

1 Supplementary Information for The Carbonyl-Lock
2 Mechanism Underlying Non-Aromatic Fluorescence
3 in Biological Matter

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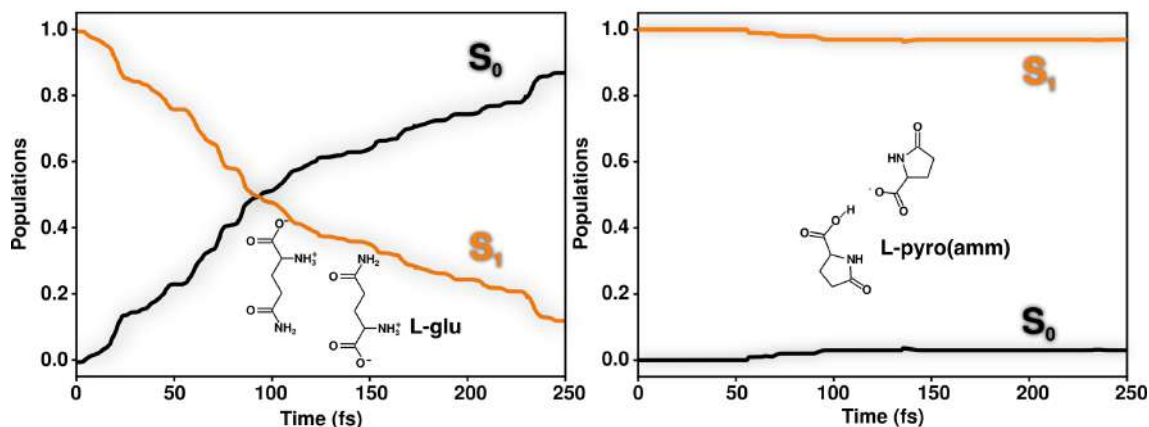
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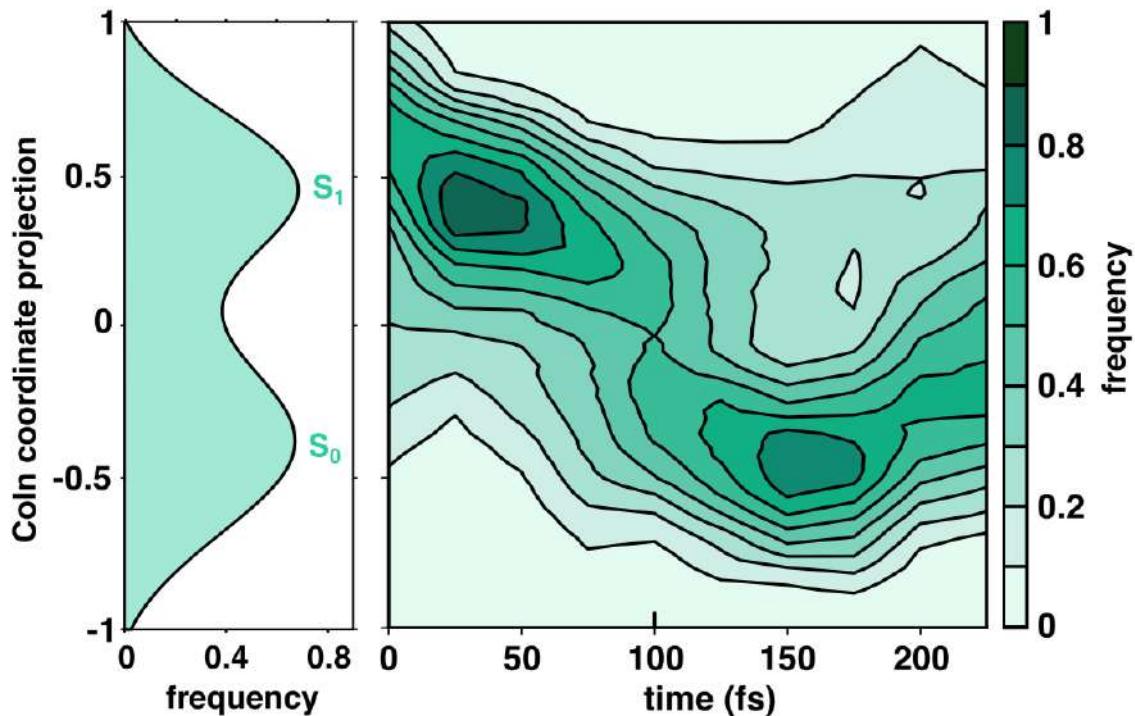
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26 The left panel of Supplementary Figure 1 shows that during the 250 fs after
27 excitation L-glu has almost completely decayed to the ground state (99% of the
28 trajectories relax to the S_0 state in this period. The non-radiative relaxation to the
29 ground state occurs between 60-150 fs. This extremely short lifetime is explained
30 by the passage through an S_1 - S_0 CoIn. Conversely, L-pyro(amm) remains in the S_1
31 state with an almost negligible population transfer to the ground state (only 2% of
32 the trajectories relax to the



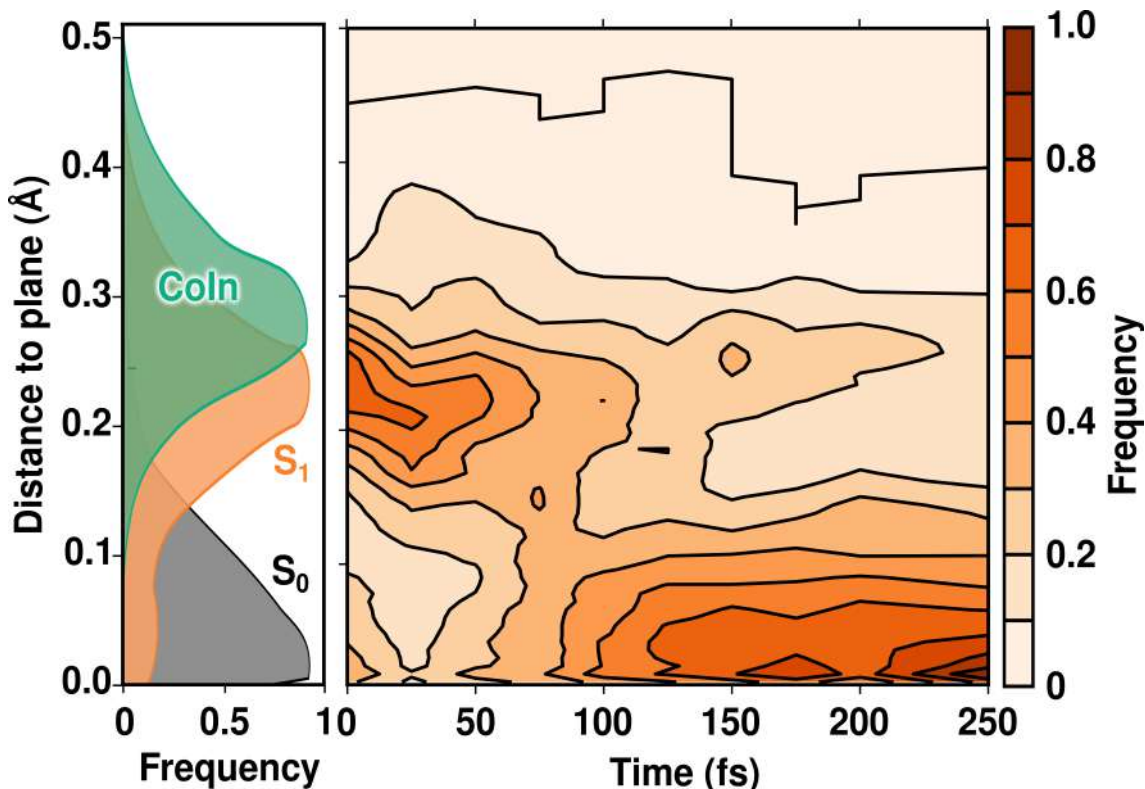
Supplementary Figure 1: Average populations of S_1 (orange) and S_0 (black) electronic states for the L-glu (left) and L-pyro(amm) (right) systems.

33 Supplementary Figure 2 shows the distribution of the AIMD projections in the
34 $S_1 \rightarrow S_0$ relaxation pathway, as well as the time evolution of these projections. The
35 observed distribution is bimodal with one maximum corresponding to the S_0 state
36 and the other to the S_1 state.



Supplementary Figure 2: Relaxation pathway histogram. The left panel represents the overall histogram of the AIMD projection in the relaxation pathway vector, discriminating the components associated with S_0 (black), S_1 (orange), and CoIn (green) dynamics. The right panel shows the time evolution of the relaxation pathway projected AIMD histogram.

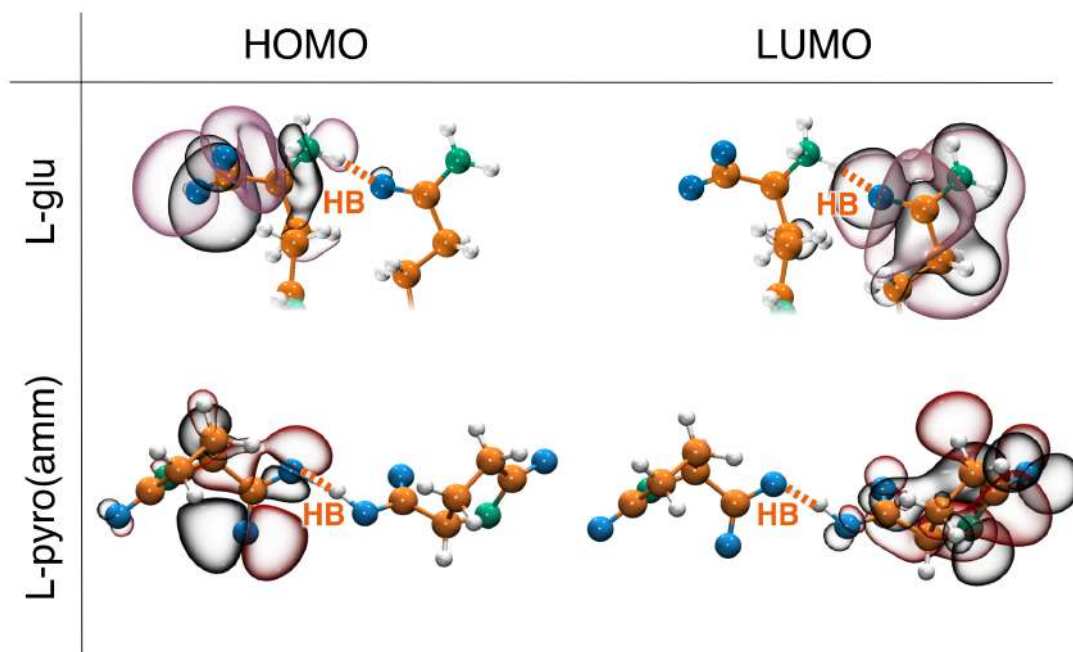
37 Supplementary Figure 3 shows the displacements along the amide deplanariza-
 38 tion degree of freedom during the NAMD simulations of L-glu. The mode was
 39 described computing the carbonyl C distance to the plane formed by N, O and C
 40 neighboring atoms. In the S_1 state the amide plane is strongly distorted, and the
 41 decay to the ground state is accompanied by a re-planarization.



Supplementary Figure 3: Distance of the C atom of the carbonyl to the plane formed by the atoms neighbors in the amide group of L-glu. Top panel show the evolution time in all the NAMDs and lower panel show the planarization as a histogram in the different electronic states as well as in the CoIn. (The histograms were normalized independently.)

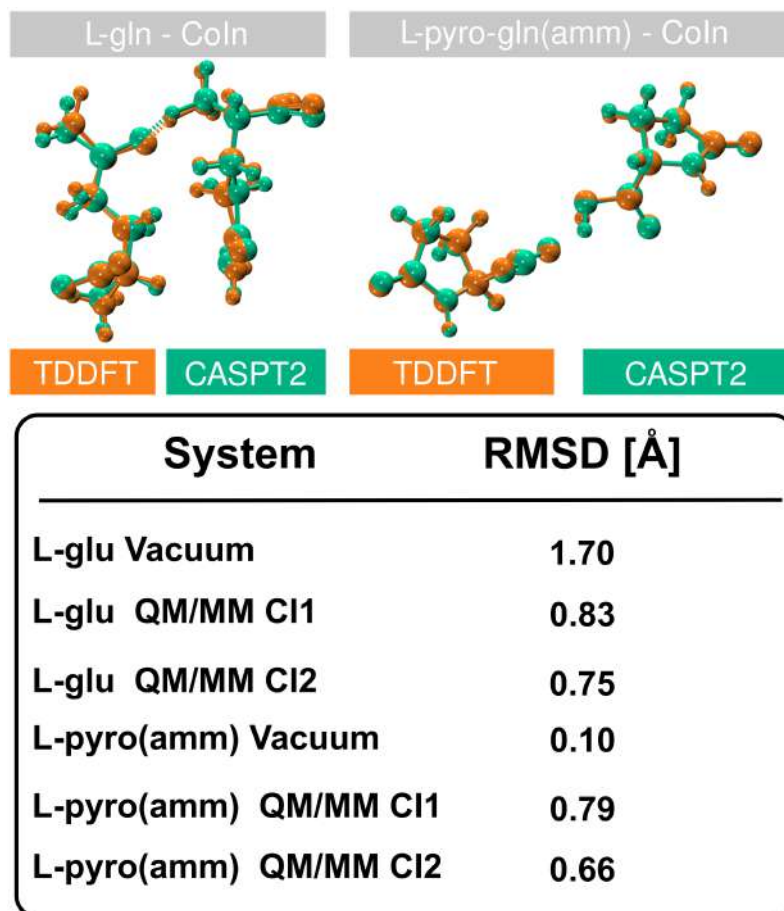
42 L-glu system exhibits a deplanarization of the amide group in the S_1 state as a
 43 consequence of the stretching of the carbonyl group. Upon stretching, the carbonyl
 44 loses its double bond character breaking the characteristic planar arrangement of
 45 its neighbors. In the CoIn, L-glu increases its deplanarization, which is consistent
 46 with the observations made by Grisanti et al[1], showing that the deplanarization
 47 minimizes the energy gap during the excited state dynamics of several amyloid model
 48 systems.

49 Supplementary Figure 4 depicts the HOMO and LUMO orbitals of L-glu and L-
50 pyro(amm). The transition between these two is the primary component of the S_1
51 state. In both L-glu and L-pyro(amm) the S_1 state corresponds to a charge transfer
52 transition between two hydrogen-bound molecules.



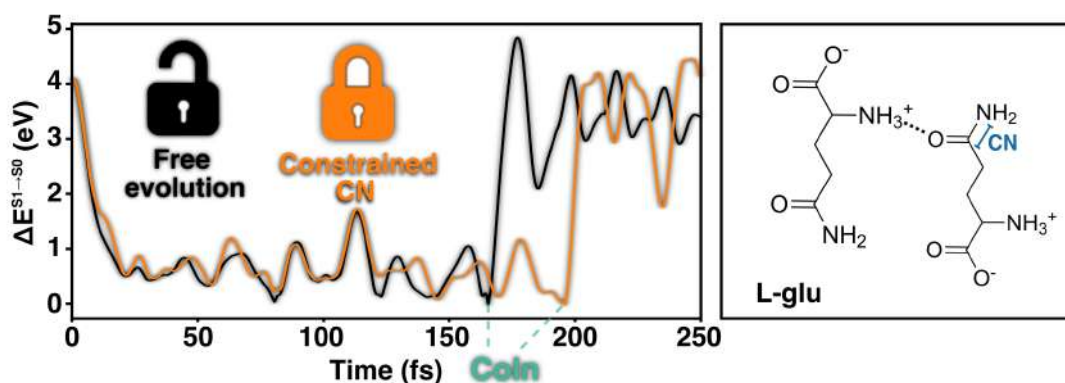
Supplementary Figure 4: HOMO and LUMO orbital representation of L-glu and L-pyro(amm).

53 Supplementary Figure 5 shows a comparison between the average CoIn structures
54 obtained from TDDFT-based NAMD simulations and CASPT2 CoIn optimizations
55 (see methods section). The resemblance between the two levels of theory serves as
56 a validation for the TDDFT description near the S_1 - S_0 CoIn.



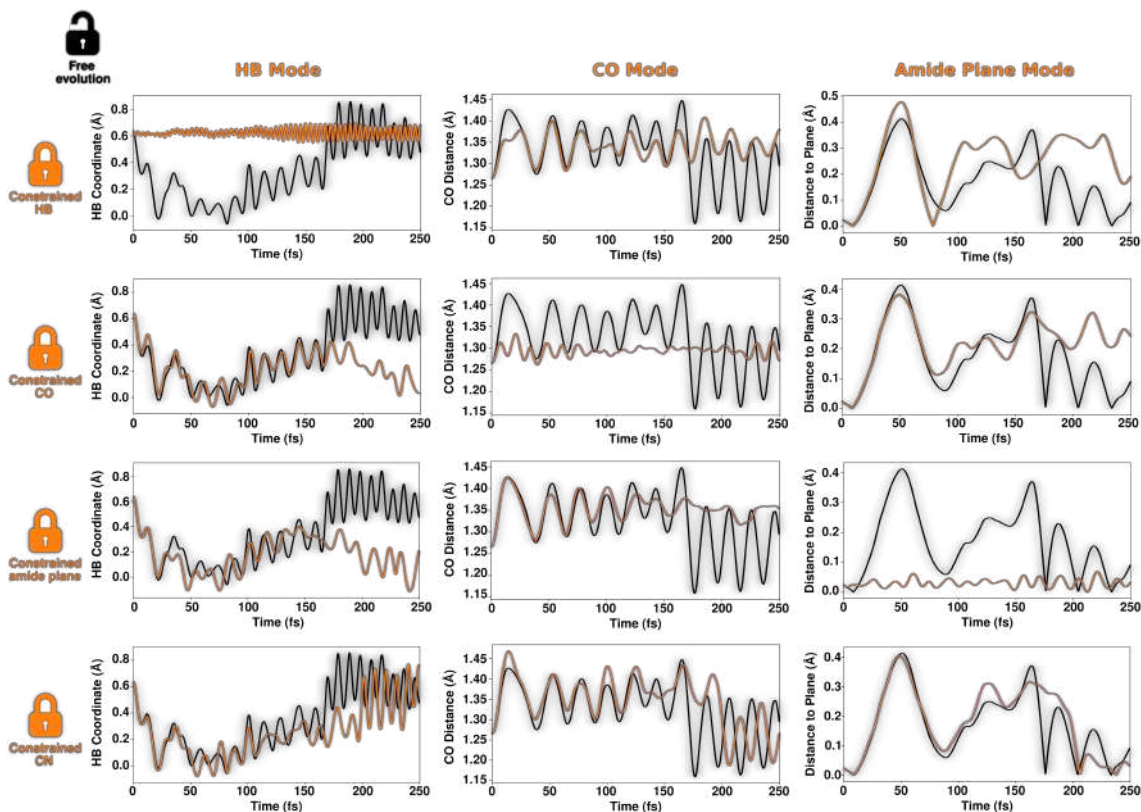
Supplementary Figure 5: Validation of our TDDFT NAMD simulations in the vicinity of the CoIns. The left panel shows the structural comparison between the average CoIn geometry visited by L-glu in our TDDFT-based NAMD simulations (orange plot), and our CASPT2 CoIn optimizations. The right panel shows an analogous comparison for the case of L-pyro(amm).

57 Supplementary Figure 6 shows the S_1-S_0 energy gap as a function of time for a
 58 selected trajectory. The black curve corresponds to a freely evolving system decaying
 59 to the ground state at ≈ 160 fs, and the orange curve corresponds to the same
 60 trajectory with an harmonic constraint on the CN DoF. The latter is in contact but
 61 not directly involved with the $S_0 \rightarrow S_1$ relaxation pathway DoFs. As a consequence,
 62 the applied constraint does not delay significantly the relaxation, and the system
 63 decays to the ground state only ≈ 30 fs later than the unconstrained trajectory.



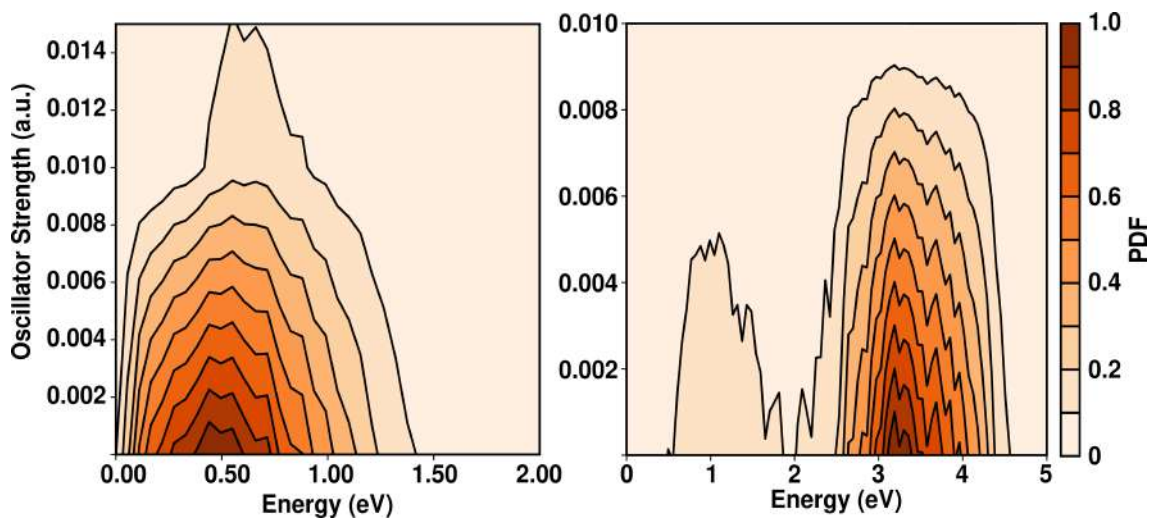
Supplementary Figure 6: Energy gap for a selected L-glu trajectory evolving freely (black curve) or applying an armonic constraint (orange curve) on the CN distance. Both trajectories reach the CoIn with a difference of ≈ 30 fs.

64 Supplementary Figure 7 shows the interplay between the different degrees of
 65 freedom (DoFs) involved in the $S_1 \rightarrow S_0$ relaxation pathway of L-glu. Each plot
 66 illustrates the displacement of a given DoF during a NAMD trajectory. The figure
 67 reveals the crossed influence between the different DoFs, i.e. the extent by which
 68 a constraint in a given DoF affects the displacement on another DoF. This data is
 69 employed to build the bar chart in Figure 2 in the main text.



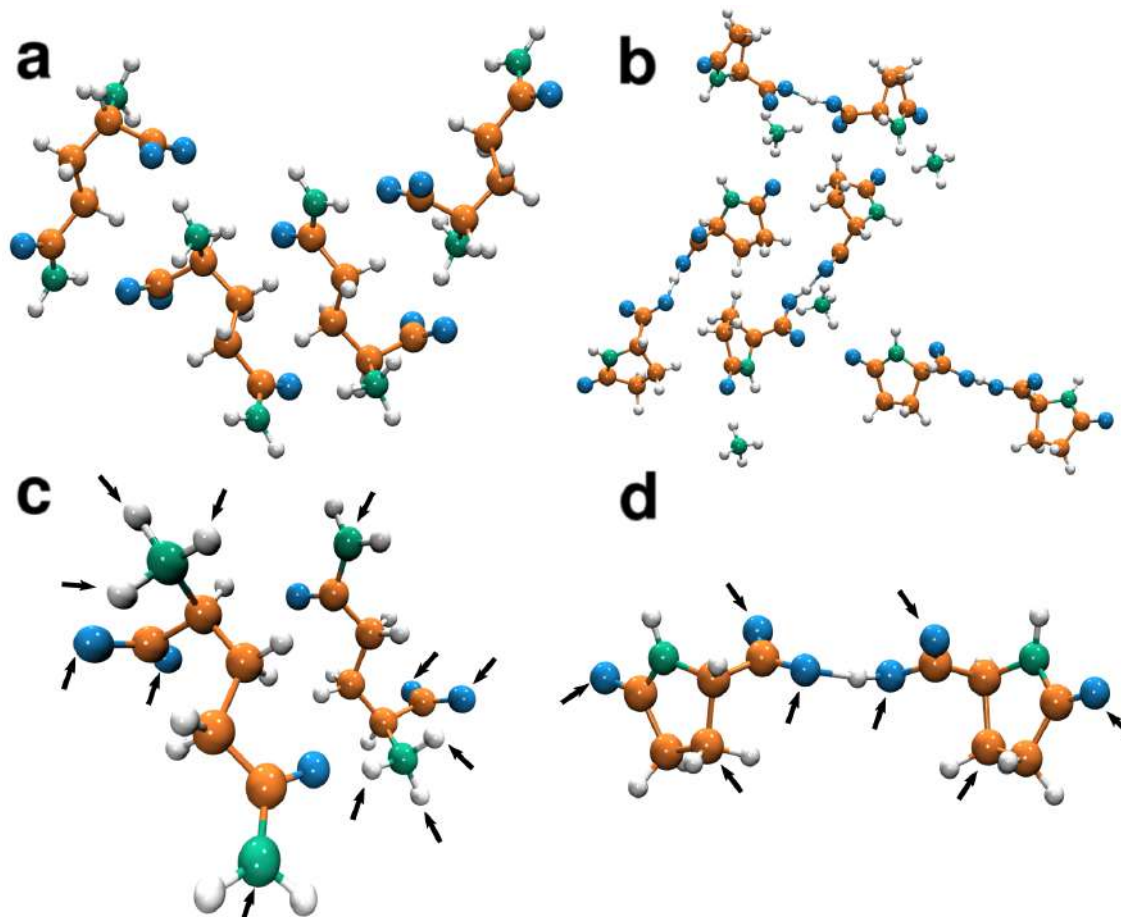
Supplementary Figure 7: Temporal evolution for the degrees of freedom integrating the $S_1 \rightarrow S_0$ relaxation pathway in L-glu trajectory evolving freely (black lines) or under an harmonic constraint applied on selected degrees of freedom. Left panels show the evolution of the HB coordinate. Mid panels show the evolution of the CO distance. Right panels show the evolution the amide planarization mode. The orange curves in the first (top) row plots illustrate the time evolution of the corresponding degrees of freedom under an applied harmonic constraint in the HB mode. The orange curves in the second row are the result of applying a constraint in the CO distance. In the third row the applied constraint is on the amide plane, and in the forth row the applied constraint is on the CN distance.

70 Supplementary Figure 8 shows the distribution of instantaneous radiative $S_1 \rightarrow S_0$
71 transition probabilities, along the transient dynamics in the S_1 state of L-glu (left
72 panel) and L-pyro(amm) (right panel). The instantaneous radiative decay probabil-
73 ities are determined employing the oscillator strength of the vertical transitions at
74 every nuclear configuration sampled in the MD simulations. Both distributions of
75 L-glu and L-pyro(amm) show a similar order of magnitude in the oscillator strength,
76 indicating that the origin of fluorescence in L-pyro(amm) is primarily due to the S_1
77 lifetime increase rather than an enhancement of the instantaneous radiative decay
78 probability.



Supplementary Figure 8: Oscillator strength distribution associated to the radiative $S_1 \rightarrow S_0$ transition for L-glu (left panel) and L-pyro(amm) (right panel).

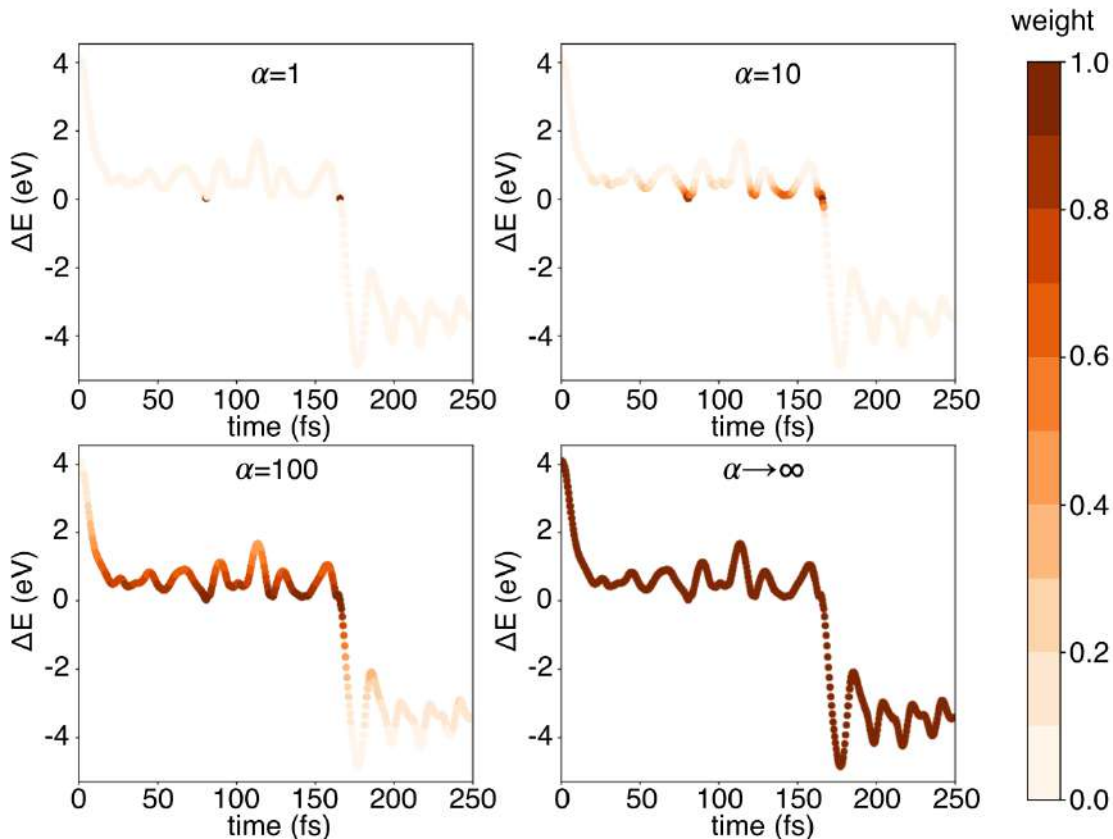
79 Supplementary Figure 9 illustrates the crystal structures of the L-glu and L-
80 pyro(amm) obtained by Stephens et al, [2] as well as the dimer model systems
81 employed throughout this work. In order to preserve the molecular arrangement
82 of the crystal structure we applied soft harmonic constraining potentials (a force
83 constant of 100 Kcal/molÅ) to the atoms indicated in Figure 9.



Supplementary Figure 9: Molecular structures. a) L-glutamine, b) L-pyroglutamine-ammonium, c) Model system of L-glu, d) system model for L-Pyro-amm, named L-pyro(amm) since it does not contain the ammonium ion explicitly but it preserves the structural arrangement of the system in presence of ammonium. The black arrows on system models (panel c and d) represent the positions where the soft constraints were applied.

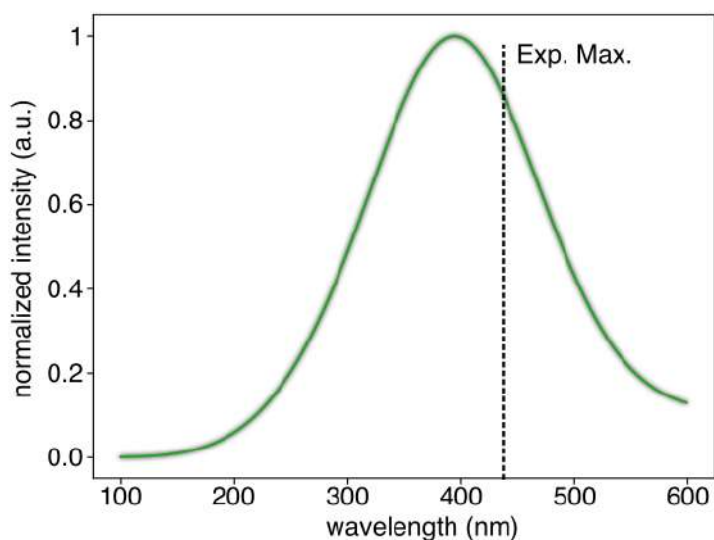
84 Supplementary Figure 10 shows the influence of the α parameter selection in the
85 relative statistical weight of the different geometric fluctuations during the NAMD
86 trajectory of L-glu. When $\alpha \rightarrow \infty$ the Arrhenius term in equation 7 in the main
87 text is equal to 1, and all the configurations explored during the NAMD trajectories
88 are equally weighted in the relaxation coordinate. As discussed in the methods
89 section, this introduces elements to the total variance that are not necessarily related

90 to the decay process itself. In contrast, when $\alpha = 1$ only configurations that are in
 91 the vicinity of the CoIn have significant contribution to the overall coordinate. In
 92 this case the fluctuations that lead to the CoIn proximity are neglected in the final
 93 relaxation coordinate. With $\alpha = 100$ the energy fluctuations that occur near the
 94 S_1-S_0 CoIn are considered in the relaxation coordinate estimation.



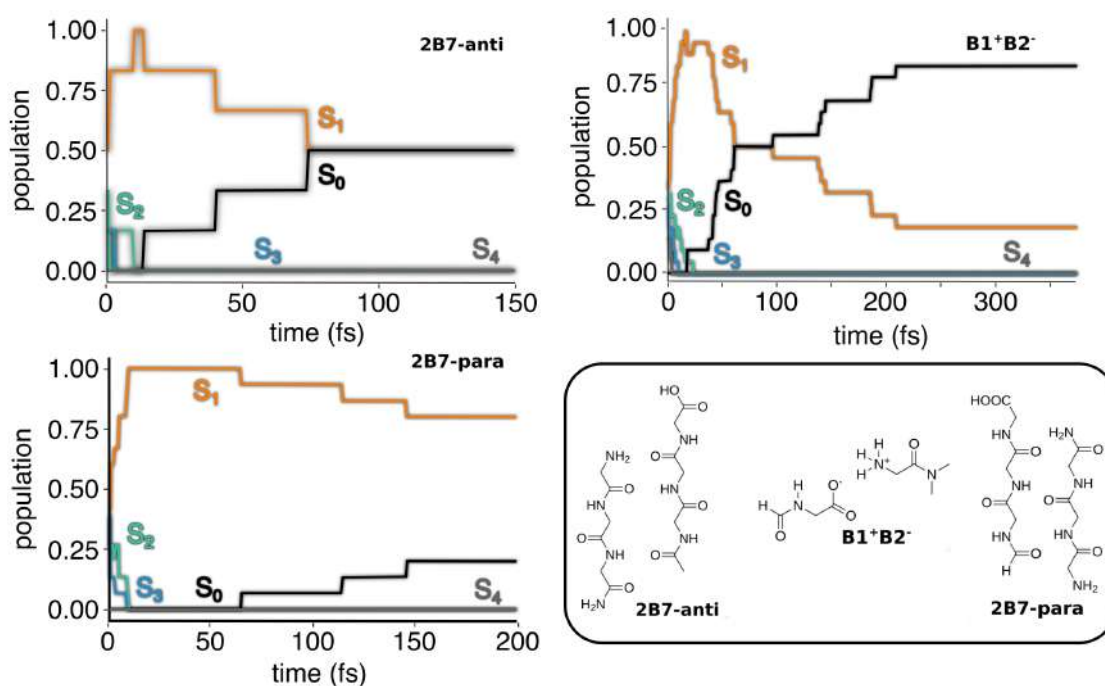
Supplementary Figure 10: Variation of the α parameter in the $S_1 \rightarrow S_0$ relaxation coordinate for a selected NAMD trajectory of L-glu. The vertical axis in the four panels show the energy difference between the S_1 and S_0 states, and the horizontal is the time evolution axis. The dot colors represent the normalized value of the Arrhenius term in expression 7 in the main text, which can be interpreted as a statistical weight of the corresponding geometry fluctuation in the relaxation coordinate.

95 Supplementary Figure 11 shows the simulated fluorescent spectrum of L-Pyro(amm)
 96 model, in reasonable agreement with the experimental result (the simulated maxi-
 97 mum is only shifted ≈ 30 nm/0.2 eV from the experimental one).



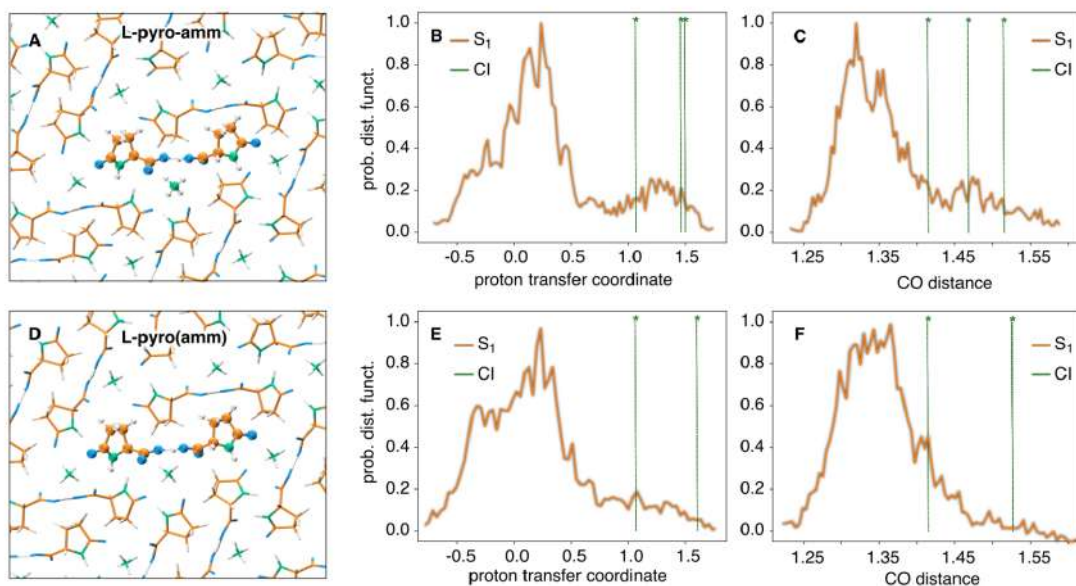
Supplementary Figure 11: Fluorescence spectrum of the L-pyro(amm) isolated dimer model system.

98 Supplementary Figure 12 shows the different time-dependent excited state pop-
 99 ulations of the small model systems, inspired in the amyloid sequence $A\beta_{30-35}$ [3].



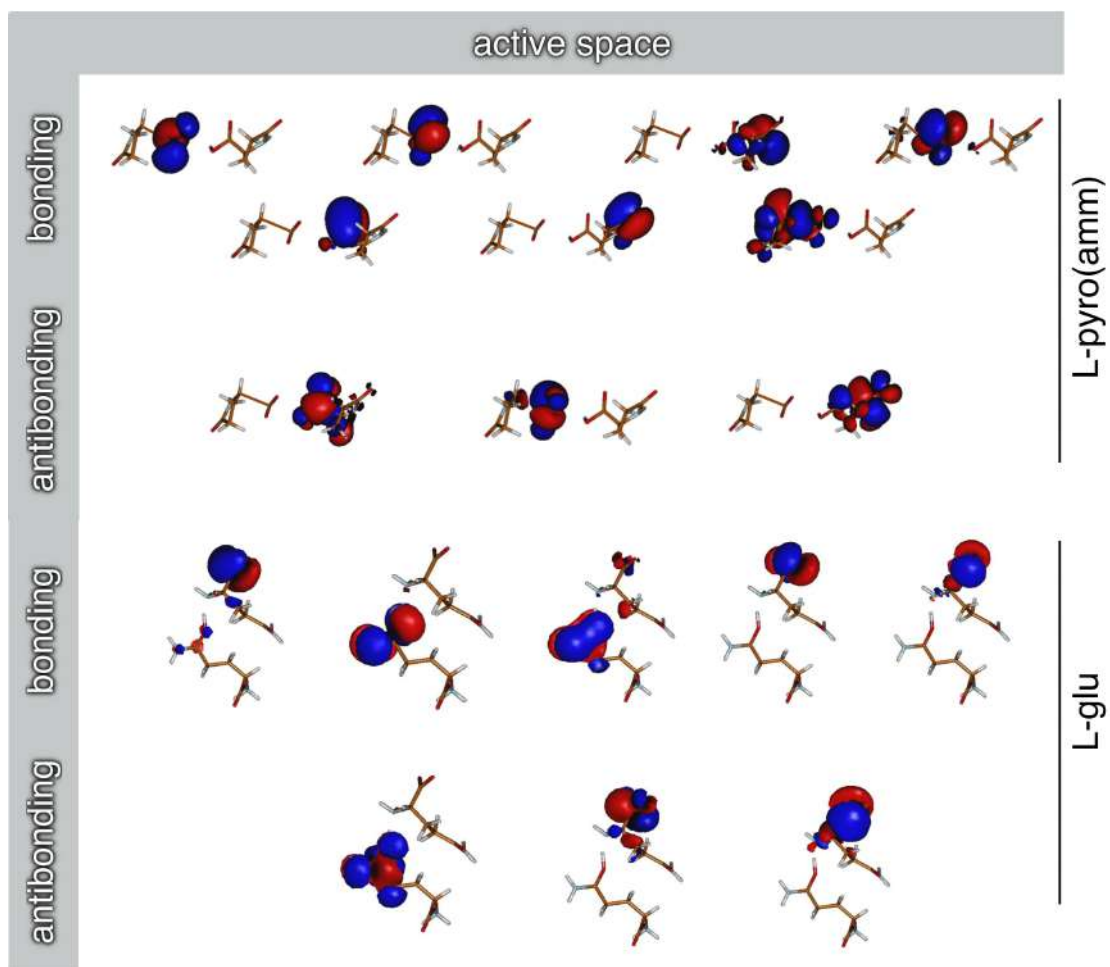
Supplementary Figure 12: Average populations of S_1 (orange) and S_0 (black), S_2 (green), S_3 (blue), S_4 (gray) electronic states for the 2B7-anti (left panel), 2B7-para (middle panel), and $B1^+B2^-$ systems.

100 We performed QM/MM non-adiabatic dynamics simulations comparing two lim-
 101 iting cases: (a) when the ammonium is included in the MM region, and (b) when
 102 the ammonium ion is included in the QM region (see Figure 13). In both cases
 103 we observe a marginal decay to the ground state, in agreement also with the iso-
 104 lated dimer models. Furthermore, no significant change in the relevant excited state
 105 modes is observed. We have now included this important discussion in the new
 106 version of the manuscript.



Supplementary Figure 13: Influence of ammonium ion in the non radiative decay of L-pyro system. The QM/MM models for L-pyro-amm and L-pyro(amm) are shown in panel A and D, respectively. The QM region is shown with a balls and sticks representation while the MM region is depicted only with sticks. The difference between the two models is that in L-pyro-amm the ammonium ion is included in the QM region, while in L-pyro(amm) it is included in the MM region. Panels B and E show the excited state distribution of the proton transfer coordinate for L-pyro-amm and L-pyro(amm), respectively. Panels C and F show the excited state distribution of the CO distance, defined as $d_{O-H} - d_{O'-H}$ (where O and O' identify the two carboxyl oxygens involved in the HB) for L-pyro-amm and L-pyro(amm), respectively.

107 Supplementary Figure 14 depicts the active space employed for the CASPT2
108 calculations, both at the full-QM or QM/MM level.



Supplementary Figure 14: Active space employed for our CASPT2 calculations.

109 **Supplementary References**

- 110 [1] Grisanti, L.; Sapunar, M.; Hassanali, A.; Došlić, N. Toward Understanding
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- 117 [3] Pinotsi, D. et al. Proton Transfer and Structure-Specific Fluorescence in Hydro-
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