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Mini Review

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Photochemical Characterization of Mechanisms Underlying Multiphoton-Induced Reactions in Optically-Dense Media, Including Important New Insights for Nonlinear Photodynamic Therapy

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ABSTRACT

In this mini-review, we summarize a novel method recently published by Masthay for characterizing the rate constants 'k' and photonicities 'n' of multiphoton-induced reactions in systems of high optical density. In their method, the conventional incident, initial n^{th}-order intensity $I^n_{0,0}$ is replaced by a longitudinally and temporally averaged n^{th} order intensity $\overline{I^n_{x,t}} = \frac{1-10^{-n\bar{A}}}{2.303n\bar{A}} I^n_{0,0}$ which accounts for the exponential decrease in intensity across the optical pathlength due to the average absorbance $\bar{A}$ over an irradiation interval of duration t. These authors demonstrate that the resulting longitudinally and temporally averaged rate constants $k_{x,t}$ and photonicities $n_{x,t}$ are more reliable than the incident, initial rate constants $k_{0,0}$ and photonicities $n_{0,0}$ for the 532 nm, two-photon induced photodegradation of ß-carotene in CCl_4 solvent. They also demonstrate that ß-carotene has an exceptionally large two-photon absorption cross section (δ_{532}~10^7 GM) because it undergoes sequential a two-photon absorption process mediated by an eigenstate intermediate

such processes typically have larger cross sections than conventional instantaneous two-photon processes, which are mediated by virtual state intermediates. Based on this research, we present below a previously unreported strategy for the use of sequentially absorbing two-photon photodynamic therapy agents which could prove uniquely efficient at treating solid tumors at deep tissue depths.

Keywords: ß–carotene; Beer's Law absorbance; Multiphoton; Optical penetration depth; Two-photon absorption; Photodynamic therapy; Photonicity; Rate constant

INTRODUCTION

Recently, Masthay and coworkers published an article [1], in which they detail the impact of optical density on the characterization of the rate constants 'k' and photonicities 'n' in n-photon processes, with an emphasis on the significance of their photochemical model for multiphoton–induced synthesis [2,3] and multiphoton-induced Photodynamic Theory (PDT) applications. In this brief mini-review we summarize many of their findings and detail the relationship of their model to recent work by other researchers, including one especially important potential new PDT application which has not yet been discussed in the literature [4-13].

LITERATURE REVIEW

Mechanistic characterization of nonlinear optical processes using longitudinally and temporally averaged intensities

Midre in optically dense samples irradiated with laser beams, the beam intensity declines exponentially across the pathlength, according to

$$I_{x,t} = I_{0,0}\,10^{-A} = I_{0,0}\,10^{-\varepsilon c_t x}. \qquad (1a)$$

In which $I_{x,t}$ and $I_{0,0}$ are positionally and temporally specific and incident, initial intensities, respectively, in units of photons cm^{-2} sec^{-1}, $A=\varepsilon cx$ is the one-photon Beer's Law absorbance, ε is the molar extinction coefficient in M^{-1} cm^{-1} at actinic wavelength λ, c_t is the average concentration in moles $liter^{-1}$ across the optical pathlength after t seconds of irradiation and x is the optical penetration depth in cm.

$$I^n_{x,t} = I^n_{0,0}\,10^{-nA} = I^n_{0,0}\,10^{-n\varepsilon c_t x} \qquad (1b)$$

The n^{th} order intensity declines even more rapidly than the first order beam intensity given in Eq. (1a) with increasing x (Figure 1) and rises or falls with time depending on the impact of the photochemistry on c_t.

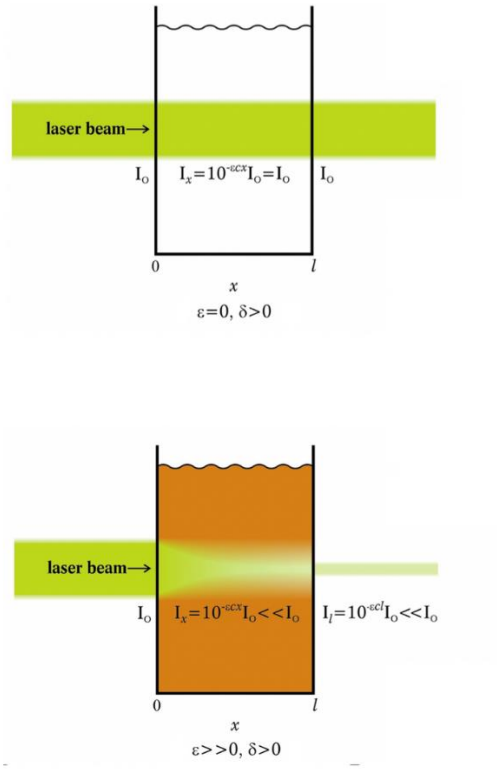

Figure 1: Impact of optical density A=εcx on beam intensity I_x in (top) optically transparent (A=0) and (bottom) strongly one photon absorbing (AS>> 0) samples.

Since the probability of *n*-photon absorption by a single molecule is proportional to $I_{x.t}^{n}$, multiphoton processes occur most efficiently at low optical penetration depths; multiphoton processes thus occur more rapidly near the front (*x* small) than near the rear (*x* large) of an optical pathway. The *x*- and *t*-dependencies in $I_{x,t}^{n}$ thus make the characterization of '*n*' and '*k*' in optically dense media challenging. Accordingly, the rate laws and mechanisms of multiphoton processes are typically characterized using optically diffuse (*i.e.*, small *A*) solutions, which are weakly one-photon absorbing (have small ε) and/or dilute (have small c_t), as such samples have nearly equal intensity across the entire pathlength. Even so, in certain important applications-most notably multiphoton–induced synthesis [2,3] and multiphoton induced Photodynamic Theory (PDT) [4-13] it is advantageous to use concentrated, high absorbance samples, as such samples increase the product yield (in synthetic applications) and photodynamic dose and efficacy (in PDT applications). It is thus essential to understand the impact of *A* on measurements of *k* and *n* in high *A* samples when mechanistically characterizing optically nonlinear processes, which obey rate laws of the form for pathlengths of length [l].

$$R = -\frac{dc}{dt} = -\frac{dA}{\varepsilon l\, dt} = kA\mathrm{I}^n \qquad (2)$$

In this regard, Masthay, *et al.*, irradiated optically dense (A_{532}=0.2 and 1.0), orange solutions of β-carotene in carbon tetrachloride solvent with the 532 nm second harmonic of a pulsed Nd:YAG laser [1]. These samples were optically dense because ε_{532}=3,630 ± 100 M^{-1} cm^{-1} for the $1^1A_g^- \xrightarrow{h\nu_{532}} 1^1B_u^{+*}$ one-photon transition of β-carotene from its ground electronic state $1^1A_g^-$ to its long–lived (τ=10^{-13} seconds), lowest–lying one–photon allowed excited state $1^1B_u^{+*}$ in CCl_4 [1]. These solutions had extremely large two–photon cross sections (δ_{532}= (1.71 ± 0.88) × 10^7 GM, in which 1 Göppert-Mayer (GM) =1 × 10^{-50} cm^4 sec $molecule^{-1}$ $photon^{-1}$ is the standard unit of two–photon absorptivity) [14] because β–carotene undergoes sequential two–photon absorption *via* a $1^1A_g^- \xrightarrow{h\nu_{532}} 1^1B_u^{+*} \xrightarrow{h\nu_{532}} m^1A_g^{-*}$ process, in which the intermediate electronic state $1^1B_u^{+*}$ is a long–lived eigenstate of the system which is *in resonance* with the actinic light because 532 nm lies within the one-photon absorption band of the $1^1B_u^{+*}$ band of β-carotene. In general, two-photon cross sections are large in resonance-enhanced processes because the one-photon transitions between eigenstates have much larger extinction coefficients than those involving short-lived ($\tau \leq 10^{-15}$ sec), non-resonant virtual states, which mediate two-photon absorption in *instantaneous two-photon absorption* processes of the form $\Psi_0 \xrightarrow{h\nu} \Psi_v \xrightarrow{h\nu} \Psi_f$, in which the light is not in resonance with the $\Psi_0 \xrightarrow{h\nu} \Psi_v$ transition [1,15,16]. β–carotene has the additional photodynamically useful property of having a highly photolabile final state $m^1A_g^{-*}$ which rapidly photdegrades to non-absorbing, colorless products in CCl_4 solvent. This photolability can potentially facilitate the photodynamic treatment of solid tumors-a property which has not been employed in clinical PDT applications to date, as we detail below [17].

$$\overline{\mathrm{I}_{x,t}^n} = \mathrm{I}_{0,0}^n \frac{\int_0^{\ell}\int_0^{t} 10^{-nA}\, dx\, dt}{\int_0^{\ell}\int_0^{t} dx\, dt} = \frac{1-10^{-n\bar{A}}}{2.303 n\bar{A}}\mathrm{I}_{0,0}^n \qquad (3)$$

To facilitate their characterization of the rate constants and photonicities of multiphoton processes in optically dense samples, Masthay, et al., [1], utilized *longitudinally and temporally averaged* n[th]-order intensities $\overline{\mathrm{I}_{x,t}^{n_{x,t}}}$ [15], which account for non-uniform intensities across the optically pathlength *x* for irradiation intervals *t*. By combining Eqs. (2) and (3), these authors showed that the incident averaged rate constant $\overline{k'_{0,0}}$ manifests a strong inverse dependence on the absorbance, whereas the longitudinally–and–temporally averaged rate constant $\overline{k'_{x,t}}$ is only very weakly dependent on optical density.

Similarly, $\overline{k'_{x,t}}$, the photonicities $n_{0,0}$ and $n_{x,t}$ are both nearly independent of absorbance. Hence, reliable photonicities may be obtained regardless of the optical density and reliable rate constants can be obtained provided they are longitudinally and temporally averaged at least for the β-carotene solutions with actinic absorbances of 1.0 and 0.2 utilized by these researchers [1] (Figure 2).

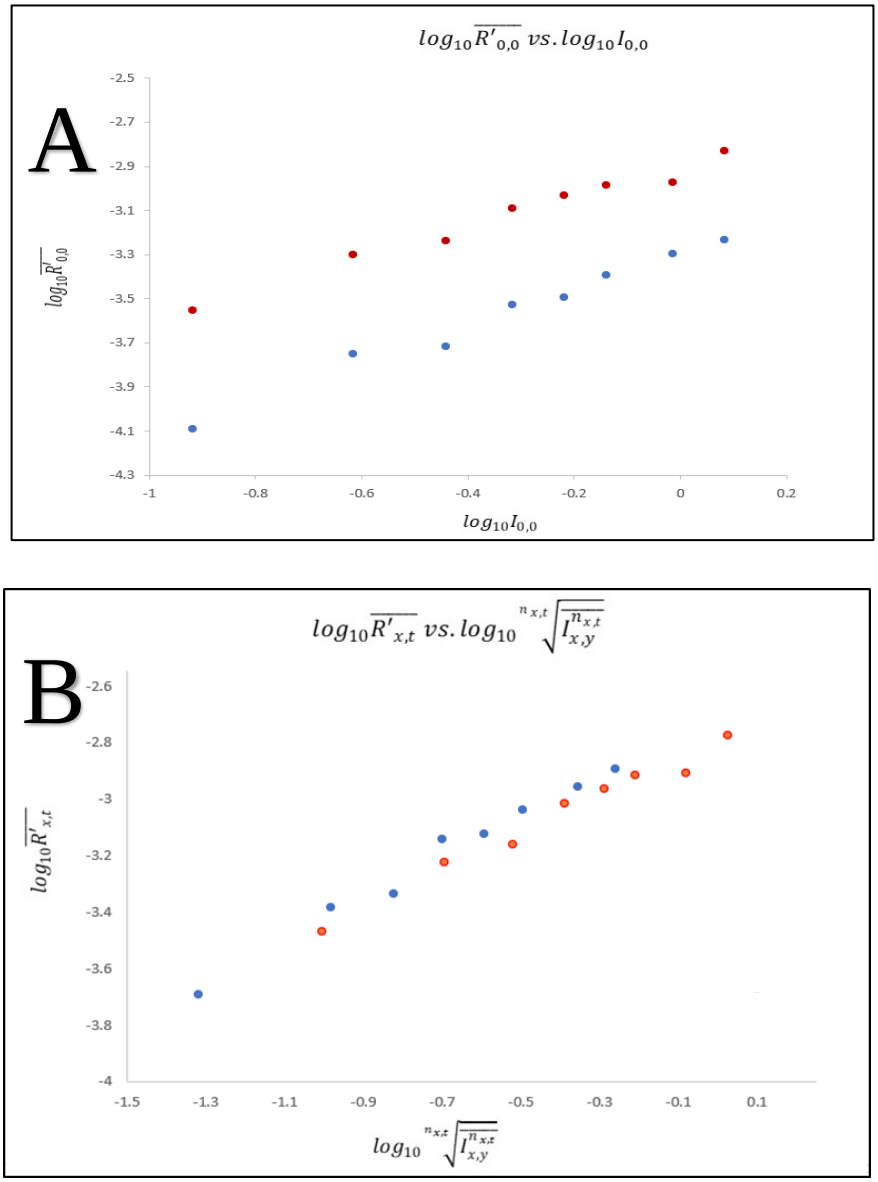

Figure 2: LLS plots of equations for βC-CCl_4 solutions, illustrating A) The inverse dependence of $\overline{k'_{0,0}}$ on the actinic absorbance A_{532}; B) the effective independence of $\overline{k'_{x,t}}$ on A_{532} and (A and B) The effective independence of both $n_{x,t}$. Note: (●) High Absorbance; (●)Low Absorbance.

Intensity averaging and multiphoton–induced photodynamic therapy

Nonlinear Photodynamic Therapy (PDT) for treating cancer and other disorders is challenging for two principal reasons: 1) the presence of a variety of endogenous chromophores in human tissue which collectively absorb light of wavelengths ranging from 200 to 10,000 nm [4,18], 2) because human tissues scatter light with high efficiency [18]. Conventional "linear" Photodynamic Therapy (PDT), which is mediated by 1PE, can thus only be used to treat disorders which require shallow tissue penetration depths (and hence can be accessed with light directly, *via* fiber optic devices and *via* other special procedures) [4]. The maximum therapeutic depth attained to date using linear PDT is ~2 cm [19].

Deeper therapeutic depths are in principle attainable *via* nonlinear PDT by capitalizing on the "transparency window" of human tissue from 700-1100 nm, in which light absorption and scattering are minimal [4,18]. To date, the deepest *in vitro* and *in vivo* cell killing depths attained with 2PE-induced nonlinear PDT (using 150–200 fs, 790 nm laser pulses) are 4 cm and 1 cm, respectively [5]. Greater penetration depths are in principle attainable if 2PE-activated PDT agents with large $\delta_{\sim 1,100}$ values can be developed.

Utilization of longitudinally–and–temporally averaged intensities $\sqrt[n_{x,t}]{\overline{I_{x,t}^{n_{x,t}}}}$ can in principle overcome the substantial challenges [4,6] encountered when characterizing the behavior of nonlinear PDT agents *in vivo*. For example, the rate constants, efficiencies and actinic doses for deep tumor shrinkage mediated by nonlinear PDT agents should be more accurately characterized with $\sqrt[n_{x,t}]{\overline{I_{x,t}^{n_{x,t}}}}$ than with incident, initial intensities $I_{0,0}$.

A new strategy for developing effective nonlinear pdt agents

First One of the most challenging issues for nonlinear PDT is the development of compounds which absorb strongly at longer wavelengths preferably within the human tissue transparency window of 700-1100 nm to facilitate therapeutic effects at deep tissue penetration depths [4]. As noted in Section II above, molecules which undergo resonance-enhanced, sequential two-photon absorption manifest exceptionally large two-photon cross sections (δ_λ values on the order of 10^6 GM or more, many orders of magnitude greater than conventional, instantaneous two-photon absorbing agents) at actinic wavelengths λ. If, like β-carotene [1], such molecules also photodegrade and bleach upon two-photon excitation, they could prove remarkably effective in treating solid tumors with nonlinear PDT, for a combination of reasons anticipated by Kogan and coworkers [7]. First, they would absorb light very strongly and hence would generate a strong photodynamic effect. Second, they would absorb at long wavelengths which penetrate deeply into tissue, providing for therapeutic effects at deep optical depth. Third, because they photodegrade and because multiphoton absorption is most effective near the incident edge of an optical path where the intensity is especially high, the light could progressively penetrate the tumor to deeper depths as the irradiation proceeds eventually providing for the therapeutic effects across the entire diameter of the tumor. Fourth, because tumors tend to be hypoxic and hence not amenable to the generation of singlet oxygen *via* Type II photodynamic effects, it would be especially useful if these agents acted *via* Type I (oxygen-independent) mechanisms [20].

CONCLUSION

Regardless of whether sequential two-photon absorbing PDT agents can be developed, the longitudinal and temporal average method recently reported by Masthay and co-authors should prove useful in helping to characterize the mechanisms responsible for nonlinear photodynamic agents in general, including recent strategies to photodynamically target specific cellular organelles such as mitochondria, lysosomes and the endoplasmic reticulum. These strategies could prove particularly useful, as they could allow for the simultaneous targeting of different organelles using a single photodynamic dye and with a single actinic wavelength. The method developed by Masthay and co-authors, may also prove particularly useful for calculating the photodynamic doses associated with such nonlinear PDT agents.

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