

On the wavelength dependence of UV induced Thymine photo-lesions: A Synchrotron Radiation Circular Dichroism study.

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Electronic Supplementary Information (ESI)

This ESI contains all of the UV absorption and synchrotron radiation circular dichroism (SRCD) data collected at various irradiation doses and grouped into the individual laser irradiation wavelengths 325, 300, 285, 270, 260, 255, 250, 240, 230, 220 and 210 nm.

Together with the spectra for each irradiation wavelength, the PCA coefficients dependence on absorbed dose are shown for each species: undamaged thymine (T-T), cyclobutane pyrimidine dimer (CPD, T<>T) and 6,4 pyrimidine-pyrimidone photoadduct (64PP). These coefficients represent the amount of each species in the sample at a given dose.

S1. Irradiation at 325 nm.

Both absorption and CD data show no changes when the sample is irradiated by 325 nm light, see figures S1 and S2 a) where the measured spectra coincide so that only the last (fourth) spectrum is visible. From the absorption spectrum of undamaged thymine it is expected that no light is absorbed at this wavelength.

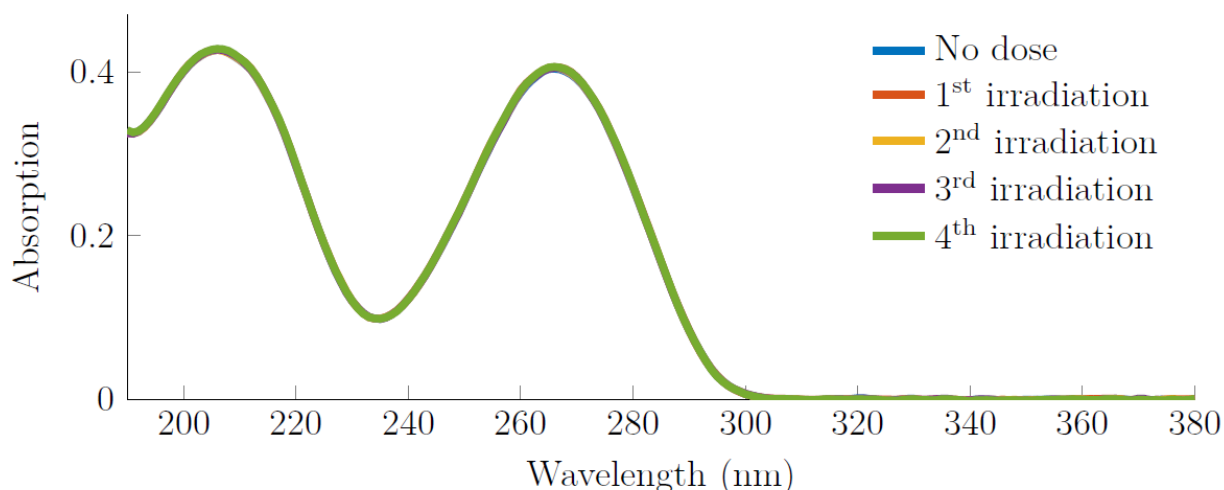


Figure S1: Measured absorption spectra from irradiation at 325 nm irradiation ranging from no dose to a maximum transmitted dose of 1.60 J/cm². The spectra are coinciding, so only the last measured spectrum is visible.

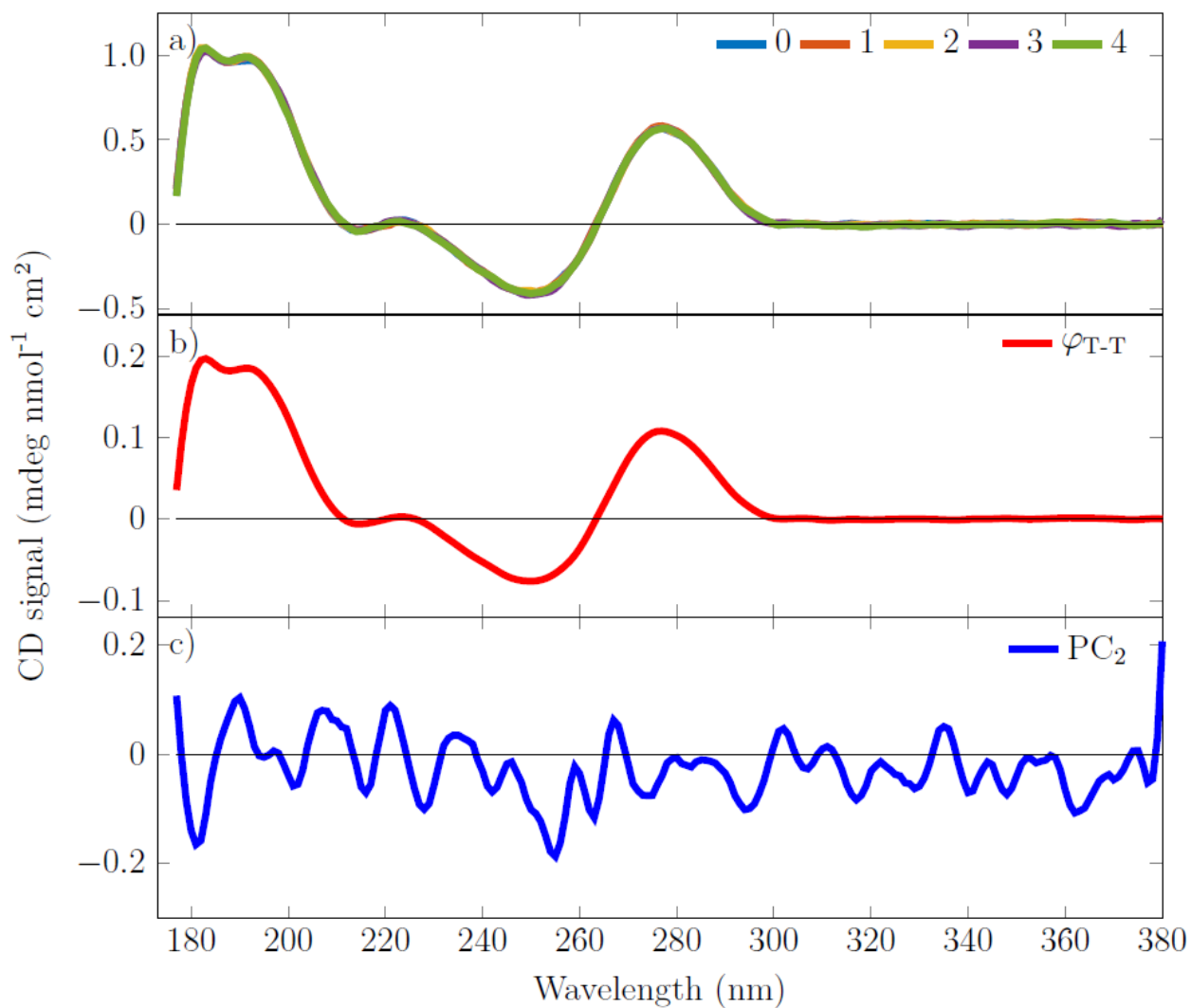


Figure S2: a) The raw CD data for irradiation at 325 nm, ranging from no dose (0) to a maximum transmitted dose of 1.60 J/cm² (4). The spectra are coinciding, which means that only the last measured spectrum is visible. b) The obvious basis spectrum of undamaged T-T. In c) it is clear that there is no second basis spectrum, only noise.

S2. Irradiation at 300 nm.

The basis spectra of the 300 nm dataset have been presented in the Results section. For completeness they are included here as well. In figure S3 the measured absorption spectra are presented. The most interesting information from these data is the absence of an absorption band at 325 nm. Figures S4 b) and c) show two principal components, the CD basis spectrum of undamaged stacked thymine and the cyclobutane dimer. These two spectra are used as the true T-T and CPD spectrum in the analysis of all the other datasets. It is possible to produce basis spectra from the lower irradiation wavelength datasets that are more or less similar to these true basis spectra, but in cases where a third and fourth product are produced at low irradiation doses, it is difficult to separate the basis spectra in a meaningful way.

The pertaining coefficients of φ_{T-T} and $\varphi_{T \leftrightarrow T}$ in figure S4 are shown in figure S5. The formation rate of the dimer follows the reduction of the T-T. This is further confirmation that the dimer is the only product formed. It is worth noting that the total absorbed dose is quite low, which is a result of the low absorption at 300 nm of both T-T and the dimer.

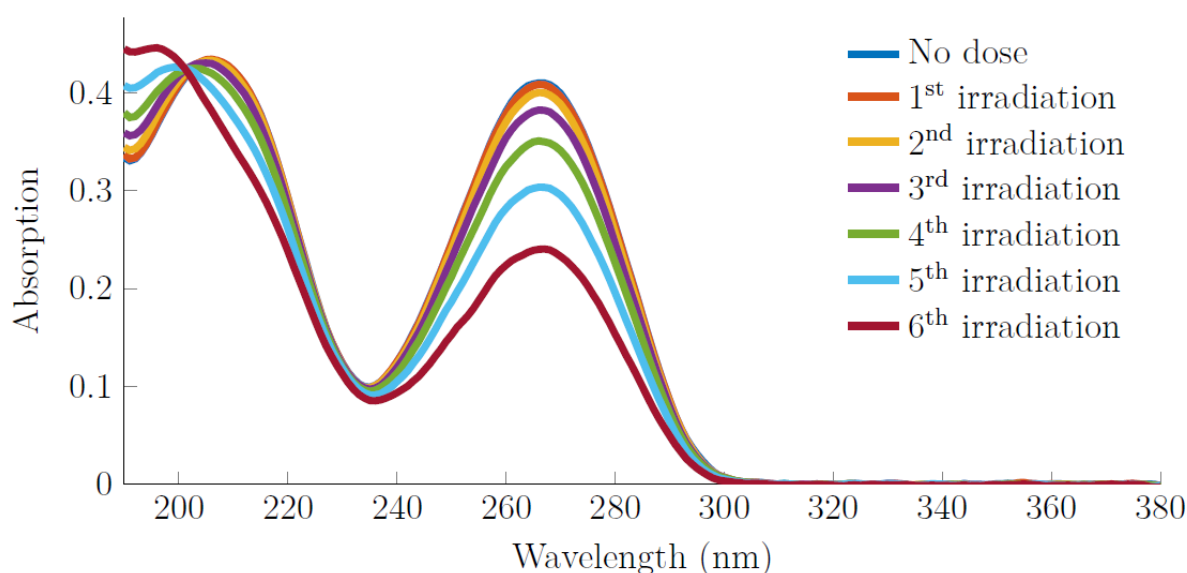


Figure S3: Measured absorption spectra for irradiation at 300 nm ranging from no dose to a maximum absorbed dose of 0.21 J/cm².

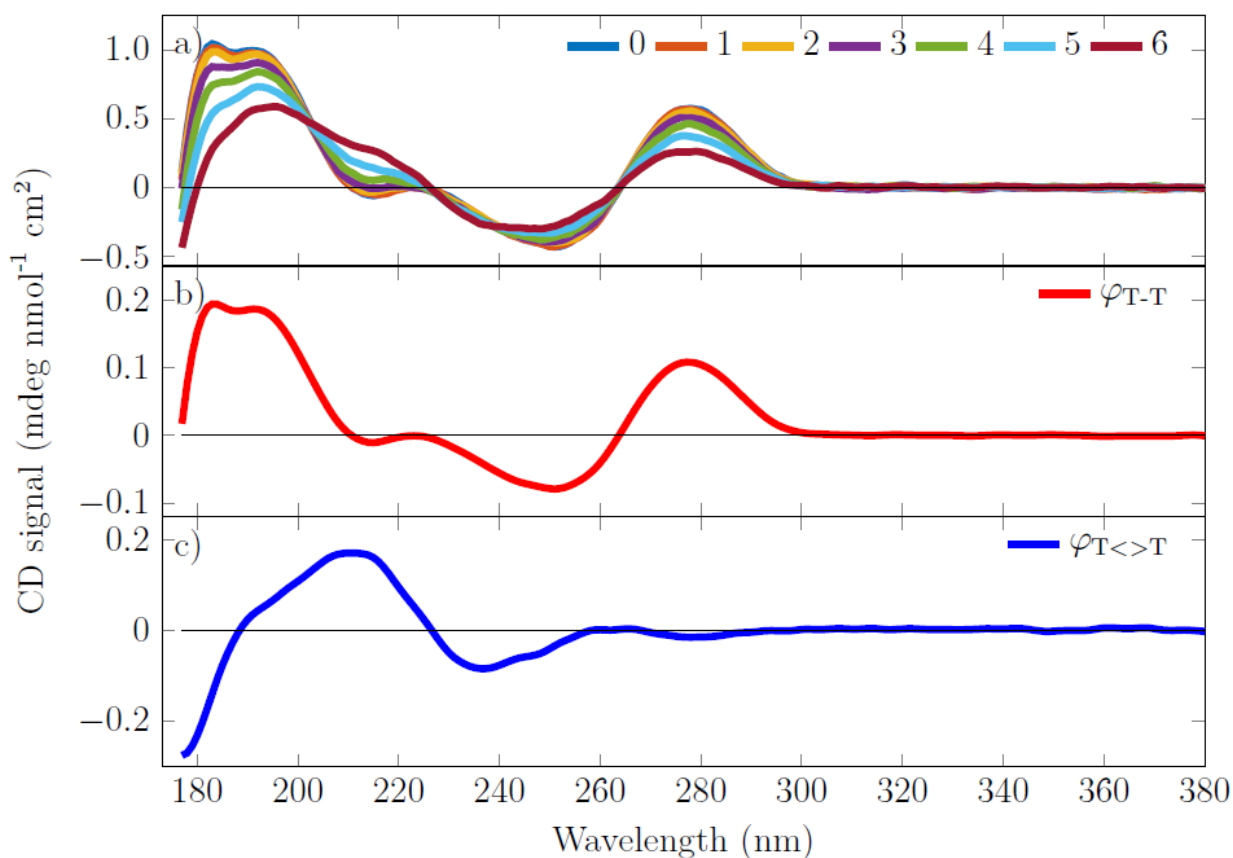


Figure S4: a) SRCD data for irradiation at 300 nm, ranging from no dose (0) to a maximum absorbed dose of 0.21 J/cm² (6). b) Characteristic basis spectrum belonging to the undamaged thymine strand, T-T. c) Characteristic basis spectrum belonging to the photo product, the thymine CPD product, T<>T.

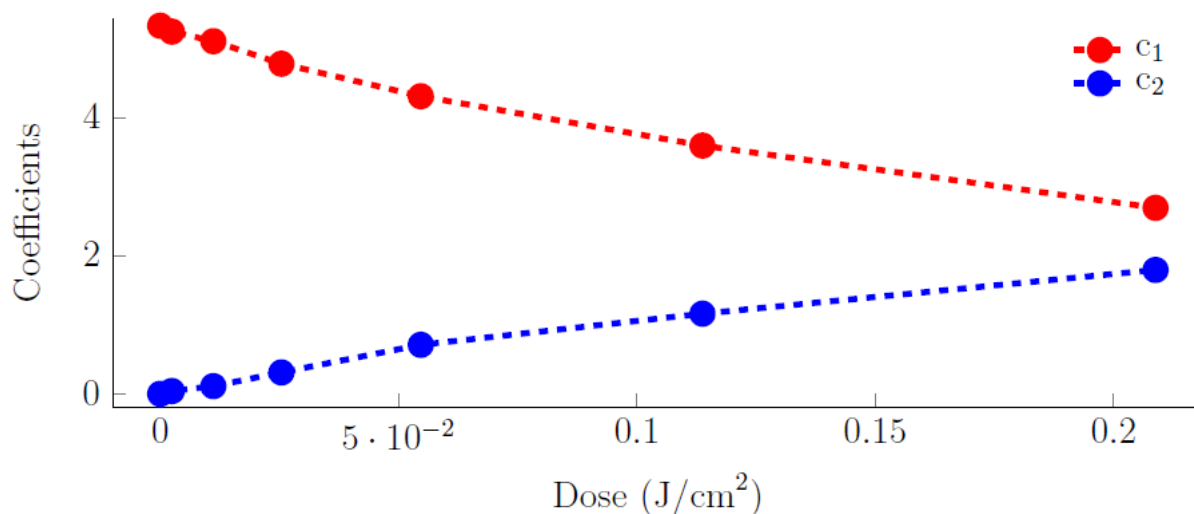


Figure S5: Coefficients of the true basis spectra for the T-T (red) and CPD product (blue) found from the 300 nm irradiation dataset. The dimer product formation increases with larger accumulated irradiation dose at the same rate as the destruction of T-T.

S3. Irradiation at 285 nm.

The absorption and CD spectra datasets are presented in figures S6 and S7. Using the φ_{T-T} and $\varphi_{T<>T}$ basis spectra from the 300 nm dataset, the third component is computed. All φ_3 s are collected in the Results section of the paper. The coefficients belonging to the three components are plotted in figure S8. In figure S9 it is clear that the third component is a very small, but non-vanishing, contribution to the total CD spectra.

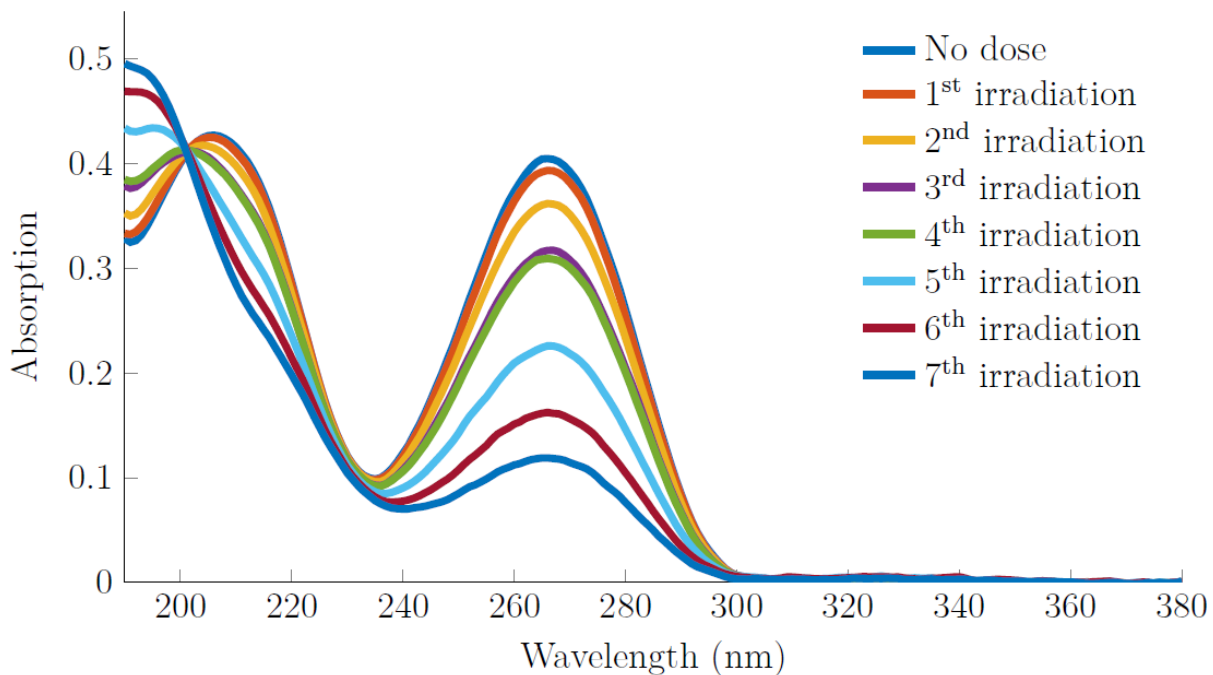


Figure S6: Measured absorption spectra for irradiation at 285 nm ranging from no dose to a maximum absorbed dose of 1.61 J/cm².

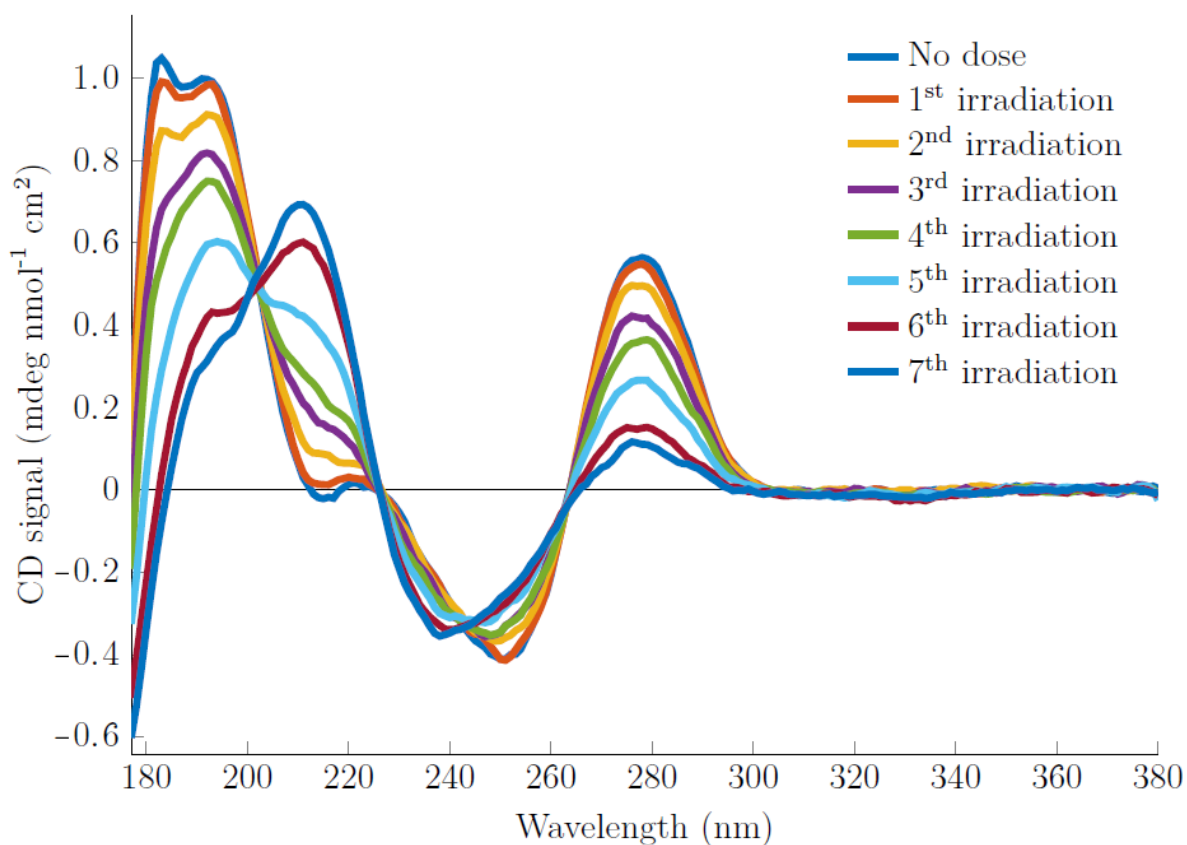


Figure S7: Measured CD spectra for irradiation at 285 nm, ranging from no dose to a maximum absorbed dose of 1.61 J/cm².

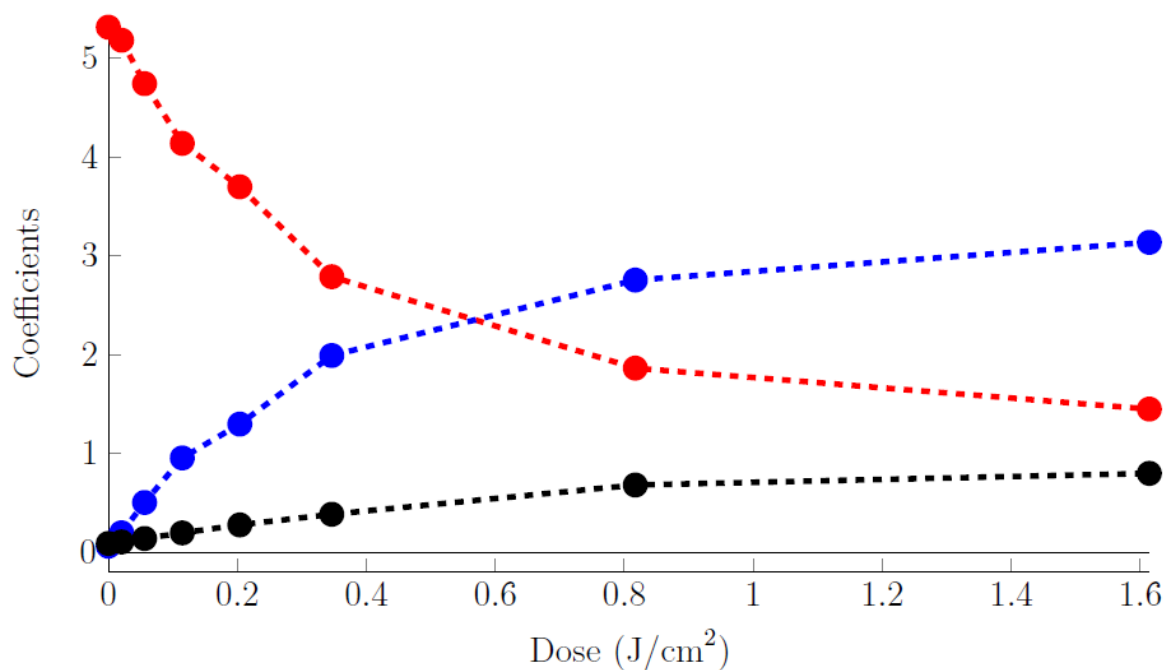


Figure S8: Coefficients for the 285 nm irradiation dataset for thymine T-T (red), dimer T<>T (blue) and the 64PP photoadduct (black).

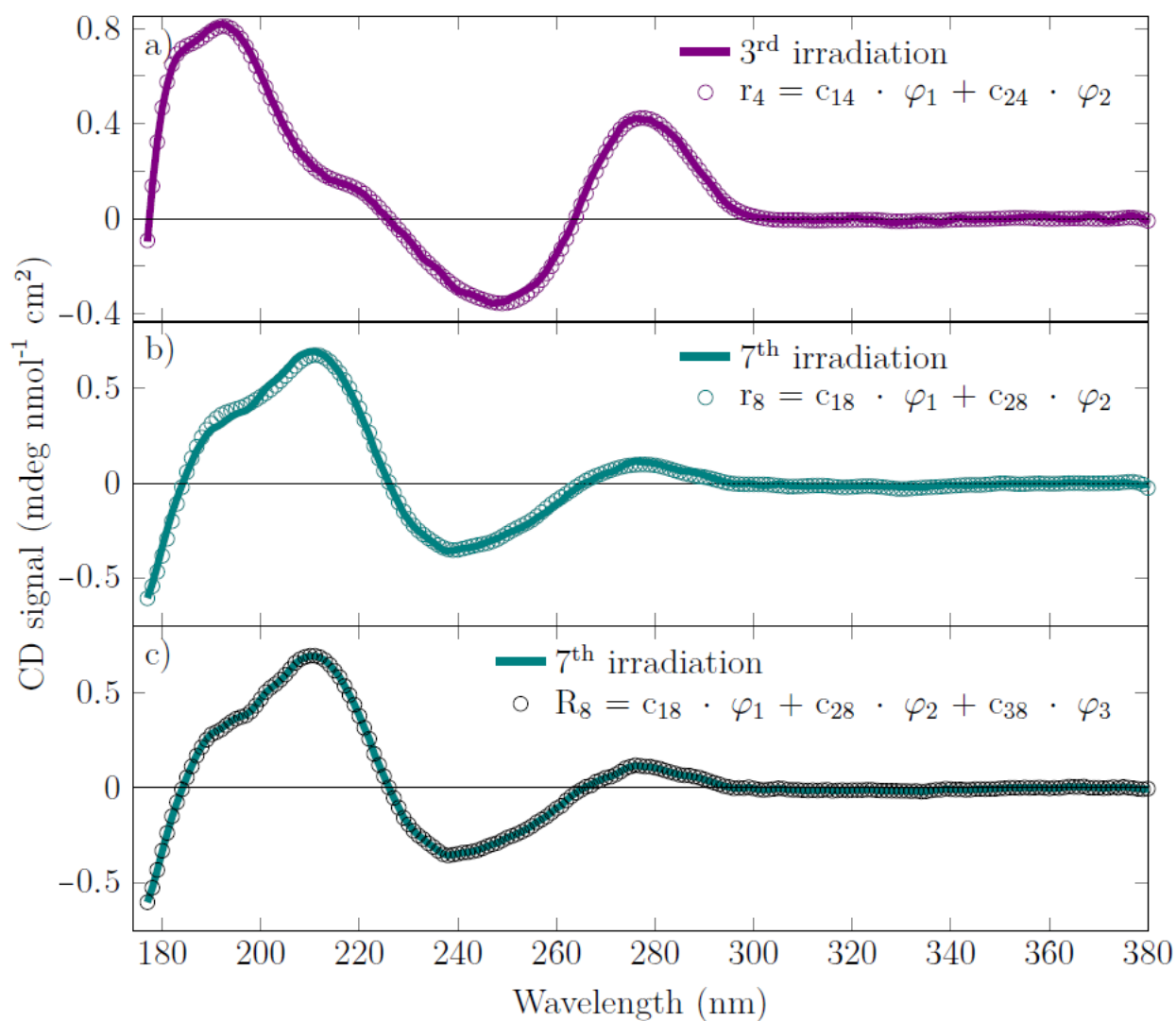


Figure S9: Reconstructed spectra (circles) plotted with the measured CD spectra (lines) from selected irradiations at 285 nm. The reconstruction is based on a PCA of the CD data: φ_1 , φ_2 and φ_3 are the obtained basis spectra and their coefficients c_{1i} , c_{2i} and c_{3i} , for the i -th spectrum, i.e. the $(i-1)$ -th irradiation. While the fourth spectrum (3rd irradiation) is well represented by only the first two components as shown in a), it is apparent from b) and c) that adding the third basis spectrum improves the reconstruction for the spectrum of the 7th irradiation.

S4. Irradiation at 270 nm.

The absorption and CD spectra for irradiation at 270 nm are presented in figures S10 and S11. The third component is more distinct at this irradiation wavelength. The coefficients belonging to the three components are plotted in figure S12.

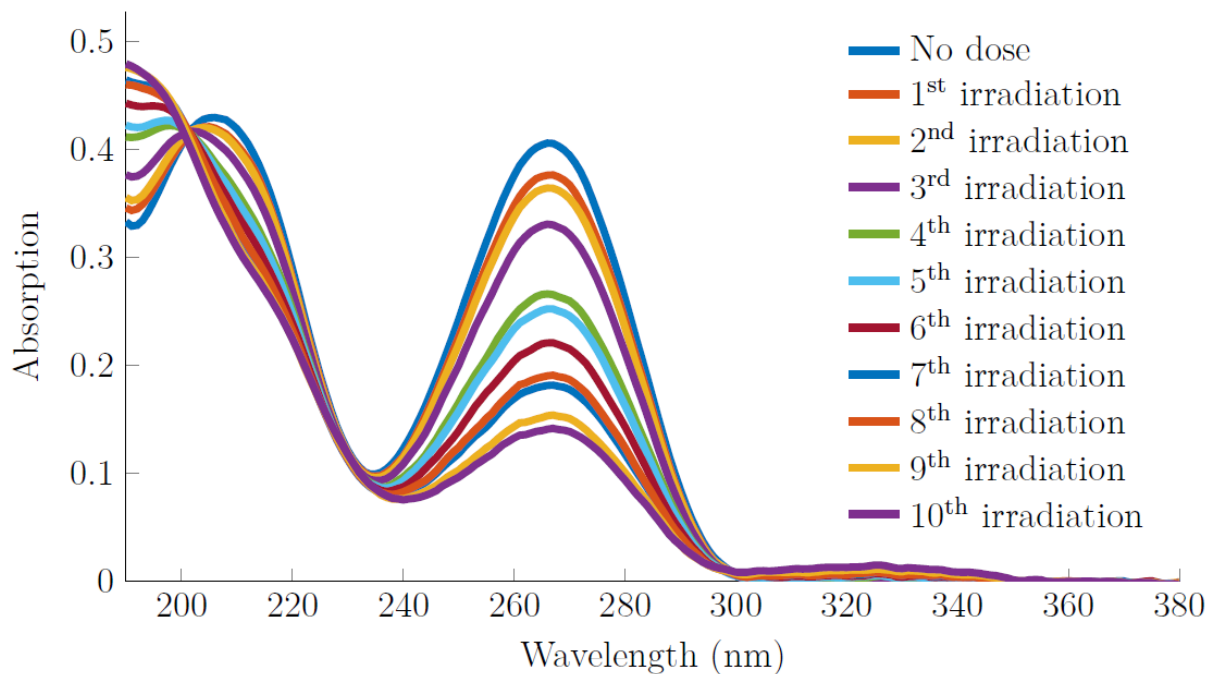


Figure S10: Measured absorption spectra for irradiation at 270 nm irradiation, ranging from no dose to a maximum absorbed dose of 1.51 J/cm².

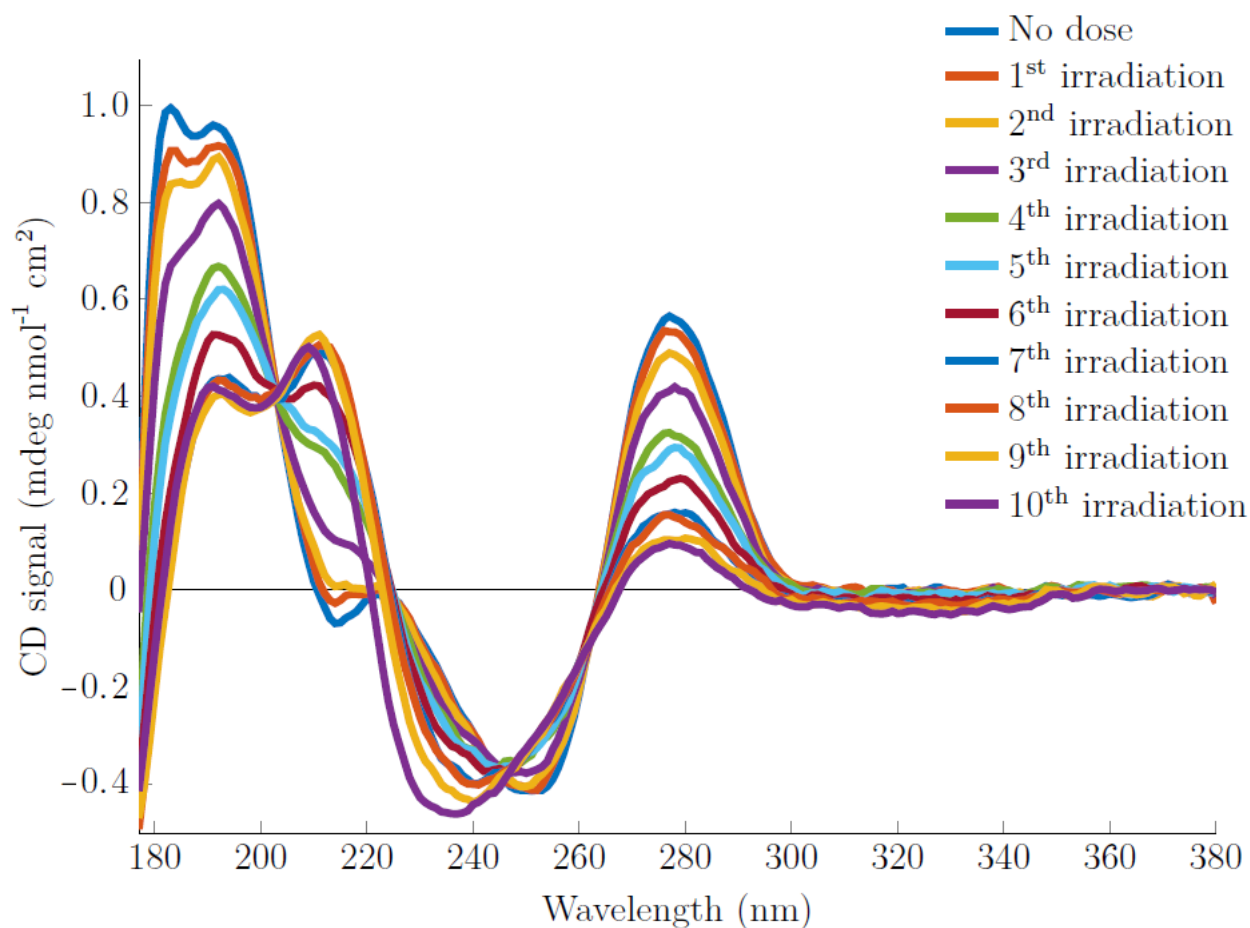


Figure S11: Measured CD spectra for irradiation at 270 nm, ranging from no dose to a maximum absorbed dose of 1.51 J/cm².

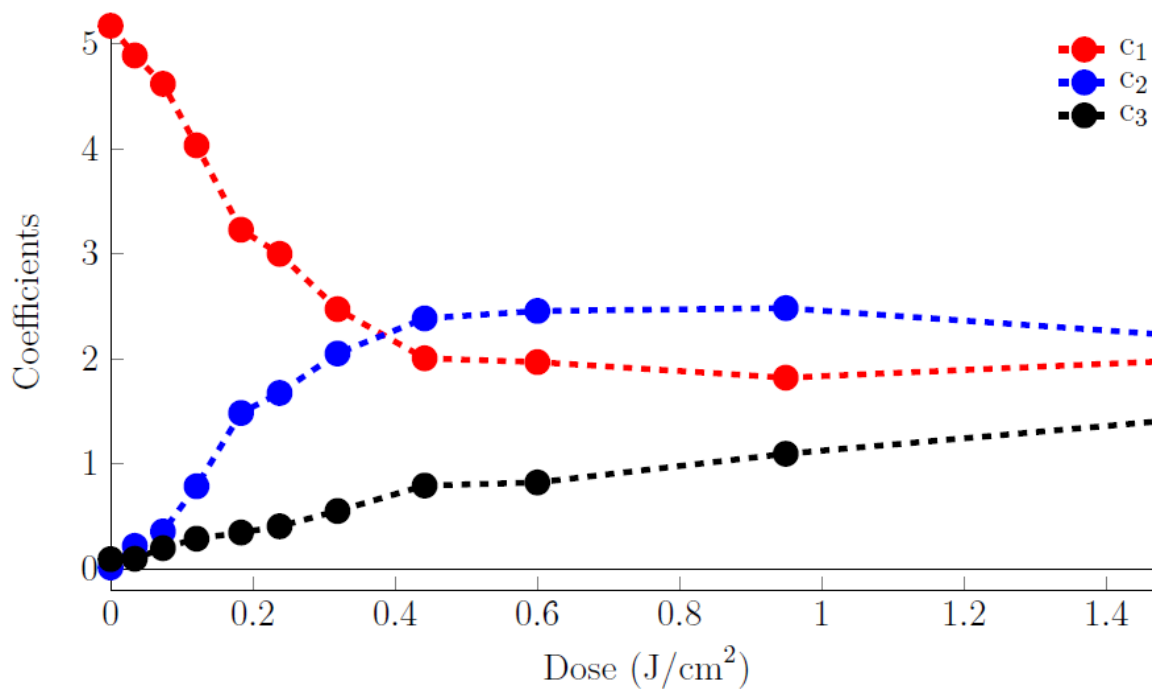


Figure S12: Coefficients for the 270 nm irradiation dataset for thymine T-T (red), dimer T<>T (blue) and the 64PP photoadduct (black).

S5. Irradiation at 260 nm.

The absorption and CD spectra for irradiation at 260 nm are shown in figures S13 and S14. The coefficients of the three products are plotted in figure S15. At a dose of 1.6 J/cm² the third coefficient surpasses the second coefficient, corresponding to the 64PP concentration being higher than the dimer concentration.

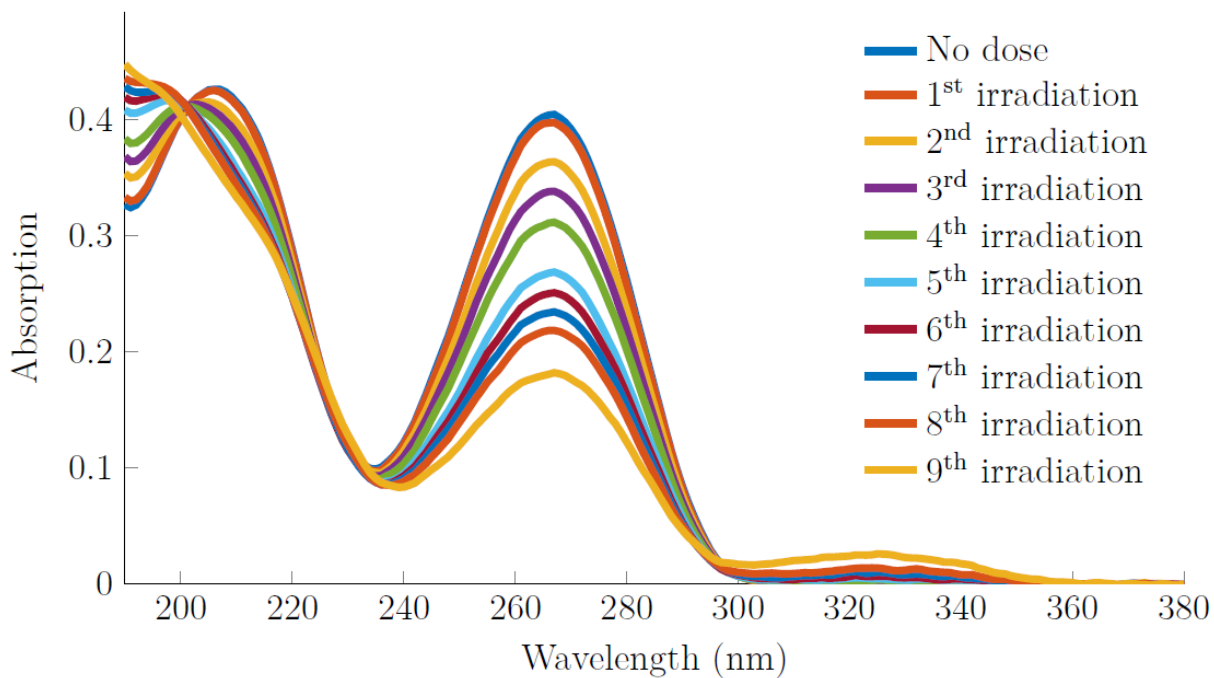


Figure S13: Measured absorption spectra for irradiation at 260 nm ranging from no dose to a maximum absorbed dose of 2.30 J/cm².

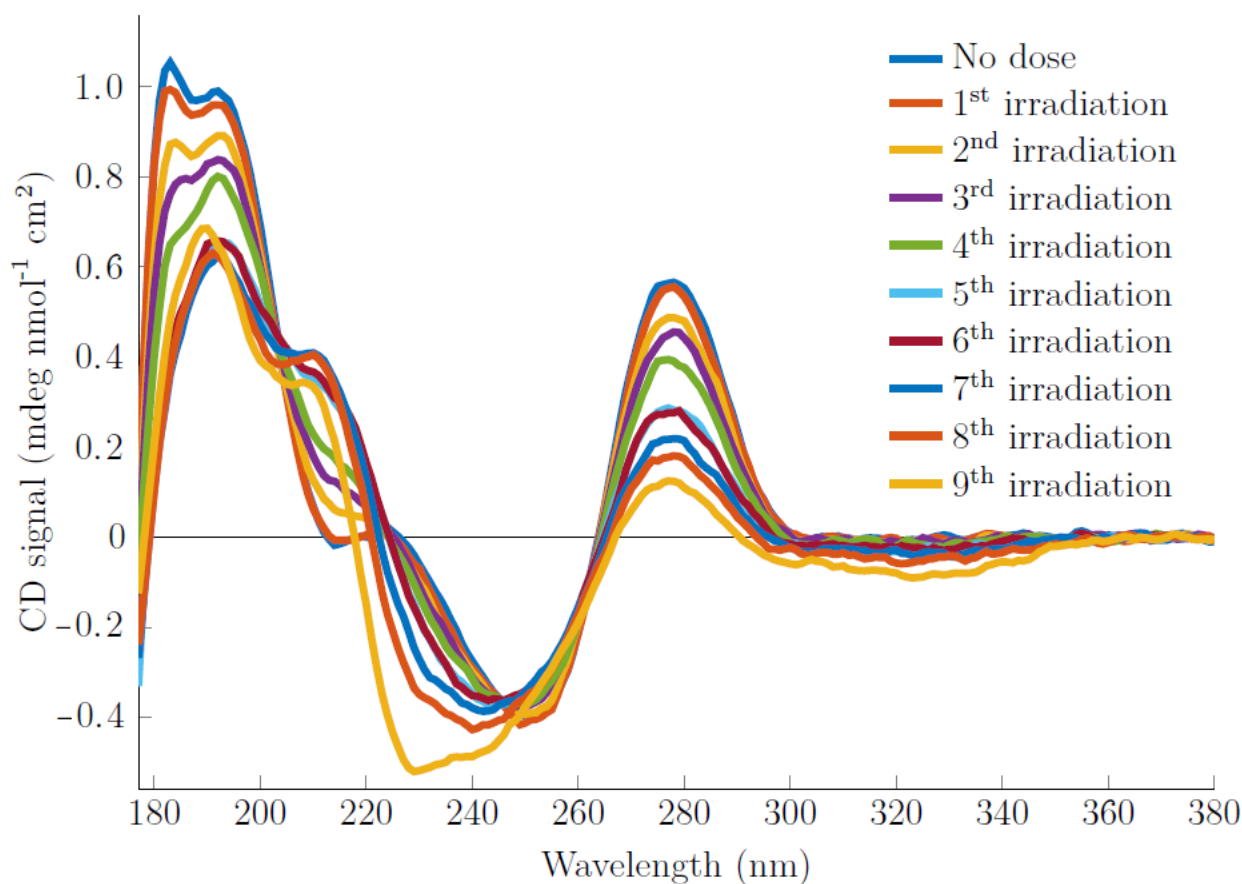


Figure S14: Measured CD spectra for irradiation at 260 nm, ranging from no dose to a maximum absorbed dose of 2.30 J/cm².

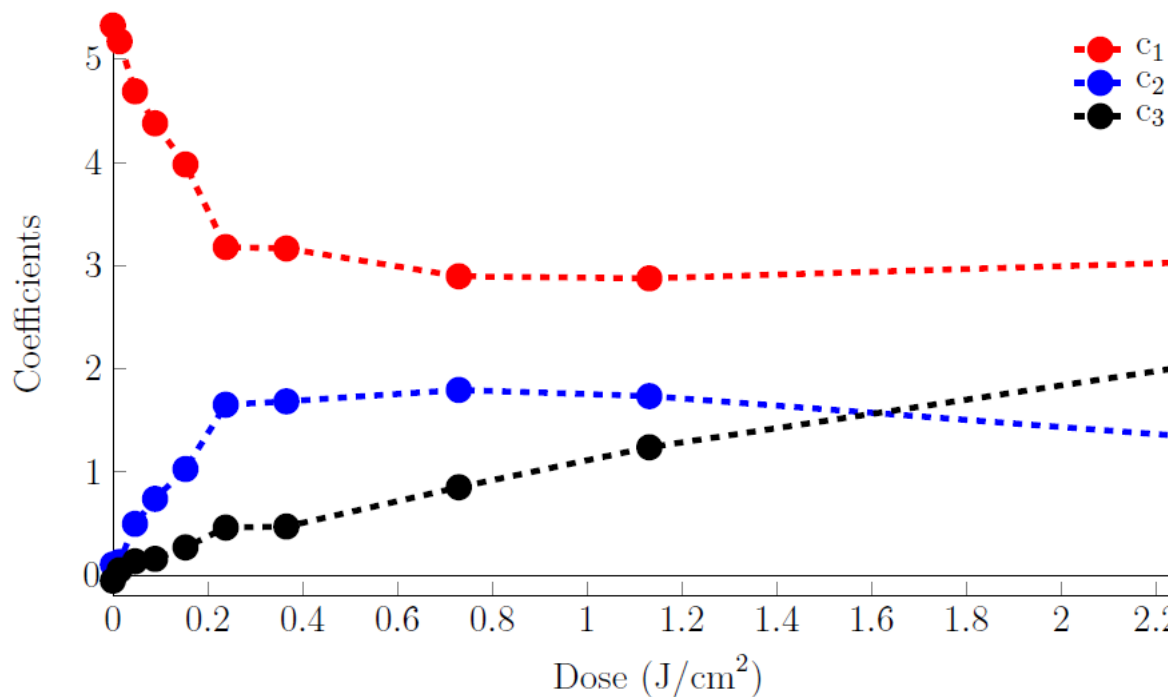


Figure S15: Coefficients for the 260 nm irradiation dataset for thymine T-T (red), dimer T<>T (blue) and the 64PP photoadduct (black).

S6. Irradiation at 255 nm.

The absorption and CD spectra for irradiation at 255 nm are shown in figures S16 and S17. The coefficients are plotted in figure S18. The 64PP concentration surpasses the CPD concentration at a dose of 0.6 J/cm^2 , which is a much lower dose than for irradiation at 260 nm. The CPD concentration also decreases faster at this irradiation wavelength of 255 nm, and is close to zero at the last measurement at a dose of 2.78 J/cm^2 .

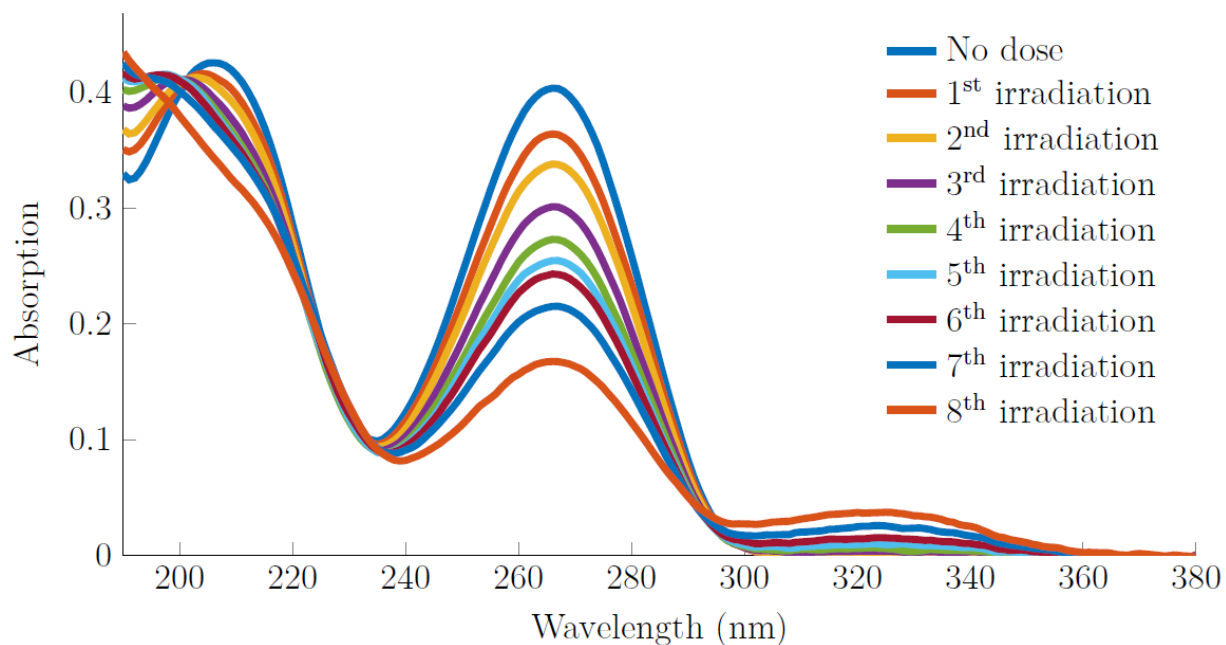


Figure S16: Measured absorption spectra for irradiation at 255 nm ranging from no dose to a maximum absorbed dose of 2.78 J/cm^2 .

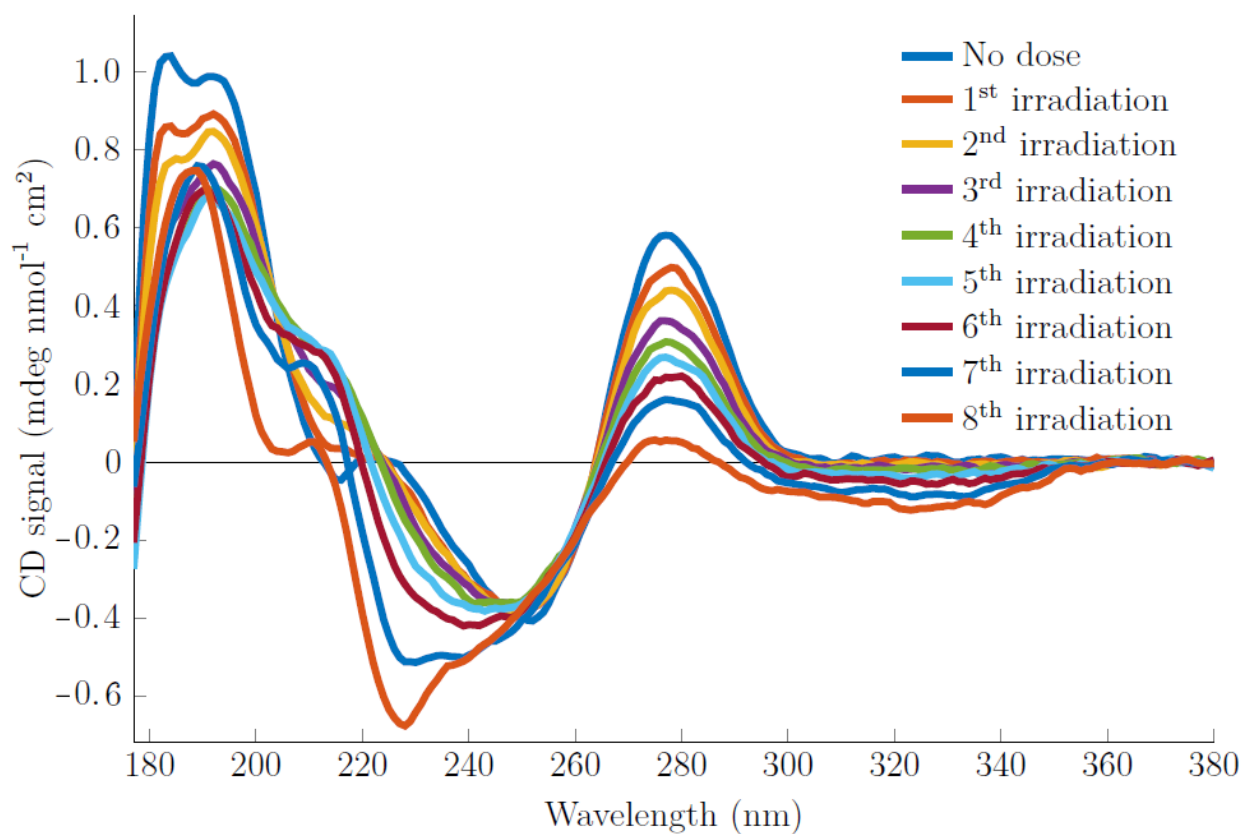


Figure S17: Measured CD spectra for irradiation at 255 nm, ranging from no dose to a maximum absorbed dose of 2.78 J/cm^2 .

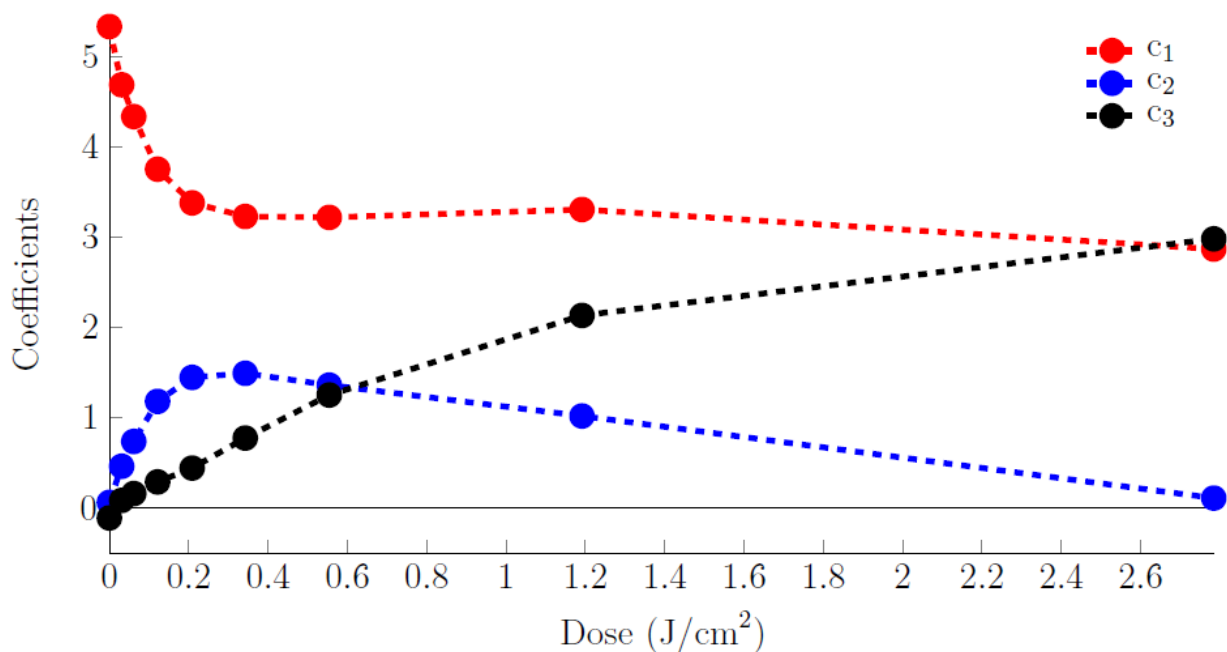


Figure S18: Coefficients for the 255 nm irradiation dataset for thymine T-T (red), dimer T<>T (blue) and the 64PP photoadduct (black).

S7. Irradiation at 250 nm.

The absorption and CD spectra for irradiation at 250 nm are shown in figures S19 and S20. The coefficients are plotted in figure S21. The 250 nm data are very similar to the 255 nm data. The CPD concentrations reach a maximum around a dose of 0.2 J/cm^2 . However at a dose of 0.4 J/cm^2 the 64PP concentration exceeds the CPD and at a dose of 2 J/cm^2 the CPD concentration is again close to zero at the last measurement. In both cases the 64PP concentration exceeds the undamaged thymine.

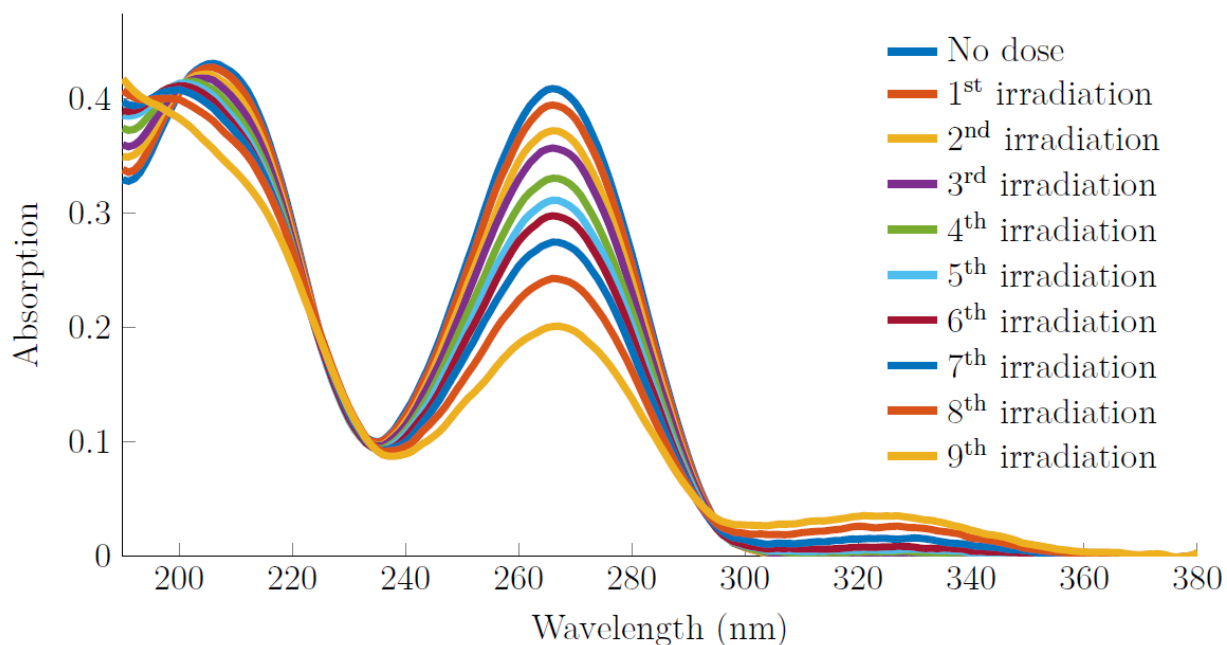


Figure S19: Measured absorption spectra for irradiation at 250 nm ranging from no dose to a maximum absorbed dose of 2.06 J/cm^2 .

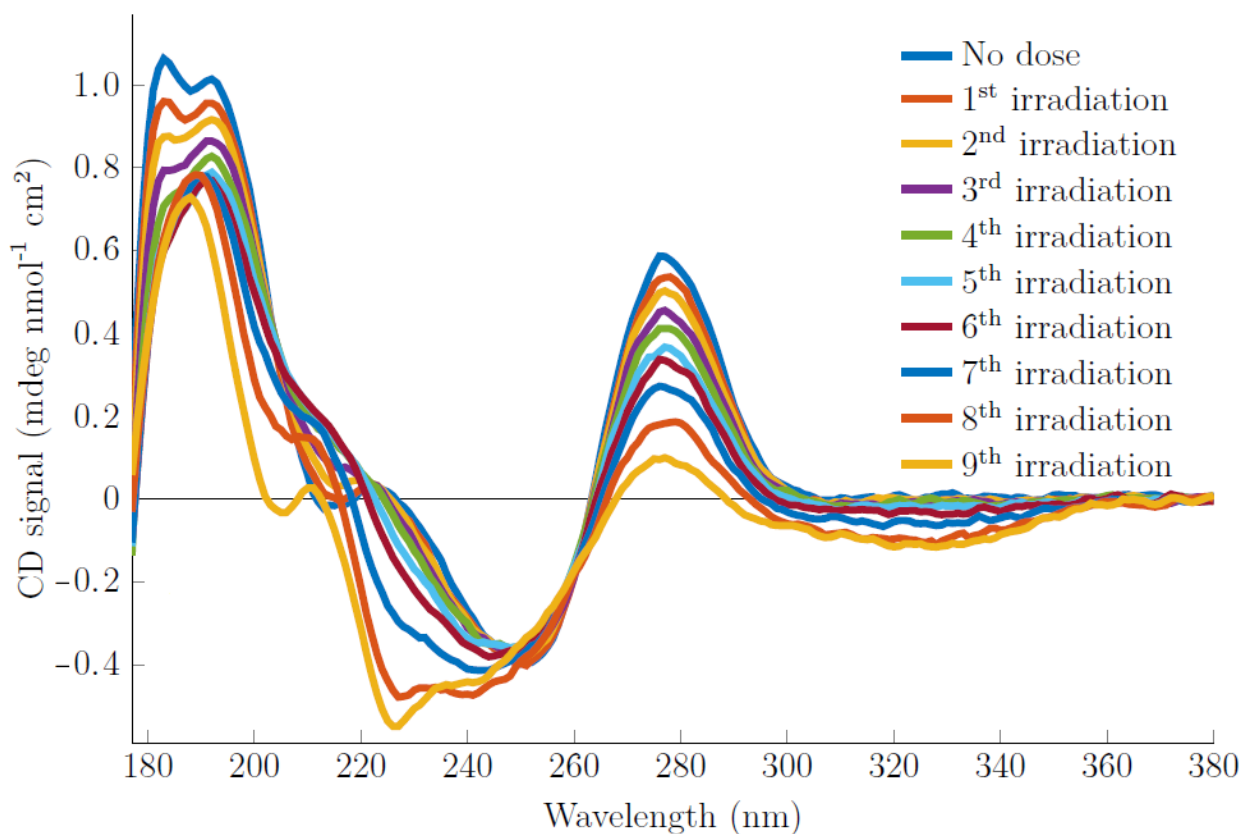


Figure S20: Measured CD spectra for irradiation at 250 nm, ranging from no dose to a maximum absorbed dose of 2.06 J/cm^2 .

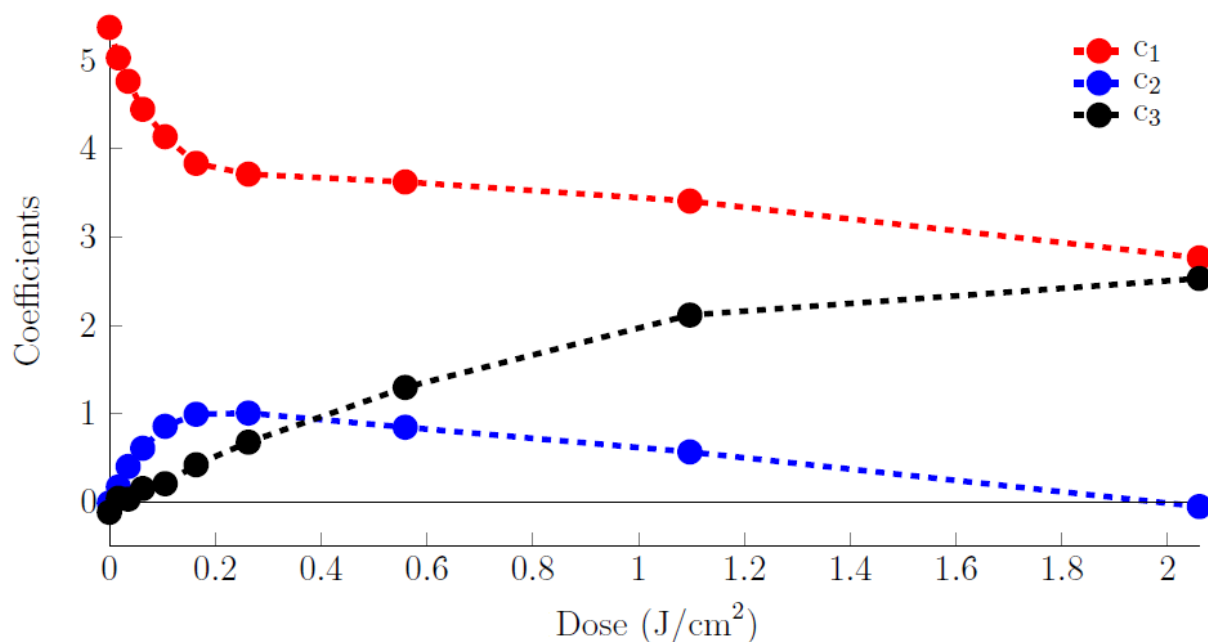


Figure S21: Coefficients for the 250 nm irradiation dataset for thymine T-T (red), dimer T<>T (blue) and the 64PP photoadduct (black).

S8. Irradiation at 240 nm.

From figures S22 and S23 it is evident that the absorption of 240 nm irradiation is small, which is also reflected in the plot of the coefficients figure S24. The first coefficient (red) indicates that the dT_5 concentration decreases very slowly. The second coefficient (blue) shows CPD formation to a limited degree. The third coefficient (black) shows some 64PP formation. At 0.1 J/cm² the 64PP coefficient surpasses the CPD coefficient.

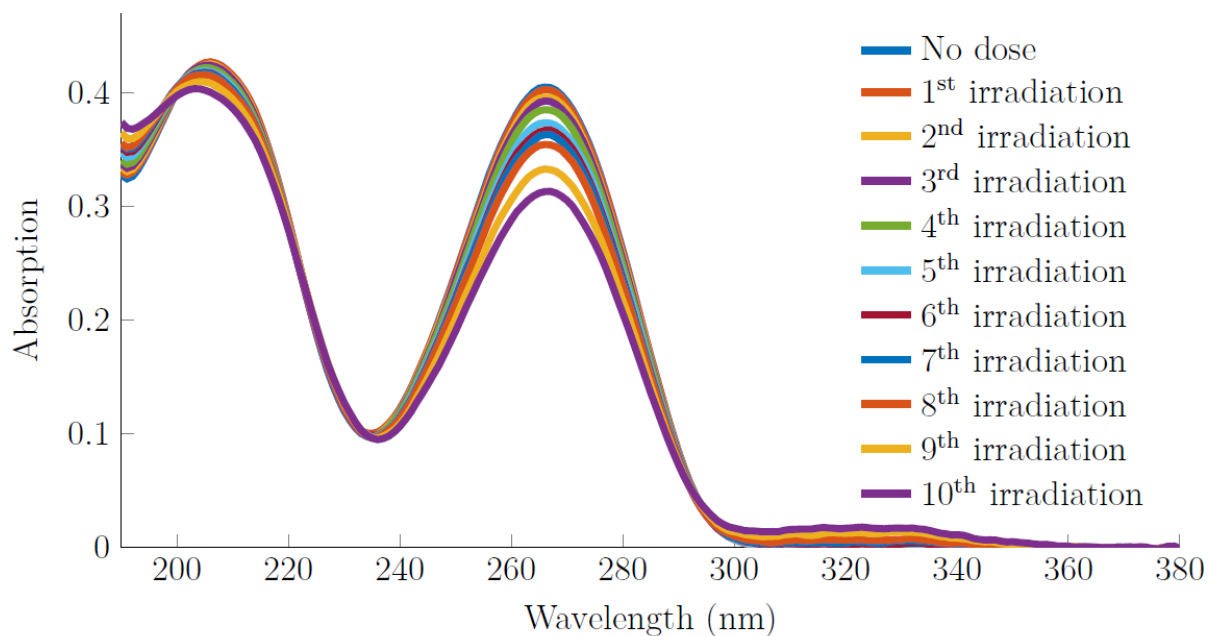


Figure S22: Measured absorption spectra for irradiation at 240 nm, ranging from no dose to a maximum absorbed dose of 0.97 J/cm².

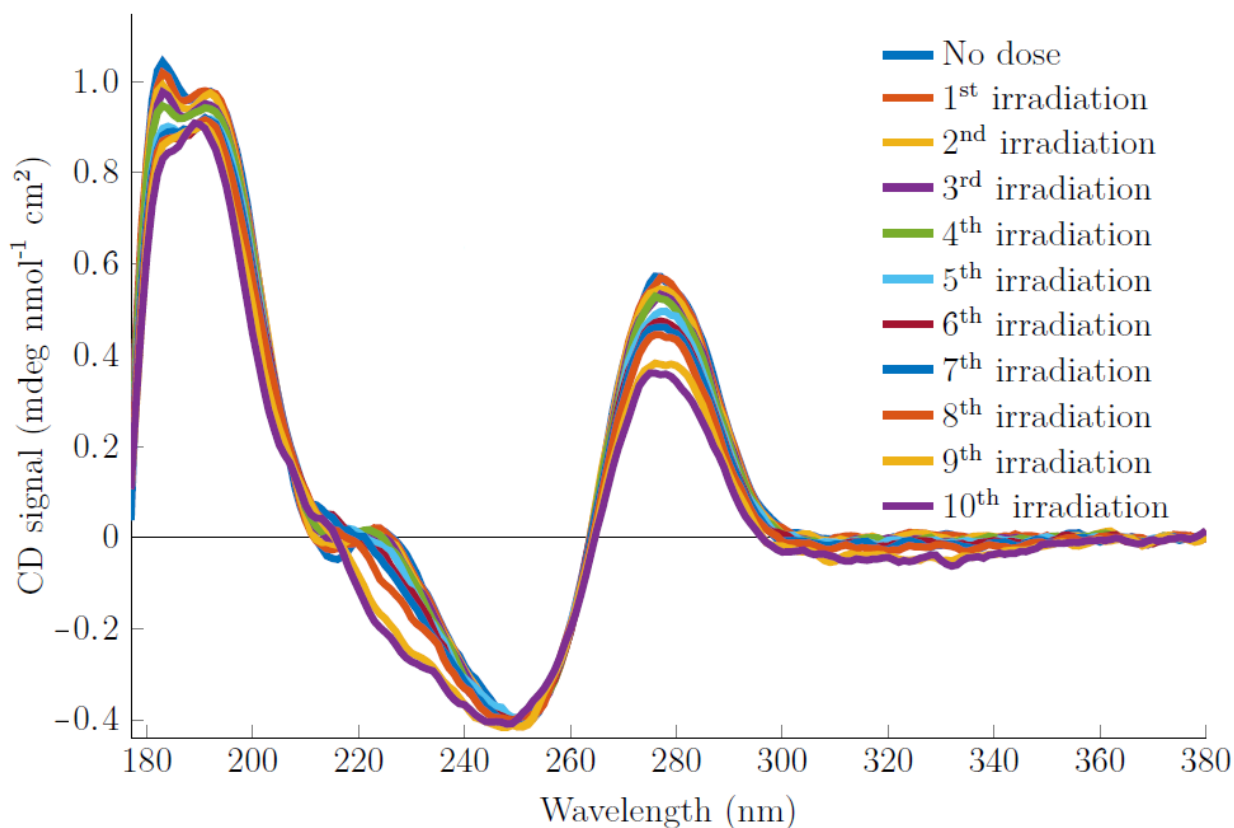


Figure S23: Measured CD spectra for irradiation at 240 nm, ranging from no dose to a maximum absorbed dose of 0.97 J/cm².

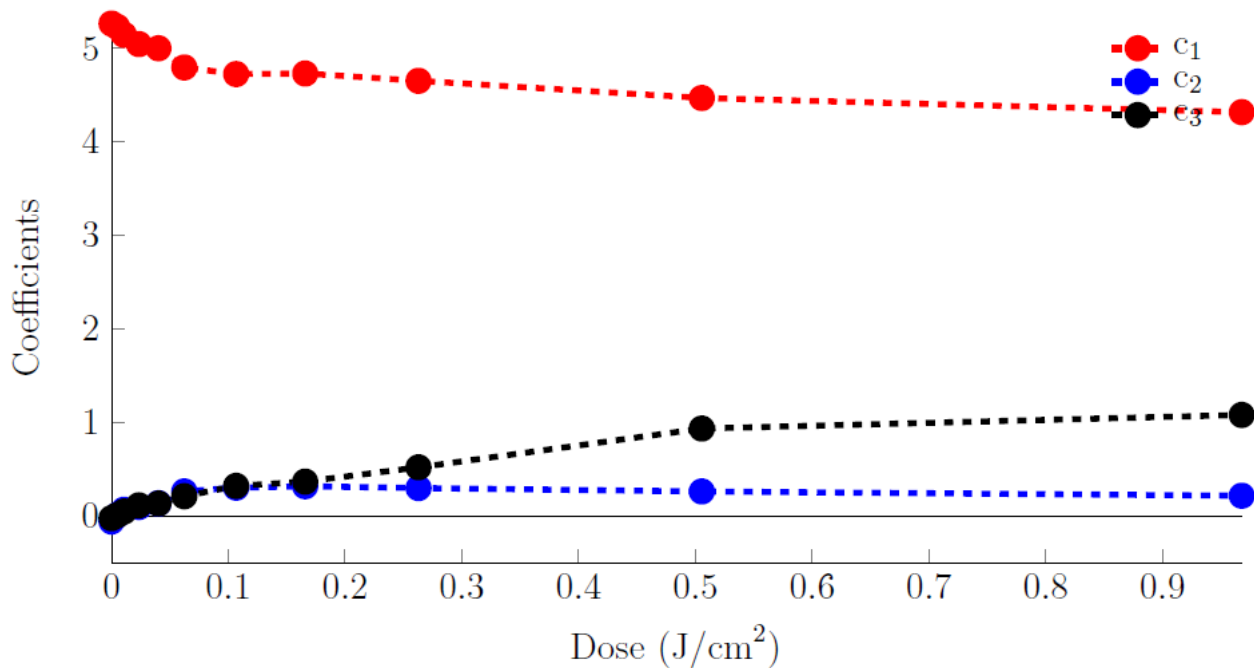


Figure S24: Coefficients for the 240 nm irradiation dataset for thymine T-T (red), dimer T<>T (blue) and the 64PP photoadduct (black).

S9. Irradiation at 230 nm.

The absorption and CD spectra for irradiation at 230 nm are shown in figures S25 and S26. The coefficients are plotted in figure S27. The second (blue) coefficient shows that the CPD formation is insignificant. Despite a large accumulated absorbed dose, the third coefficient (64PP, black) does not increase after 1 J/cm². However the first (red) coefficient continues to decrease, which indicates a continued decrease of undamaged thymine, i.e. the backbone of the single strand is cleaved.

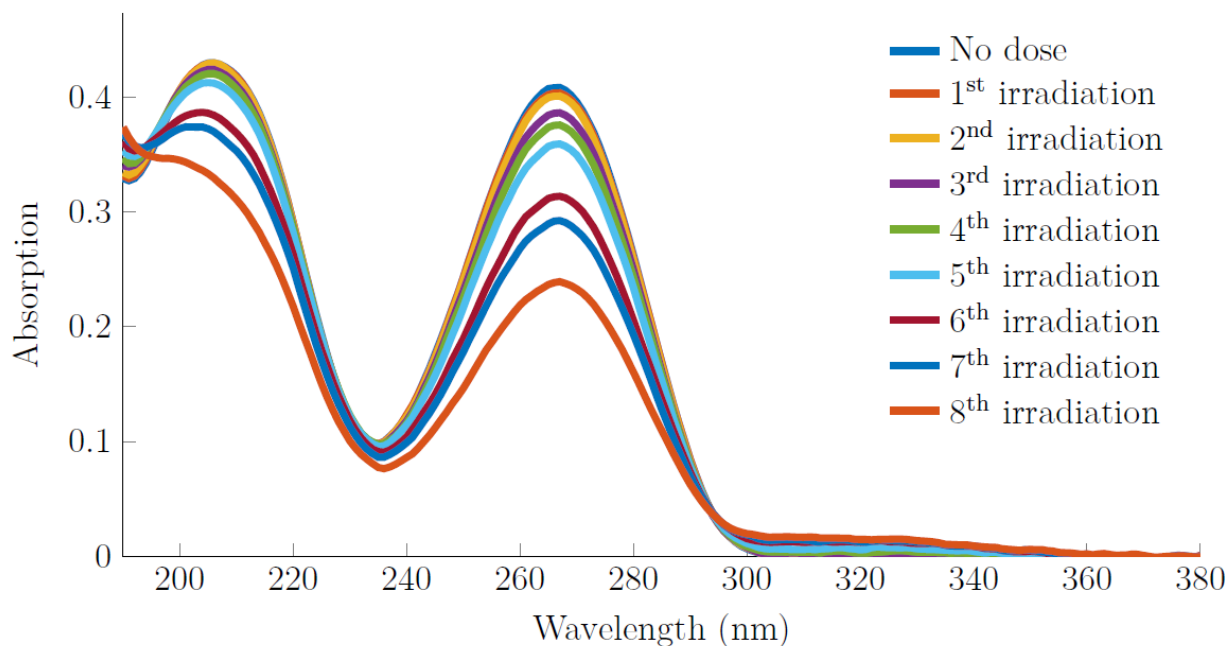


Figure S25: Measured absorption spectra for irradiation at 230 nm, ranging from no dose to a maximum absorbed dose of 3.73 J/cm².

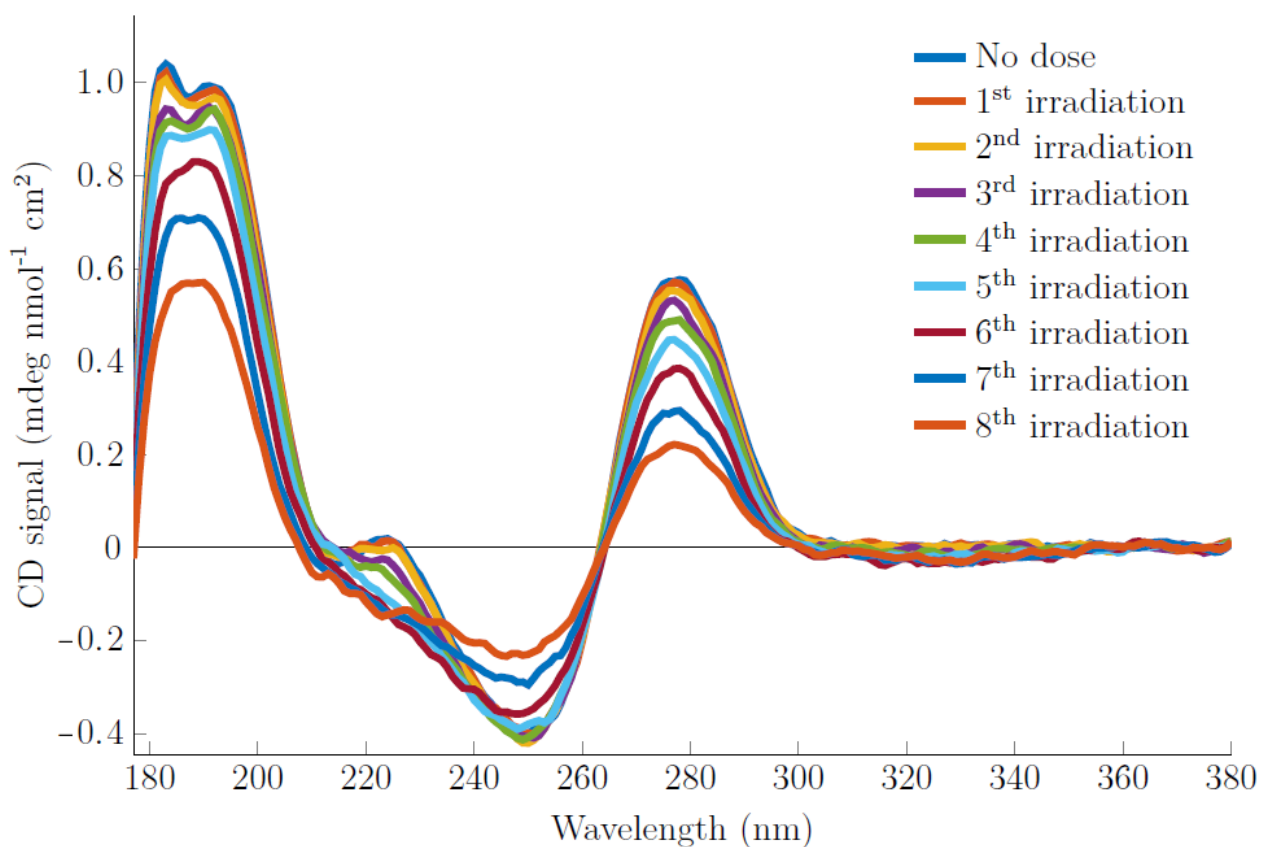


Figure S26: Measured CD spectra for irradiation at 230 nm, ranging from no dose to a maximum absorbed dose of 3.73 J/cm².

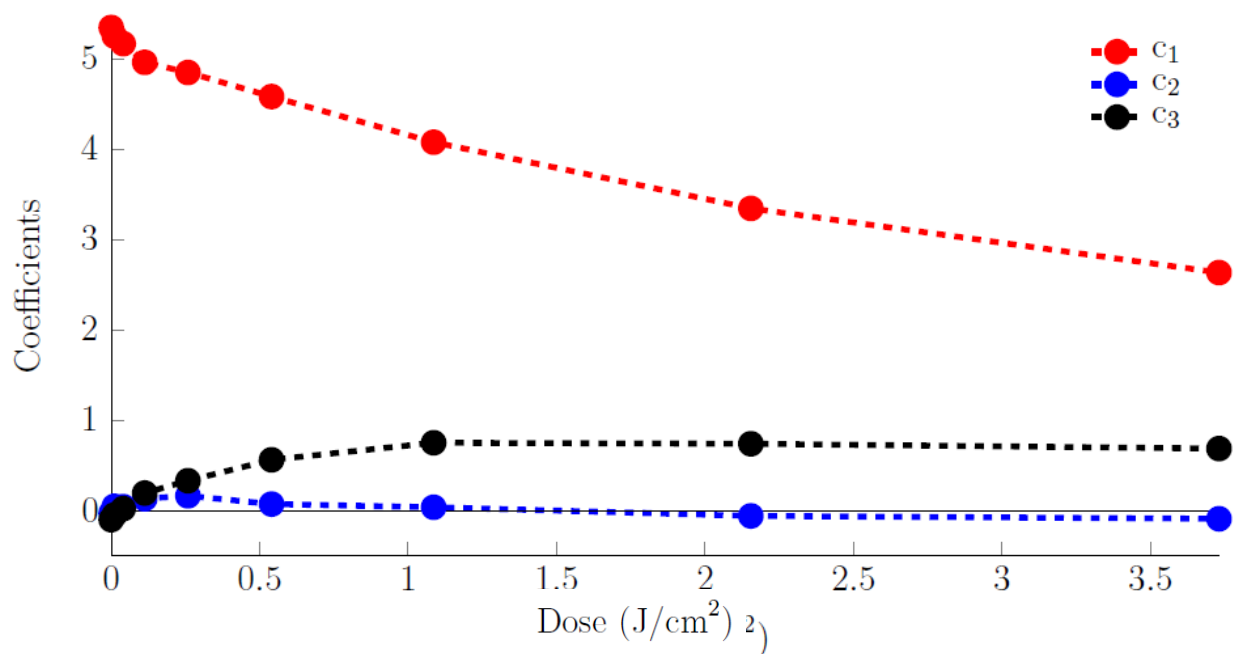


Figure S27: Coefficients from 230 nm irradiation dataset for thymine T-T (red), dimer T<>T (blue) and the 64PP photoadduct (black).

S10. Irradiation at 220 nm.

In figures S28 and S29 the absorption and CD spectra for irradiation at 220 nm are shown. It is noticeable that the CD signal magnitude is much reduced for the last measurement. The coefficients are plotted in figure S30. The first (red) coefficient decreases quickly up to and including the last measurement. The second coefficient (CPD, blue) is insignificant. Some 64PP is formed as indicated by the third coefficient, but the formation does not correspond to the decrease of undamaged thymine, due to strand backbone cleavage.

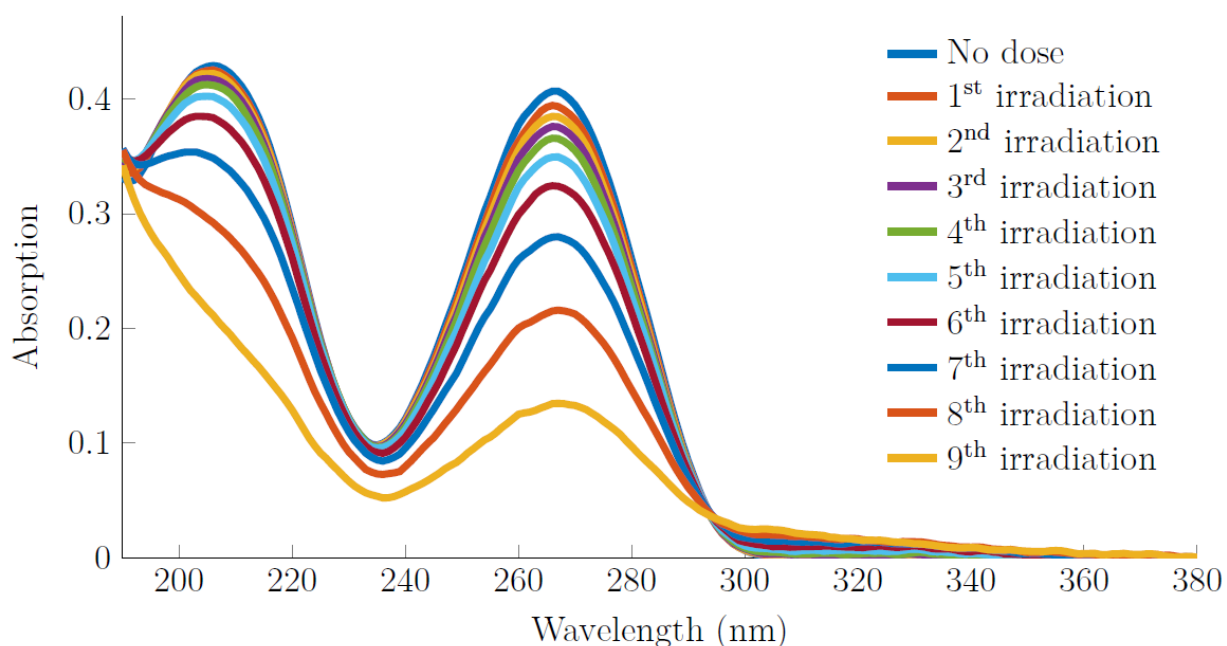


Figure S28: Measured absorption spectra for irradiation at 220 nm, ranging from no dose to a maximum absorbed dose of 1.99 J/cm².

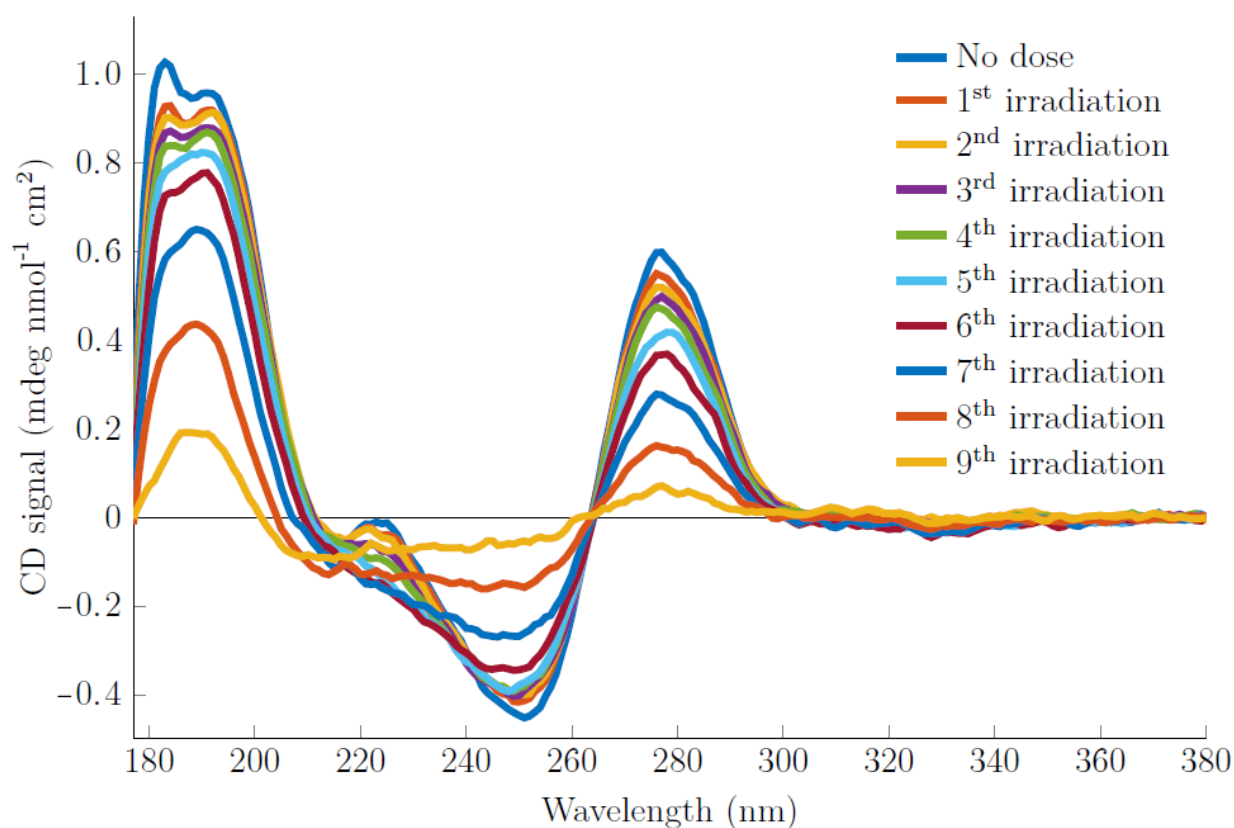


Figure S29: Measured CD spectra for irradiation at 220 nm, ranging from no dose to a maximum absorbed dose of 1.99 J/cm².

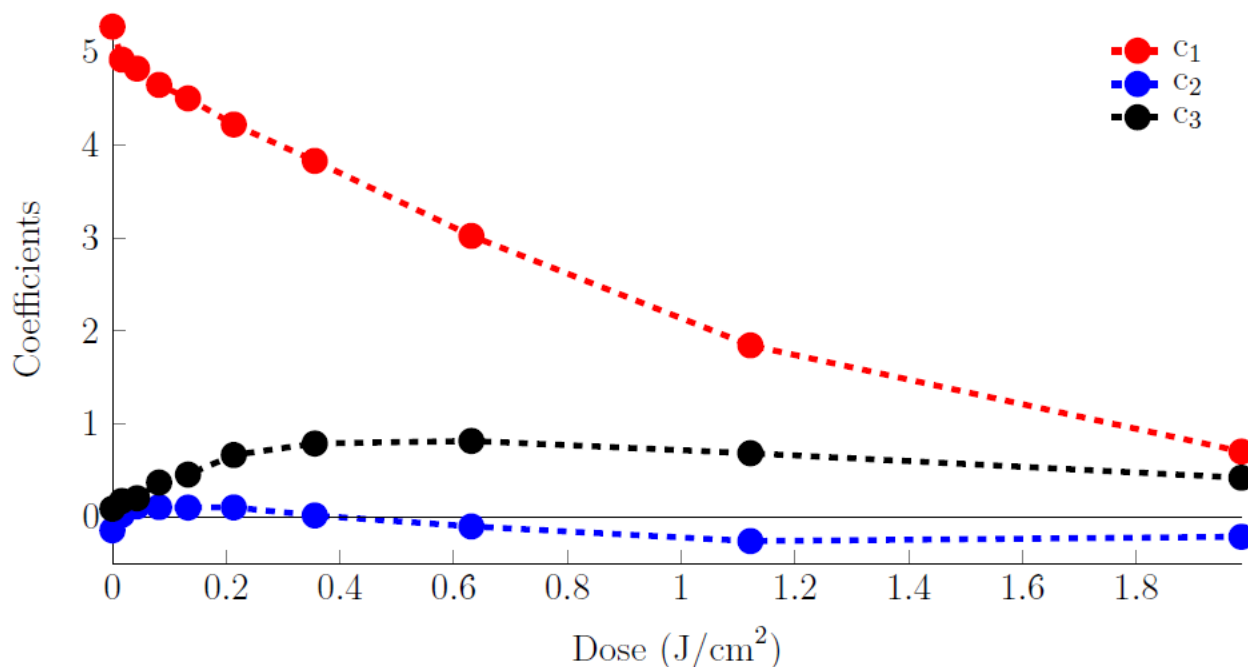


Figure S30: Coefficients for the 220 nm irradiation dataset for thymine T-T (red), dimer T<>T (blue) and the 64PP photoadduct (black).

S11. Irradiation at 210 nm.

The absorption and SRCD spectra for irradiation at 210 nm are shown in figures S31 and S32. The features of the spectra are very similar to those for irradiation at 220 nm. The coefficients are plotted in figure S33. The second (blue) coefficient is very close to zero in this dataset, which shows that there is an insignificant amount of CPD produced.

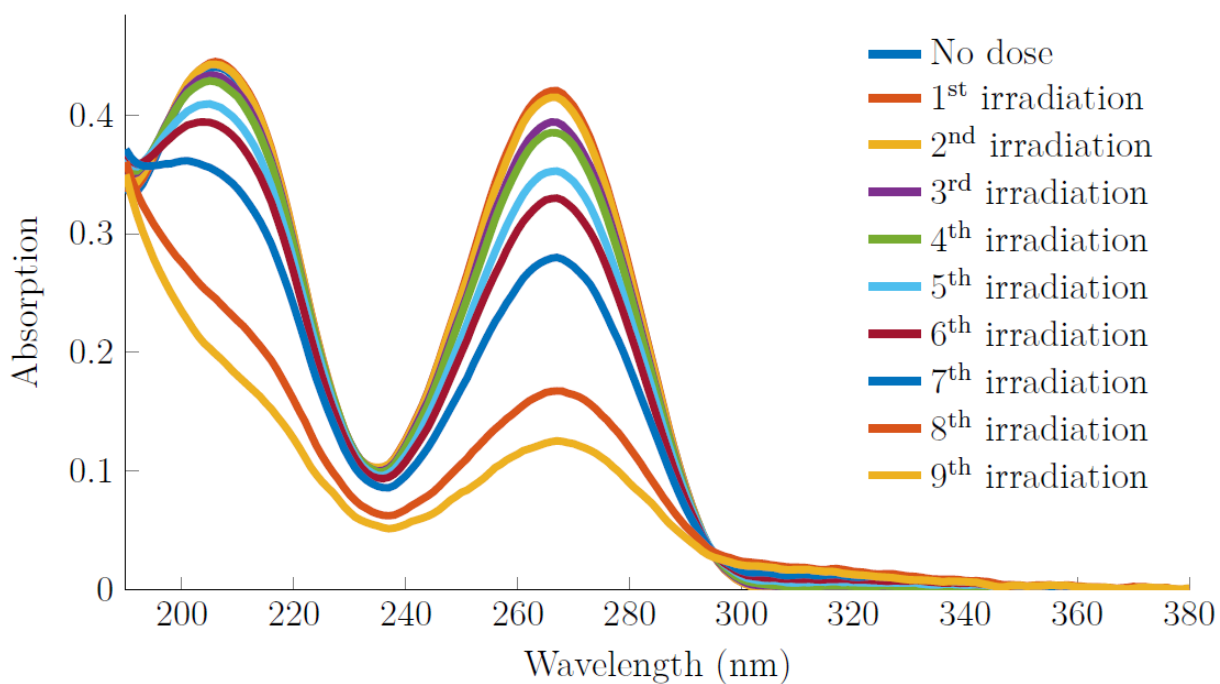


Figure S31: Measured absorption spectra for irradiation at 210 nm, ranging from no dose to a maximum absorbed dose of 4.52 J/cm².

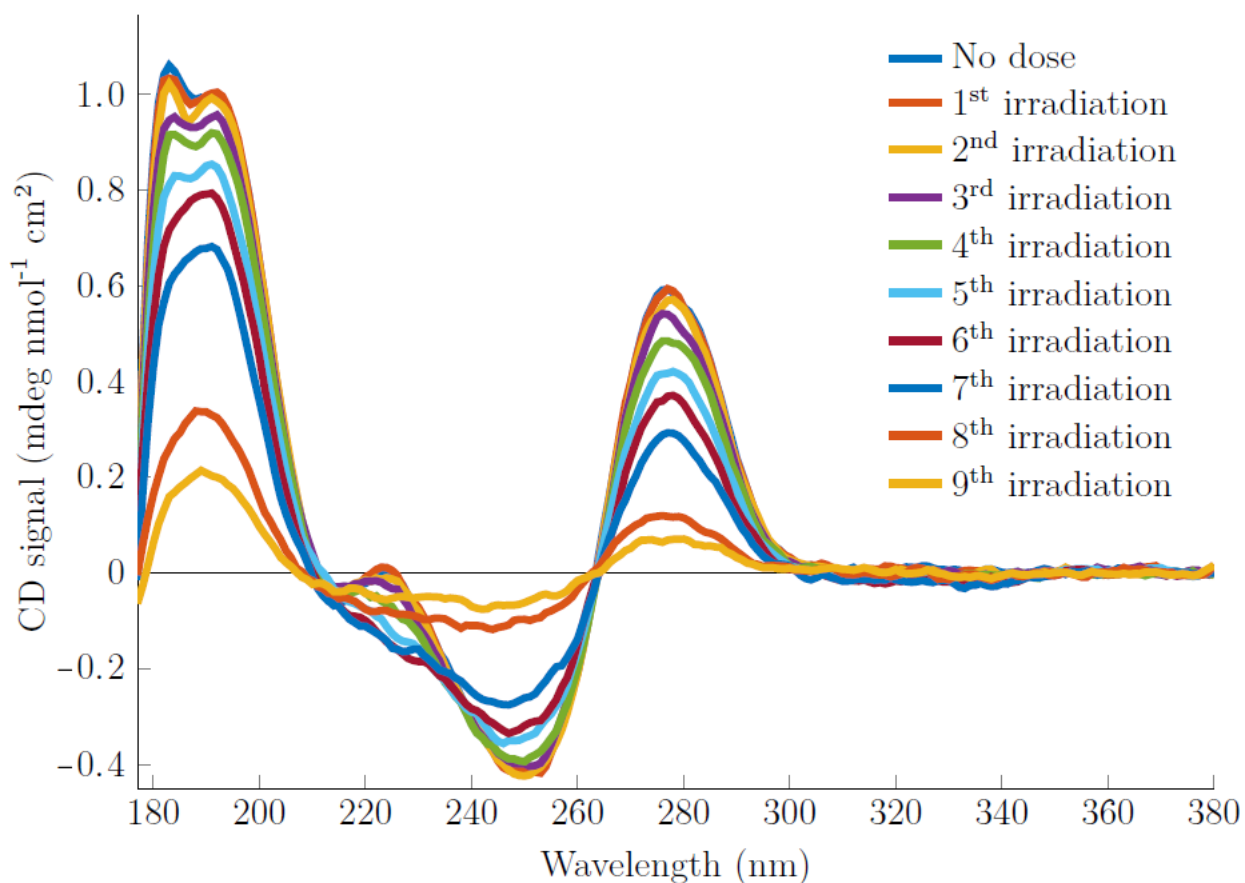


Figure S32: Measured CD spectra for irradiation at 210 nm, ranging from no dose to a maximum absorbed dose of 4.52 J/cm^2 .

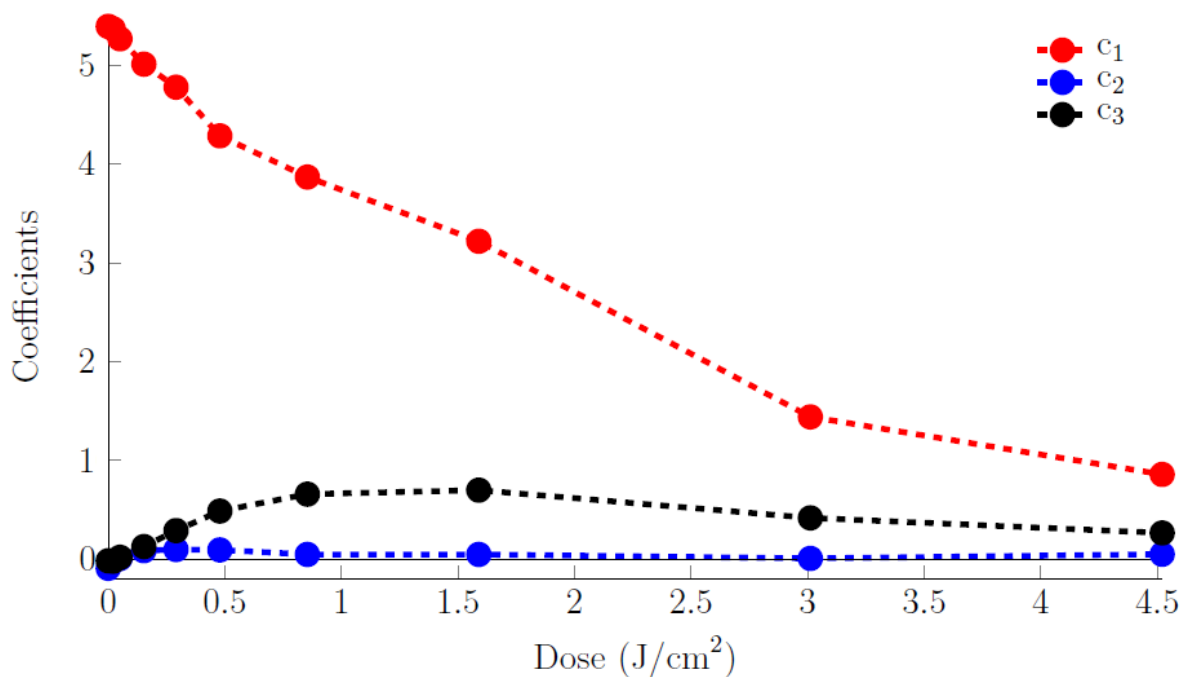


Figure S33: Coefficients for the 210 nm irradiation dataset for thymine T-T (red), dimer T<>T (blue) and the 64PP photoadduct (black).

S12. Single irradiation wavelength PCA analysis for the 255 nm dataset

If a PCA analysis is only performed on the first four spectra of the SRCD data from the 255 nm irradiation dataset (Fig. S17), two significant components, denoted v_1 and v_2 in figure S34, are found. Ideally these would represent the undamaged thymine as well as the CPD photoproduct. However, v_1 is not exactly a normalized ($v_1 \cdot v_1 = 1$) CD spectrum of the un-irradiated thymine. By refining the PCA as described in the Results section of the paper, the two new basis spectra φ_1 and φ_2 now represent the undamaged thymine and photoproducts respectively. The third component spectrum, φ_3 , can then be formed via the subtraction method described in the Results section. Using this method the third component spectrum has a clear negative band near 325 nm, where the 64PP photoadduct has an absorption band (see figure S16). The same band is also noticeable in the second component (φ_2), and this component is thus not a ‘clean’ representation of the CPD photoproduct, which does not absorb above 260 nm. The use of the first few CD spectra in an irradiation series to determine basis spectra representing undamaged thymine and CPDs is therefore not ideal. Using the full 300 nm dataset to derive the true basis spectrum for the CPD photoproduct (denoted $\varphi_{T \rightarrow T}$) is much more reliable due to the lack of 64PP photoadduct formation at this wavelength, and is free of any artifact near 325 nm. The $\varphi_{T \rightarrow T}$ basis spectrum can then be used for all subsequent analyses of CD spectra to find the amount of CPD photoproduct in the sample.

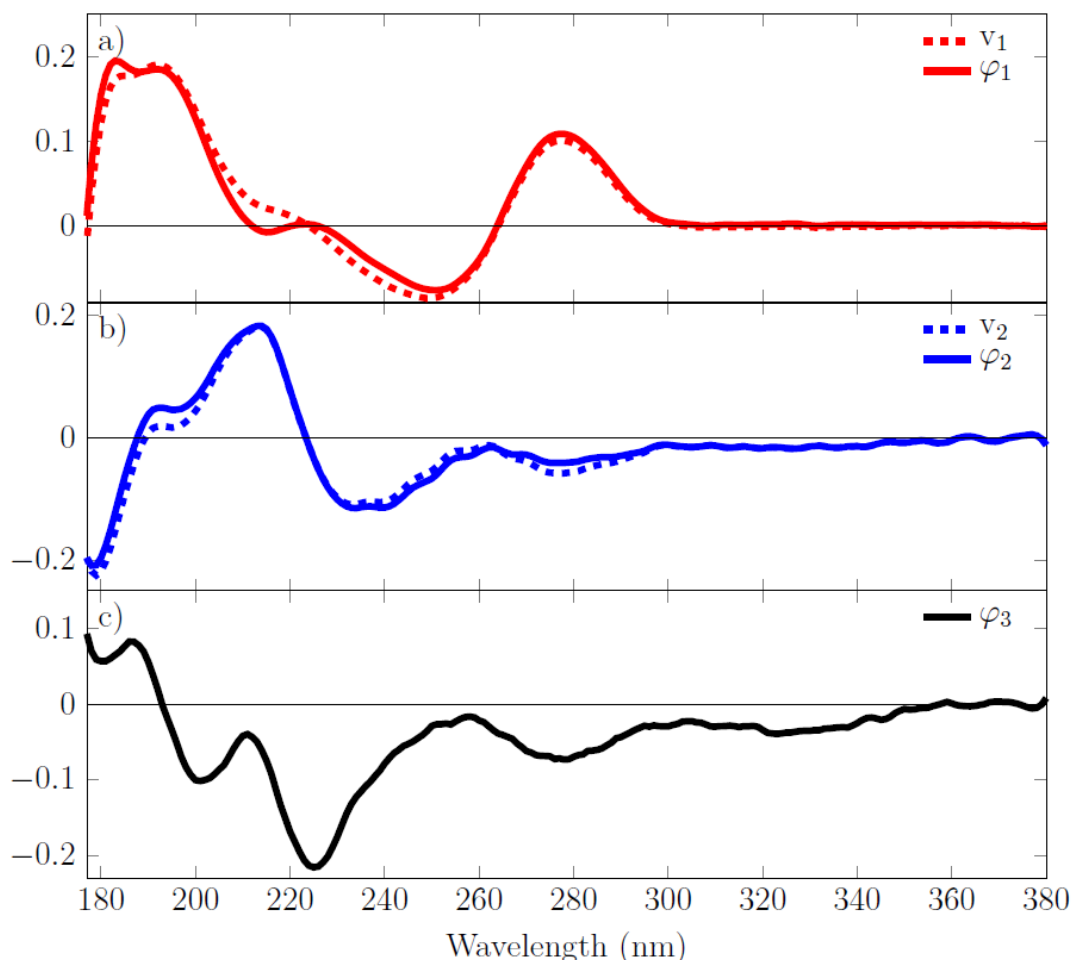


Figure S34: a) and b) The two basis spectra, v_1 and v_2 , resulting from a raw PCA analysis of the first four 255 nm dataset CD spectra. After refinement φ_1 and φ_2 represent the undamaged thymine and photoproducts. c) φ_3 is the third component spectrum from a full analysis of the entire 255 nm dataset.