**Expanding the molecular computing tool-kit: iteration, smart biosensing, small molecule detection, and in vivo therapeutics**

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**Introduction**

Biocompatible information processing systems are critical for advancing biological devices, such as wearable or implantable medical equipment. One approach towards wireless information processing is the use of oligonucleotide strands that silicomimetically perform logical operations (Macdonald, 2006; Poje, 2014; Harding, 2018). However, several key functions are still critically required for bio-integration of silicomimetic logic gates, including reusability, integration with native biological components, and in vivo processing. Here we report development of these new functionalities using deoxyribozyme-based logic gates and/or aptamer-based logical control.

**Methods**

An iterative deoxyribozyme-based logic gate system was developed by addition of complementary and quencher-labelled oligonucleotides to remove previously activated circuits. Integration with native biological components was demonstrated via logic circuits containing novel mixed-based logic gates and ANDNOT functionality for discrimination between *lyssavirus* families (Vijayakumar, 2017), as well as incorporating an RNA aptamer complement into NOT gates for detection of the small molecule theophylline. Finally, logical control in-vivo was achieved through aptamer integration into RNA-interference (RNA-i) mediated gene silencing, to dynamically control expression of an enzyme (Cytochrome P450 1A2) in mammalian cell culture (293 cells).

**Results**

We demonstrated, for the first time, the ability for deoxyribozyme-based logic gates to have their activity toggled, returning to 90-125% of original activity over multiple reset-reuse cycles. Detection of natural biological components was demonstrated in two ways: firstly, by correct discrimination of the large diversity of sequences in the seven *Lyssavirus* genus families (Vijayakumar, 2017), displaying results in a bio-powered dot-matrix text output; and secondly, by detection of the small molecule theophylline, with 6 to 16-fold allosteric responsive detection. Finally, in vivo logical control of an enzyme therapeutic was demonstrated in cell cultures by 2.9 – 3.3 fold activation of a Cytochrome P450 enzyme after supply of our toxic small molecule theophylline.

**Conclusion**

The ability to reset, detect native biological nucleic acid sequences, and interact with small molecule signaling events, provides significant steps forward towards the bio-integration of logical control in vivo. Future work is considering not just in vivo integration in cell culture, but how to effectively embed systems and provide communication in more complex three-dimensional tissue structures.

**References**

Harding & Macdonald (2018) *In* Knopf (ed.) *Smart Biosensor Technology*, 2nd Ed., Boca Raton: Taylor & Francis, 2018; Macdonald & Stefanovic *et al.* (2006) *Nano Letters* 6:2598-2603; Poje, Stefanovic & Macdonald *et al.* (2014) *Angewandte Chemie* 53:9222-9225; Vijayakumar & Macdonald (2017) *ChemPhysChem* 18:1735-1741.

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