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DISCUSSIONS

Energy and charge-transfer in natural photosynthesis: general discussion

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Andrew Marcus opened a discussion of the introductory lecture by Majed Chergui: My question pertains to the theoretical interpretation of the interesting time-resolved circular dichroism (CD) experiments on DNA that you presented. The determination of nucleic acid base conformation from CD data is a fairly complex and challenging problem, which has been studied for many years. In an oligonucleotide with many bases, the CD spectrum depends on the numerous non-degenerate couplings between the many transition dipole moments of the bases, which in turn depends on their relative distances and orientations (*i.e.* base conformation). In general, there is no unique mapping between a given CD spectrum and a specific oligonucleotide conformation. How might this issue be addressed for the structural interpretation of time-resolved CD experiments on oligonucleotides?

Majed Chergui responded: This is a very good question and I agree with you that the modelling of CD spectra is far from trivial for systems like nucleic acid bases or even peptides. What I showed in my talk aimed at starting to resolve this problem, at least in the case of peptide chains, using thioamide substitution. We investigated a dipeptide whose two carbonyl oxygen atoms were replaced by sulfur, so that the strong lowest-lying pi–pi* transitions are shifted to the red. This not only brings them in the range of our time-resolved CD set-up but more importantly, it offers a site specific labelling of the peptidic chains.

Similar substitutions are possible with DNA strands and they are already used in either fluorescence or CD studies, *e.g.* 2-aminopurine dinucleotides, which we are planning to use.

From a more general perspective, there are still huge challenges to be overcome in order to make time-resolved CD a reliable and easy to implement method:

extending the spectral range, developing labels that can be incorporated into chains and, very importantly, developing the theory. However, I see this as a very promising avenue because there are not many experimental tools that allow you to look into the conformational changes of systems such as peptides or DNA/RNA (that consist of light atoms) in their physiological media and with the adequate temporal resolution. The only other method I can think of is time-resolved X-ray solution scattering, which I believe is extremely challenging with such systems and no less prone to heavy modeling. Furthermore, one needs to carry out the X-ray experiments at large scale installations (synchrotrons and X-ray free electron lasers), whereas time-resolved deep-UV CD can be performed in the lab.

Bern Kohler commented: You drew a contrast between excitons in the dinucleoside monophosphate $(dA)_2$ and in the 20-mer $(dA)_{20}$. Because base stacking is only weakly stabilized at room temperature, domains of well-stacked bases are interrupted by one or more unstacked bases with the result that the average number of bases in a stacked domain can be much less than the total number of residues in a strand.[1,2] For this reason, it would be very interesting to study strands of intermediate length as the maximum extent of excitonic delocalization could be reached for an oligonucleotide of length much less than 20.

1 C. Su, C. T. Middleton and B. Kohler, *J. Phys. Chem. B*, 2012, **116**, 10266–10274.
2 B. Kohler, *Excited States of Single-Stranded DNA Revealed by Femtosecond Transient Absorption Spectroscopy. In Ultrafast Dynamics at the Nanoscale: Biomolecules and Supramolecular Assemblies,* ed. I. Burghardt and S. Haacke, Pan Stanford Publishing Pvt Ltd, Singapore, 2017, pp. 3–64.

Eric Bittner opened the discussion of the paper by Alexandra Olaya-Castro: In your paper, you present a fully quantum model of a system driven by a fully quantum ensemble of oscillators. However, it would seem that a classical bath model would also produce synchronization simply by symmetry. At what point does it matter if the bath is quantum or classical and does this really matter for realistic biological systems?

Alexandra Olaya-Castro replied: The model assumes a quantum description for the excited states of each chromophore and for the local vibrational motions interacting with each chromophore. The influence of the remaining the thermal environment is assumed to be Markovian. As we discussed during the presentation, synchronicity of the local vibrational motions is preceded by a transient where motions are anti-synchronised. This corresponds to the dynamics as a collective relative motion that assists the energy transfer. We have previously shown that such a collective mode exhibits non-classical thermal fluctuations, that is fluctuations that are narrower than Poissonian and can only be described *via* a quasiprobability distribution that exhibit negativities, thereby eliminating any classical description.[1] We have also shown that a classical description of such high energy vibrations will not appropriately describe the directionality of the energy transfer.

1 E. O'Reilly and A. Olaya-Castro, *Nat. Commun.*, 2014, **5**, 3012.

Eric Bittner asked: The exciton/phonon model you present is much like that used to study molecular dimers in J- and H-aggregate systems. As such is it not

anticipated that the synchronization between oscillators is simply reflecting that coupling? In essence, the steady-states of the system must always be in symmetric or anti-symmetric irreducible representations. Furthermore, is it possible to construct a phase diagram in terms of the coupling parameters, exciton gap, and phonon lineshape?

Alexandra Olaya-Castro replied: Our model differs from H- and J- aggregates as in this case the on-site energies are not identical. Most importantly, the synchronisation dynamics that we describe here are not between the electronic states of the heterodimer we consider but between vibrational displacements of local quasi-coherent modes coupled to each local excited state. The vibrational modes do not interact directly, yet their synchronisation dynamics are mediated by the interaction between electronic states. The emergence of anti-synchronisation and synchronisation of such displacements does reflect the formation of collective vibrational motions during energy transfer. The vibrational motions are assumed to be of equal frequency and locally coupled to each electronic state, with the same strength. Hence, in the short time regime, a collective anti-correlated relative motion is formed and drives energy transfer dynamics.[1,2] Given that vibrational coherence lasts longer, in the long time regime, the centre-of-mass correlated collective motion survives and leads to synchronised motions.

1 E. J. O'Reilly and A. Olaya-Castro, *Nat. Commun.*, 2014, **5**, 3012.
2 V. Tiwari, *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**(4), 1203–1208.

Jeffrey Cina asked: In some famous measurements of polarized fluorescence up-conversion from LH-1 in the middle nineties[1] vibrational quantum beats in the perpendicular channel were shown to appear in-phase with those in the parallel channel. These results suggested that coherent intra-chromophore vibrations in the energy-transfer acceptors were somehow synchronized with those in the donor chromophore. Graham Fleming and I later found an explanation in a surface-crossing picture of electronic excitation transfer.[2] In this description, it is seen that in a homodimer the crossing seam between donor-excited and acceptor-excited electronic states passes through the Franck–Condon points of both surfaces. Consequently, when a nuclear wave packet is launched by short-pulse excitation to the donor potential, energy-transfer is most efficient each time it returns to this point, and the Franck–Condon excited nuclear wave packet in the acceptor is therefore synchronized with that in the donor. Does this sound like a phenomenon that is related to the synchronization revealed in your paper?

1 S. E. Bradforth, R. Jimenez, F. van Mourik, R. van Grondelle and G. R. Fleming, *J. Phys. Chem.* 1995, **99**, 16179.
2 J. A. Cina and G. R. Fleming, *J. Phys. Chem. A*, 2004, **108**, 11196–11208.

Alexandra Olaya-Castro responded: This is a very interesting observation. Thank you for pointing out these works and it is fantastic to see that there is a direct experimental approach that will allow us to confirm our predictions of negative and positive synchronisation of Franck–Condon active vibrations during energy transfer. We have shown that as excitonic states are more delocalised, the

period of negative synchronisation is shorter and vibrational displacements reached positive synchronisation faster. We therefore expect that in the case of a homodimer, with fully delocalised excitonic states, positive synchronisation will dominate all the way through.

Animesh Datta queried: Can we prove that the synchronisation effect studied here is uniquely quantum? Coupled harmonic oscillators, which the vibrations are, such as pendulums can be synchronised classically as well. Here the mediator is a two-level system. Is that what makes this quantum?

Alexandra Olaya-Castro replied: The mediators here are two collective electronic states (not just a two level system). As we have discussed in ref. 1, it is the exchange of exactly one quantum of energy between these collective electronic states and the emergent collective vibrational states that leads to truly quantum behaviour with a non-classical analogue for the vibrational motions in this system. In other words, it is the formation of collective vibronic coherences and their decoherence mechanisms that leads to the synchronisation dynamics observed. Different from the classical case, the oscillatory pattern of the observables being synchronised emerges due to quantum interference among different coherences, and not by simply natural oscillating frequencies of the oscillators involved. These are all features indicating that the spontaneous synchronisation dynamics described in our manuscript is uniquely quantum.

1 E. J. O'Reilly and A. Olaya-Castro, *Nat. Commun.*, 2014, **5**, 3012.

Vanessa Huxter asked: Can you comment on the physical interpretation of negative or positive synchronization?

Alexandra Olaya-Castro answered: Thanks for this question. Negative synchronisation indicates that as energy transfer happens the oscillating displacement of one site is anti-correlated with the ones of these other sites. The message this gives is that a collective relative displacement mode has been activated during exciton energy transfer. This relative mode promotes both quantum superposition of the two excitons and coherent exciton population transfer in the short time-regime. In our case, electronic dephasing is faster than vibrational dephasing, then, as time evolves, the phases of these anti-correlated motions get destroyed due to electronic dephasing and the complementary collective mode (centre of mass motion) is activated *via* exciton population transfer, which carries information on the surviving local vibrational phase thereby promoting synchronisation.

Jennifer Ogilvie commented: In your paper you find that negative synchronization occurs simultaneously with coherent energy transfer and that there is a positive correlation between coherent transport and the time it takes for positive synchronized coherence to emerge. Your vibronic dimer model is related to the work of Tiwari and Jonas that also considers a dimer model with an intramolecular vibration common to both monomers and resonant in frequency to an electronic energy gap in the Fenna-Matthews Olson complex.[1] Are the positive and negatively synchronized coherences in your paper related to Tiwari and Jonas's

correlated and anti-correlated vibrations, of which they propose that the latter can influence energy transfer?

1 V. Tiwari, W. K. Peters and D. M. Jones, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110(4)**, 1203–1208.

Alexandra Olaya-Castro responded: Thanks for this great question. The answer is yes. Our negative and positive synchronised motions are consistent with the anti-correlated and correlated vibrational motions discussed by Tiwari and Jonas[1] and with the non-classical behaviour of such collective relative motions that we discuss in ref. 2. Our main contribution in this case is that we quantify and follow the dynamics of such synchronisation while establishing a direct relation to the coherence dynamics.

1 V. Tiwari, W. K. Peters and D. M. Jones, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110(4)**, 1203–1208.
2 E. J. O'Reilly and A. Olaya-Castro, *Nat. Commun.*, 2014, **5**, 3012.

Amro Dodin said: The energy eigenstate analysis in this paper provides a great deal of insight into how environment-induced relaxation drives synchronization. Can this mechanism be complementarily thought of using excited state forces? In this picture, when the exciton is in an in-phase superposition between the two sites (Fig. 1(A)) the excitonic coupling would produce an attractive force acting on the two oscillators and an out-of phase superposition (Fig. 1(B)) would produce a repulsive force. Would it be correct, in this case, to think of the exciton dynamics providing an oscillatory driving force on the two oscillators as the mechanism for synchronization? In that case, does negative synchronization cause enhanced electron transfer rates by bringing the two sites into closer proximity, increasing their coupling?

Alexandra Olaya-Castro answered: Vibrational motions induce fluctuations of the on-site energies. Hence, if the on-site energies are very different (as it is in our case), negative synchronised displacements will bring electronic levels into resonance and therefore promote energy transfer from one site to another. So your picture of bringing sites on close proximity is correct as long as we under-stand is proximity in energy and not in space. It is correct to think of the electronic interactions as the driving force of the vibrational negative and positive synchronisation. And it is important to bear in mind that synchronisation is a collective phenomenon. The electronic interactions mediate the collective response of the oscillators. Negative synchronisation is associated with the
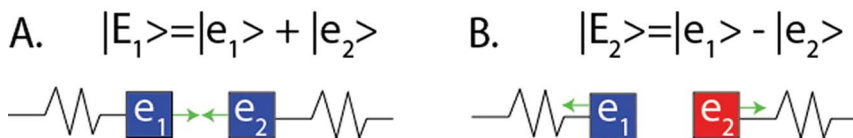
Fig. 1 Potential mechanism for synchronisation using excited state forces. (A) shows an attractive force acting on two oscillators due to an in-phase superposition and (B) shows a repulsive force acting on two oscillators due to an out-of-phase superposition.

emergence of collective relative displacement motions while positive synchronisation will be associated to the emergence of a collective centre of mass motion, just as in the case of two coupled oscillators. So instead of the idea of a repulsive and attractive force, the picture of relative and centre of mass motion is more appropriate.

Yin Song asked: How does the preparation of the initial state affect the synchronization and energy transfer rate? Does this study also suggest that there is an approach to regulate energy transfer using selective laser pulse excitation?

Alexandra Olaya-Castro replied: The preparation of the initial state affects both the synchronisation dynamics of local displacements and the electronic energy transfer, as discussed in Sections 3.1 of our paper (DOI: 10.1039/c9fd00006b) and therefore, yes, this suggests selective laser pulse excitations can be used to regulate energy transfer. If the initial state is such that energy will be transferred from one quasi-localised exciton to another one *via* the mode, then short time negative synchronised motions will precede positive synchronisation of local vibrational displacements, as we have explained. If the initial state is such that now one has a population distribution between the excitons (not single excited states) and the vibrational motions start out in thermal equilibrium, this will naturally result in slow population dynamics as the joint exciton-vibration system is closer to its thermal equilibrium distribution. In this case one will still expect some transient dynamics for the displacements with a very fast emergence of positive synchronisation. In a separate work developed by one of my master's students, we have shown that if the initial states of the local vibrations are coherent states (instead of thermal states) energy transfer from one exciton to the other is enhanced (in comparison to the thermal vibrations). In this case we expect rich synchronisation dynamics as well. In short, selective laser pulse excitation can create different initial vibrational states which, in combination with an approach that selectively excites the highest exciton state, offers a way to regulate energy transfer.

Karen Morenz questioned: I understand that with increased coupling you get increased coherent energy transfer and so you reach synchrony sooner, but I'm wondering, with the way that you've defined synchrony as a constant phase difference between the two states *i.e.* a constant Pearson correlation value, what is the mathematical definition of perfect asynchrony? Or is there a way to quantify the extent of synchrony or asynchrony?

Alexandra Olaya-Castro responded: This is an important question as it touches on the conceptual differences between defining perfect synchronous or asynchronous states in the quantum regime. We have only investigated synchronisation of one observable. But, does one need to measure every observable and therefore the entire density matrix representing the quantum state to assert perfect synchronisation?

But in the spirit of your question (having in mind the classical picture synchronisation), we have considered using the rate of change of the Pearson correlation to quantify the 'degree of synchronicity'. This would have a nice zero value for any constant phase difference. However, I don't imagine there would be

a value for a maximum asynchrony as two signals could be made to be arbitrarily different.

Animesh Datta addressed Alexandra Olaya-Castro: Related to my previous question, synchronicity can be attained between classical coupled pendulums. So can we make, as the paper says, the 'negative synchronicity' something stronger than a 'signature of excitonic coherence and coherent energy transfer'?

In other words is negative synchronicity necessary and sufficient for excitonic quantum coherence? A separate question: This work (such as Fig. 8 in 10.1039/c9fd00006b) shows correlations between population transfer and synchronicity. Is this statistically significant? This can be shown by calculating the p-value. We did a similar study for a related problem in ref. 1.

1 G. C. Knee, P. Rowe, L. D. Smith, A. Troisi and A. Datta, *J. Phys. Chem. Lett.*, 2017, **8**(10), 2328–2333.

Alexandra Olaya-Castro responded: I will address the question:

"Is negative synchronicity necessary and sufficient for excitonic quantum coherence?" For the initial state considered, *i.e.* the system is in a separable state with the electronic system in the highest exciton state and the quasi-coherent vibrations in thermal states, negative synchronisation of local displacements emerges due to the formation of a collective vibronic coherence that involves the two excitonic states (quasi-localised in different chromophores) and different local vibrational states. Such vibronic coherence will lead necessarily to a non-zero excitonic coherence. Notice that all of this is satisfied when the energy quanta of the identical vibrations commensurate with the inter-exciton energy gap.

Jahan Dawlaty commented: This question is not on our paper. However, I find the topic very interesting and have a quick comment to add. Two classical oscillators can and do couple with a classical coupling term. Franck–Condon active modes that are excited as wave-packets behave as classical oscillators, at least for a short length of time. The coupling term between two chromophores is dipole–dipole interaction (which is classical in nature). Therefore, it would be interesting to isolate the classical and quantum manifestations of the reported synchronicity.

Tom Oliver asked: Does synchronisation between the two chromophores only occur for the case that the thermal bath fluctuations surrounding each molecule are correlated?

Alexandra Olaya-Castro said: I am not sure what you mean. The thermal baths and the vibrations in question are assumed local and, to begin with, uncorrelated. What we show is that, due to the electronic interaction between local excited states, these specific, local vibrational motions can become anti-synchronised or synchronised depending on the local decoherence and dissipation mechanisms taking place at different time scales.

Jeffrey Cina remarked: If I understand correctly, your calculations proceed by transferring to the excited electronic state the quiescent nuclear wave function

from the electronic ground state. Have you considered turning things around by using external sources to drive coherent nuclear motion in the electronic ground state prior to short-pulse electronic excitation? The thought would be that doing so might allow the inter-mode synchronization you investigate to serve as a means of influencing the time-course of electronic energy-transfer dynamics, or excited-state dynamics more generally. Jason Biggs and I recently explored some simple consequences of vibrational pre-excitation for electronic excitation transfer.[1]

1 J. D. Biggs and J. A. Cina, *J. Phys. Chem. A*, 2012, **116**, 1683–1693.

Alexandra Olaya-Castro responded: We have not yet considered this scenario but it certainly sounds like an interesting avenue to approach. Thanks for the suggestion.

Graham Fleming commented: In light of your demonstration of vibrational synchronization in a vibronic dimer the 2DVE (2D vibrational-electronic) spectroscopy developed by Munira Khalil might be very interesting.[1] Here the vibrational degree of freedom is excited first, followed by the electronic excitation.

1 T. L. Courtney, Z. W. Fox, K. M. Slenkamp and M. Khalil, *J. Chem. Phys.,* 2015, **143**, 153201.

Alexandra Olaya-Castro responded: One of our motivations to investigate observables of the vibrations, came precisely from knowing there was an experimental technique that can probe concomitantly the interplay between electronic and vibrational motions such as the 2D vibrational-electronic spectroscopy. We expect that the non-thermal equilibrium states that are formed *via* excitation of the vibrational degree of freedom will affect the electronic dynamics for as long as the vibrations affected have energies comparable to the excitonic gap. I definitely think it will be extraordinary to use 2DEV to demonstrate without ambiguity the emergence of negative and positive vibrational synchronisation during exited state dynamics.

Natercia das Neves Rodrigues communicated: Your studies describe synchronisation between different systems, *i.e.* a "through-space" coherence between the vibrational states of different molecular units—is this correct? However, the vibrational states of each one of these individual molecular units may themselves couple to each other, so that there is intramolecular coherent energy dissipation. I was wondering, then, how this potential intramolecular coherence may affect the intermolecular case (can energy get trapped in molecular unit 1 if there is coherence, unable to then transfer to molecular unit 2, for example?) and, if so, how do you account for these effects in your calculations?

Alexandra Olaya-Castro communicated in reply: Yes, our work describes the transient synchronisation dynamics of some specific vibrations located at different chromophores, so it is correct to think of these as correlations between the oscillatory patterns of displacements of different molecular units. Notice that a key fact in our systems is that the energy of the vibrations involved commensurate with the exciton energy splitting. In other words, the mechanism of synchronisation involves direct energy exchange of energy between excitons and

the vibrational states. This is important, as it means that other vibrations which do not satisfy those energy conditions will not be involved in such energy exchange. These vibrations can however affect both the energy transfer dynamics and will effectively broaden the mode in question. The way we account for these in our calculations is that we consider those as part of the thermal bath inducing incoherent transitions in the system. If the coupling of single excitation states to those local vibrations is very different and very strong, the emergence of positive synchronisation will be delayed, if it happens at all.

Jahan Dawlaty opened discussion of the paper by Graham Leggett: Adding a layer of dielectric to the metal can shift the plamson frequency. Is that accounted for in the assigning the splitting?

Graham Leggett replied: Adding a dielectric layer to the surface of a plasmonic nanoparticle changes the local refractive index, and causes a small shift in the position of the plasmon band in the extinction spectrum. However, that is a very different phenomenon from the one described in our paper; we do not see a small shift, but rather a dramatic splitting of the plasmon mode. The observation of splitting demands a different explanation than simply the modification of the local refractive index. The type of splitting observed here is a type of asymmetric Fano resonance, and it is explained by a hybridisation of the confined optical mode (the localised surface plasmon resonance) with an ensemble of excitons (the chlorins and carotenoids in the light-harvesting complexes).

Jahan Dawlaty asked: The coupling between plasmons and the molecular excitons on the overlayer is likely not describable by dipole–dipole coupling. However, an electrostatic approach may be used to estimate that coupling from simpler arguments. Can one justify the 0.25 eV electronic coupling in this way?

Graham Leggett responded: Strong plasmon–exciton coupling is a quantum optical phenomenon in which a localised electromagnetic mode is coupled to an ensemble of quantum emitters. In our case, the confined light mode is a localised surface plasmon resonance, and the emitters are pigment molecules in light-harvesting proteins. There are a number of approaches to the modelling of such interactions. In a particularly important paper, Gallinet and Martin performed a first-principles analysis of strong coupling, but then demonstrated that in the special case where a broad mode (the LSPR in our system) is coupled to a narrow mode (the transition in the pigment), the predicted spectrum is also obtained by modelling the system as coupled harmonic oscillators.[1] The fact that our spectra are effectively modelled using this approach is evidence for strong coupling.

However, we can apply a number of other tests for whether we are really in the strong coupling regime. The most basic is that the coupling energy is greater than or equal to the square root of the products of the linewidths of the coupled modes. In Fig. 3 of 10.1039/c8fd00241j the data do seem to suggest an avoided crossing. Unfortunately it was not possible to fabricate arrays with LSPR energies greater than the exciton energy, making the figure look a little lop-sided. However, the energy difference between the upper and lower polariton branches for the plasmonic array with the LSPR of highest energy was approximately 0.25 eV,

suggesting that there was indeed an avoided crossing with a Rabi energy large enough for us to be in the strong coupling regime.

1 B. Gallinet and O. J. F. Martin, *Phys. Rev. B: Condens. Matter Mater. Phys.*, 2011, **83**, 235427.

Tom Oliver remarked: I presume that wild-type LH2 and LH1 proteins preferentially orient onto the gold nanostructures with their rings aligned parallel to the gold surface, however, is any alignment inferred for wild-type LHCII? Would the lack of LHCII protein orientation with respect to the electric field associated with the localised surface plasmon resonance explain (at least in part) the smaller bulk coupling energy for LHCII compared to LH2 and LH1?

Graham Leggett replied: The gold nanoparticles in our arrays are functionalised with a self-assembled monolayer of alkylthiolates. In particular, the monolayer is derivatised with nitrilotriacetic acid (NTA) terminal groups, which after complexation with Ni^{2+}, enable us to bind His-tagged proteins site-specifically. For proteins that cannot be His-tagged (*e.g.* LH1) there is still expected to be some control of orientation, because the NTA functionalised surfaces tend to be negatively charged, with the consequence that binding to the cationic face of the protein predominates. The fact that naturally occurring light-harvesting complexes are membrane proteins is helpful in this respect, because one face contains the C-termini, and the other the N-termini, meaning that they posses an intrinsic anisotropy of interaction with the surface.

Andrew Marcus questioned: Based on the various light harvesting complexes that you have examined, can you say what the effects of structural inhomogeneity are, if any, on the plasmon–exciton coupling strength? Is the inhomogeneity due mostly to disorder of the attachment sites, or rather internal to the protein scaffolds?

Graham Leggett answered: An important type of inhomogeneity is found in the orientations of the transition dipole moments. Of course in LHCII, the pigment complement is diverse, and the transition dipole moments have a variety of orientations. However, in LH2 from bacterial antenna complexes, there is important aniosotropy. In particular, the transition dipole moments for the bacteriochlorophyll Qx transitions and the carotenoid S0 to S2 transitions are aligned perpendicular to the membrane, or the gold surface in our nanostructures, *i.e.* they lie in the electric field direction. In contrast, the Qy transitions are orthogonal to the Qx transitions. It is notable that we see strong coupling of the plasmon mode to the Qx transitions but not to the Qy transitions. Thus the introduction of the plasmon field as a reference direction leads to a sensitivity to orientation in the excitons.

There is likely to be some disorder in the protein attachment sites at the surface, but it is hard to gauge the significance of this. For His-tagged proteins measurements of the amount of adsorbate using spectroscopic ellispsometry are consistent with the formation of close-packed monolayers, and in such systems the disorder in the attachment sites relative to the plasmon field direction is not likely to be significant. However, it is hard to be categorical because these are difficult systems to characterise at the level of spatial resolution really required to answer the question unequivocally.

Jennifer Ogilvie said: It is very interesting in LHCII that you extract an exciton energy of 2.24 eV which does not match any of the main exciton transitions in the LHCII pigment molecules. In 2DIR studies of vibrational polaritons there have been reports of the observation of "dark states" of molecules that can be accessed when the vibrational states of the molecule are strongly coupled to a cavity.[1] Could this be a similar effect and if so do you have any ideas of what the dark states could be?

1 B. Xiang *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2018, **115**(19), 4845–4850.

Graham Leggett replied: This is a very interesting question, and one that we did not consider. If there was coupling to "dark" states then it might be very interesting. It is physically feasible that this could be the case, but, bearing in mind the comparative simplicity of our current model, I would be reluctant to want to conclude that nevertheless. Our current model does not allow us to include multiple different excitons in the coupled system, which we know is the case for LHCII because of its diverse complement of caroteinoids. In LH2, where there is a single carotenoid, and a large difference between the transition dipole moments of the bacteriochlorophyll Qx and carotenoid S2 transitions, it is reasonable to assume that coupling to the S2 transition may dominate the strongly coupled system. However, for LHCII the situation is much less clear because of its diverse pigment complement. It would be useful to improve the model so that we could address such systems in a more meaningful way. Then we might be able to give a better answer to the question!

Vasilios Stavros asked: Have you considered examining the influence of plasmonic resonances from other metal surfaces on the substrate?

Graham Leggett responded: This is an interesting question. To date we have predominantly worked with arrays of gold nanoparticles. However, we have recently done some work with arrays of aluminium nanostructures fabricated using interferometric lithography. Al is an attractive material because it is cheap and abundant, but the fabrication presents greater challenges. In preliminary experiments we appear to have demonstrated strong coupling of Al plasmon modes to light harvesting complexes, but this work is currently in its early stages. Certainly there are other materials that are plasmonically active and some of these may also be suitable for use in studies of strong coupling of plasmon modes to excitons in light harvesting systems.

Naomi Ginsberg asked: What is the impact of the relative size of the gold particles and the light harvesting complexes on top of them?

Graham Leggett replied: The morphology of the gold nanostructures is important, because it determines the plasmon energy. The size is important – the larger the structures the smaller the energy of the plasmon mode – but their geometries are also important (needle-like nanostructures have longitudinal and transverse modes). In addition, the packing of nanostructures can be important because of the possibility for coupling between nanostructures at short range (see Fig. 5 of DOI: 10.1039/c8fd00241j). The interferometric lithography technique (from ref. 32 of DOI: 10.1039/c8fd00241j) that we use to fabricate our arrays (each

of which covers nearly 1 cm^2) allows us to vary these parameters systematically, and we have built up a library of hundreds of arrays with different characteristics.

Minjung Son queried: In the paper (DOI: 10.1039/c8fd00241j), you state that the coupling strength was dependent on the density of the bacterial light-harvesting and synthetic maquette proteins. Have you tried the same experiment for the case of LHCII? Furthermore, LHCII proteins are known to exhibit strong protein–protein interactions by self-aggregation when multiple LHCIIs are closely packed together, which likely happens at a high protein concentration (*i.e.*, density). Have you considered the possibility of these protein–protein interactions might be interfering with your signal of interest, *i.e.* the LHCII-gold nanostructure interaction? Do you think you would be able to tell that by looking at the changes in the UV/Vis spectra?

Graham Leggett responded: This is a very interesting question. In strong plasmon–exciton coupling the plasmon mode couples to an ensemble of emitters, and the coupling energy is thus proportional to the square root of the density of excitons (eqn 2 in our paper (DOI: 10.1039/c8fd00241j)).

We did not model the coupling as a function of fractional coverage for LHCII, unfortunately. It would certainly have been good to do so. However, these measurements are very hard to do, because to be sure that spectral changes are due solely to the change in coverage, it is best to re-use the same plasmonic array. We have done this successfully for LH2 and maquettes by cleaning the array between each adsorption using piranha solution (a mixture of concentrated sulfuric acid and hydrogen peroxide) but it takes its toll on the arrays and we lost a number along the way! Having been through this process for other proteins, we are now quite confident of our model, and so would now feel that a good fit of the experimental spectrum to the calculated one is sufficient evidence that strong coupling is observed. But in an ideal world we would certainly have done a study of coverage-dependence.

We did not consider LHCII aggregation at the time we carried out the work presented. I do not have a detailed understanding of the aggregation phenomena described, but note from a quick survey of the literature that these are predominantly phenomena that have been studied in bulk phases (in solution) and that aggregation is thought to be strongly influenced by the nature and concentration of lipid in solution. It is important to note that the conditions at the surface are probably very different. There is a substantial literature on protein adsorption and based on our knowledge of this phenomenon, and our ellipsometric data, we believe that LHCII forms close-packed monolayers at the gold surface. One might argue that our samples consist of macroscopic aggregates, therefore! This may in fact be rather important. My understanding is that aggregation is thought to alter the rates of energy transfer processes in the protein; I do not know whether this would have an effect on plasmon–exciton coupling. There are some suggestions in the literature that new transitions may be observed in aggregates; it might be that this would have an effect, if these transitions coupled to the plasmon mode.

Our measurements do not currently have any time resolution, so we cannot measure affects related to changes in energy transfer rates at present. However, changes in the energies of transitions are potentially detectable. A distinguishing

feature of our measurements is that the directionality of the plasmon field (perpendicular to the gold surface) means that couplings in that direction have particular importance. Thus aggregation effects would become evident if they led to changes of transition energies or transition dipole moments able to couple to the plasmon field. Perhaps with a more sophisticated model than our current two-oscillator model we might be able to explore that better. It would certainly be interesting to know whether there was any evidence for coverage-dependent changes in the transition energies. However, at the moment, I do not think I can give a more detailed answer to the question, interesting though it is.

Bern Kohler remarked: You write that the exciton density is low (10^{17} m^{-2}), yet this value appears to be roughly equal to the surface density of carotenoids in your sample. How can every carotenoid molecule be excited by the low light intensities used in your experiments?

Graham Leggett replied: A very important characteristic of strong plasmon–exciton coupling is that the confined optical mode (the plasmon) couples collectively to an ensemble of excitons to form a type of quasiparticle called a plasmon–exciton polariton. Theoreticians talk about the excited state being delocalised across the nanoparticle/exciton ensemble.

The consequence of all of this is that the coupling depends on the organisation of excitons within the plasmon mode volume; for example the coupling energy depends on the square root of the density of excitons (eqn 2 in the paper (DOI: 10.1039/c8fd00241j)). As shown in our earlier Nano Letters paper,[1] a reduction in the fractional coverage of proteins at the surface from 1.0 to 0.6 leads to a visible reduction in the splitting of the plasmon mode in the extinction spectrum.

As you say, the light intensities in our experiment are very low compared to those used in the other types of experiment described at this meeting. However, I'd argue that this is not a problem because of the physics of strong plasmon–exciton coupling. If the coupling was not a quantum optical phenomenon, then we might need a photon for every carotenoid. But in our plasmon–exciton polaritons, we do not need a photon for every carotenoid because the plexcitons are delocalised modes.

The delocalised nature of the excitation in these plasmon–exciton polaritons is what gives rise to their coherence, and makes them such attractive prospects for the design of new kinds of photonic materials and devices.

1 A. Tsargorodska, M. L. Cartron, C. Vasilev, G. Kodali, O. A Mass, J. J Baumberg, P L. Dutton, C N. Hunter, P. Törmä and G. J. Leggett, *Nano Lett.*, 2016, **16**(11), 6850–68456.

Jeffrey Cina opened a discussion of the paper by Benjamin Fingerhut: Is your one-dimensional plot of exciton-state energies and bridge and electron-acceptor states meant to be merely schematic? I would have imagined that the reaction coordinates for the excitonic states and those serving as bridge and acceptor for electron transfer would be distinct.

Benjamin Fingerhut replied: The one-dimensional plot of exciton-state energies and bridge/electron-acceptor states (Figure 2 in the talk) is schematic in order to highlight the tight integration of exciton-states and charge transfer states in

bRC. In the performed simulations a multidimensional system is employed. In particular, we consider a 3-dimensional system, where the excitonic states are coupled to an independent heat bath each along a single dimension. The lower exciton state P− serves as donor state towards charge transfer states (bridge: $P^+ B_A^-$, acceptor: $P^+ H_A^-$) that are displaced along the collective coordinate of electron transfer reactions, which is treated as the third dimension. The position along this collective coordinate affects the reorganization energy of respective the charge transfer reaction (see Section 3.2 of the main text (DOI: 10.1039/c8fd00189h) for DVR coordinate values).

Alexandra Olaya-Castro commented: During your presentation you argue the idea that for photosynthetic reaction centres, including vibrational motions *via* narrow features in spectral density did not necessarily result in an improved performance in terms of energy transfer efficiency. Could you elaborate on the physical underlying mechanisms supporting your conclusions? Your conclusions are consistent with the analysis we have done for one of the energy transfer pathways in PSIIRC where we demonstrate that coupling to such local vibrational motions does not necessarily results in an improve performance in terms of increased rate of population transfer.[1] We argue the main reason is the asymmetry of the system-bath coupling for excitation states and for charge transfer states. The much stronger coupling between charge transfer states and such local vibrations guarantees directional and step-wise charge transfer process absolutely necessary for reactions centres, but this doesn't mean it accelerates the process. This is different from what is expected for light-harvesting complexes with no charge transfer states.

1 R. Stones *et al.*, *Chem. Sci.*, 2017, **8**, 6871–6880.

Benjamin Fingerhut replied: I would like to clarify that our simulations show that the performance of energy transfer efficiency within the electronic coherence time (memory time) can indeed be affected and manipulated by the details of the spectral density. This is demonstrated in Fig. 4b and 5a of the paper (*cf.* dashed and solid green and orange lines) where the resonance frequency ω_0 shows profound impact on excitonic dynamics. In contrast, we argue that upon strong coupling of states to the environment (*e.g.* due to the polaronic character of charge transfer states) the impact on directional and irreversible trapping dynamics appears to be moderate in the investigated parameter regime of bRC. The physical mechanism for such different behavior is rooted in the separation of timescale of excitonic and charge transfer dynamics. The latter proceeds largely outside the memory time and consequently phase relations between pathways are evaded.

Compared to the findings of Stones *et al.*,[1] we show that the coupled excitation energy-charge transfer dynamics is particularly stable with respect to details of the spectral density even upon coupling to non-equilibrium exciton states, in particular with respect to the low (∼3) ps timescale of charge transfer between the special pair and accessory bacteriochlorophyll A. Such findings are consistent with a picture where the strong coupling between charge transfer states and local vibrations guarantees directional and step-wise charge transfer with moderate accelerating impact on the dynamics.

1 R. Stones *et al.*, *Chem. Sci.*, 2017, **8**, 6871–6880.

Shuming Bai commented: The parameters of orange, red and green lines in Fig. 5(c) of the paper (DOI: 10.1039/c8fd00189h) are not clear. In the Marcus theory method, is it true that only V_{SE} from superexchange through bridge is considered, while $V = 738$ cm^{-1} in eqn (20) is not included? Since the reason of the invalidity of Marcus theory here is clarified, is it possible to modify the original Marcus theory for Donor–Bridge–Acceptor systems, or it is beyond the limits?

Benjamin Fingerhut responded: In descriptions of population dynamics with Marcus theory, charge transfer from an equilibrated donor state is inherently assumed, which precludes the coupling to the coherently evolving special pair exciton states. We thus employ a simplified three-state Donor–Bridge–Acceptor model system, as described in the Appendix (Sec. 5.2 Marcus theory) with the Hamiltonian given in eqn 27 (see DOI: 10.1039/c8fd00189h). As correctly noted, this model does not include the strongly coupled excitonic states ($V = 738$ cm^{-1}) but a single donor state that is coupled to the bridge *via* the direct coupling element V_{DB} and to the acceptor *via* the superexchange coupling element V_{SE}. In Fig. 5(c) different 'flavors' of Marcus theory are compared to results obtained with the MACGIC-iQUAPI method.[1] Employing the celebrated Marcus rate formula (eqn 29, $V_{SE} = 0$), quadratic dependence of transfer rate with driving force is obtained (orange line in Fig 5b), which deviates qualitatively from the MACGIC-iQUAPI results for bridge energies $E_{Bridge} > 10700$ cm^{-1}. Accounting for a multimode picture in Franck–Condon factors (eqn 31, $V_{SE} = 0$) improves on the qualitative agreement with the MACGIC-iQUAPI results for bridge energies 11700 cm$^{-1} > E_{Bridge} > 10700$ cm^{-1} (red line in Fig 5b). The incoherent description of CT dynamics employing different 'flavors' of Marcus theory thus allows us to demonstrate the relevant contributions to the transfer rate as demonstrated for the first-order super-exchange correction to the transfer rate (green line in Fig 5b) that only becomes relevant for $E_{Bridge} > 11700$ cm^{-1} (eqn 31, $V_{SE} > 0$).

A modification of Marcus theory to rigorously account for a coupling of populations and coherences in non-equilibrium population dynamics has to the best of our knowledge not yet been realized, apart from elaborate numerical methods like MACGIC-iQUAPI or HEOM. An intermediate and numerically more efficient level of theory is given by the generalized Redfield theory[2] that reduces to the second order perturbation theory of Marcus-type treatments in the respective limits, but the time evolution of populations and coherences evolves decoupled, precluding the description of, *e.g.*, coherence to population transfer that is rigorously accounted for by the MACGIC-iQUAPI method.

1 M. Richter *et al.*, *J. Chem. Phys.*, 2017, **146**, 214101.
2 W. Zhang *et al.*, *J. Chem. Phys.*, 1998, **108**, 7763–7774.

Jennifer Ogilvie commented: You cite the recent work of Ishizaki and coworkers wherein they explore the effect of electronic-vibrational resonance on the charge separation in a model dimer inspired by the Photosystem II reaction center.[1] Can you comment on how your study compares to this one? How does it differ in its approach, the approximations that are made and the parameters that are chosen?

1 Y. Fujihashi, M. Higashi and A. Ishizaki, *J. Phys. Chem. Lett.*, 2018, **9**, 4921–4929.

Benjamin Fingerhut replied: Charge separation in the model dimer inspired by the PSII RC as investigated by Ishizaki and coworkers (ref. 23 in DOI: 10.1039/c8fd00189h) is described with fixed couplings on the order of 70 cm^{-1} and reorganization energies in a range 100–500 cm^{-1}, parameters derived from TDDFT calculations. The resulting population transfer dynamics thus appears as incoherent, justifying a treatment with second order perturbation theory. Herein instantaneous medium relaxation and charge separation from an equilibrated donor state is inherently assumed. In the current work, we numerically investigate the impact of excitonic coherence and intramolecular vibrations on charge separation dynamics. The explored parameter regime (reorganization energies > 500 cm^{-1}, couplings > 30–700 cm^{-1}) goes beyond the validity of perturbation theory and covers a regime of coherent oscillatory population dynamics within exciton states. The investigated bRC inspired model thus covers extended dynamics that connects the initially coherent dynamics to an incoherent trapping of population within low energy charge separated states. Moreover, medium reorganization is considered to occur on comparable timescales to system dynamics. We find that distinct vibrational modes can substantially affect the population dynamics among strongly coupled exciton states but a moderate effect on the charge separation process is found. In summary, our results suggest a moderate impact of vibrational modes on transfer dynamics and a decoupling of timescales and of coherent EET and CT dynamics even if a finite timescale of medium relaxation is taken into account and charge separation from a coherent non-equilibrium state is considered. In this view, molecular vibrations primarily assure the robustness of optimal CT reactions in bRC, in agreement with conclusions from Ishizaki and coworkers for charge separation in PSII.

Yuan-Chung Cheng commented: I think this work using a reliable and accurate method to look into the coupled excitation energy transfer-charge transfer dynamics is very enlightening and important for clarifying several important issues in the literature. I am a little bit surprised by the result that the high frequency vibrational mode only has a minor effect on the charge transfer rates. So, I am wondering if the energy resonance effect has been fully explored. What are the respective energy gaps between the donor and acceptor states of the two charge transfer processes? Were the dynamics affected by the vibrational mode as the mode frequency matches the energy gap? I also notice that the highest frequency examined in Fig. 5(d) of DOI: 10.1039/c8fd00189h is around 1100 cm^{-1}, why not try higher frequency modes? In pi-conjugated systems the often strongly coupled carbon–carbon stretching mode is around 1400–1600 cm^{-1}. It will be nice if some comments on the resonance effect can be provided.

Benjamin Fingerhut replied: Frankly, we were similarly surprised by the finding that resonant vibrational modes (represented *via* structured spectral density function centered around ω_0) show only moderate impact on the charge transfer dynamics. The adiabatic energy gap of the low energy exciton state P− and primary CT state is 517 cm^{-1} and thus well within the investigated range of vibrational mode resonance frequencies ω_0. The resulting impact on charge transfer dynamics is found to be moderate (*cf.* solid and dashed red lines in Fig.

4b of the paper). For the secondary ET step the adiabatic energy gap is ~1100 cm^{-1} and thus also comparable to the investigated resonance frequency ω_0. Here, similar conclusions apply for the impact on dynamics apply (*cf.* solid and dashed violet lines in Fig. 5a of the paper). In contrast, for the excitonic system (P− and P+ special pair states, $\Delta E = 1704$ cm^{-1} upon diagonalization of eqn (16)) we find pronounced effects of the resonance frequency ω_0 on the dynamics already in the investigated 0–1100 cm^{-1} range, properly described with the nonperturbative MACGIC-iQUAPI method.

Sharon Hammes-Schiffer asked: Does this work provide any insight into the functional asymmetry that leads to unidirectional electron transfer in photosynthetic reaction centers?

Benjamin Fingerhut responded: The presented model of exciton coupled charge transfer accounts for electron transfer along the active A-branch of bRC in order to demonstrate the general applicability of the MACGIC-iQUAPI algorithm to both regimes of excitation energy transfer (intermediate system-bath coupling strength) and charge transfer dynamics (strong system-bath coupling strength) subject to structured spectral density functions. Furthermore, model parameters are reliably available only for the A-branch with substantial uncertainty for the B-branch.

The favorable numerical scaling of the MAGCIC-QUAPI algorithm in principle allows for an extension of the model to system sizes that account for the inactive B-branch. The current work thus establishes a benchmark for further investigations. The established methodology should allow to establish boundary conditions for unidirectional charge separation originating from the functional and structural asymmetry of bRC.

Jennifer Ogilvie said: Can you comment on how sensitive your conclusions are to the parameters of your model? For example, you employ a Huang Rhys factor that is considerably larger than many other treatments such as the recent work by Ishizaki.[1] In addition, the separation you use between upper and lower exciton states of the special pair is considerably larger than we recently found from our two dimensional electronic spectroscopy experiments on Rhodobacter *Capsulatus*.[2] Your spectral densities are also quite simple, containing a single vibrational mode that is relatively broad. How might these various parameter choices affect the generality of your conclusions?

1 Y. Fujihashi, M. Higashi and A. Ishizaki, *J. Phys. Chem. Lett.*, 2018, **9**, 4921–4929.
2 A. Niedringhaus *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2018, **115**(14), 3563–3568.

Benjamin Fingerhut responded: The employed model parameters intend to reflect the bRC B. *viridis*. The current system Hamiltonian is derived from ref. 1 that was shown to reproduce the temperature dependent linear absorption spectrum of bRC B. *viridis* with high accuracy. Modeled special pair exciton states are found at P+ = 11 852 cm^{-1} and P− = 10 148 cm^{-1}, corresponding to the weak and strong Q_Y absorption observed at 850 and 980 nm, respectively. Other bRC, like Rb. *capsulatus*[2] and Rb. *Sphaeroides* are characterized by smaller P+/P− energy gaps. We note that the energy of the P+ state closely resembles recent reports of the P+ state in Rb. *Capsulatus*[2] in the direct vicinity of the BA absorption

band. The parameters of the current bRC B. *viridis* model can be adopted to other bRC, in particular the reduced excitonic splitting can be addressed efficiently using the MACGIC-iQUAPI method. For such reduced excitonic splitting, vibrations could have enhanced impact on excitonic equilibration dynamics as the reduced energetic splitting of P− and P+ states ($\approx$ 780–650 cm^{-1}) closely matches the vibrational resonance frequency ω_0 in the spectral density. However, as charge separation proceeds *via* couplings on the order of 30–60 cm^{-1} we do not expect that the charge separation dynamics is significantly altered by EET dynamics.

Concerning the coupling strength to the environment (as reflected in the magnitude of Huang Rhys factors) our bRC model employs established parameters from experimental mutation studies with $-\Delta E \approx \lambda = 500$ cm^{-1} for the initial charge transfer reaction P B $\to$ P$^+$ B$_A^-$ in bRC.[3] The substantial driving force of the sequential CT steps allows to realize efficient (> 90%) charge separation, which is a prerequisite to suppressed thermal repopulation of exciton donor states (see also ref. 4–6).

Given that sequential charge transfer at room temperature proceeds with highly conserved dynamics in the various bRC, we do not expect the generality of our conclusions to be affected when changing the bRC B. *viridis* Hamiltonian parameters to *e.g.* Rb. *capsulatus* or Rb. *Sphaeroides* (see also the answer to Jennifer Ogilvie's previous question).

1 B. Fingerhut *et al.*, *J. Phys. Chem. Lett.*, 2012, **3**, 1798–1805.
2 A. Niedringhaus *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2018, **115**(14), 3563–3568.
3 M. Bixon *et al.*, *Chem. Phys.,* 1995, **197**, 389–404.
4 A. Warshel and D. W Schlosser, *Proc. Natl. Acad. Sci. U. S. A.,* 1981, **78**, 5564, 1981.
5 B. Fingerhut *et al.*, *Chem. Phys. Lett.,* 2008, **466**, 209–213.
6 B. Fingerhut *et al.*, *Phys. Chem. Chem. Phys.,* 2010, **12**, 422–432.

Vasilios Stavros addressed Benjamin Fingerhut and Alexandra Olaya-Castro: Your calculations assume (as far as I can tell) that the excitation is instantaneous. Can you comment on the importance of the pulse profile on the dynamics and how easy (or difficult) it would be to include the pulse profile in your calculations?

Alexandra Olaya-Castro answered: We are interested in investigating spontaneous and transient synchronisation of vibrational displacements while excitation energy transfer takes place. Hence, the experimental scenario we consider is indeed that in which a particular initial state is created and allowed to evolve over time. In this case we assume the initial state is formed instantaneously and is such that only the highest exciton state is populated. Introducing pulsed excitation will not be difficult for the simplified model we have considered and it will effectively be the same as considering a different initial state for the system of interest. We expect the specific synchronisation dynamics (*i.e.* their evolution in time) will naturally be different, yet the mechanisms underlying the emergence of negative or positive synchronisation will still be as we have described in our manuscript: the formation and decay of specific collective vibronic coherences.

Benjamin Fingerhut responded: The excitation is indeed assumed to be instantaneous occurring *via* a delta excitation pulse. Including realistic (Gaussian) pulse shapes in our simulations could occur *via* two complementary

approaches: (i) *via* a perturbative approach in a response function formalism and (ii) by directly including the matter–electric field interaction (within dipole approximation) into the system Hamiltonian. Within approach (i) convolution of dynamics with realistic pulse profiles is accounted for, see ref. 1 for a recent overview. Approach (ii) further accounts for phase sensitivity of the excitation process and opens the prospects of coherent control *via* affecting off-diagonal coherences and strong field effects. This approach requires the implementation of the QUAPI algorithm with a time-dependent Hamiltonian, to be diagonalized under the action of the electric field in every time step. While such developments are in principle feasible, important properties of the QUAPI algorithm are lost (*e.g.* norm conservation that provides an important indicator of convergence) and a substantially higher numerical effort is expected due to the required reduction in time step size. We would like to note that the simulated, oscillatory dynamics of exciton states is readily observed with current laser technology as demonstrated in pump probe studies of exciton coherences with high temporal resolution[2] and more recently *via* oscillation in 2D signals.[3,4]

1 M. Kowalewski *et al.*, *Chem. Rev.* 2017, **117**, 12165–12226.
2 D. C. Arnett *et al.*, *J. Phys. Chem. B*, 1999, **103**, 2014–2032.
3 A. Niedringhaus *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2018, **115**(14), 3563–3568.
4 F. Ma *et al.*, *J. Phys. Chem. Lett.*, 2018, **9**, 1827–1832.

Graham Fleming commented: The question of whether quantum coherence is important in systems driven incoherently by natural sunlight is very challenging. Analysis of photon statistics is an attractive method of investigating this question as demonstrated by Prof. Olaya-Castro's paper (DOI: 10.1039/c9fd00006b) on g2(t, ω1,ω2) in a vibronic system.

Benjamin Fingerhut responded: I would like to add that spectroscopy with entangled biphoton states[1] increases the information content and the prospects of control over observables, paving a route towards addressing questions about the importance of quantum coherence. For example, the unique temporal and spectral features of entangled photons may be employed for nonlinear spectroscopy in order to reveal multiexciton properties in the bacterial reaction centre. The absorption of photon pairs with short entanglement time allows suppression of population transport in the intermediate single-exciton manifold, thus allowing the manipulation of the distribution of two-exciton states. Here, the quantum nature of light is essential for achieving such a degree of control. While classical light is fundamentally limited by the frequency-time uncertainty, entangled photons provide independent temporal and spectral characteristics not subjected to this uncertainty.

1 F. Schlawin *et al. Nat. Commun.*, 2013, **4**, 1782.

Jennifer Ogilvie said: I think theory has a critical role to play in guiding the experiments that can test the functional relevance of electronic-vibrational resonance to photosynthetic energy transfer and charge separation. Do you have ideas for any 'smoking gun' experiments, or further simulations for that matter, that could definitively prove or disprove the functional relevance?

Benjamin Fingerhut answered: In order to demonstrate functional relevance of electronic-vibrational resonances spectroscopic measurements in my opinion should aim at successively expanding on the information content of the observed signals (see also ref. 1 for an overview). Near future challenges are the improvement in pulse bandwidth to cover the energy scale of special pair exciton states and/or two-color experiments with improved (broadband) probing towards the near-IR region. Such experiments address the direct probing of $P^+ B_A{}^-$ and $P^+ H_A{}^-$ charge separated states with possibly imprinted coherence effects in the transient signals.

The use of polarized pulse sequences further provides a route to increase contrast (for prospects of ambitious experiments relying on spectroscopy with entangled biphoton states see answer to the previous question). The combination of parallel and orthogonal polarized pulse sequences (as suggested in ref. 2) provides increased selectivity towards the orientation of dipole moments in RC. The signal contribution of parallel-aligned exciton transitions can be largely suppressed, with the prospect of highlighting weaker signal features and allowing us to dissect the kinetic components of charge separation from EET and possible (coherent) coupling of both.

1 M. Kowalewski *et al. Chem. Rev.*, 2017, **117**, 12165–12226.
2 B. Fingerhut *et al. J. Phys. Chem. Lett.,* 2012, **3**, 1798–1805.

Alexandra Olaya-Castro asked: Have you carried out your analysis for the different transfer pathways that are argued to exist in PSIIRC?

Benjamin Fingerhut replied: We have not yet performed simulations for the different transfer pathways proposed in PSIIRC but plan to do so in the near future. The current study on bRC model Hamiltonians serves to establish the methodological groundwork for such investigations, *i.e.*, the treatment of structured spectral densities and non-equilibrium exciton coupled charge separation with the MACGIC-iQUAPI method.

Alexandra Olaya-Castro commented: One recurrent subject of discussion over the years has been whether the conclusions we are drawing from experiments done with a laser applied to the situation in which these biomolecules are illuminated with incoherent sunlight as happens in physiological conditions. It is about time for the community to achieve conceptual and experimental clarity on the issue so that we can move on! It seems to me that the whole discussion in the field comes mostly from our inability, and sometimes unwillingness, to listen to each other's scientific arguments carefully.

My take on the issue is the following. It is clear that coherent superpositions are formed when illuminating with lasers and, therefore, experiments are probing how those superpositions evolve in time. What we are after is a clear understanding of the mechanisms that support such coherent dynamics because such coherent mechanisms will definitely play a role under incoherent illumination. Under incoherent input and output of excitation one expects non-thermal equilibrium steady states to be formed, and how far these states are from thermal equilibrium will depend fully on the coherent mechanism that may be present.

Jennifer Ogilvie said: I would like to comment that I think experiments on model systems such as dimers, in which electronic energy gaps and vibrational modes can be independently varied and more easily modeled than photosynthetic complexes, will be important to ultimately answer the question of the relevance of electronic-vibrational resonance to photosynthetic function.

Graham Fleming opened the discussion of the paper by Yuan-Chung Cheng: How does the cutting method you describe relate to the systematic (and unique) reduction of the rate matrix into kinetic domains described by Mino Yang?[1]

1 M. Yang *et al., J. Chem. Phys*, 2003, **119**, 5614.

Yuan-Chung Cheng replied: The kinetic domain approach proposed by Mino Yang and yourself focus on the trapping time of each site and iteratively constructs a kinetic domain by identifying the sites that are closest to the trap site(s). Therefore, the method is based on finding the most rapid pathways towards the traps. On the other hand, the minimum-cut approach used in our work is based on a philosophy of finding the bottleneck and applying time-scale separation to construct coarse-grained cluster models. In our experience, this approach produced better coarse-grained models compared to methods that focus on rapid pathways. In my opinion, these various coarse-graining methods might contain complementary information and it would be interesting to carry out a comparative study for complex photosynthetic energy transfer networks.

Benjamin Fingerhut said: An empirical scaling factor is applied to the time correlation function in order to derive a scaled time correlation function (eqn 19 in DOI: 10.1039/c8fd00205c). Could you elaborate on the procedure used to obtain the scaling factor f?

Yuan-Chung Cheng responded: A scaling factor of 5 was applied to the site-energy fluctuations calculated from the charge-density-coupling method and MD structures in order to reproduce the absorption spectra of the PSII-cc subunits. Basically we tested different values of the scaling factor, and found that scaling the energy shift by a factor of 5 and the energy-gap fluctuation time-correlation function by a factor of 5^2 best produces the spectra shown in Fig. 3 and 5 of the paper (DOI: 10.1039/c9fd00006b). The rationale of applying the scaling factor is that the site-energy shifts are calculated from partial charges of the protein residues and waters defined in the AMBER forcefield, however the classical forcefield was never meant to be used to simulate spectroscopic data. Nevertheless, the protein dynamics should be captured reasonably well by the AMBER forcefield. Therefore, we choose to neglect the magnitude of the charge-density-coupling interactions by introducing the scaling factor, whereas the information on the protein dynamics is kept in the time-dependent profile of the time-correlation function. Indeed, our results yields site-energy fluctuations with $\sim$30 cm^{-1} standard deviation at the room temperature, which is too small. After scaling, the standard deviation for energy fluctuation is $\sim$150 cm^{-1}, which is in the expected scale of the thermal energy and better reproduces the spectra as well as the dynamics.

Benjamin Fingerhut queried: The empirical scaling factor f of the time correlation function accounts for deficiencies of both, the employed partial charge model and the simulation method of the optical density (relying on modified Redfield theory, eqn 15–18 in DOI: 10.1039/c9fd00006b) in an average fashion. Could you comment on the magnitude of both sources of error and discuss a strategy to disentangle the two?

Yuan-Chung Cheng answered: I think this is a good point. In our study, we believe the modified Redfield method yields accurate dynamics, so the larger error comes from the spectral density. In fact, I do not think it is possible to fully disentangle the uncertainties in the spectral density (Hamiltonian) and the errors in the methods used to carry out the dynamical simulations (the modified-Redfield approach in this study). In particular, whenever a fitting to empirical data is used to determine the parameters in the model, the resulting Hamiltonian and the dynamical method should be bound together, *i.e.* the parameters best describe the system when the same dynamical method is used. There, using the same parameters with a higher-order or even numerically exact dynamical method does not mean the results would be a better representation of the real system. At least it is more likely that the results will deviate more from the experiments. Hence, fitting to determine empirical parameters in a consistent level of theory is important. Having said that, we still hope to reduce the ambiguities as much as possible. That is why we perform this study to utilize MD simulations in our parametrization of the Hamiltonian. Our results also serve the purpose of verifying the approximations in conventional models used to describe energy transport in photosynthesis, and so far we conclude that the typical assumptions such as identical independent harmonic baths and diagonal system-bath couplings do work reasonably well.

Vytautas Balevicius Jr. asked: In the paper you mention that the time-correlation function (TCF) of the energy gap fluctuations is calculated within a 10 ps window of the MD trajectory, which allows the TCF to capture the fast/dynamical aspects of fluctuation as opposed to the static disorder. However, the motivation for the specific number (10 ps) is presented in a somewhat vague manner. The question is then: is there a specific measure to help extract the duration value of 10 ps, or is this number purely empirical and based on attempts to obtain TCFs within different windows?

Yuan-Chung Cheng replied: The 10 ps window was chosen based on the relevance of the timescale for population transfer in photosynthetic energy conduction, *i.e.* 95% quantum yield means a total mean trapping time of about 50 ps. We did experiments with different numbers ranging from 5 ps to 100 ps with our MD data, and we found that the resulting TCFs were only slightly different and the 10 ps window consistently yields results that have the static part of the fluctuations eliminated.

Jennifer Ogilvie said: Renger and coworkers propose that the energy funnel in the PSII CC is not a simple one in which the lowest energy states are located in the reaction center.[1] In particular they propose a specific low energy site in CP47 that could be important for photoprotection. Can you comment on how your work differs in this regard and what the origin of the differences might be?

1 Y. Shibata *et al.*, *J. Am. Chem. Soc.*, 2013, **135**(18), 6903–6914.

Yuan-Chung Cheng responded: Our model is in general in agreement with Renger's model, although the low-energy site in CP47 in our model is different from that in Renger's model. We also emphasize the complex nature of the energy funnel in the PSII-cc and hence the function of the combined CP47 subunit for energy pooling and to enhance the robustness of the dimeric PSII-cc. Actually, our MD data also show significant energy fluctuations in long times that scramble the order of energies of the sites across the PSII-cc, and as a result, the different low-energy sites proposed by Renger and coworkers can be explained as different realizations of the system. We are currently investigating the details of the fluctuations captured by the MD simulations and their implications on the energy transfer dynamics. Regarding the implications for NPQ, there are many speculations on the molecular mechanisms and explicit energy quenching sites, but we do not think our data so far would allow us to comfortably comment on this. We will work towards building a full PSII supercomplex microscopic model in order to test various NPQ hypothesis.

Vanessa Huxter questioned: How does the system change when both reaction centers are closed?

Yuan-Chung Cheng answered: I do not know about any structure changes accompanying the closing down of both reaction centers. In terms of the change in energy transfer dynamics, the dimeric PSII core complex is only a small portion of the larger photosynthetic apparatus on the thylakoid membrane. It is known that excitation energy can visit more than one reaction center *via* long-range transport across PSII supercomplexes. So, I suspect when both reaction centers are closed, the excitation energy could reach an open reaction center in a different supercomplex, although in a much longer timescale and lower quantum efficiency.

Graham Fleming said: To study the regulation of light harvesting *via* non-photochemical quenching requires a much larger region of the thylakoid membrane than a single super complex to be modeled. We made a model of a 200 × 200 nm patch of the membrane and find that the quantity that is being controlled is the diffusion length.[1] In fact any combination of the density of quenchers and intrinsic quenching rate that produces the same diffusion length gives the same fluorescence lifetime.

The classic experiments of Joliot and Joliot[2] show the high degree of connectivity in the membrane and our model reproduces the hyperbolic shape of their plot of photochemical yield *vs.* fraction of open reaction centers extremely well.

1 G. Fleming *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2019, **116**, 3385–3390.
2 A. Joliot and P. Joliot, *Comptes Redues*, 1964, **258**, 4622–4625.

Yuan-Chung Cheng answered: Thank you very much for the informative comment. I also agree that our current model is too small and a much larger area of the photosynthetic membrane must be studied in order to realistically describe the regulation of light harvesting *via* non-photochemical quenching. Your

theoretical model (and the works by you and Doran Bennett[1–3]) is still the state-of-the-art and the only one that provides a full perspective on the energy regulation problem. We are working on constructing models for the full PSII supercomplex and beyond, and hopefully I will be able to report our results and comparisons to your model in the near future.

1 D. I. G. Bennett, K. Amarnath and G. R. Fleming, *J. Am. Chem. Soc.*, 2013, **135**, 9164.
2 K. Amarnath, D. I. G. Bennett, A. R. Schneider and G. R. Fleming, *Proc. Natl. Acad. Sci. U. S. A.*, 2016, **113**, 1156.
3 D. I. G. Bennett, G. R. Fleming and K. Amarnath, *Proc. Natl. Acad. Sci. U. S. A.*, 2018, **115**, E9523.

Jahan Dawlaty asked: Given that circular dichroism experiments are likely to be more information rich compared to linear absorption, is it possible to simulate those experiments?

Yuan-Chung Cheng responded: Yes, we plan to simulate circular dichroism and other spectroscopic signals (fluorescence, 2D, *etc.*) in future work in order to better verify the Hamiltonian obtained from the MD trajectories. Note that we did compare our Hamiltonian with that calculated by Renger and coworkers.[1] For the most part, our site energies and couplings are consistent with each other. Renger and coworkers have calculated the CD spectrum and shown that their theoretical results are in good agreement with experiments. So we believe our model should also capture the CD spectrum of the PSII-cc system.

1 Y. Shibata *et al.*, *J. Am. Chem. Soc.*, 2013, **135**(18), 6903–6914.

Tom Oliver commented: How would the molecular re-arrangements associated with various forms of non-photochemical quenching, for example the xanthophyll cycle,[1] re-organisation of pigment protein complexes[2] or state transitions,[3] affect the excitonic energy landscape and thus the electronic energy transfer rates and pooling onto CP47?

1 B. Demmig-Adams and W.W. Adams, *Trends Plant Sci.,* 1996, **1**, 21.
2 A.V. Ruban *et al.*, *Nature*, 2007, **450**, 575.
3 J. Minagawa, *Biochim. Biophys. Acta Bioenerg.,* 2011, **1807**, 897.

Yuan-Chung Cheng replied: I think this is a fair and important question, but at this point, I do not think we have an answer. The phenomena related to NPQ occur in a bigger length scale and timescale, and we will need to, at the minimum, build a model of the PSII supercomplex in order to have any data meaningful for NPQ. We are currently working on that.

Amro Dodin asked: Has this study looked at differences in energy transfer pathways for different realizations of the system? In particular, are these dominant pathways (*e.g.* in Fig 11 of DOI: 10.1039/c9fd00006b) conserved for different realizations of static disorder?

Yuan-Chung Cheng answered: This is an excellent question. In the present work, we have focused on the time-averaged Hamiltonian that should give a better overall picture of the energy transfer pathways. Nevertheless, the MD simulation

results do provide us the opportunity to investigate energy transfer pathways for different realizations of the PSII-cc system. This further analysis is currently in progress and will be published in a separate paper. Generally speaking, the dominant pathways presented in the 7-cluster model are very conservative (robust) against static disorders, but the magnitude of the rates do vary significantly across different realizations. I want to point out that the data presented in Fig. 11 in the current work (DOI: 10.1039/c9fd00006b) are already indicative of this. The two monomeric subunits (M1 and M2) are symmetric, but the rates belonging to the same pathways on different subunits shown in Fig. 11 of the paper can be significantly different, meaning the static disorders do affect the magnitudes of the rates.

Yumin Lee said: Thank you for the great presentation and work. I just wonder why does this work exclude the ultrafast relaxation component with a sub-ps ultrafast timescale which was previously observed in 2D-ES measurement. Do you think this component is not related to this work?

Yuan-Chung Cheng responded: I think they are related, but at this point, we intend to first focus on the general behavior of the system by studying the time-averaged Hamiltonian. Note that we do explicitly include the spectral density representing the averaged dynamical fluctuations obtained from the MD data in our dynamics and spectral calculations. There a bath correlation of ~100 fs in the dynamical fluctuations is observed. So I do believe some of the relaxation dynamics observed in 2DES is described in our simulations. Our MD data also have the influence of structural relaxations and high-frequency vibrational modes, which we will include in our dynamical simulations in future work. We also plan to simulate 2DES spectra and have a more solid comparison with the experimental observations.

Naomi Ginsberg asked: Although I appreciate that it is generally expected based on bulk measurements that an exciton may make multiple 'trips' to a reaction centre prior to quenching, could you explain the logic behind the statement that the rate of quenching and concentration of quenchers (reaction centres) can simply trade off because they are multiplied together and the exciton diffusion length is large?

Graham Fleming responded: First, the Photosystem II antenna is highly connected as shown by the classic experiments of Joliot and Joliot[1] in which the photochemical yield was measured as a function of the fraction of open reaction centers. The dependence is hyperbolic - in other words the yield is greater than 50% of the value when all RCs are open when 50% of the RCs are closed. This can only arise from a high degree of connectivity. Our model[2] reproduces this curve quantitatively, suggesting we are capturing the connectivity adequately. Our model is sufficiently detailed to enable us to see what is the longest time and excitation spends on a specific chlorophyll on LHCII. The median dwell time on Chl 610-612 is 2.8 ps. As long as the intrinsic quenching time is longer than 10 ps a 2D Diffusion picture holds and for quenching times between 10 ps and 100 ps we find that a wide range of quenching times and quencher densities fit the experimental fluorescence decay curves. We further find that any combination of these two quantities that fit a given decay curve at a specific degree of quenching produce the

same diffusion length for the excitation. In other words the single quantity that is controlled is the diffusion length. This arises because the quenching is weak (multiple visits before quenching) combined with the very large diffusion length in the thylakoid membrane (50 nm for dark adapted thylakoids). If the quenching rate were much faster than the inverse of 10 ps then this picture would not hold as well.

1 A. Juliot and P. Juliot, *Comptes Rendus*, 1964, **258**, 4622–4625.
2 D. I. G. Bennett *et al. Proc. Natl. Acad. Sci. U. S. A.*, 2018 **115**, E9523–E9531.

Alexandra Olaya-Castro commented: The complexity of simulating with atomistic level the full photosynthetic membrane is too great and probably unnecessary. Can we identify a minimal functional unit that will suffice to understand the energy transfer and conversion in photosynthetic membrane? For instance, in ref. 1 we investigate the performance of a minimal membrane subarea containing the fewest possible LH1s, such that both the LH2:LH1 ratio and the typical local core environment observed in the larger membrane are maintained. Remarkably, the energy transfer performance (quantum yield, excitation life-time, fluorescence yield) of this minimal subunit compare very well with the energy transfer performance in the full membrane. Will this be a relevant unit to investigate at the atomic level? What insight are we expecting to gain form such coarse-grained description?

1 F. Fassioli *et al.*, *Biophys Journal*, 2009, **97**, P2464–P2473.

Andrew Marcus opened the discussion of the paper by Graham Fleming: My question pertains to the central line slope analysis of your 2D electronic vibrational spectra of LHCII. You mentioned that the long-lived correlation that you observe between an initially excited electronic coherence and a subsequently excited vibrational coherence defies expectations due to the rapid energy and population transfer in LHCII. Does this long-lived correlation imply that there is a quantum coherent pathway that connects the initial electronic coherence to the final vibrational coherence, or instead could this correlation be due to a pathway of classical incoherent processes that are, nevertheless, statistically meaningful?

Graham Fleming answered: I believe that the reciprocal nature of the Center Line Slopes (CLS) in donor acceptor systems arises in part from the mixing of the levels, but as you suggest there may also be a contribution coming from a bias in energy transfer rates within the distributions of the two states. It is true, in our model calculations, that the magnitude of the CLS is strongly influenced by the magnitude of the reorganization energy.

Tom Oliver said: In your calculated two-dimensional electronic-vibrational spectra, the centre line slope appears to oscillate as a function of the waiting time- what does this physically correspond to?

Graham Fleming replied: The oscillation frequency is at the energy gap of the heterodimer v=0 levels.

Tom Oliver said: In the 2DEV experiments do you see any evidence for anharmonic coupling between the low-frequency wavepackets initiated with your

short visible pump pulses and the high frequency vibrational modes probed in the mid-infrared region?

Graham Fleming answered: We have not yet modeled spectra with this type of coupling in detail but preliminary calculations in Nick Lewis's thesis suggest such coupling distorts the line shape of the higher frequency modes in the 2DEV spectrum

Jeffrey Cina queried: In her poster, my student Alexis Kiessling demonstrates that at least in the case of two-dimensional electronic spectroscopy, it can be helpful to plot the data as time-*versus*-time, rather than frequency-*versus*-frequency. This approach helps to expose the underlying nuclear wave-packet dynamics. Has your group considered using time-*versus*-time plots for your two-dimensional electronic-vibrational interferograms?

Graham Fleming responded: This is a good idea. Thanks for the suggestion.

Jennifer Ogilvie said: You mentioned in your paper that partial motivation for developing the 2DEV approach was to gain insight into the role of mixed electronic-vibrational states in energy transfer and charge separation. Many of the vibrational modes that have been suggested to have possible functional importance are in the THz regime. Do you envision extending the method to employ a THz probe? In addition, what specific signatures in 2DEV spectra do you think will be particularly illuminating for addressing the possible functional role of mixed electronic-vibrational states in photosynthesis?

Graham Fleming answered: Because the phase differences are only imposed on the visible pulses, provided appropriate sources and detectors are available, the 2DEV method could be used in the THz region. Having an effective way to distinguish vibronic coherences from ultrafast population transfer peaks will likely be important in unraveling the complex question of vibronic *vs.* electronic *vs.* vibration coherences.

Jahan Dawlaty asked: Can you comment on the similarities and differences between the 2D-EV and femtosecond stimulated Raman scattering (FSRS)? What type of information can be obtained from 2D-EV that can not be retrieved from FSRS?

Graham Fleming responded: This is an interesting question but not one I have considered in any detail. I suspect some of the time resolution issues in FSRS are less of a problem in 2DEV. We are currently working on a detailed comparison of 2DES and 2DEV.

Alexandra Olaya-Castro remarked: Two-dimensional (2D) electronic as well as 2D electronic-vibrational spectroscopy have allowed us to gain a wealth of information on the ultrafast excitation dynamics in light-harvesting systems but since the signals are congested their interpretation is not entirely clear. Which other techniques do you think will help us gain complementary insight into the quantum dynamics of complex molecular systems?

Graham Fleming replied: Experimental and complementary theoretical studies of the photon statistics from light harvesting complexes have the potential to open new windows into the dynamics. It may be possible to determine if an incoherently excited system evolves coherently, whether a complex system can generate its own coherent dynamics, and what the difference (if any) is between microscopic and macroscopic quantum yields. It has also been suggested by Mukamel and coworkers[1] that combined high time and spectral resolution may be achieved in congested spectra with rapid relaxation rates through the use of correlations between entangled photon pairs, perhaps used in conjunction with "classical" ultrashort light pulses.

And a second area, which may also involve photon statistics, is the use of micro cavities to study polariton spectra and dynamics.

1 K. E. Dorfman, F. Schlawin and S. Mukamel, *Rev. Mod. Phys.* 2016, **88**, 045008.

Animesh Datta commented: It seems that temporal correlations in the emitted light in the form of g^2 measurements may be the way to resolving quantumness in EET. Do we have any estimate of how much statistics this would take, given that we are seeking two-photon coincidences? And how does that compare to nonlinear spectroscopy statistics?

Graham Fleming responded: This is an important experimental question to which we do not yet have a complete answer.

Yin Song asked: In 2DES, rephasing and non-rephasing spectra are often used to separate signals from different pathways, *e.g.* differentiating electronic and vibrational coherences in complex systems. Is there a similar protocol that can be applied to 2DEV to shed light on the interplay of electronic and vibrational states and corresponding dynamics?

Graham Fleming responded: Because of the frequency difference between the infrared and visible pulses the rephasing effect in 2DEV spectra is not large, see ref. 1.

1 H. Dong *et al.*, *J. Chem. Phys.*, 2015, **142**, 17429.

Karen Morenz remarked: You seemed to be concerned that when doing a second order correlation experiment with a pulsed laser, you would be limited in time resolution by the detector deadtime. The way that people usually get around this is by using a beam splitter and two detectors; this wastes 50% of the data when both coincident photons go to the same detector within the detector deadtime, but the other half of the time they go to two different detectors, and so you can still build up statistics over time, and you are not limited by the detector deadtime. Perhaps I didn't quite grasp the concern with time resolution.

Graham Fleming answered: There is an issue here to do with whether one is thinking of single molecule or ensemble measurements. If the latter I suspect that the g(2) needs to be measured one photon at a time (with an entangled pair source) so that the sample only ever has one photon in it at once. This then means

one would like to run the experiment at the maximum possible repetition rate. This was the point I was making.

Jennifer Ogilvie asked: Can you comment on the potential of your approach in other frequency regimes? For example, 2D UV excitation, combined with vibrational probing could provide an exciting new probe of protein and nucleic acid structure and dynamics.

Graham Fleming replied: Yes there is great potential for the method in protein and nucleic acid studies.

Naomi Ginsberg addressed Graham Fleming and Alexandra Olaya-Castro :How does one go about 'scaling up' from a model dimer system in an experiment that employs pair of correlated photons as a probe?

Alexandra Olaya-Castro replied: I find the question unclear but I can make the following comment. The possibility of probing ultrafast dynamics with quantum light (entangled photons) was mentioned. Given that entangled photons offer different frequency and time resolutions, this can provide an advantage for isolating excited state features in two-dimensional spectroscopy, for instance. Systematic studies showing the specific advantaged offered are needed.

Graham Fleming responded: Multiple systems have be discussed in the context of cavities.[1,2]

1 J. Yuen-Zhou and V. M. Menon, *Proc. Natl. Acad. Sci. U. S. A.*, 2019, **116**, 5214.
2 Z. Zhang *et al. J. Chem. Phys.*, 2018, **148**, 074302.

Conflicts of interest

There are no conflicts to declare.