The assignment of $Q_y(1,0)$ vibrational structure and $Q_x$ for chlorophyll $a$

Joseph L. Hughes, a,b* Brendon Conlon, c Tom Wydrzynski, c Elmars Krausz b

a Laboratoire d’Optique et Biosciences, INSERM U696 – CNRS UMR7645 Ecole Polytechnique, 91128 Palaiseau, France
b Research School of Chemistry, The Australian National University, Canberra ACT 0200, Australia,
c Photobioenergetics, Research School of Biological Sciences, The Australian National University, Canberra ACT 0200, Australia

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Abstract

We used non-photochemical persistent spectral hole-burning at 1.4 K to investigate the $Q_y(1,0)$ vibrational structure of Chl $a$ in a water-soluble chlorophyll-binding protein (WSCP) which exhibits resolved structure in its broadband optical spectra. Franck-Condon vibrational overlap factors were determined from the vibrational hole-burning data and used to simulate the $Q_y(1,0)$ spectra. The simulations were not able to accurately reproduce the details of the $Q_y(1,0)$ spectrum. This indicates a breakdown of the approximations used for the analysis and demonstrates that vibrationally induced mixing of electronic states (vibronic coupling) is active for Chl $a$. By considering the inhomogeneous broadening and vibrational hole-burning phenomena in the $Q_x$ and $Q_y(1,0)$ region of Chl-WSCP in addition to magnetic circular dichroism data, we favor the traditional placement of $Q_x$ at $\lambda\sim570-590$ nm rather than the alternate assignment underneath the $Q_y(1,0)$ absorption near $\lambda\sim615-630$ nm.

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1. Introduction

The assignment of $Q_x$ and $Q_y(1,0)$ transitions for chlorophyll $a$ (Chl $a$) have been regularly debated throughout the history of spectroscopic investigations of Chl. The broadband optical spectra for Chl bound in a recently studied water-soluble chlorophyll-binding protein (Chl-WSCP) [1] exhibit higher resolution when compared to solvent-based Chl systems, as well as a minimal number of Chl per WSCP complex compared to most other Chl-protein systems. This can provide a greater constraint on descriptions of the $Q_x(1,0)$ and $Q_y$ spectra, as discussed in the present report. Methods have been established [2-4] to utilize non-photochemical hole-burning (NPHB) spectra obtained by burning into vibrational sidebands to obtain the Franck-Condon factors (FCFs) for (bacterio)chlorophyll

*Corresponding author. Tel.: +33-1-6933-5049; fax: +33-1-6933-5084.
E-mail address: joseph.hughes@polytechnique.edu

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(B)Chl Qₜ(1,0) transitions. Recently, there has been discussion [5] regarding the validity of the assumptions used in such analyses. The methods were justified based on studies of (B)Chl spectra in glassy matrices. However, solvent-based (B)Chl systems and some (B)Chl-protein complexes can suffer from significant (>300 cm⁻¹) inhomogeneous broadening, leading to a broad and rather featureless linear Qₜ(1,0) electronic-vibrational sideband region. (B)Chl-protein systems can exhibit spectral components with narrower inhomogeneous broadening (<200 cm⁻¹), as evidenced by zero-phonon hole-burning action spectra, but there are typically numerous spectral components, corresponding to the many (B)Chl pigments bound to each protein subunit. This makes a stringent test of the FCF values obtained, and the method for obtaining them, difficult. The work presented in this article uses a relatively simple Chl-protein system (discussed below) to address such analyses of FCFs at a greater level of detail than previously possible.

The original assignment of the Qₓ band for 5-coordinate Chl a placed it at near ~570-580 nm, at higher energy than both Qₓ and Qₓ(1,0) [6-9]. Subsequent assignments have placed it underneath the Qₓ(1,0) vibrational region, near ~615-630 nm [10-12]. This latter assignment has recently been revisited and favored [13] based on temperature-dependent broadband and low-temperature line-narrowed optical spectra. Conversely, we recently assigned the Qₓ band for Chl a near 570-580 nm based on the magnetic circular dichroism (MCD) spectra [1] of Chl-WSCP. Here we provide further discussion of these assignments based on inhomogeneous widths and vibrational hole-burning data.

In this work we have studied a recombinant water-soluble chlorophyll-binding protein (WSCP) (~22 kDa) from cauliflower (Brassica oleracea var. Botrys) that forms tetrameric protein units upon binding Chl [14, 15]. This Chl-WSCP complex has been reported to bind only two Chl per protein tetramer [15]. The steady state absorption, circular dichroism, magnetic circular dichroism, and emission spectra were interpreted [1] as due to a Chl a dimer. Transient absorption [16] and fluorescence [17] measurements on Chl-WSCP systems have also been interpreted within a refined exciton dimer framework for Chl-WSCP [18].

2. Materials and Methods

The Chl-WSCP complex was prepared as described in Hughes et al. [1]. Sample preparation for optical measurements was performed under dim green light, as described in Hughes et al. [1]. For the measurements presented in this work we used ethylene glycol:glycerol (1:1, v/v) as the glassing agent, diluted to ~40% in the final sample mix in the optical cell. Samples were cooled from room temperature to ~4 K over a time period of ~40 seconds. All measurements were performed in superfluid helium at 1.4 K.

The spectral hole-burning measurements were performed in transmission and for hole readout we used a scanning monochromator system described in detail elsewhere [19]. For narrow-band excitation we used a SpectraPhysics model 375 dye laser operating with DCM dye, pumped by a SpectraPhysics model 171 Ar⁺ laser. A 3-plate birefringent filter was used for wavelength selection, which resulted in a laser linewidth that approximately matched the resolution of the monochromator, ~1.3-1.7 cm⁻¹. The dye laser output was propagated ~11 meters to the spectrometer from a Littrow reflector to minimize the fluorescence pedestal at the sample. Typical illumination fluences were ~50-150 mW/cm², and reduced with neutral density filters where necessary.

3. Results

3.1. Vibrational sideband hole-burning and Franck-Condon factors

Fig 1 shows a representative hole-burning ΔA spectrum (thin grey trace) where the laser frequency was tuned to the Qₓ(1,0) vibrational sideband region. The pre-burn absorption spectrum is also presented (black trace). The upper and lower exciton components of the Chl a dimer are observed as the main band at ~672 nm and the shoulder at ~681 nm, respectively. There is a distribution of pseudo-ZPHs that span the region of the lower exciton absorption band. The distribution of zero-phonon lines for this absorption was identified by the hole-burning action spectrum (data not shown) obtained for constant burn fluence (1.4 J/cm²) across the Qₓ(0,0) absorption region. The
zero phonon action spectrum was described well by a Gaussian function of FWHM=177 cm\(^{-1}\) centered at 14661 cm\(^{-1}\) (682.08 nm). The energy separation between the pseudo-ZPHs and the resonant vibrational hole provides the excited state vibrational frequencies. The broad increase in absorption peaking around 670 nm may be associated with photoproduct. The derivative-type features in the \(Q_y(1,0)\) region that are broader than the resonant vibrational hole may be associated with this \(Q_y(0,0)\) origin feature.

![Figure 1](image-url)

**Fig 1.** A representative vibrationally hole-burned spectrum obtained at 1.4 K after excitation with 173 J/cm\(^2\) (115 mW/cm\(^2\), 25 minutes) at 631.8 nm. The sharp structure in the \(Q_y(0,0)\) region are pseudo-ZPHs that span the region identified by the action spectrum. The pre-burn absorption spectrum (thick black trace) is also shown for comparison.

We follow the method introduced by Small and co-workers [2-4] to determine Franck-Condon vibrational overlap factors (FCFs) for the \(Q_y(1,0)\) state. The justifications for this approach are outlined in the Appendix. The key features of the analysis are (i) non-Condon effects can be neglected, (ii) the electron-vibrational coupling is weak, so that the Franck-Condon factors (FCFs) can be approximated by the Huang-Rhys parameter, S < 0.1, and (iii) the intensity of the \(Q_y(0,0)\) excitation is invariant for Chl sites in the ensemble with different transition energy.

Table 1 lists the 64 excited state vibrational frequencies that we were able to identify in this work. Also listed are our estimates for the Franck-Condon vibrational overlap factors (FCFs). Our FCFs are of the same order of magnitude as those reported in the literature for Chl \(a\) [4, 20] and BChl \(a\) [2], and good agreement is found throughout the Chl \(a\) \(Q_y(1,0)\) region for a number of modes. However, it was found that the relative FCFs obtained from different vibrational burns exhibited significant variation, up to a factor of ~3 or more. This is also the case for reported literature values [4, 20] for FCFs of some Chl \(a\) modes. These variations are outside the maximum uncertainty expected (+20%, see Appendix) when using the approximations for weak Huang-Rhys parameters, S < 0.1. The uncertainty in our determination of the pseudo-ZPH areas for the smallest pseudo-ZPHs is also ~ ±20%.

### 3.2. \(Q_x\) and \(Q_y(1,0)\) spectra

We were not able to observe any significant vibrational hole-burning in Chl-WSCP for burn wavelengths shorter than ~620 nm. We also note that at wavelengths shorter than ~610 nm, the sharper structure observed in the
broadband absorption spectra is no longer apparent, and instead there are only two broad features. This can be seen directly from the broadband absorption and MCD spectra in Fig 1 of Hughes et al. [1]. These two phenomena are relevant to the renewed debate [13] regarding the assignment of Q_x and Q_y(1,0) addressed in the Discussion section.

Table 1: Q_y(1,0) excited state vibrational frequencies

| \( \nu_j/\text{cm}^{-1} \text{ (±4 cm}^{-1}) \) | FCF_j | \( \nu_j/\text{cm}^{-1} \text{ (±4 cm}^{-1}) \) | FCF_j | \( \nu_j/\text{cm}^{-1} \text{ (±4 cm}^{-1}) \) | FCF_j |
|-----------------|--------|-----------------|--------|-----------------|--------|
| 162             | 0.002  | 685             | 0.018  | 1122            | 0.019  |
| 178             | 0.004  | 709             | 0.033  | 1136            | 0.039  |
| 192             | 0.006  | 734             | 0.013  | 1176            | 0.014  |
| 207             | 0.010  | 740             | 0.027  | 1184            | 0.005  |
| 232             | 0.011  | 747             | 0.016  | 1196            | 0.003  |
| 266             | 0.006  | 755             | 0.017  | 1228            | 0.003  |
| 280             | 0.004  | 766             | 0.009  | 1243            | 0.013  |
| 304             | 0.006  | 776             | 0.013  | 1265            | 0.002  |
| 327             | 0.023  | 783             | 0.014  | 1280            | 0.004  |
| 352             | 0.015  | 797             | 0.015  | 1286            | 0.002  |
| 360             | 0.017  | 803             | 0.013  | 1296            | 0.002  |
| 371             | 0.003  | 816             | 0.013  | 1314            | 0.003  |
| 385             | 0.014  | 833             | 0.014  | 1320            | 0.002  |
| 406             | 0.007  | 877             | 0.023  | 1350            | 0.010  |
| 429             | 0.010  | 920             | 0.047  | 1378            | 0.006  |
| 469             | 0.011  | 984             | 0.039  | 1422            | 0.003  |
| 482             | 0.004  | 1000            | 0.011  | 1433            | 0.003  |
| 494             | 0.003  | 1005            | 0.006  | 1495            | 0.005  |
| 520             | 0.023  | 1032            | 0.032  | 1517            | 0.009  |
| 572             | 0.012  | 1054            | 0.015  | 1543            | 0.002  |
| 580             | 0.010  | 1072            | 0.024  |                  |        |
| 604             | 0.014  | 1106            | 0.016  |                  |        |

4. Discussion

4.1. \( Q_x \) and \( Q_y(1,0) \) assignments and inhomogeneous widths

As discussed recently by Rätsep et al. [13], one reason for the debate over the assignment of \( Q_y(1,0) \) and \( Q_x \) is that the early absorption data [6-9] provided little information about the vibrational structure of these transitions. This led to the application of line-narrowing techniques to address this question [10], which was recently revisited by Rätsep et al. [13]. In the present work, we have noted that unlike solvent-based Chl systems, in the broadband absorption and MCD [1] for Chl-WSCP, vibrational structure can be readily observed without recourse to line-narrowing techniques (Fig 1 of Hughes et al. [1]).

An assignment of the main absorption in the region at \( \lambda < 610 \text{ nm (FWHM ~400-500 cm}^{-1}) \) to \( Q_y(1,0) \) transitions [13] must then also explain why these particular electronic-vibrational transitions exhibit greater inhomogeneous
broadening than those at longer wavelengths, \( \lambda > 610 \) nm (FWHM ~200 cm\(^{-1}\)). A significantly different inhomogeneous broadening for transitions that are built upon the same electronic origin seems very unlikely.

As we pointed out previously [1], the MCD profile in the 570-610 nm region matches the absorption profile very well, while in the 600-660 nm range it is a very poor match. The assignment placing only \( Q_y(1,0) \) and \( Q_x(1,0) \) electronic-vibrational transitions throughout the 570-610 nm region would also need to explain why the MCD of these transitions follow the absorption profile, but those electronic-vibrational transitions in the 600-660 nm range do not. Such an explanation at present is lacking.

The assignment of \( Q_x \) to the 570-600 nm region and \( Q_y(1,0) \) to 600-660 nm was interpreted [1] to indicate that vibronic coupling is active for \( Q_y \), thereby rendering the polarization of the \( Q_y(1,0) \) transitions variable. This would result in a poor match of the MCD profile to the absorption profile in the \( Q_y(1,0) \) region, as observed [1]. We also note that the \( Q_y(1,0) \) region for pheophytin \( a \) exhibits minimal MCD intensity compared to absorption [9]. The significant change in \( Q_y(1,0) \) MCD structure for the two related systems establishes that (i) vibronic coupling is active and (ii) vibronic activity varies significantly between these two chlorins. The assignment of vibronic activity to the \( Q_y(1,0) \) transitions is also supported by our attempts to simulate the \( Q_y(1,0) \) region using the FCFs and excited state frequencies obtained from vibrational hole-burning, which is addressed in the following section.

Further rationale for our arguments is found by noting that the \( Q_y \) and \( Q_x \) electronic transitions are expected to exhibit poor correlation. The differential effects in \( Q_y \) and \( Q_x \) of Mg ligation [13, 21] and H-bonding to the 13\(^1\)-keto substituent [21, 22], for example, are evidence for this. The lack of vibrational hole-burning in the 570-600 nm region is then also consistent with the assignment of this region to \( Q_x \). This is because the lack of correlation results in a distribution of pseudo-ZPHs across the \( Q_y(0,0) \) SDF region, where their small area and overlap with the photoproducts then precludes their observation in the NPHB spectra.

Finally, we note that in the region we attribute to \( Q_x \) there are two broad absorption features. Chl bound to WSCP is mono-ligated [23] (also identified by our own unpublished X-ray structural results), thus this is not due to the Chl ligation state. It is possible that the two broad features are due to vibrational structure built upon \( Q_x \), or an additional electronic absorption.

![Fig 2](image-url) Simulations (colored traces) of the 1.4 K \( Q_y(1,0) \) absorption spectrum (black trace) for Chl \( a \)-WSCP. The simulations were performed using the FCFs determined for Chl \( a \)-WSCP (purple), for Chl \( a \) in the Photosystem I antenna complex [4] (dotted red trace) and for Chl \( a \) in the CP29 minor antenna complex from Photosystem II [20] (dotted green trace). See text and Appendix for details. The Chl \( a \)-WSCP absorption spectrum in the \( Q_y(0,0) \) region has been truncated for clarity of presentation.
4.2. Simulating the Qy(1,0) absorption spectrum from experimentally determined FCFs

A test of the validity of the FCFs determined by vibrational hole-burning is to reconstruct the Qy(1,0) absorption spectrum. Our simulation is based on the same assumptions used for estimating the FCFs, i.e. the Condon approximation and weak electronic-vibrational coupling, S < 0.1. The Qy(1,0) structure is built upon the Qy(0,0) origins for the two exciton components, which are both assumed to have the same vibrational structure. The details can be found in the Appendix.

The simulated Qy(1,0) region is shown in Fig 2 (thin traces). The overall magnitude of the simulated Qy(1,0) absorption is in agreement with the experimental spectrum. The solid purple trace shows the result using the vibrational frequencies and FCFs determined in this work, while the dotted red and green traces were simulated using the results from vibrational hole-burning of Chl a in the antenna complex of Photosystem I [4] and the CP29 minor antenna complex isolated from Photosystem II [20], respectively. It is clear that there is significant deviation in all the simulated Qy(1,0) profiles from the actual absorption spectrum for Chl a-WSCP. An SDF with FWHM = 500 cm⁻¹ results in a simulated Qy(1,0) spectrum that is featureless, while an SDF with FWHM = 300 cm⁻¹ shows only marginal structure (simulations not shown). In these latter cases, the distinction between the various sets of FCFs is less obvious than for Chl a-WSCP (i.e. SDF FWHM = 177 cm⁻¹).

In the work of Zazubovich et al. [2] vibrational frequencies and FCFs for BChl a in glycerol:water/LDAO (0.5%) and in neat triethylamine were determined using the same vibrational hole-burning method as used here. They reconstructed the Qy absorption spectra using the electronic-vibrational and electron-phonon parameters determined from the hole-burning data, and found reasonable agreement with the experimental absorption spectrum. However, the simulation could not be compared to the experimental absorption spectrum for vibrational frequencies greater than ~1000 cm⁻¹, due to an impurity absorption. The significantly broader SDF for BChl a in that system (FWHM ~600 cm⁻¹) compared to Chl a-WSCP (FWHM =177 cm⁻¹) lead to a featureless Qy(1,0) spectrum, as discussed above. Thus, we find that reconstruction of the Chl a-WSCP Qy(1,0) spectrum provides a far more stringent test of the FCFs determined by vibrational hole-burning than the broad and featureless spectra of (B)Chl systems in amorphous hosts, and in other protein systems.

If the Condon approximation no longer holds, the relative intensities of the pseudo-ZPHs in vibrational hole-burning spectra that correspond to the same vibrational modes may in general be different for different burn frequencies. As discussed above, this is what we have observed for some of the electronic-vibrational modes for Chl a in this work. The effect is most clearly demonstrated in simulated Qy(1,0) spectra, and this interpretation is supported by the magnetic circular dichroism (MCD) spectrum of Chl a-WSCP [1] which suggests that the Chl a Qy(1,0) region cannot be described within the Condon approximation, and vibronic coupling is active.

5. Conclusion

Consideration of inhomogeneous widths and vibrational hole-burning phenomena of Chl-WSCP suggest the original assignment of the Qx absorption for Chl a near ~570-590 nm, rather than the alternate assignment as hidden underneath the Qy(1,0) absorption near ~620-630 nm, as favored recently [13]. For the latter assignment to hold, significant fundamental variations Qy(1,0) vibrational structure, including vibronic coupling activity and inhomogeneous broadening mechanisms need to be invoked. We cannot exclude this situation, but prefer the simpler explanation (original assignment) in the absence of positive evidence. Simulation of the Qy(1,0) broadband spectrum from FCFs obtained via vibrational hole-burning leads to the conclusion that vibronic coupling is active for Chl a.

6. Acknowledgements

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References

[1] J.L. Hughes, R. Razeghifard, M. Logue, A. Oakley, T. Wydrzynski and E. Krausz, J. Am. Chem. Soc. 128 (2006) 3649-3658.
[2] V. Zazubovich, I. Tibe and G.J. J. Phys. Chem. B 105 (2001) 12410-12417.
[3] J. Pieper, J. Voigt and G.J. J. Phys. Chem. B 103 (1999) 2319-2322.
[4] J.K. Gillie, G.J. Small and J.H. Golbeck, J. Phys. Chem. 93 (1989) 1620-1627.
[5] M. Ratsep and A. Freiberg, J. Lumin. 127 (2007) 251-259.
[6] L.L. Shipman, T.M. Cotton, J.R. Norris and J.J. Katz, J. Am. Chem. Soc. 98 (1976) 8222-8230.
[7] T.A. Evans and J.J. Katz, Biophys. Acta 396 (1975) 414-426.
[8] D. Zevenhuijzen and P.J. Zandstra, Biophysical Chemistry 19 (1984) 121-129.
[9] C. Houssier and K. Sauer, J. Am. Chem. Soc. 92 (1970) 779-791.
[10] I. Renge, K. Mauring, P. Sarv and R. Avarmaa, J. Phys. Chem. 90 (1986) 6611-16.
[11] R.A. Avarmaa and K.K. Rebane, Spectrochimica Acta 41A (1985) 1365-1380.
[12] M. Umemtsu, Z.-Y. Wang, M. Kobayashi and T. Nozawa, Biochim. Biophys. Acta 1410 (1999) 19-31.
[13] M. Rätsep, J. Linnanto and A. Freiberg, The Journal of Chemical Physics 130 (2009) 1-11.
[14] S. Krawczyk, Biophys. Acta 976 (1989) 140-149.
[15] D. Horigome, H. Satoh, N. Itoh, K. Mitsunaga, I. Oonishi, A. Nakagawa and A. Uchida, J. Biol. Chem. 282 (2007) 6525-6531.
Appendix A. Franck-Condon vibrational overlap factors for the Q_y(1,0) state

The Condon approximation is assumed and is nominally valid for allowed electronic transitions, such as chlorophyll π-π* transitions. Within the harmonic oscillator approximation and at low temperature, the total intensity of an electronic transition that is accompanied by r quanta of the ith vibrational mode is modulated by the FCF,

$$ FCF_r = \sum S_i e^{-S_i r!} / r! , $$

where S is the Huang-Rhys parameter. The Huang-Rhys parameter is used as it identifies the average number of vibrational quanta that accompany the electronic transition. This parameter is in turn related to the Debye-Waller factor (α), by $S = -\ln(\alpha)$, and is a measure of the strength of the electronic-vibrational coupling for the mode in question. The Debye-Waller factor is given by the ratio of the intensity of the 0-quantum transition ($I_{0,0}$) to the sum of the intensities over all the r-quanta transitions, i.e. for $r > 0$

$$ e^{-S} = I_{0,0} / (I_{0,0} + \sum I_{0,r}). $$

Note that the purely electronic transition ($r = 0$) intensity is modulated by $e^{-S}$. This reflects the intensity redistribution that occurs when an electronic transition couples to vibrational modes. For weak electronic-vibrational coupling ($S < 0.1$) the majority (99%) of the absorption intensity is contained in the 0- and 1- vibrational quantum electronic-vibrational transitions. In this regime, the sum over $I_{0,r}$ can be approximated by the 1-quantum term $I_{0,1}$, and thence $FCF_{r1} = S e^{-S} \sim S_i$. Thus, where the electronic-vibrational coupling is weak ($S < 0.1$), the FCF is approximated well by the Huang-Rhys parameter.

Within these approximations, the ratio of the hole-areas of the pseudo-ZPHs for the ith and jth vibrational modes can be obtained very simply by determining the pseudo-ZPH-area ratios $i/r$, of the intensities over all the r-quanta transitions, i.e. for $r > 0$

$$ S_i e^{-2S_i} / S_j e^{-2S_j} , $$

which for $S_{ij} < 0.1$ is approximately $S_i / S_j$. If the upper limit of the electronic-vibrational coupling for a mode is $S = 0.1$, then the maximum error that this method introduces for that mode will be less than 20%. Thus, the relative FCFs for the ith and jth vibrational modes can be obtained very simply by determining the pseudo-ZPH-area ratios in vibrational satellite hole-burned spectra. As the pseudo-ZPHs represent zero-phonon transitions, determination of the relative intensities of the pseudo-ZPHs in the vibrational satellite hole-burned spectra requires normalization of the ΔΔ spectra to the profile of the hole-burning action spectrum [2]. For this purpose we used our Gaussian fit to the action spectrum (see text).

From equation (2), the absolute value for the Huang-Rhys parameter can be determined from the ratio of a resonantly burned ZPH and the sum of the ZPH and the vibrational satellite holes for the ith mode, which can be approximated well by the 1-quantum term only. For completeness, we note that the overlap factor for an r-quantum transition, $S e^{-S r!} / r!$, applies for each vibrational mode and thus there will be a reduction of the intensity in the electronic origin due to each Franck-Condon active vibrational mode. If the contributions are additive, then the total (normalized) intensity that the purely electronic origin will lose is $(1/n) \sum e^{-S_i}$, where n is the number of Franck-Condon active modes. However, the method is justified [20] given the weak electronic-vibrational coupling, and for values of $S < 0.1$ it introduces less than 10% error.

To determine absolute values for the FCFs, we used a burn at ~682 nm, and the method described above. We found a FCF for the 385 cm⁻¹ mode of 0.014, which agrees very well with the FCF of 0.015 for the 390 cm⁻¹ mode as determined by Gillie et al. [4]. This mode also exhibits good mirror symmetry between the ground and excited state, and is therefore considered to be a reliable benchmark for determining the absolute values of the FCFs. This procedure was then used to determine the absolute values for the relative FCFs that we obtained from the 666 nm...
vibrational satellite burn. These absolute values were found to have reasonable agreement with those of Gillie et al. [4]. There were however some modes that exhibited significantly different FCFs when determined from different vibrational burns, with variations outside the ±20% range expected from this method of analysis. This is discussed in the main text. Thus, for the successive normalization of the FCFs obtained by sequential vibrational satellite burns, where the burn frequency was varied throughout the \( Q_y(1,0) \) region, we compared the normalization using all the available modes found within a given burn. Those that gave relative normalizations that matched with other burns the best were used for the absolute FCF determination. For the vibrational modes that appeared in more than one vibrational satellite hole-burned spectrum, the FCFs presented in Table 1 of the main article were obtained by averaging the values.

Appendix B. Simulation of the \( Q_y(1,0) \) absorption spectrum from FCFs

To fully reproduce the spectra we need to consider the homogeneous profiles, as well as the effect of inhomogeneous broadening. We consider only the 1-quantum vibrational transitions that are built upon the electronic origin in the usual Franck-Condon manner, and use the vibrational frequencies and Franck-Condon vibrational overlap factors determined above. Vibrational relaxation times are on the order of a few ps, leading to lifetime-limited Lorentzian linewidths of the order of a few cm\(^{-1}\), as evidenced by the resonantly burned vibrational holewidths (data not shown). The inhomogeneous broadening is accounted for by convolution of the SDF profile (Gaussian, FWHM = 177 cm\(^{-1}\)) with the homogeneous absorption band.

The profile of the \( Q_y(1,0) \) absorption region is approximated by a sum of Gaussian bands (FWHM=177 cm\(^{-1}\)) centered to higher energy from the origin transition energy by the vibrational frequencies. With our approximations, these \( Q_y(1,0) \) Gaussian bands are weighted by the FCFs, with respect to the intensity of their \( Q_y(0,0) \) origin, and since the FCFs are small, the \( Q_y(0,0) \) transition can just be approximated to 1, i.e. \( \Sigma c^{S_i} \approx 1 \). This results in two series of \( Q_y(1,0) \) bands for the \(|\pm\rangle\) and \(|\mp\rangle\) exciton components. The asymmetry in the \(|\mp\rangle\) origin band due to electron-phonon coupling is neglected, and it is modelled as the SDF Gaussian determined from the action spectrum. We consider this a reasonable simplification here, given the other approximations. The \(|\mp\rangle\) intensity is given by the ratio of the \(|\pm\rangle\) and \(|\mp\rangle\) absorptions. This allows all the \( Q_y(1,0) \) bands, \(|\pm\rangle_{1,0}\), to be simply scaled from the \(|\mp\rangle_{0,0}\) peak height.

An estimate needs to be made of the peak height for the \(|\mp\rangle_{0,0}\) absorption. The actual absorption value at the long wavelength should be an upper limit, which has been estimated to decrease by a factor of roughly ~2 when the lifetime broadening of the \(|\pm\rangle\) component is accounted for [18]. This is because the fit of the spectra the \(|\pm\rangle\) component has a significant absorption tail in the long wavelength region. Likewise, the relative intensity of the \(|\pm\rangle\) and \(|\mp\rangle\) transitions needs to be estimated. From our previous simple Gaussian band fit of the \( Q_y(0,0) \) absorption, this ratio was found to be 3.7:1, and in the fit using a lineshape theory by Renger et al. [18], it increases by a factor of approximately ~2, i.e. ~7-8:1. Thus, the \( Q_y(1,0) \) region is given by:

\[
Q_y(1,0) = I_0 \times \Sigma \{ FC_F \times SDF[\omega - (\omega_0 + \omega_j)] + R_{\pm} \times FC_F \times SDF[\omega - (\omega_0 + 2J+ \omega_0)] \} \tag{4}
\]

where \( I_0 \) is the peak height of the \(|\mp\rangle_{0,0}\) absorption band, SDF[x] is the Gaussian describing the \(|\mp\rangle_{0,0}\) absorption, \( \omega \) is energy (cm\(^{-1}\)), J is the transition-dipole–transition-dipole coupling (cm\(^{-1}\)), \( R_{\pm} \) is the ratio of the intensities for the \(|\pm\rangle_{0,0}\) and \(|\mp\rangle_{0,0}\) transitions, and the sum \( \Sigma \) is over all the Franck-Condon vibrational modes, i. The \( Q_y(1,0) \) spectrum was simulated using \( J = 90 \) cm\(^{-1}\), an SDF centered at 14660 cm\(^{-1}\) with FWHM = 177 cm\(^{-1}\) and an estimate for the \(|\mp\rangle_{0,0}\) absorption peak height and \(|\pm\rangle_{0,0}\) to \(|\mp\rangle_{0,0}\) intensity ratio consistent with the analysis by Renger et al. [18]. The latter two estimates mostly serve to determine the absolute magnitude of the simulated \( Q_y(1,0) \) absorption (which is in general agreement with the actual spectrum, see Fig 2 of main article), whereas the structured profile is determined by the relative FCFs and is of primary interest to us in this analysis.