# De‘feet’ing Barriers to Binding: An Analysis of Toeholds in LOCKR

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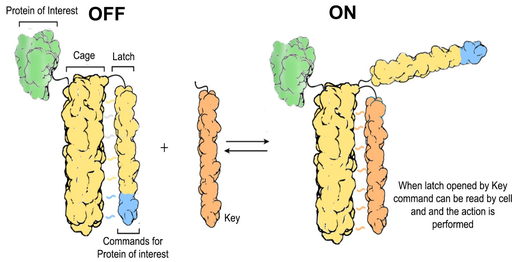
## INTRODUCTION:

LOCKR (Latching Orthogonal Cage-Key pRoteins) is a protein switch that includes a Cage and a Latch. The Latch contains a **bioactive, response-eliciting sequence**. This sequence is in the ”OFF” position until it is turned “ON” by opening the Latch segment using a protein **Key** (1).




The length of the Latch can vary in what we call toeholds. The larger the toehold, the shorter the Latch is relative to the Cage. Different Latch lengths can change the efficiency of activating LOCKR into the “ON” state (1).


## GOAL:

To investigate the impact of Latch toeholds on LOCKR activity and identify the toehold length which achieves the optimal dynamic range of “OFF” and “ON” states in LOCKR.

## HYPOTHESIS:

We hypothesize larger Latch toeholds, up to an unknown threshold, decrease Cage and Latch interactions and thus show higher binding affinity with the Key.

## CONCLUSIONS + FUTURE WORK:

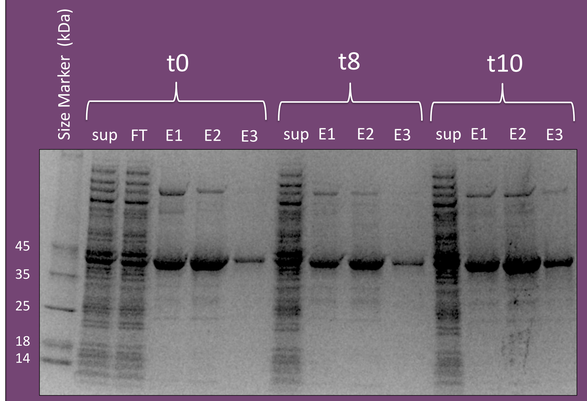
Preliminary data shows a strong influence of Key concentration below 2-fold excess of Key; it also suggests that a 16-residue toehold is too large for selective LOCKR function. Further assays will investigate the impact of a broader selection of toehold variants on Key-LOCKR binding within the 0 to 2-fold Key excess range.

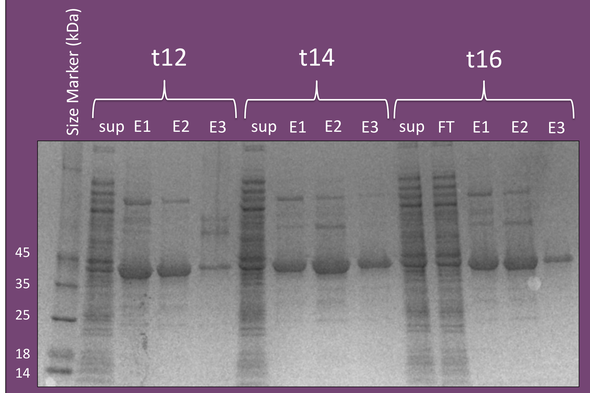
## REFERENCES:

1) Langan, R.A., Boyken, S.E., Ng, A.H. et al. De novo design of bioactive protein switches. Nature 572, 205–210 (2019).
2) Kim, Y., Ho, S. O., Gassman, N. R., Korlann, Y., Landorf, E. V., Collart, F. R., & Weiss, S. (2008). Efficient site-specific labeling of proteins via cysteines. Bioconjugate Chemistry, 19(3), 786–791. https://doi.org/10.1021/bc7002499

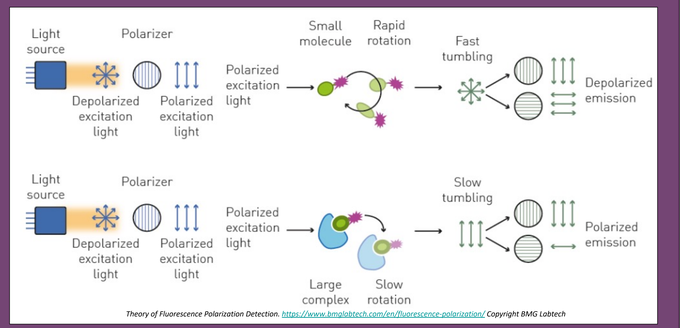
# Establishing a **fluorescence polarization** method to **optimize** Key and Switch **binding.**

## Purification of LOCKR Toehold Variants



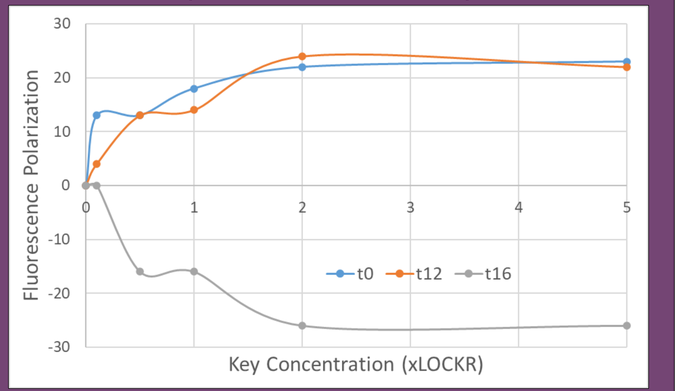




## Fluorescence Polarization Overview



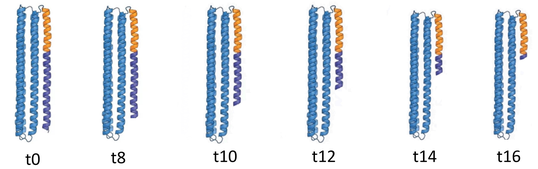

*Theory of Fluorescence Polarization Detection. https://www.bmglabtech.com/en/fluorescence-polarization/ Copyright BMG Labtech*

## Fluorescence Polarization of LOCKR Toehold Variants (t0, t12, and t16)

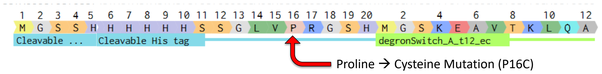



## METHODS:

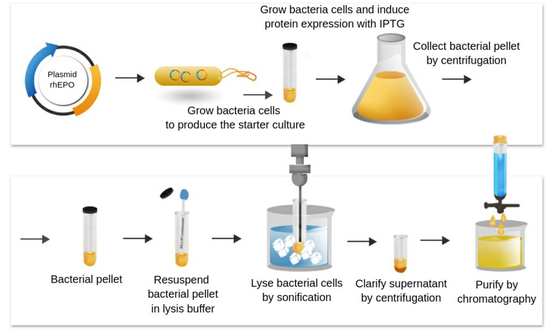
1. Obtain samples of LOCKR protein variants with different toeholds.




2. Use molecular genetics to insert a dye tagging site into LOCKR toehold variants: digest LOCKR DNA with restriction enzymes and use Gibson Assembly to insert a cysteine mutation.

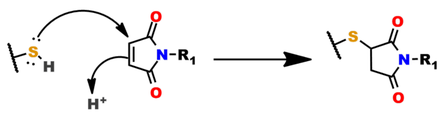



3. Express mutated LOCKR toehold variants in bacteria and purify using metal affinity chromatography.




*Bacteria Expression Systems. https://theory.labster.com/bacteria-expression-systems/ Copyright Labster*

4. Use purified cysteine-containing LOCKR proteins to tag different toehold Switches with a bright fluorescent dye molecule (2).




*Maleimide Reaction Chemistry. https://www.quantabiodesign.com/maleimide-reaction-chemistry/ Copyright Quanta Biodesign Ltd.*

5. Combine dye-tagged LOCKR with different concentrations of Key. Measure the rate of protein tumbling to characterize Key-LOCKR binding (see Fluorescence polarization of LOCKR toehold variants).

## ACKNOWLEDGEMENTS:

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