

Optically Accessible Long-Lived Electronic Biexcitons at Room Temperature in Strongly Coupled H- aggregates



Open Access This file is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. In the cases where the authors are anonymous, such as is the case for the reports of anonymous peer reviewers, author attribution should be to 'Anonymous Referee' followed by a clear attribution to the source work. The images or other third party material in this file are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit

<http://creativecommons.org/licenses/by/4.0/>.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

This paper contrasts the intra-aggregate exciton-exciton annihilation (EEA) behavior from that due to excitons migrating between different aggregates, using single-aggregate and chemically-connected aggregates of zinc phthalocyanine (ZnOPPC). This molecule aggregates in an H-type configuration, which has previously been shown theoretically and experimentally to radically suppress EEA. Similar observations are made in the present manuscript, where single-aggregate EEA is virtually absent even at high fluences. When hydrogen-bonded organic frameworks (HOFs) are made out of such aggregates, a new inter-aggregate degree of freedom is enabled, which gives rise to a pronounced EEA contribution. These results suggest ways of enhancing and inhibiting the formation of stable biexcitons, enabling the integration of exciton reservoirs within systems used for light harvesting.

I find this paper to be timely and interesting, and of relevance to the readership of Nature Communications. It follows in the footsteps of various recent works showing the importance of excitonic phase relations to EEA, but differs in its clever approach to controlling the different annihilation pathways. The ramifications of this photophysical process are far-reaching, including optoelectronics and photosynthesis, adding to the urgency of the submitted work. However, there are several shortcomings in the presentation of the manuscript that need to be addressed:

1: It would be helpful if the authors include a schematic of the packing structure of the ZnOPPC HOFs. My reading of the manuscript is that the formation of such HOFs relies on pi-pi stacking of the ZnOPPC molecules, upon which such stacks are hydrogen bonded through the eight peripheral carboxylic acid groups, but I fail to see how this could give rise to tubular structures similar to the previously-reported porphyrin J-aggregates from ref 25.

2: I wonder if the EEA dynamics extracted from the fluence dependent data shown in fig 4 can be made more quantitative. The manuscript mentions that annihilation is observed within 2 ps at a fluence of 86 nJ, which indeed seems to be borne out from this figure. However, I suppose some global analysis could provide numbers to back this up.

3: I am confused by the 2D spectral interpretations. For example, in line 134 it is mentioned that the PIA feature is observed at the earliest times and that therefore the biexciton is immediately formed. However, to my understanding the time-resolved dynamics happens entirely between the excitation and detection pulse-pairs, and therefore reflects the manifold of single excitons, and it is only instantaneous transitions to the biexciton that are being recorded. I'm happy to be proven wrong here, but would appreciate the inclusion of Feynman diagrams to support the author's interpretations.

4: I also encourage the authors to include energy level diagrams to support the discussion on the energetics in lines 150-156.

5: Lastly, I have a few minor corrections/clarifications:

- 5a: What is meant by "strong-coupling limit" in line 42? (What strong coupling criterium is referred to?)
- 5b: In line 26, with "without significantly changing underlying electronic structure", I suppose the authors are referring to the molecular electronic structure? I would argue that tweaking inter-aggregate couplings in order to switch between annihilating and non-annihilating regimes would impact the (collective) electronic structure of the HOFs.
- 5c: Band structure "valleys" are mentioned in the introduction and conclusion, but throughout the rest of the article it remains unclear what this refers to.
- 5d: In the caption of fig 2, I believe it should be "a) isolated ZnOPPC H aggregate and b) HOF"
- 5e: In line 132, "PIA" is not previously defined.
- 5f: In line 157 I am not sure what is meant by the "negligible spectral redshift". With respect to what is this redshift determined?

Reviewer #2 (Remarks to the Author):

In this paper, the authors characterize the ultrafast exciton dynamics in H aggregates of phthalocyanines by pump-probe and 2D electronic spectroscopy. Through the comparison between the response of isolated H-aggregates and H-bonded H-aggregates, they extract important information on the mechanism of exciton-exciton annihilation (EEA). The main conclusion is that EEA requires inter-aggregate interactions.

In general, I believe that the results obtained from the study are meaningful and have the potential to offer useful insights into ways of enhancing the optoelectronic properties of these materials. Specifically, minimizing EEA could lead to an increased dynamic range. The data are carefully taken, and the methodology is sound. However, the interpretation of the data is not fully convincing. Therefore, I cannot recommend publication in Nature Comm in the current form, but I would be willing to reconsider a revised version where the presentation of the data and the interpretation and discussion of the results are improved, as detailed below.

1) My first difficulty was with the contents flow. I found it difficult to follow the flow of the discussion, thus I recommend to re-organize partially the contents (especially of the intro and discussion).

1a) On page 1 the authors quickly state that:

‘Coherent suppression of exciton-exciton annihilation is attributed to highly oscillatory spatial wavefunctions in H-aggregate bands that make the sum of wavefunction coefficients over all chromophores vanishingly small in the strong-coupling limit’.

While these are already published results, the sentence is very cryptic and difficult to understand, especially for a non-expert. In effect, a better explanation is given later in the ‘Discussion’ section, but I believe that it is too delayed. In my opinion, the introductory section is too brief and not informative enough. On the other hand, most of the descriptions and the concepts reported in the ‘Discussion’ section are, in fact, introductory concepts (the idea of the oscillatory spatial behavior of the wave function, the typical properties of phthalocyanine H-aggregates, etc.). I suggest that the authors move the information from

the discussion section to the introductory section and use the discussion section to establish a more robust connection between experimental data and interpretation.

1b) Several concepts, fundamental for the understanding of the results, are taken for granted. For example:

- the difference between two-excitons and bi-excitons states (as they are important for the interpretation of nonlinear ultrafast measurements)
- the power dependence of third and fifth-order responses
- explain why a second order kinetic is expected in the case of EEA
- ...

I understand that these concepts have been covered in previous literature and the letter format may not allow for lengthy introductions. However, I believe that the manuscript would benefit from a brief introduction of these concepts to enhance clarity.

2) My major concern is about data discussion and interpretation.

2a) It is not fully clear to me what the main conclusions extracted from 2DES data are. It is clear to me that the dynamics in the first ps (both in all-linear and in the cross-peak-specific polarization) are the same for isolated and H-bonded aggregates. But is not clear if the discussion reported in lines 131-156 refers to both samples or only to the H-bonded one. In this last case, the differences among samples should be better discussed.

It is also obvious to me that, if excitons are formed, there must be a common ground state. It is also well-known that tetrapyrroles have degenerate orthogonal dipole moments. So I do not understand what are the implications of the sentence: 'This observation suggests a ground-state coupling (a common ground state) between non-parallel dipoles' and why this could be interpreted as a signature of biexcitons.

The negative PIA feature in 2DES was attributed to the instantaneous formation of bi-exciton (line 135). Later (lines 157-159), it is said that 'the negligible spectral redshift of the biexciton photoinduced absorption feature in this system suggests that the biexciton binding energy is low and the excitons are relatively noninteracting'. Thus, would it be more reasonable to attribute the PIA to two-exciton states? While bi-excitons are invoked if no interaction between excitons is seen?

Overall, to summarize, I do not understand what signatures appearing in the 2D spectra should be attributed to the formation of biexcitons and if there is any relevant difference between isolated and H-bonded aggregates. Is this what the author would expect based on their interpretation?

2b) About the pump-probe results.

What do the authors mean when they write that for isolated aggregates: 'No fluence second order kinetics is seen'? I guess the main point is that in isolated aggregated they do see a power-dependent amplitude but no change in the values of the time constants. In any case, this is a crucial point and should be clarified.

Most importantly, it is not fully clear to me the connection between pump-probe and 2DES measurements. I was expecting more comments from the authors about the complementarity of the data obtained with the two techniques.

3) My last comment is about the conclusions.

I found the conclusion a bit understated. In their current form, they basically represent a summary of what is already explained. I believe that a paper in Nature Comm should highlight the implications of the obtained results and suggest possible future directions. For example, I found it very interesting (even though this was not commented by the authors) that chemical stimuli (a pH or a solvent change) were employed to modify the hierarchical inter-aggregate structure of the aggregates promoting/removing inter-aggregate interactions by H-bonds, in turn also modifying the EEA regime. (See also: <https://doi.org/10.3390/molecules28083553>)

This was mentioned quickly in the abstract, but I think this concept would deserve to be emphasized more also in the manuscript conclusions.

4) Additional observations:

4a) In figure 2, panels c and d are missing the numeric scale on the y-axis.

4b) Lines 163-165 report: 'We calculate the degree of coupling in the aggregates using the nearest-neighbor approximation to be $V_0 \approx +1000 \text{ cm}^{-1}$ which is much larger than the static disorder seen in the monomer from negligible dynamic center-line slope change (Supplementary Information Figures S13, S14).'

However, in Figure S13 and S14 there is no data relative to the monomer, nor any CLS analysis.

4c) There is something wrong with the caption of Figure 4. I also think that in the same figure the traces are normalized to their max (or to the value at 10 ps)? This should be clearly stated.

Again about Figure 4, the comparison between the traces for the isolated (yellow) and H-bonded (red) aggregates would have been more elegant by choosing the same fluence values...

4d) In lines 148-149 the authors quickly mention that 'These spectra show a faint 920 cm^{-1} vibronic beating which is not clearly resolved in the parallel polarization spectra suggesting that...'. However, there is no beating analysis in the main text nor in the SupplInfo. If beatings are mentioned and attribution is attempted, a better analysis should be done (power spectra, Fourier maps, etc.), and a comparison between the beating behavior in all-parallel and cross-peak-specific configurations for both samples should be made.

Reviewer #3 (Remarks to the Author):

The manuscript by Sohoni et. al. reports difference in exciton behavior of an octa carboxylic derivative of phthalocyanine (namely, Zinc(II)-2,3,9,10,16,17,23,24-octa(4-carboxyphenyl)phthalocyanine) in alkaline and at neutral pH. Authors observed a few contrasting behavior in alkaline and neutral pH. Based on the observations author claim

absence of exciton annihilation in alkaline pH, but dominant exciton annihilation dynamics in neutral pH. While exciton annihilation is obvious in extended aggregates of this kind of molecule, author highlights biexciton formation in alkaline medium which inhibit annihilation. Authors have proposed small disconnected aggregates exists in alkaline pH gets connected in neutral pH (termed as HOF). While HOF (at neutral pH) sample has been well characterized by DLS, AFM and TEM, aggregate structure in alkaline solution is not characterized. Further formation of biexciton in alkaline pH is not rationalized. Analysis of the 2D electronic spectroscopic results is also appears incomplete. More detailed 1D pump-probe experiments are also required. Authors highlight suppressed exciton annihilation in aggregate (alkaline pH) is similar to that recent report by Prof. Huang and Coworkers, which showcases influence of destructive interference between excitons in H-aggregates suppressing exciton annihilation. However, the case in present manuscript is different. If tightly bound biexciton persist in a small dimer (or small oligomer), there would be no annihilation for obvious reason of absence of another exciton/biexciton to interact. Overall, there are several shortcomings in the manuscript with respect to characterization, spectroscopic measurements, data analysis and data interpretation and hence not recommended for publication in Nature Communication. Following are the comments in detail.

1. What is the nature of the aggregates at pH 11. Due to deprotonation of carboxylic acid groups at alkaline pH, it is understandable very small aggregates will be formed. Are they just dimer, trimer or small oligomer? What DLS measurement say? AFM or TEM is not suitable for such scenario as agglomeration would happen upon solvent drying. If very small aggregates (like dimer or trimer) form at pH 11, it is obvious that only one exciton would reside per aggregate and no annihilation would happen. In case of bound biexciton (as proposed by authors), same is the expectation.
2. What is the pKa of carboxylic acid groups in the molecule? Typically, $pK_a < 6$ is expected for carboxylic acid group. It is surprising to see that at pH = 7, authors claim neutral CO₂H groups which helps extended hydrogen bonded framework (HOF). A pH titration curve of absorption and fluorescence spectral behavior can provide clear transition from small aggregate to HOF structure.
3. It is intriguing to see quadratic power dependence of TA signal of unconnected aggregates which prompted authors to consider biexciton formation. What drives (selection rule) biexciton formation instead of single exciton? Authors are advised to propose energy level scheme to illustrate how pump excitation lead to biexciton generation. What are the spectroscopic signatures of biexciton which distinguishes from exciton? How much is the biexciton binding energy?
4. Given the absorption feature of both disconnected small aggregates and HOF is quite similar, quadratic power dependence behaviour is expected for HOF sample as well. Such data is not presented. Is it concluded that biexciton formation is not there in HOF sample?
5. There is no reason to assume intermolecular arrangement in isolated aggregate and extended HOF structure will be same. At alkaline pH, only π - π stacking will dominate for aggregation without hydrogen bonding network. On the other hand, at neutral or acidic pH, extended H-bonding leads to HOF structure where intermolecular arrangement is expected to significantly differ.
6. Before proceeding to advanced 2D-electronic spectroscopy, transient pump-probe (1D) results for monomer and two different aggregate regimes must be reported and analysed in detail. Comparison of 1D pump-probe results of monomer and two different aggregate

regimes is essential to bring out most significant differences in the spectroscopic properties in the two aggregated state. It is imperative to analyse 1D pump-probe data of two different types of aggregates and monomer in comparative manner to establish biexciton formation and its spectroscopic manifestation. In addition, 1D TA experiment up to enough time delay should be reported to measure fate and lifetime of exciton or biexciton (as claimed) in the two types of aggregates.

7. In HOF system, fluence dependent TA kinetics has been reported but no analysis has been attempted to quantify the exciton diffusion parameters. Given the present system is proposed to exhibit long range ordering promoted by hydrogen bonding network, long range exciton diffusion is expected. Evaluation of exciton diffusion parameter and a comparison with reported phthalocyanine systems is necessary to bring out important insights.

8. Referring to supporting figure S13 and S14, authors propose 'We calculate the degree of coupling in the aggregates using the nearest-neighbor approximation to be $V_0 \approx +1000 \text{ cm}^{-1}$ which is much larger than the static disorder seen in the monomer from negligible dynamic center-line slope change'. Author should detail (in Supporting data) how V_0 and magnitude of static disorder is calculated.

9. Reported analysis of 2D electronic data seems incomplete. It is not clear what new information 2D electronic data brought out in present manuscript? Authors report 'These spectra show a faint 920 cm^{-1} vibronic beating.....' but FFT spectrum is not reported. What is the origin of the beating? Why is only this single mode (920 cm^{-1}) observed? Why are other high frequency (intramolecular) and low frequency (intermolecular) modes absent?

10. Discussion section only describes general aspects of exciton behaviour in organic aggregates without bringing the results of present manuscript into context.

11. Minor point: Figure 3 caption is incomplete. Each figure caption should be elaborated.

Response to Reviewers' comments

We thank the Reviewers for their insightful comments on our work. Below, we have attempted to address the comments in detail. Reviewers' comments are shown in **Black**. Our responses are shown in **Red**. Quotes from the manuscript/SI are shown in **Tan**.

Reviewer #1 (Remarks to the Author):

This paper contrasts the intra-aggregate exciton-exciton annihilation (EEA) behavior from that due to excitons migrating between different aggregates, using single-aggregate and chemically-connected aggregates of zinc phthalocyanine (ZnOPPC). This molecule aggregates in an H-type configuration, which has previously been shown theoretically and experimentally to radically suppress EEA. Similar observations are made in the present manuscript, where single-aggregate EEA is virtually absent even at high fluences. When hydrogen-bonded organic frameworks (HOFs) are made out of such aggregates, a new inter-aggregate degree of freedom is enabled, which gives rise to a pronounced EEA contribution. These results suggest ways of enhancing and inhibiting the formation of stable biexcitons, enabling the integration of exciton reservoirs within systems used for light harvesting.

I find this paper to be timely and interesting, and of relevance to the readership of Nature Communications. It follows in the footsteps of various recent works showing the importance of excitonic phase relations to EEA, but differs in its clever approach to controlling the different annihilation pathways. The ramifications of this photophysical process are far-reaching, including optoelectronics and photosynthesis, adding to the urgency of the submitted work. However, there are several shortcomings in the presentation of the manuscript that need to be addressed:

Response: We thank the Reviewer for these comments about our work.

1: It would be helpful if the authors include a schematic of the packing structure of the ZnOPPC HOFs. My reading of the manuscript is that the formation of such HOFs relies on pi-pi stacking of the ZnOPPC molecules, upon which such stacks are hydrogen bonded through the eight peripheral carboxylic acid groups, but I fail to see how this could give rise to tubular structures similar to the previously-reported porphyrin J-aggregates from ref 25.

Response: We have removed the word 'tubular' and have now rewritten that sentence in the main text:

"Rod-like framework structures, ~35 nm in diameter and > 200 nm in length are formed in contrast to tubular porphyrin J-aggregate structures reported earlier²⁸."

While the aggregates are elongated, they are not hollow inside to the best of our knowledge, so we agree that they should not be called tubular. Based on the TEM images and XRD pattern, we believe that the four arms of the ZnOPPC molecule participate in hydrogen bonding to form the hydrogen-bonded framework, and π -stacking between the faces of the phthalocyanines creates H-like coupling. Further X-ray scattering and high-resolution electron microscopy beyond the scope of this work will be required to obtain an exact structural model. We have, however, optimized the ZnOPPC monomer using DFT calculations and have proposed a HOF structure that we have added to Section 6 of the Supplementary Information and have shown

below. These calculations were done by Zitong Wang, and we have now added them as an author in the manuscript.

“6. Electronic structure calculation to estimate the HOF lattice arrangement

A Density Functional Theory (DFT) calculation was carried out using Gaussian 16, Revision A. 03.⁸ The structure of a ZnOPPC monomer was optimized by using the B3LYP functional and the D3(BJ) dispersion correction in gas phase. The 6-31G* basis set was used for C, H, O and N atoms and SDD was used for the Zn atoms. Frequency analysis was conducted at the same level of theory to verify that we obtained an energetic minimum.

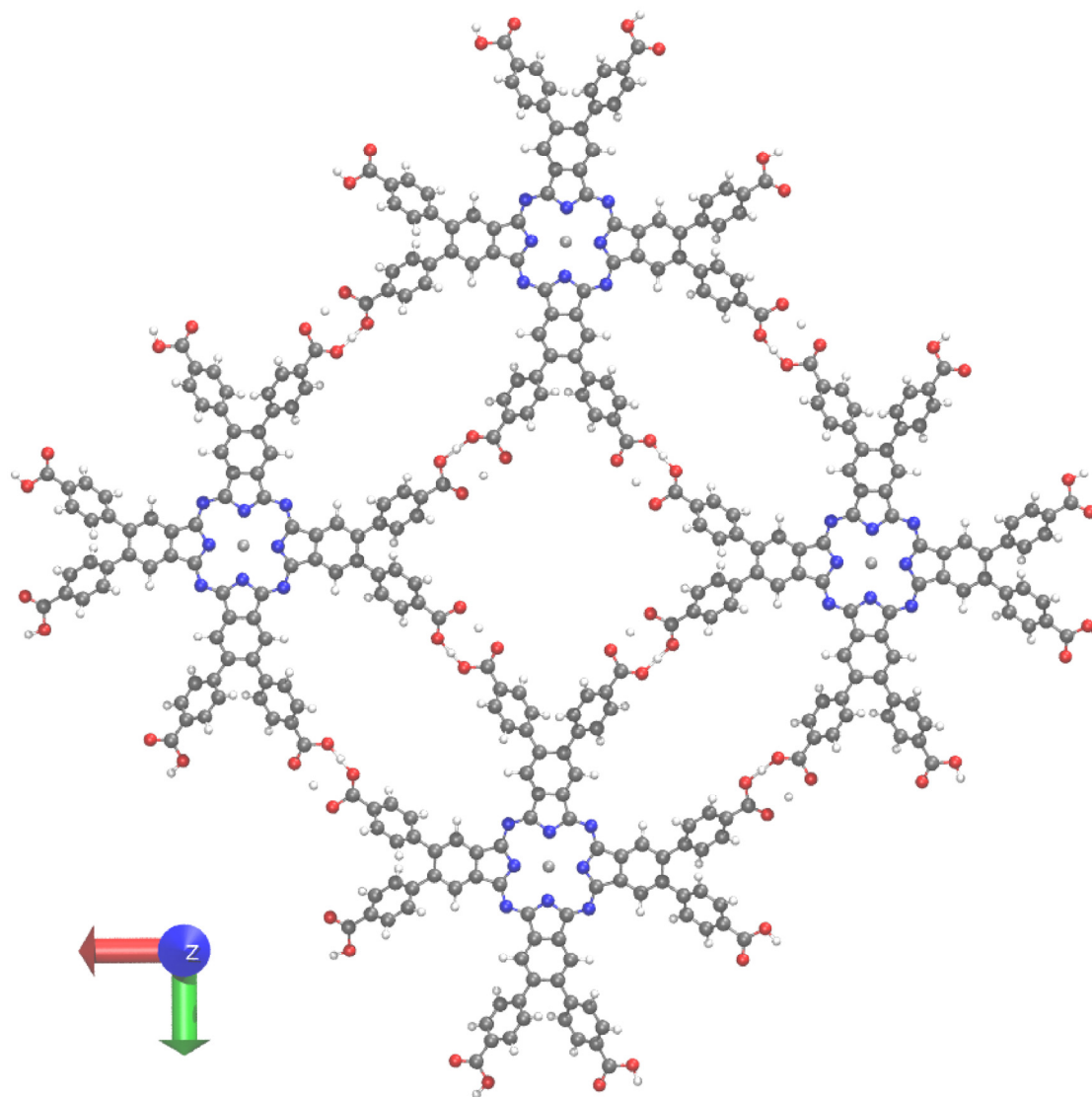


Figure S39: Orthographic top view of π -stacked units of four H-bonded ZnOPPC monomers.

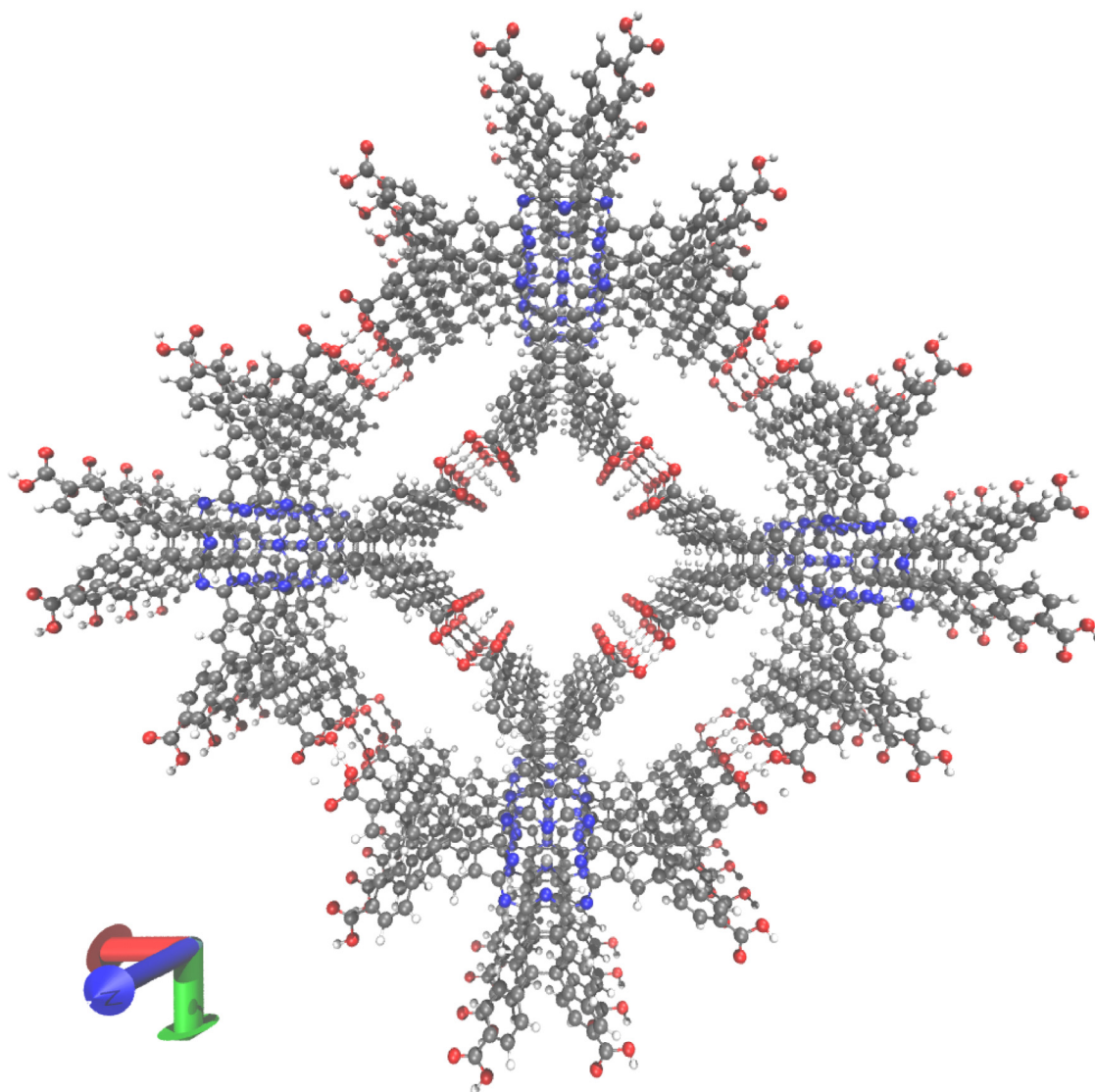


Figure S40: Perspective top view of π -stacked units of four H-bonded ZnOPPc monomers.

After the structure of the ZnOPPc monomer was optimized, a hydrogen bonding framework structure was proposed, which allowed the maximum number of hydrogen bonding between adjacent ZnOPPc monomers. In this model, the a value (lattice parameter) of the lattice was measured to be 25.6 Å, which was consistent as the estimated a value from the PXRD pattern. Different layers were arranged in a π -stacked fashion, and the c value of the lattice was set to be 4 Å.

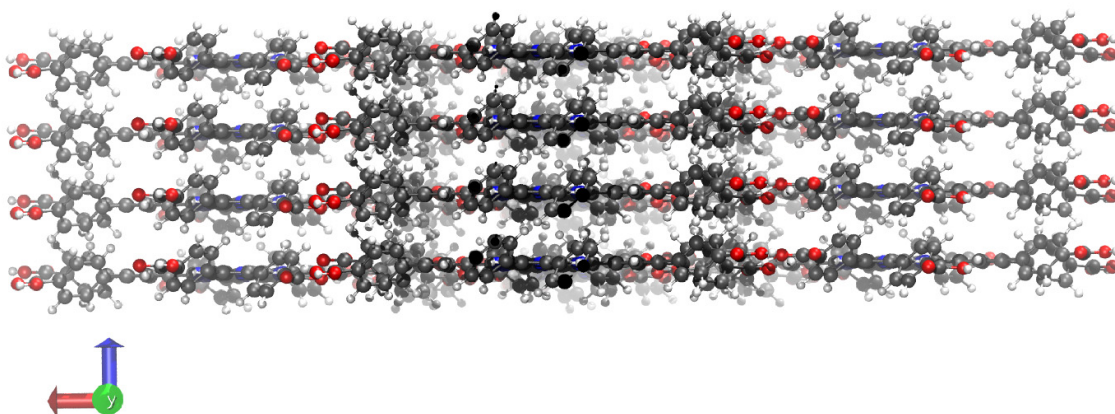


Figure S41: Orthographic side view of π -stacked units of four H-bonded ZnOPPc monomers.”

We have also provided a schematic for the aggregate based on UV-Vis, TA and current EM, AFM, XRD and DLS measurements in a reworked Figure 3:

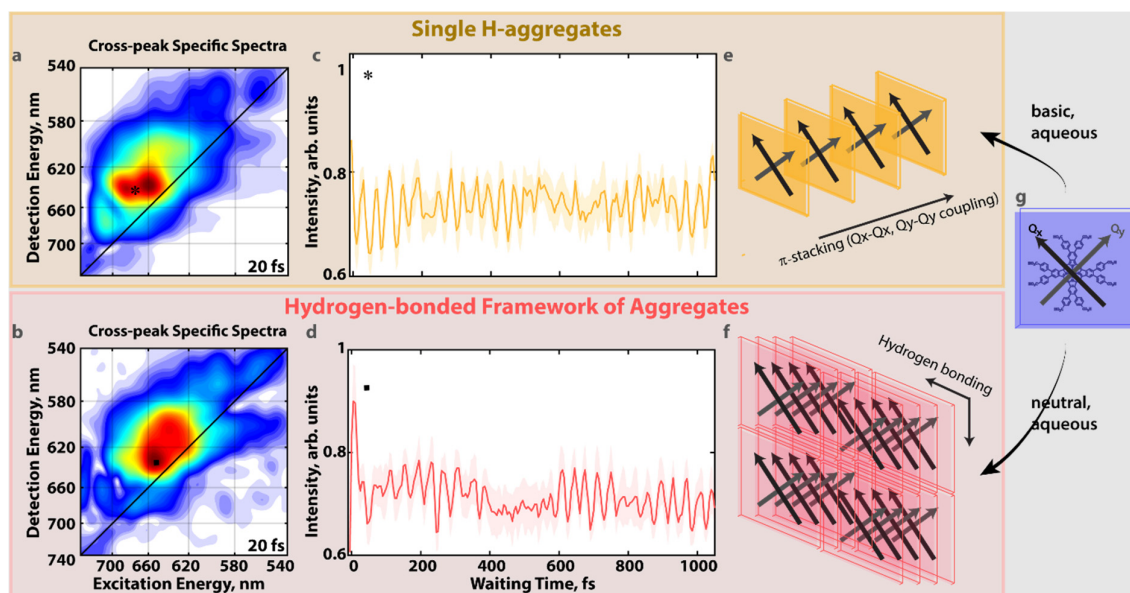


Figure 3: Cross-peak specific 2DES spectra in the $\langle 90, 60, 120, 0 \rangle^\circ$ polarization sequence of **a)** single and **b)** connected aggregates at 20 fs. Time evolution of the main cross-peak across 1 ps is shown for **c)** single and **d)** connected aggregates. **e)** and **f)** show the formation of orthogonal bands upon phthalocyanine π -stacking which leads to coupling of orthogonal Q-band dipole moments. **g)** shows the orthogonal Q_x and Q_y phthalocyanine dipole moments.”

2: I wonder if the EEA dynamics extracted from the fluence dependent data shown in fig 4 can be made more quantitative. The manuscript mentions that annihilation is observed within 2 ps at a fluence of 86 nJ, which indeed seems to be borne out from this figure. However, I suppose some global analysis could provide numbers to back this up.

Response: We have now performed second order fits to the transient absorption time traces of the framework of aggregates and first order fits for those of the basic aggregate. The following

details of the fitting process can be found in Sections 4.2 and 4.3 of the Supplementary Information.

“4.2. Calculation for coherence size in HOFs and number of excitations per HOF absorbing unit

The number of monomers in an aggregate across which an exciton is delocalized is called the coherence size. If an exciton is delocalized across N monomers, each with a transition dipole strength of μ_{mono} , the transition dipole of the aggregate exciton scales as $\sqrt{N}\mu_{mono}$. all Coherence sizes have been computed previously by Scherer and co-workers for the LH2 photosynthetic protein using the following equation⁴.

$$\frac{S_{B850}}{S_{B800}} = \frac{18/N}{9} * \frac{(\sqrt{N}\mu_{Bchl})^4}{\mu_{Bchl}^4} \quad \text{Equation S4.1}$$

In Equation S4.1, S_{B850} and S_{B800} are the laser power normalized integrated transient absorption signals for the B800 and B850 rings of the LH2 protein. It is assumed that the excitons are not delocalized in the B800 ring and those in the B850 ring are. The B850 ring contains 18 bacteriochlorophyll (BChl) monomers while the B800 ring has 9 BChl monomers; this explains the normalization factor on the right-hand side of Equation S4.1. The right-hand side is simplified to just $2N$ i.e., the normalization factor times N .

In our case, the fluence dependent (HOFs) and independent (monomer) dynamics are in different datasets, unlike the case in LH2 as described above. Therefore, we need to calculate the number of monomers in the phase-matched excitation volume (overlap volume between the pump and probe beams inside the sample cell) for the HOF and ZnOPPC monomers transient absorption experiments – this will contribute to our normalization factor. Therefore, the coherence size, N , of the HOF is given by:

$$N = \frac{S_{HOF}}{S_{mono}} * \frac{n_{mono}}{n_{HOF}} \quad \text{Equation S4.2}$$

In Equation S4.2, n_{mono} and n_{HOF} are the number of monomers in the phase-matched volume of the monomeric and HOF solutions respectively. Equation S4.2 can be rewritten as:

$$N = \frac{S_{HOF}}{S_{mono}} \frac{c_{mono} * V}{c_{HOF} * V} * \frac{N_A / M}{N_A / M} \quad \text{Equation S4.3}$$

In Equation S4.3, c_{mono} and c_{HOF} are the concentration of the samples in g/mL; V is the phase-matched volume; N_A is Avogadro's number, and M is the molecular weight of the monomer. This equation is simplified to the following:

$$N = \frac{S_{HOF}}{S_{mono}} * \frac{c_{mono}}{c_{HOF}} \quad \text{Equation S4.4}$$

The concentration of the samples for which transient absorption data are reported in Figures S26 and S28 are 3.06×10^{-5} g/mL (c_{HOF}) and 6.21×10^{-5} g/mL (c_{mono}). The laser power normalized integrated transient absorption signal is obtained by fitting a sum of Gaussians to the transient absorption data and integrating the area under the Gaussian that fits the positive transmittance feature. The quality of fits for the spectra at a waiting time of 250 fs for a fluence of 62 nJ is shown in Figure S33. The coherence size is evaluated for the three highest pump fluences i.e., 40, 62 and 86 nJ. The coherence size obtained is $N = 11 \pm 3$.

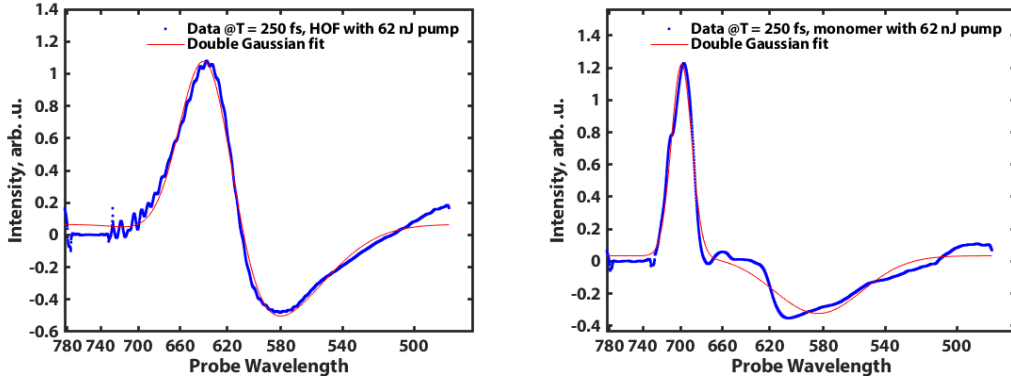


Figure S33. Fit quality shown for HOF (left) and monomer (right) spectra at $T = 250$ fs for a 62 nJ fluence.

To calculate the number of excitations per unit HOF, we perform the computation adapted from Dostal and co-workers⁵. The following equation is used to compute excitations per HOF absorbing unit:

$$n_{0,f} = \frac{E_{\text{pump}}}{A_{\text{overlap}}} * \frac{\int P(\lambda) * \lambda * (1 - 10^{-A(\lambda)}) d\lambda}{hc \int P(\lambda) d\lambda} * \frac{1}{CN_A d} \quad \text{Equation S4.5}$$

Here, E_{pump} is the average pump energy; A_{overlap} is the overlap area between the pump and probe pulses (see Figure S37). $P(\lambda)$ is the pump laser spectrum (see Figure 1); $A(\lambda)$ is the linear absorption spectrum of the HOF (see Figure 1); h is the Planck's constant; c is the speed of light; C is the molar concentration of the aggregate; d is the sample cell path length of 200 μm . The molar extinction coefficient of the monomer¹ is scaled by the square root of the coherence size ($N = 11$) to obtain the molar extinction coefficient of the HOF. This is used in Beer's law to obtain C . The number of excitations per rod obtained from Equation S4.5 is reported in the following table.

| f (nJ) | $n_{0,f}$ |
|----------|-----------|
| 27 | 0.47 |
| 40 | 0.70 |
| 62 | 1.09 |
| 86 | 1.51 |

Table S4.1

4.3. Fitting of time traces from transient absorption data of aggregates

4.3.1 Isolated aggregates

Transient absorption spectroscopy on the isolated aggregates did not show any fluence-dependent kinetics. Therefore, we sought to fit monoexponential kinetics of the form:

$$s(t) = Ae^{-\frac{t}{\tau}} + c \quad \text{Equation S4.6}$$

to datasets up to 100 ps, where $s(t)$ is the signal at waiting time t and τ is the exponential time constant. Within 95% confidence intervals, PIA time constants for three fluences are the same (Figure S34).

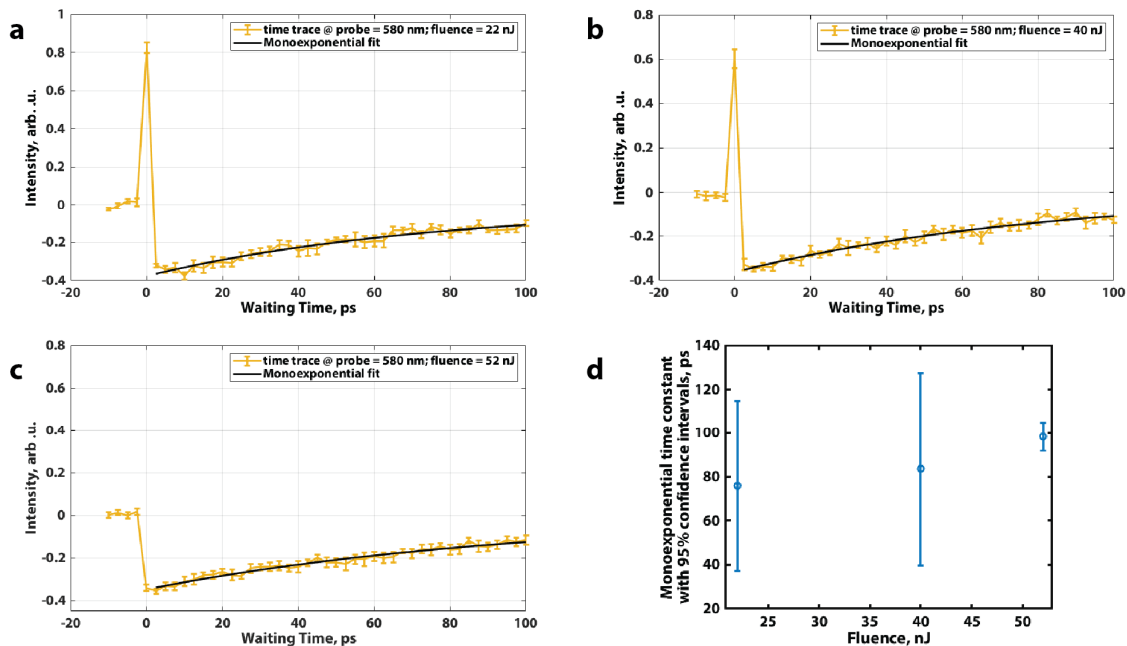


Figure S34. **a**, **b**, and **c** show transient absorption time traces of isolated aggregates up to 100 ps for a probe wavelength of 580 nm and fits with Equation S4.6. **(d)** Time constants from the fits plotted with 95% confidence intervals.

4.3.2 Hydrogen-bonded framework of aggregates

We conduct second order kinetic fits to the transient absorption time traces up to 10 ps of the framework of aggregates at probe wavelength of 580 nm. The average lifetime from the three time-constants plotted in Figure S34d is 85.3 ps. Since this lifetime of the aggregate exciton is much longer than 10 ps, that lifetime will not manifest itself in the 10 ps transient absorption datasets for the framework of aggregates. Therefore, a single second order rate equation is justified for fitting for data below 10 ps.

$$s_f(t) = A \left(\frac{n_{0,f}}{1+n_{0,f}t/\tau} \right) + c \quad \text{Equation S4.7}$$

The suffix f in Equation S4.7 denotes a certain pump fluence. n_0 is the initial number of excitations per absorbing unit, which in this case is ~ 11 monomeric units. These equations have been used to model 5th order nonlinear signal before.^{6,7} Calculation for the $n_{0,f}$ values is reported in section 4.2 of the Supplementary information.

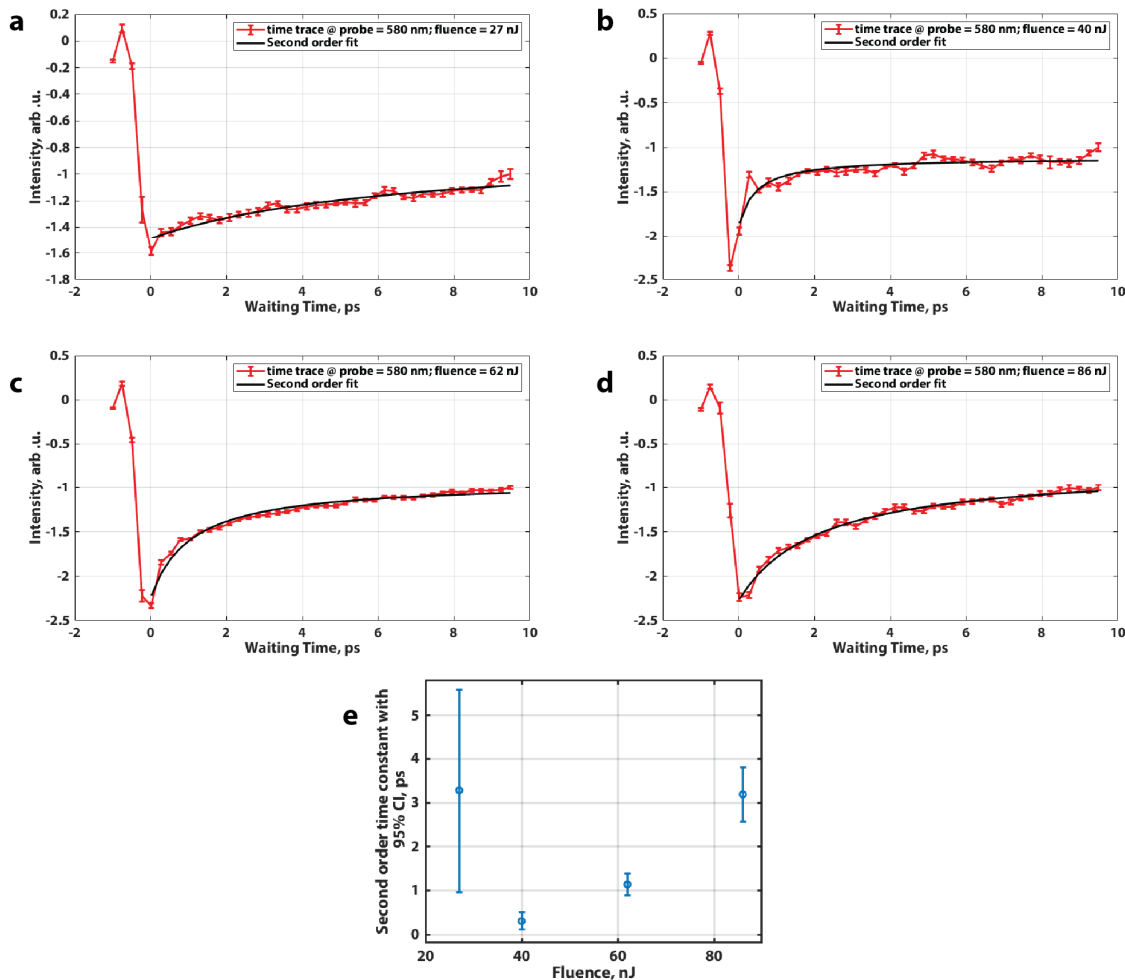


Figure S35. a, b, c, and d show transient absorption time traces of isolated aggregates up to 10 ps for a probe wavelength of 580 nm and fits with Equation S4.7. e shows time constants from the fits plotted with 95% confidence intervals.

It is evident from the poor quality of a second order fit to the lowest (27 nJ) fluence that the 5th order signal contribution in that dataset is minimal (see Figure S35e). Overall, the 5th order signal decays at a time scale of 2-3 ps.

To further check the amount of 5th order signal in the framework of aggregates, one can subtract the 3rd order signal component from the fluence dependent time traces and plot the 5th order (in general, higher than 3rd order) residuals and confirm the presence of multiexciton dynamics in the sub-10 ps timescale. To do this, we fit Equation S4.6 to the time traces by fixing the time constant to 85.3 ps (mean value of time constants shown in Figure S34d) and only varying the parameters A and c .

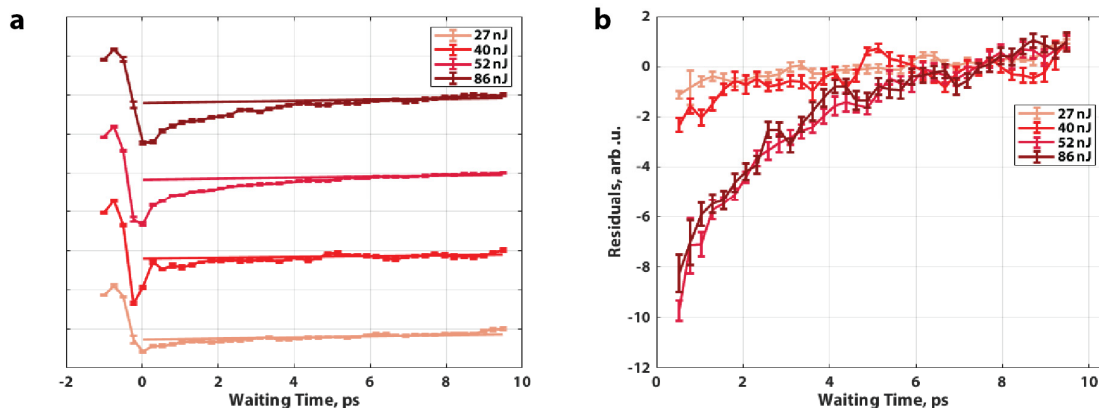


Figure S36. (a) Framework of aggregate time traces for probe wavelength of 580 nm at different fluences fit with Equation S4.6. Fit starting point is 1 ps for 27 nJ and 5 ps for higher fluences. (b) Residuals after the fits were subtracted from the time traces in **a**. Data is normalized to the last time point.

The fit starting point is chosen to be 1 ps since that signal is assumed to be predominantly 3rd order, as also seen from the poor fit quality in Figure S35 for 27 nJ. The same fitting is done to the three higher fluence time traces, but the fit starting point is 5 ps, so that the fit is insensitive to the early time 5th order kinetics. The fits are plotted in Figure S36a and the residuals (after subtracting the fit from the data) are shown in Figure S36b. This shows that 5th order signal contribution increases with pump fluence. This can also be compared with the trend obtained from SVD analysis as shown in Figure S31.”

We have also added the following sentences in the Fluence Dependent Pump-Probe Spectroscopy section of the main text:

“In our kinetic analysis, we use the pump-probe spectra at the earliest positive waiting time to first calculate the coherence size i.e., the number of monomers in a HOF across which the exciton is delocalized immediately after exciting with a pump pulse. We compute the coherence size of the HOF using the formalism described by Scherer and co-workers⁴⁶ to be 11 ± 3 . This quantity is then used to compute the number of excitations per HOF absorbing unit for each pump fluence by following the method described by Dostal and co-workers⁸, and we obtain values of 0.5, 0.7, 1.1 and 1.5 excitations per HOF absorbing unit for pump fluences of 27, 40, 62 and 86 nJ respectively. These calculations are detailed in **Supplementary Information Section 4.2**. This is used in the integrated second order rate equation¹⁰ to fit the PIA signal of the HOFs that yield time constants of 1-3 ps and the calculation is shown in **Supplementary Information Section 4.3**.”

Our analysis reveals time-constants of EEA that are in agreement with previous measurements of Wasielewski and coworkers in donor-acceptor peryleneimide-zinc phthalocyanine aggregates. In this material, they also observe aggregate-to-aggregate exciton transfer and annihilation (Ref 24 in main text).

3: I am confused by the 2D spectral interpretations. For example, in line 134 it is mentioned that the PIA feature is observed at the earliest times and that therefore the biexciton is immediately formed. However, to my understanding the time-resolved dynamics happens entirely between the excitation and detection pulse-pairs, and therefore reflects the manifold of single excitons, and it is only instantaneous transitions to the biexciton that are being recorded. I'm happy to be proven wrong here, but would appreciate the inclusion of Feynman diagrams to support the author's interpretations.

Response: We agree with the Reviewer that in a third order PIA spectrum, the dynamics happen in the single exciton manifold during the time between excitation and detection pulses. In the largely annihilation-free regime in which our 2DES measurements are performed (10 nJ/pulse), the two exciting pulses create population in the single exciton manifold that is then probed with the third pulse and gives rise to a PIA feature. We see this PIA feature at T=0 meaning that excitation to the biexciton is possible within the time duration of our pulse (6 fs, IRF of 9fs). We emphasized the instantaneous access to biexciton states because in other materials, biexciton formation is not necessarily instantaneous. For example, in monolayer MoS₂, biexciton formation requires ~50 fs (<https://pubs.acs.org/doi/full/10.1021/acs.jpcclett.0c00169>). It is possible that in MoS₂, relaxation of the single exciton is required before a biexciton can be formed, disallowing instantaneous biexciton formation. From our pump-probe measurements, we see that signal scales nonlinearly with excitation fluence and EEA is prominent, proving that biexcitons are formed and no spectral evolution is observed after ~20 fs suggesting that they are formed instantaneously. The observation of instantaneous PIA made us comment that in this material, the formation of biexcitons does not require a delay after single exciton formation. This is important because in a fifth order measurement, we can directly create multiple excitons using the initial excitation pulses. If the PIA feature were not instantaneously present, we would not be able to optically access the biexciton state with our excitation pulse. To clarify this point in the manuscript, we have now added the following sentence in the second paragraph of the 2DES section of the main text:

“In other materials, like monolayer MoS₂, biexciton features do not appear in third order experiments until the system has evolved for about 50 fs following direct excitation precluding direct optical excitation of biexcitons with a single short (6 fs) laser pulse.”^{32,,}

In summary, the $|ee\rangle$ state is accessed through the probe pulse in third-order 2DES and pump-probe measurements at 0 fs separation between excitation and detection pulses, showing that it can be optically accessed. The quadratic dependence of the signal with fluence in pump-probe measurements shows that this state is also accessed through multiple pump interactions in high-fluence pump-probe spectra. We have also included a Feynman pathway in Figure 2 (shown in response to the next comment) for the PIA feature that shows the $|ee\rangle$ state being accessed instantaneously in waiting time i.e., between the pump and probe pulse pairs.

4: I also encourage the authors to include energy level diagrams to support the discussion on the energetics in lines 150-156.

Response: We have added a band diagram and associated Feynman pathways to a reworked Figure 2. We assume bandlike electronic structure based on the strong-coupling limit description of Hestand and Spano of H- aggregation.
“

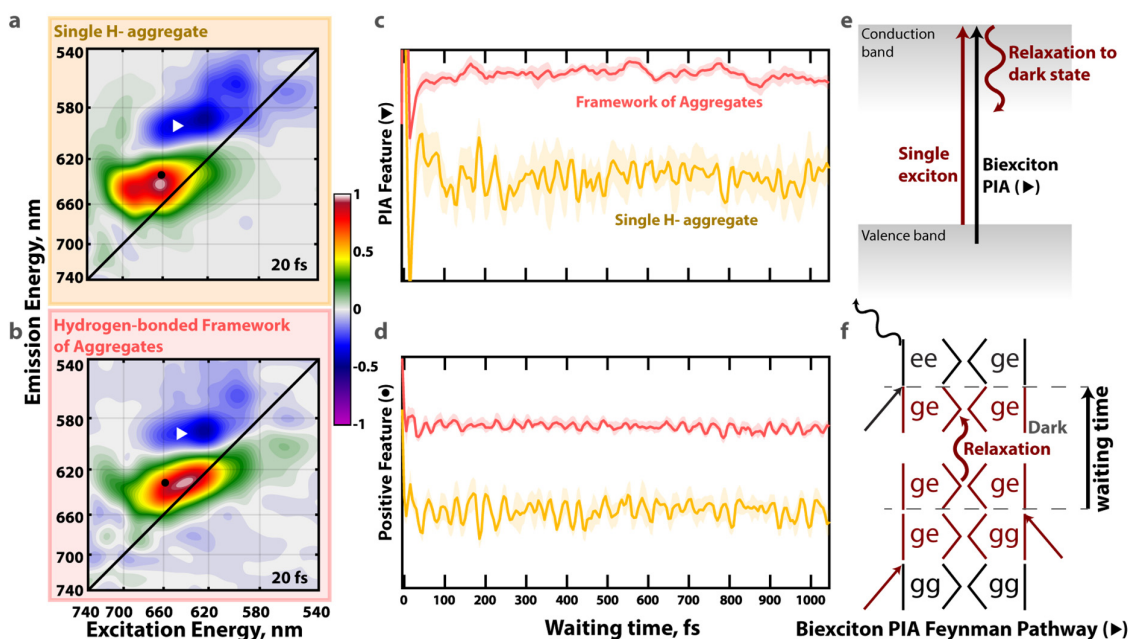


Figure 2: Representative 2DES spectra of **a)** isolated ZnOPPc aggregates and **b)** HOF at 20 fs. **c)** normalized and offset photoinduced absorption and **d)** ground-state bleach feature time traces from 2DES data from the marked points in **a** and **b**. Pump, probe and local-oscillator polarizations are kept parallel to each other when collecting this data. **e)** Energy-level diagram of the aggregate bands to depict biexciton formation and the negative PIA feature observed in both aggregates in **a** and **b**. The corresponding biexciton ($|ee\rangle$) formation Feynman pathway is shown in **f**. Pump interactions are shown in maroon. The probe interaction, or biexciton formation process, is shown in black.”

5: Lastly, I have a few minor corrections/clarifications:

5a: What is meant by "strong-coupling limit" in line 42? (What strong coupling criterium is referred to?)

Response: We use the phrase, “strong coupling” as per the work of Spano and coworkers. We have now added the following sentence in the first paragraph:

“Chromophores are said to be strongly coupled when the coupling energy is much larger than the energies of active vibrational modes of the chromophores^{12,13}.”

We have cited the review by Hestand and Spano that describes the electronic structure and strength of coupling in H- aggregates. Comparing our samples to the many aggregates studied in their review, we find a coupling of $\sim 1000 \text{ cm}^{-1}$ based on the blueshift of the aggregate from the monomer, and a nearest-neighbor coupling assumption. For this value of coupling, the theory suggests that vibrational fine structure should be largely wiped out and material-like bands should largely describe the electronic structure.

5b: In line 26, with "without significantly changing underlying electronic structure", I suppose the authors are referring to the molecular electronic structure? I would argue that tweaking inter-aggregate couplings in order to switch between annihilating and non-annihilating regimes would impact the (collective) electronic structure of the HOFs.

Response: We meant that the electronic structure of single aggregates is not changing significantly upon being connected to other aggregates as is revealed by the absorption spectrum. We have now changed the “the underlying electronic structure” to “the electronic structure of single aggregates.”

5c: Band structure "valleys" are mentioned in the introduction and conclusion, but throughout the rest of the article it remains unclear what this refers to.

We have now added the phrase: “valleys in the excited-state band” in our description of the 2DES results. We have also described the valleys in the description of Figure 3 as stated earlier (response to comment #1). We have also used a band picture in the energy level diagram in Figure 2 shown in response to comment #4.

5d: In the caption of fig 2, I believe it should be "a) isolated ZnOPPc H aggregate and b) HOF"

Response: We have now changed the title of Figure 2 to the following:

“Figure 2: Representative 2DES spectra of **a)** isolated ZnOPPc aggregates and **b)** HOF at 20 fs. **c)** normalized and offset photoinduced absorption and **d)** ground-state bleach feature time traces from 2DES data from the marked points in **a** and **b**. Pump, probe and local-oscillator polarizations are kept parallel to each other when collecting this data. **e)** Energy-level diagram of the aggregate bands to depict biexciton formation and the negative PIA feature observed in both aggregates in **a** and **b**. The corresponding biexciton ($|ee\rangle$) formation Feynman pathway is shown in **f**. Pump interactions are shown in maroon. The probe interaction, or biexciton formation process, is shown in black.”

5e: In line 132, "PIA" is not previously defined.

Response: We have now added a few sentences describing the spectral signatures observed in 2DES to the introduction of the 2DES section:

“Positive features in 2DES spectra arise from ground state bleach (GSB) and stimulated emission (SE) Feynman pathways. Negative features in 2DES spectra arise from photoinduced/pump-induced absorption (PIA). Excited-state absorption of pump-excited chromophores and ground-state absorption of coupled chromophores creates PIA signals in 2DES spectra.”

5f: In line 157 I am not sure what is meant by the "negligible spectral redshift". With respect to what is this redshift determined?

Response: We observe that the PIA from biexciton formation has a negative peak in 2DES spectra at a wavelength bluer (600 nm) than the excitation wavelength and the absorption maximum of the aggregate at 650 nm.

Reviewer #2 (Remarks to the Author):

In this paper, the authors characterize the ultrafast exciton dynamics in H aggregates of phthalocyanines by pump-probe and 2D electronic spectroscopy. Through the comparison between the response of isolated H-aggregates and H-bonded H-aggregates, they extract important information on the mechanism of exciton-exciton annihilation (EEA). The main conclusion is that EEA requires inter-aggregate interactions.

In general, I believe that the results obtained from the study are meaningful and have the potential to offer useful insights into ways of enhancing the optoelectronic properties of these materials. Specifically, minimizing EEA could lead to an increased dynamic range. The data are carefully taken, and the methodology is sound. However, the interpretation of the data is not fully convincing. Therefore, I cannot recommend publication in Nature Comm in the current form, but I would be willing to reconsider a revised version where the presentation of the data and the interpretation and discussion of the results are improved, as detailed below.

1) My first difficulty was with the contents flow. I found it difficult to follow the flow of the discussion, thus I recommend to re-organize partially the contents (especially of the intro and discussion).

1a) On page 1 the authors quickly state that:

‘Coherent suppression of exciton-exciton annihilation is attributed to highly oscillatory spatial wavefunctions in H-aggregate bands that make the sum of wavefunction coefficients over all chromophores vanishingly small in the strong-coupling limit’.

While these are already published results, the sentence is very cryptic and difficult to understand, especially for a non-expert. In effect, a better explanation is given later in the ‘Discussion’ section, but I believe that it is too delayed. In my opinion, the introductory section is too brief and not informative enough. On the other hand, most of the descriptions and the concepts reported in the ‘Discussion’ section are, in fact, introductory concepts (the idea of the oscillatory spatial behavior of the wave function, the typical properties of phthalocyanine H-aggregates, etc.). I suggest that the authors move the information from the discussion section to the introductory section and use the discussion section to establish a more robust connection between experimental data and interpretation.

1b) Several concepts, fundamental for the understanding of the results, are taken for granted. For example:

- the difference between two-excitons and bi-excitons states (as they are important for the interpretation of nonlinear ultrafast measurements)
- the power dependence of third and fifth-order responses
- explain why a second order kinetic is expected in the case of EEA
- ...

I understand that these concepts have been covered in previous literature and the letter format may not allow for lengthy introductions. However, I believe that the manuscript would benefit from a brief introduction of these concepts to enhance clarity.

Response: We thank the Reviewer for this valuable comment. We have taken this comment seriously and have reworked the introduction section of the paper. We have now added sections to describe the concepts involved. We start with a description of delocalization:

“Electronic excitations created in materials upon photoexcitation delocalize over several aggregated molecules when the electrostatic coupling between them is strong¹⁻³. Delocalization changes the photophysical properties of the aggregate relative to the single chromophore and can alleviate trapping processes that halt exciton diffusion⁴; energetic relaxation⁵ and excimer formation⁶. However, it also facilitates exciton-exciton annihilation⁷⁻¹¹, a process that limits the dynamic range and efficiency of light-harvesting at high photon fluence. Chromophores are said to be strongly coupled when the coupling energy is much larger than the energies of active vibrational modes of the chromophores^{12,13,14}.”

We then describe photophysical properties of H- aggregates:

“Positive electrostatic coupling between chromophores results in a blueshift of the absorption spectrum of the aggregate from the constituent monomer’s absorption. Positively coupled aggregates are commonly referred to as H- aggregates¹². Upon positive coupling, the highest-energy eigenstate of the aggregate is optically bright whereas the lowest-energy state is optically dark. This lowest-energy state in H- aggregates is dark because its spatial wavefunction is highly oscillatory, with adjacent monomers in the aggregate having alternating positive and negative wavefunction coefficients. The oscillatory wavefunction of the lowest-energy excited-state makes the transition to it from the electronic ground-state forbidden by selection rules¹².”

Next, we describe the process of exciton-exciton annihilation and how it is typically studied by spectroscopy:

“Exciton-exciton annihilation (EEA)⁷⁻¹⁰ can occur when two excitons collide. It reduces the efficiency of light harvesting at high fluences. EEA is a second-order kinetic effect because it is dependent on two-particle collisions¹⁴ and it results in one exciton decaying to the ground state non-radiatively, thereby lowering the quantum yield of the photovoltaic process. Circumventing EEA could be a possible design route towards increasing the dynamic range in photovoltaics and other optoelectronics.

EEA is routinely probed with fluence-dependent third-order nonlinear spectroscopy^{1,7-10,14-17}. Two-interactions between an exciting pulsed electric field and the material create exciton populations. These populations are then probed with a third field interaction in third-order nonlinear spectroscopy. Upon increasing the fluence of the exciting pulses, the likelihood of fifth-order interactions increases because more excitons are created in the same material, increasing the cross-section of exciton-exciton collisions. At high fluences, exciton dynamics often show a distinct second-order population decay that arises from exciton-exciton collisions leading to annihilation. In nonlinear spectroscopy, a second order effect results from four interactions with the exciting electric field as opposed to two interactions. The likelihood of four interactions increases with increasing pump fluence because more excited-state particles are created within the same sample.”

After having described properties of H- aggregates and exciton-exciton annihilation, we describe how the process can be suppressed in H- aggregates:

“Recently, Tempelaar and coworkers and Huang and coworkers showed theoretically and experimentally that the oscillatory property of H- aggregate wavefunctions could be used to suppress EEA^{16,18}. The EEA rate was shown to be proportional to the square of the sum of wavefunction coefficients along the sites of aggregates by Tempelaar and coworkers¹⁸.”

We have also explained why EEA is still expected to occur at room temperature:

“Huang and coworkers found however that EEA occurs at room temperatures in H- aggregates because exciton diffusion allows collisions between excitons of many different spatial phases¹⁶. At low temperature however, they observed that EEA was strongly suppressed because exciton diffusion slowed down.¹⁶”

We have also explained the difference between biexciton and two-exciton states in the second paragraph of the 2DES section:

“When excitons share a common ground state, excitation into the two-exciton manifold creates a biexciton state of two coupled excitons, which we depict as $|ee\rangle$ in the Feynman pathway in **Figure 2f**, and as an energy-level diagram in **Figure 2e**. This state is different from a state with two non-interacting excitons that are not Coulombically coupled to each other, and from excitation into a higher energy level/band from the conduction band. The ground-state coupling of the two excitons of the biexcitonic

state yields a photoinduced absorption feature in 2DES, yielding a dispersive lineshape along the detection axis^{30,34}. In other materials, like monolayer MoS₂, biexciton formation is not instantaneous and it is possible that some relaxation of the single exciton state is required before the biexciton state can be accessed with third-order interactions³².”

2) My major concern is about data discussion and interpretation.

2a) It is not fully clear to me what the main conclusions extracted from 2DES data are. It is clear to me that the dynamics in the first ps (both in all-linear and in the cross-peak-specific polarization) are the same for isolated and H-bonded aggregates. But is not clear if the discussion reported in lines 131-156 refers to both samples or only to the H-bonded one. In this last case, the differences among samples should be better discussed.

It is also obvious to me that, if excitons are formed, there must be a common ground state. It is also well-known that tetrapyrroles have degenerate orthogonal dipole moments. So I do not understand what are the implications of the sentence: ‘This observation suggests a ground-state coupling (a common ground state) between non-parallel dipoles’ and why this could be interpreted as a signature of biexcitons.

The negative PIA feature in 2DES was attributed to the instantaneous formation of bi-exciton (line 135). Later (lines 157-159), it is said that ‘the negligible spectral redshift of the biexciton photoinduced absorption feature in this system suggests that the biexciton binding energy is low and the excitons are relatively noninteracting’. Thus, would it be more reasonable to attribute the PIA to two-exciton states? While bi-excitons are invoked if no interaction between excitons is seen?

Overall, to summarize, I do not understand what signatures appearing in the 2D spectra should be attributed to the formation of biexcitons and if there is any relevant difference between isolated and H-bonded aggregates. Is this what the author would expect based on their interpretation?

Response: We have now made clear in these lines that we are referring to the spectra of both connected and single aggregates. First, to clearly state our findings for all-parallel 2DES, we have appended a paragraph at the end of the 2DES section with a summary of our findings from these experiments.

We have also performed center-line slope analysis on the ground-state bleach features of the basic and neutral aggregates. We find no appreciable time-dependent changes in the center-line slope after the first 50 fs in either condition, further confirming that the electronic structure of the aggregates does not evolve over the exciton lifetime. In summary, 2DES spectroscopy and its analysis confirm the instantaneous formation of a non-annihilating biexciton state accessed by the probe pulse in both connected and isolated aggregates. This state can also be accessed through higher pump fluences as is shown by the nonlinear signal dependence in pump-probe spectroscopy. We have added sentences clearly explaining the observations from 2DES in the summary section:

“Finely sampled 2DES measurements reveal that the electronic structure of the aggregate and its ultrafast excited state dynamics do not change significantly upon being connected to/disconnected from other aggregates. They also reveal that the excited-state electronic structure does not change significantly after ~50 fs from excitation ruling out processes like excited-state absorption, excimer formation, and trapping, while strongly suggesting the formation of a biexciton upon probe interaction in both samples. Center-line slope analysis of the main ground state bleach feature of the 2DES spectra of both connected and isolated aggregates further confirms that pH change does not impact the excited-state dynamics of the system (Supplementary Figure S23).”

The Supplementary Figure S23 showing the CLS analysis is shown below.

“

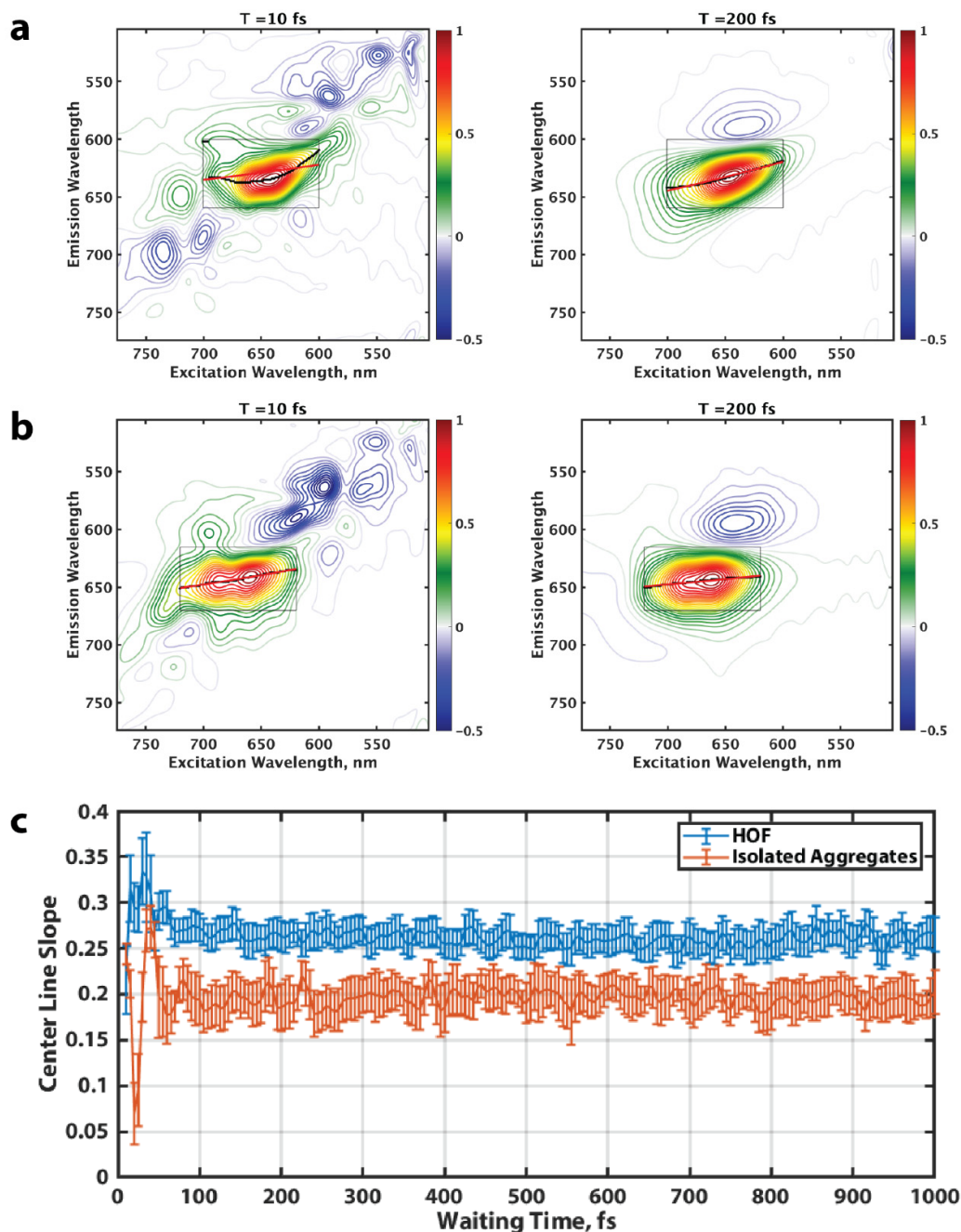


Figure S23. CLS fits to the ground state bleach feature for the (a)framework of aggregates and (b) isolated aggregates for one experimental run. (c) CLS as a function of waiting time in the sub-ps timescale for both aggregate systems.”

We have then added the following sentence to the beginning of the cross-peak specific 2DES discussion (3rd paragraph in the 2DES section) to clearly delineate what we want to observe with these experiments:

“We perform cross-peak-specific 2DES on connected and dispersed H- aggregates to eliminate the prominent ground-state bleach feature to selectively observe the dynamics of the PIA feature.”

We have also added a more detailed description of cross-peak specific 2DES results in the 2DES section:

“In cross-peak-specific spectra of both samples, we see a cross-peak on the diagonal at the location of the ground-state bleach and along the observed PIA feature in all-parallel spectra (see **Supplementary Information Figure S22** for overlaid spectra). This peak is present from $T = 0$, and does not show any appreciable spectral evolution over the finely sampled first picosecond. The instantaneous observation of a cross-peak displaying negligible spectral evolution over delay time in both connected and single aggregates points strongly towards a ground-state coupling between non-parallel transition dipole moments within single H- aggregates. These observations suggest that the PIA feature is unlikely to be excimer formation because no spectral evolution is observed over either 1 ps or the lifetime of the aggregates (see **Supplementary Information Figures S26**).”

We close the 2DES section with the following sentences:

“Our 2DES experiments reveal an instantaneous PIA feature in both aggregate samples which we attribute to biexciton formation upon probe interaction. 2DES allows us to observe the ultrafast formation of this manifold without the pulse overlap artefact that is a limitation in pump-probe spectroscopy. Cross-peak specific 2DES reveals that the biexciton manifold can be instantaneously accessed through non-parallel pump- and probe- interactions, suggesting multiple orthogonal band ‘valleys’ created through Q_x and Q_y π - stacking interactions between adjacent stacked phthalocyanine chromophores within a single aggregate. Both 2DES pulse sequences reveal that this electronic structure is not substantially changed upon association with other aggregates through hydrogen bonding.”

Finally, we have revised Figures 2 and 3 that contain the 2D data to incorporate our key findings and claims into them. Figure 2 now includes a proposed energy level scheme. Figure 3 now includes the formation of orthogonal Q_x and Q_y valleys upon aggregation in both basic and neutral medium.

2b) About the pump-probe results.

What do the authors mean when they write that for isolated aggregates: ‘No fluence second order kinetics is seen’? I guess the main point is that in isolated aggregated they do see a power-dependent amplitude but no change in the values of the time constants. In any case, this is a crucial point and should be clarified.

Most importantly, it is not fully clear to me the connection between pump-probe and 2DES measurements. I was expecting more comments from the authors about the complementarity of the data obtained with the two techniques.

Response: We have now changed the sentence to:

“Although we observe a quadratic increase in pump-probe signal with pump fluence in single aggregates, we do not observe a change in the time-dependent exciton dynamics in single aggregates as a function of pump fluence.”

We have now discussed the pump-probe and 2DES results in conjunction with each other at the end of the section on the pump-probe experiments:

“Our 2DES experiments reveal an instantaneous PIA feature in both aggregate samples which we attribute to biexciton formation upon probe interaction. 2DES allows us to observe the ultrafast formation of this manifold without the pulse overlap artefact that is a limitation in pump-probe spectroscopy. Cross-peak specific 2DES reveals that the biexciton manifold can be instantaneously accessed through non-parallel pump- and probe- interactions, suggesting multiple orthogonal band ‘valleys’ created through Q_x and Q_y π - stacking interactions between adjacent stacked phthalocyanine chromophores within a single aggregate. Both 2DES pulse sequences reveal that this electronic structure is not substantially changed upon association with other aggregates through hydrogen bonding.”

3) My last comment is about the conclusions.

I found the conclusion a bit understated. In their current form, they basically represent a summary of what is already explained. I believe that a paper in Nature Comm should highlight the implications of the obtained results and suggest possible future directions.

For example, I found it very interesting (even though this was not commented by the authors) that chemical stimuli (a pH or a solvent change) were employed to modify the hierarchical inter-aggregate structure of the aggregates promoting/removing inter-aggregate interactions by H-bonds, in turn also modifying the EEA regime. (See also: <https://doi.org/10.3390/molecules28083553>)

This was mentioned quickly in the abstract, but I think this concept would deserve to be emphasized more also in the manuscript conclusions.

Response: We have endeavored our Conclusions section more assertive and forward-looking. We have added the following paragraph at the end of the conclusions section:

“Using a post-synthetic strategy of changing the pH of the aggregates’ medium, we show a switching between annihilating and non-annihilating regimes using a chemical stimulus⁶⁷ at room temperature. This post-synthetic strategy allows us to access both states without significantly modifying the electronic structure of single aggregates and by directly controlling exciton diffusion rather than other properties that are frequently tuned to promote/hinder diffusion like bandgap alignment⁶⁸ and defect placement⁴. The annihilating and non-annihilating regimes could be dynamically accessed with pH changes in future works akin to plant photoresponses that also rely on pH-dependent aggregation⁶⁹.”

We have cited the article suggested by the Reviewer.

4) Additional observations:

4a) In figure 2, panels c and d are missing the numeric scale on the y-axis.

In this panel, the traces are neither normalized, nor plotted on the same scale. We only want to draw attention to the fact that in both cases, the dynamics are identical and that in both cases, the dynamics are relatively non-evolving after ~50 fs.

4b) Lines 163-165 report: ‘We calculate the degree of coupling in the aggregates using the nearest-neighbor approximation to be $V_0 \approx +1000$ cm⁻¹ which is much larger than the static disorder seen in the monomer from negligible dynamic center-line slope change (Supplementary Information Figures S13, S14).’

However, in Figure S13 and S14 there is no data relative to the monomer, nor any CLS analysis.

Response: We incorrectly used the term CLS in the earlier version. This sentence has been changed to:

“We calculate the degree of coupling in the aggregates using the nearest-neighbor approximation³ to be $V_0 \approx +1000 \text{ cm}^{-1}$ which is much larger than the static disorder seen in the monomer from the difference between the diagonal and antidiagonal linewidths of the monomer 2D spectra (Supplementary Information Figure S24)²⁹.”

It has also been moved to the description of the assembly of the aggregates in the larger restructuring that the Reviewer suggested we do.

To calculate the inhomogeneous broadening contribution in the monomer, we calculated the difference between the diagonal and antidiagonal linewidths of the monomer bleach feature at 710 nm at waiting time zero. The diagonal linewidth (full width at half maximum – FWHM from a Gaussian fit) representing the total broadening is 295 cm^{-1} and the antidiagonal linewidth representing the homogeneous broadening is 207 cm^{-1} . This results in an inhomogeneous broadening of 88 cm^{-1} . The following Supplementary Figure 24 shows this analysis:

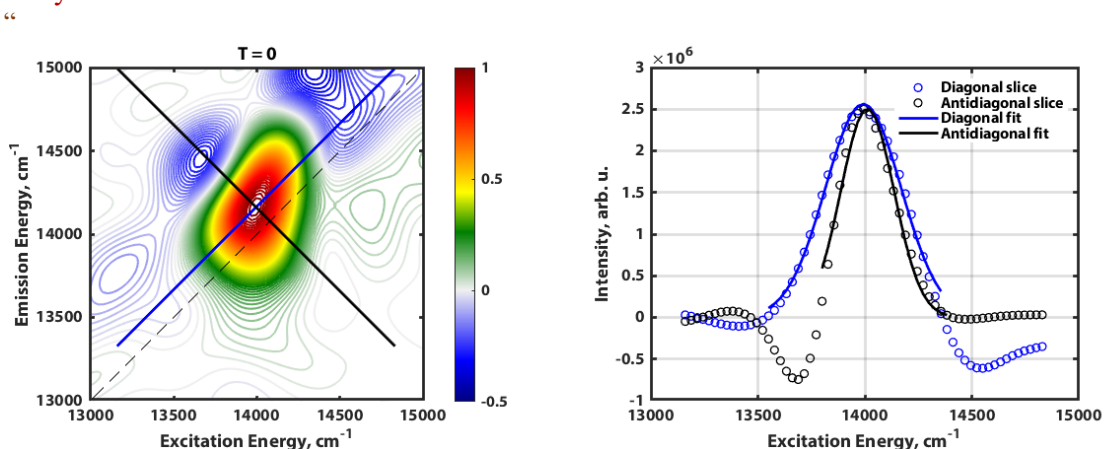


Figure S24. Gaussian fits of the diagonal and antidiagonal slices of the ground state bleach feature of the monomer 2DES spectrum at waiting time zero. The FWHM values from the diagonal and antidiagonal fits are 295 cm^{-1} and 207 cm^{-1} respectively – indicating an inhomogeneous broadening of 88 cm^{-1} .”

4c) There is something wrong with the caption of Figure 4. I also think that in the same figure the traces are normalized to their max (or to the value at 10 ps)? This should be clearly stated. Again about Figure 4, the comparison between the traces for the isolated (yellow) and H-bonded (red) aggregates would have been more elegant by choosing the same fluence values...

Response: We thank the Reviewer for pointing out this omission to us. We have now changed the caption of figure 4. It now reads:

“Dynamics of the pump-probe excited-state absorption feature at 580 nm are shown as a function of delay time for **a**) isolated aggregates and **b**) connected aggregates. In the inset of **a**, the fluence dependence of the signal is shown. It is observed that the magnitude of the signal increases quadratically with pump-fluence, indicating the multi-exciton regime at higher fluences. All traces are normalized to 10 ps. **c**) A schematic showing the difference between connected and isolated aggregates’ dynamics: in connected aggregates, exciton diffusion allows excitons of many different spatial phases to meet whereas in isolated aggregates, the excitons remain on the same quantum site without annihilating.”

We would have also liked to report identical fluences for both samples, but the data were collected in two separate weeks so that the laser power for the same ND values were slightly

different. We measured pulse power to depict accurate power after every measurement to report values accurately. We performed the isolated aggregate experiment with many more fluences to obtain a reliable second-order curve as compared to the connected aggregate experiment, in which we readily found annihilation dynamics consistent with literature:

Kakade, S., Ghosh, R. & Palit, D. K. Excited state dynamics of zinc–phthalocyanine nanoaggregates in strong hydrogen bonding solvents. *J. Phys. Chem. C Nanomater. Interfaces* 116, 15155–15166 (2012).

and,

Li, X., Sinks, L. E., Rybtchinski, B. & Wasielewski, M. R. Ultrafast aggregate-to-aggregate energy transfer within self-assembled light-harvesting columns of zinc phthalocyanine tetrakis(perylenediimide). *J. Am. Chem. Soc.* 126, 10810–10811 (2004).

4d) In lines 148-149 the authors quickly mention that ‘These spectra show a faint 920 cm⁻¹ vibronic beating which is not clearly resolved in the parallel polarization spectra suggesting that...’. However, there is no beating analysis in the main text nor in the SuppInfo. If beatings are mentioned and attribution is attempted, a better analysis should be done (power spectra, Fourier maps, etc.), and a comparison between the beating behavior in all-parallel and cross-peak-specific configurations for both samples should be made.

Response: We realize that an in-depth beating analysis of the spectra is beyond the scope of this work, and we have now removed any discussion of vibronic/vibrational beating. Initially, we only invoked this beating mode to point out that the spectroscopic data obtained in all-parallel and cross-peak-specific spectra are actually different and can be seen when their waiting time traces are compared, but in light of many other previous works, we feel that we do not need to justify this fact further with the beating argument.

Reviewer #3 (Remarks to the Author):

The manuscript by Sohoni et. al. reports difference in exciton behavior of an octa carboxylic derivative of phthalocyanine (namely, Zinc(II)-2,3,9,10,16,17,23,24-octa(4-carboxyphenyl)phthalocyanine) in alkaline and at neutral pH. Authors observed a few contrasting behavior in alkaline and neutral pH. Based on the observations author claim absence of exciton annihilation in alkaline pH, but dominant exciton annihilation dynamics in neutral pH. While exciton annihilation is obvious in extended aggregates of this kind of molecule, author highlights biexciton formation in alkaline medium which inhibit annihilation. Authors have proposed small disconnected aggregates exists in alkaline pH gets connected in neutral pH (termed as HOF). While HOF (at neutral pH) sample has been well characterized by DLS, AFM and TEM, aggregate structure in alkaline solution is not characterized. Further formation of biexciton in alkaline pH is not rationalized. Analysis of the 2D electronic spectroscopic results is also appears incomplete. More detailed 1D pump-probe experiments are also required. Authors highlight suppressed exciton annihilation in aggregate (alkaline pH) is similar to that recent report by Prof. Huang and Coworkers, which showcases influence of destructive interference between excitons in H-aggregates suppressing exciton annihilation. However, the case in present manuscript is different. If tightly bound biexciton persist in a small dimer (or small oligomer), there would be no annihilation for obvious reason of absence of another exciton/biexciton to interact. Overall, there are several shortcomings in the manuscript with respect to characterization, spectroscopic measurements, data analysis and

data interpretation and hence not recommended for publication in Nature Communication. Following are the comments in detail.

1. What is the nature of the aggregates at pH 11. Due to deprotonation of carboxylic acid groups at alkaline pH, it is understandable very small aggregates will be formed. Are they just dimer, trimer or small oligomer? What DLS measurement say? AFM or TEM is not suitable for such scenario as agglomeration would happen upon solvent drying. If very small aggregates (like dimer or trimer) form at pH 11, it is obvious that only one exciton would reside per aggregate and no annihilation would happen. In case of bound biexciton (as proposed by authors), same is the expectation.

Response: We thank the Reviewer for this insight. We have now added the DLS measurements for basic aggregates to the SI (Figure S12) as shown below.

“

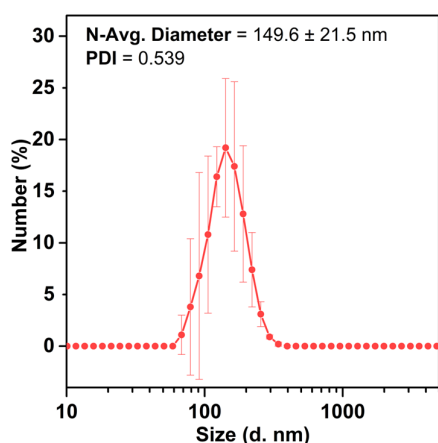


Figure S12. Number-averaged diameter and polydispersity index (PDI) for a water dispersion of the ZnOPPC basic aggregate, measured by DLS.”

We observe that the basic aggregates are ~150 nm in size, which is smaller but still similar to the size of neutral aggregates (~215 nm from DLS – Figure S9). The direct comparison is a bit fraught because the aspect ratio of the particles seems to differ in the electron microscopy. Perhaps more important than the physical size measured by hydrodynamic volume is the effective size of the electronic delocalization. We have now calculated this size to be 11 ± 3 monomeric units (see Supplementary Information section 4.2 and the response to comment #2 of Reviewer 1). We conclude that the effective number of monomeric units across which the electronic state is initially delocalized in the basic and neutral aggregates are the same because of their similar absorption spectra (Figure 1 of main text) and excited state dynamics (Figure 2 and 3 of main text). If aggregates formed in basic conditions have less electronic delocalization, this would change the ground-state electronic structure which would result in appreciable changes to the UV-Vis spectrum that we do not observe in our data. From their nearly identical excited-state dynamics seen in our 2DES results, we can conclude that the neutral and basic aggregates do not substantially differ in their excited-state electronic structures either. Lastly, the signal’s quadratic dependence on pump fluence in pump probe measurements (while holding probe fluence constant) confirms that our excitation pulse is creating more than one exciton residing on the basic aggregates, as this would not have occurred if pump-induced biexciton formation was absent. Therefore, the suppression of exciton-exciton annihilation in

the basic aggregates cannot be the result of only one exciton residing on the basic aggregates after photoexcitation.

2. What is the pKa of carboxylic acid groups in the molecule? Typically, $pK_a < 6$ is expected for carboxylic acid group. It is surprising to see that at $pH = 7$, authors claim neutral CO_2H groups which helps extended hydrogen bonded framework (HOF). A pH titration curve of absorption and fluorescence spectral behavior can provide clear transition from small aggregate to HOF structure.

Response: We agree with the Reviewer that we expect the carboxylic acid groups of the ZnOPPc acid monomer to be at least partially deprotonated at neutral pH due to typical pK_a values for organic acids. However, the energetic benefit of extended HOF formation in aqueous conditions will compete with this deprotonation and would likely change the pK_a values of the hydrogens on the carboxylic acid groups of ZnOPPc monomers that make up the extended HOF. We have now conducted linear absorption measurements for the HOF and isolated aggregates as a function of concentration. These experiments (the rationale detailed in Section 1.5 of the Supplementary Information) show that monomers are not in solution when the solvent contains water. Fluorescence is suppressed upon H-aggregation; therefore, absorption spectroscopy would be the most informative characterization of changes in spectral behavior due to pH. We have conducted titrations of ZnOPPc aggregates in conjunction with linear absorption measurements at different pH values during the titration. As explained below, we were not able to conduct pK_a measurements for the monomer.

“1.5. Aggregate linear absorption spectra as a function of pH

Additional titrations were performed in conjunction with linear absorption measurements to spectroscopically characterize the effect of changing pH on the ground-state electronic structure of the ZnOPPc aggregates. A pH electrode (Oakton pH 5 Acorn series) was submerged in an aqueous neutral ZnOPPc aggregate solution, and the pH of the solution was adjusted to approximately 4 through the addition of aqueous HCl ($pH\ 1.4$). During the titration, small amounts of aqueous NaOH ($pH\ 12.9$) were added to the solution under stirring while the pH of the solution was measured. At selected pH values, an aliquot of the ZnOPPc aggregate solution was pipetted into a 1 mm quartz cuvette and its UV-Vis absorption spectrum was taken on a Cary 5000 UV-Vis-NIR spectrometer. After the absorption spectrum was taken, the ZnOPPc solution in the cuvette was transferred back to the original solution and titration with NaOH was resumed. There was some drift of the pH electrode from the time the UV-Vis spectrum was taken and the time the titration was resumed; however, this pH drift was relatively small, with the maximum magnitude being 0.15. For consistency, we have reported the pH values read at the time that the UV-Vis absorption spectrum was taken for all spectra.

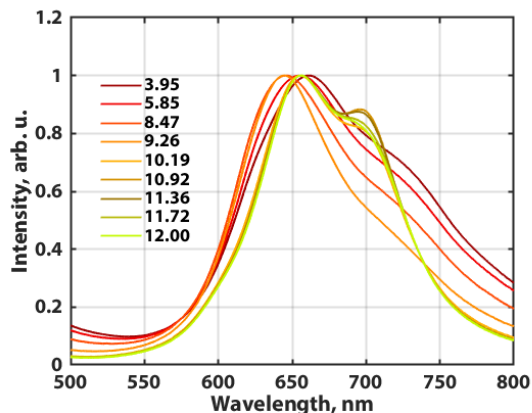


Figure S14. Normalized linear absorption spectra of the aggregate solution as a function of pH.

Figure S14 shows normalized linear absorption spectra of aqueous ZnOPPc aggregates taken at different pH points during titration with NaOH. All spectra consist of the main aggregate absorption peak at approximately 650 nm as well as a vibronic shoulder located at 700 nm. From pH 3.95 to 9.26, an increase in pH results in a blueshift of the aggregate absorption maximum from 662 nm to 647 nm. The magnitude of the blueshift of the aggregate absorption maximum from the monomer absorption maximum (700 nm) can be interpreted as the magnitude of coupling between monomers through face-to-face π -stacking in the ZnOPPc aggregates from pH 3.95 to 9.26. Although we cannot definitively assign a cause to this increase in coupling, we speculate that at the most acidic pH, protonation of nitrogen atoms on the phthalocyanine ring of ZnOPPc may lead to positive charge buildup on the monomers. This increased charge would result in electrostatic repulsion which would increase the distance between monomers and decrease coupling. As the pH is raised from 3.95 to 9.26, decreased protonation would lead to the increased H-aggregation evident in our absorption spectra. From pH 9.26 to 10.19, there is an abrupt redshift in the absorption maximum from 647 nm to 655 nm. We speculate that in this pH range, the carboxylic acids that hold together the HOF finally deprotonate leading the aggregates to separate and the increased charge on the monomers in these isolated aggregates decreases coupling leading to the observed redshift. From pH 10.26 to 12.00, there are only slight changes in the UV-Vis absorption spectra of the ZnOPPc aggregates, suggesting that changes to electronic structure with increasing pH are negligible in this regime.

The consistent appearance of a shoulder peak around 700 nm (the expected position of the monomer Q-band transition) in the linear absorption spectrum of both neutral and basic ZnOPPc aggregate solutions leads us to question whether aggregates and monomers coexist in appreciable amounts in aqueous solution. We conducted linear absorption measurements on neutral and basic aggregate solutions of different concentrations that were prepared through serial dilution. The reasoning behind the experiment is noted below.

We observe narrowing of the spectral features when changing from pH 9.26 to 10.19. We attribute this narrowing to decreased static disorder. We cannot definitively assign the origin of this disorder, but it likely arises from one of two sources in the HOF samples: either disorder amongst the aggregate environments within the extended HOF or disorder within individual aggregates due to the partial protonation of the monomer carboxylic acid groups. Destruction of the extended HOF and the resulting full deprotonation of the carboxylic acid groups reduces the static disorder leading to narrowing of the aggregate peaks in the linear absorption spectrum.

We calculate that each H-aggregate in the extended HOF contains 11 ± 3 ZnOPPc monomers. This value for the electronic delocalization represents a lower bound for the physical size of the aggregate. Therefore, we can write an approximate equilibrium expression for the process of disaggregation as follows:

$$\begin{aligned} \text{ZnOPPc}_{\text{aggregate}} &\rightarrow 11 \text{ ZnOPPc}_{\text{monomer}} \\ K_{eq} &= \frac{[\text{ZnOPPc}_{\text{monomer}}]^{11}}{[\text{ZnOPPc}_{\text{aggregate}}]} \end{aligned} \quad \text{Equation S1.1}$$

Suppose a solution of ZnOPPc aggregates and monomers at equilibrium was diluted by a factor of n . We can calculate the reaction quotient, Q , for this process as follows:

$$Q = \frac{\left(\frac{[\text{ZnOPPc}_{\text{monomer}}]}{n}\right)^{11}}{\left(\frac{[\text{ZnOPPc}_{\text{aggregate}}]}{n}\right)} = K_{eq} \left(\frac{1}{n}\right)^{10} \quad \text{Equation S1.2}$$

Upon dilution, Q would become less than K_{eq} . To restore equilibrium, we would expect that more monomer would be formed. This evolution of free monomer would manifest in our absorption spectra as the growth of a relatively sharp peak at 700 nm compared to the main aggregate peak at approximately 650 nm. We do observe such a peak as we transition from an acidic/neutral aggregate (HOF) to a basic aggregate (isolated aggregate) as is evident in Figure 1 of the main text and in Figure S14. However, titrations performed below in Figure S15 suggest that no free monomer population exists in our solution, meaning that that feature is likely vibronic structure amplified by H-aggregation.

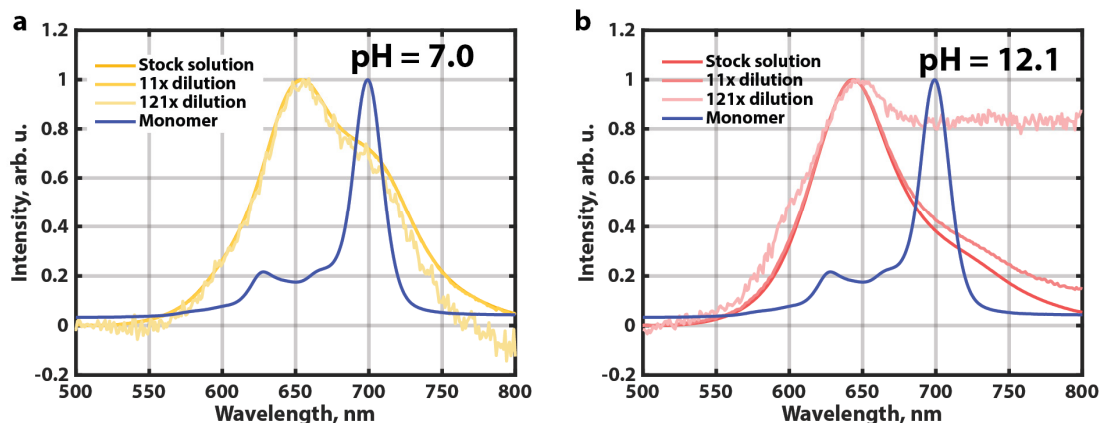


Figure S15. Normalized aggregate linear absorption spectral variation with concentration at (a) pH 7.0 and (b) pH 12.1. The normalized monomer spectrum is shown in both figures for context.

Figure S15 shows normalized linear absorption spectra of a series of aqueous neutral and basic aggregate solutions prepared to be different concentrations through serial dilutions by a factor of 11. The neutral aggregate solutions were prepared and diluted with phosphate-buffered saline (HyQ[®] DPBS), while basic aggregate solutions were prepared and diluted with an NaOH stock solution of pH 12.1. The pH values of all neutral and basic aggregate solutions were in the ranges of 6.97-7.00 and 12.09-12.14, respectively, which eliminates potential effects of changing pH on the ground state electronic structure. Peaks at 650 nm and the red shoulder at ~700 nm are visible in the absorption spectra of both basic and neutral aggregates even after dilution by a factor of 121, although the spectra of aggregate solutions diluted by a factor of 121 display visible noise. Upon diluting the neutral aggregate solution, there is a slight increase in relative intensity of the 700 nm peak; however, the shape of the spectrum does not significantly change. Thus, we attribute this increase in relative intensity of the 700 nm to a drift of the baseline during the experiment which is evident from the increased absorbance in the 700-800 nm region of the spectrum in more dilute solutions. Upon dilution of the basic aggregates, we see no increase in relative intensity of the 700 nm peak compared to the 650 nm peak. In fact, the spectra of the basic aggregate stock solution and the basic aggregate solution diluted by a factor of 11 are nearly identical. Therefore, we conclude that the peak at 700 nm in the aggregate spectrum is a vibronic shoulder and not due to monomer absorption. Furthermore, we conclude that aggregates and monomers do not coexist in appreciable amounts in aqueous solution.”

As a result, we cannot titrate the monomer with aqueous NaOH or HCl to determine its pKa because aggregation persists even at low concentration. Therefore, we addressed the Reviewer's comment by performing titrations of ZnOPPC aggregate solutions starting from acidic and basic conditions. These titration curves and their interpretations have been added to Section 1.4 of the Supplementary Information and are also included below:

“1.4. Acid-Base titration of the ZnOPPC aggregates

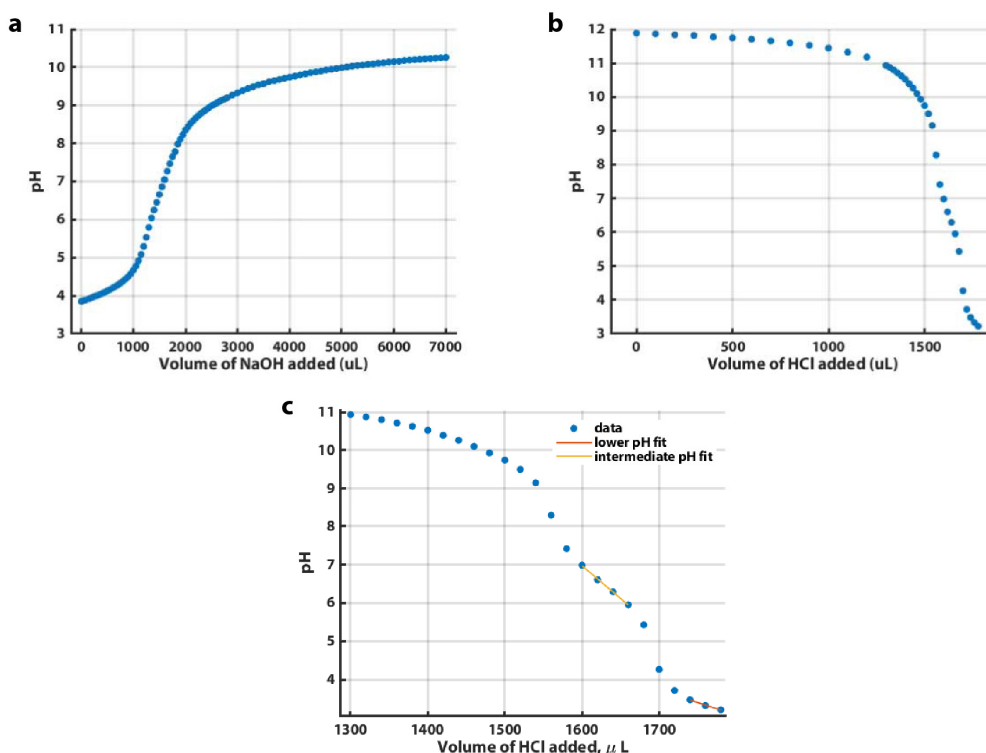


Figure S13. (a) Titration of acidic ZnOPPc aggregates at an initial pH of 3.85 with aqueous NaOH (pH = 10.92). (b) Titration of basic ZnOPPc aggregates at an initial pH of 11.88 with aqueous HCl (pH = 1.26). (c) Inflection regions of the data shown in part b shown with linear fits to the buffer regions with R^2 values of > 0.99 .

Acid-base titrations were performed on the ZnOPPc aggregate to better understand the pH dependent chemical changes to our system. ZnOPPc acid powder (3.43 mg) was first dissolved in a minimal amount of DMSO and sonicated at room temperature in a water bath for 90 minutes. A concentrated neutral ZnOPPc aggregate solution was then prepared by adding 5 drops of the ZnOPPc acid monomer solution and 10 drops of deionized water in a large vial followed by sonication for 5 minutes. The neutral ZnOPPc aggregate solution was subsequently diluted through addition of 15 mL of deionized water. Immediately prior to the titrations, aqueous HCl or aqueous NaOH was added to two neutral ZnOPPc aggregate solutions to modify the pH to be 3.85 and 11.88, respectively. A pH electrode (Oakton pH 5 Acorn series) was then submerged in the acidic or basic ZnOPPc aggregate solution and the pH was recorded after small amounts of aqueous HCl or NaOH stock solutions were added under stirring. The titration curve of acidic ZnOPPc aggregates with aqueous NaOH is sigmoidal in shape with an equivalence point at pH 7 (Figure S13a). This resembles what is expected for the titration of free HCl in solution with aqueous NaOH. Thus, there is no obvious deprotonation of the carboxylic acid groups of the acidic ZnOPPc aggregates upon addition of NaOH. This is in stark contrast with the titration curve of the basic ZnOPPc aggregates with aqueous HCl, which shows two clear equivalence points at pHs of approximately 5 and 8 as well as two linear buffer regions from pH 3.22-3.48 and 5.95-6.98 (Figure S13b). The difference in shape between the titration curves of acidic and basic ZnOPPc aggregates suggests that we are titrating two different chemical species. Figure S13a likely corresponds to the titration of acidic ZnOPPc aggregate framework which eventually breaks up in high pH conditions at the end of the titration. The titration of basic ZnOPPc aggregates should initially start with disordered aggregates in solution; however, it is possible that these disordered aggregates are in a kinetic trap in which the H-bonded framework expected for neutral ZnOPPc aggregates is not restored upon decreasing

the pH during the titration. If this is the case, the titration of the disordered basic ZnOPPC aggregates which do not have hydrogens involved in the formation of a hydrogen-bonded framework should better reflect the acid-base chemistry of the free ZnOPPC acid monomer. Performing a linear regression analysis of the buffer regions (pH 3.22-3.48 and 5.95-6.98) of the basic ZnOPPC titration curve and calculating the expected pH at the midpoints of these buffer regions, we estimate pKa values of 3.34 and 6.46 (Figure S13c). These pKa values are in the expected range for hydrogens of carboxylic acid groups and therefore likely correspond to protonation of the hydrogens on the carboxylic acid groups of the ZnOPPC acid monomers of the disordered aggregates.”

3. It is intriguing to see quadratic power dependence of TA signal of unconnected aggregates which prompted authors to consider biexciton formation. What drives (selection rule) biexciton formation instead of single exciton? Authors are advised to propose energy level scheme to illustrate how pump excitation lead to biexciton generation. What are the spectroscopic signatures of biexciton which distinguishes from exciton? How much is the biexciton binding energy?

Response: We have now proposed the energy-level scheme for biexciton formation in Figure 2 as shown in the response to comment #4 of Reviewer 1.

The spectroscopic signature of the biexciton in 2DES and pump-probe is the rise of a PIA feature at $T = 0$ that does not show any spectral evolution over 1 ps in 2DES and 100 ps in PP. We have also explained how a biexciton is different from two excitons in the main text:

“When excitons share a common ground state, excitation into the two-exciton manifold creates a biexciton state of two coupled excitons, which we depict as $|ee\rangle$ in the Feynman pathway in Figure 2f. This state is different from a state with two non-interacting excitons that are not Coulombically coupled to each other, and from excitation into a higher energy level/band from the conduction band. The ground-state coupling of the two excitons of the biexcitonic state yields a photoinduced absorption feature in 2DES, yielding a dispersive lineshape along the detection axis^{30,34}. In other materials, like monolayer MoS₂, biexciton formation is not instantaneous and it is possible that some relaxation of the single exciton state is required before the biexciton state can be accessed with third-order interactions³².”

We estimated the biexcitonic binding energy to be around 400 cm⁻¹. This was obtained by subtracting the energy corresponding to the linear absorption peak maximum from the emission energy of the cross peak maximum (from the cross-peak specific spectrum). We have added this information to the penultimate paragraph of the 2DES section of the main text.

“The biexcitonic binding energy can be estimated from the difference between the emission energy of the cross peak maximum (biexciton signature) and the linear absorption peak and is calculated to be around 400 cm⁻¹.”

We attribute the PIA feature to biexcitons since both all-parallelly polarized and cross-peak specific 2DES spectra reveal that the PIA feature is only slightly blue-shifted from the ground state bleach feature. As noted above, our best estimate of the biexcitonic binding energy is 400 cm⁻¹, but the difference in lineshapes between the PIA and GSB features make it hard to accurately assign this value from the 2DES alone necessitating comparison to linear spectra.

4. Given the absorption feature of both disconnected small aggregates and HOF is quite similar, quadratic power dependence behaviour is expected for HOF sample as well. Such data is not presented. Is it concluded that biexciton formation is not there in HOF sample?

Response: This data is not explicitly presented because the HOF sample already shows fluence-dependent annihilation (a second order effect) in agreement with previous reports (<https://doi.org/10.1021/ja047176b>), confirming already that second-order effects are occurring in this sample. This data is now fit to a second order rate expression as shown in Supplementary Information Section 4, confirming that while at $T = 10$ ps, the signals are identical for all fluences, they decrease in a second-order fashion between $T = 0$ ps and $T = 5$ ps. We refrain from plotting the quadratic dependence of the signal because we slightly adjusted the ND filter before the camera to avoid camera saturation from small drifts due to laser fluctuations. Data was collected in a staggered fashion with one run of each fluence before moving to the next set of all fluences to ensure that laser fluctuations did not yield incorrect dynamics. For the basic aggregates, we were careful to not adjust any ND filter (camera and probe) between all runs of all fluences. We also wanted to take more than four fluences (as is the case for the HOF dataset) to show the parabolic dependence of the signal with fluence more clearly. This is also why the fluences don't match as we wanted to fit eight fluences within the power limits of the spectrometer on the day of the experiment.

5. There is no reason to assume intermolecular arrangement in isolated aggregate and extended HOF structure will be same. At alkaline pH, only π - π stacking will dominate for aggregation without hydrogen bonding network. On the other hand, at neutral or acidic pH, extended H-bonding leads to HOF structure where intermolecular arrangement is expected to significantly differ.

Response: We share the Reviewer's concern here. However, the UV-vis spectrum would indicate discrepancies between stacking because the degree of coupling would be very sensitive to it. Both aggregates show nearly identical UV-vis spectra and excited state dynamics, indicating that the packing is not significantly different in the two conditions. We have also changed the title to eliminate the word "confined" which we know to be correct for isolated aggregates, but we cannot prove for excitons within the HOF.

6. Before proceeding to advanced 2D-electronic spectroscopy, transient pump-probe (1D) results for monomer and two different aggregate regimes must be reported and analysed in detail. Comparison of 1D pump-probe results of monomer and two different aggregate regimes is essential to bring out most significant differences in the spectroscopic properties in the two aggregated state. It is imperative to analyse 1D pump-probe data of two different types of aggregates and monomer in comparative manner to establish biexciton formation and its spectroscopic manifestation. In addition, 1D TA experiment up to enough time delay should be reported to measure fate and lifetime of exciton or biexciton (as claimed) in the two types of aggregates.

Response: We have now performed an in-depth kinetic analysis of the PP data. We also report the 2DES (Figure S19) and transient absorption third-order spectroscopy data (Figure S28). Experiments have now been performed to 100 ps for both aggregates and added to the SI (Figure S26). Supplementary Sections 4.1 shows the singular value decomposition analysis.

“

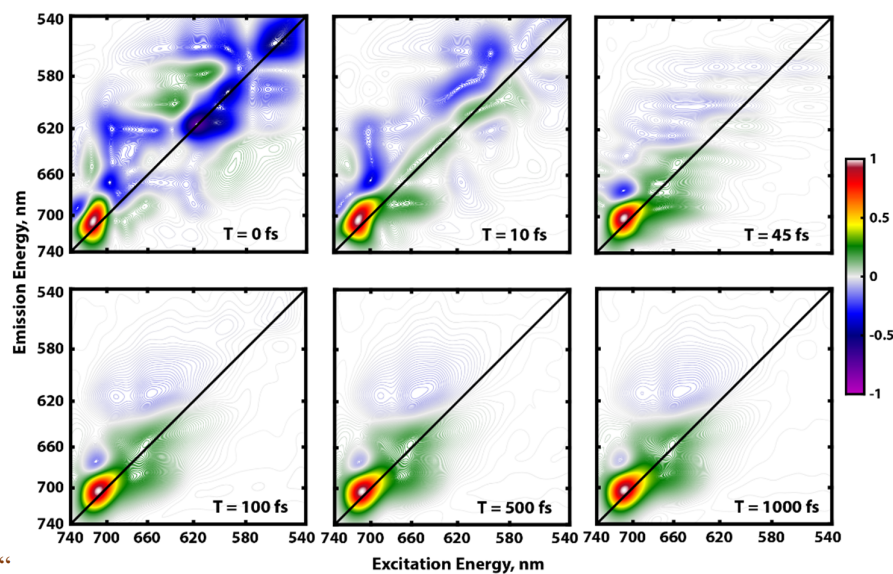


Figure S19. 2DES frames of the ZnOPPC monomer in DMSO with all-parallel pulse sequence. Each frame is normalized to itself.”

4. Transient absorption data and kinetic analysis

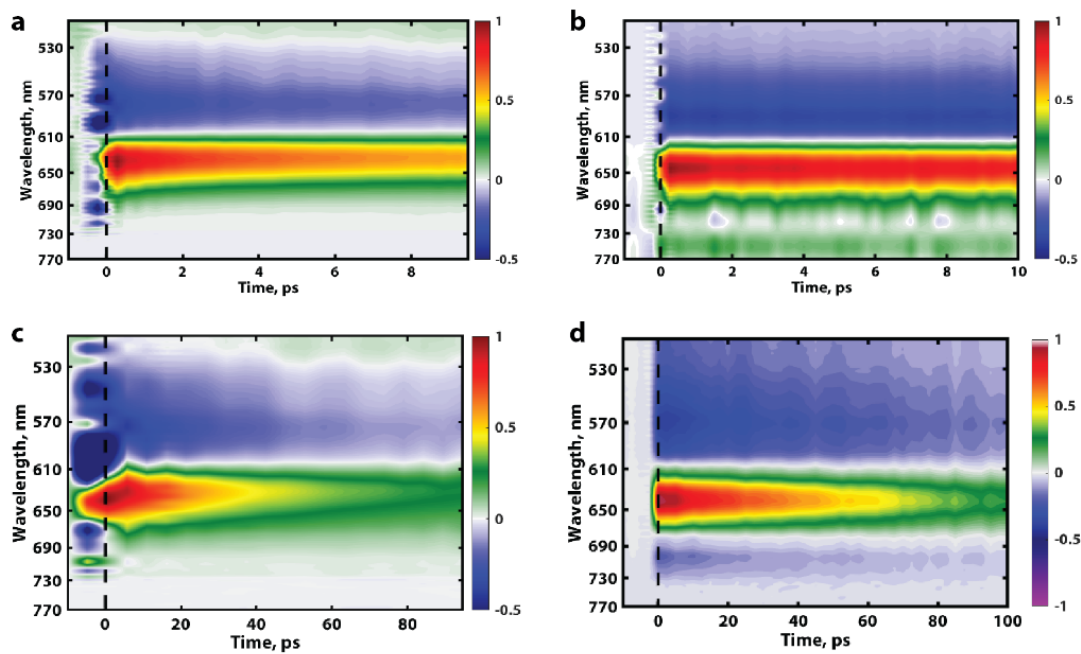


Figure S26. Transient absorption spectrum of the framework of aggregates with a pump fluence of 86 nJ up to (a) 10 ps and (c) 100 ps. Transient absorption spectrum of isolated aggregates with a pump fluence of 52 nJ up to (b) 10 ps and (d) 100 ps.

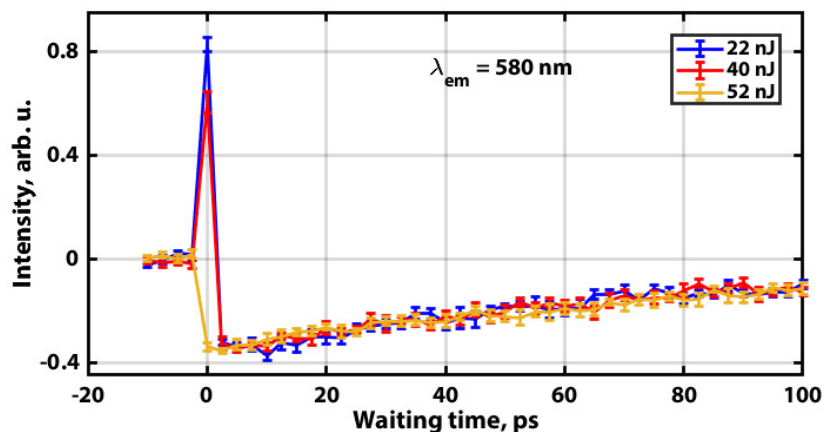


Figure S27. Time traces with standard error plotted for the probe wavelength of 580 nm for isolated aggregates. No fluence dependence is observed during the lifetime of isolated aggregates.

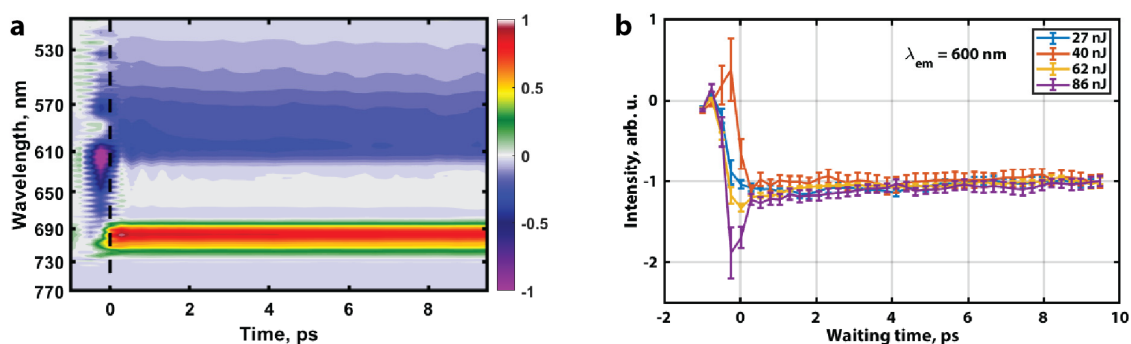


Figure S28. (a) Transient absorption spectrum of ZnOPPC monomers in anhydrous DMSO with a pump fluence of 62 nJ up to 10 ps. (b) Time traces with standard error plotted for the probe wavelength of 600 nm for ZnOPPC monomers. No fluence dependence is observed.

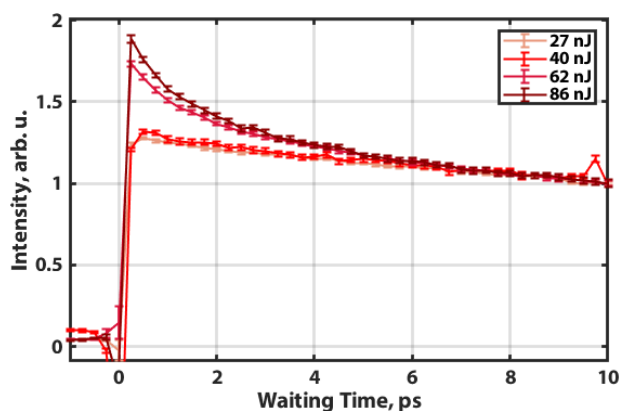


Figure S29. Time traces (normalized to last time point) with standard error plotted for the probe wavelength of 640 nm for HOFs. The positive feature also shows fluence dependence.

4.1. Singular value decomposition of transient absorption data of aggregates up to 10 ps

Singular value decomposition was done on transient absorption data of isolated and framework of aggregates to deconvolve the important kinetic components of the spectra. The first component

largely captures the complete spectroscopic information of the dataset recorded up to 10 ps, as seen from the singular values in Figures S31 and S32.

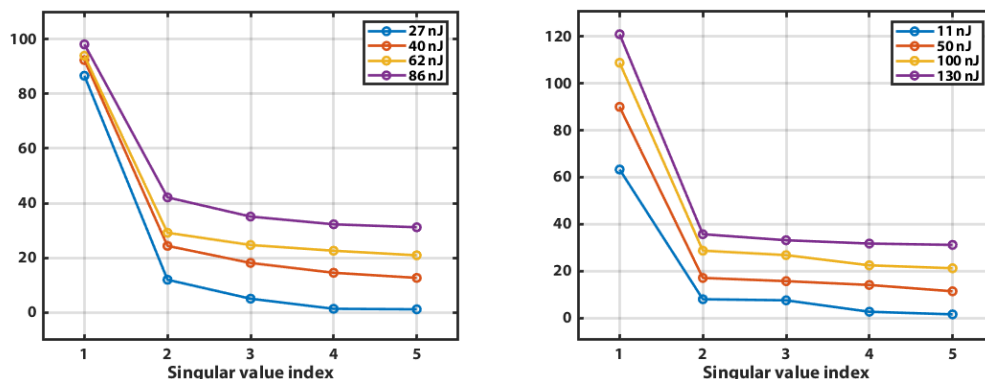


Figure S30. The first five singular values are plotted for the framework of aggregates to the left and for isolated aggregates to the right. The different fluences are separated vertically by 10 units for clarity. For example, to read the singular values of the isolated aggregates at 100 nJ, subtract 20 units from the values plotted.

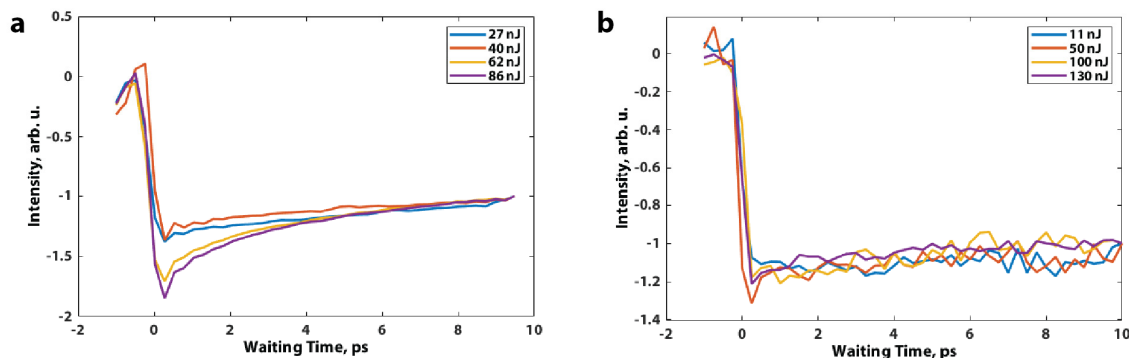


Figure S31. The first kinetic component of the framework of aggregates (a) and for isolated aggregates (b) for different pump fluences. This completely captures the behavior shown in Figure 4 in the main text.

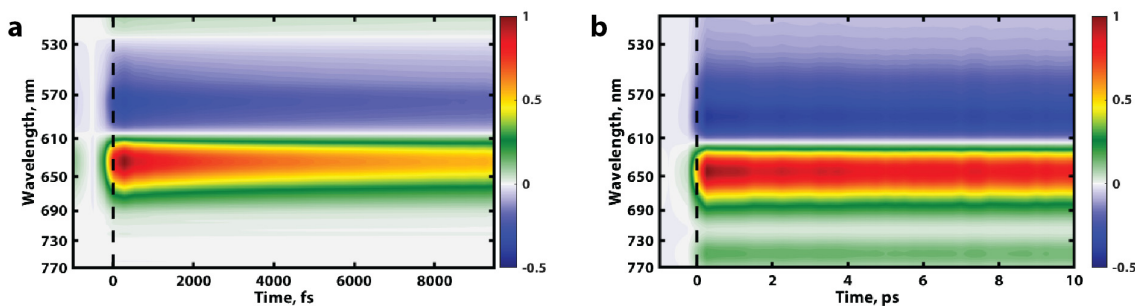


Figure S32. First SVD component for the framework of aggregates with 86 nJ pump fluence (a) and that for isolated aggregates with 130 nJ pump fluence (b). This shows that the first component largely captures the dynamics in the first 10 ps. See Figure S26 for the experimental dataset.

We have also done first and second order fits to time traces of the aggregate pump-probe datasets that have been added to Supplementary Information Sections 4.2 and 4.3 and in the Fluence Dependent Pump-Probe Spectroscopy section of the main text – these are printed in the response to comment #2 of Reviewer 1.

“Singular value decomposition (SVD) of pump-probe data up to 10 ps suggests that there is only one principal component that captures almost all of the signal. This component reproduces the fluence dependence as a function of waiting time in HOF and its absence in separated aggregates as shown in **Supplementary Information Section 4.1**. This justifies our reasoning for fitting only a second order expression to the HOF time traces instead of a sum of second order and first order expressions.”

7. In HOF system, fluence dependent TA kinetics has been reported but no analysis has been attempted to quantify the exciton diffusion parameters. Given the present system is proposed to exhibit long range ordering promoted by hydrogen bonding network, long range exciton diffusion is expected. Evaluation of exciton diffusion parameter and a comparison with reported phthalocyanine systems is necessary to bring out important insights.

Response: We have now calculated the coherence size (the number of monomers across which the HOF excitons are delocalized) following the methodology suggested by Book and co-workers (*J. Phys. Chem. B* **2000**, *104* (34), 8295–8307. <https://doi.org/10.1021/jp000485d>). We obtain a coherence size of 11 ± 3 . We use this number to then calculate the transition dipole strength of HOF which then informs the computation of the number of excitations per HOF absorbing unit (*Nat. Commun.* **2018**, *9* (1), 2466. <https://doi.org/10.1038/s41467-018-04884-4>). Finally, this number is used in the second order rate expression to fit the HOF pump-probe time traces. We have added Supplementary Information Section 4.2 that details the coherence size and excitations per HOF absorbing unit calculation – also printed in the response to comment #2 of Reviewer 1.

We note that the coherence size obtained is much smaller than the number of monomers that are on average in one HOF assembly (as seen in TEM and DLS data). This calculation gives a quantitative picture of the aggregate exciton delocalization in the HOF structures. We have added this information in the main text as shown in the last part of the response to the previous comment.

8. Referring to supporting figure S13 and S14, authors propose 'We calculate the degree of coupling in the aggregates using the nearest-neighbor approximation to be $V_0 \approx +1000 \text{ cm}^{-1}$ which is much larger than the static disorder seen in the monomer from negligible dynamic center-line slope change'. Author should detail (in Supporting data) how V_0 and magnitude of static disorder is calculated.

Response: We have obtained the value of the coupling from the difference between the monomer and aggregate absorption maximum as suggested by Hestand and Spano and we have cited their seminal review and added the following sentence:

“The coupling is calculated as the difference between the aggregate and monomer absorption maxima as prescribed by Hestand and Spano¹².”

We have now calculated the static disorder of the monomer sample by the difference of the diagonal and antidiagonal linewidths and reported it (88 cm^{-1}). The analysis leading to this number is now shown in Figure S24 and printed in the last part of the response to comment #4b of Reviewer 2.

9. Reported analysis of 2D electronic data seems incomplete. It is not clear what new information 2D electronic data brought out in present manuscript? Authors report 'These spectra show a faint 920 cm⁻¹ vibronic beating.....' but FFT spectrum is not reported. What is the origin of the beating? Why is only this single mode (920 cm⁻¹) observed? Why are other high frequency (intramolecular) and low frequency (intermolecular) modes absent?

Response: We have now removed all mention of vibrational beating from the manuscript and the SI. We intend to pursue a detailed analysis of vibrational dynamics separately. We refer the Reviewer to a similar response that we provided to comment #4d of Reviewer 2.

10. Discussion section only describes general aspects of exciton behaviour in organic aggregates without bringing the results of present manuscript into context.

Response: We have now added multiple paragraphs discussing our work in context of other previous works in the 2DES and Conclusions section and have made the Intro section more pedagogical from the suggestions of Reviewer 2. For example, we have now added the following paragraphs:

“Our 2DES experiments reveal an instantaneous PIA feature in both aggregate samples which we attribute to biexciton formation upon probe interaction. 2DES allows us to observe the ultrafast formation of this manifold without the pulse overlap artefact that is a limitation in pump-probe spectroscopy. Cross-peak specific 2DES reveals that the biexciton manifold can be instantaneously accessed through non-parallel pump- and probe- interactions, suggesting multiple orthogonal band ‘valleys’ created through Q_x and Q_y π - stacking interactions between adjacent stacked phthalocyanine chromophores within a single aggregate. Both 2DES pulse sequences reveal that this electronic structure is not substantially changed upon association with other aggregates through hydrogen bonding.”

“When taken together, observations from 2DES and fluence-dependent pump-probe spectroscopy show that biexcitons can instantaneously form on isolated and connected H- aggregates upon interaction with pump or probe light, but that the formation of a biexciton is not sufficient to observe exciton-exciton annihilation. From 2DES, we argue that the biexciton is non-annihilating because the individual excitons within an aggregate are quantum mechanically non-interacting, possibly due to the orthogonal bands formed upon stacking of phthalocyanines, and due to the highly oscillatory spatial phases of the delocalized H- aggregate exciton wavefunction. Pump-probe experiments then confirm that if the non-interacting excitons in the single-aggregate, multi-exciton regime are allowed to diffuse, they lead to exciton-exciton annihilation due to increased sampling of spatial phases and valleys during exciton-exciton collisions.”

11. Minor point: Figure 3 caption is incomplete. Each figure caption should be elaborated.

Response: We have changed the caption of the updated figure 3. It now reads:

“Cross-peak specific 2DES spectra in the (90, 60, 120, 0)° polarization sequence of a) single and b) connected aggregates at 20 fs. Time evolution of the main cross-peak across 1 ps is shown for c) single and d) connected aggregates. e) and f) show the formation of orthogonal bands upon phthalocyanine π -stacking which leads to coupling of orthogonal Q-band dipole moments. g) shows the orthogonal Q_x and Q_y phthalocyanine dipole moments.”

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

The authors did a thorough job addressing my previous comments, leading to significant improvements of their manuscript.

I particularly appreciate the addition of the HOF structure, as well as the author's clarification that this structure should be referred to as rod-like rather than tubular.

I also welcome the expanded analysis of the fluence dependent data shown in fig 4. As to this addition, I wonder if the authors could clarify how exactly the RHS of eq S4.5 depends on the fluence f . I suppose this dependence is contained in the average pump energy E_{pump} , but a clarification at this point would help.

As to the newly-added sec 4.3.2, I wonder if it would be better to do a multi-exponential fit over the entire time range. Presumably, this would identify the same early-time constant, while it omits introducing a (potentially arbitrary) time cut-off.

While I appreciate the addition of the energy-level diagram in fig 2, I believe this representation could be improved. For example, it does not clearly show the band-gap, which is a critical feature of semiconductor materials such as studied here. Also, the diagram does not show the "valleys in the excited-state band".

Lastly, as to the definition of "strong coupling", I would suggest to rephrase the relevant sentence to "Chromophores are said to be strongly coupled to one another when..." which would remove any further ambiguity.

Reviewer #2 (Remarks to the Author):

The authors have addressed all the referees' concerns by including new material, analyses, and figures. The manuscript now reads much better, and the paragraphs that raised objections from the referees have been revised or clarified. Although I still have reservations about the final interpretation of the optical response in the two types of aggregates, I acknowledge the high value of the collected data, the likelihood of the interpretation, and overall, I believe the manuscript deserves publication.

Reviewer #3 (Remarks to the Author):

In the revised manuscript by Sohoni et. al., many of the questions raised in first review have been addressed. However, there are some crucial and important points have not been well addressed. Hence, a major revision is recommended. The main claim in the manuscript is persistent non-annihilating biexciton in isolated aggregate, but not in extended HOF. In figure 4A, they have shown quadratic fluence dependence of transient signal in isolated aggregate, an important observation in favour of the claim that biexciton is generated in

isolated aggregate. However, the same experimental data is not shown in Fig 4b for extended HOF. As the authors claim that there is no biexciton in HOF sample, fluence dependence of transient signal at earliest time must be shown in Fig. 4b. Author has tacitly avoided this comment raised in previous review (Comment 4) by citing ND filter issue in experiment. However, this is an important experimental information which must be provided and highlighted in favour of the claim made in the manuscript. It also remains intriguing and puzzling at the same time; why would biexciton be only formed in isolated aggregate but not in HOF? Providing a molecular level insight would definitely increase the quality of the manuscript.

Response to Reviewers' comments

We thank the Reviewers for their insightful comments on our work. Below, we have attempted to address the comments in detail:

Reviewer 1

The authors did a thorough job addressing my previous comments, leading to significant improvements of their manuscript.

I particularly appreciate the addition of the HOF structure, as well as the author's clarification that this structure should be referred to as rod-like rather than tubular.

I also welcome the expanded analysis of the fluence dependent data shown in fig 4. As to this addition, I wonder if the authors could clarify how exactly the RHS of eq S4.5 depends on the fluence f . I suppose this dependence is contained in the average pump energy E_{pump} , but a clarification at this point would help.

Response: We apologize for the confusion. E_{pump} is the same as f . We have now replaced E_{pump} with f in Eqn. S4.5 in the Supplementary Information.

$$n_{0,f} = \frac{f}{A_{\text{overlap}}} * \frac{\int P(\lambda) * \lambda * (1 - 10^{-A(\lambda)}) d\lambda}{hc \int P(\lambda) d\lambda} * \frac{1}{CN_A d} \quad \text{Equation S4.5}$$

As to the newly-added sec 4.3.2, I wonder if it would be better to do a multi-exponential fit over the entire time range. Presumably, this would identify the same early-time constant, while it omits introducing a (potentially arbitrary) time cut-off.

Response: We acquired new data to attempt this fitting approach and now have recorded transient absorption data at pump fluences where we finely sampled up to 10 ps and sparsely sampled up to 120 ps to allow this sort of analysis. We note that a multi-exponential fit doesn't accurately capture second order kinetics so we attempted to use the equation reported by Huang and co-workers (DOI: 10.1039/c5nr00383k) to conduct our analysis. We indeed obtain reasonable agreement with the same second order time constants. We show this analysis at the end of section of 4.3.2 of the Supplementary Information, as shown below.

We also recorded two fluence-dependent transient absorption data where sub-10 ps data were finely sampled (250 fs steps) and then sparsely (5 ps steps) till 120 ps. This data was acquired to capture the second order kinetics (annihilation) and the first order kinetics (exciton lifetime) in one dataset. Using Eq 4.5 and the procedure detailed in Section 4.2, we calculated the number of excitations per rod ($n_{0,f}$) for pump fluences 60 nJ and 98 nJ to be 1.05 and 1.72 respectively. Following the approach of Huang and coworkers, we assume a time independent rate constant of annihilation and an infinite number of absorbers such that the following solution to the differential rate equation⁸ can be used to describe the complete kinetics of the HOF aggregates.

$$n_f(t) = \frac{n_{0,f} e^{-t/\tau_1}}{1 + \tau_1 \gamma_2 n_{0,f} [1 - e^{-t/\tau_1}]} \quad \text{Equation S4.8}$$

In this equation, $n_f(t)$, τ_1 and γ_2 are respectively the exciton density at time t , the first order time constant, and the second order rate constant. Equation S4.8 can be rewritten in a more linear fashion.

$$\frac{1}{n_f(t)} = \left(\frac{1}{n_{0,f}} + \tau_1 \gamma_2 \right) e^{t/\tau_1} - \tau_1 \gamma_2 \quad \text{Equation S4.9}$$

This equation is linear in e^{t/τ_1} . Fixing τ_1 to 85.3 ps (the exciton lifetime – see Figure S34), we fit Equation S4.9 to the inverse of the signal time traces at a probe wavelength of 580 nm for two fluences; 60 and 98 nJ. We obtain second order rate constants of $1/(2.6 \text{ ps})$ and $1/(2.4 \text{ ps})$ for the pump fluences 60 and 98 nJ respectively. The results are shown in Figure S37. These results are in reasonable agreement with the analysis shown in Figure S35. However, it is important to note that this continuous differential equation is not rigorously appropriate for finite sized molecular systems because second order dynamics cease to be operative when only a single excitation exists on each aggregate. This issue has been studied extensively in the context of photosynthetic antenna complexes but applies generally.⁹ Unfortunately, the finite sized system formulation cannot be analytically solved, but has been shown to match the differential rate laws in all important limits such as short time (second order kinetics), long time (first order kinetics), as well as the limit above of an infinitely connected system with continuous excitation density. For these reasons, we favor extracting the chemically intuitive rate and time constants from early dynamics when second order processes dominate and later times when first order processes dominate.

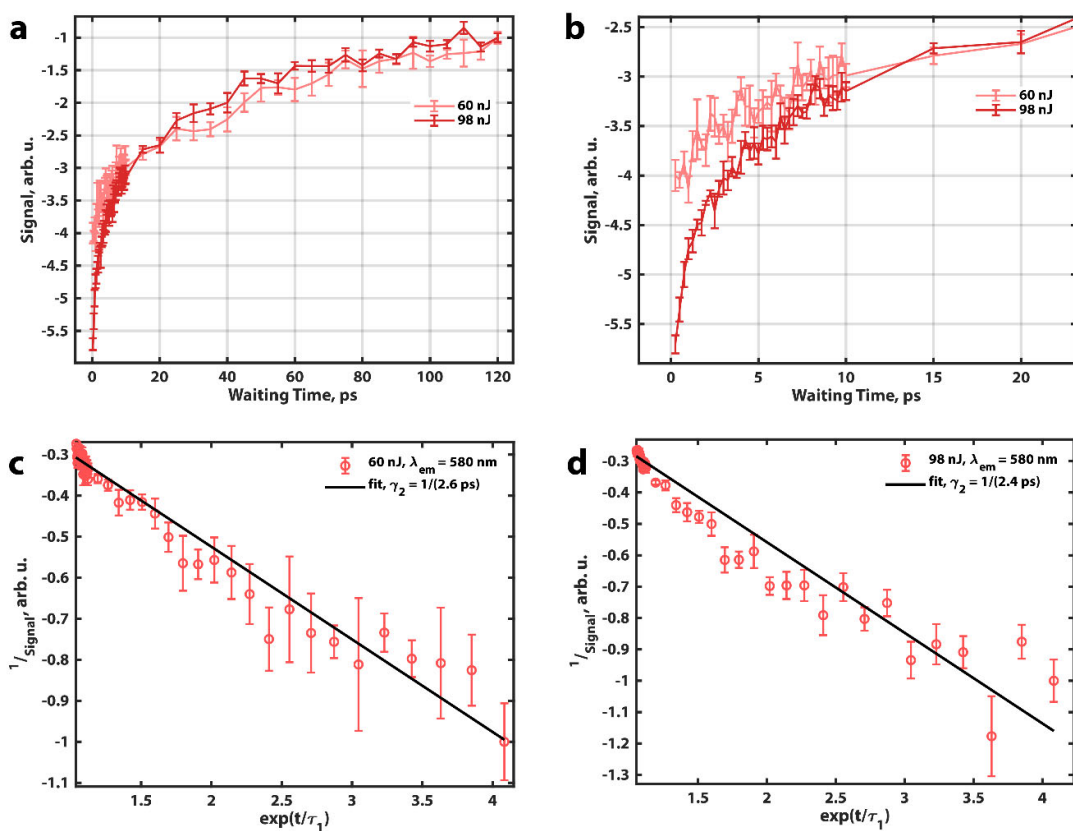


Figure S37: (a) Time traces at $\lambda_{em} = 580 \text{ nm}$ for fluences 60 nJ and 98 nJ normalized to the last time point. (b) Panel a zoomed in the first 20 ps. (c) Quality of fit of Equation S4.9 to data at 60 nJ yielding a second order rate constant of $1/(2.6 \text{ ps})$. (d) Quality of fit of Equation S4.9 to data at 98 nJ yielding a second order rate constant of $1/(2.4 \text{ ps})$.

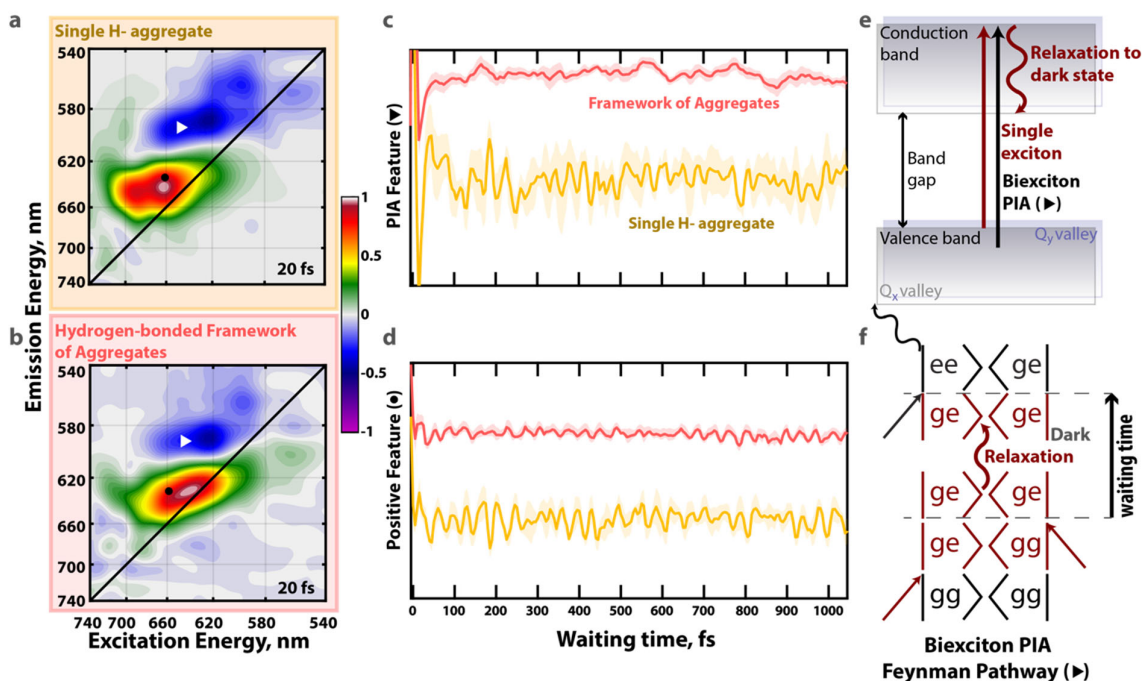
We have added the following citations relevant to the quoted text above in the Supplementary Information References section:

(8) Yuan, L.; Huang, L. Exciton Dynamics and Annihilation in WS_2 2D Semiconductors. *Nanoscale* 2015, 7 (16), 7402–7408. <https://doi.org/10.1039/C5NR00383K>.

(9) Gülen, D. Theory of Exciton Annihilation in Complexes of a Finite Number of Molecular Sites. *Math. Biosci.* 1990, 102 (1), 21–39. [https://doi.org/10.1016/0025-5564\(90\)90054-3](https://doi.org/10.1016/0025-5564(90)90054-3).

While I appreciate the addition of the energy-level diagram in fig 2, I believe this representation could be improved. For example, it does not clearly show the band-gap, which is a critical feature of semiconductor materials such as studied here. Also, the diagram does not show the "valleys in the excited-state band".

Response: We have incorporated these labels into a revised Figure 2:



Lastly, as to the definition of "strong coupling", I would suggest to rephrase the relevant sentence to "Chromophores are said to be strongly coupled to one another when..." which would remove any further ambiguity.

Response: We have changed the sentence to:

Chromophores are said to be strongly coupled to one another when the coupling energy is much larger than the energies of active vibrational modes of the chromophores^{12,13}.

Reviewer 2

The authors did a thorough job addressing my previous comments, leading to significant improvements of their manuscript.

The authors have addressed all the referees' concerns by including new material, analyses, and figures. The manuscript now reads much better, and the paragraphs that raised objections from the referees have been revised or clarified. Although I still have reservations about the final interpretation of the optical response in the two types of aggregates, I acknowledge the high

value of the collected data, the likelihood of the interpretation, and overall, I believe the manuscript deserves publication.

Response: We thank the Reviewer for these comments. Similar apprehensions were raised by Reviewer 3. We have now attempted to address these issues by collecting the data on the connected aggregated again and plotting the second order curve. We show this in the response to comments from Reviewer 3.

Reviewer 3

In the revised manuscript by Sohoni et. al., many of the questions raised in first review have been addressed. However, there are some crucial and important points have not been well addressed. Hence, a major revision is recommended. The main claim in the manuscript is persistent non-annihilating biexciton in isolated aggregate, but not in extended HOF. In figure 4A, they have shown quadratic fluence dependence of transient signal in isolated aggregate, an important observation in favour of the claim that biexciton is generated in isolated aggregate. However, the same experimental data is not shown in Fig 4b for extended HOF. As the authors claim that there is no biexciton in HOF sample, fluence dependence of transient signal at earliest time must be shown in Fig. 4b. Author has tacitly avoided this comment raised in previous review (Comment 4) by citing ND filter issue in experiment. However, this is an important experimental information which must be provided and highlighted in favour of the claim made in the manuscript. It also remains intriguing and puzzling at the same time; why would biexciton be only formed in isolated aggregate but not in HOF? Providing a molecular level insight would definitely increase the quality of the manuscript.

Response: We thank the Reviewer for these comments. We have now taken transient absorption data at specific waiting time points for seven pump fluences to illustrate that biexcitons are also formed in the HOF. However, unlike the isolated aggregates, these biexcitons annihilate in 2-3 ps and, therefore, the quadratic component of the fluence dependent signal is significantly diminished by a waiting time of 5 ps. We have added the following figure in the Supplementary Information to illustrate this point:

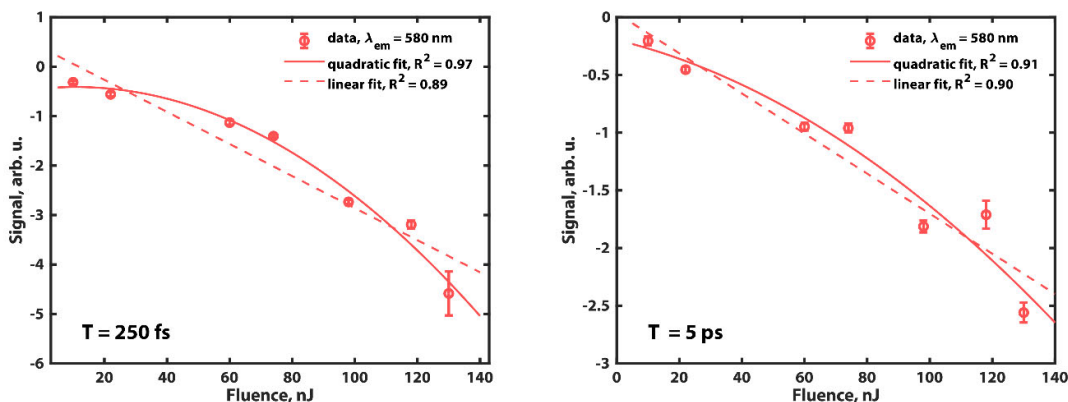


Figure S40. Figure showing the probe intensity normalized signal intensity recorded at a waiting time of 250 fs and 5 ps for each fluence at a detection wavelength of 580 nm. The quadratic component of the signal with fluence is significantly diminished by a waiting time of 5 ps due to annihilation of the biexcitons in a timescale of 2-3 ps.

We have also updated the figure showing the signal fluence dependence of the isolated aggregates in the Supplementary Information to include signal behavior in 10 ps. At this waiting time point, the signal also shows appreciable nonlinearity, and, therefore, indicating presence of biexcitons in late waiting times. This updated figure is shown below.

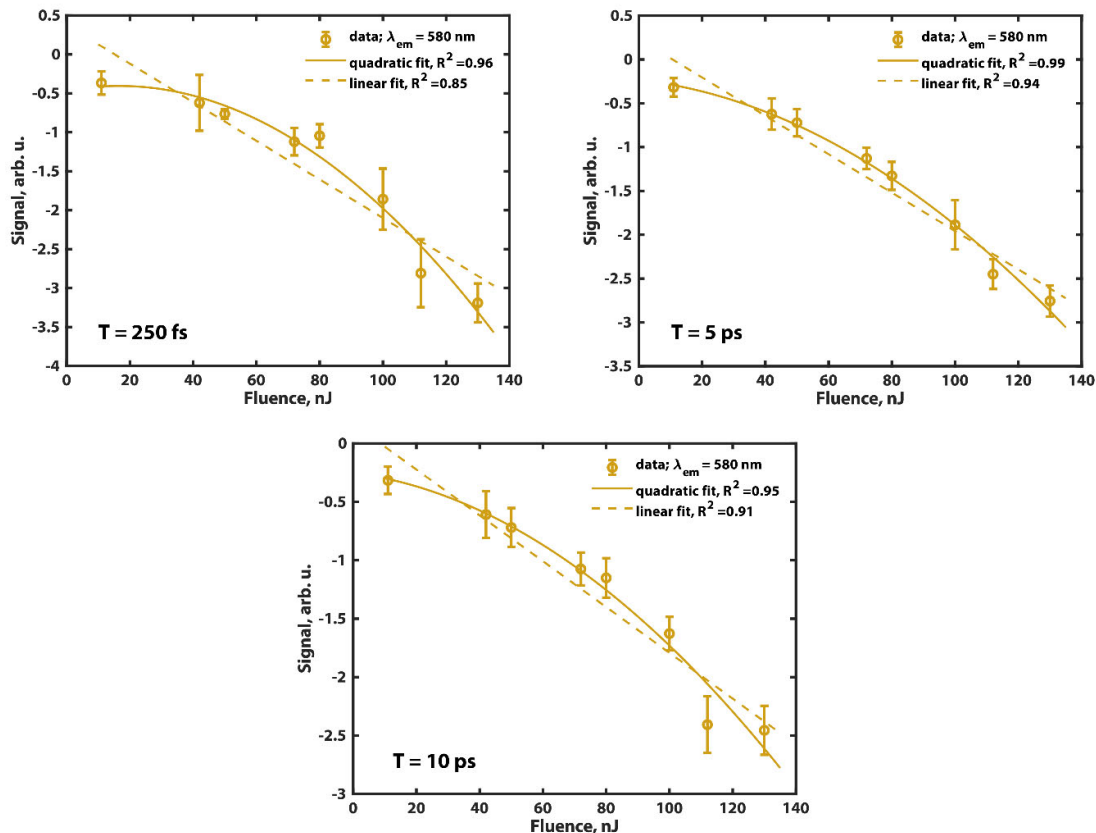


Figure S39. Figure showing the probe intensity normalized signal intensity recorded at a waiting time of 250 fs, 5 ps and 10 ps, with pump fluence at a detection wavelength of 580 nm for the isolated aggregates. The nonlinear signal dependence with power confirms that multiple excitations are created and sustained in the picosecond timescale.

In figure 4b of the main text, we have now added the fluence dependent signal for a 250 fs waiting time as an inset to the panel that shows the HOF fluence dependent time traces. We have changed the captions accordingly to state that quadratic dependence is also shown as an inset in a. This now shows that biexcitons are formed in early times for both the HOF and the isolated aggregate systems. The only difference is that annihilation of the biexcitons take place in the HOF along the H-bonded dimensions in 2-3 ps and is absent in the isolated aggregates.

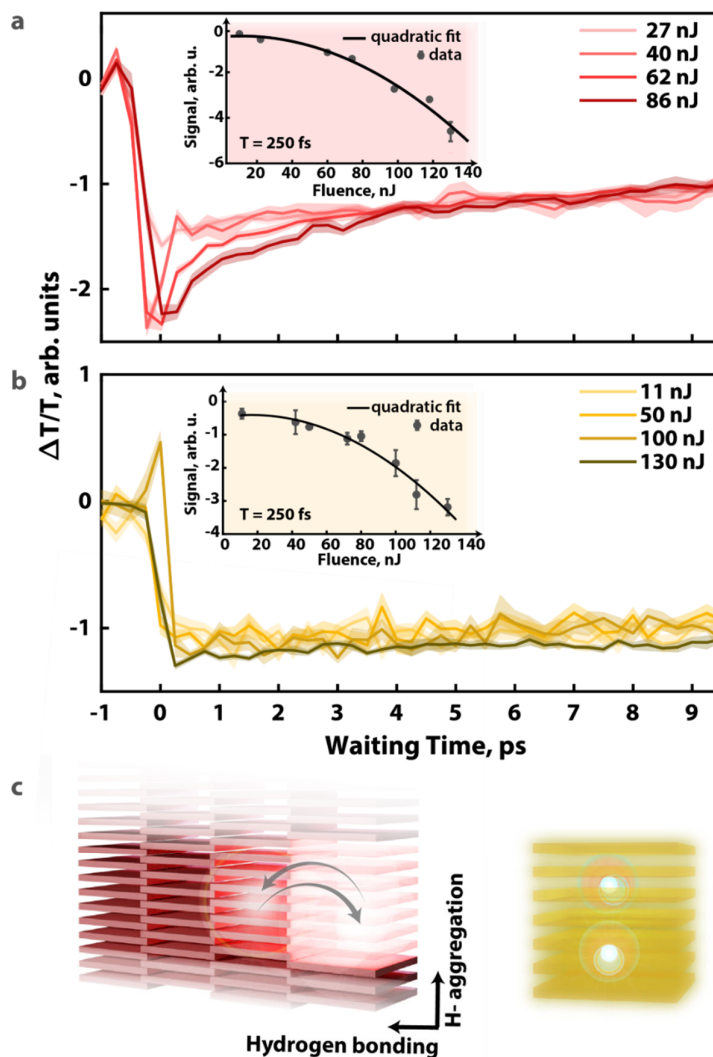


Figure 4: Dynamics of the pump-probe excited-state absorption feature at 580 nm are shown as a function of delay time for **a)** connected aggregates and **b)** isolated aggregates. In the inset of **a** and **b**, the fluence dependence of the signal is shown. It is observed that the magnitude of the signal increases quadratically with pump-fluence, indicating the multi-exciton regime at higher fluences for both aggregate types. All traces are normalized to 10 ps. **c)** A schematic showing the difference between connected and isolated aggregates' dynamics: in connected aggregates, exciton diffusion allows excitons of many different spatial phases to meet whereas in isolated aggregates, the excitons remain on the same quantum site without annihilating.

The insets of a and b in Figure 4 show that biexcitons form in both isolated and chemically connected (hydrogen bonded) aggregates. However, they only annihilate in connected aggregates because diffusion is possible. They do not annihilate in isolated aggregates and remain biexcitons because lateral diffusion is not possible.

For completeness, we also show the linearity of the monomer signal with fluence at early waiting time indicating an absence of any multiexcitonic dynamics in the monomer.

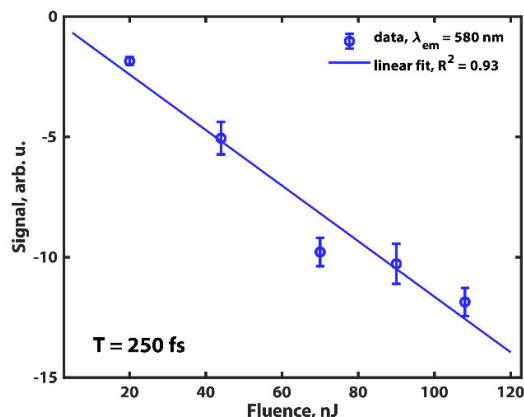


Figure S41. Figure showing the probe intensity normalized signal intensity recorded at a waiting time of 250 fs with pump fluence at a detection wavelength of 580 nm for the monomer. The linearity of the signal indicates an absence of multiparticle dynamics.

Lastly, we have also added the following sentences to the main text:

In **Supplementary Information Figure S39**, we show that the signal is a quadratic function of fluence even at 5 ps and 10 ps in isolated aggregates, when annihilation in the HOF is complete. **Figure 4a** inset shows that biexcitons are formed at high fluences in the HOF sample as well. However, this signal becomes linear with fluence at a waiting time of 5 ps, as shown in **Supplementary Information Figure S40**, indicating negligible biexciton presence at this later waiting time, because annihilation induced by diffusion is complete.

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

The authors have adequately addressed my previous comments. I also applaud the addition of the fluence-dependent plots at given time delays, in response to Reviewer 3. I support publication of this manuscript.

Reviewer #3 (Remarks to the Author):

Authors have done the suggested experiments and made the necessary revision in the manuscript. Hence the revised manuscript may be accepted for publication.