Bio LOGIC

Logic Orthogonal gRNA-Implemented Circuits

ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

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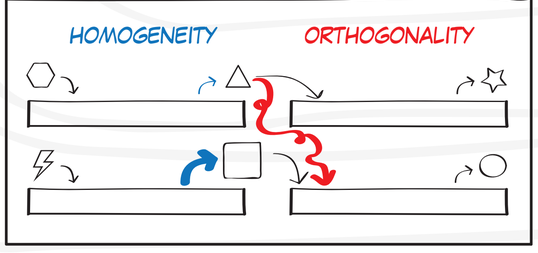
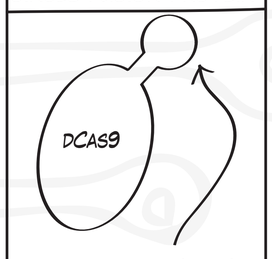
BACKGROUND

BUILDING COMPLEX BIOLOGICAL CIRCUITS IS DIFFICULT BECAUSE EACH STEP REQUIRES A DIFFERENT TRANSCRIPTION FACTOR (TF) THAT SHOULD MEET THE FOLLOWING REQUIREMENTS {1} :

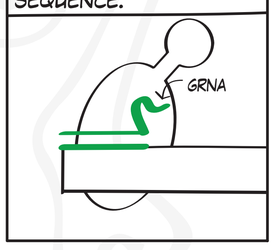


BioLOGIC IS BASED ON A SINGLE FUSION PROTEIN

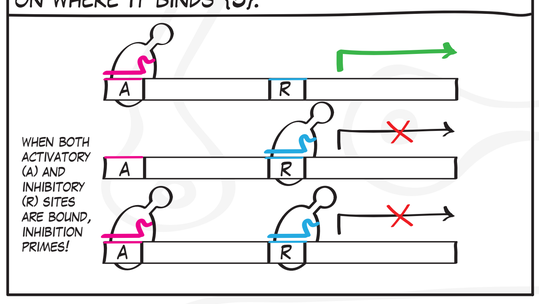


RNA POLYMERASE (RNAP) RECRUITING ELEMENT {2}

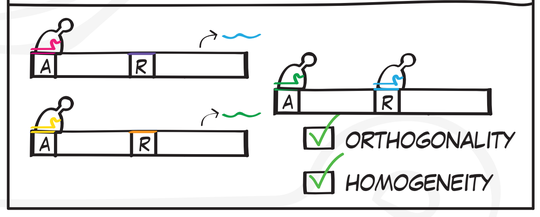
DCAS9 BINDS A GUIDE-RNA (GRNA) THAT WILL GUIDE IT TOWARDS A COMPLEMENTARY DNA SEQUENCE.



DCAS9 CAN ACT ON PROMOTERS BOTH AS AN ACTIVATOR OR A STERIC INHIBITOR, DEPENDING ON WHERE IT BINDS {3}.



THE Bio LOGIC TRANSISTOR CAN BE SEEN AS A PROMOTER SEQUENCE THAT PROCESSES THE PRESENCE OF CERTAIN GRNAS AND THAT CONSEQUENTLY PRODUCES (OR NOT) ANOTHER GRNA. BY CHAINING THESE TRANSISTORS ONE COULD IMAGINE BUILDING MORE COMPLEX CIRCUITS.



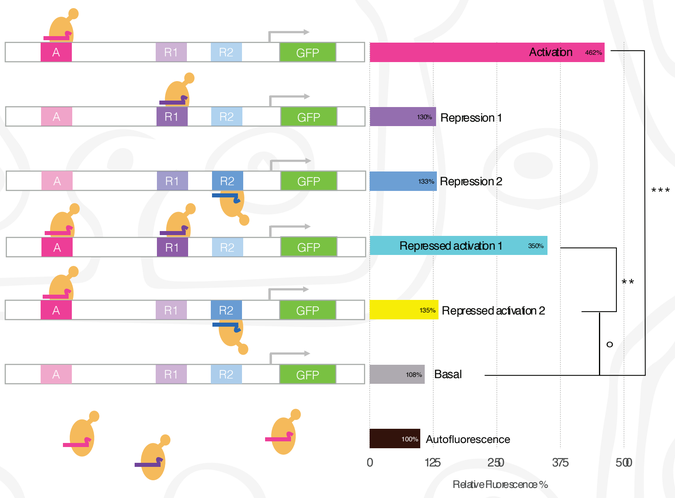
OUR TEAM TESTED THE FOLLOWING PROPERTIES OF THE BioLOGIC TRANSISTOR: RESPONSE, MULTIPLICABILITY, CHAINABILITY A ND ORTHOGONALITY. ASSEMBLY OF BioLOGIC TRANSISTORS INTO A NAND GATE W AS M ODELED.

RESULTS

BBa_K1723001 TRANSISTOR RESPONSE

We showed in E. coli that fusion dCas9 targeted to site 'A' **activates** expression and turns the transistor '**on**'. Targeting 'R1' and 'R2' sites preserves basal expression: the transistor is still '**off**'. **Simultaneous** targeting of 'A' and 'R2' sites sets the transistor to '**off**' as well.

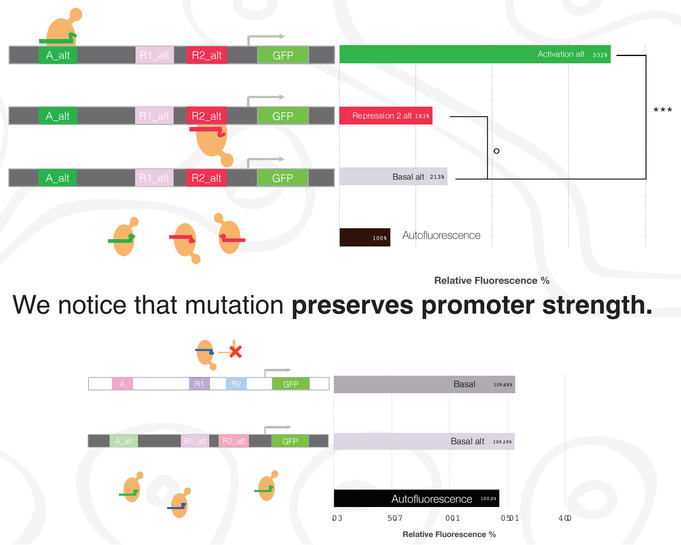
Overall, this promoter mimics an **electronic PNP transistor** (for 'A' the emitter, 'R2' the base and 'GFP' the collector).



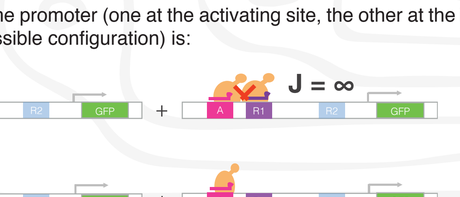
MULTIPLICABILITY

Building on the transistor response experiment, we **mutated** BBa_K1723001 transistor regulatory sites to make the BBa_K1723005 transistor.

We observed that regulation patterns depend mainly on the **location** - not the sequence - of the targeted site : the fluorescence response of the new transistor is similar to that of the original one.



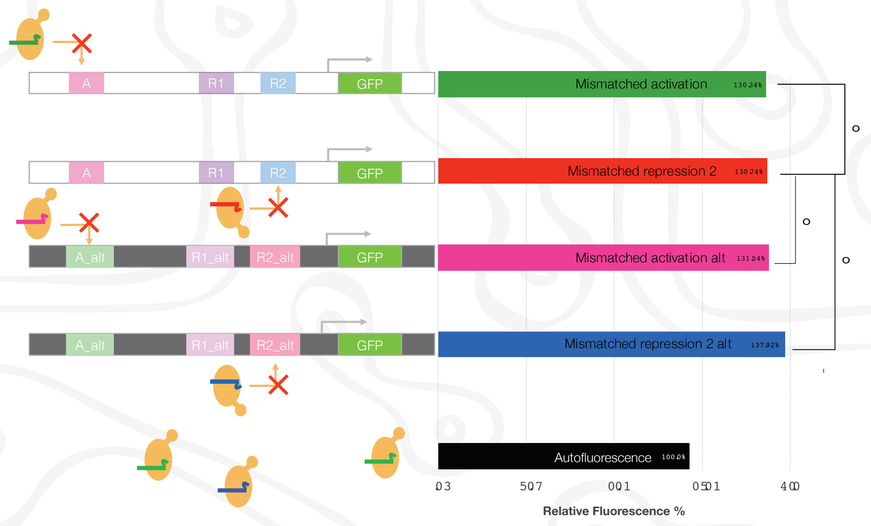
We notice that mutation **preserves promoter strength.**



ORTHOGONALITY

Due to the binding properties of dCas9, promoters should **not** be **activated nor inhibited** by gRNAs that are **not complementary** to their regulation sites.

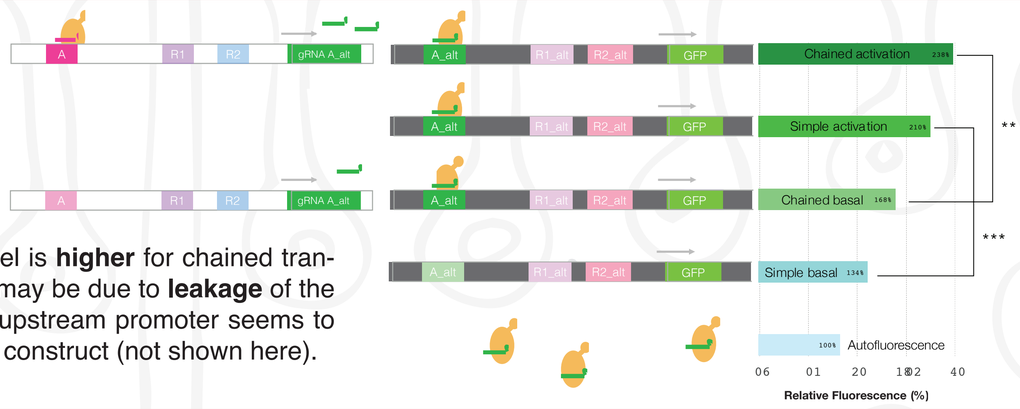
We tested this in the lab and obtained fluorescence **levels close to autofluorescence** that are not significantly different from each other. This suggests that transistors can be regulated specifically.



CHAINABILITY

We determined it is possible to chain transistors: promoter **activation** was **successfully transmitted** from one transistor to another.

We noticed that **basal** fluorescence level is **higher** for chained transistors than for a single transistor. This may be due to **leakage** of the upstream promoter as inhibition of the upstream promoter seems to reduce basal expression of the chained construct (not shown here).



METHODOLOGY

- Promoter strength of the transistor according to gRNA input was assessed by fluorometry. The transistors were set up on a reporter plasmid to produce GFPmut2 in JEN202 *E. coli* expressing dCas9-ω and different gRNAs (results in S. cerevisiae were produced but are omitted here).
- Fluorescence was measured for 8 to 10 hours with a microplate reader in relative fluorescence units (RFU) and normalised by optical density at 600nm (OD600). Measurements are given at steady state.
  Autofluorescence is the measure of JEN202 + dCas9-ω without reporter plasmid, whereas basal has the reporter plasmid but lacks dCas9-ω.
- For each construct, we measured three biological replicates for which the median of three technical replicates was used.
  The error bars represent the standard deviation of the biological replicates.
- o, *, **, *** represent p values >= 10%, < 10%, < 5%, < 1% respectively for unilateral Welch's t-tests on the population means.

MODELING

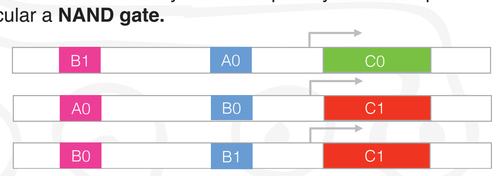
We used **statistical mechanics** in order to compute the binding probability of two dCas9 on the same promoter (one at the activating site, the other at the inhibiting site). We used a lattice model and the **partition function** (the sum of unnormalized probabilities for each possible configuration) is:

$$Z = 1 + K_a[A] + K_a[R] + K_a^2[A][R]e^{-\beta J}$$

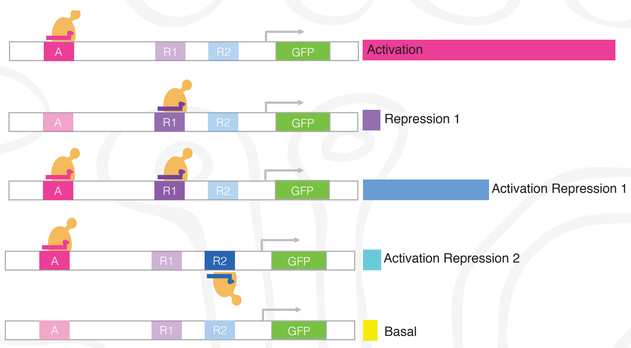
Z = 

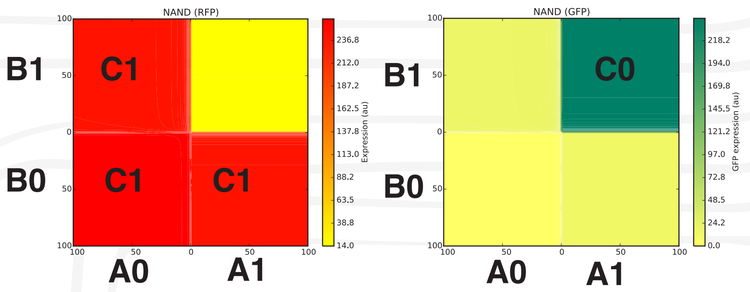
J is the **interaction energy** between two dCas9. In the limiting case J=0, we have independent binding. For J=∞ the binding is exclusive (only one dCas9 can bind to the promoter). Binding probabilities (derived from Z) allowed us to compute the response of the transistor in function of dCas9 concentrations. At steady state we were able to **reproduce all wet lab results** solely by using experimental activation to basal and inhibition to basal ratios!

We also used our model to study more complex systems composed of multiple transistors and in particular a **NAND gate.**



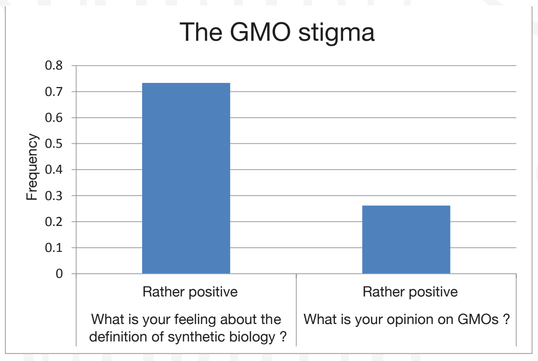
Using the model of independent binding (J=0), we were able to **reproduce the truth table of this gate in silico!** Our model is deterministic and is a simplification of a real biological system. This is nevertheless an encouraging result calling for supplementary experimental effort to build a NAND gate in vivo.





HUMAN PRACTICES

Through a public survey in the streets of Lausanne, we observed **enthusiasm** for the term «synthetic biology» but a clear **hostility** to the acronym «GMO», which led us to investigate the matters of communication and interaction between the general public and researchers.



We addressed this with **eleven experts** from various fields: ethics, biology, law, politics, journalism, religion and industry. We discussed their points of view in an **article** accessible on our wiki.

This led us to inviting fifty high school students to our campus, in order to communicate and interact. In addition to showing them around our **lab**, we confronted them with the **brainstorming of an iGEM project** and the **ethical questions** linked with gene regulation and modification technologies through role-playing.

References:
[1]Alec AK Nielsen & Christopher A Voigt (2014). Multi-input CRISPR/Cas circuits that interface host regulatory network. Molecular systems biology, 10(11), 763.
[2]Bikard, D., Jiang, W., Samai, P., Hochschild, A., Zhang, F., & Marraffini, L. A. (2013). Programmable repression and activation of bacterial gene expression using an engineered CRISPR-Cas system. Nucleic acids research, 41(15), 7429-7437.
[3]Farzadfard, F., Perli, S. D., Lu, T. K. (2013). Tunable and Multifunctional Eukaryotic Transcription Factors Based on CRISPR Cas. ACS Synth. Biol., 2 (10), pp 604–613.

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