

One- and two-photon absorption of highly conjugated multiporphyrin systems in the two-photon Soret transition region

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This study presents a detailed investigation of near-infrared one- and two-photon absorption (TPA) in a series of highly conjugated (porphinato)zinc(II) compounds. The chromophores interrogated include *meso*-to-*meso* ethyne-bridged (porphinato)zinc(II) oligomers (PZn_n species), (porphinato)zinc(II)-spacer-(porphinato)zinc(II) (PZn-Sp-PZn) complexes, PZn_n structures featuring terminal electron-releasing and -withdrawing substituents, related conjugated arrays in which electron-rich and -poor PZn units alternate, and benchmark PZn monomers. Broadband TPA cross-section measurements were performed ratiometrically using fluorescein as a reference. Superficially, the measurements indicate very large TPA cross-sections (up to $\sim 10^4$ GM; 1 GM = 1×10^{-50} cm⁴ s photon⁻¹) in the two-photon Soret (or *B*-band) resonance region. However, a more careful analysis of fluorescence as a function of incident photon flux suggests that significant one-photon absorption is present in the same spectral region for all compounds in the series. TPA cross-sections are extracted for the first time for some of these compounds using a model that includes both one-photon absorption and TPA contributions. Resultant TPA cross-sections are ~ 10 GM. The findings suggest that large TPA cross-sections reported in the Soret resonance region of similar compounds might contain significant contributions from one-photon absorption processes. © 2009 American Institute of Physics. [DOI: 10.1063/1.3089795]

INTRODUCTION

New materials with large two-photon absorption (TPA) cross-sections are critical for a variety of optical and electro-optic technologies including optical data storage,¹ fluorescence microscopy,² and lithographic microfabrication.³ Of the many candidate materials for these applications, porphyrins are among the most highly polarizable organic molecules reported to date, and because of their enhanced nonlinear susceptibilities,^{4–9} they are promising molecules for optical limiters,^{10–14} for photodynamic therapy,¹⁵ and as contrast agents for *in vivo* imaging and microscopy.^{16–19}

Highly conjugated multiporphyrin chromophores possess large third-order nonlinear susceptibilities, $\chi^{(3)}$.^{6,20} The imaginary part of $\chi^{(3)}$ is responsible for the process of two-photon absorption, and recent studies have reported enormous TPA cross sections (i.e., up to $\sim 10^4$ GM) in conjugated, *meso*-to-*meso* ethyne- and butadiyne-bridged (porphinato)zinc(II) (PZn) dimers and related structures that feature ethyne-arene-ethyne PZn–PZn linkages.^{21,22} The linear absorption spectra of these conjugated chromophores consist of a high oscillator strength $S_0 \rightarrow S_2$ absorption manifold in the visible regime, called the Soret or *B*-band, and a weaker $S_0 \rightarrow S_1$ *Q*-band manifold in the near infrared (NIR).

Large molecular absorptive cross-sections have been reported in the two-photon resonance of the Soret band, and resonance enhancement due to *Q*-band transitions has been suggested to be the root cause of these large two-photon cross-sections.^{21–24} The *B*-band two-photon resonances in highly conjugated (porphinato)metal (PM) oligomers (~ 800 – 950 nm) are of particular interest because of their very large reported TPA cross-sections and because this wavelength range spans a spectral region readily accessible to the mode-locked Ti:sapphire laser.

Typically, one-photon absorptivity is negligible in the two-photon Soret resonance region of PM monomers and ethyne- and butadiyne-bridged PM oligomers. For two-photon absorption-based applications, however, the extremely high peak illumination intensities provided by mode-locked lasers can lead to significant one-photon absorption, as well as other photophysical processes such as excited-state absorption and photobleaching. In fact, several investigations of the linear and nonlinear optical properties of porphyrins have reported sizeable excited-state absorption effects at relatively low excitation intensities.^{25–27} Furthermore, comprehensive studies of highly conjugated PZn oligomers highlight that broad, high oscillator strength NIR excited-state absorptive transition manifolds ($S_1 \rightarrow S_n$, $T_1 \rightarrow T_n$) are a common characteristic for *meso*-to-*meso* macrocycle-macrocycle linkage topologies that include ethyne, butadiyne, and related

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proquinoidal bridging motifs.^{14,28–31} For TPA-based applications such as two-photon microscopy, the simultaneous presence of one-photon absorption or excited-state absorption processes can undermine these gains. Two-photon fluorescence microscopy, in particular, will suffer from undesirable increases in excitation volume and photobleaching^{32,33} in the presence of such processes.

In this report we present results of broadband TPA cross-section measurements in conjugated multiporphyrins that utilize ultrashort light pulses from an argon-ion laser-pumped mode-locked Ti:sapphire laser for excitation at 750–960 nm. The family of molecules explored includes *meso-to-meso* ethyne-bridged PZn_n oligomers, (porphinato)zinc(II)-spacer-(porphinato)zinc(II) (PZn-Sp-PZn) complexes where the conjugated Sp features proquinoidal character, PZn_n structures that feature terminal electron-releasing and -withdrawing substituents, related conjugated arrays in which electron-rich and -poor PZn units alternate, and benchmark PZn monomers.^{4,29,30,34–38} The emission-based measurements employed a popular ratiometric technique,³⁹ which involves significantly fewer measurements per wavelength compared with the Z-scan measurement technique⁴⁰ but requires that TPA be the only significant source of sample absorption. Additionally, we present fluorescence linearity tests, which reveal the relative contributions of one- and two-photon absorption. Extremely large “apparent” TPA cross-sections were measured ratiometrically, consistent with previous reports. However, a careful analysis, including the above mentioned linearity tests, suggests that significant linear absorption is present in the two-photon Soret-band resonances of these structures. Although nonlinear absorption increases were observed for a number of highly conjugated multiporphyrin systems and PZn monomers at wavelengths progressively longer than the Q-band absorption, the effect was discovered to be a small perturbation on an otherwise linear fluorescence intensity function with respect to excitation power. Our results therefore suggest that in the absence of a clearly demonstrated intensity-squared absorption dependence, the enormous TPA cross-sections reported in similar highly conjugated multiporphyrin structures, as well as comparable structures involving related macrocycles, may reflect significant one-photon absorption contributions in the Soret resonance region.

EXPERIMENTAL

Materials

Syntheses and characterization data for the compounds in this study are described in Refs. 29, 37, and 38. Structural diagrams for the 15 porphyrin compounds investigated are shown in Fig. 1. Samples of these compounds for spectroscopic interrogation were prepared at concentrations of $\leq 100 \mu\text{M}$ in tetrahydrofuran solvent; following two-photon cross-section measurements, linear absorption spectroscopy was used to verify compound integrity. The reference sample for “apparent” TPA cross-section measurements was fluorescein in H₂O at pH 13. Two-photon cross-sections are known for this reference sample.^{39,41}

Measurement procedures

The background theory and procedure used for measuring two-photon excitation (TPE) cross-section spectra have been previously described.⁴² Ideally, the technique directly measures the TPE cross-section, $\sigma_{2\text{-photon}}$, which is equal to the product of the TPA cross-section, Σ_2 , multiplied by the two-photon fluorescence quantum yield, q_2 : $\sigma_{2\text{-photon}} = q_2 \Sigma_2$. The two-photon fluorescence quantum yield, q_2 , is defined as the number of photons emitted by the molecule per pair of absorbed photons. TPA and TPE cross-sections both have units of $\text{cm}^4 \text{s photon}^{-1}$, but the TPA cross-sections can be substantially higher, depending on the magnitude of q_2 . The informal unit for Σ_2 and $\sigma_{2\text{-photon}}$ is the Göppert-Mayer (GM), where $1 \text{ GM} = 1 \times 10^{-50} \text{ cm}^4 \text{ s photon}^{-1}$. In this study, one-photon fluorescence quantum yield data were available for 9 of 15 samples, and corresponding apparent TPA cross-sections were calculated, assuming that the one-photon and two-photon fluorescence quantum yields were the same, i.e., $q_1 = q_2$. Results are specified as either TPA or TPE cross-section on the y-axis labels of Fig. 2.

The input power dependence of the fluorescence signals was obtained by measuring the integrated fluorescence as a function of excitation intensity. Excitation intensities were measured with a calibrated power meter. The results were plotted on a log-log scale and fit to a line; the slope of this line yielded a power-law exponent for the optical process.

Extraction of TPE cross-sections from power-law tests

The data available from the power-law tests can be used to derive approximate values for the TPE cross-section via polynomial regression analysis,⁴³ despite the simultaneous presence of one-photon absorption. Assuming *only* one- and two-photon absorption, the time-averaged fluorescence signal detected by the system, $\langle F(t) \rangle$ (photons s^{-1}) is the sum of the respective fluorescence contributions, i.e.,

$$\langle F(t) \rangle = \langle F(t) \rangle_{1\text{-photon}} + \langle F(t) \rangle_{2\text{-photon}}. \quad (1)$$

The time-averaged fluorescence signal, $\langle F(t) \rangle$, is thus modeled as a quadratic polynomial function of the incident time-averaged power $\langle P(t) \rangle$ (photons s^{-1}) with unknown one- and two-photon coefficients c_1 and c_2 , respectively,

$$\langle F(t) \rangle = c_1 \langle P(t) \rangle + c_2 \langle P(t) \rangle^2. \quad (2)$$

The coefficients c_1 and c_2 can be derived from experiment by fitting the power-law test data (Figs. 3 and 4) to the function in Eq. (2) using polynomial regression. The coefficients c_1 and c_2 can, in turn, be used to derive the one- and TPA cross sections utilizing known relationships between input power $\langle P(t) \rangle$ and fluorescence contributions $\langle F(t) \rangle_{1\text{-photon}}$ and $\langle F(t) \rangle_{2\text{-photon}}$.

For one-photon absorption, the fluorescence signal detected is *linearly* dependent on the excitation intensity integrated over the focal volume, i.e.,

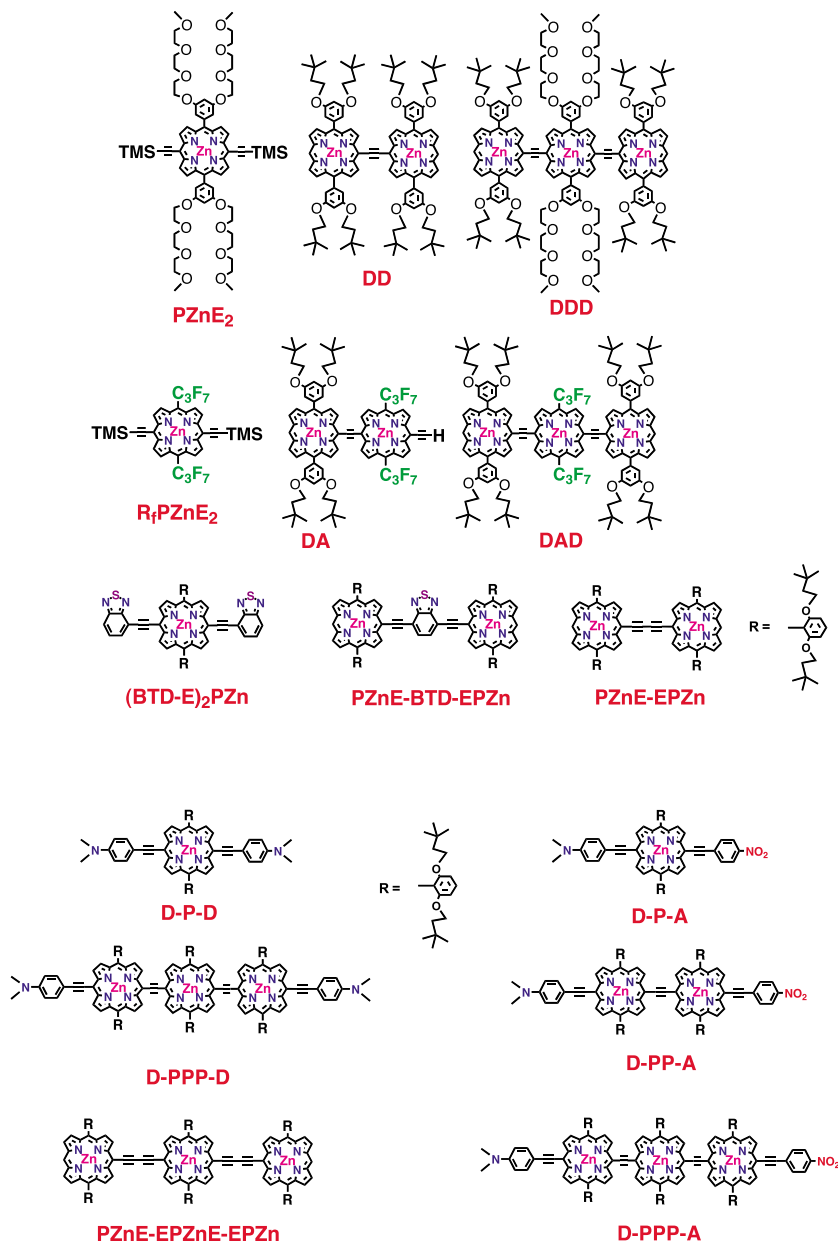


FIG. 1. (Color online) Structures of [(porphinato)zinc(II)]-based monomers, dimers, and trimers used in this study.

$$F(t)_{1\text{-photon}} = q_1 \varphi \sigma_{1\text{-photon}} C \int_V I(\vec{r}, t) dV, \quad (3)$$

where q_1 is the one-photon fluorescence quantum yield, φ is the system fluorescence collection efficiency, $\sigma_{1\text{-photon}}$ is the one-photon absorption cross-section, C is the number density (cm^{-3}) of molecules in the sample, and the excitation intensity, $I(\vec{r}, t)$, is integrated over the focal volume. We assume that the sample concentration is spatially homogeneous. For one-photon absorption, the integral of $I(\vec{r}, t)$ can be approximated as the time-varying intensity at the focus, $I(t)$, multiplied by the focal volume, V_{focal} ; thus

$$F(t)_{1\text{-photon}} \approx q_1 \varphi \sigma_{1\text{-photon}} C V_{\text{focal}} I(t). \quad (4)$$

By approximating V_{focal} as a cylinder with diameter equal to the diffraction-limited spot size, i.e., $2.44\lambda/2NA$, and length equal to twice the depth of field around the focal point, i.e., $4\lambda n/NA^2$, we can relate the time-averaged detected fluorescence to the input power as follows:

$$\langle F(t) \rangle = c_1 \langle P(t) \rangle \approx q_1 \varphi \sigma_{1\text{-photon}} C \frac{4\lambda n}{NA^2} \langle P(t) \rangle, \quad (5)$$

where NA is the numerical aperture of the objective and n is the linear refractive index of the sample at the excitation wavelength, λ . Equating the coefficients of $\langle P(t) \rangle$, we can rearrange and solve for the one-photon absorption cross-section, $\sigma_{1\text{-photon}}$, yielding

$$\sigma_{1\text{-photon}} = \frac{c_1}{q_1 \varphi C} \times \frac{NA^2}{4\lambda n}. \quad (6)$$

The one-photon absorption cross-section can be used to calculate the molar absorptivity, ε ($\text{M}^{-1} \text{cm}^{-1}$), using the relationship $\varepsilon = (\sigma_{1\text{-photon}}/2.303)(C/C_m)$, where C_m is the decadic molar concentration. ε was measured as a function of excitation wavelength for all of the compounds in this study.

The time-averaged two-photon fluorescence signal $\langle F(t) \rangle_{2\text{-photon}}$ detected by a spectroscopic system with collec-

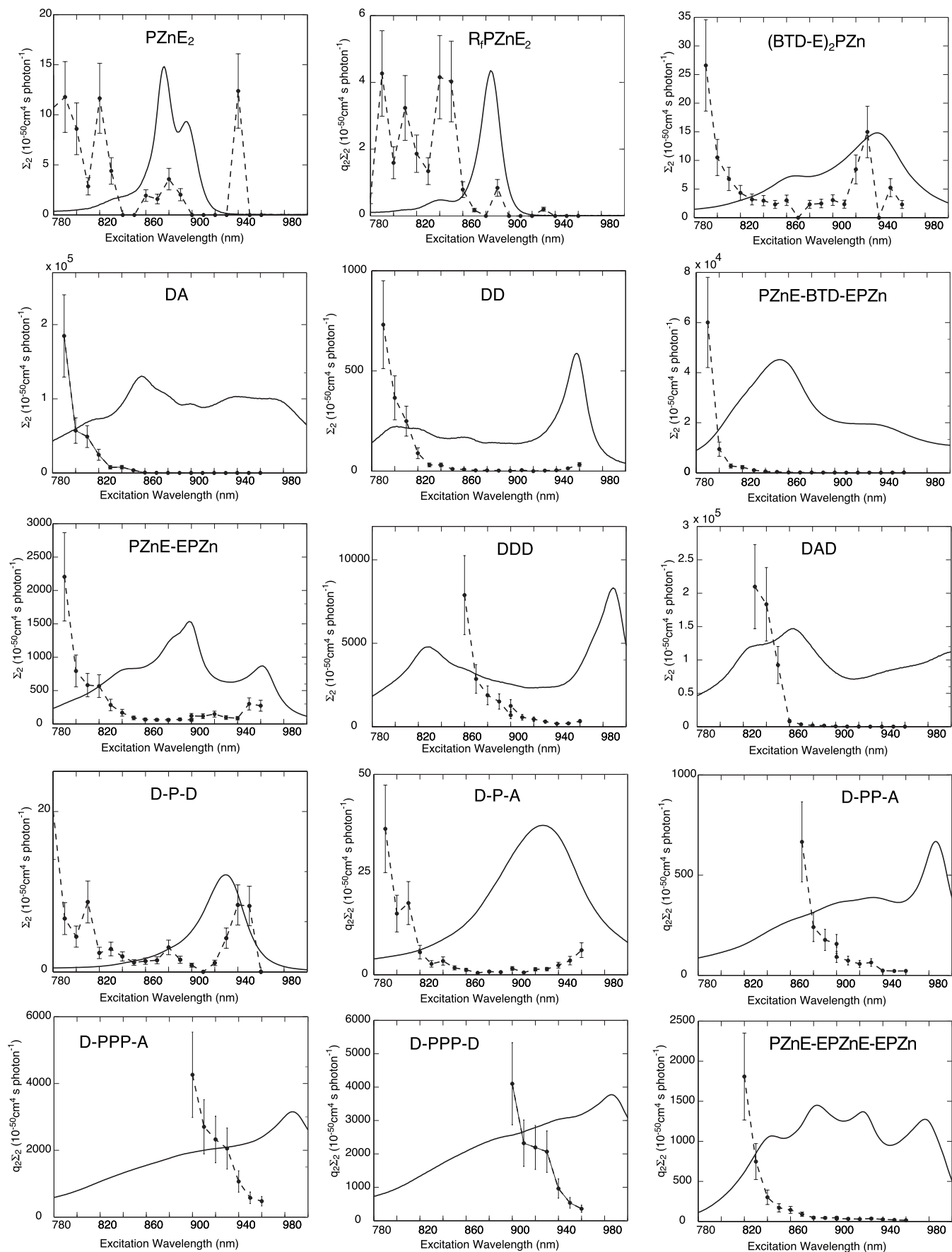


FIG. 2. "Apparent" TPA, Σ_2 , and TPE, $q_2\Sigma_2$, cross-sections measured for all 15 porphyrin compounds. Labels on y-axes indicate either Σ_2 (TPA) or $q_2\Sigma_2$ (TPE). Solid lines: one-photon absorptivity (a.u., linear y-axis scale not shown) as a function of $2 \times$ the one-photon excitation wavelength (nm). Dashed lines connecting data points: "Apparent" TPA or TPE cross-sections (GM) as calculated using a ratiometric emission-based measurement technique. Error bars ($\pm 30\%$) represent the estimated systematic error.

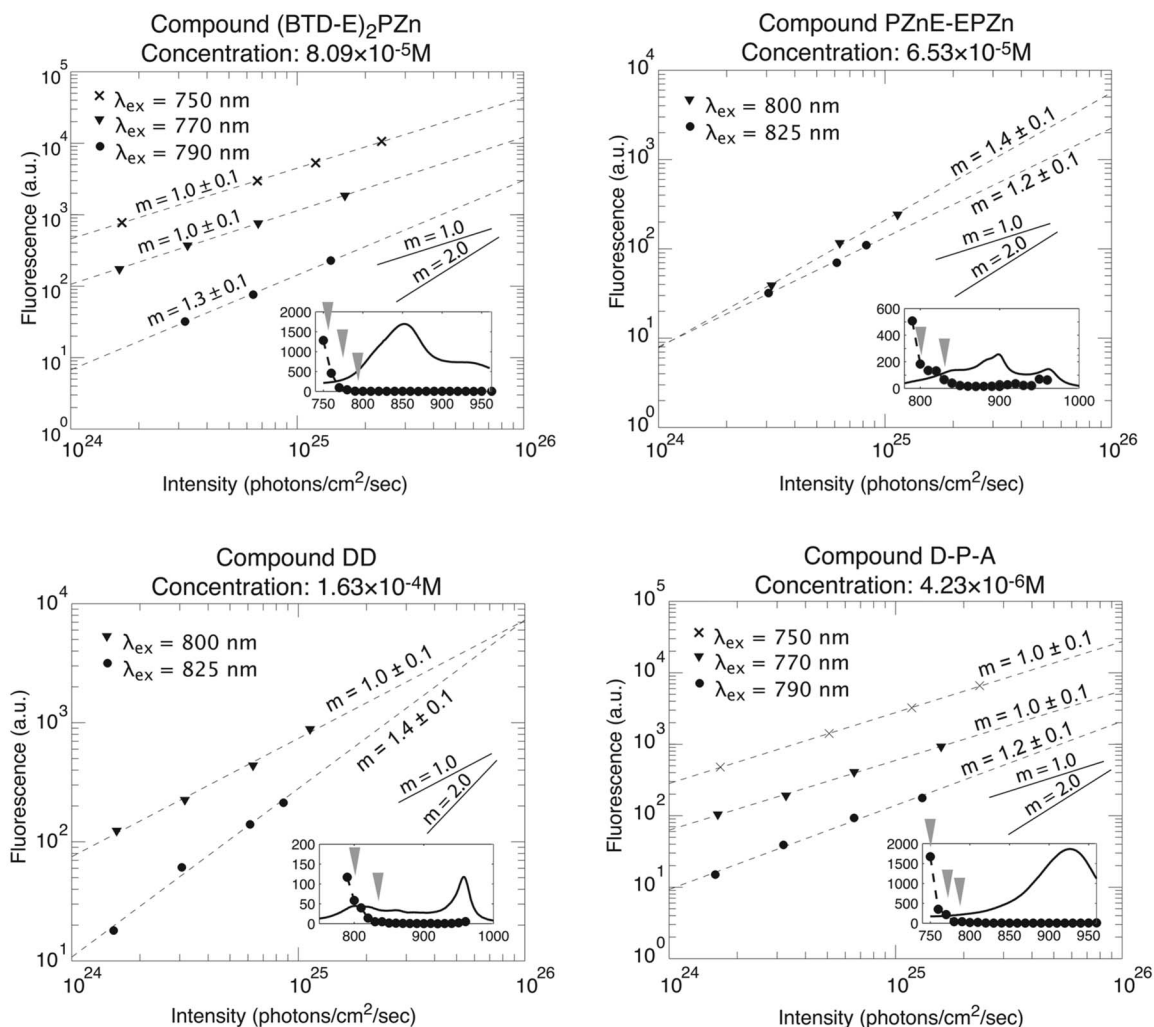


FIG. 3. Fluorescence intensity as a function of incident excitation laser beam intensity and wavelength. The four plots correspond to four different compounds. Within each plot, power-law tests at more than one excitation wavelength are shown. The straight dotted lines show linear regression fits of the measured data points on a log-log scale plot. The values of excitation laser wavelength (nm) are indicated in the chart legend. The slope fit values (m) are indicated above each dotted line. Line segments with slopes of 1 and 2 are shown for reference. Inset: Data points on the dashed line show apparent TPE cross-section spectra (GM) as a function of wavelength (nm); the solid line shows one-photon absorption spectra (a.u., y-axis scale not shown) as a function of $2 \times$ the incident laser excitation wavelength (nm).

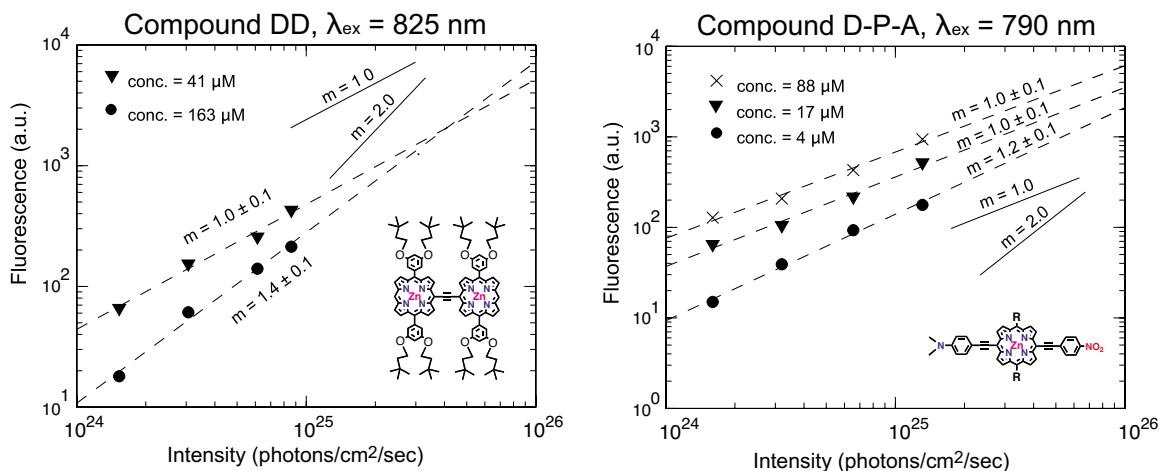


FIG. 4. (Color online) Fluorescence intensity as a function of incident excitation laser beam intensity and sample concentration. The straight dotted lines show linear regression fits of the measured data points on a log-log scale plot. Values of sample concentration are indicated in the chart legend. Slope fit values (m) are indicated above each dotted line. Line segments with slopes of 1 and 2 are shown for reference. Inset: compound structural diagrams.

TABLE I. Extracted values of TPA cross-sections for selected compounds. Data from Figs. 3 and 4 were fit to the function $\langle F(t) \rangle = c_1 \langle P(t) \rangle + c_2 \langle P(t) \rangle^2$, and values of ε and $\sigma_{2\text{-photon}}$ were calculated from c_1 and c_2 , respectively. The systematic error of 50% in the extracted values represents uncertainty in collection efficiency and data fitting. The measured values of ε are presented in order of magnitude range due to the proportionally large measurement errors at these excitation wavelengths (see text).

Compound	λ_{ex} (nm)	λ_{em} (nm)	Slope	Extracted ε ($\text{M}^{-1} \text{cm}^{-1}$)	Measured ε ($\text{M}^{-1} \text{cm}^{-1}$)	Extracted $q_2 \Sigma_2$ (GM)	P -value of $\langle P(t) \rangle^2$ fit term
DD	825	713	1.4 ± 0.1	25 ± 13	$\sim 10^1 - 10^2$	10 ± 5	$P=0.002$
PZnE-EPZn	800	732	1.4 ± 0.1	30 ± 15	$\sim 10^2$	25 ± 13	$P=0.053$
(BTD-E) ₂ PZn	790	689	1.3 ± 0.1	37 ± 19	$\sim 10^1$	17 ± 9	$P=0.001$

tion efficiency φ and utilizing mode-locked laser sources with time-averaged illumination power $\langle P(t) \rangle$ is

$$\langle F(t) \rangle_{2\text{-photon}} = c_2 \langle P(t) \rangle^2 \approx \frac{1}{2} \varphi q_2 \Sigma_2 C \frac{g_p}{f\tau} \frac{8n \langle P(t) \rangle^2}{\pi\lambda}. \quad (7)$$

Here C is the number density of fluorophores in the sample (cm^{-3}), f is the repetition rate of the pulsed laser, τ is the laser pulse width, g_p is a numerical constant of order unity, λ is the center wavelength of the laser pulse, and n is the real part of the linear index of refraction of the sample.⁴⁴ Equating the coefficients of $\langle P(t) \rangle^2$ in Eq. (7), we can solve for the TPE cross section, $q_2 \Sigma_2$,

$$q_2 \Sigma_2 = \sigma_{2\text{-photon}} = 2c_2 \frac{f\tau}{g_p} \frac{1}{C} \frac{\pi\lambda}{8n\varphi}, \quad (8)$$

where all parameters on the right-hand side are known except for the system collection efficiency, φ . In this report, the reference sample power-law test data were employed to approximate φ using Eq. (7) since all values except for φ were known for fluorescein in H₂O at pH 13.

In the absence of one-photon absorption, cross-sections extracted from the polynomial fits have larger error compared with those measured via the ratiometric technique. These higher uncertainties arise from possible errors implicit in an approximation of the illumination intensity distribution (errors that cancel out in the ratiometric measurement). The observed level of significance (P -value) for the quadratic term in the data fits used to extract the cross-sections presented in Table I was generally significant, ranging from $P=0.001$ to $P=0.053$.

RESULTS

“Apparent” TPA cross-section spectra

“Apparent” TPA and TPE cross-section spectra measured ratiometrically for all 15 compounds are plotted as a function of excitation wavelength in Fig. 2. Most of the compounds exhibit large “apparent” cross-sections over the shortest wavelength regime probed. Spectral features possibly corresponding to the two-photon Soret resonance are found in three of the monomers [R_f PZnE₂, (BTD-E)₂PZn, and D-P-D]. These putative nonlinear absorption spectral features are blueshifted in R_f PZnE₂. Spectral features exhibiting a slight plateau before rising to very high values at wavelengths approaching 750 nm are found in one monomer (D-P-A), three dimers (DA, PZnE-EPZn, and D-PP-A), and one trimer (D-PPP-A). These plateaus occur at wavelengths

slightly shorter than twice the one-photon Soret resonance wavelength. An increase in absorption toward the longest excitation wavelength examined (960 nm) was observed in the monomer D-P-A, the dimers PZnE-EPZn and DD, and the trimer DDD.

Power-law tests

The input power dependence of the fluorescence was measured at various laser excitation wavelengths for selected samples. The results are shown in Fig. 3. A slope of 1 on a log-log scale indicates purely linear absorption, and a slope of 2 indicates TPA. A slope between 1 and 2 is most easily understood as due to both one- and two-photon processes. Significant changes in the fitted slope as a function of wavelength were observed in four compounds (shown in Fig. 3): monomers (BTD-E)₂PZn and D-P-A, and dimers PZnE-EPZn and DD. The largest slope determined among all samples at any wavelength was $m=1.4$, measured for PZnE-EPZn at 800 nm and DD at 825 nm. For all other samples, the measured slopes were equal to 1 within the estimated fit error (± 0.1). In three out of the four compounds exhibiting a mixture of linear and nonlinear absorption (i.e., all but PZnE-EPZn), an incrementally higher slope was found at longer wavelengths (~ 800 – 825 nm) compared to that evident near the shortest excitation wavelength examined (750 nm). The observed slopes did not continue to increase beyond 825 nm, and at the longest wavelength studied (960 nm) the absorption rates were too small to provide sufficient signal-to-noise ratio for cross-section measurements. While the other larger compounds did have appreciable levels of absorption up to 960 nm, the fitted slopes were equal to 1 within the estimated fit error.

Increased TPA as a result self-aggregation has been experimentally measured in butadiyne-bridged PZn dimers;⁴⁵ we thus also performed power-law tests at various sample concentrations. Figure 4 shows the results of fluorescence emission versus input power at different sample concentrations for two compounds, with the excitation wavelength held constant. While the monomer D-P-A shows little significant change in power law, the dimer DD does show a significant increase in nonlinear absorption at a higher concentration (i.e., $m=1.4$ at a concentration of 1.63×10^{-4} M).

Extracted values of ε and $\sigma_{2\text{-photon}}$

Extracted ε and $\sigma_{2\text{-photon}}$ ($=q_2 \Sigma_2$) values are tabulated in Table I for samples with the largest power-law slopes [DD,

PZnE-EPZn, and (BTD-E)₂PZn]. When studied using a conventional spectrophotometer, the three samples exhibit extremely small one-photon absorption at the excitation wavelengths used for two-photon cross-section measurements. Consequently, values of ϵ measured in this region were frequently near or equal to “zero” due to the spectrophotometer’s limited dynamic range and, presumably, read-out noise of the spectrophotometer’s charge coupled device (CCD) detector. Measured values of ϵ are therefore presented in order of magnitude because of this large uncertainty. Extracted ϵ for compounds DD and (BTD-E)₂PZn were approximately equal to these measured values, while the extracted ϵ for PZnE-EPZn was an order of magnitude less than the measured value. All three samples had values of extracted $q_2\Sigma_2$ of order 10 GM, with a relatively large estimated uncertainty (50%) due to approximation errors.

DISCUSSION

Exhaustive spectroscopic studies are required in searches for materials with large TPA cross-sections. While methods for quantifying TPA such as the Z-scan technique are accurate and can discriminate linear from nonlinear absorption processes, these techniques are labor intensive, requiring several measurements per wavelength. On the other hand, the increasingly popular emission-based ratiometric measurement technique³⁹ accurately measures TPA with a single fluorescence measurement relative to a reference sample, thereby significantly simplifying high-resolution spectral measurements. Motivated by the technique’s utility in the previous work of our group and others, we had hoped the ratiometric method would be similarly effective for TPA measurements involving these highly conjugated PZn structures, but it was not.

The largest “apparent” TPA cross-sections measured ratiometrically in our study are on the order of 10^4 – 10^5 GM, comparable to the largest values measured to date by ratiometric methods that probed TPA in closely related highly conjugated porphyrin oligomers. Note also that the general spectral trends of these apparent cross-section measurements (Fig. 2) closely match early investigations of *meso-to-meso* ethyne- and butadiyne-bridged PZn dimers.^{21,22} The results from fluorescence power-law tests of our compounds suggest, however, that a significant portion of the detected fluorescence is the result of other (linear) absorptive processes.

By fitting the power-law intensity dependencies to a model that assumed one- and two-photon absorption processes, we extracted TPE cross-sections on the order of 10 GM. While such fitting techniques introduce fairly large errors,⁴³ these values are likely to be close to the *true* TPA contributions of these compounds since the fits account for the presence of simultaneous one-photon absorption. Extracted values of (one-photon) molar absorptivity, ϵ ($M^{-1} \text{ cm}^{-1}$), were equal to or within one order of magnitude of the spectrophotometrically measured ϵ (the one-photon absorption spectra for these compounds typically span five orders of magnitude between visible and NIR excitation wavelengths).

All methods for calculating TPA cross-sections based on

measuring intensity-dependent absorption or emission require assumptions about the relevance of different physical processes. Previous studies of this compound class have typically assumed an absence of one-photon absorption and excited-state absorption; this assumption is suspect given the fact that these conjugated multiporphyrin structural motifs have been established to give rise to high oscillator strength $S_1 \rightarrow S_n$ transition manifolds that span an unusually broad spectral domain of the NIR.^{14,28–31} Likewise, the TPA and TPE cross-section ratiometric measurement techniques used in this report and in other studies (e.g., see Refs. 22 and 46) require that TPA is the only significant source of absorption. Our power-law tests show clearly that these assumptions are not always sound and are inappropriate for this particular class of chromophores.

The results of power-law tests as a function of wavelength (at constant concentration) in Fig. 3 exhibit significant departures from pure TPA even at wavelengths seemingly beyond the one-photon absorption tail based on one-photon absorption spectra. The results suggest that while there may indeed be large TPA at wavelengths approaching the two-photon Soret resonance, other processes such as one-photon absorption and excited-state absorption must be considered. The finding of significantly increased nonlinear absorption at higher concentrations in the dimer DD but none in the monomer D-P-A (Fig. 4) is consistent with reports of increased optical nonlinearities due to self-aggregation effects in similar compounds.^{45,47,48}

CONCLUSION

The increasing interest in nonlinear optical applications of porphyrins and highly conjugated multiporphyrin structures has led to a demand for accurate measurements of nonlinear optical properties. While certain optical applications (e.g., optical limiting devices) may not be hindered by the occurrence of excited-state absorption and can take advantage of chromophoric species that are outstanding sequential one-photon absorbers,^{9,14,28–30} other applications (e.g., two-photon microscopy) require pure *intensity-squared* dependence of TPA. From the viewpoint of synthesis, one of the goals of investigating the nonlinear optical properties of porphyrins has been to enable predictions about TPA patterns based on one-photon absorption spectra. The results presented in this report suggest that while some general TPA properties may be predicted, experimental care must be taken to consider and account for other photophysical processes that may occur in electronically complex molecules.

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