

Abstract Preparation Guidelines for the Eurosenors 2026

Yukina Partington¹, Beatrice Dalla Via¹, Fabrice Abgottspon¹, Isaure de Geyer¹, Piet Cools², Andrew J. deMello¹, Daniel A. Richards^{*1}

¹ ETH Zürich, DCHAB, Vladimir-Prelog-Weg 1, 8093, Zürich, Switzerland

² Ghent University, Faculty of Medicine, Corneel Heymanslaan 10, 9000, Ghent, Belgium
daniel.richards@chem.ethz.ch

Summary:

Base Gap switches are programmable transcriptional regulators activated DNA, which can be used to sense disease. In this talk, I will detail how these switches are easy to install, exert strong control over expression, and are highly orthogonal. I will show that Base Gap switches can be combined with Recombinase Polymerase Amplification to achieve single-copy detection of targets and be leveraged to differentiate single-nucleotide mismatches. Finally, I will discuss how we will apply Base Gap switches to detect drug-resistant infections. This talk will discuss the results of our recently published work.[1,2]

Keywords: Synthetic gene circuits, IVTT, multiplexing, transcriptional regulation, SNVs.

Title

Base Gap Switches: Exploiting the Central Dogma of Molecular Biology to Detect Disease

Background, Motivation and Objective

Proteins are ideal signalling molecules. Their chemical diversity enables a broad range of readily detectable biochemical processes, including fluorescence, luminescence, reduction, oxidation, hydrolysis, ligation, and more. As such, linking protein expression to the presence of disease markers presents a powerful opportunity for sensing. Such methods rely on manipulating the replication–transcription–translation pathway (the “central dogma” of molecular biology). This is achieved using regulatory components, commonly termed “switches”, that control various mechanisms essential for protein expression.[3] Unfortunately, contemporary switches are hampered by poor sensitivity and specificity, largely due to expression in the absence of the target, a phenomenon we call “leakage”. The objective of this work was to develop less leaky methods for manipulating protein expression in response to disease targets.

Description of the New Method or System

The most common tools for controlling protein expression in response to disease targets are toehold switches.[4] These rely on target RNA binding inducing a conformational change in an mRNA, which in turn enables *translation* of this mRNA into a functional protein. These leak because of conformational changes that occur even in the absence of the target mRNA. Thus,

we reasoned that removing the reliance on conformational changes would reduce leakage. To do this, we developed a DNA-based switch that controls *transcription*. In these switches, we introduce a gap into the template strand of a gene encoding a signalling protein. This gap prevents elongation by RNA Polymerase, halting transcription and thus protein expression. This gap is engineered to be specific to a DNA target, which can “fill” the gap and allow transcription to resume (**Fig. 1A_i**). Since this gene-silencing method does not rely on conformational changes, but rather total physical disruption of transcription, we hypothesised that it would reduce leakage. Moreover, since these switches are built from DNA, rather than RNA, we reasoned that they would be easier to work with and more robust. We termed these new transcriptional switches “Base Gap” switches.

Results

As a proof-of-concept, we installed a Base Gap switch onto a gene encoding the fluorescent protein mNeonGreen (**Fig. 1A_{ii}**). We observed target-dependent expression as a function of single- and double-stranded DNA targets (switch ON) (**Fig. 1B**). In this proof-of-concept experiment, we observed no leakage (Switch OFF). We also performed cross-validation of seven orthogonally designed Base Gap switches, demonstrating excellent orthogonality (**Fig. 2A**). We exploited this orthogonality by installing three orthogonal switches onto genes encoding for blue, orange, and red fluorescent proteins, and demonstrated one-pot multiplexing of three tar-

gets (**Fig. 2B**). Next, we combined Base Gap switches with Recombinase Polymerase Amplification (RPA) to achieve single-copy-number sensitivity for *Trichomonas vaginalis* (**Fig. 3A**). Finally, we showed that Base Gap switches could be used to detect Single Nucleotide Variants (SNVs) within circulating tumour DNA (ctDNA) associated with Epidermal Growth Factor Receptor (EGFR) mutations (**Fig. 3B**).

Illustrations, Graphs, and Photographs

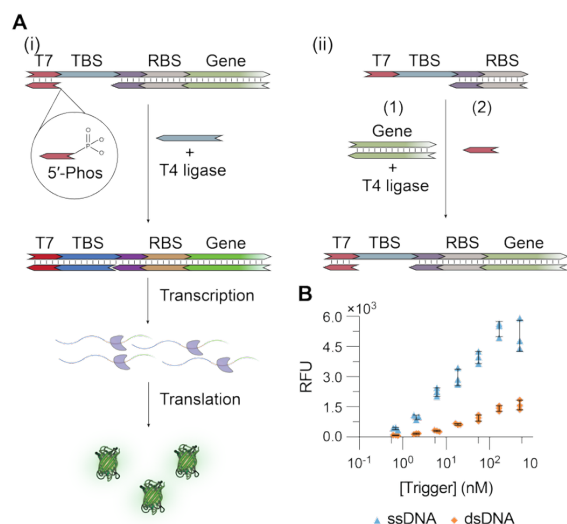


Fig. 1. (A) (i) A diagrammatic representation of the mechanism of Base Gap switches. (ii) The ligation-based installation of Base Gap switches onto genes. (B) Expression of mNeonGreen under the control of a Base Gap switch in response to single-stranded (ssDNA) and double-stranded (dsDNA) DNA. Figures are adapted from our published work [1,2].

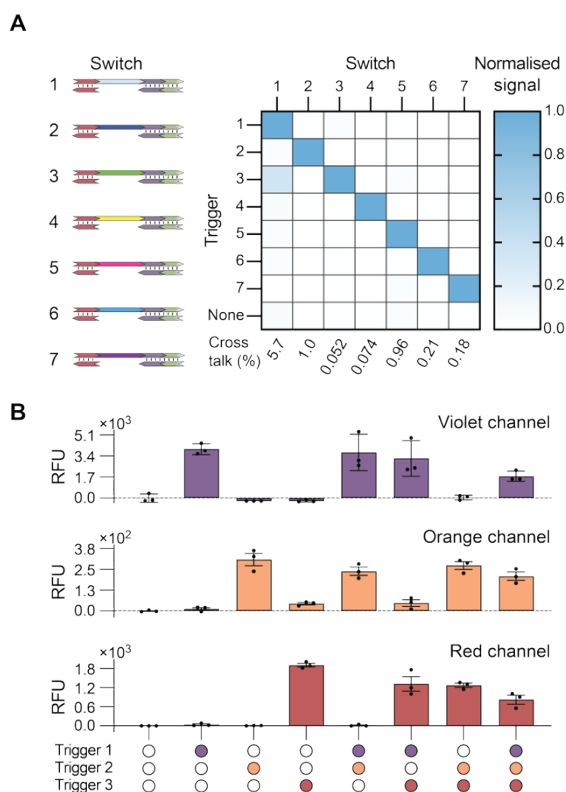


Fig. 2. (A) Cross-validation of seven targets against seven Base Gap switches to demonstrate orthogonality. (B) One-pot multiplexing of three targets using three Base Gap switches controlling the expression of blue, orange, and red fluorescent proteins. Figures are adapted from our published work [1,2].

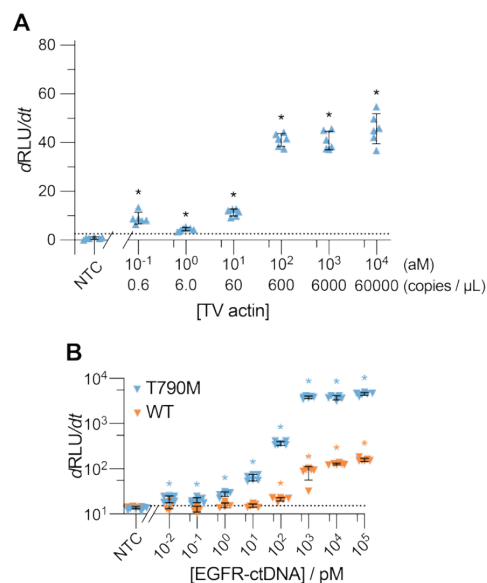


Fig. 3. (A) Detection of the actin gene of *Trichomonas vaginalis* (TV) using Base Gap switches, combined with RPA. (B) Differentiation of the T790M and wild type (WT) EGFR gene in ctDNA. Figures are adapted from our published work [1,2].

References

- [1] Y. Partington, B. Dalla-Via, F. Abgottspon, I. de Geyer, P. Cools, A. J. deMello, D. A. Richards, Base Gap Switches as Transcriptional Regulators and Their Applications as Biosensors, *Nature Sensors*, just accepted. (2026)
- [2] Y. Partington, B. Dalla-Via, F. Abgottspon, I. de Geyer, P. Cools, A. J. deMello, D. A. Richards, Base Gap Switches as Transcriptional Regulators and Their Applications as Biosensors, *ChemRxiv*. (2026) <https://doi.org/10.26434/chemrxiv.15001046/v1>
- [3] F. Sedlmayer, D. Aubel, M. Fussenegger, Synthetic Gene Circuits for the Detection, Elimination and Prevention of Disease. *Nat. Biomed. Eng.* 2, 399–415. (2018) <https://doi.org/10.1038/s41551-018-0215-0>.
- [4] A. A. Green, P. A. Silver, J. J. Collins, P. Yin, Toehold Switches: De-Novo-Designed Regulators of Gene Expression. *Cell*, 159, 925–939. (2014) <https://doi.org/10.1016/j.cell.2014.10.002>.