Supporting Information for: The Effect of Ions on the Optical Absorption Spectra of Aqueously Solvated Chromophores

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S1 Potential of mean force

The ion pair formation of Na\(^+\) with the phenolate oxygen was investigated by generating potential of mean force (PMF) along the Na\(^+\)-O1 distance reaction coordinate in the absence
of any NaCl in the solution environment. Thirty-six windows were generated between Na\(^+\)-O1 distances of 2.1 Å to 11.0 Å. The O1 and Na\(^+\) positions were restrained with a harmonic potential with a 1,000 kJ/(mol*nm\(^2\)) force constant. Simulation for each window was run for 2 ns with 1 ps sampling. Simulation details are provided in section II A of the main article. The same conditions were applied for the PMF with the exception that the 86 Cl\(^-\) and 86 of the 87 Na\(^+\) were removed and the simulation box volume was re-equilibrated in the NPT ensemble for 2 ns. The PMF was generated from the window sampling using the weighted histogram analysis method (WHAM).\(^1\) The resulting PMF is plotted in Figure S1, where the contact ion pair (CIP), solvent-separated ion pair (SSIP), and the double solvent-separated ion pair (2xSSIP) are identified and illustrated. Because the 2xSSIP is a shallow free energy minimum, and less likely to impact the electronic structure of the chromophore, the analysis in this study considers the role of the CIP and SSIP only.

![Figure S1: The plot of potential of mean force for the O1-Na\(^+\) distance reaction coordinate. The free energy minima for each type of ion pair are highlighted: in red is the contact ion pair (CIP), in green is the solvent-separated ion pair (SSIP), and in blue is the double solvent-separated ion pair (2xSSIP). Corresponding snapshots taken from the window sampling are included to illustrate the ion pairing.](image)
S2 Structural Analysis of Ion Pairing from MD Simulation

A dihedral angle was defined to measure how the CIP and SSIP Na$^+$ ion resides relative to the phenolate (O1) and imidazolinone (O2) ring. The plane of each ring is defined by the positions of the covalently bonded C-C-O atoms. The angle-defining atoms are shown in the insets in Figure S2A. The inset of Figure S2B illustrates the angle values for the sodium ion relative to its position above or below the plane of the phenolate ring. The distributions of the observed dihedral angles for the CIPs and SSIPs for each oxygen site are plotted in Figure S2A and S2B, respectively. For both oxygen sites, the CIPs showed a stronger preference for lying above or below (angle values near 90$^\circ$) the plane of the imidazolinone or phenolate ring as compared to the SSIPs. Additionally, that preference is enhanced for the imidazolinone oxygen site compared to the phenolate oxygen site.

Figure S2: The probability distributions of a CIP (A) or SSIP (B) forming at a given C-C-O...Na$^+$ dihedral angle. The insets in panel A show the atoms defining the dihedral angles for an example CIP at the O1 (phenolate) site and an example SSIP at the O2 (imidazolinone) oxygen site. The inset in panel B depicts how the dihedral angle is defined from the C-C-O plane, including its 180$^\circ$ symmetry. Data for the phenolate oxygen is drawn in blue and data for the phenolate oxygen in red.
Impact of Ion Force Field on Ion Pairing and Chromophore Hydrogen Bonding

In addition to the ion potentials described in the main text, two additional force fields were simulated for 80 ns under the same conditions: updated AMBER ion parameters given by Joung and Cheatham\(^2\) and parameters applying an electronic continuum correction to account for polarization developed by Kohagen, Mason and Jungwirth.\(^3\) The resulting ion pairing probabilities and effect on water hydrogen bonding at the chromophore oxygen sites are given in Table S1.

Table S1: The average chromophore-water hydrogen bond distance (O1–H\(_W\), O2–H\(_W\)), angle (O1...H\(_W\)–O\(_W\),O2...H\(_W\)–O\(_W\)), and number are calculated for snapshots classified as a CIP, SSIP or no IP at each chromophore oxygen site. Below those are the probabilities of occurrence of each ion pair type for both oxygen sites. The top half of the table are resuls using the ion parameters developed in ref. 2 and the bottom table from ref. 3.

<table>
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<th>Hydrogen Bond</th>
<th>Ref. 2 Ion Parameters</th>
<th>Ref. 3 Ion Parameters</th>
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<tr>
<td></td>
<td>CIP</td>
<td>SSIP</td>
</tr>
<tr>
<td>O1 Length [Å]</td>
<td>1.86</td>
<td>1.88</td>
</tr>
<tr>
<td>O2 Length [Å]</td>
<td>1.83</td>
<td>1.85</td>
</tr>
<tr>
<td>Angle [degrees]</td>
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</tr>
<tr>
<td>Number</td>
<td>1.61</td>
<td>0.95</td>
</tr>
<tr>
<td>% Occurrence</td>
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<td>11.0 ± 2.6</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Hydrogen Bond</th>
<th>CIP</th>
<th>SSIP</th>
<th>No IP</th>
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<tr>
<td>O1 Length [Å]</td>
<td>1.84</td>
<td>1.87</td>
<td>1.82</td>
</tr>
<tr>
<td>O2 Length [Å]</td>
<td>1.82</td>
<td>1.85</td>
<td>1.81</td>
</tr>
<tr>
<td>Angle [degrees]</td>
<td>158.8</td>
<td>156.9</td>
<td>161.0</td>
</tr>
<tr>
<td>Number</td>
<td>1.95</td>
<td>1.20</td>
<td>3.12</td>
</tr>
<tr>
<td>% Occurrence</td>
<td>6.4 ± 1.6</td>
<td>6.9 ± 1.6</td>
<td>31.6 ± 6.6</td>
</tr>
</tbody>
</table>
S4  Non-covalent interaction (NCI) analysis between chromophore and water molecules

Fig S3 shows the isosurface plot of the reduced density gradient obtained using Multiwfn 3.6,\textsuperscript{4} revealing the non-covalent interactions in real space. See ref.\textsuperscript{5} for theoretical details of the method. The analysis shows that the solvated GFP chromophore forms three hydrogen bonds at the O1 site.

![Figure S3: Reduced density gradient isosurface plot for GFP chromophore and three water molecules forming hydrogen bonds at the phenolate oxygen (O1). The dark-blue density region between the O1 oxygen and the Water 1 molecule indicates a strong hydrogen bond and the light-blue density regions located between O1 and Water 2 and 3 indicate weak hydrogen bonds.](image)

S5  Excited state calculations

S5.1  QM solvent shell

Fig. S4 shows an example of a QM solvent shell where the ions at the boundary are not completely solvated. A simple 8Å region cutoff radius for both solvent molecules and ions
Figure S4: Snapshot of a QM region that had poor SCF convergence. The carbon atoms are cyan, nitrogens atoms are blue, oxygens atoms are red color, hydrogens atoms are white, sodium ions are yellow, and chlorine ions are purple.

results in a significant number of snapshots where ions are located at the boundary of the QM region. Such snapshots result in poor SCF convergence. The convergence can be improved by setting selection criteria for the QM region such that ions at the boundary are also screened by water molecules. The details are discussed in section II C of the main article.

S5.2 Electron density difference

Fig. S5 shows the ground to excited state density difference plot for one snapshot with a CIP at the O1 site. The isosurface is plotted at an isovalue of 0.00005. The green isosurface represents positive density difference and the orange isosurface represents negative density difference. On excitation, the electron density is transferred from green to orange. The distance for this CIP is 2.57 Å.

S6 Screening of distant ions by QM and MM solvent

Fig. S6 compares the ensemble absorption spectra simulated from snapshots of the trajectory with a single Na⁺ ion to that from snapshots with no ion pairing from the 1M NaCl MD trajectory for the large QM region and for an all MM treatment of the environment. For both solvent treatments, the spectra are almost identical, which shows that solvent treated
as fixed point charges effectively screens the chromophore from the distant ions. The MM spectra are blue shifted compared to the QM/MM spectra as has been seen in previous work. The fixed MM point charges tend to over-polarize the QM electron density, leading to over-stabilization of the ground state and a blue shift of the excitation energy.

S7 Analysis of the effect of ions on the geometry of the chromophore

Table S2 presents the mean and standard deviation of four key bond lengths of the chromophore. Bond labels are given in Fig. S7. Similar means and standard deviations for CIP configurations at O1 and O2 indicate that there is no significant change in geometry upon CIP formation for two different sites of the chromophore that would cause the observed spectral shifts.

To further confirm that the chromophore geometry does not contribute to the spectral shifts observed for the two sets of CIP configurations, we computed ensemble absorption spectra with the environment from CIP O1 and CIP O2 snapshots removed. If the geometries were different for the two sets of chromophore configurations, we would expect a shift in
Figure S6: Computed ensemble absorption spectrum for the GFP chromophore anion in aqueous solution with a single Na\(^+\) counter ion (500 snapshots) and the chromophore in concentrated 1 M NaCl aqueous solution using configurations with no ion pairing (1015 no IP snapshots). The chromophore is treated with QM, but the environment is treated with fixed point charges electrostatically embedded into the QM region. (a) The large QM region shown in the text is used. (b) The chromophore is treated with QM, but the environment is treated with fixed point charges electrostatically embedded into the QM region.

spectra similar to Fig. 4 (main text). However, the spectra obtained from the two sets of CIP configurations (green and pink) computed for the chromophore in vacuum are very similar, suggesting that the geometry of the chromophore is not responsible for the spectral shifts.

S8 Analysis of direct effect of the ions on the electronic structure of the chromophore

We analyzed the direct effect of the ion on the chromophore’s excitation energy by first examining how the proximity of the Na\(^+\) ion within the CIP correlates with the excitation
Table S2: The mean($\mu$) and standard deviation($\sigma$) for C1-O1, C2-C2, C3-C4 and C5=N1 bonds measured for CIP at O1, CIP at O2 and for all the snapshots (20 ns trajectory) The bond length parameters under the heading combined included all the possible configuration of CIP, SSIP and no IP. Bond lengths were measured in Å. Refer to Fig. S7 for bond labels.

<table>
<thead>
<tr>
<th>Bond Label</th>
<th>CIP at O1</th>
<th>CIP at O2</th>
<th>Combined</th>
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<tr>
<td></td>
<td>$\mu$</td>
<td>$\sigma$</td>
<td>$\mu$</td>
</tr>
<tr>
<td>C1-O1</td>
<td>1.2336</td>
<td>0.0223</td>
<td>1.2349</td>
</tr>
<tr>
<td>C2-O2</td>
<td>1.2210</td>
<td>0.0220</td>
<td>1.2213</td>
</tr>
<tr>
<td>C3-C4</td>
<td>1.5003</td>
<td>0.0271</td>
<td>1.5009</td>
</tr>
<tr>
<td>C5=N1</td>
<td>1.3475</td>
<td>0.0236</td>
<td>1.3475</td>
</tr>
</tbody>
</table>

energy. A scatter plot of the computed excitation energy versus the distance of the ion from the oxygen site is shown Fig. S9. There is a significant degree of noise in the data due to the heterogeneous distribution of the solvent environment along with the changes in the chromophore geometry during the MD. It is therefore difficult to determine any correlation between excitation energy and ion distance for this distribution of snapshots. In the main text we isolate the effect of the ion on the excitation energy by freezing all atoms except the ion and moving it closer to and further from the CIP oxygen site. This scan showed that the change in excitation energy due to the ion was opposite that seen for the CIP MD configurations.

To better understand the reverse trend observed in excitation energy when the ion is
Figure S8: The ensemble absorption spectra of GFP chromophore anion for two different configurations of CIP, simulated with the environment (red and blue color spectra) and without environment (vacuum) (maroon and yellow spectra). Using the same snapshots with the environment stripped away, the corresponding spectra for the chromophore in the vacuum are shown in light green and magenta.

moved closer or further from the electronegative oxygen site of the chromophore, we analyzed the partial atomic charges on O1 oxygen and O2 oxygen. The charges are calculated using the Mulliken population analysis scheme. Fig S13 shows how the charge on O1 and O2 site of the chromophore changes with distance. For both CIP sites, the oxygen becomes more electronegative as the ion moves closer to the oxygen. This change in charge supports our hypothesis that the positive ion stabilizes the negative charge at the oxygen site, which is greater for O1 in the ground state and greater for O2 in the excited state.

S9  Analysis of the indirect effect of the ion on the chromophore excitation energy: Changes in hydrogen bonding

To determine why the computed ensemble spectrum for ion pairs shifts in the opposite direction for what would be expected based on the excitation energies obtained from scanning the Na+ distance from the ion pairing site, we next analyze the indirect changes in the solvent
environment caused by ion pair formation. Following a similar analysis as we performed for Na\(^+\), we looked for correlation of hydrogen bond strength with excitation energy. We show in Figs. S11 and S12 the computed excitation energy versus the bond length of the shortest of the hydrogen bonds formed between water and the oxygen site of the CIP. There is again a considerable degree of scatter in the excitation energies, as would be expected for a heterogeneous environment.

In Fig. S13 we show that the charge distribution on the O1 oxygen of the chromophore correlates with the excitation energy. The O1 oxygen has a more negative Mulliken charge for the configurations with no ion pairing compared to the CIP configurations. Computed Van Alsenoy charges show the same trend as the Mulliken charges. This change in charge is likely due to the presence of the stronger H-bonding environment in the absence of ion pairing. This stronger H-bonding environment leads to a higher excitation energy, presumably from better stabilization of the ground state that has the negative charge mostly localized at the phenolate O1 oxygen site.
Figure S10: The Mulliken charges on O1 and O2 sites of the chromophore versus Na\(^+\)-O distances. Values are shown for six randomly selected snapshots from the CIP configurations.

Figure S11: Scatter plot showing how excitation energy (eV) varies with distance of hydrogen bond from the O1 oxygen site for the strongest hydrogen bond.
Figure S12: Scatter plot showing how excitation energy (eV) varies with distance of hydrogen bond from the O2 oxygen site for the strongest hydrogen bond.

Figure S13: Contour plot of excitation energy (eV) versus charge on the O1 site of the chromophore. The charges are calculated using Mulliken population analysis scheme implemented in the TeraChem software package. The black contour shows correlation between charges and excitation energies calculated for snapshots with no ion pair. Red contour shows the same quantities calculated for the CIP O1 snapshots.
References


