Structural enhancement of two-photon sensitizers for photodynamic therapy

Cyrille Monnereau and Chantal Andraud

A systematic engineering method improves the spectroscopic and structural properties of molecules that can be triggered by two-photon excitation to induce cancer cell death.

Photodynamic therapy (PDT) has been gaining importance over the past 20 years as an often beneficial alternative to surgery and conventional chemotherapy for treating various skin and epithelial cancers. It involves using a chemical dye (the photosensitizer) that when activated by light undergoes a transition between molecular electronic states called intersystem crossing (ISC) to form an excited triplet state. In this long-lived high-energy state, the photosensitizer transfers energy to surrounding cellular oxygen, converting it into excited singlet state oxygen, which is a highly reactive and thus extremely cytotoxic species: see Figure 1(a). When administered selectively to tumoral tissue, PDT often leads to complete regression of the tumor, with relatively low adverse effects. The benefits of PDT compared to other cancer therapies include an almost painless treatment, no need for sedation, much less severe side effects, better and faster recovery, and a very satisfactory long-term appearance of the scar tissue.¹

However, clinical application of PDT is still in its infancy, and only a handful of photosensitizers have thus far been approved for use in cancer treatment: see Figure 1(b).² Consequently, the use of PDT in clinical practice remains limited, and wet age-related macular degeneration is the only condition for which it has become a first-line therapy. A major obstacle to wider use of PDT is that the currently approved photosensitizers all have significant shortcomings for this application. First, their activation wavelengths are generally in the 600–690 nm range, which falls outside—or at best barely intersects—the biological transparency window. This makes them difficult to use in deep tissue, allowing only superficial tumors to be treated to good effect. Second, although all photosensitizers have fairly good selectivity to tumors, their accumulation is not perfectly exclusive. This means patients undergoing PDT also experience a general photosensitizing effect that requires them to take various precautions against exposure to sunlight. Accordingly, most

Figure 1. (a) One-photon (blue arrows) or two-photon (red arrows) absorption activates the photosensitizer from its ground state (S₀) to an excited singlet state (S₁), and thence via intersystem crossing to a long-lived excited triplet state (T₁). Energy released from the photosensitizer in the triplet state excites cellular oxygen from the ground state (³Σg) to a highly reactive and cytotoxic singlet state (¹Σg), in some cases via a shorter-lived higher-energy singlet state (¹Σg). (b) Typical clinically approved photosensitizers for (one-photon) photodynamic therapy. Me: Methyl group.

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current research efforts on optimizing photosensitizers focus on tackling those two limitations. One promising approach for achieving deep-tissue and selective activation of a photosensitizer is by two-photon absorption (TPA). Typically, two-photon absorbing chromophores feature absorption in the 750–1000nm range, which corresponds perfectly with the maximum transparency of organic tissue. Also, whereas linear absorption occurs uniformly along the optical path of the laser beam, the nonlinear character of TPA makes it possible to activate the photosensitizer exclusively at the focal point, to the benefit of treatment selectivity. An impressive proof of concept for in vivo two-photon PDT was reported in 2008 by H. L. Anderson and B. C. Wilson, with a porphyrin-based photosensitizer.\(^3\) Using two-photon irradiation, they were able to selectively close off a blood vessel located at the focal point of the laser.

Figure 2. (a) Structure of our first series of chromophores, with \(\alpha\) and \(\beta\) positions shown in red on 00000, and the dibromo-phenyl substituent shown in blue. Br: Bromine. Hex: Hexyl group.

(b) Variations of some photophysical parameters within the series: fluorescence quantum yield (\(\phi_f\)), singlet oxygen generation quantum yield (\(\phi_A\)), and fluorescence lifetime (\(\tau_f\)).

To further investigate the potential of TPA, our group conducted an experiment to better understand what parameters influence photosensitization efficiency in two-photon absorbing chromophores.\(^4,\)\(^5\) We were particularly interested in determining how singlet oxygen generation efficiency could be related to the substitution pattern of an ISC-promoting group (a heavy atom such as bromine) on the chromophore. To study this, we designed a series of related chromophores sharing a common conjugated backbone, on which we systematically varied the number and positioning of a dibromophenyl group: see Figure 2(a). We then analyzed the variations of the main photophysical parameters within this series, and determined that singlet oxygen generation quantum yield (\(\phi_A\)) could be straightforwardly related to ISC efficiency.

Somewhat unexpectedly, we found that ISC efficiency seemed to depend only on the position of the ISC promoters along the chromophore backbone, with substitution in \(\alpha\)—chromophores 02020 and 02000 in Figure 2(b)—more efficient than in the \(\beta\) position (00200), irrespective of their total number. It was also striking that substituting the chromophore in both its \(\alpha\) and \(\beta\)
position (02220) was not only ineffective but turned out to impair the molecule’s ISC efficiency, suggesting a complicated interplay between the substitution parameters.

We also showed that, as well as strongly influencing singlet oxygen generation efficiency, the bromine substitution pattern can be used to tune the spectroscopic properties of the chromophore. First, increasing the number of bromine groups, which are electron-deficient, causes a bathochromic shift in the one-photon absorption and emission spectra, placing the latter in the biological transparency window. This is important for practical applications since it eases in vivo fluorescence detection of the photosensitizer in the tumor tissue prior to its photoactivation. Second, the bromine groups also produce marked changes in the chromophores’ electron symmetry—possibly due to a distortion of its conjugated backbone—and thus in the selection rules of two-photon absorption. We consequently observe a strong red-shift and broadening of the TPA band to cover the whole biological transparency window, with an average cross-section above 300GM (Goeppert-Mayer units), which thus facilitates activation in deep tissue.

In summary, our work has shown that subtle variations in the bromine substitution pattern of two-photon absorbing chromophores can be used to consistently improve their potential for two-photon PDT applications, obtaining a photosensitizer that can be selectively activated at greater tissue depths. We are currently seeking to extend this molecular engineering work by varying additional parameters, such as conjugation length and molecule symmetry, to better understand the relation between two-photon-induced electronic transitions and the ISC process. In parallel, we are also developing various macromolecular platforms as vectors for delivering such chromophores into target cells, with promising preliminary results (see Figure 3).6–8

This work has been supported by a grant from the NanoPDT program of the French National Research Agency (ANR).

Author Information

Cyrille Monnereau and Chantal Andraud
Laboratory of Chemistry
École Normale Supérieure Lyon (ENSL) - University of Lyon 1
Lyon, France

Cyrille Monnereau is an assistant professor in the Chemistry for Optics group at ENSL. His current research interests are the molecular engineering of fluorophores for biophotonics, the study of their excited state photophysics, and the development of polymeric platforms as vectors for their delivery to cells.

Chantal Andraud is a grade 1 senior researcher at the French National Center for Scientific Research (CNRS), head of the Chemistry Laboratory at ENSL, and leader of the Chemistry for Optics group. She is the author of 120 publications, six chapters, nine patents, and 65 invited presentations, and has chaired or co-chaired eight international conferences in the fields of molecular engineering and spectroscopy in optics and nonlinear optics.

References

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