**Article**

**Meso-Zn(II)porphyrins of Tailored Functional Groups for Intensifying the Photoacoustic Signal**

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Development of efficient molecular photoacoustic contrast agent plays significant role in the next generation biomedical imaging techniques. This article demonstrates a design criterion for small molecules to exhibit large photoacoustic effect by density functional theory (DFT) and its experimental validation. The method relies on controlling the effect of resonating structures on the vibrational energies of small molecules such that rapid thermalization pathways exist near higher-order unoccupied molecular orbitals. A series of Zn(II)porphyrin derivatives (Por-C<sub>n</sub>-RA<sub>m</sub>, where n=12 or 8 and m=1–4) is designed as a model system by DFT and demonstrated a systematic variation in the absorption coefficients of C-H vibrational modes occurring at high-frequency spectral region. A systematic decrease in absorption coefficients was observed; and therefore, a similar variation in the photoacoustic signals is predicted. To validate the theoretical results, four Zn(II)porphyrin based molecules showing systematic variation in absorption coefficients, viz. Por-C<sub>12</sub>-RA<sub>4</sub>, Por-C<sub>12</sub>-RA<sub>3</sub>, Por-C<sub>8</sub>-RA<sub>4</sub>, and Por-C<sub>8</sub>-RA<sub>3</sub> are synthesized in good yields (40–70%) and their optoelectronic properties are systematically studied as well as the effect of resonating structures in these molecules in determining the vibrational energies are discussed. Theoretical predictions are validated by photoacoustic coefficients measurements and photoacoustic tomography. The photoacoustic coefficients and tomographic intensities decreased in the order Por-C<sub>12</sub>-RA<sub>4</sub> > Por-C<sub>12</sub>-RA<sub>3</sub> > Por-C<sub>8</sub>-RA<sub>4</sub> > Por-C<sub>8</sub>-RA<sub>3</sub> as predicted by DFT. Large photoacoustic coefficients are observed for the Por-C<sub>12</sub>-RA<sub>4</sub>, which is superior to that of the existing small molecules.

Besides offering a superior molecule for photoacoustic tomography, the present criterion adopted here would enable to design simpler molecules with superior photoacoustic and other nonlinear optical properties.

Photoacoustic imaging (PAI) is an emerging in vivo biomedical imaging technique that offers high spatial resolution images with excellent optical contrast. In general, PAI can be described as ‘light in and sound out’ instead of ‘sound in and sound out’ as in ultrasound imaging and ‘light in and light out’ as in optical imaging. PAI utilizes laser pulses to penetrate into the tissue, which is absorbed by contrast agents (either endogenous biomolecules or exogenous materials) and detect the generated acoustic waves using an ultrasound transducer. Thus, PA signals are optically generated and ultrasonically detected; therefore, PAI combines advantages of optical imaging contrast with deep penetrating and spatial resolution of ultrasound. Even though light penetration depth in a tissue is limited, compared to endogenous contrast agents such as hemoglobin, deoxyhemoglobin, melanin, and other biological molecules, an exogenous contrast agent with higher photoacoustic yield will likely provide high signal to noise ratio images, thereby enabling probing deeper into the tissue. Therefore, exogenous contrast agents such as organic molecules, clusters or nanocrystals whose photophysical properties can be tuned to exhibit large PA coefficients by absorbing the more light for deep tissue PAI are drawing major attention. An ideal contrast agent should have high absorption cross-section in the biological window (~680 – 980 nm) where the light penetration in the tissue is maximum. In addition, they should have relatively low fluorescence such that the photoexcited electrons are rapidly relaxed through non-radiative pathways, causing a transient rise in temperature around its closest vicinity thereby leading to a localized thermoelectric expansion followed by acoustic waves.

In recent years, many micro- or nano-scale materials, besides organic molecules, such as nanoparticles, nanofibers, nanorods, nanocages and nanotubes have been studied as contrast agents. Nanostructures such as gold nanoparticles or carbon nanotubes offer high absorption cross-section and superior thermal effects than organic molecules. However, they impose severe restrictions on clinical use due to toxicity and metabolism. Alternative approaches are the use of organic dyes assembled into vesicles, such as porphysomes, and photoacoustic probes. In these approaches, organic or metalorganic dye molecules are either covalently attached
on the external surface, non-covalently encapsulated inside a core/shell structure, or doped within suitable scaffolds or matrices, which are optically transparent to excitation or emission light. Moreover, molecular organic dyes with strong absorption in the biological window, such as cyanine,54–56 porphyrin57 and BODIPY derivatives,56 are used as PA contrast agents. However, a class of CY2,5 cyanine dyes, known as ICG dye is the only contrast agent approved by FDA for clinical PAI applications due to its excellent absorption in NIR region especially in biological transmittance wavelength window (700–800 nm). Although, the ICG derivatives are used as rare molecular PAI contrast agents, they possess various other photo-physical parameters that make this class of dyes not suitable as contrast agents for PAI. For example, ICG dye is fluorescent and a portion of the absorbed light is not completely transformed into PA signal, reducing its effectiveness for PAI applications. The optical properties of ICG are concentration dependent and can be change dramatically by varying its concentration due to aggregation in aqueous environment and by binding with intercellular molecules.47, 48 ICG is also restricted to the vasculature space and it rapidly clears (t1/2 < 3 min), making its usage complicated for longitudinal in-vivo studies.49 Irrespectively to ICG dye, literature studies have shown that the porphyrin derivatives could be far better than ICG due to their stability, non-toxicity, tunable optical properties, long lived S1 singlet excitation states, biocompatibility, higher contrast imaging and can also be used for lifetime-based PA imaging to probe the molecular environment surrounding the sample.33, 50 Therefore, the focus of this work is majorly on porphyrins. Although several attempts have been made, the design and development of small molecule PA contrast agents is perhaps limited because the factors influencing the PA effect have rarely been systematically studied, resulting in a lack of guidance.2, 52, 53 Conspicuously rare in the literature are small molecular porphyrin contrast agents designed for PAI. Especially efforts to develop common metalloporphyrins such as Zn(II)porphyrins as molecular contrast agents by tailoring their absorption spectra and correlating their absorption with that of phonon emission have not been thoroughly investigated. Herein, by employing the state-of-the-art density functional theory (DFT), a molecular guideline to design and develop the Zn(II)porphyrins as contrast agents is reported. Metalloporphyrins are chosen as a model system because of their natural existence in many biological systems as well as the availability of a large number of synthetic porphyrins offering diverse applications, from drug delivery systems to solar energy conversion medium. Moreover, porphyrins provide enormous possibilities to tailor their properties through chemical modification of their core structure and deriving a homologous series by appropriate substitution and anchoring groups.32, 54 The molecules are designed by judicious placing of functional groups to manipulate their molecular geometry such a way that thermalization pathways are provided near to the emitting states of the molecules; a major fraction of the photoexcited electrons are to be thermalized through non-radiative pathways. The designed Zn(II)porphyrins show a systematic variation in their vibrational energies of C-H groups placed near higher order unoccupied molecular orbitals (considering a region of thermalization) and a systematic change in the IR absorption coefficient is also observed with relative change in the positions of functional groups. The carboxylic group at the para-position of the meso-substituted phenyl group of porphyrin core (Figure 1) showed the highest absorption coefficient than the corresponding groups; therefore, it is expected to give the best PA performance among the designed Zn(II)porphyrins. To validate the theoretical assumption, these Zn(II)porphyrins are then synthesized, characterized and studied their PA properties as contrast agents. A systematic variation in PA intensity was observed with the highest being as predicted by DFT. The PA intensity of the molecule developed in this process showed superior performance than the reference and many of the materials reported in the literature. The design criterion presented here could be employed to establish superior contrast agents for photoacoustic tomography (PAT) and photoacoustic microscopy (PAM) in simpler molecular systems.

Results and discussion

Molecular designing of Por-Cn-RAn porphyrins:

The general criterion for designing, synthesis and characterization of a series of Zn(II)porphyrin (Por-Cn-RAn, where n = 12 or 8 and m = 1–4) and their utility to PAI is reported here. The molecules are designed such that their geometry support a significant fraction of the photoexcited electrons to be thermalized through non-radiative pathways to exhibit enhanced PA emission. It is observed that the lowest unoccupied molecular orbital (LUMO) and few higher order molecular orbital (MO) energies of most porphyrins lie at their core; if the vibrational energies of the functional groups in these orbitals are manipulated such that they scatter more energies, a major fraction of the excited energy can be thermalized. To prove this hypothesis, Zn(II)porphyrin containing four substituted phenyl groups at its symmetrical meso-positions have been chosen. Zn metal has the closed-shell valence electrons (d10) orbital. Therefore, it does not affect the geometry of porphyrin core more. The phenyl groups at the 10, 20-meso-positions of porphyrin core have been linked through ethynyl group to increase the p-conjugation of molecules, correlating the effect of resonance on PA signals. The introduction of extended chain alkoxy phenyl groups on 5, 15-meso-position of Zn(II)porphyrins is to reduce the aggregation as well as to improve the stability of the molecule and also to manipulate the emission states such that the excited electrons are relaxed thermally. Two functional groups, one carboxylic and the other 2-carboxy-2-cyanoethyl group, are then attached at the meta- and para-positions of 10, 20-meso-substituted ethynylphenyl groups symmetrically and unsymmetrically and observed the intensities of the vibrational/rotational/translational energies by changing their resonance using DFT. A systematic change in the IR absorption coefficient was observed with relative change in the positions of these groups (Figure 1a). The carboxylic group at the para-position of the meso-substituted phenyl group on porphyrin showed the highest absorption coefficient as this position is less resonating than the corresponding groups at its meta-position; therefore, it is expected to give the best PA performance among the four designed porphyrin molecules. These designed molecules are then
synthesized, characterized and used them as contrast agents in PAI to validate the DFT results. The details for structural design of Por-C\textsubscript{n}-RA\textsubscript{m} complexes and their optoelectronic properties are in the Supplementary Information (Figure S1).

**Figure 1.** (a) Molecular structural representations for Por-C\textsubscript{12}-RA\textsubscript{1}, Por-C\textsubscript{12}-RA\textsubscript{2}, Por-C\textsubscript{12}-RA\textsubscript{3}, Por-C\textsubscript{8}-RA\textsubscript{4}, ZnTPP and ICG obtained by ChemDraw Ultra; (b) Vibrational absorption coefficient (y-axis) versus thermalization (y-axis) of (i) Por-C\textsubscript{12}-RA\textsubscript{1}; (ii) Por-C\textsubscript{12}-RA\textsubscript{2}; (iii) Por-C\textsubscript{12}-RA\textsubscript{3}; (iv) Por-C\textsubscript{8}-RA\textsubscript{4}; (v) ZnTPP and (vi) ICG. The onset graphs represent the absorption coefficient in the region of ~0.36–0.42 eV that is considered to be involved in thermalization of the molecules; (c) The representation of electronic distribution in higher order unoccupied MOs (LUMO) of Por-C\textsubscript{12}-RA\textsubscript{1}; (i); Por-C\textsubscript{12}-RA\textsubscript{2}; (ii); Por-C\textsubscript{12}-RA\textsubscript{3}; (iii); Por-C\textsubscript{8}-RA\textsubscript{4}; (iv); ZnTPP (v) and (vi) ICG that are involved in thermalization (non-radiative decay) due to the effect of resonance; (d) The higher order unoccupied MOs having energies in ~0.36–0.42 eV region that is equivalent to that of C-H vibrational mode energies i.e., LUMO+9 of Por-C\textsubscript{12}-RA\textsubscript{1}; (i); LUMO+9 of Por-C\textsubscript{12}-RA\textsubscript{2}; (ii); LUMO+10 of Por-C\textsubscript{12}-RA\textsubscript{3}; (iii); LUMO+15 of Por-C\textsubscript{8}-RA\textsubscript{4}; (iv) and LUMO+7 of ICG (v). For ZnTPP, there is no higher order unoccupied MOs in this region. The LUMO+11 of ZnTPP (0.885 eV) is the closest to the vibrations of C-H stretching.
Vibrational mode studies & higher-order unoccupied MOs contribution to PA signals:

Structures of the \textit{Por-C_{n}-RA_{m}} molecules are provided in the Supplementary Information (Figure S1a). Harmonic frequency calculations confirm that all the structures are optimized. Figures S1b and S2a in the Supplementary Information represent the experimental FTIR data and theoretical DFT results for most of the finger print (low-frequency) spectral region (600–1650 cm$^{-1}$ or 0.074–0.204 eV) as well as the high-frequency (4000–2900 cm$^{-1}$ or 0.49–0.36 eV) spectral region; the calculated IR spectra of the molecules are in good agreement with the experimental spectra. Dynamics of the molecules generated from the Graphical User Interface (GUI, Gauss View)\textsuperscript{55} of the Gaussian program package\textsuperscript{56} are in the AVI file uploaded, where visuals of specific C–H vibrations are provided. A number of experimental as well as DFT studies have been published in detail about IR and Raman spectra of substituted diphenyl and tetraphenyl porphyrins\textsuperscript{57,58} at finger print spectral region (0.074–0.204 eV) and the assignment of several IR bands has been proposed. However, there is very limited literature focused on the IR vibrational bands at the high-frequency spectral region of porphyrin molecules, thus ignoring the effect of high-frequency vibrational modes on the thermalization. As observed, the vibrational bands at high-frequency region are more important to interpret because of their direct effect on photoacoustic properties of a porphyrin molecule. To characterize the different thermalization pathways of the molecules, calculated absorption coefficients of the vibrational energies (normalized for comparison) of the molecules are presented in Figure 1b. The Figure 1b also shows the vibrational spectra of the experimental control used in this work (zinc tetraphenylporphyrin; ZnTPP) and the FDA approved dye for fluorescent and photoacoustic imaging (indocyanin green; ICG) for comparison.

As noted from calculated vibrational energy spectra, there are 699, 699, 714 and 585 modes of vibrations in compounds \textit{Por-C_{12}-RA_{1}}, \textit{Por-C_{12}-RA_{2}}, \textit{Por-C_{12}-RA_{3}} and \textit{Por-C_{8}-RA_{4}} respectively, and the vibrational spectra of all molecules are similar (Figure S1b). However, some obvious differences could be very informative in the high-frequency region of C–H bands providing evidence for non-radiative decay. In the calculated IR spectrum of \textit{Por-C_{12}-RA_{1}}, out of 699 transitions, there are 122 stretching vibration modes which belong to C–H groups in the region 0.411–0.373 eV (considered as a region of thermalization). The highest frequencies at ~0.411–0.407 eV correspond to C_{β}–H stretching vibrations of the pyrrole rings. The calculated C–H stretching vibrations in benzene rings substituted

Scheme 1. Synthesis of Por-C_{n}-RA_{m} porphyrins
with porphyrin core either directly or through ethynyl groups are observed in the region of ~0.403–0.398 eV. The absorption coefficients of the C–H vibrations are higher than that of C–H stretching in benzene rings. The C–H stretching vibration modes of the long alkoxy groups are observed in the interval ~0.389–0.373 eV. As it is clearly observable, the absorption coefficients of the C–H stretching vibration modes for long alkoxy chain are of much higher intensity than that of C–H stretching in benzene rings and C–H vibrations of pyrrole rings. Similar trends are observed in the calculated vibrational energy spectra of Por-C12-Ra, Por-C12-Ra, and Por-C12-Ra. Likewise, there are 122, 123 and 124 C–H stretching vibration modes for Por-C12-Ra, Por-C12-Ra, and Por-C12-Ra, respectively in the region 0.411–0.373 eV. However, the intensity of C–H stretching vibrations of Por-C12-Ra complexes increase in the order of Por-C12-Ra > Por-C12-Ra > Por-C12-Ra > Por-C12-Ra. A comparison of the relative changes in the vibrational absorption spectra in C–H stretching region of these four molecules is presented in the Figure S1c (Supplementary Information). The absorption coefficients of Por-C12-Ra in C–H vibrational bands are the highest compared to other complexes, indicating the higher density of phonons emission in Por-C12-Ra (Figure S1c) which is also in line with time-resolved photoluminescence (TRPL) finding, thereby expecting amplified PA signals as compared to other complexes. As the absorption coefficients of C–H vibrational modes decrease in the order of Por-C12-Ra < Por-C12-Ra < Por-C12-Ra < Por-C12-Ra, it is predicted that the PA signals also follow the same order. Interestingly, energies of these vibrations (~0.36–0.42 eV) fall at that of some higher order unoccupied molecular orbitals: LUMO+9 of Por-C12-Ra; LUMO+9 of Por-C12-Ra; LUMO+10 of Por-C12-Ra and LUMO+11 of Por-C12-Ra, as shown in Figure 1c-d. The vibrational spectra of ZnTPP and ICG display a similar relationship, the LUMO+7 of ICG (0.362 eV) LUMO+11 of ZnTPP (0.885 eV) is close to the vibrations of C–H stretching. Probably, these indicate a generality of photoacoustic emission from molecular materials; but such conclusions require data from a large number of compounds – which is beyond the scope of the present work. However, a comparison of higher unoccupied MOs energy values of designed molecules is tabulated in Table 1, indicating the higher order unoccupied MOs that could contribute in photon emission. Nevertheless, the systematic variation of the oscillation strengths of the vibrational energies and their relationship with the higher order unoccupied molecular orbitals motivate us to synthesize them and examine their optical and PA properties.

**Synthesis of Por-C12-Ra** porphyrin:

The detailed synthesis of Por-C12-Ra complexes are in the Supplementary Information. Briefly, the synthesis of Por-C12-Ra complexes are described in the Scheme 1, which involved the acid-catalyzed condensation of dipyrromethane (9) with aldehyde derivatives 6a and 6b to give the free-base porphyrins 10a and 10b, respectively. The free-base porphyrins (10a & 10b) were then brominated using N-bromosuccinimide to get porphyrins 11a and 11b, followed by metalation with zinc acetate to afford 12a and 12b in 90% yields. Sonogashira coupling with triisopropylsilyl (TIPS) acetylene gave the porphyrins 13a and 13b in 80% and 78% yields, respectively, which were in turn coupled with various carboxylphenyl iodides under mild conditions via a Sonogashira coupling reaction (14a and 14b) that included an in situ deprotection step with tetrabutylammonium fluoride (TBAF), to afford good yields (40-70%) of Por-C12-Ra porphyrins.

**Time-Resolved photoluminescence measurements:**

The thermalization (non-radiative decay) in Por-C12-Ra complexes is further proven by photoluminescence (PL) and Time-Resolved photoluminescence measurements (TRPL) measurements. Figure 2a (left) shows the TRPL decays of the Por-C12-Ra porphyrins at room temperature.

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Table 1. LUMOs energies, Molar absorption coefficients (ε) at 532 nm & 680 nm, fast component PL decay and life-time decay (1τ) of Por-C12-Ra series porphyrins

<table>
<thead>
<tr>
<th></th>
<th>Por-C12-Ra</th>
<th>Por-C12-Ra</th>
<th>Por-C12-Ra</th>
<th>Por-C12-Ra</th>
<th>ZnTPP</th>
<th>ICG</th>
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<td>LUMO</td>
<td>5.10 eV</td>
<td>5.10 eV</td>
<td>5.10 eV</td>
<td>5.21 eV</td>
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<td>-2.32 eV</td>
<td>-2.46 eV</td>
<td>-2.82 eV</td>
<td>-2.35 eV</td>
<td>-1.51 eV</td>
</tr>
<tr>
<td>LUMO+X</td>
<td>LUMO+8= -0.14 eV</td>
<td>LUMO+9= -0.16 eV</td>
<td>LUMO+9= -0.09 eV</td>
<td>LUMO+10= -0.17 eV</td>
<td>LUMO+10= -0.09 eV</td>
<td>LUMO+6= -0.26 eV</td>
</tr>
<tr>
<td>Extinction</td>
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<td>1.05 x 10^4</td>
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<td>0.42 x 10^4</td>
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<tr>
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<td>-</td>
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<td>Fast component PL</td>
<td>314.5</td>
<td>93.00</td>
<td>131.5</td>
<td>126.9</td>
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Figure 2. (a) TRPL decays (left) of the Por-C₁₂-RA₃ complexes at room temperature that indicate that the Por-C₁₂-RA₃ exhibits the long-lived S₁ excited state relative to other complexes of same series; and TRPL fast and slow time components decay (right) for four Por-C₁₂-RA₃, the initial fast decay represents the thermalization carrier dynamics and the slow decay involves radiative process. TRPL decay curves are fitted using the double exponential decay model, which provides excellent fit to the experimental data of the multiple recombination characteristics.

\[ I(t) = A e^{-\frac{t}{\tau_{slow}}} + B e^{-\frac{t}{\tau_{fast}}} \]  

where \( I(t) \) is the time-dependent intensity, \( \tau_{slow} \) and \( \tau_{fast} \) are the slow and fast decay lifetimes, respectively, and \( A' \) and \( B' \) are the initial states of the slow and fast exponential decay curves. It is notable from the Figure 2a (right) that the fast component decay for Por-C₁₂-RA₃ (314.5 ps) is many orders of magnitude higher than the other studied porphyrins (Table 1), suggesting that the recombination is predominantly non-radiative in Por-C₁₂-RA₃. This observation is consistent with the predictions that a high fraction of

![Diagram](image_url)
Optical and photoacoustic Z-scan studies:

The optical PA absorption and PA emission responses as a function of laser fluence for the Por-C_{12}-RA\textsubscript{m} molecules as well as ZnTPP (reference material) are measured using the 532 nm laser as shown in Figure 2b (left and middle) respectively. As seen in Figure 2b (left), all the molecules including the reference show typical reverse saturable absorption behavior.\textsuperscript{24} Since the generated PA signal is linearly proportional to optical absorbance, the corresponding PA signal for ZnTPP and all of the newly synthesize Por-C_{12}-RA\textsubscript{m} dyes show an increase with the incident laser fluence. Relative to the well-studied ZnTPP, a ~7-fold enhanced PA emission is observed for the Por-C_{12}-RA\textsubscript{3} at the highest laser fluence of ~410 mJ/cm\textsuperscript{2}; whereas the rest of the molecules exhibited a ~4-fold enhancement (Figure 2b, middle). PA signal generation as a function of molar concentration at fixed low and high laser fluence is also measured (Figure 2c). Possible additional factors contributing to the PA signal are (i) the thermal dependence of the Grüneisen coefficient at high laser fluence, (ii) the concentration dependence of heat distribution around absorbing molecule,\textsuperscript{29} and (iii) fluorescence quenching due to concentration. All these factors contribute to a nonlinearity of PA amplitude to the optical absorption. It is worth to mention that in a concentrated dye sample, quenching of radiative emission may occur which in turn leads to an increase in PA (non-radiative) emission. In the Por-C_{12}-RA\textsubscript{m} samples, the PA signal is linearly proportional to their optical absorption at lower fluence (<200 mJ/cm\textsuperscript{2}). The curve shapes for Por-C_{12}-RA\textsubscript{3}, for instance, show similar trends in PA signal and optical absorption at the laser fluence up to 200 mJ/cm\textsuperscript{2} (Figure S6c & S6d), whereas the nonlinear optical properties come into effect at laser fluence >200 mJ/cm\textsuperscript{2}. A similar behavior is observed for other Por-C_{12}-RA\textsubscript{m} dyes too.

A major requirement for contrast agents to be utilized for PAT is to have absorption in the biological window (680–980 nm), although the new molecules have limited absorption in this region, the absorption of their Q-bands could be extended to the biological window through appropriate structural modification. In fact, the extinction coefficients for these molecules are higher at 680 nm compared to 532 nm (Table 1). To evaluate the usefulness of the new molecules at biological experiments, PA signals as a function of fluence as well as concentration were also measured using 680 nm excitation at similar conditions as that using the 532 nm laser but with an OD of ~0.3 at 680 nm, shown in Figure 2b (right) & Figure 2c (right), respectively. Similar to 532 nm results, dyes display reverse saturable absorption property. Strongest PA signal emission is observed from Por-C_{12}-RA\textsubscript{3}, which is a ~7-fold enhancement over the weakest PA emitter (Por-C_{3}-RA\textsubscript{3}). As ZnTPP does not absorb at 680 nm, it is not included for this part of the study.

It is worth noted that a systematic variation Por-C_{12}-RA\textsubscript{3}>Por-C_{12}-RA\textsubscript{2}>Por-C_{12}-RA\textsubscript{1}>Por-C_{9}-RA\textsubscript{3}>Por-C_{9}-RA\textsubscript{2}>Por-C_{9}-RA\textsubscript{1} in PA intensity was observed with the highest being as predicted by DFT i.e., Por-C_{12}-RA\textsubscript{3}. The PA intensity of the molecule developed in this study showed superior performance than the reference and many of the materials reported in the literature (Figure S8 in Supplementary Information).\textsuperscript{24}

Dye concentration dependence – low laser fluence: To effectively estimate the photoacoustic emission strength of each dye, PA signal generation as a function of optical density/molar concentration at fixed low and high laser fluence is examined. This study tells about the potential of each dye as a PA contrast agent with respect to both the high and the light dosage. The optical Z-scan absorption and corresponding PA emission at low laser fluence (20 mJ/cm\textsuperscript{2}, which is below the maximum permissible exposure limit at 352 nm) are presented in Figure 2c (top left & top middle).

At low incident laser fluence, these dyes behave as linear absorbers, a stark contrast to the results at high laser fluence. The expected linear correlation with the observed slopes is proportional to the corresponding molar absorption coefficients (\(\epsilon\)) at 532 nm (Table 1) – increase of slope with \(\epsilon\). The corresponding PA emission at 532 nm is also linear in accordance with the linear relationship between the optical absorption and the PA signal generation. Results effectively illustrate the dosage vs PA response profile for each dye.

Dye concentration dependence – high laser fluence: Studies at high laser fluence could be useful for ex vivo applications such as photoacoustic microscopy.\textsuperscript{60} The nonlinear PAM technique will also allow for high resolution optical imaging.\textsuperscript{61} Plots of optical absorption and PA amplitude vs concentration at a high laser fluence of 400 mJ/cm\textsuperscript{2} at 532 nm are presented in Figure 2c (bottom left & bottom middle). Although the plots of optical absorbance vs dye concentration appear to be linear, a close observation shows slight nonlinearity. Despite having excited state absorption, albeit low, at a fixed laser fluence and upon increasing of dye concentration, greater numbers of molecules are competing for the same number of photons in the ground state. Hence, there is less probability for excited state absorption thus, reduced nonlinear absorption contribution at increased dye concentration.

The amplitude of PA emission vs dye concentrations recorded at high laser fluence of 532 nm is plotted in Figure 2c (bottom middle). A linear enhancement of PA signal amplitude is observed with the increase in concentration for all the studied dyes. The PA amplitude soars sharply with concentration for Por-C_{12}-RA\textsubscript{3} and Por-C_{12}-RA\textsubscript{2}, closely followed by Por-C_{9}-RA\textsubscript{3} and Por-C_{9}-RA\textsubscript{2} exhibiting a weak dependence of PA emission on dye concentration. The substantial growth of PA signal in these dyes is conforming to their extinction coefficients. This confirms that the nonlinear contribution is negligible under the experimental conditions. At high laser fluence, Por-C_{12}-RA\textsubscript{3} featuring strong RSA behavior shows superior characteristics in the conversion of absorbed photons into acoustic signal.

PA emission at high laser fluence is not warranted for photoacoustic tomography. Thus, the photoacoustic emission strength studies are limited to fixed low laser fluence only at 680 nm. Figure 2c (right) denotes the variation of PA signal strength with molar concentration of all the dyes. While the slopes for Por-C_{12}-RA\textsubscript{3} and Por-C_{9}-RA\textsubscript{3} have increased, in comparison to 532 nm studies, Por-C_{9}-RA\textsubscript{3} shows a drastic change.

Photoacoustic tomography:
Despite all the necessary precautions are taken to avoid any ambiguity in demonstrating a systematic variation of incident laser fluence and molar concentration with the strength of PA emission in Por-C_{RA} complexes, a comparison of each dye as a PA imaging contrast agent is necessary for validation. Figure 2d and 2e compare the PAT reconstructed images at 532 nm and 680 nm lasers, respectively, with that of blank solvent (DMF). To correlate the Z-scan results with tomography data, imaging was conducted at 20 ml/cm² and 400 ml/cm² at 532 nm λ_{ex}. After the reconstruction of images, entire data set was normalized with respect to the optimum signal i.e., with the signal of Por-C_{12}-RA₁ at high fluence. Both for the low and high fluence PAT, Por-C_{12}-RA₁ displays superior PA contrast agent as shown from Figure 2d (left and right), agreeing with the PAZ scan data as well as DFT prediction. PAT using 680 nm laser was also performed with fixed low laser intensity fluence. Consistent with the molar extinction coefficient values (Table 1), within the samples at 680 nm as well as when compared to 532 nm, the PA emission of Por-C_{RA} complexes is much stronger at 680 nm laser (Figure 2e).

Correlation of phonon emission of Por-C_{RA} molecules with resonating structures:

The observed PA intensities are correlated to that of the resonating structures of the molecules under consideration as shown in Figure 3. To our best knowledge, the effect of resonating structures on phonon emission through vibrational IR absorption by non-radiative decay of porphyrin molecules is reported here for the first time. Among the four molecules, the compound Por-C_{4}-RA₁ has the most resonating structures due to the presence of 2-carboxy-2-cyanoethenyl groups; and therefore, has more electron density bonds of alkoxy long chain (Figure 3b). This influences the C-H bonds to act as less intense IR energy absorbers in the region ~0.389–0.373 eV, causing the phonon emission of lower intensity and thereby reducing the non-radiative decay (PA signals strength). While in Por-C_{12}-RA₁ complex, one of the 2-carboxy-2-cyanoethenyl groups on meso ethynyl substituted phenyl ring was replaced by the carboxylic group, lowering π-conjugation of the Por-C_{12}-RA₁ molecule and reducing the electron withdrawing ability in carboxyl acid compared to that of 2-carboxy-2-cyanoethenyl group. Hence, the electron cloud increases onto the porphyrin core and on C-H bond regions, generating a more intense PA signal (Figure 3c) due to the high intensity of energy quanta absorption relative to that of Por-C_{4}-RA₁ complex. Similarly, compared to Por-C_{12}-RA₂, compound Por-C_{12}-RA₃ has shown more intense PA signals due to the less electron withdrawing ability of carboxylic acid groups as compared to that of 2-carboxy-2-cyanoethenyl groups (Figure 3d). On the other hand, the compound Por-C_{12}-RA₄, comprising of two carboxyl groups at 3-position of meso-ethynyl substituted phenyl rings, showed the strong PA signals relative to other compounds (Figure 3e). This could be explained by inductive effect on Por-C_{12}-RA₄, the carboxyl groups on 3-position of phenyl group have less ability to attract the electronic cloud and therefore have greater electron density on the C-H bond region, causing PA signals to be enhanced compared with that of other complexes.

The above discussion can also be correlated with the stability and the energies of LUMO levels, which would affect the PA properties of a molecule. As observed, higher energy of LUMO and more stable higher order unoccupied MOs (LUMO+Y, where Y=1,2,3,...) with energies in the 0.36-0.42 eV range cause higher PA signal. In this regard, the LUMO with energy -2.72 eV for Por-C_{12}-RA₄ are more compared to other compounds. High electronegativity of nitrile and carboxylic acid groups reduces the electron density of the porphyrin core and thereby results in reduction of electron density at C-H contributed by the porphyrin core and the LUMO+9 and LUMO+10 having energies of 0.334 eV and 0.389 eV respectively, make this molecule more effective for PA enhancement (Table 1). While in the

Figure 3. Contribution of electronic cloud (resonating structures) to phonon emission (thermalization) and PA intensity enhancement for (a) ZnTPP; (b) Por-C_{4}-RA₁; (c) Por-C_{4}-RA₂; (d) Por-C_{4}-RA₃; and (e) Por-C_{12}-RA₁. Molecular structural representations along with their electronic cloud distribution obtained by Gausssview, red and blue color marks indicate the more electronegative and electropositive parts inside the molecules. The electronic distribution is increased by changing the electronegativity of functional groups from 3-carboxyphenyl groups in Por-C_{12}-RA₁ to (2-cyanopropenoic acid)phenyl groups in Por-C_{4}-RA₃.
case of Por-C12-RA2, LUMO+9 and LUMO+10 with energy values 0.320 eV and 0.374 eV respectively contribute in PA signal while LUMO with -2.87 eV energy is comparatively localized by (4-carboxyphenyl) ethynyl groups causing the PA signal to reduce. A (4-(2-cyanopropenoic acid) phenyl) ethynyl group on meso position of the porphyrin core causes more stabilization of LUMO (-3.01 eV) in the case of Por-C12-RA3 complex, therefore generating the less intense PA signal. Furthermore, the MO of energy 0.396 eV is for LUMO+10, which is higher than that of Por-C12-RA1 and Por-C12-RA2. This indicate that more energy is required to excite electrons to that level; and therefore, reducing the probability of thermalization compared to Por-C12-RA1 and Por-C12-RA2. Likewise, in the case of Por-C8-RA4, LUMO with energy -3.21 eV and LUMO+15 (0.347 eV) are responsible for lowering the PA signal due to the presence of two (4-(2-cyanopropenoic acid)phenyl)ethynyl groups. 

Absorption spectra of Por-Cn-RAm porphyrins & vibrational levels splitting in PA signals enhancement:

The absorption spectra of the Por-Cn-RAm series of molecules are shown in Figure 4a. Por-C12-RA1 functionalized with (3-carboxyphenyl)ethynyl group at meso- position features an absorption spectrum typical of porphyrins with maxima from the Soret band at 453 nm and minima from the two Q-bands, Q(0,0) & Q(1,0), at 605 nm and 660 nm. The introduction of (4-carboxyphenyl)ethynyl unit had a significant impact on the absorption spectrum of Por-C12-RA3 due to the inductive effect, the carboxyl group at para-position of phenyl contributes more π-electrons in conjugation, leading to the bathochromic shift of Soret band at 458 nm. However, there is a slight change in Q-bands (606 & 661nm) compared to that of Por-C12-RA1. Similarly, the Por-C12-RA3 functionalized with (4-(2-cyanopropenoic acid)phenyl)ethynyl and (4-carboxyphenyl)ethynyl units and Por-C8-RA4 functionalized with (4-(2-cyanopropenoic acid)phenyl)ethynyl units exhibited more red-shifted Soret-band at 461 and 464 nm respectively, and Q-bands at 617 and 667 nm for Por-C12-RA3 and at 593 and 670 for Por-C8-RA4 clearly indicating more effective electronic interaction between the

![Figure 4](image_url)

**Figure 4.** (a) Absorption spectra (continuous spectra) and oscillation strengths (vertical lines) of (i) Por-C12-RA1 (purple); (ii) Por-C12-RA2 (red); (iii) Por-C7-RA3 (green) and (iv) Por-C7-RA4 (blue); (b) The contribution of vibrational levels in excited states S1 and S0 in the absorption and emission spectra of Por-C12-RA1 (i); Por-C12-RA2 (ii); Por-C7-RA3 (iii) and Por-C7-RA4 (iv). The vibrational levels (ν0, ν1, ν2... νn) of each states are represented in various colored levels. The distance between these level represents their contribution in PAI. This analysis is generated from a comparison of experimental and calculated data.
porphyrin core and meso-substituents as compared with that of Por-C_{12}RA_{4}. A most common assumption is that a molecular dye showing more absorption in NIR region must act as a suitable PA contrast agent because of deep penetration in tissue and having the same wavelength window as that of tissue. According to this respect, Por-C_{12}RA_{4} could have more promising molecular photoacoustic properties among the all studied dyes. Contrary to this assumption, it is aimed to demonstrate how a dye with less absorption or no absorbance in NIR region can display enhanced PA signals. It is noticed in the spectra of the synthesized complexes that the molar absorption coefficients (ε) of the Soret-band increases in the order of Por-C_{12}RA_{4} > Por-C_{12}RA_{2} > Por-C_{12}RA_{3} > Por-C_{9}RA_{4} and the lowest energy Q-band increases in the order of Por-C_{9}RA_{4} > Por-C_{12}RA_{2} > Por-C_{12}RA_{3}. As observed the ε value of Soret-band is higher for Por-C_{12}RA_{4} as compared to that of other dyes indicating that more electrons are excited from S_{0}→S_{1} (HOMO to LUMO) in Por-C_{12}RA_{4} dye, therefore more phonons can be released by thermal expansion causing higher photoacoustic signals. Hence the decrease of ε values of the Soret-band from Por-C_{12}RA_{4} to Por-C_{9}RA_{4} also decrease the photoacoustic signal in similar order of Por-C_{12}RA_{4} > Por-C_{12}RA_{2} > Por-C_{12}RA_{3} > Por-C_{9}RA_{4}. A similar manner is also observed in the Q-bands region with a little variation where the molar absorption coefficients decreases in order of Por-C_{9}RA_{4} > Por-C_{12}RA_{2} > Por-C_{12}RA_{3} > Por-C_{12}RA_{4} i.e.; opposite to that of ε of the Soret-bands, showing the more S_{0}→S_{1} (HOMO to LUMO) electron transitions with less photoacoustic effect in Por-C_{9}RA_{4} dye. This can be further proved by calculating the discrete transition energies (oscillation strengths) of all the dyes by using LR-TD-DFT/IEF-PCM calculations (See Supplementary Information) for the first 50 excited singlet states by using the B3LYP functional with LANL2DZ basis set. A noticeable difference in their oscillation strengths are observed. The oscillation strength in Soret region is higher as compared to Q-bands region in Por-C_{12}RA_{4} indicating the probability of electrons to excite in Soret-band to be higher compared to Q-bands. While the probability of electrons to go to the excited state in Q-bands is increasing in order of Por-C_{9}RA_{4} > Por-C_{12}RA_{2} > Por-C_{12}RA_{3} > Por-C_{12}RA_{4} showing that the dye with more population of electrons in the Soret-band compared to that of the Q-bands have stronger PA signals. The wavelength of absorption bands alone with their molar extinction coefficients and oscillation strengths for all the studied Zn(II)porphyrins are displayed in Supplementary information (Table S1). It is worth noting that HOMO-LUMO gaps are decrease in order of Por-C_{12}RA_{4} > Por-C_{12}RA_{2} > Por-C_{12}RA_{3} > Por-C_{9}RA_{4} suggesting that a higher energy gap causes phonon thermalization in Por-C_{12}RA_{4} due to relatively long S_{1} excited state.

A most common assumption is that a molecular dye showing a low Φ_{r} would make suitable PA contrast agent. According to this respect, Por-C_{9}RA_{4} having the low Φ_{r} could act as a promising molecular PA contrast agent among all the studied complexes. Contrary to this assumption, it is demonstrating how a dye with high Φ_{r} can display enhanced PA signals. In a recent article, it is demonstrated how a strongly fluorescent bis-styryl (MeOPh)-BODIPY dye (Φ_{r} = 0.719; τ = 5.41 ns) displayed an enhanced PA signal. More importantly, it was reported that a dye having long-lived S_{1} excited state along with a high Φ_{r} facilitate a strong PA emission because of an excited-state sequential absorption mechanism. However, as shown from Figure 4b, the life-time of S_{1} excited state for Por-C_{9}RA_{4} complexes increases in the order of Por-C_{12}RA_{4} > Por-C_{9}RA_{3} > Por-C_{12}RA_{2} > Por-C_{9}RA_{2} and PA signals increase in the order of Por-C_{12}RA_{4} > Por-C_{12}RA_{2} > Por-C_{9}RA_{4} > Por-C_{9}RA_{2}. According to above assumption, the Por-C_{12}RA_{4} showing relatively long-lived S_{1} excited state (41.79 ps) generates high PA signal due to excited-state sequential absorption mechanism. While, the Por-C_{12}RA_{2} having the low S_{1} excited state lifetime (21.94 ps) produces the enhanced PA signal relative to that of Por-C_{9}RA_{2} and Por-C_{12}RA_{2} complexes whose S_{1} lifetimes are 23.87 ps and 31.27 ps respectively. Hence, by considering the splitting of vibrational levels of S_{1} excited state, the distance between the vibrational states are also contributing to PA enhancement. As shown from the Figure 4b, the splitting between ν=1 vibrational level contributing for Q(1,0) excitation and ν=0 vibrational level contributing for Q(0,0) excitation in S_{1} excited state decreases in the order of Por-C_{9}RA_{4} < Por-C_{12}RA_{4} < Por-C_{12}RA_{2} < Por-C_{9}RA_{2}, indicating that raising the distance between ν=1 and ν=0 vibrational levels of excited state favors the non-radiative decay and thereby enhancement of PA signals.

Conclusions

In conclusion, a design protocol is demonstrated herein for small molecules to exhibit large photoacoustic coefficients by molecular structure engineering. As hypothesized, a major fraction of the excited electron energy converts into phonons through the judicious placing of functional groups of higher vibrational energies near to the higher order unoccupied molecular orbitals. Using Zn(II)porphyrins as a model system and employing the state of the art DFT, four molecules (Por-C_{9}RA_{4}, where n = 12 or 8 and m = 1–4) are designed with systematic variation in the vibrational energies of C-H groups placed near higher order unoccupied molecular orbitals (which appeared in the 0.36–0.42 eV region in this case). It is observed that the stability of LUMO and the energies of higher order unoccupied MOs that are closer to C-H stretching vibrations (~0.36–0.42 eV) indicate their contribution to photon emission. The absorption coefficients of C-H vibrational modes decrease in the order of Por-C_{12}RA_{4} > Por-C_{12}RA_{2} > Por-C_{9}RA_{4} > Por-C_{9}RA_{2}. Therefore, a similar variation in the PA signals is anticipated. These molecules are synthesized in good yields (40-70%) by acid-catalyzed oxidation, Knoevenagel condensation, MacDonald (2+2) condensation, bromination, metatlation, deprotection, and Sonogashira coupling reactions. Structural and optical properties of the molecules are systematically studied and reported. Theoretical predictions are validated by photoacoustic coefficients measurements and photoacoustic tomography. The PA coefficients and tomographic intensities decreased in the order Por-C_{12}RA_{4} > Por-C_{12}RA_{2} > Por-C_{9}RA_{4} > Por-C_{9}RA_{2}, as predicted by DFT. The absorption coefficients in alkoxy C-H vibrational bands, the ε value (3.82×10^{7} M^{-1} cm^{-1}) of Soret-band, the oscillation strength in Soret region (1.0656) compared to Q-bands (0.7772), LUMO stability, long-lived S_{1} excited state (46.88 ps) along with a high Φ_{r} (0.52), HOMO–LUMO gap <700 nm and the fast component PL decay (314.5 ps) for Por-C_{12}RA_{4} are many order of magnitude higher than that of conventional ZnTPP and the other studied porphyrins, suggesting that the recombination is predominantly non-radiative in Por-C_{12}RA_{4}, which has been further correlated to the resonating structures. Por-C_{12}RA_{4} consisting of the 3-carboxyphenyl groups of meso-ethynyl substituted porphyrin have
less ability to attract the electronic cloud towards itself due to inductive effect as compared to Por-C12-RA because of the bulky substituents. Furthermore, the porphyrins are rigid structures which would increase the splitting between ν=1 and ν=0 vibrational energy transitions, indicating the similar trend in PA signals enhancement. Further purification was carried out through pre-TLC method to afford the corresponding porphyrins. Finally, the products were recrystallized in dry CH3OH to give pure product as a green solid. Por-C12-RA: Yield: 50%. 1H NMR (CD2OH, 500 MHz): δ 3.95 (d, δ = 4.5 Hz, 4H), 8.53 (d, δ = 4.5 Hz, 4H), 8.08 (d, δ = 8.45 Hz, 4H), 7.90 (d, δ = 8.45 Hz, 4H), 7.60 (t, δ = 8.55 Hz, 2H), 6.94 (d, δ = 8.6 Hz, 4H), 3.73 (t, δ = 6.1 Hz, 8H), 1.10-1.04 (m, 8H), 0.92-0.79 (m, 12H), 0.74-0.63 (m, 48H), 0.52-0.49 (m, 8H), 0.22-0.17 (m, 8H). 13CNMR (CD2OH, 125 MHz): δ 174.61, 159.90, 151.51, 150.60, 132.70, 131.03, 129.55, 129.31, 123.36, 115.02, 114.56, 105.16, 100.66, 97.66, 83.35, 68.35, 31.62, 29.38, 29.35, 29.15, 29.04, 28.85, 28.53, 28.45, 25.17, 22.37, 13.15. IR: 3422 (O-H), 2932 (C-H), 2849 (C-H), 2192 (C≡C), 1725 (C=O), 1568 (C=C), 1387 (C-O).

Por-C12-RA: Yield: 40%. 1H NMR (CD2OH, 500 MHz): δ 9.49 (d, δ = 4.5 Hz, 4H), 8.60 (d, δ = 4.5 Hz, 2H), 8.58 (d, δ = 4.5 Hz, 2H), 8.02 (s, 1H), 8.01 (d, δ = 8.2 Hz, 4H), 7.95 (d, δ = 8.1 Hz, 2H), 7.84 (d, δ = 8.15 Hz, 2H), 7.59 (t, δ = 8.5 Hz, 2H), 6.93 (d, δ = 8.55 Hz, 4H), 3.74 (t, δ = 6.0 Hz, 8H), 1.03-0.97 (m, 8H), 0.93-0.85 (m, 8H), 0.81-0.72 (m, 8H), 0.67-0.58 (m, 52H), 0.48-0.42 (m, 8H), 0.19-0.13 (m, 8H). 13CNMR (CD2OH, 125 MHz): δ 174.63, 159.83, 151.61, 151.45, 148.80, 131.33, 130.36, 130.14, 129.75 129.31, 115.26, 105.05, 99.86, 88.89, 86.25, 31.65, 29.21, 29.15, 29.08, 28.92, 28.77, 28.46, 25.16, 22.39, 13.16. IR: 3453 (O-H), 2927 (C-H), 2847 (C≡C), 2194 (C≡C), 1625 (C=O), 1583 (C≡C), 1389 (C-O).

Por-C12-RA: Yield: 50%. 1H NMR (CD2OH, 500 MHz): δ 9.49 (d, δ = 4.4 Hz, 4H), 8.61 (d, δ = 4.45 Hz, 4H), 8.07 (s, 2H), 8.06 (d, δ = 8.35 Hz, 4H), 7.98 (d, δ = 8.1 Hz, 4H), 7.55 (t, δ = 8.45 Hz, 2H), 6.88 (d, δ = 8.6 Hz, 4H), 3.67 (t, δ = 5.25 Hz, 8H), 1.22-1.13 (m, 8H), 1.07-1.02 (m, 8H), 0.95-0.89 (m, 8H), 0.86-0.80 (m, 8H), 0.73-0.64 (m, 12H), 0.55-0.52 (m, 52H), 0.26-0.21 (m, 8H). 13CNMR (CD2OH, 125 MHz): δ 179.47, 178.04, 162.08, 155.85, 154.75, 147.54, 143.80, 133.00, 131.99, 128.41, 124.16, 123.08, 123.02, 117.09, 112.45, 109.36, 103.07, 101.67, 87.97, 85.70, 83.32, 62.15, 52.21, 30.93, 30.61, 29.25, 29.03, 22.32, 22.06, 20.70, 18.92, 17.44, 15.45, 13.07. IR: 3446 (O-H), 2916 (C≡C, H), 2847 (C≡C), 2194 (C≡C), 1635 (C=O), 1583 (C≡C), 1362 (C-O).

Synthesis of Por-C12-RA: To a degassed solution of compound 12 (100 mg, 0.071 mmol), 3-ethylbenzoic acid (41.50 mg, 0.284 mmol) and CuI (3.86 mg, 0.020 mmol) in anhydrous THF (10 mL), NEt3 (1 mL) was added under N2 gas flow. The Pd(PPh3)3Cl2 (9.96 mg, 0.014 mmol) used to the reaction mixture and it was stirred at 85°C for 8 h under nitrogen atmosphere. The reaction mixture was cooled at room temperature and THF was removed under reduced pressure. The crude product was purified by column chromatography (silica gel) using CH2Cl2: CH3OH (1:7) as an eluent.

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9.91. IR: 3448 (O-H), 2926 (-C=H), 2847 (-C-H), 2187 (C≡C), 1690 (-C=O), 1578 (-C=C), 1376 (-C-O)

Photoacoustic Studies:

The PA signal generated due to the irradiation of the laser pulse can be expressed as

\[ PA = \varepsilon_C C'_T \Phi' \] (2)\n
where '\( \varepsilon'_C \)' is the ground state molar extinction coefficient at the incident wavelength, '\( C'_T \)' is the ground state concentration of dye molecules, '\( \Phi' \)' is the Grüniesen coefficient, 'l' is the incident photon fluence, and '\( \Phi_0 \)' is the quantum yield for non-radioactive decay. The 'l' is a constant that quantifies a medium's ability to conduct sound efficiently and is defined as

\[ l = \frac{v^2 \alpha}{C_p} \] (3)\n
where 'v' is the velocity of sound, '\( \alpha \)' is the thermal expansion coefficient of the medium, '\( C'_T \)' is the specific heat of the medium at constant pressure, '\( C'_0 \)' is the specific heat of the medium at constant volume, respectively.\(^{15}\) While Eq. 1 holds true in a linear optical absorption regime, it is recently demonstrated\(^{16}\) that photoacoustic transients are produced due to the interaction between the incident laser fluence and the nonlinear absorbing medium

\[ PA = \varepsilon_C C'_T \Phi' + \varepsilon_C C'_T \Phi' l + \varepsilon_C C'_T \Phi' l^2 + \ldots + \varepsilon_C C'_T \Phi' l^{n+1} \] (4)\n
where '\( \varepsilon'_C \)' is the first excited state molar extinction coefficient at the incident wavelength and '\( C'_T \)' is the concentration of excited state dye molecules.\(^{16, 15}\) In this case, consistent with Kasha's rule, '\( \Phi_0 \)' should equal unity for any excited state relaxation process equivalent to \( S_0 \rightarrow S_2 \) or \( T_1 \rightarrow T_1 \). Hence, '\( \Phi_0 \)' is only relevant for the first (linear) term in Eq. 3. Thus, nonlinear absorption processes contribute quantitatively to the PA response resulting in a nonlinear PA amplification for a fluorescent dye even with a significantly large fluorescence ('\( \Phi_0 \)'), or indeed phosphorescence ('\( \Phi_0 \)ph'), quantum yield, pending that the excited state absorption cross section is non-negligible.\(^{14}\) It is also important to note that the concentration of excited state dye molecules will vary with time and thus the laser pulse width is also an important consideration when optimizing such a nonlinear PA amplification with respect to the lifetime of the excited state chromophore.\(^{19}\)

For effective correlation of the PA emission to its corresponding photophysical properties, optical and PA Z-scan (PAZ-scan) experiments were performed for all the samples.\(^{24}\) All the samples were dissolved in DMF solvent and using a 2.0 mm path length quartz cuvette, their optical density was adjusted to 0.1 at 532 nm excitation wavelength. The same sample cuvette was used for all the measurements to avoid ambiguity. Sample preparation and PAZ-scan arrangement are detailed in the Supplementary Information.

Conflicts of interest

There are no conflicts to declare.

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Keywords: DFT methods • phonon emissions • photoacoustic contrast agents • porphyrins • sonogashira cross coupling reactions

Notes and references

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