Generation of Aptamer-Molecularly Imprinted Polymer Hybrid Materials

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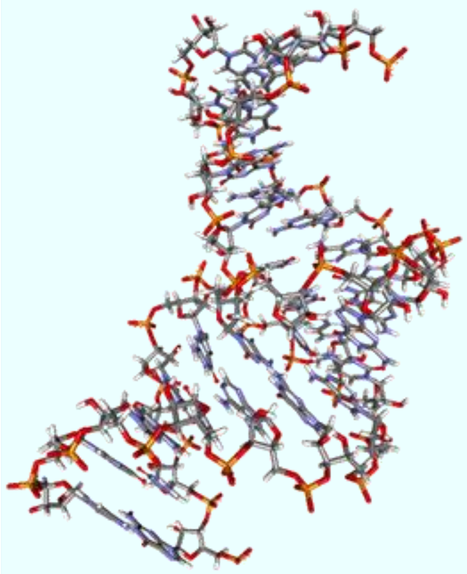
Summary

Aptamers are novel tools (made of nucleic acids) capable of binding with high affinity and selectivity to a variety of targets, ranging from small molecules to proteins. These characteristics make them attractive alternatives to antibodies for diagnostic/sensing purposes, even though they are quite susceptible to enzymatic and chemical degradation. By slightly changing the chemical structure of the aptamer DNA, aim of this work is to develop the first hybrid aptamer-imprinted polymer system, in which the aptamer acts as the recognition part of a molecularly imprinted polymer (MIP). MIPs involve the formation of a small binding pocket in a polymer which is chemically and shape specific for the target compound. These "smart materials" offer the robustness to work in extreme environmental conditions but usually cannot equal the affinity/specificity of their biomolecular counterparts. Such a hybrid system can potentially improve the selectivity of the aptamer but also protect the DNA strands from enzymatic and chemical degradation.

A bit of history: aptamers *VS* MIPs

Aptamers

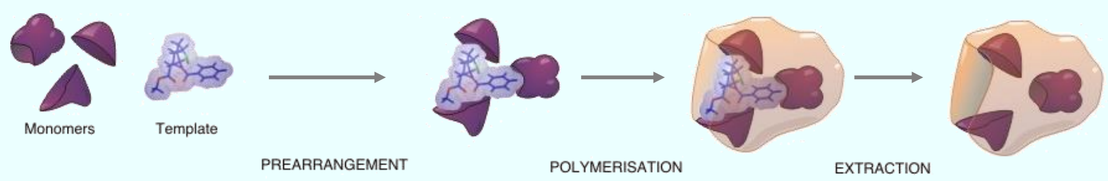


The term “aptamer” derives from the Latin word “*aptus*”, which means “fitting”, and the Greek word “*meros*”, which means “particle”. Aptamers are short (15–60 nucleotides) single-stranded nucleic acid (DNA or RNA) oligomers with a specific and complex three-dimensional shape, which allows them to recognize a variety of targets ranging from small organic molecules to large protein complexes.[1-3] Aptamers are synthesised entirely *in vitro* through the generation of combinatorial libraries and the subsequent stringent selection process with the immobilised target. The selected sequences are amplified by polymerase chain reaction (PCR) and used in several selection/amplification cycles with increasingly stringent selection conditions in a process called SELEX (systemic evolution of ligands by exponential enrichment).[1-3]

Advantages	Disadvantages
Similar affinity and selectivity to natural biomolecules	SELEX processes are quite lengthy and labor intensive (unsuccessful automation)
More stable than antibodies	Much lower stability than MIPs (susceptible to enzymatic and chemical degradation)
Can be used in high density arrays	Need a suitable solid support to modify the format
Relatively cheap	More expensive than MIPs
Easy post-modification (e.g., fluorescent labelling)	Commercialisation hindered by exclusive ownership of IP by a small number of companies

MIPs

First observed in 1931 by Polyakov, molecular imprinting as we known it today was re-discovered by Wulff and Sarhan in 1972, and by Arshady and Mosbach in 1981.[4] The general theory is simple. The target molecule (template) interacts with complementary functional monomers, forming a complex. This is then trapped within a polymeric matrix. The template is removed leaving a specific binding cavity within the polymer.

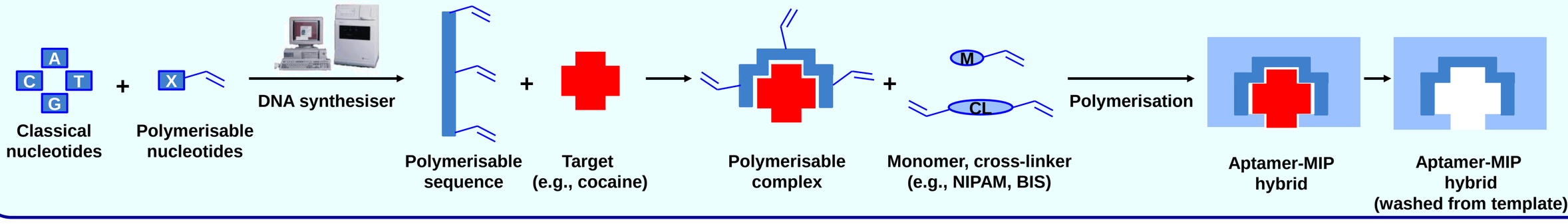


The simplicity and generic nature of molecular imprinting has made it viable alternative to biological recognition elements. Today the technique has blossomed into an established and commercially viable method of producing artificial recognition materials. Traditionally limited to small molecules (<1500 Da) imprinting of macromolecules (proteins, carbohydrates, DNA) is on the increase as the full potential of the technique is realised.[5]

Advantages	Disadvantages
Good affinity and selectivity compared with antibodies	Aptamers generally exhibit better recognition properties
Stable in extreme environments (low/high pH, pressure and temperature, organics)	Generally poorer performance in aqueous and gas phase
High versatility and availability of formats	Production can be lengthy and labor intensive depending on the format (e.g., bulk monoliths)
Relatively easy post-derivatisation (e.g., fluorescent labelling)	Careful preservation of binding sites needed during post-modification
Inexpensive	Cost of template can be prohibitive unless analogues or solid-phase synthesis are used

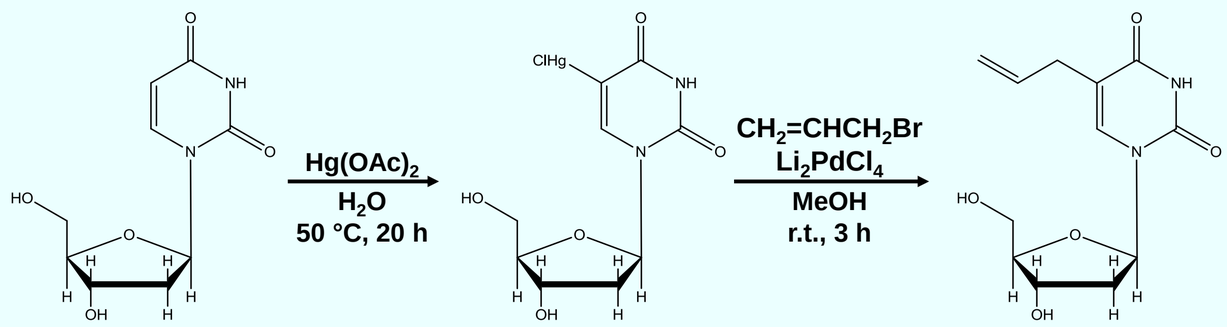
The idea

Using DNA synthesiser, monomeric bases (A, C, G, T) and suitable modified bases (X) are assembled to create a modified aptamer. This aptamer is then introduced to its target molecule forming a stoichiometric complex. The complex is then polymerised with acrylic monomers and cross-linkers (M, CL), with the modified bases on the aptamer allowing the strand to be incorporated directly via covalent bonds into the polymer network. The target molecule (template) is then removed leaving a cavity “imprint” behind that bears a single aptamer within it as the recognition element. As the aptamer is entrapped within the polymer it should retain its conformation offering correct steric positioning for rebinding of the target. The 1:1 ratio between the aptamer and template should ensure the formation of only high-affinity binding sites.



Where are we?

Currently we have attempted at least three different strategies in order to introduce an polymerisable moiety in position 5 of a 2’-deoxyuridine nucleoside, followed by the production of an active phosphoramidite suitable for being used in automatic DNA synthesis. Among the approaches tested, the one here represented has been the most successful, in which 2’-deoxyuridine **(a)** has been transformed first into 5-HgCl-2’-deoxyuridine **(b)**[6] and then derivatised with allyl bromide to give 5-allyl-2’-deoxyuridine **(c)**[7,8] using Pd catalysed chemistry in good yield (product structures have been confirmed by TLC and ^{1}H-NMR).



Challenge accepted: towards the first hybrid aptamer-MIP!

Summarising, we have successfully synthesised suitable nucleosides to prepare polymerisable DNA and aptamer sequences which can be covalently incorporated into a polymeric matrix. Future work will involve the development of polymerisation and imprinting strategies to produce the very first hybrid aptamer-MIP system. The astonishing potential of these materials is clear, considering that they will bring the best of two worlds into a single technology, offering both the robustness and chemical stability to work in extreme environmental conditions and the affinity/specificity of the biological systems. With these new developments, we foresee a time when the application of natural antibodies in diagnostics and therapeutics will be facing strong competition by the appearance of new sensor devices, assays and even drugs and drug delivery systems based upon stable, more “green” and inexpensive aptamer-MIP hybrids.

References

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