Supporting Information

An AIMD study of CPD repair mechanism in water:reaction free energy surface and mechanistic implications

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1 Configurational properties

We now detail the change in nuclear coordinates that occur during the bond splitting process. Specifically we monitor the changes in the C5-C6 (C5′-C6′), C6-N1 (C6′-N1′) and C=O (carbonyl oxygen) distances which are illustrated in Fig. S1 at the four points shown in Fig. 4. Umbrella sampling trajectories at these four points were run for at least $10ps$ longer than the other umbrella sampling windows, to enhance sampling of the distributions shown below. Because the distributions are very similar for both bases we only show the distributions for the bonds on one thymine base. Also shown in the figure is the C5-C6-C6′-C5′ dihedral angle which exhibits some unique features near the transition state.

The C5-C6 and C5′-C6′ distances show a decreasing trend from 1.53 to 1.40Å over the course of the splitting process. This is consistent with the fact that as the C5-C5′ and C6-C6′ bonds split, the C5-C6 and C5′-C6′ bonds develop double bond character. At the AM1 level of theory, Voityuk and co-workers[1] suggested a qualitative scheme for the underlying mechanism of CPD dimer splitting in which the double bond is formed only on one base, say C5′-C6′, as the C6-C6′ bond splits. The excess electron would then be localized on the C6 carbon atom once both the C5-C5′ and C6-C6′ bonds are split. We can test the mechanism proposed by Voityuk *et al.* by tracking bond length changes that are indicative of double bond formation. Shown in Fig. S2 is the evolution of the C5-C6 and C5′-C6′ bonds for 4 different non-equilibrium trajectories that were begun in the minima where the the C5-C5′ bond was already split and whose C6-C6′ bond split within 4.5ps. The data shows that as the C6-C6′ bond splits, the double bond character in the C5(C5′)-C6(C6′) bond develops simultaneously without any noticeable lag between them.

The data shown in Fig. S2 also suggest a revision of MacFarlane and Stanley's[2] interpretation of their experiments on the repair of the CPD unit in the active site of photolyase. Stanley and co-workers use the absorption of light at 265nm to monitor the formation of the C5-C6 and C5′-C6′

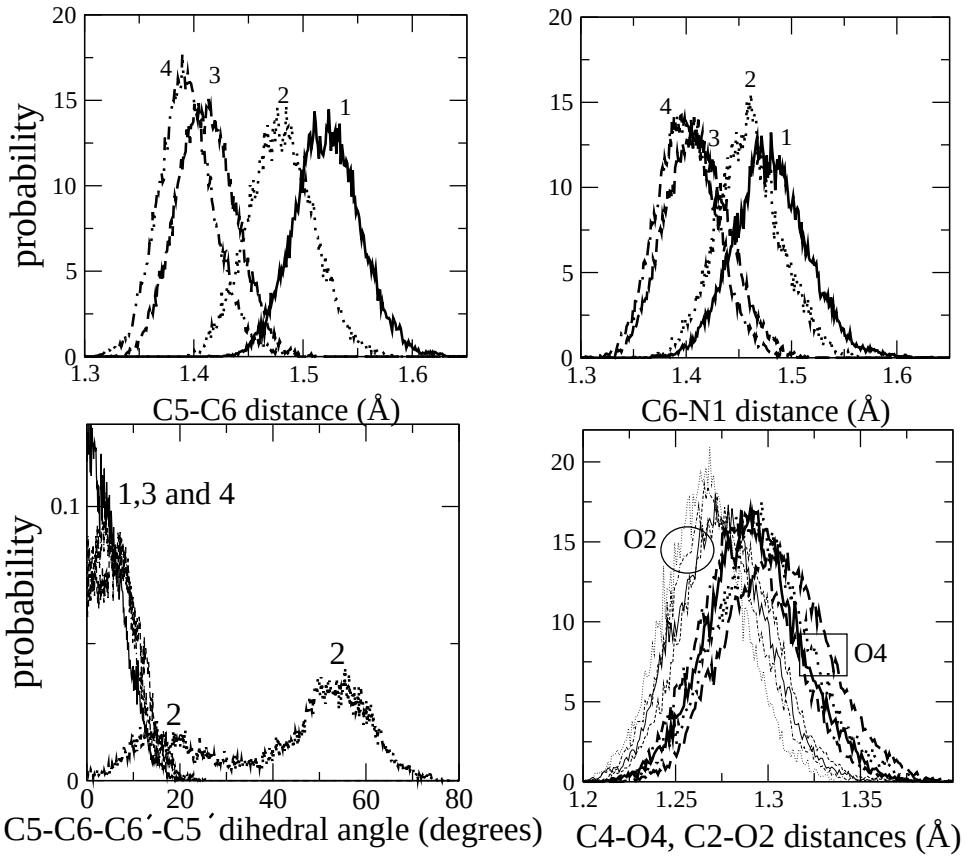

Figure S1: Configurational changes during the splitting of the dimer.

double bonds. They propose that the cleavage of the first bond is limited by the electron transfer lifetime and occurs within 60ps which indicates the formation of only one double bond between the carbon atoms. Furthermore, they also find an increase in the transient absorption signal on a timescale of 1500ps which they suggest indicates the splitting of the second bond and the formation of the second double bond between the carbon atoms. However our results shown in Fig. S2 shows that as the C6-C6′ bond splits, the double bond forms simultaneously in both bases. Thus, if the presence of a double bond is used as a signature for splitting of the C5-C5′ and C6-C6′ bonds, its formation implies that both bonds have broken. Masson *et al.*[3] have recently critiqued MacFarlane and Stanley's[2] interpretation of their experiments. However, Masson *et al.* suggest that because the spin density of the electron is localized on the C6 or C6′ atoms, the formation of the second double bond after splitting of the bonds could be unfavorable. The mechanism is evidently different in our simulations, either because the aqueous environment is different from the enzyme, or because we treat the solvent electrons quantum mechanically. Fig. S2 shows that for our system, the C5-C6 and C5′-C6′ double bonds form at the same time.

The C6-N1 and C6′-N1′ distances show a similar decreasing trend, very similar to the C5-C6 bond distance, as the bond splits. The carbonyl oxygen distances do not show any significant distance changes as the bond splits. However we do find that the C4-O4(C4′-O4′) carbonyl distances are

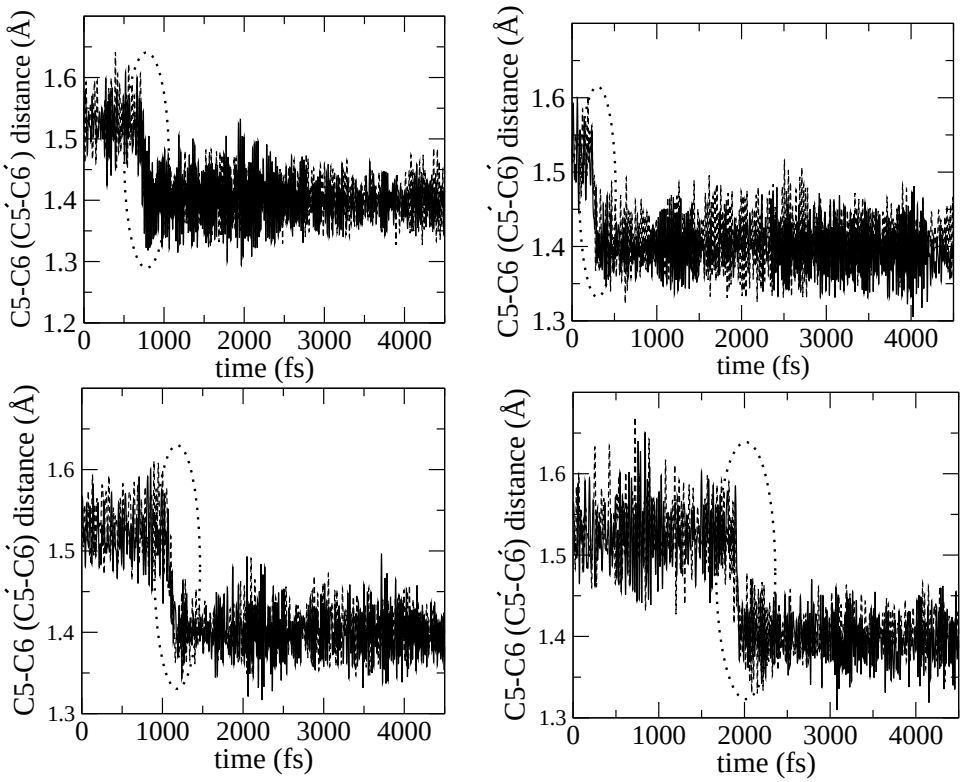

Figure S2: C5-C6 (solid curve) and C5′-C6′ (dashed curve) bond lengths over time for four splitting trajectories. The sharp decrease in bond length reflects formation of double bond character, which is seen to be simultaneous in both bases, as splitting of the C6-C6′ bond occurs. The circled regions indicate where the C6-C6′ bond splits.

slightly longer than those of the C2-O2(C2′-O2′) across points 1 through 4. Finally the C5-C6-C6′-C5′ dihedral angle exhibits dramatic changes as it approaches the transition state. The dihedral angle can change from 2-3 degrees before the transition state to about 50 degrees near the transition state and thereafter relaxes back to 6 degrees after crossing this region. This kind of distortion at the transition state has been observed in previous work although they were done with gas-phase and small cluster calculations.[1,4,5] Fig. S1 shows that at Point 2, there is a small peak in the dihedral angle at 15 degrees and a larger peak at about 50 degrees. During the course of our relatively short simulations, we observe that the dihedral angle changes from about 15 degrees at the beginning of the run to 50 degrees and does not revert back to the population at the smaller dihedral angle. At this point with our limited simulation lengths, we cannot establish whether the small peak at 15 degrees results from configurations not yet equilibrated along the dihedral angle or whether this is a real sub-population near the transition state. Four representative snapshots along points 1-4 are illustrated in Fig. S3 to demonstrate the configurational changes that occur during splitting and the open configuration that can occur near the transition state region.

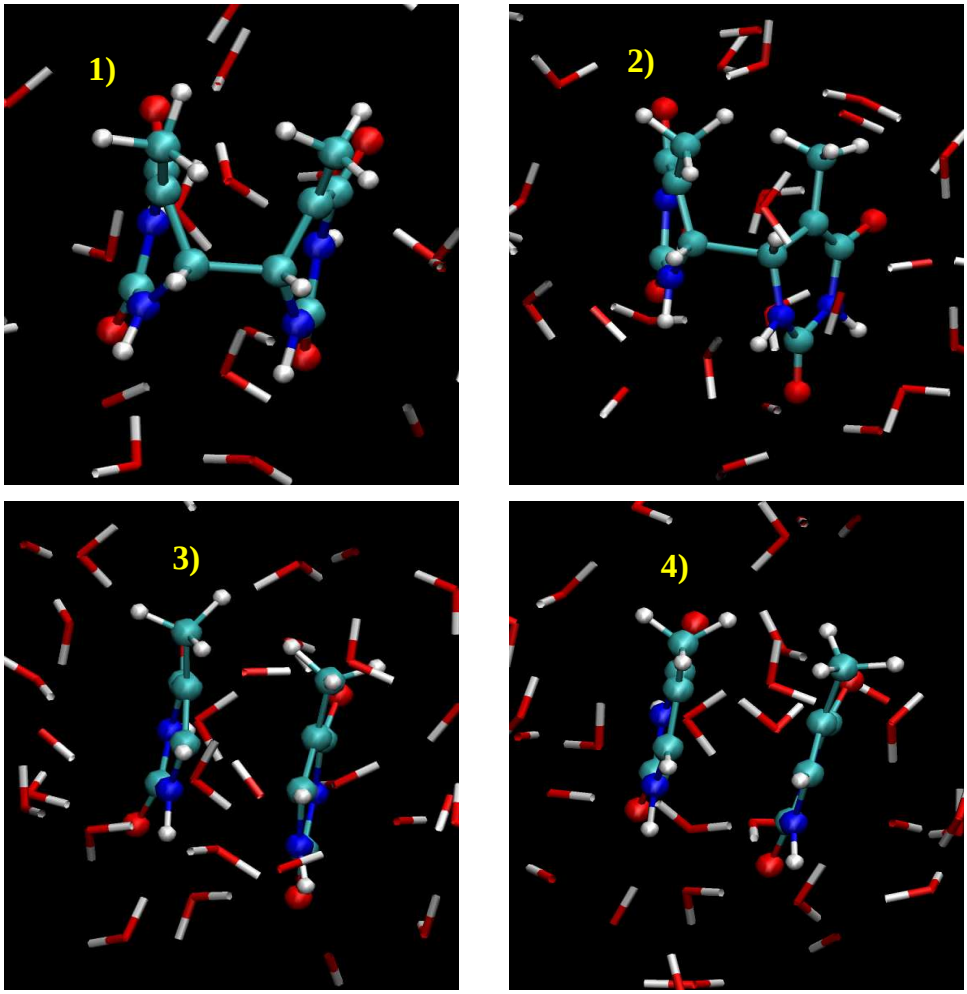

Figure S3: Snapshots of typical configurations analyzed along umbrella sampling C6-C6′ coordinate. These configurations are typical of the thymine dimer anion at points 1,2,3, and 4 along the C6-C6′ free energy surface shown in Fig.4.

References

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