315 Conditional Guide RNAs: Logical control of CRISPR gene editing system

Asset Overview

Product Type	Gene therapy
Indication	Various diseases
Current Stage	Hit to lead optimization
Target(MoA)	CRISPR/Cas9 constructs with the use of this modified guide-RNAs (gRNA) containing the blocking module
Brief Description	CRISPR is described as "the technology of the century" and brings the hope of treatments for currently incurable diseases, by excising or silencing genes that drive tumor proliferation, repair of faulty genes, or by killing diseased cells. However, unwanted DNA cuts, resulting from nonspecific delivery to off-target cells, remain a serious problem in the successful clinical translation of CRISPR based therapeutics. Exact spatio-temporal control of CRISPR/Cas9 activity would lead to the development of new therapies and new tools for biotechnology with great translational potential.
Organization	University of Oxford

Differentiation

□ **RNA-guided CRISPR effectors**

- A guide RNA (gRNA) directs the function of a CRISPR protein effector to a target gene of choice, providing a versatile programmable platform for engineering diverse modes of synthetic regulation (edit, silence, induce, bind)
- However, The fact that gRNAs are constitutively active places limitations on the ability to confine gRNA activity to a desired location and time

□ **Engineer conditional guide RNAs (cgRNAs)**

- The scRNA paradigm of programmable conditional regulation based on dynamic RNA nanotechnology
- cgRNAs that change conformation in response to an RNA trigger X to conditionally direct the function of dCas9 to a target gene Y

□ **Main applications of conditional regulation**

- Use in biomedical applications for cell-type-specific activation of the CRISPR/Cas9 system
- Tool for synthetic biology: genetic circuits or nucleic-acid nanotechnology
- Production of other conditionally activated CRISPR/ Cas9 systems based on the use of other activation molecules, such as proteins or small molecules

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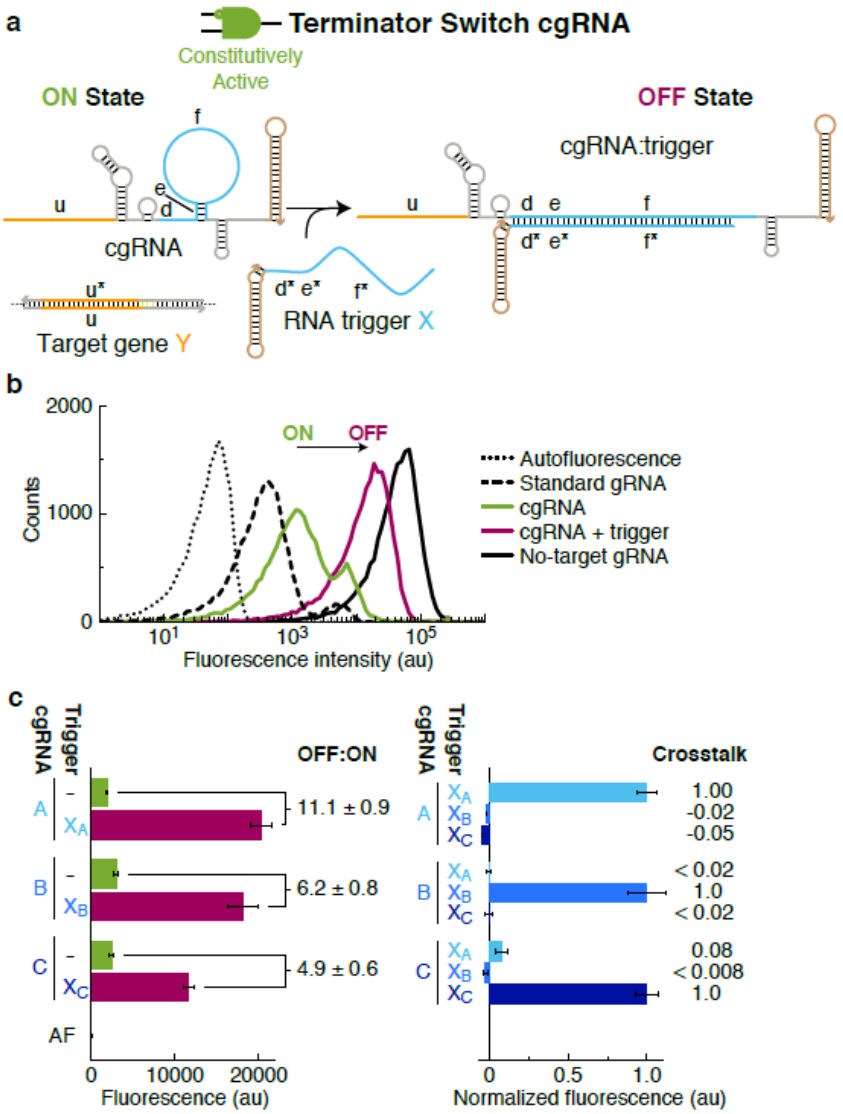
Key Data

Conditional gene silencing using cgRNA (ON > OFF logic)



(a) Mechanism schematic. The constitutively active cgRNA is inactivated by hybridization of RNA trigger X. (b) Expression of RNA trigger X (40 nt + synthetic terminator) toggles the cgRNA from ON!OFF, leading to an increase in fluorescence. Single-cell fuorescence intensities via flow cytometry. (c) Programmable conditional regulation using 3 orthogonal cgRNAs (A, B, C). Left: ON>OFF conditional response to cognate trigger (OFF:ON ratio = [OFF-AF]/[ON-AF]). Right: crosstalk between non-cognate cgRNA/trigger pairs ([non-cognate trigger - no trigger]/[cognate trigger - no trigger]).

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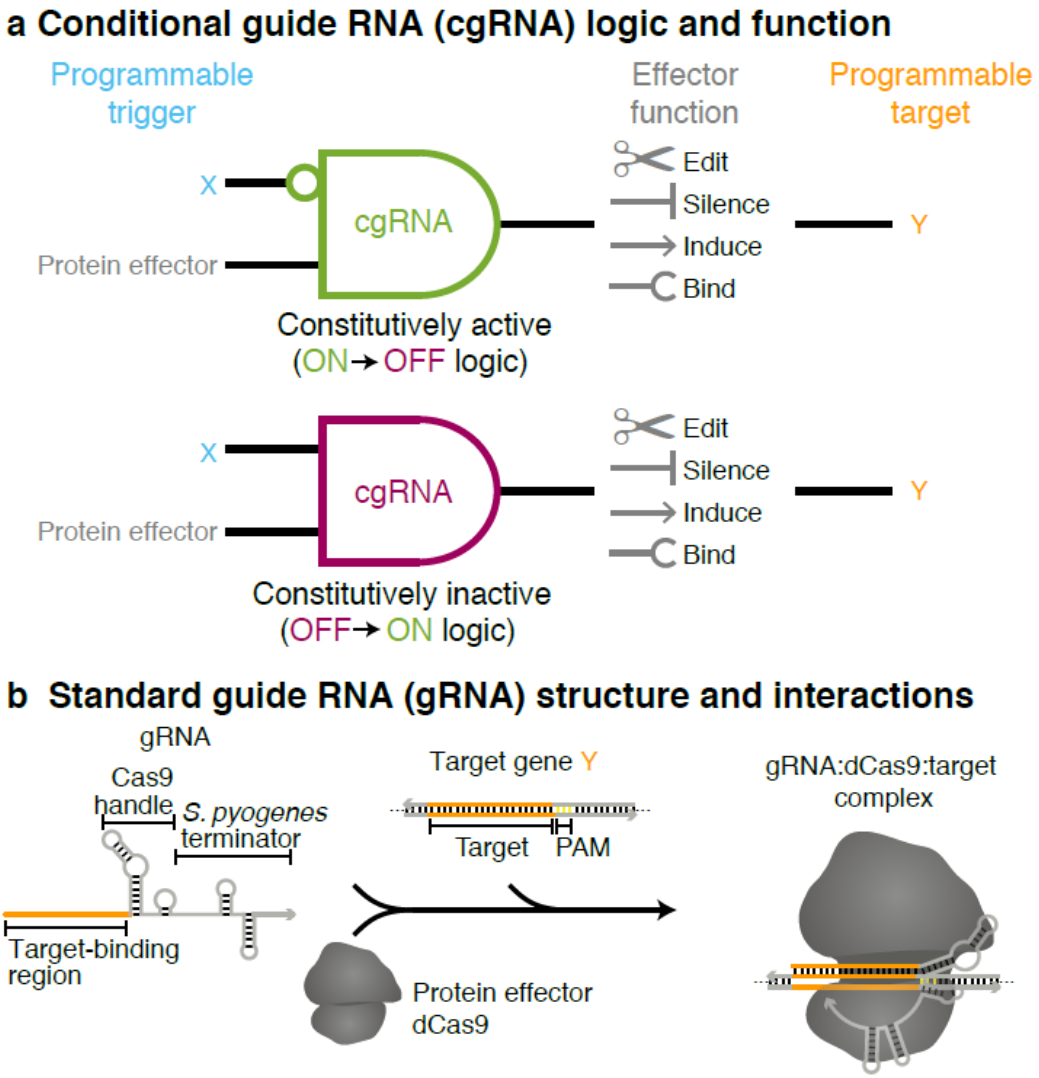
Programmable regulators



(a) A conditional guide RNA (cgRNA) changes conformation in response to a programmable trigger X to conditionally direct the activity of a protein effector to a programmable target Y. Top: a constitutively active cgRNA is conditionally inactivated by X (ON>OFF logic). Bottom: a constitutively inactive cgRNA is conditionally activated by X (OFF>ON logic). (b) A standard guide RNA (gRNA) is constitutively active, directing the function of protein effector dCas9 to a target gene Y; different dCas9 variants implement different functions (edit, silence, induce, bind).

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Intellectual Property

Patent No.	
Application Date	
Status	
Country	

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