.12 V) in the excited state.

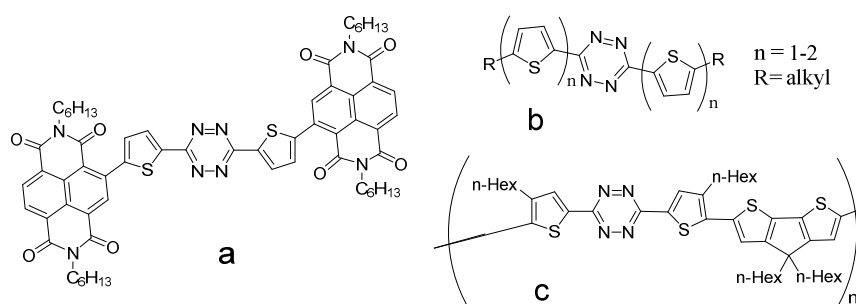
4) Materials featuring tetrazines, and their properties.

Motivations to include tetrazines in materials are numerous, many of them searching simply at disposing of a supported matrix, possessing the characteristics of the molecular tetrazines, aiming at the same applications; we will e. g. just recall the possibility of supported photocatalytic reactions by Tayebie (53) and Biswas (49, 50). However, in a few cases, the matrix plays a cooperative role, with the tetrazines, and leads to unprecedented properties. This is for example the case when the tetrazine is incorporated into a conducting polymer, or oligomer, or inserted in an inorganic matrix, like a metal oxide framework (MOF).

a) Conducting polymers and related oligomers.

Because of the electron withdrawing character of the tetrazines, but also of their fluorescence, it was tempting to introduce this moiety, in order to produce, either low band-gap oligomers and polymers, or electrofluorochromic organic materials, or nonlinear optical molecules. Besides D-A type polymers, the electrofluorochromism of tetrazines is so important, that a special section has been devoted to it in this review.

The first attempt to incorporate a tetrazine moiety into a conducting polymer, namely polythiophene, was from our group in 2004 (32); however, the polymer was not emitting, whatever its redox state, and did not show anything special outside the fact that the charge transfer followed a very different mechanism for holes (variable range hopping typical of a classical conducting polymer) than for electrons (slow localized hopping between tetrazines). The situation paced for a while, before J. Ding (61-63), A. Pron (64), Y. Tu (65) and N. Avarvari (66), published series of oligomers of classical heterocycles (thiophenes, EDOT... Fig. 9) and investigated their optical properties, and



efficiencies in organic photovoltaic (OPV) and/or electronic (OED) devices.

Fig. 9: Selected examples of oligomers and polymers (from: a, B. Kippelen (67) b A. Pron (68) and c J. Ding (61))

Their first results were promising, but one especially interesting result was obtained by B. Kippelen and S. Marder, in collaboration with us (67), who demonstrated that an OED transistor based on a tetrazine lying between two organizing naphthalimide groups, displayed a very good mobility for electrons of $0.1 \text{ V}^{-1}\text{cm}^2\text{s}^{-1}$. This mobility was intrinsically good, while not exceptional, but it was very stable, both in the absence or the presence of oxygen. This feature, linked to the fact that tetrazine anion-radicals are only sluggishly oxidized by oxygen, was unique of in the field of OED at the time.

Another desirable goal in the field is to switch the emission of the fluorophore included in the conducting polymer (See examples Fig. 10). Such a feature was actually never fully realized; however, we made two recent attempts in this direction. We successively prepared a poly(SNS) (33) and, in coll. with F. Serein-Spirau a poly(TBT) (69), both functionalized by a tetrazine pending group (SNS = thiophene-pyrrole-thiophene, TBT = thiophene-benzene-thiophene). Actually, the poly(SNS) polymer, while showing both full electroactivity of the chain and the pending tetrazines, was totally non-emissive, the excited state of the fluorophore being totally quenched by charge transfer to the chains, whatever their oxidation state (even when the chains were already oxidized, an overoxidation process could take place). On the other hand, due to its higher redox potential, the poly(TBT) displayed a weak and switchable luminescence in the oxidized state; however, the tetrazine luminescence was still quenched to a large extent, and the modulation by the redox state was therefore partial. On this same track, we also published recently the tetrazine fluorescence reversible quenching through the intermediacy of the well-known color (blue to red chains) transition in a grafted polydiacetylene (70) in collaboration with T. Barisien.; polyacetylene is actually not a redox polymer, but undergoes phase transition due to two possible chain conformations. In this case, the fluorophore quenching is due to energy exchange solely, instead of charge transfer.

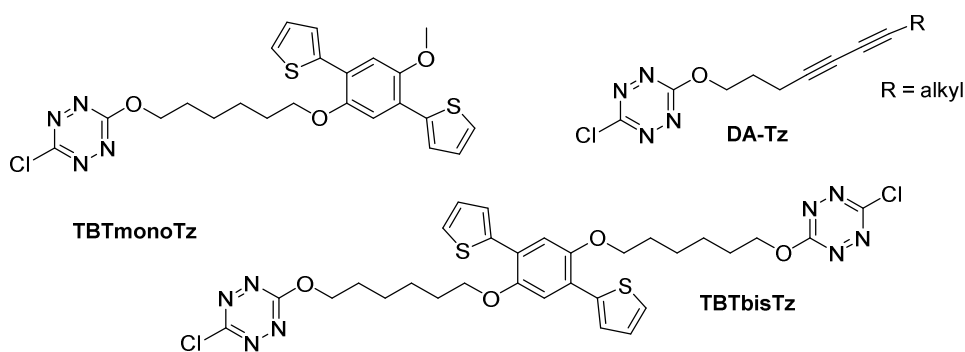


Fig. 10 : Examples of polymerizable monomers bearing tetrazines pending groups, from our works (Refs (69, 70))

Finally, quadrupolar D-A-D type molecules were developed by our group, and found to have interesting third order non-linear optical properties. With a ferrocene as a donor, frequency tripling could be observed to some extent, as found by I. Ledoux. (71). However, when the donor was an oligothiophene, two-photon absorption, up to almost 1000 GM were measured, in coll. with J. Perry's group, a relatively high value for a molecule of this family (72).

b) Inorganic materials

Because of the desirable properties of tetrazines, and the robustness of inorganic frameworks, several groups have tried and succeeded to produce composites from these two components. Actually, it is very easy to produce major symmetrical tetrazine containing ligands, containing, either two pyridines, or two carboxylate units, using the classical Pinner synthesis (according to the target, a couple of simple additional steps may sometimes be required). Therefore, tetrazine containing MOF-frameworks have been produced for the first time as early as in 2011 (73) by S. Feng, then in 2015 by C. Zhang and M. Humphrey, (74) from the well-known 3,6-bis(pyridin-4-yl)-tetrazine, followed shortly after by tetrazine-3,6-dicarboxylate, introduced via the dihydro derivative (75), further oxidized in the MOF. T. Devic and C. Serre published in coll. with us an example of Zr-based MOF, built with a bis(2-thioacetic acid)-tetrazine (76), displaying fluorescence, which could be quenched by the presence of electron-rich molecules like phenols with a good sensitivity. The most common metals used for these MOF's were copper, zinc and zirconium, because of their redox compatibility with the tetrazine.

We were able to graft tetrazines onto silica NP's, though a siloxane link, by post-functionalization (77). The amount of grafted tetrazine was intrinsically limited and the color of the particles was not discernable with naked eye. However, they did emit an intense yellow fluorescence. By using two types of functionalization, we were able to graft a naphthalimide in the core and bulk of nanoparticles, and tetrazines on the outskirts (78). The core imides were blue emitting, and the ones nearer from the surface were able to transfer their energy to the tetrazines on the surface. Therefore, the NP's did emit a mixture of blue and yellow light which, tuning cautiously the synthetic conditions (fluorophore ratio) could be almost pure white (Fig. 11). Since the particles were not colored, we had in our hands white, white emitting objects. We could also, in collaboration with E. Kim, decorate TiO₂ nanoparticles which displayed tunable fluorescence (79).

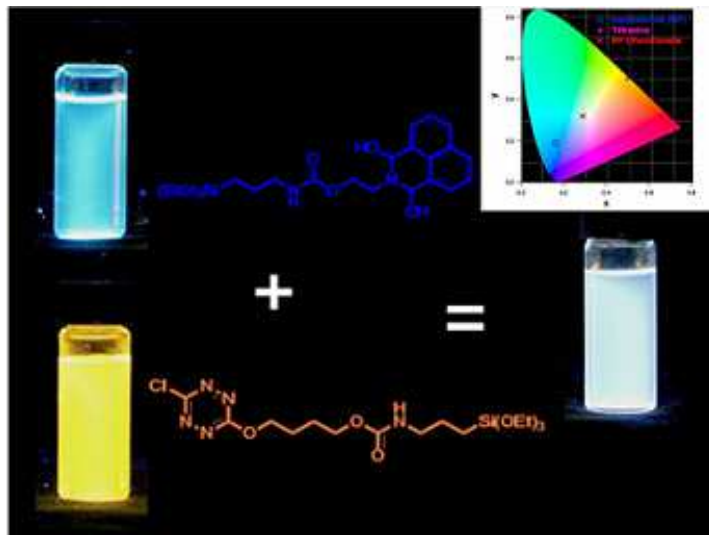


Fig. 11: Fluorescence color of grafted NP's; bottom left, with only tetrazine, top left, with only naphthalimide, and bottom right, with an adequate mixture of the two fluorophores, producing almost pure white. Insert top right, show of the color path available changing the fluorophore proportions, on the color diagram (from ref. (78)).

In collaboration with A. Walcarius, we were able to graft tetrazines on porous silica layers (80) organized on an electrode (Fig. 12). The tetrazines inside silica remained fluorescent, and could also be addressed electrochemically, although electron transfer through the layer was slow.

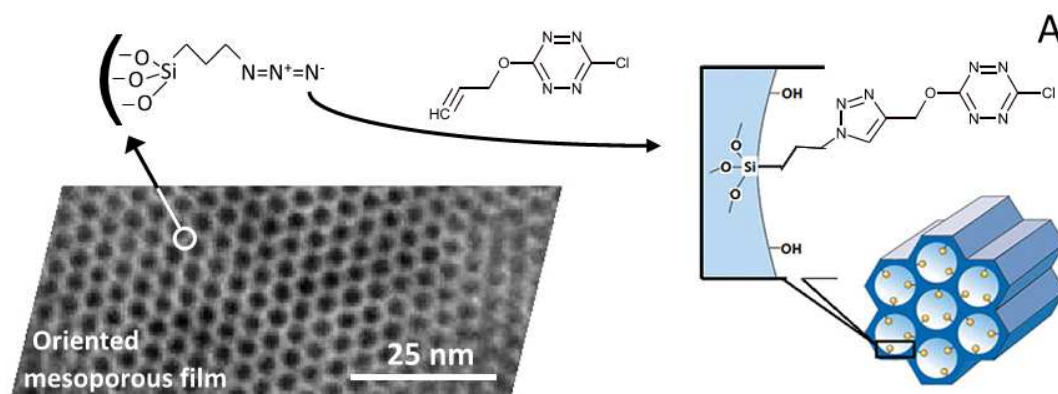
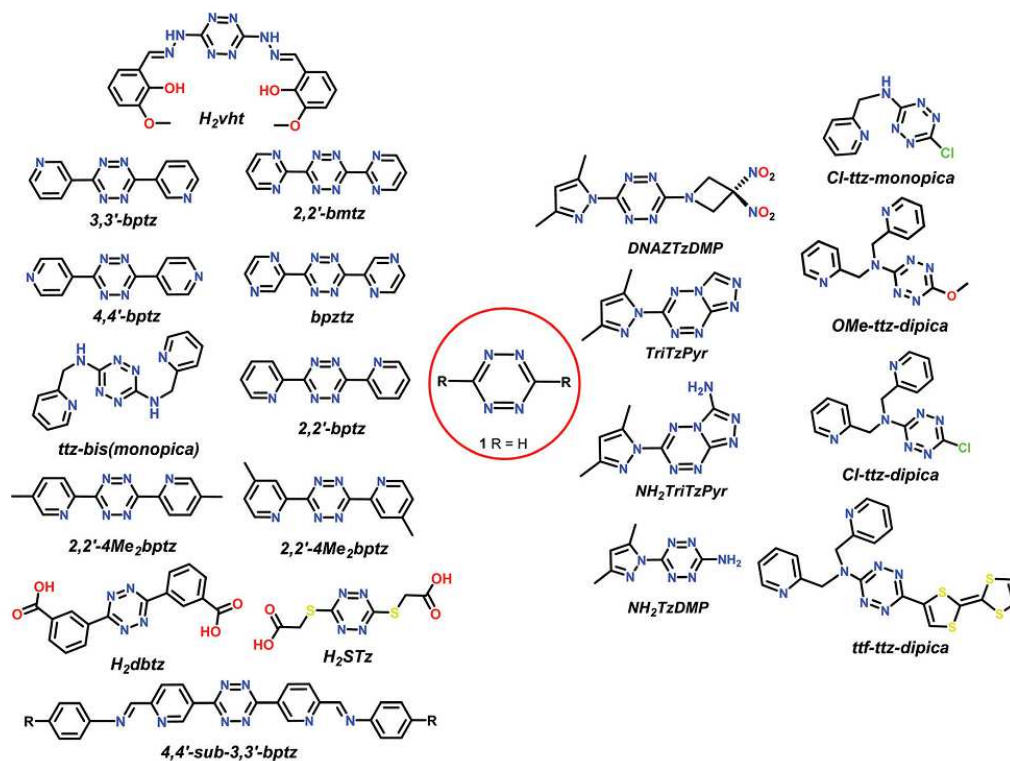


Fig. 12: Grafting of porous silica nanotubes with tetrazines by standard click chemistry (81).

Another key domain involving tetrazines is coordination chemistry, and especially co-ordination (co-coordinating sites, since the very low basicity of the tetrazines' nitrogens forbids their use as main ligands) N. Avarvari wrote a very recent review devoted to this special topic (82), which makes an extensive record on the building blocks used up to now, which we remind in the Scheme 4 below. However, entering in details this chemistry, not only would be out of the scope of this review, but has been in addition perfectly reported by the authors of this nice review (78).



Scheme 4: Extensive record of the tetrazine ligands reported to date, from ref. (82)

II. Electrofluorochromism of tetrazines

Electrofluorochromism (EF) is the property of compounds having multiple luminescence states depending of their various redox states and the possibility to switch reversibly between them(83, 84). This phenomenon is the pendant of electrochromism, known from more than 50 years, where the color change due to emission replaces the one due to absorption. The most common cases in that field are found in systems where an 'on' emissive state can be quenched in an 'off' non-emissive one upon oxidation or reduction. The reverse transformation from an 'off' state to an 'on' state is also possible, especially in dyads associating a redox unit and a fluorophore in close interaction, allowing either electron or energy transfer between them. Examples of molecular, macromolecular or nanoscopic systems have demonstrated their efficiency as electrofluorochromic (EFC) materials(85).

Tetrazine derivatives are electron-deficient compounds facilitating their reduction and the subsequent stabilization of the electrogenerated anion radical in aprotic media. When tetrazines are fluorescent, which requires a specific order between the n and π occupied orbitals(3), it is expected that a reversible switch is observed between the neutral emissive form of the molecule and the anion radical if this latter is non-emissive, which is the case of most open-shell compounds. This was demonstrated for the first time in 2006 by the joint work of our group and E. Kim(86), who built the first electrofluorochromic window based on a tetrazine active unit (figure 13). The effect was evidenced in a two-electrode cell where a bias voltage was able to tune the luminescence of the device. After this pioneering work, several examples of EFC devices can be found in the recent literature involving tetrazines either in solution (vide infra), in polymers(69, 87, 88) or on oxide nanoparticles(79). When combined with another fluorophore, tetrazines have been used to design multicolor EFC systems(89, 90). In one of these examples, naphthalimide-tetrazine dyads were synthesized with naphthalimide playing the role of an antenna in order to enhance the absorption properties and thus the brilliance of the device(89). Tetrazines were also incorporated in dyads with a redox-active unit, with the idea of controlling the emission by changing the redox state of the redox-active unit. Tetrazine-triphenylamine dyads were designed to check this concept and the influence of the linker(91).

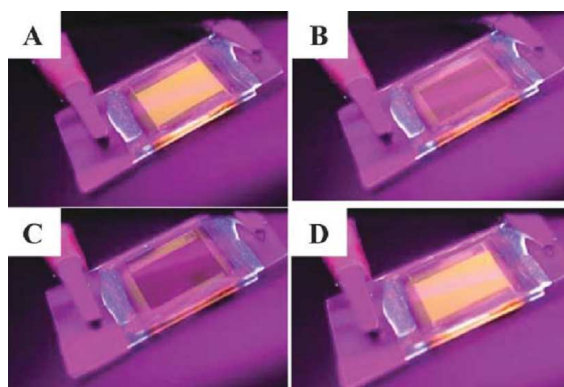


Fig. 13: The first electrofluorochromic window based on tetrazine. Images recorded at various bias: A) 0 V; B) -1.25 V; C) -3 V; D) +1 V (reproduced from ref. (86))

To go further in the investigation of the phenomenon, it was necessary to use a three electrode electrochemical cell where the potential of one electrode in particular (the working electrode) can be controlled independently of the reaction processing at the other electrode (the counter electrode). This potential is of course measured vs. a reference electrode, in presence of an

electrolyte solution containing also the redox active compound. The aim being to measure the fluorescence characteristics (emission intensity or wavelength) as the working electrode potential is changed (by sweeping, or applying steps), several experimental set-ups can be envisioned. Inspired by spectroelectrochemical studies based on absorption properties, one might use OTTE (Optically Transparent Thin Layer Electrodes) that can be inserted in the cuvette place of a spectrophotometer(92-95). By recording the emitted light either perpendicularly to the excitation direction or in front face when a significant Stokes shift exists, the emission intensity or emission spectra and current or charge can be simultaneously acquired under an electrochemical control. Despite of the relative simplicity of this experiment, several limitations may occur especially the rate at which the potential can be varied, because a complete conversion of the fluorophores present in solution is required to extract the 'off' state signal with a good sensitivity. Another issue to deal with is the reaction occurring at the counter electrode that may interfere electrochemically or optically. Therefore, we developed another set-up based on fluorescence microscopy where the substrate is both the working electrode and the place where excitation is focused and emission is collected(96). This allows extracting the signal coming from the electrode vicinity with a very good sensitivity, whatever the potential scan rate. Moreover, as the counter electrode can be put far away from the substrate, no interference occurs as it is no more encompassed in the region where the fluorescence is measured. We can mention that thin layer spectroelectrochemistry and fluorescence microscopy were also combined in the same experiment by our group to highlight the possibility of measuring all the fluorescence features (intensity, spectra, lifetimes) in a single experiment(97) (see figure 14).

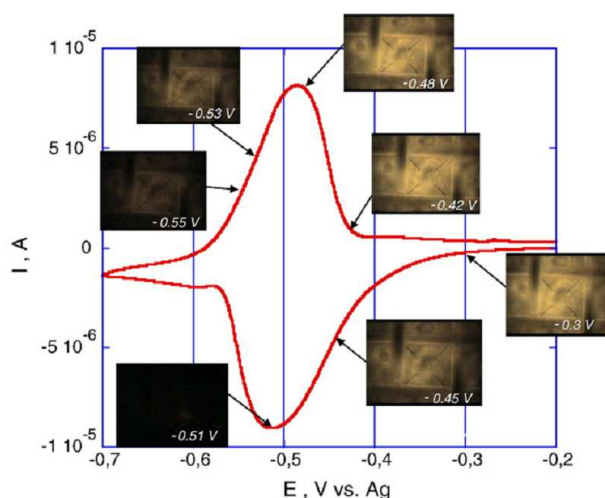


Fig. 14 : Fluorescence images of chloromethoxytetrazines at various electrode potentials recorded by coupling thin layer electrochemistry and fluorescence microscopy (reproduced from ref. (97)).

Coupling fluorescence microscopy and electrochemistry in a single experiment with a pulsed laser source allowed us to investigate the mechanism of electrofluorochromism and demonstrate for the first time the involvement of a quenching reaction due to an electron transfer between the excited fluorophore and the transiently electrogenerated anion radical(98). This results in an efficient and rapid extinction of emitted light as soon as the electrochemical reaction starts. Tetrazines were also used to investigate the behavior of fluorescent redox molecules in the vicinity of plasmonic electrodes made of gratings of gold nanoparticles of various sizes(99, 100). Our group demonstrated that upon plasmon excitation the emitted and reflected lights are tuned electrochemically in

opposite directions for tetrazines. This effect is not observed with EFC units switching in the reverse way (i.e. oxidative luminescence quenching like iridium complexes).

A recent development in the hyphenated fluorescence and electrochemical techniques consisted in combining epifluorescence microscopy with Scanning ElectroChemical Microscopy (SECM), a technique used to investigate the electrochemical reactivity of surfaces at the micro scale. SECM requires a redox probe in solution and a microelectrode tip scanning the surface of a substrate at a certain distance in the order of magnitude of its radius. Using an electrofluorochromic unit as the probe allows a simultaneous determination of the fluorescence intensity and electrochemical current in the region between the tip and the substrate, when varying the tip-substrate distance and the tip potential (and possibly the substrate potential when the substrate is conductive). Chloromethoxytetrazine in acetonitrile was used as an EFC probe to demonstrate, as a proof of concept, that these parameters can actually control the fluorescence intensity and that the amplitude of the fluorescence modulation can be used to construct optical approach curves, with a higher sensitivity than the electrochemical ones(101). Fluorescence images clearly show that the tip and substrate potentials can control the intensity of emission when the tip is positioned at a fixed distance from the substrate (see figure 15).

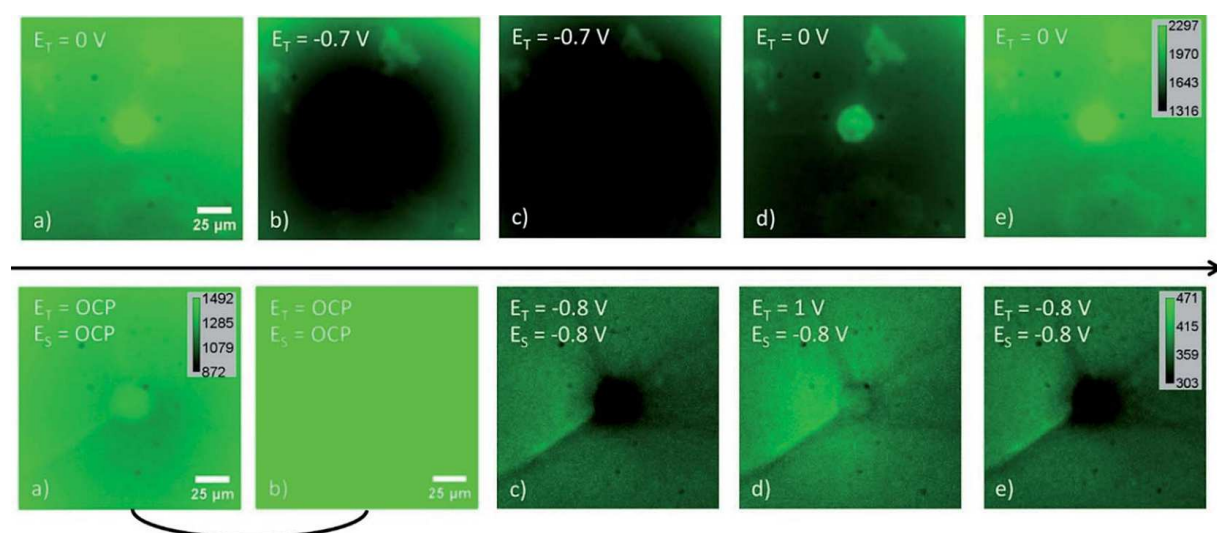


Fig. 15: Fluorescence images recorded in the region of interest around the tip (seen as a brilliant spot in a)) in the combined SECM-fluorescence microscopy experiment. The tip is polarized at various potentials (E_T values) and positioned close to an insulating (top) or conducting (bottom) substrate. In the latter case, the substrate can be polarized independently as well (E_S values). OCP: Open circuit potential. (Reproduced from ref. (101))

In summary, tetrazines displaying efficient emission in the visible range and redox properties at moderate potentials are very good candidates for electrofluorochromism. The phenomenon was investigated in various forms (solutions, polymer films, nanoparticles) and at various scales including the micron ones using fluorescence and electrochemical microscopies. It can be applied in emitting devices (electrofluorochromic windows) where it can be smartly coupled with electrochromic units to make low energy consuming displays or information coding systems.

III – iDDA reactions, to introduce fluorophore and/or turn on the fluorescence by converting a non-emitting tetrazine into another fluorophore.

As previously mentioned in the introduction, iDDA reactions have been along the last 150 years since the tetrazines discovery, a breadcrumb trail that was never left over by researchers in the field. However, since 20 years, the scope of this research has extended a lot from simple pyridazines synthesis. The first objective was the so-called bio-click chemistry, since between partners like H-tetrazines and reactive alkenes, the iDDA reaction is so complete and tolerant, that applications in biology have soared up, as a privileged way to link various biochemical objects together. This has been extensively reported in a few reviews (56, 102-106), and is beyond the scope of this report. However, in some occasions, optical properties, like fluorescence, can be brought on by the click reaction. This happens, either when one partner already is attached to a fluorophore, or, more interestingly, when the click reaction itself produces a new emitter. When a fluorophore is already attached, usually the click reaction, destroying the tetrazine, restores, or at least enhances, the emission of the already present fluorophore. This can be obtained, either by a slight change in the fluorophore core, or simply by stopping the energy transfer between the non-emitting tetrazine and the fluorophore.

1) Click reaction bringing on a fluorophore.

The first attempt to our knowledge, to attach an already present fluorophore, was proposed by N. Devaraj in 2013 (107). An H-tetrazine was attached by a spacer to a BODIPY type fluorophore. The H-tetrazines do not belong to the class of fluorescent tetrazines, but they are colored, nevertheless, and therefore can quench efficiently the BODIPY emission, as we also formerly demonstrated, by the way, in a closely related case (108). Therefore, the tetrazine-BODIPY dyad is not emissive. When the tetrazine is converted into a colorless dihydropyridazine (or a pyridazine in case of a follow-up oxidation process) by the click reaction, the fluorophore emission is restored. The same group published similar articles, based on the same demarche, changing the fluorophore for a rhodamine. Recently, they published a number of analogous molecules, including fluorescein, new BODIPYs and xanthenes (109) (selected examples in Figure 16).

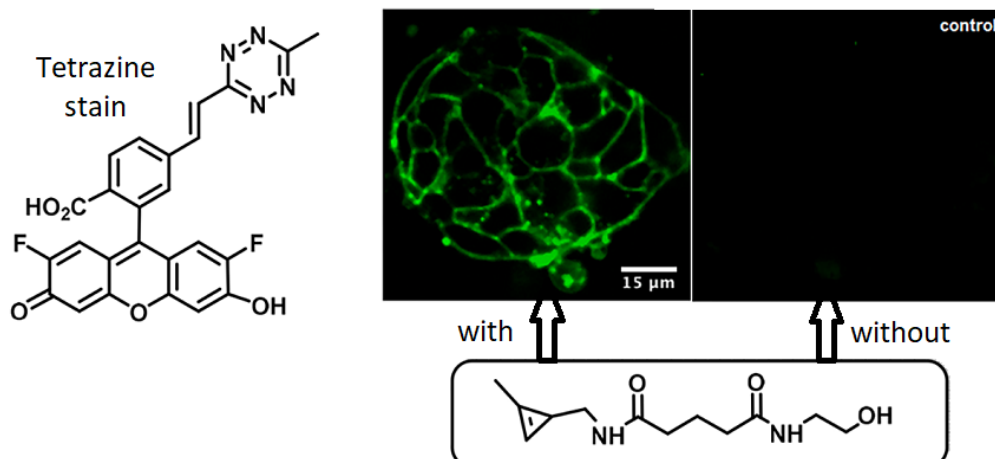
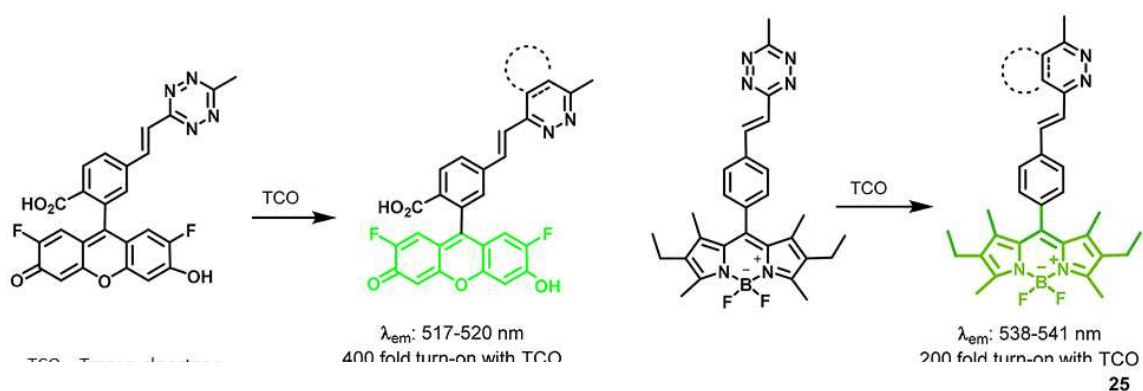
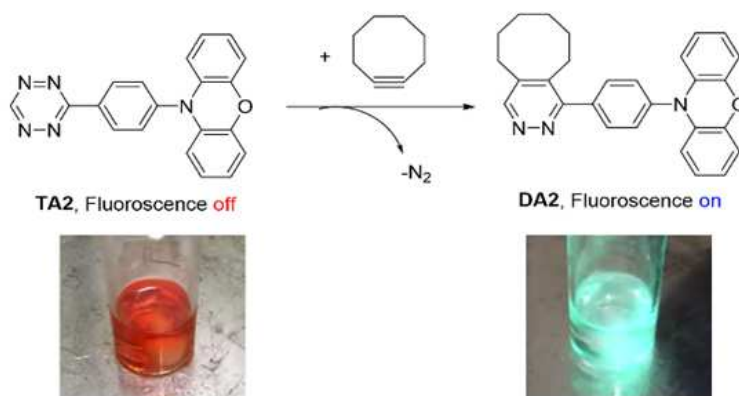


Fig. 16: Top: Example of tetrazines conjugated with standard fluorophores, not emissive with the tetrazine, which turn green emitters after TCO clicking. Bottom: Example of a cell stained by the tetrazine represented on the left; Right image, without addition of the cyclopropene (the tetrazine itself is not fluorescent); Left image, after cyclopropene addition (the pyridazine formation restores fluorescence). Adapted from ref. (103)

Besides the previous approach, a very interesting way to use the tetrazine is to build up the fluorophore, the first example by J. Guo dating from 2016 (110). This has been achieved by two approaches. First, by using the cycloaddition to promote the formation of a fluorescent conjugated dihydropyridazine or pyrazine (Guo's approach and M. Vrabel later on (111, 112)). Vrabel also found out a nice variant where a non-emissive fluorophore core is transformed into an emissive one, by converting an enol ether into a keto group (113) through the rearrangement following the iDDA process, and sequential breaking of the fluorophore-dihydropyridazine bond. In the same direction as Guo, but working on another different class of fluorophores, we were able to demonstrate the first example of preparation of a delayed fluorescence (TADF) fluorophore, by coupling of a H-tetrazine to a phenothiazine, followed by cycloaddition. Like in all previous cases, the initial tetrazine is non emissive, while the twisted pyridazine obtained (after *in situ* oxidation following the iDDA) exhibited a nice green fluorescence. At the difference from the first situation, the fluorophore itself, in our case



a TADF fluorophore, is intrinsically formed during the click reaction (Figure 17).

Fig. 17: Formation of a green TADF fluorophore through click chemistry (adapted from ref (114))

IV- Applications

As exposed in the introduction, besides fundamental studies, the unusual physico-chemical properties of tetrazines have raised the apparition of a few non-standard applications. Interestingly, the most striking applications came straight from the fact that the tetrazine core has an extremely low molecular weight for an organic fluorophore; actually, they can be considered as the lightest efficient organic fluorophores known to date (115). This feature has two remarkable consequences on their applications: they can be evaporated *in situ* for the revelation of latent fingerprints, and they can be used to prepare the less viscous (by far) intrinsically fluorescent liquids, with a unique application to friction measurements at solid-solid interfaces.

1) Fingerprint revelation

Since the mid-seventies, latent fingerprints in forensic investigations are revealed by a new method (since that time, and as compared to the powder methods used before) by evaporation in controlled conditions of temperature and moisture in appropriate chambers of cyanoacrylate (Superglue), which polymerizes at the fingerprint surface, making it visible. This system works well, but a drawback is that the glue forms a white crust at the surface of the fingerprint, which becomes visible only on dark substrates. A privileged way to surmount this is to impregnate with a fluorescent dye, but it raises other difficulties (blurring of the print, deterioration of DNA...) while consuming working time.

In our group, in collaboration with a French company (CST), we have discovered a tetrazine which can be mixed with a certain type of glue (cyanoacrylate plus additives), and be used in exactly the same conditions by the forensic services. We have developed a new commercial product, the Lumicyano™ (116) which permits, in one single step, the direct revelation of the fingerprints fluorescent, without any change in the standard process and chambers. In addition, the fingerprint details are better kept, and the DNA preserved (117). The synthesis of the key tetrazine is very simple, but a careful choice of its physical characteristics (especially the molecular weight and the melting point) had to be defined and realized. The high definition of the one-step developed fingerprint can be appreciated in Figure 18 below.



Fig. 18: Typical fingerprint developed with Lumicyano®. Left, under visible daylight; right, under UV (365 nm broad band) illumination.

2) Low viscosity fluorescent liquids and friction measurements.

Some intrinsically organic molecular fluorescent liquids have been developed these last 20 years. However, while these compounds are indeed liquids in terms of their physics (they flow at a measurable speed) the term “liquid” can also appear like an approximation, since the best performing compounds, while already representing a nice achievement, (118, 119) in terms of viscosity still flow slightly slower than standard honey. In the quasi totality of the cases described, the same approach is followed, with a central fluorescent classical core, decorated with carefully chosen alkyl chains, so that the final molecules do not crystallize, nor turn into a gel.

We reasoned that the same approach would be even more gratifying with a very small and weakly interacting core, like tetrazine, rather than standard fluorophore. Among the possible choices of alkyl chains, the 2-ethylhexyl chain (sometimes called “magic chain”) seems to be most performing, likely because it possesses an asymmetric carbon which prevents easy crystal formation. We easily prepared 3-chloro-6-((2-ethylhexyl)oxy)-tetrazine and bis-(2-ethylhexyl)oxy)-tetrazine, which were both fluorescent liquids (Figure 19). While fluorescent in pure state, they both displayed incredibly low viscosities of resp. 28 and 58 mPa.s (much less than olive oil, around 88 mPa.s)! (120)

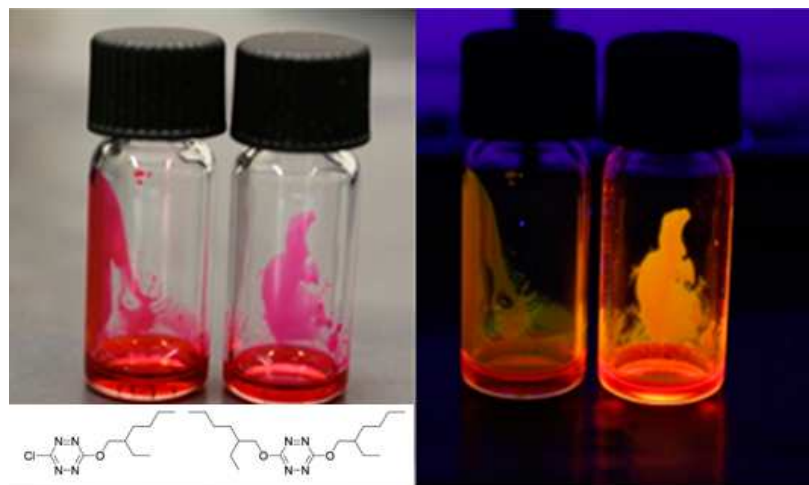


Fig. 19: Pictures showing the two liquid tetrazines, left under visible light, right under UV (365 nm broadband) light. Left, under the vials, are the formulae of the tetrazines (adapted from ref (120)).

A. M. Brouwers in Amsterdam was able to apply these new liquids to the investigations of lubrication between two surfaces separated only by the fluorescent liquid, acting as the lubricant. A first study was able to directly and quantitatively visualize the interfacial gap between two surfaces, using 3D mapping of fluorescence emission intensity(121) (this is possible, not only because of the low viscosity of the tetrazines, but also of its transparency to the emission, caused by its large Stokes shift). It reveals especially information about deformation of the surface roughness with the force applied, which is crucial in tribology (Fig. 20). Further investigations allowed to calculate precisely the volume of the interfacial volume, in function of the gliding speed in the case of a sphere-plane contact. These precise measures, obtained for the first time, were found to match exactly the theoretical predictions (122).

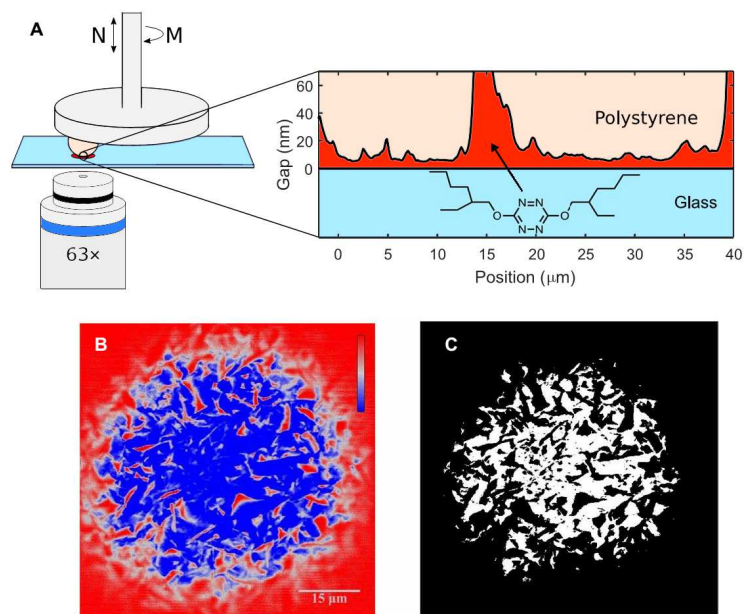


Fig. 20: Principle of the imaging of surface contacts using a fluorescent liquid. The fluorescent liquid actually acts as the lubricant and allows precise “in situ” imaging of the changing contact points. (adapted from ref. 119)

3) Sensors using tetrazines.

Y. Li et al have used a tetrazine containing cyclophane for sensing fluoride ions by colorimetry; the tetrazine helps as a π -binding site, in addition to classical H-bonds moieties already present in the cyclophane, thus increasing the selectivity (123). D. O’Shea very recently published a nice article, where an IR emitting aza-BODIPY, linked to a tetrazine, sees its fluorescence restored by clicking of the tetrazine, providing a sensor for iDDA reaction (124).

As previously mentioned, tetrazines can be incorporated into materials for sensing. An especially interesting feature is their incorporation in aqueous medium, which can be achieved through the intermediacy of cyclodextrin, introducing for example adamantanyl groups on the tetrazines. We were able to solubilize strongly emitting tetrazines in aqueous media in collaboration with S. Cosnier(41). Later on, we could realize modified electrodes usable in water incorporating the same tetrazines, continuing on the same track but attaching a pyrrole onto the cyclodextrin, that could be polymerized in a second step(125). R. Borsali was able to incorporate NITZ emitters into self-assembled polystyrene-cyclodextrin NP’s (Figure 21). The particles were quite monodisperse and brilliant (showing both fluorescence from the imide and the tetrazine, as a result of restricted motion of the encapsulated fluorophores), as well as electroactive, which is the basis of a dual fluorescence sensor (126).

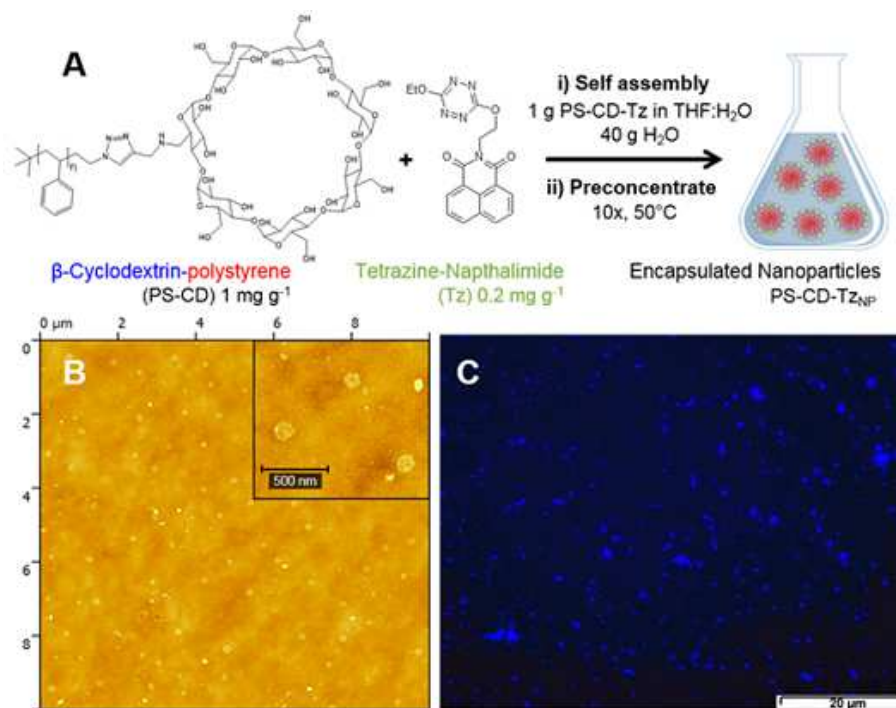


Fig. 21: Self-assembly of cyclodextrin-polymer NP's including the activated tetrazine NITZ. A) Synthetic scheme, B) AFM picture (1 Hz tapping mode), C) Fluorescence (DAPI filter). From ref. (126)

Another approach is grafting on “hard” substrates like oxides. We have been the first to prepare silica NP's functionalized by tetrazines, whose fluorescence was quenched by phenols or aliphatic amines (77). MOF-containing tetrazines, the fluorescence of which is progressively quenched by the substrate addition, which is usually an electron rich compound, or a nucleophile, can also be used as a sensor basis.

Tetrazine click chemistry can also be used for biosensing purposes. The group of R. Weissleder has used this reaction to bind magnetic fluorescent nanoparticles to antibodies(127). In another clever attempt, they used click chemistry to attach crystal violet moieties to magnetic nanoparticles (MNP's). Crystal violet is known long ago to selectively stain (+)-Gram bacteria (through the crystal violet binding interaction). After *in situ* clicking the MNP's to crystal violet, the (+)-Gram bacteria selectively incorporate the MNP's (128).

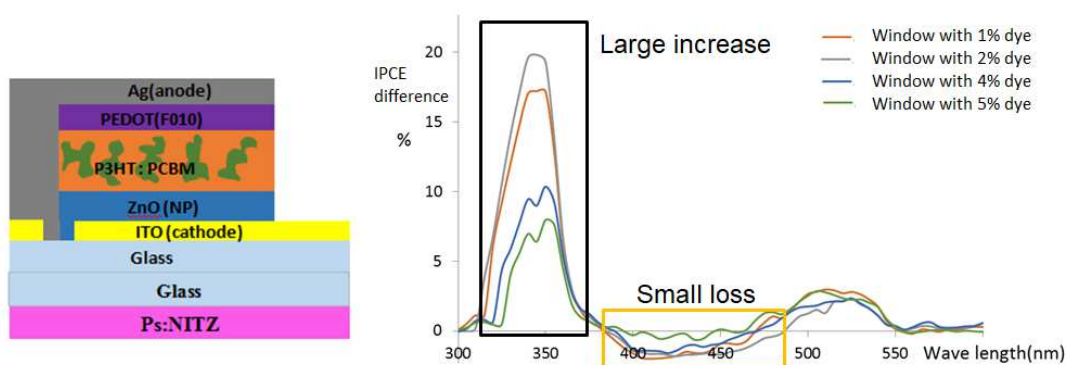
4) Miscellaneous applications

A few very recent applications of tetrazines are emerging in the domain of active applied materials. As mentioned in the introduction, tetrazines can exchange up to two electrons, on a small 6-membered ring. This is interesting for batteries and accumulators. In collaboration with us, S. Y. Park, was able to demonstrate the efficiency of tetrazines as active materials in batteries (129).

Modification of graphene and related materials with tetrazines is another important issue in materials chemistry. However, it was only in 2014 that Schirmacher published the first example of the modification of pyrolytic graphite through tetrazines cycloaddition (130). Quickly following, I. Aksay and us were able to incorporate tetrazines into graphene. The adducts could be obtained by nucleophilic substitution on hydroxylated graphene oxide (131), or by cycloaddition on reduced graphene (132). Their electrochemical behavior showed remarkable performances of these materials for energy storage (133).

A last application, still in the domain of active materials, is photon down conversion for organic photovoltaic devices (OPV). It is well known that, for reasons linked to the absorption spectra of components, standard OPV cells (including a classical polymer like P3HT or MEHPPV and fullerenes) do not use UV photons. An ancient idea is to put a layer in front of the cell, that not only protects it from UV degradation, but also is able to reemit light at a frequency usable by the device (134). Most of the photon-shifting layers published up to now used lanthanide emitters (135, 136), because all of them absorb UV light (through the ligand absorption) and emit visible light on the metal ion transitions, the color depending on the metal, with therefore huge Stokes shifts. However, while adapted to the task, lanthanide emitters suffer often from low to average quantum yield, and are expensive (long syntheses and/or expensive starting materials).

Because of their insufficient Stokes shift, purely organic dyes are, in most of the cases, not adapted to photon shifting; however, our super emissive “antenna” tetrazine NITZ described in part I-1-a absorbs near UV through its imide antenna, and emits yellowish orange light like all fluorescent tetrazines. Therefore, it represented a pleasant alternative to the classical lanthanide downshifters. We were able to fabricate a PMMA layer containing dissolved NITZ, which was able to convert the UV photons into yellow photons, and improve by about 5% the overall cell yield, making use of the otherwise lost UV light (137) (Figure 22). The same result could be confirmed in the case of perovskite cell (138), where the shifting layer was in addition able to extend the cell life-time because



of UV protection.

Fig. 22: Illustration of the increase of the efficiency (IPCE) of a standard OPV cell, with a downshifting window containing NITZ tetrazine in front of the window. Left cell scheme. Right relative variations of the IPCE of the cell with the wavelength. Adapted from ref. (137)

Conclusion

In our former review on tetrazines, in 2010 (3) we wrote: “(...) we have shown that tetrazines display a panel of rare and useful characteristics that make them usable for several types of applications in various domains, far beyond their traditional use in explosives and flame-retardant technology, (...) especially in the case where their unusual fluorescence is observed. We emphasize more particularly the starting field of research, which is the study of the interplay of redox chemistry and fluorescence.” Eleven years later on, this has become completely obvious, evidenced by the fast uprising of publications linked to the tetrazine’s and tetrazines derived materials optical and electrochemical properties, and their electrofluorochromism. A striking fact is the commercial

application (the Lumicyano[®]) based on their luminescence. Meanwhile, additional far-sighted fields have emerged during the past 10 years, and among the most promising in our eyes, photoredox catalysis and composites based on carbon-based materials. Photocatalysis with *high nitrogen content heterocycles* and derived materials, like tetrazines, and especially the emerging heptazines (139-141), are nowadays opening a wide new domain of chemistry, possibly solving in the end incredible industrial problems like photochemical water splitting.

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