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Glycoconjugated porphyrin dimers as robust ratiometric temperature sensors†

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We report the properties of glycoconjugated porphyrin dimers behaving as highly sensitive ratiometric temperature sensors in water. This effect results from interactions between carbohydrate and water altering molecular relaxation kinetics leading to temperature sensitive dual emission. These dimers are robust ratiometric fluorescent probes over a large temperature window (20–90 °C).

Over the last few years, the development of fluorescent sensors has been the subject of significant interest. Fluorescence allows easy, sensitive and non-invasive detection of a specific substance (metal ions, pollutants, and biomolecules)[1] as well as measurement of physical parameters like pH,[2] viscosity[3] or electric field.[4] A wide range of mechanisms have been discovered that allow the detection of specific stimuli both at molecular[5] and supramolecular levels.[6] Among the local quantities that can be monitored by fluorescence sensing is temperature. Some of the reactions taking place in living cells are exothermic, among which adenosine triphosphate (ATP) hydrolysis is the most frequent.[7] This reaction leads to a slightly higher temperature in mitochondria compared to the other organelles. In addition, cancer cells are known to have a significantly higher temperature than healthy ones.[8] These observations contribute to make temperature monitoring a strategic stake in biological media and biocompatible fluorescent probes could prove useful in several domains where classical methods cannot be used. The design and development of molecular thermometers at the nanometric scale have received increasing attention.[9] Several fluorescent nanomaterials, such as semiconductor quantum dots (QDs),[10] gold nanoclusters,[11] rare earth-doped nanoparticles,[12,13] polymer-based nanogels[14] and temperature dye indicators such as rhodamine B,[15] have already shown great potential for nanothermometry in biological systems. Thermally sensitive fluorescent indicators for molecular thermometry have been proposed to monitor temperature changes in tumor cells which have a perturbed metabolism implying lower or higher physiological temperatures than normal ones.[16]

For this purpose, some devices have been developed among which polymer based sensors have shown superior properties in terms of precision[17] or photobleaching.[18] However the transition involved in temperature sensing often takes place within a few degrees thereby limiting their performance.[19–22] In this particular aspect, molecular approaches seem to be more promising with ''slower'' transitions (spin transition, ''Twisted Intramolecular Charge Transfer'' or intramolecular rotation) allowing accuracy over a larger temperature range.[23,24]

Conjugated porphyrin dimers were first reported by Anderson *et al.* because of their superior two-photon absorbing properties and later allowed strategic breakthroughs in the development of two-photon activated Photodynamic Therapy (2PA-PDT), a promising technique in oncology.[25–29] These authors showed that the fluorescence emission and singlet oxygen production were both controlled by the intramolecular rotation of the dimers[30,31] and that both could be modulated by the viscosity of the media, opening the way to the imaging of micro-viscosity in cells.[32]

The rotation of the dimers along the butadiynyl axis leads to a dual fluorescence of the plane conformation (λ > 700 nm) and of the twisted one (around λ = 650 nm) and the ratio between the two intensities indicates the local viscosity. Such probes, called ratiometric, avoid the dependence of the signal on the local concentration and are therefore of great interest.

Inspired by Anderson's approach of 2PA-PDT sensitizers, we developed a family of glycoconjugated porphyrin dimers (Fig. 1) optimized for the targeted 2PA-PDT treatment of retinoblastoma

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† Electronic supplementary information (ESI) available: All methods and products used for the synthesis of compound **1**. UV/Vis spectra were recorded using an Agilent Cary 300 spectrophotometer. Fluorescence spectra were recorded by using a Cary Eclipse spectrophotometer. Both apparatus were equipped with a Cary temperature controller. For every measurement, temperature was checked using a thermocouple. All curves were smoothed following the Savitzky–Golay method (5 points). See DOI: 10.1039/c4cc03367a

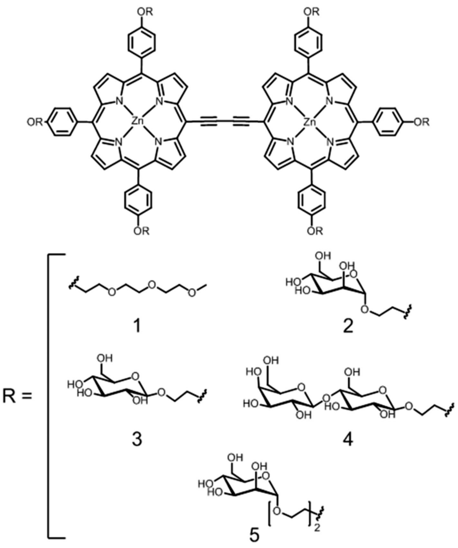

Fig. 1 Structures of compounds **1–5**.

through specific interactions between carbohydrates and specific lectins overexpressed at retinoblastoma cell membranes.[33–35] The glycosyl moieties also provide the aqueous solubility of the otherwise highly aromatic and hydrophobic porphyrin dimers. Despite a high two-photon cross section and high singlet oxygen production in organic solvents, the dimers showed no phototoxicity toward cancer cells during *in vitro* assays due to poor cell internalization.[35]

Water is known for being a highly structured solvent because of the existence of numerous intermolecular H bonds.[36] Carbohydrates, thanks to their multiple hydroxyl functions, are excellent H bond donors and acceptors, which allow them to insert into the network created by water molecules resulting in their excellent solubility. It has recently been shown that carbohydrate molecules can influence neighbouring water molecules up to twice the thickness of the solvatation layer.[37,38] The dissolution of dimers bearing six carbohydrate moieties will presumably lead to a strong organization of water at their proximity. The results described previously prompted us to investigate and use these interactions.

This publication presents the influence of solvent and temperature on the emission properties of the glycosylated dimers **2–5**, previously designed as photosensitizers for one-photon and two-photon excited photodynamic therapy,[35] compared with the properties of the non-reported non-glycosylated analog **1** bearing six {2-[2-(2-methoxyethoxy)ethoxy]ethoxy}phenyl moieties as *meso*-substituents (synthesis described in the ESI†). We now propose these molecules as robust, ratiometric probes for temperature sensing along with an interpretation of their peculiar properties.

While all dimers showed similar absorption properties in water (see ESI,† S8), glycosyl groups had a strong impact on fluorescence emission. At 20 °C, compound **1** (red) shows a single emission band at λ = 730 nm whereas the glycosylated compounds **2–5** exhibit a dual emission peak at 630 and 740–800 nm (Fig. 2), which will be referred to as blue and red transitions respectively.

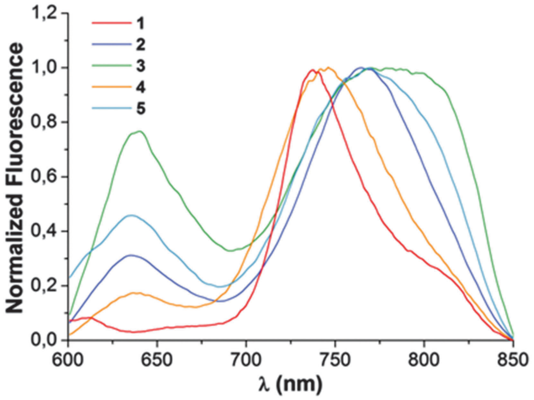

Fig. 2 Emission spectra of compounds **1–5** in water at 20 °C (1 μM, λ_{exc} = 450 nm).

Dual emission was also observed in other polar solvents such as MeOH, EtOH or DMF (see ESI,† S9) though to a lesser extent for compounds **2–5** whilst emission of compound **1** displays a single band in all tested solvents. According to Anderson's interpretation, the glycosyl moieties alter the relaxation kinetics of the dimers, allowing emission from the twisted excited state in all the tested polar solvents.

If viscosity was the driving parameter, an increase in temperature should ease the transition rate and lead to the decrease in the intensity of the blue band. This is the case in polar organic solvents (see ESI,† S9). However, the fluorescence emission of dimer **2** in water between 20 and 90 °C (shown in Fig. 3) follows the opposite trend: high temperature favors emission from the twisted state.

Under the same conditions, the absorption underwent weak modifications in the Soret area. This suggests that the observed phenomenon is mainly due to the evolution of the populations in the excited state rather than in the ground state. Following previous work on dimer analogs by Winters *et al.*,[31] an interpretation of this phenomenon is presented in Fig. 4. Excitation at 450 nm leads to the promotion in the S_2 states of both

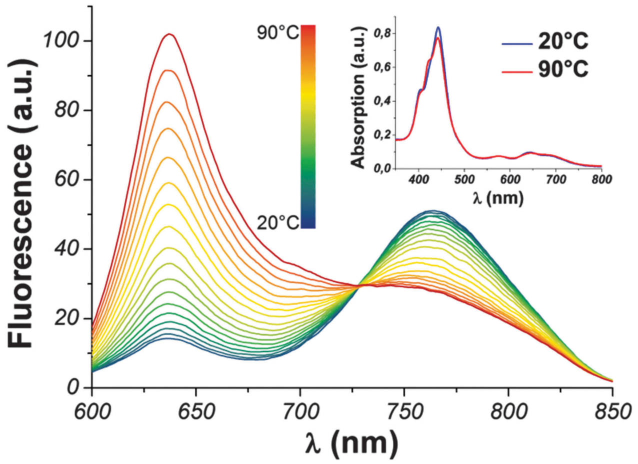

Fig. 3 Fluorescence emission of dimer **2** in water between 20 and 90 °C (1 μM, λ_{exc} = 450 nm). Inset: absorption spectra at 20 and 90 °C.

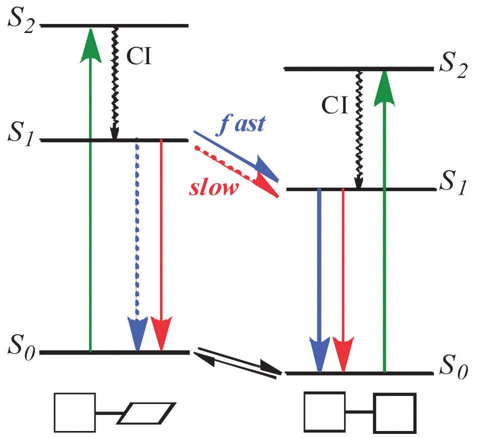

Fig. 4 Relaxation kinetics of dimer **2** in water at low (blue) and high (red) temperatures.

twisted and planar conformations. Fast relaxation of the twisted conformation toward the planar form at 20 °C leads mainly to emission in the red band. At 90 °C, the rotation is hindered and both emissions are observed.

Similar behaviors were observed for dimers **3** to **5** but not for the non-glycosylated analog **1** which quickly degraded upon heating thereby confirming the role of the carbohydrate moieties that also provide a shielding effect.

Although the exact origin of both effects has yet to be determined, we believe that H bonds between hydroxyl groups and water are at the heart of the phenomenon. Temperature modulates the number and strength of these bonds thereby altering the relaxation kinetics. They might also form a bundle around the fluorophore slowing or preventing the diffusion of reactive species, such as oxygen, at its proximity. This explanation is consistent with the absence of photosensitization by the dimers in water, even at high temperatures. To sustain this hypothesis, the behavior of the compounds is easily modified by the addition of biological components in the solution (see ESI†). Further studies are underway to determine the exact nature of this phenomenon.

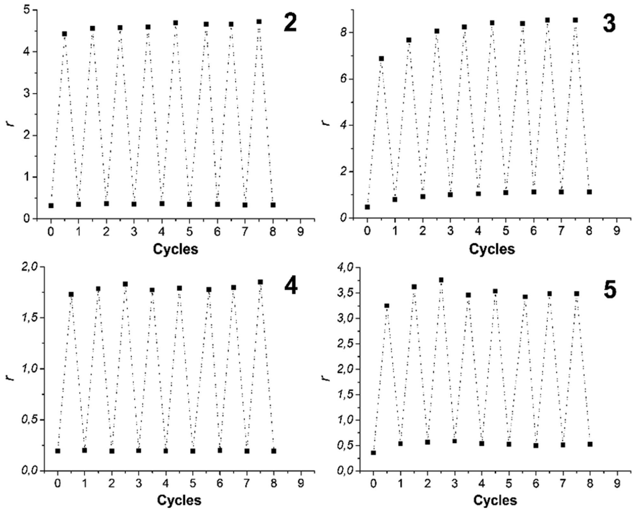

Fig. 5 Stability of the probes during heating–cooling cycles in water. Evolution of the *r* parameter *versus* cycles.

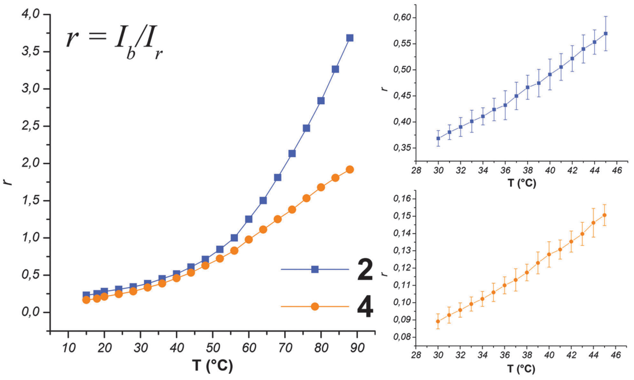

Fig. 6 Temperature characteristics between 20 and 90 °C (left) and accuracy between 30 and 45 °C (right) of **2** and **4**.

The thermal stability and accuracy of the dimers were investigated to determine the potential of these molecules as temperature probes. They were first subjected to heating–cooling cycles between 20 and 90 °C (Fig. 5). The parameter $r = I_b/I_r$, where I_b = intensity of the blue band, I_r = intensity of the red band, measured as a function of temperature was introduced. It is weak at low temperatures and increases with temperature.

The best reproducibility was observed for compounds **2** and **4** for which a stable regime was observed after the first cycle. Compound **3** also reached a similar state but needed 2 to 3 additional cycles. Dimer **5** exhibits more or less the same response after 4 cycles but its emission spectra showed important drifting which could indicate significant degradation over time. This result is consistent with the behavior of dimer **1** and shows that longer polyethyleneglycol (PEG) linkers weaken the carbohydrate shield.

r values for compounds **2** and **4** were plotted between 20 and 90 °C (Fig. 6, left). The slow evolution allows temperature sensing over the whole temperature window. The dimers also allowed precise measurement of the temperature between 30 and 45 °C (Fig. 6, right) with accuracies of ±2 °C for compound **2** and ±1 °C for **4**, making these compounds promising candidates for biological applications after encapsulation in nano-containers.

We report the influence of temperature on the spectroscopic properties of a series of glycoconjugated porphyrin dimers and hexa-PEG. These compounds, previously designed as photosensitizers for one-photon and two-photon excited photodynamic therapy,[35] exhibit unexpected dual fluorescence in polar solvents in general whose relative intensity depends on the temperature of the solvent. Water leads to a peculiar behavior where the band of highest energy is exalted whilst previous results would predict the opposite. This was attributed to the specific nature of water as an organized solvent due to the high number of H-bonds in which carbohydrates insert remarkably well.[39] These bonds modify the intramolecular rotation of the dimer leading to the dual emission and also provide an efficient shield against high temperatures.

Finally, the observed transitions are progressive over a large temperature window (20–90 °C), which could allow applications beyond biology, in media where classical temperature sensing methods cannot be applied. The high two-photon cross section of these compounds could also open the way towards 3D monitoring of the temperature.

Investigations are currently carried out in our group to encapsulate the probes within nano-capsules in order to provide them with a ''free'' environment and avoid interaction with other molecules that could distort temperature measurements in biological media.

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