OXFORD UNIVERSITY INNOVATION

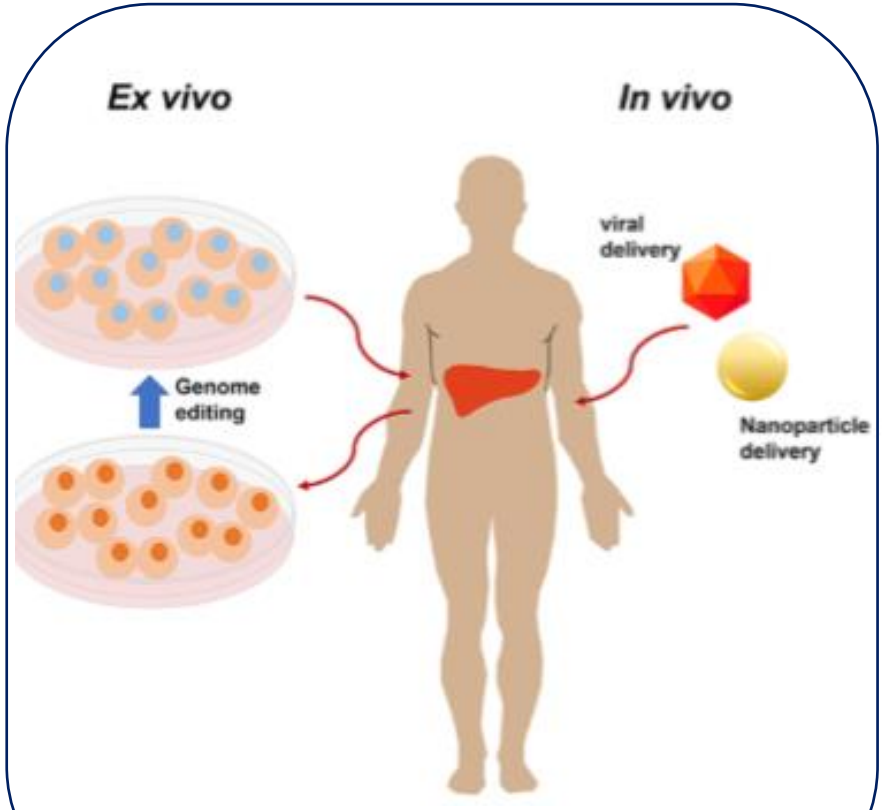
Conditional Control of CrispR Technologies

OUI Project 13916

OUI Project 14361

CrispR in Healthcare

Disruptive Technology with Many Technological Challenges



Hirakawa et al., 2020, Bioscience Report

Delivery: how to reach the right site of action and how to reduce dangerous off-target organ accumulation.

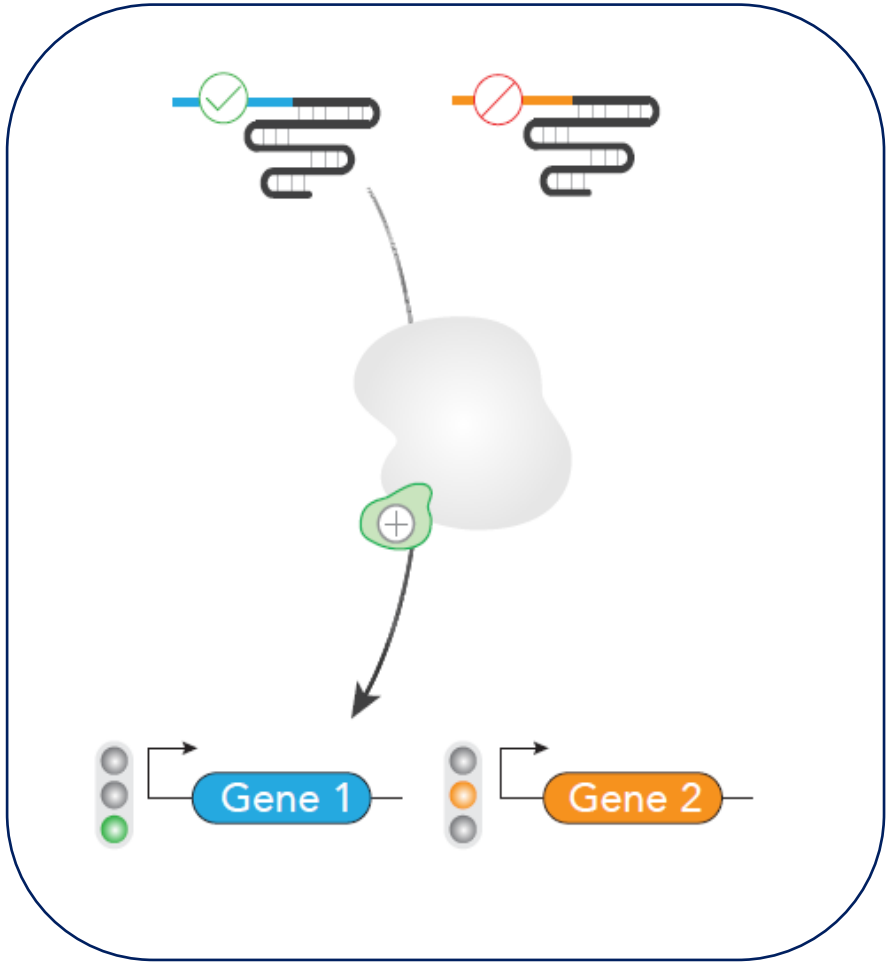
Precision: how to prevent off-target editing activity.

Undesirable effects: associated with constitutive expression.

Limited controlled activity: exogenous methods (e.g. light-activation), tissue-specific promoters.

Programmable molecular switches for CrispR Systems

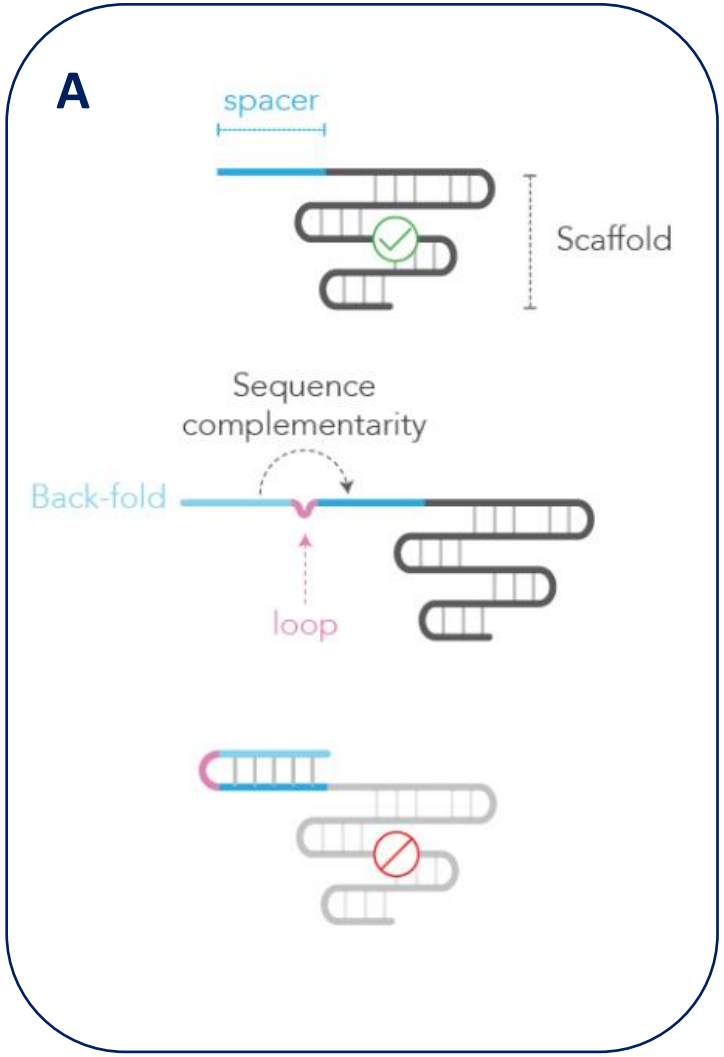
Engineering Inducible sgRNA strategies

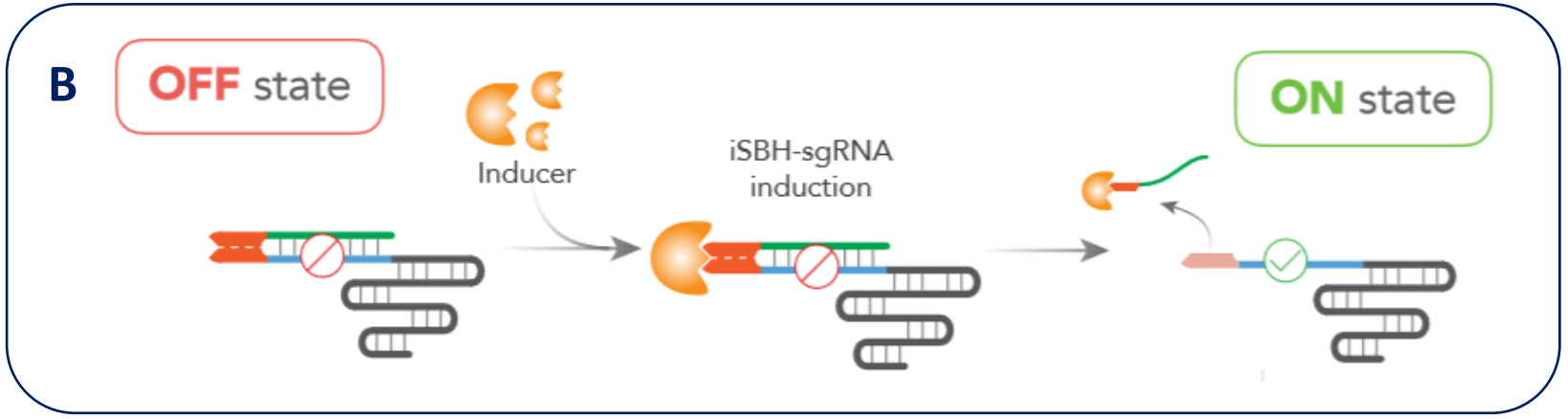


- ✓ **Delivery**: sgRNA detect endogenous molecules specific to their chosen site of action.
- ✓ **Precision**: switches are engineered sgRNA and can be used with different RNA-directed nucleases.
- ✓ **Safety**: switchable behaviour reduces the probability for undesirable activity.
- ✓ **High level of control**: versatile repertoire of activation signals (small molecules, proteins, ASOs, miRNAs).

OUI Project 13916: Spacer Blocking Hairpins

Technology Status





- Proof of concept demonstrated:

  - ✓ Validated *in vitro* and in cellular models
  - ✓ Several inducers: ASOs and proteins
  - ✓ Branching and orthogonal gene modules

Ferry et al., 2017, Nature Comm.

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Patent status

- PCT application published as WO2018/130830
- Regional/national applications pending in Europe and the US
- Claim 1 :

An **inducible CRISPR RNA** comprising:

(i) a spacer-blocking element;

(ii) a cleavable loop element; and

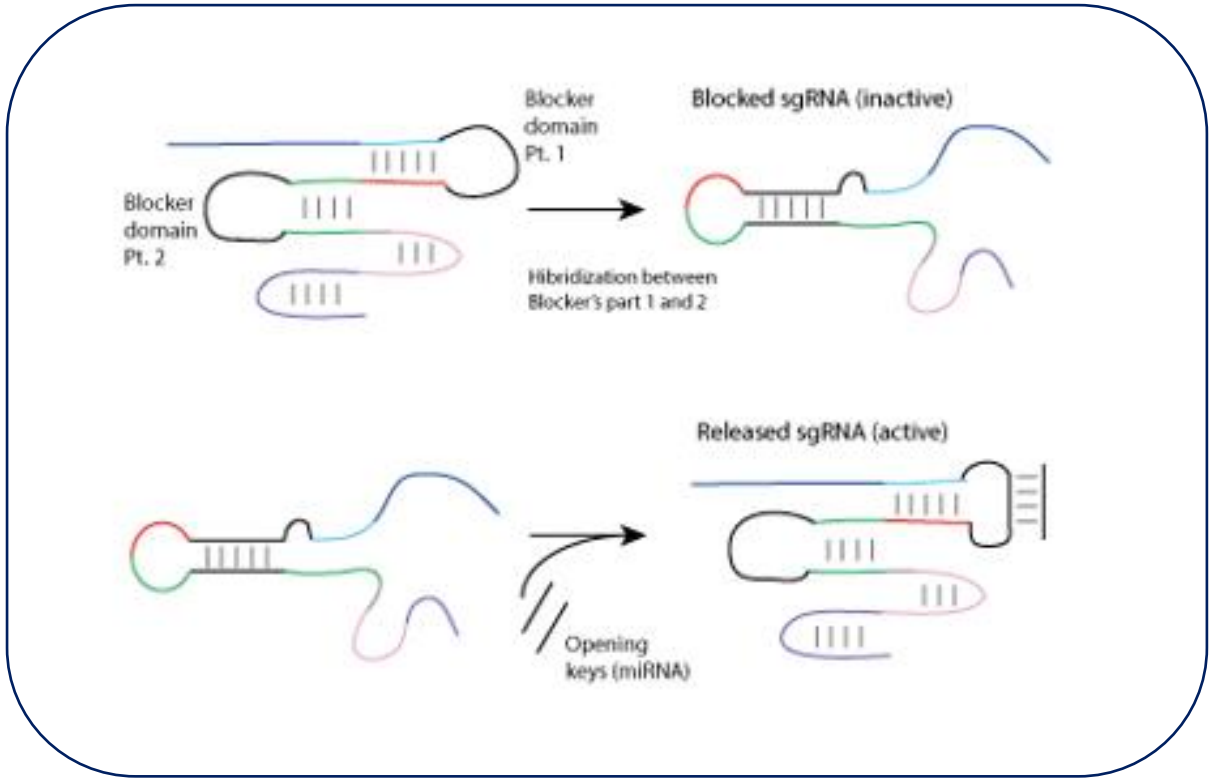
(iii) a CRISPR sgRNA comprising a spacer element;

wherein (i)-(iii) are arranged 5' - 3' in the above order in the inducible CRISPR RNA, wherein the spacer-blocking element has a nucleotide sequence which is at least partially complementary to that of the spacer element, and wherein the spacer-blocking element, cleavable loop element and spacer element are capable of forming a stem- loop structure.

- Fully owned by, and all rights assigned to, OUI

OUI Project 14361: Strand Displacement

Technology Status



- Proof of concept demonstrated:
  - ✓ Validated in cellular models
  - ✓ Several microRNA-specific RNA guide strands developed
  - ✓ Tested with Cas9 and Cpf1

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Patent status

- PCT application published as WO 2020/044039
- Claim 1 :

An **inducible sgRNA** comprising a crRNA or a crRNA linked to a tracrRNA (crRNA- tracrRNA) and a Blocker Domain, wherein the nucleotide sequences of one or more regions of the Blocker Domain are at least partially complementary to the nucleotide sequences of one or more regions of the crRNA or crRNA-tracrRNA or to an additional domain or domains inserted into the crRNA or crRNA-tracrRNA such that the Blocker Domain is capable of hybridizing to those one or more regions of the crRNA or crRNA-tracrRNA or additional domain or domains inserted into the crRNA or crRNA-tracrRNA, and wherein: (i) the position of the Blocker Domain in the inducible sgRNA, and (ii) the location(s) of the one or more regions of the crRNA or crRNA-tracRNA or domains inserted into the crRNA or crRNA-tracrRNA are selected such that the sgRNA is capable of adopting at least the following two conformations:

(A) a first conformation wherein the Blocker Domain is substantially hybridized to the one or more regions of the crRNA or crRNA-tracrRNA or domains inserted into the crRNA or crRNA-tracrRNA, and

(B) a second conformation wherein the Blocker Domain is substantially not hybridized to the one or more regions of the crRNA or crRNA-tracrRNA or domains inserted into the crRNA or crRNA-tracrRNA,

wherein in conformation (A), the sgRNA is incapable of binding to its cognate CRISPR enzyme or otherwise incapable of activating the nucleic-acid-binding or nuclease functionalities of the enzyme; and in conformation (B), the sgRNA is capable of binding to its cognate CRISPR enzyme and of activating its nucleic-acid-binding or nucleic-acid-binding and nuclease functionalities,

wherein the change in conformation from (A) to (B) is inducible by the binding of one or more Opening Keys to the Blocker Domain, and wherein the Opening Key is: (a) a non-coding RNA, or (b) a nucleic acid-protein complex.

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Further development and partnering

- Available for commercial partnering, e.g. via:
  - Option or licence
  - Translational collaboration or co-development (OUI Project 14361)
  - Spin-out formation (OUI Project 14361)

Thank you. Any questions?

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