



Project no. **222422**

Project acronym: **ECCell**

Project title: Electronic Chemical Cell

Instrument: STREP/FET OPEN

Thematic Priority: Theme 3 Information and Communication Technologies

**Deliverable n. 3.4: other, restricted (RE)**

**Autonomous activation of isothermal ligation/replication DNzyme machinery using strand displacement or pH as triggering signals.**

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Organisation name of lead contractor for this deliverable:

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# 1. Deliverable 3.4: Autonomous activation of isothermal ligation/replication DNAzyme machinery using strand displacement or pH as triggering signals.

## 1.1 Introduction

Deliverable 3.4, with the original title “**Modified electrodes that reversibly switch the pH of a solution by redox reactions**” was already achieved at M12 of the project, as a significant step beyond D3.1, and published as previously reported[1]. Since one of the applications of this capability is to replication, additional activity at HUJI was invested in developing isothermal amplification systems capable of utilizing electronic and molecular triggers. One of these is reported here, since it takes a significant step beyond the state of the art in this area.

The ligation and replication of nucleic acids is a challenging scientific goal, since the ligated product exhibits always a stronger binding affinity to the ligation template than the individual fragments. As a result, the separation of the ligated product is inhibited. Enzymatic systems overcome such inhibition with a variety of activated mechanisms including helicase functionality, but the majority of these require the complex conformational changes that large specifically evolved proteins can orchestrate. *In vitro* isothermal enzymatic replication is known in systems like Q $\beta$  replicase as well as the 3SR, NASBA and SDA reactions[2]. These systems employ some of the chemical energy associated with polymerization reaction to displace the previous product strand. Non enzymatic isothermal amplification schemes have been described, based on purely conformational transitions rather than ligation[3]. Furthermore, as exploited in WP1, isothermal amplification is possible using triplex structures for moderately long sequences and pH cycling. While heating might provide a route to separate the ligated product, and thermal cycles could lead to ligation/replication reactions (like in PCR), nonenzymatic isothermal ligation/replication processes for longer sequences without chemical cycling in some form are hard to find.

In this deliverable, as an alternative to the general disulphide and triple helix scheme being developed elsewhere in the project, we push through the development of a novel isothermal replication scheme that is halfway between the purely conformational and the purely ligation based extremes. Constructive replication is deemed more difficult and fundamental than destructive or non-constructive replication.

## 1.2 Isothermal ligation/replication DNAzyme machinery

This problem was resolved by the fundamental concept shown in Figure 3.3. The hairpin structure (**8**) includes in domains (IV), (V) and (I) the Zn<sup>2+</sup>-dependent ligation sequence, and in domains (II) and (III) of the single stranded loop, the recognition sequence for the activator (**11**). The two subunits, the imidazole-modified nucleic acid (**9**), and (**10**), act as the substrate for ligation. In the presence of the activator (**11**), hairpin (**8**) opens, resulting in the hybridization of the subunits (**9**) and (**10**) to the DNAzyme and their ligation. Since the ligated product is complementary to the loop region of (**8**), strand-displacement of the ligated product is energetically favored, leading to the generation of additional ligation DNAzyme units. The major accomplishments of this autonomous biocatalytic ligation cycle are: (i) The ligated replication product acted as reporter unit of the activator. It included a copy of the activator sequence. (ii) The strand-displacement principle provided a mechanism for separation of the ligated product and the regeneration of the ligation DNAzyme. (iii) The ligated product provided an input for the further formation of the ligation DNAzyme units. (iv) The entire system is an isothermal replication system. (v) The paradigm was extended by the pH-induced separation of the ligated product. (vi) The replication of the reporter units

represents a route to enhance the sequence of the activator, and thus, provides a means to amplify the detection of a gene (activator sequence). By the appropriate labeling of the hairpin (8) with a fluorophore/quencher pair, the autonomous ligation and opening of hairpin (8) was followed by the fluorescence of the fluorophore units. Figure 3(B) depicts the time-dependent fluorescence changes of the system upon activating the autonomous ligation process by different concentrations of the activator.

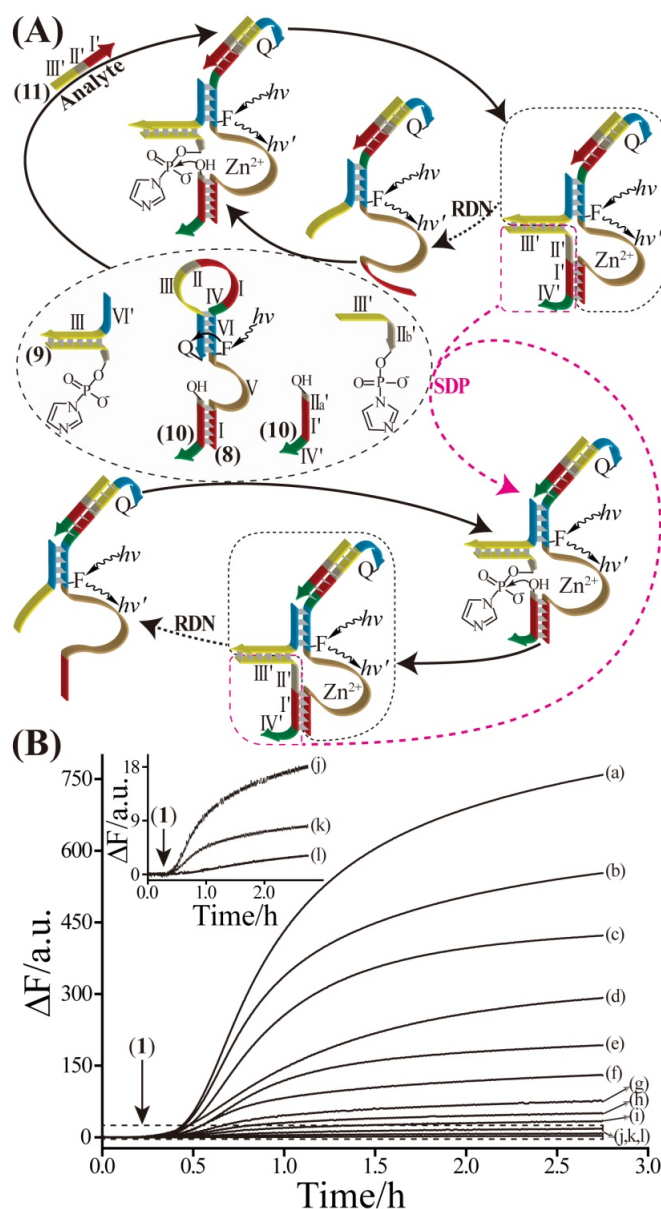


Figure 3.3: (A) Schematic autonomous replication of an analyte reporter unit by the  $Zn^{2+}$ -dependent ligation DNAzyme upon sensing the analyte and the autonomous synthesis of the DNAzyme nanostructures. Throughout the paper, domains X and X' in the respective analyte and DNAzyme or substrates subunits represent complementary base pair regions. (B) Time-dependent fluorescence changes upon analyzing different concentrations of the analyte (11) according to Figure 3(A): (a)  $4.0 \times 10^{-7}$  M, (b)  $2.0 \times 10^{-7}$  M, (c)  $1.0 \times 10^{-7}$  M, (d)  $4.0 \times 10^{-8}$  M, (e)  $2.0 \times 10^{-8}$  M, (f)  $1.0 \times 10^{-8}$  M, (g)  $4.0 \times 10^{-9}$  M, (h)  $2.0 \times 10^{-9}$  M, (i)  $1.0 \times 10^{-9}$  M, (j)  $1.0 \times 10^{-10}$  M, (k)  $1.0 \times 10^{-11}$  M, (l) 0 M. Arrow indicates the time of addition of the analyte. Inset: Enlargement of curves (j)–(l).

#### Publications:

1. F. Wang, J. Elbaz, C. Teller and I. Willner, Amplified Detection of DNA through the Autocatalytic, Catabolic Generation of the Analyte by a DNAzyme, *Angew. Chem. Int. Ed.*, **50**, 295-299 (2011).

2. F. Wang, J. Elbaz and I. Willner, Enzyme-Free Amplified Detection of DNA by an Autonomous Ligation and Replication DNAzyme Machinery, submitted for publication.

### References

- [1] M. Frasconi, R. Tel-Vered, J. Elbaz, and I. Willner, "Electrochemically stimulated pH changes: a route to control chemical reactivity.," *J Am Chem Soc*, vol. 132, no. 6, pp. 2029–2036, Feb. 2010.
- [2] R. Ehricht, T. Ellinger, and J. McCaskill, "Cooperative amplification of templates by cross-hybridization (CATCH)," *Eur J Biochem*, vol. 243, no. 1, pp. 358–64., 1997.
- [3] D. Zhang, A. Turberfield, B. Yurke, and E. Winfree, "Engineering Entropy-Driven Reactions and Networks Catalyzed by DNA," *Science*, 2007.