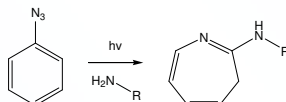


Light Directed Immobilisation of DNA

Introduction

It has been reported that phenyl azides are a group of compounds that are UV light active¹. When phenyl azides are illuminated in solution in the presence of a primary or secondary amine they undergo a rearrangement in which the azide loses N₂ gas and the remaining nitrogen atom enters the ring forming a 7 membered species. The additional amine attaches at the alpha carbon on from the nitrogen in the ring.



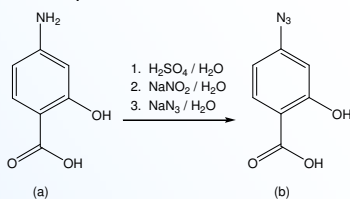
This function can be utilised to immobilise biomolecules such as: DNA, proteins and antibodies.

Experimental

Azide Synthesis²

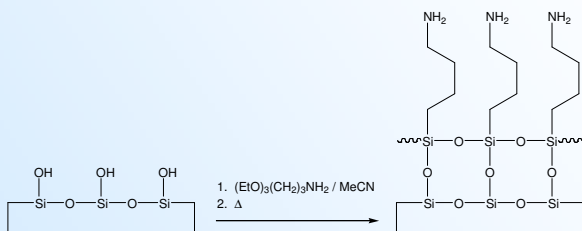
4-Azido-2-hydroxybenzoic acid was chosen as a suitable linker as it has two fundamental functionalities:

1. Phenyl azide – required for the photochemistry
2. Carboxylic acid – required for surface attachment at quartz surface

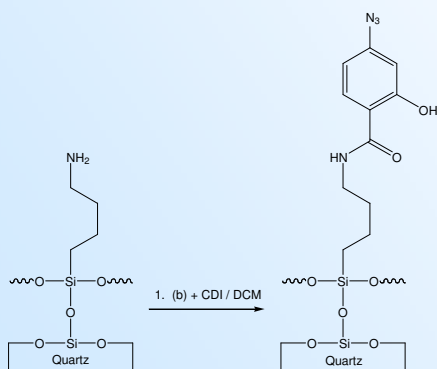


Quartz Functionalisation

1. Piranha³ of quartz – treatment of quartz glass with a solution of 3:1 H₂SO₄:H₂O₂ leaves surface free from organics and extremely hydrophilic, i.e. OH functionality on the surface. This provides a clean platform for amine functionalisation
2. Amine functionalisation⁴ – reaction with 3-aminopropyltriethoxy silane leaves the quartz with a monolayer of aminopropyl groups on the surface.

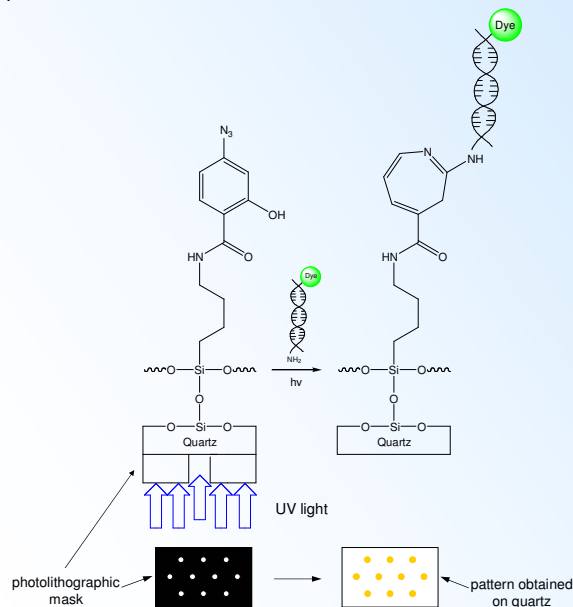


3. 4-Azido-2-hydroxybenzoic acid attachment – the amide forming reaction between the carboxylic acid of the phenyl azide and the amine on the quartz creates a photoactive surface.



Light Directed Immobilisation of DNA

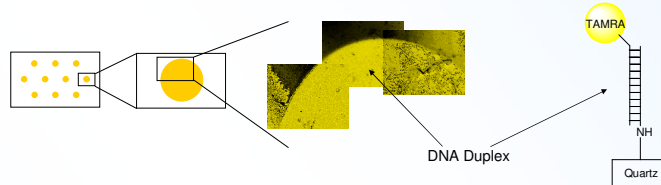
3' Amino modified single stranded DNA was immobilised on to the surface using a 300nm broad LED through a photolithographic mask with apertures of 1.8 μm.



Results

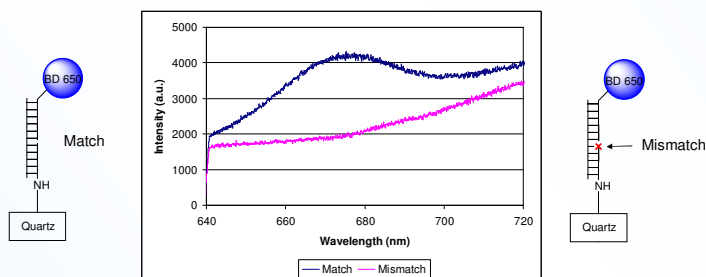
Fluorescence Microscopy

Hybridisation with 3' TAMRA modified complementary strand enables the visualisation of the duplex using fluorescence microscopy.



Fluorescence Spectroscopy

Using a sandwich hybridisation approach with 3' BD 650 modified complementary strand enables the discrimination against a single base mismatch.



References

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2. K. W. Gano, *Tetrahedron Lett.*, 2001, **42**, 2249 – 2251.
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4. S. L. Beaucage, *Curr. Med. Chem.*, 2001, **8**, 1213 – 1244.

Acknowledgements

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