DNA Photoswitching by Azobenzene

Steve Norton
(supervisor: Phillip Milnes)
DNA

Adenine

Guanine

Thymine

Cytosine
• Transcription of genes and replication of DNA

• Separation of DNA strands: must expose strands to pair new bases correctly
Azobenzene and photoswitching

• 2 benzene rings, connected by azo group
• Under UV light switches from \textit{trans} to \textit{cis} form

Switch takes on the order of picoseconds
Why combine DNA and azobenzene?

• Maybe azobenzene can replace DNA helicase in synthetic systems:
• Could form part of an artificial ribosome project, and let us open and close DNA whenever we want, without enzymes.

• Also useful in DNA origami.

• Or any system where tight control of DNA’s shape is needed, especially if it needs to change.
How can we combine them?

- DNA strands and azobenzene both modified: amine on DNA, carboxylic acids on azobenzene
• The modifications allow us to form amide bonds (called peptide bonds in proteins):

\[
\text{O} \\
\text{C} \\
\text{N} \\
\text{R'} \\
\text{R'} \\
\text{R''}
\]
Forming Amide bonds

- Azobenzene carboxylic acid
- Azo with EDCI
- Azo with HOBt
- Amine-DNA
- Compound with peptide bond
Identifying products

• High Performance Liquid Chromatography
  – Sample pushed through column of semi-porous material.
  – According to size and chemistry, molecules detected at end of column at specific times...
  – We look for new peaks
• Original HPLC:
1\textsuperscript{st} attempt

- Reacted DNA strand 1 with azobenzene.
- Used HPLC to see if product made.
- Shift in peak location => success?
Was the product right?

• Used Mass Spectrometry to estimate the mass of compounds present after the reaction.

• 3563 is correct! (the mass of DNA1 + Azo + HOBT)
Adding complementary DNA

• Tried **adding DNA strand 2 with more coupling reagents**…
  Peak for DNA1-azo (middle), DNA 2 (left), nothing else significant.

• Added **more coupling reagents**… 3rd peak seen (right)
Another method

- DNA strands 1 and 2 allowed to hybridise first
  - Should make it easier to bond with both DNA strands at once
  - Should reduce chance of other molecules forming

- Tried reaction with UV light and without UV light
New peak tested

- Mass is exactly as expected for DNA-Azo-DNA compound
Conclusions

• Using modified DNA and azobenzene, with the coupling reagents HOBt and EDCI, we can form amide bonds between DNA strands.

• These strands can be complementary, but other implications of this are yet to be tested.
Future work

• Repeat with different sizes of DNA strand.
• Introduce other techniques for characterisation (e.g. gel electrophoresis)
• Test products for azobenzene photoswitching:
  – Do we see DNA duplex being pulled apart?
  – How long a section of DNA will the azobenzene separate in this way before it is overwhelmed by the strength of hydrogen bonds?
Thanks

• Phillip Milnes
• Rachel O’Reilly
• Tom Wilkes
• ROR Group
  (www2.warwick.ac.uk/fac/sci/chemistry/research/oreilly/oreillygroup)
• EPSRC & MOAC

Main References: