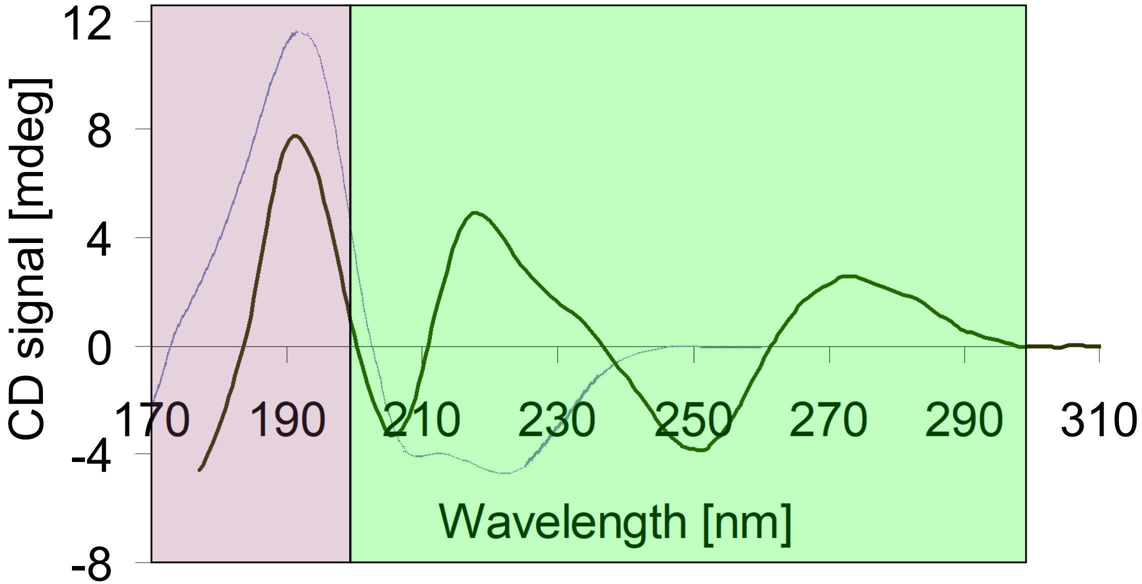
ESC1: Circular Dichroism: best practice and data analysis

Lecture 6: Examples of non-protein CD studies

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 101004806

Non protein CD: Oligo-nucleotides

- Nucleotides have strong CD signals below 300 nm
- Long wavelength range of data on conventional CD above 200 nm
- Are data below 200 nm really important?
  - As I will demonstrate: Yes!



So please keep your instrument in good condition

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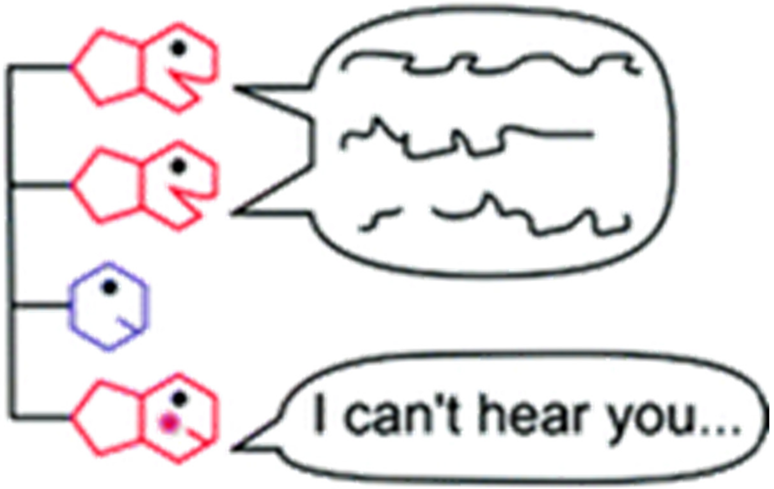
Non protein CD: Oligo-nucleotides

Graphical abstract in PCCP



Q: Does Adenines talk, and what is the influence of Thymine?

"Vacuum-ultraviolet circular dichroism spectroscopy of DNA: a valuable tool to elucidate topology and electronic coupling in DNA"
Holm A.I.S., Nielsen L.M., *Hoffmann S.V.*, Nielsen S.B. PCCP 12 (2010) 9581-9596

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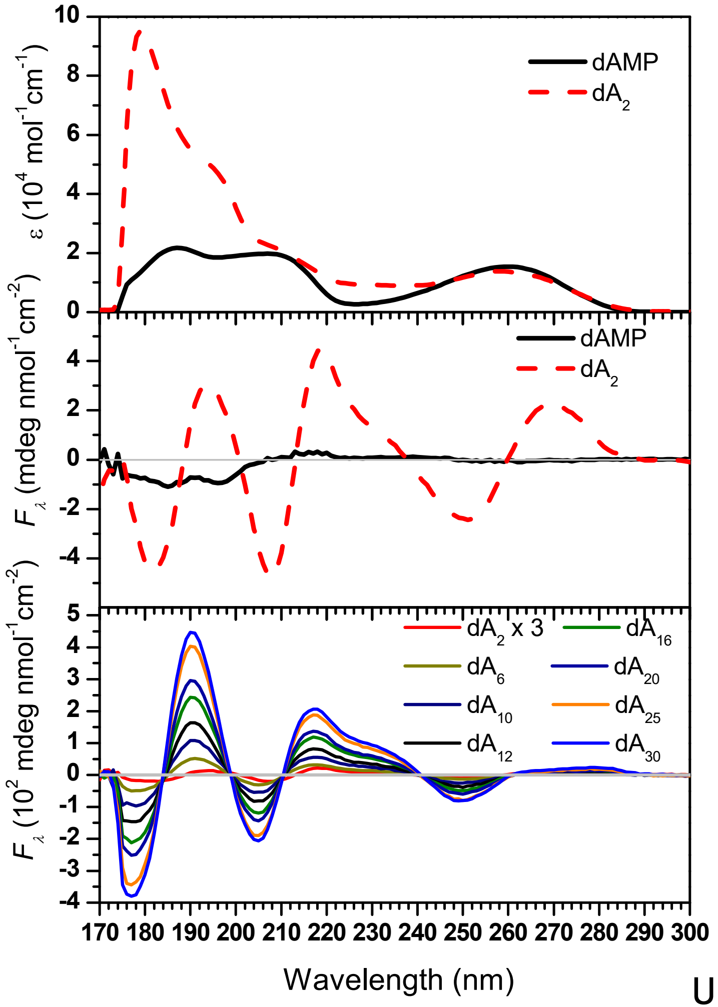
Non protein CD: strands of adenine



Single strands of adenine

dAMP *vs.* dA$_2$

Circular Dichroism:
Strong coupling between bases

dA$_n$ n = 2 - 30

The signal at 190 nm:
Doesn't increase linearly with n
(for small n)

U. Kadhane et al. PHYSICAL REVIEW E 77, 021901 (2008)

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Non protein CD: strands of adenine

The signal at 190 nm: Doesn't increase linearly with n (for small n)

Make a model:

A A

$\overset{\longleftrightarrow}{a_1}$

A A A

$\overset{\longleftrightarrow}{a_1} \quad \overset{\longleftrightarrow}{a_1}$

$\overset{\longleftrightarrow}{a_2}$

A A A A

$\overset{\longleftrightarrow}{a_1} \quad \overset{\longleftrightarrow}{a_1} \quad \overset{\longleftrightarrow}{a_1}$

$\overset{\longleftrightarrow}{a_2}$

$\overset{\longleftrightarrow}{a_2}$

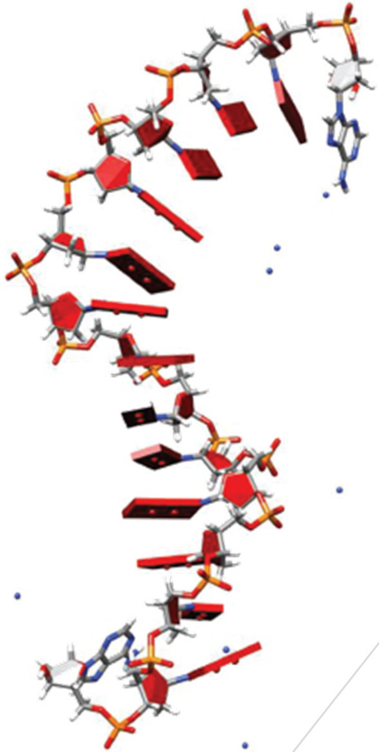
$\overset{\longleftrightarrow}{a_3}$

$a_1, a_2, a_3, \ldots$ are "coupling" terms

$$e.g.\ F_\lambda(4) = a_3 + 2a_2 + 3a_1$$

$$\text{In general: } F_\lambda(n) = \sum_{i=1}^{n} (n-i)a_i$$

$$\text{Where } a_i = \alpha \exp(-\beta i d),\ d = 3.4\ \text{Å}$$



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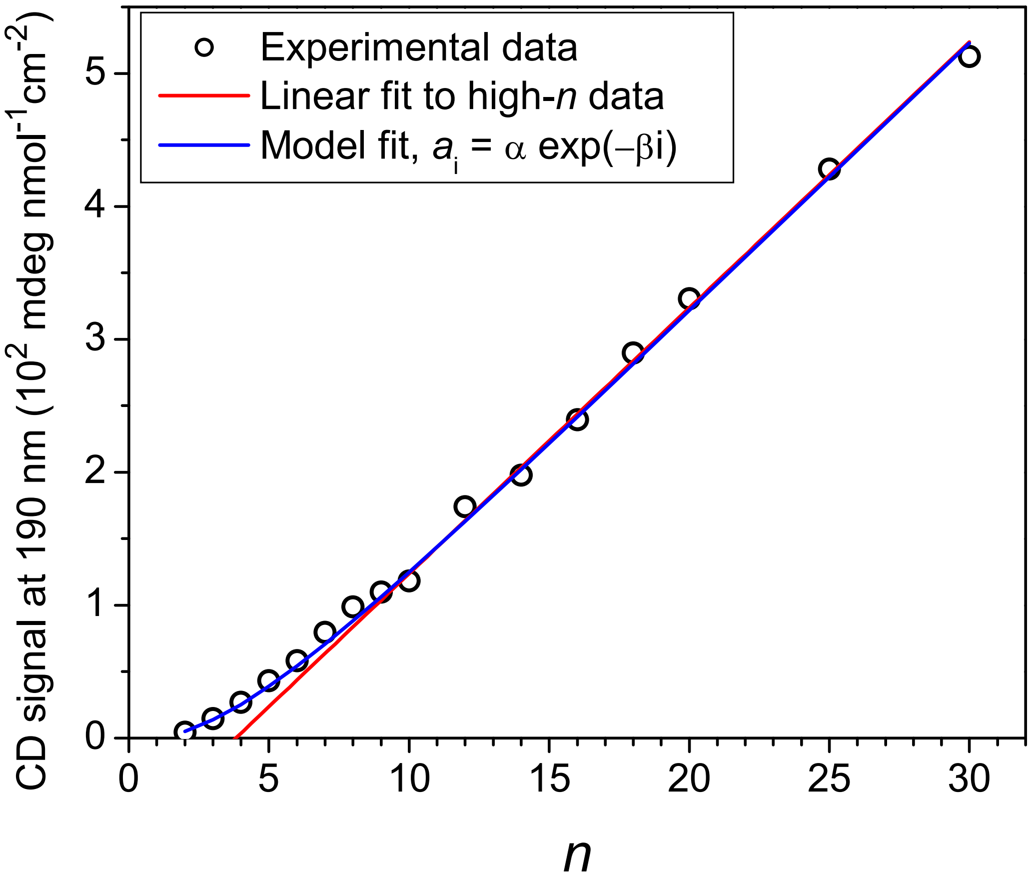
Non protein CD: strands of adenine

CD signal 190 nm



At least eight nucleobases couple.
Nearest neighbour coupling (a_1) is only about 24%

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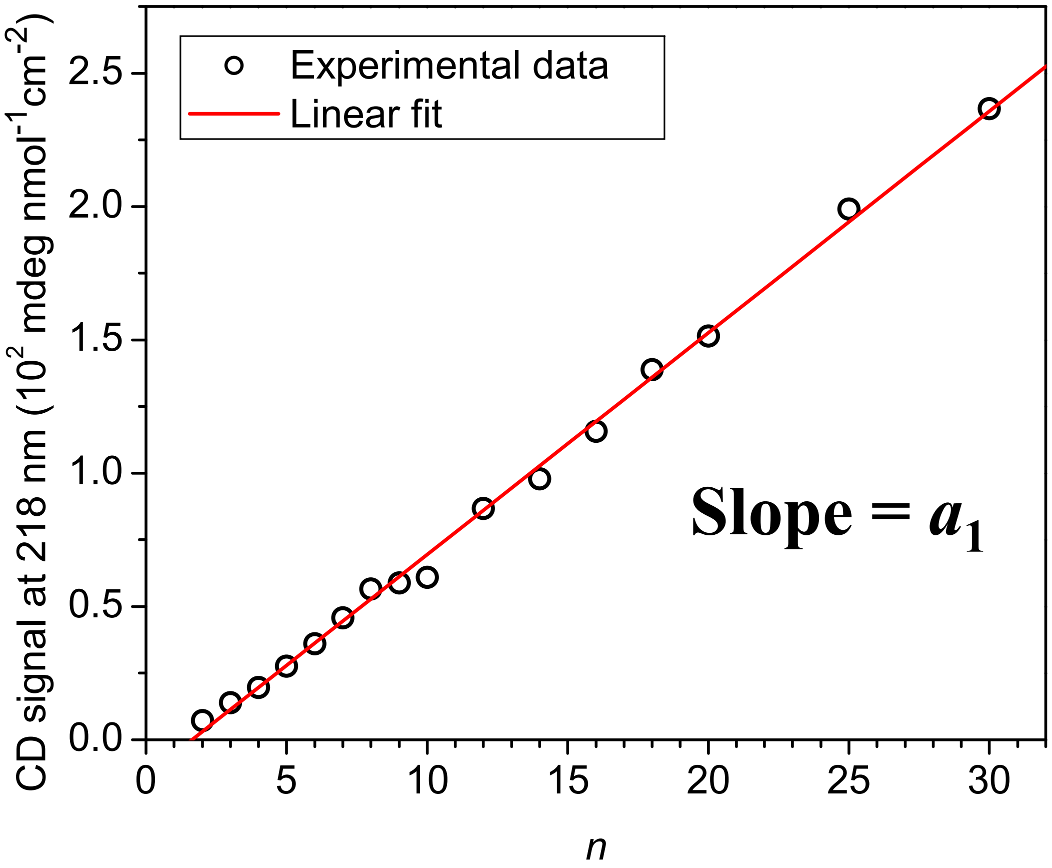
Non protein CD: strands of adenine

CD signal 218 nm



Only two adenine bases couple!

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Non protein CD: strands of adenine

Conclusion:

Electronic coupling between stacked adenine bases depends strongly on the excitation energy (wavelength)

→ Below 200 nm: At least eight adenine bases couple

→ Above 200 nm: Only two adenine bases couple

Electronic coupling between nucleobases impacts:

- Excitation energy is spread over a large spatial region

  → Self-protection mechanism of DNA: less prone to UV or VUV damage

- DNA as a conducting nanowire

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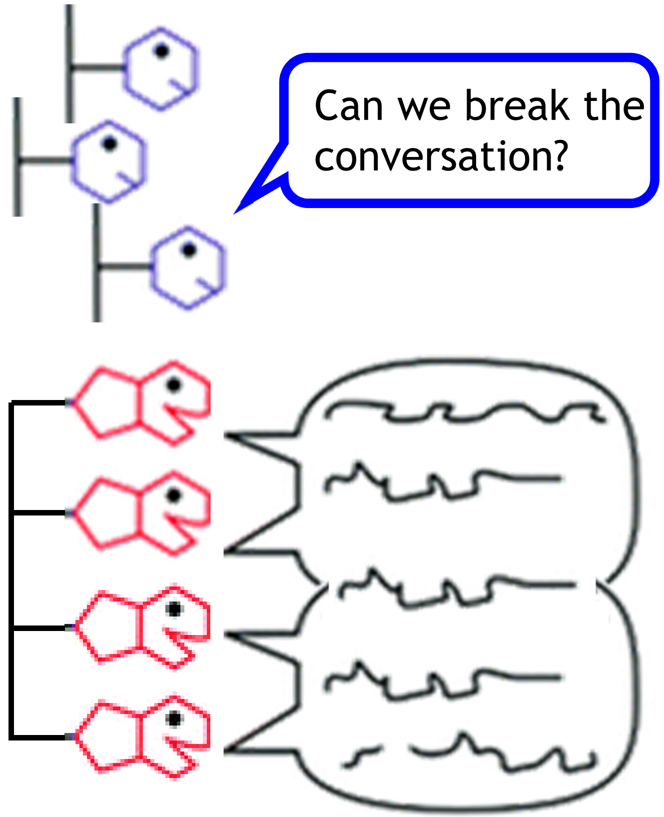
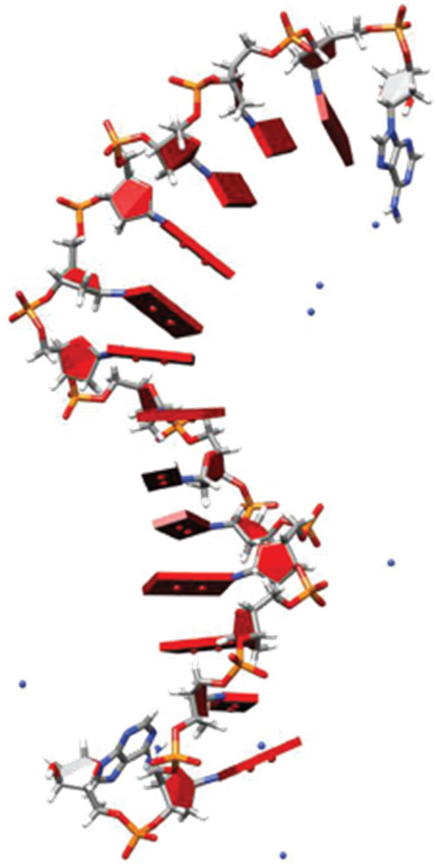
Non protein CD: strands of adenine and thymine

Can Thymine break the Adenine coupling?





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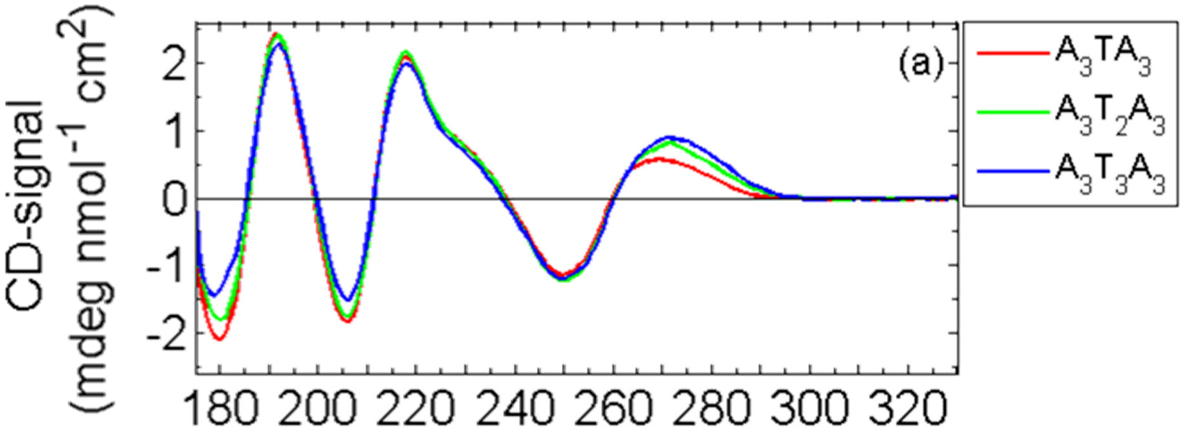
Non protein CD: strands of adenine and thymine

Dependence on Thymine



The number of thymine 'spacers' does not significantly change the spectra

→ If there is an effect, *one* thymine is enough

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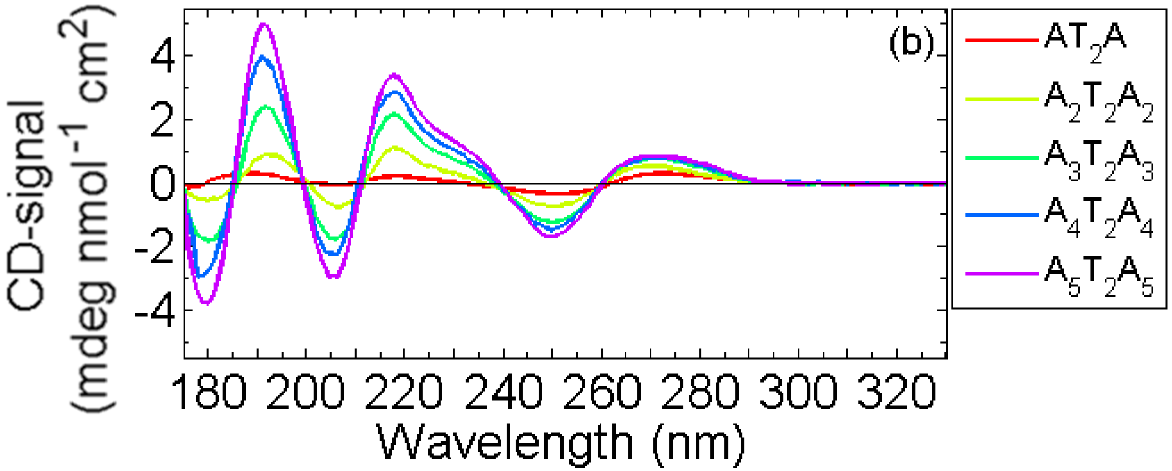
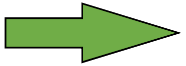
Non protein CD: strands of adenine and thymine

Dependence on Adenine



The spectra are very similar to the spectra of dA_n



Need to determine over *how many* Adenine bases coupling do occur.

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Non protein CD: strands of adenine and thymine

Does T break the A coupling?

A A A A T A A A A

dA_n coupling to dA_n ?

"Math. with A's and T's"

(1) A A A A T A A A A = ATA + AAAAAAAA

?

(2) A A A A T A A A A = ATA + 2 * AAAA

(1) ⟶ The A's couple through the T's

(2) ⟶ The T's break the coupling between the A's

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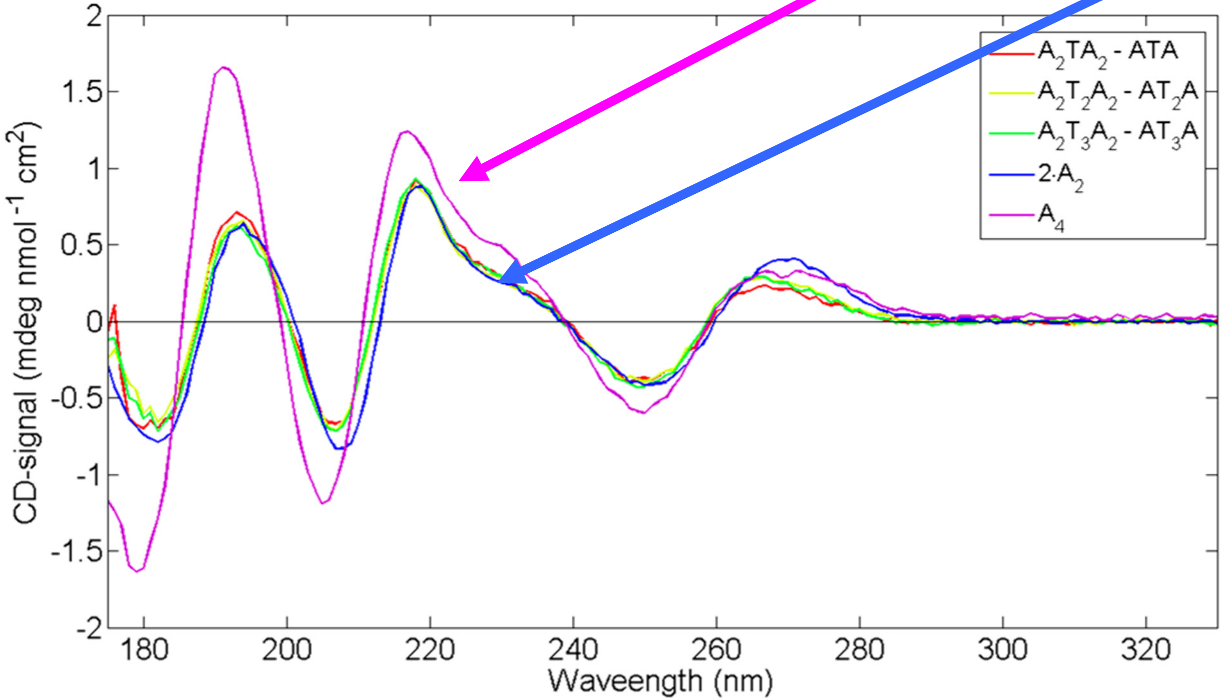
Non protein CD: strands of adenine and thymine

Do the Math: Difference spectra

$A_nT_mA_n - AT_mA$ *vs* A_{2n} and $2{\cdot}A_n$



Electronic coupling between the adenine bases is broken

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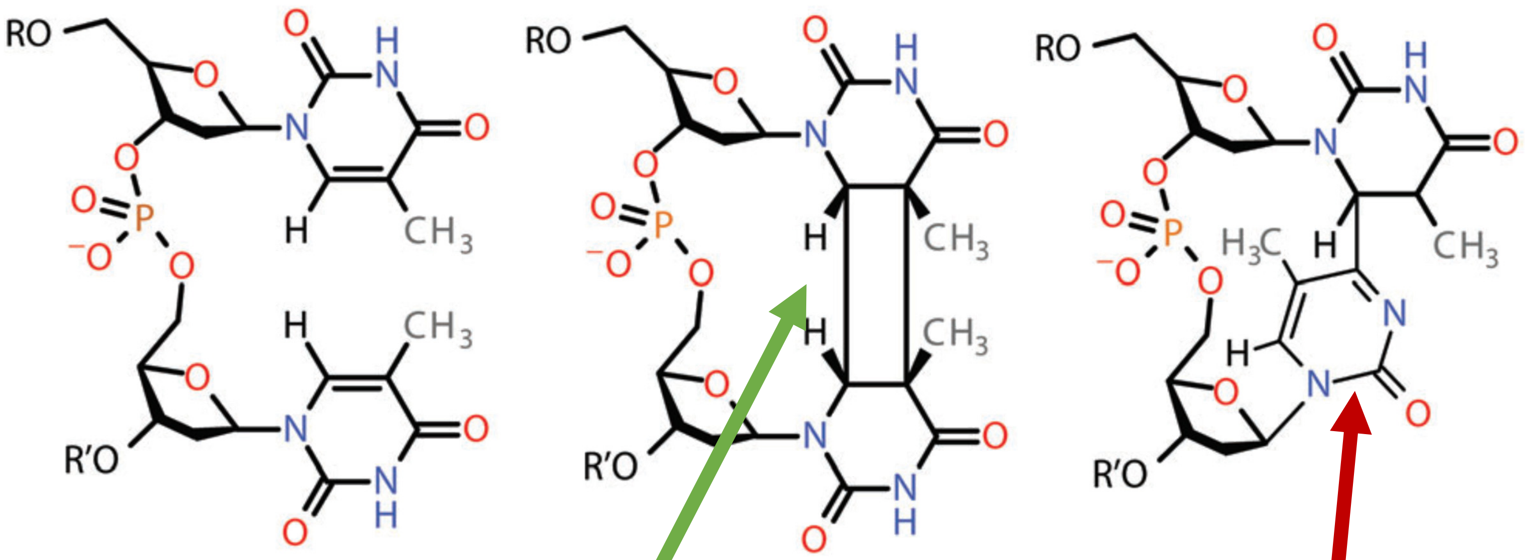
Non protein CD: UV induced thymine photolesions

Formation of thymine dimer photoproducts is a primary cause of skin cancer



Neighbouring thymine nucleobases can fuse into a cyclobutane pyrimidine dimer (CPD)

... or the pyrimidine (6-4) pyrimidone photoadduct (64PP)

Reversible process T-T ⟷ CPD

Irreversible process T-T ⟶ 64PP

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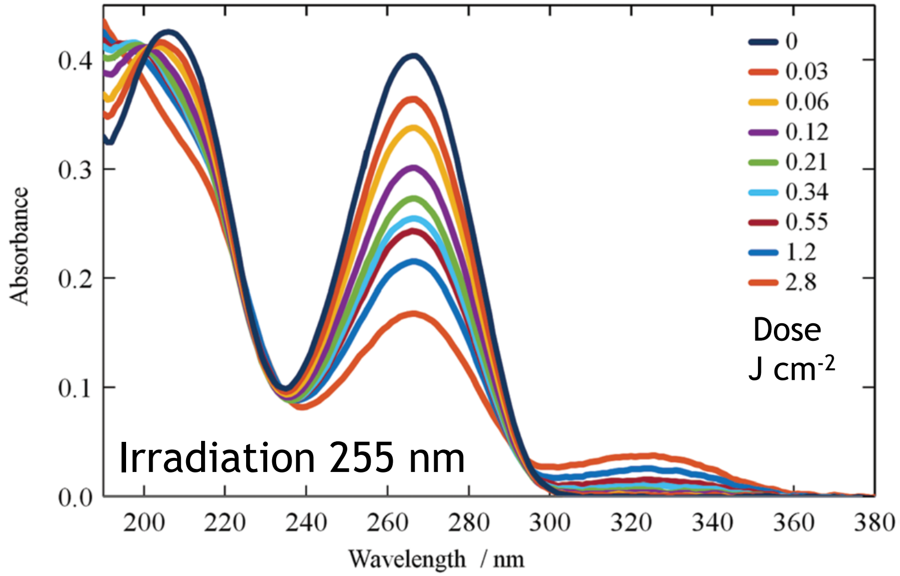

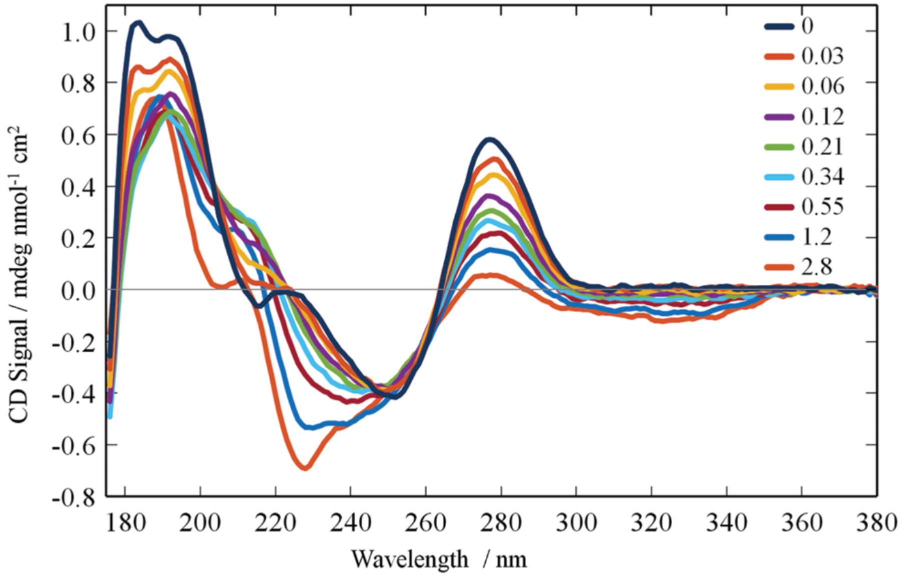

Follow the development of dT_5 via absorption

Very little spectral structural change

… or via circular dichroism

… large *spectral structural* change

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Non protein CD: UV induced thymine photolesions

Which photo lesions develop depends on irradiation wavelength

Irradiation at 300 nm only develops the cyclobutane pyrimidine dimer (CPD)

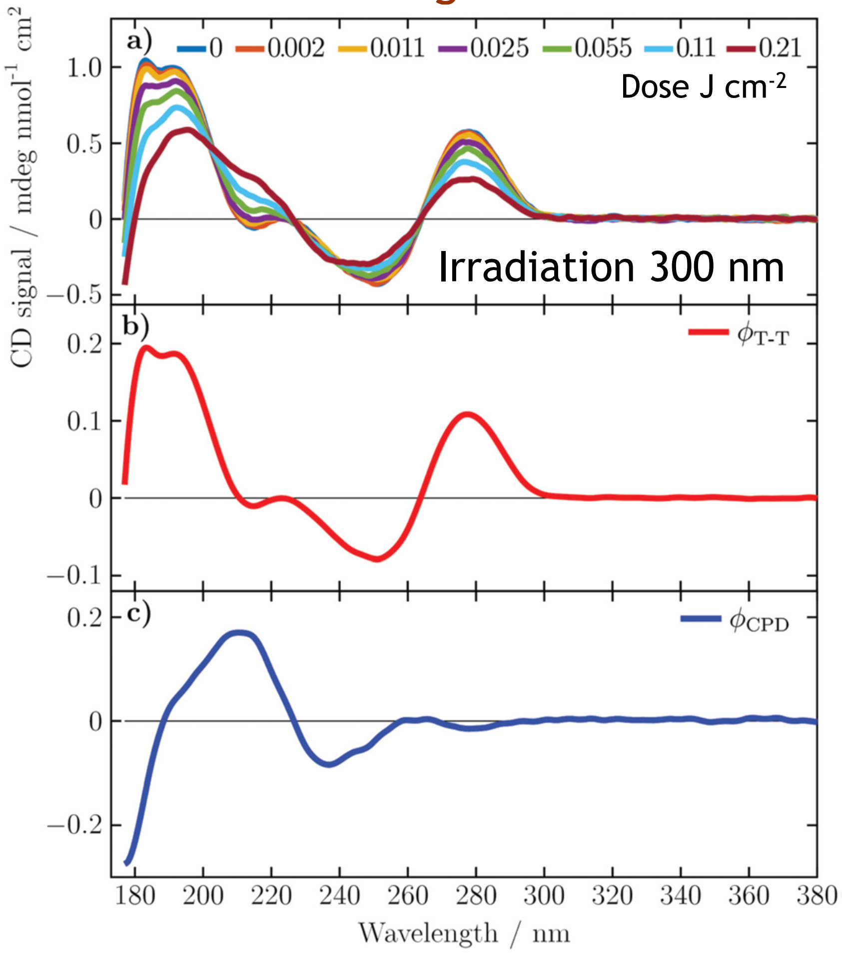
Principal Component Analysis (PCA):

- The basis spectrum for the thymine dimer, $\Phi_{T\text{-}T}$ (λ)

- The basis spectrum for the CPD, Φ_{CPD} (λ)

The two basis spectra are orthonormal:

$$\Phi_{CPD} \cdot \Phi_{CPD} = \Phi_{T\text{-}T} \cdot \Phi_{T\text{-}T} = 1$$
$$\Phi_{CPD} \cdot \Phi_{T\text{-}T} = 0$$



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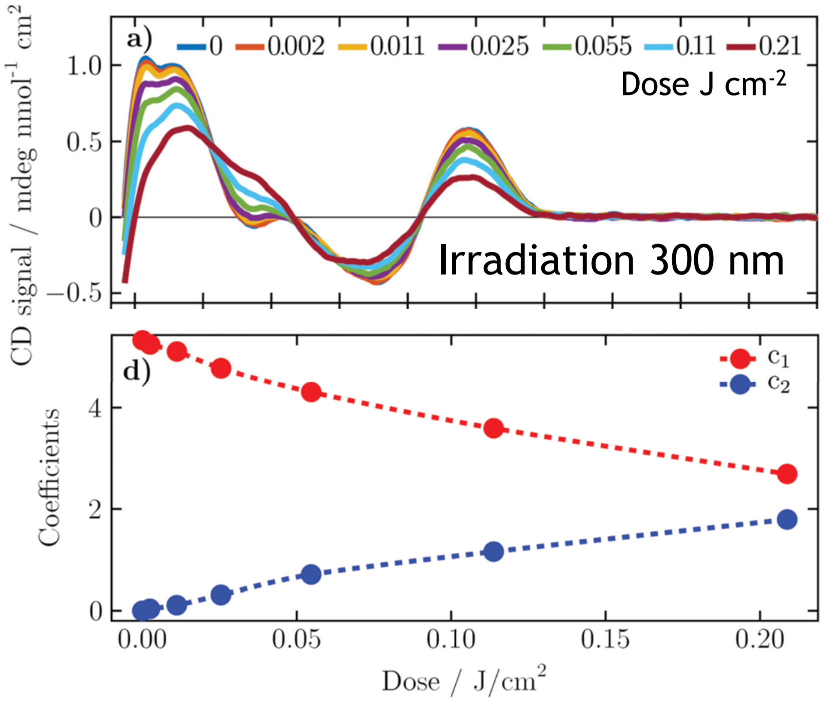
Non protein CD: UV induced thymine photolesions

Multiplying an irradiated dT_5 spectrum with these basis spectra, gives the amount (coefficient) of the **dimer** and the **CPD**

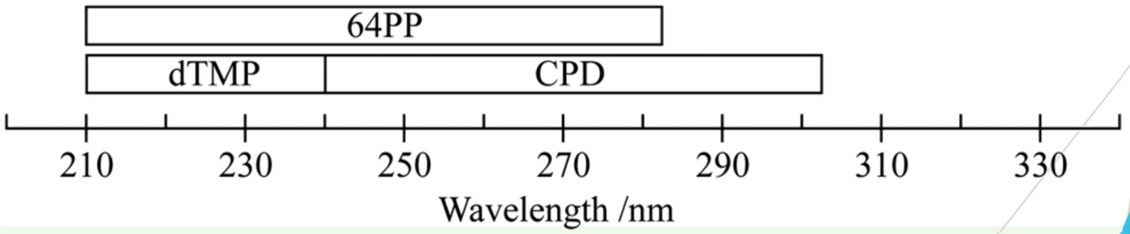
$$c_1 = CD(\lambda) \cdot \Phi_{T\text{-}T}(\lambda)$$

$$c_2 = CD(\lambda) \cdot \Phi_{CPD}(\lambda)$$



The *amount of photolesions* can be followed for any irradiated thymine strand sample

The irradiation *wavelength dependence* on the photo products formed



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