**Towards designer DNA nanomaterials for biosensing**

C. J. Whitfield, R. C. Little, E. M. Tuite, and A. R. Pike\*

1Newcastle University, Newcastle upon Tyne, NE1 7RU, UK

\*E-mail : andrew.pike@ncl.ac.uk, Tel: +44-191-2087061

**Keywords:** DNA, artificial nucleobases, functional nanomaterials

 The enzymatic synthesis of long DNA with a controllable sequence, length and functional content will be presented. A modified PCR protocol using the key components of a repeating sequence oligo-seed (ca.20 bases), the deoxynucleotide triphosphates (dNTPs), and a DNA polymerase is reported. Using a thermostable *Thermococcus gorgonarius* Family B DNA polymerase exonuclease minus variant, Z3, and 20 heat-cool cycles, long DNA up to 20,000 base pairs bearing repeating units between 1 to 40 bases can be produced.

 Incorporation of artificial nucleotides, with modifications ranging from single atom exchanges, 5-I-dCTP, 7-deaza-I-dATP, 5-Br-dUTP and 6-S-dGTP, to long chains, 5-C8-alkyne-dCTP, was demonstrated. Modifications situated in the major groove have little effect on the DNA polymerase efficiency but reduced enzymatic processivity is observed if the modification lies in the hydrogen-bonding region. By tailoring the oligo-seed, it is possible to synthesise long designer DNA to include modifications at user-defined positions. The modified DNA product lengths are similar to the unmodified DNA products.

6-S-dGTP is renowned for strong metal interactions, and was exploited for the specific localisation of Au+, Ni2+, Cd2+ and Au3+ at repeating G positions. As the final 6-S-DNA product is limited in length, an alternative thiol modification was investigated. Using phosphorothioate dNTPs, sulfur bearing DNA products similar in length to the unmodified DNA were produced after 30 heat-cool cycles. This enabled the specific positioning of Au-nanoparticles through careful oligo-seed design. DNA bearing the 5-C8-alkyne-dCTP provides alkyne anchors at sites sitting in the major groove. To demonstrate the ability to add a second layer of design, click chemistry with azide-fluor-545 was investigated. This opens up potential routes to more complex modifications via organic synthesis at precise sites within the designer DNA.