ABSTRACT BOOK

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OLIGONUCLEOTIDES FOR SPLICE-SWITCHING AND STRAND-INVASION

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Synthetic nucleic acid chemistry has made possible the generation of oligonucleotides (ONs) with special features allowing potent effects in biological systems. We have recently developed highly efficient ONs for the correction of pseudo-exon inclusions secondary to mutations in the BTK gene causing recessive, X-linked agammaglobulinemia (XLA). In XLA there is a differentiation block in the B-lymphocyte lineage resulting the absence of B-lymphocytes and plasma cell in affected individuals. Bacterial artificial chromosome (BAC) transgenic mice carrying the mutated human BTK gene were generated as a new model for the study of splice-switching. Following algorithm-based identification of suitable target sequences efficient correction of both mRNA and protein was observed using locked nucleic acid (LNA) as well as morpholino-based ONs in both reporter cell assays and in primary cells from both patients and BAC-transgenic mice.

We have also developed methods for strand-invasion into duplex DNA. The original "Zorro-LNA" construct is formed by two ONs, each targeting one of the two strands in a DNA duplex, and tethered by a hybridizing linker sequence. We have also studied single-stranded Zorro-LNAs devoid of any hybridizing region, but instead equipped with a single linker of different chemistry. Both of these compounds strand-invade DNA as evidenced by S1 nuclease assays. We have recently investigated the effect of Zorro-LNA on the expression of Huntingtin (HTT) mRNA in transfected cells. The HTT gene is mutated in patients with the dominantly inherited, neurodegenerative disorder named Huntington's disease. By scanning the HTT gene, six different regions were identified and tested. Two of them showed robust down-regulation of HTT transcripts, when targeted by Zorro-LNAs. Reduced expression was also obtained in cell-lines from patients with Huntington's disease.

Keywords: *DNA; Oligonucleotides;*

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INK-JET PRINTING FOR DRUG SCREENING BY DROPLET MICROARRAYS

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Drug screening is the complex process of retrieving chemical compounds able to modulate the activity of biological targets which are of interest for a disease. Conventional miniaturized drug screening technologies require time and reagent consuming (micro-, nanoliter scale) instrumental tools, liquid handling robotics and complex detectors. Here we show a low-cost and efficient drug screening methodology based on inkjet printing for delivering molecular systems in picoliter volumes coupled with easily-implemented detection tools for probing target-drug interaction. We firstly show up a screening platform for a model enzyme/substrate couple and we extend this approach to systems of clear interest for medicinal chemistry.

The approach was initially proved with a model enzyme system like Glucose Oxidase substrate covalently linked to a functionalized silicon oxide support.[1] On this support an enzymatic substrate (D-glucose)/inhibitor (D-glucal) couple was dispensed. A simple colorimetric detection method based on the production of a red quinoneimine dye in a reaction catalyzed by Horseradish Peroxidase proved the screening capability of the microarray at the single spot. Occurrence of competitive inhibition was verified at the solid-liquid interface with a similar behavior occurring for such system in a solution phase.[2]

Afterwards, this methodology has been extended to other systems including CYP450 enzymes like CYP3A4, one of the main targets for the phase I drug metabolism via a droplet microreactors arrays containing CYP3A4 enzyme mixed with model inhibitors (i.e. ketoconazole and erythromycin) and enzymatic chemiluminescent substrates (Luciferin-Isopropylacetate). Enzymatic activity in picoliter liquid spots was detected by using a low cost optical method. Accordingly, bioluminescence given by D-luciferin leads to a production of photons that increase spot brightness which can be quantified by Charge-coupled device camera.

References:

1. G. Arrabito, C. Musumeci, V. Aiello, S. Libertino, G. Compagnini and B. Pignataro, Langmuir, **2009**, *25*, 6312-6318.
2. G. Arrabito and B. Pignataro, Analytical Chemistry, **2010**, *82*, 3104-3107.

Keywords: *drug screening; inkjet printing; enzymes; microarrays; biotechnology;*