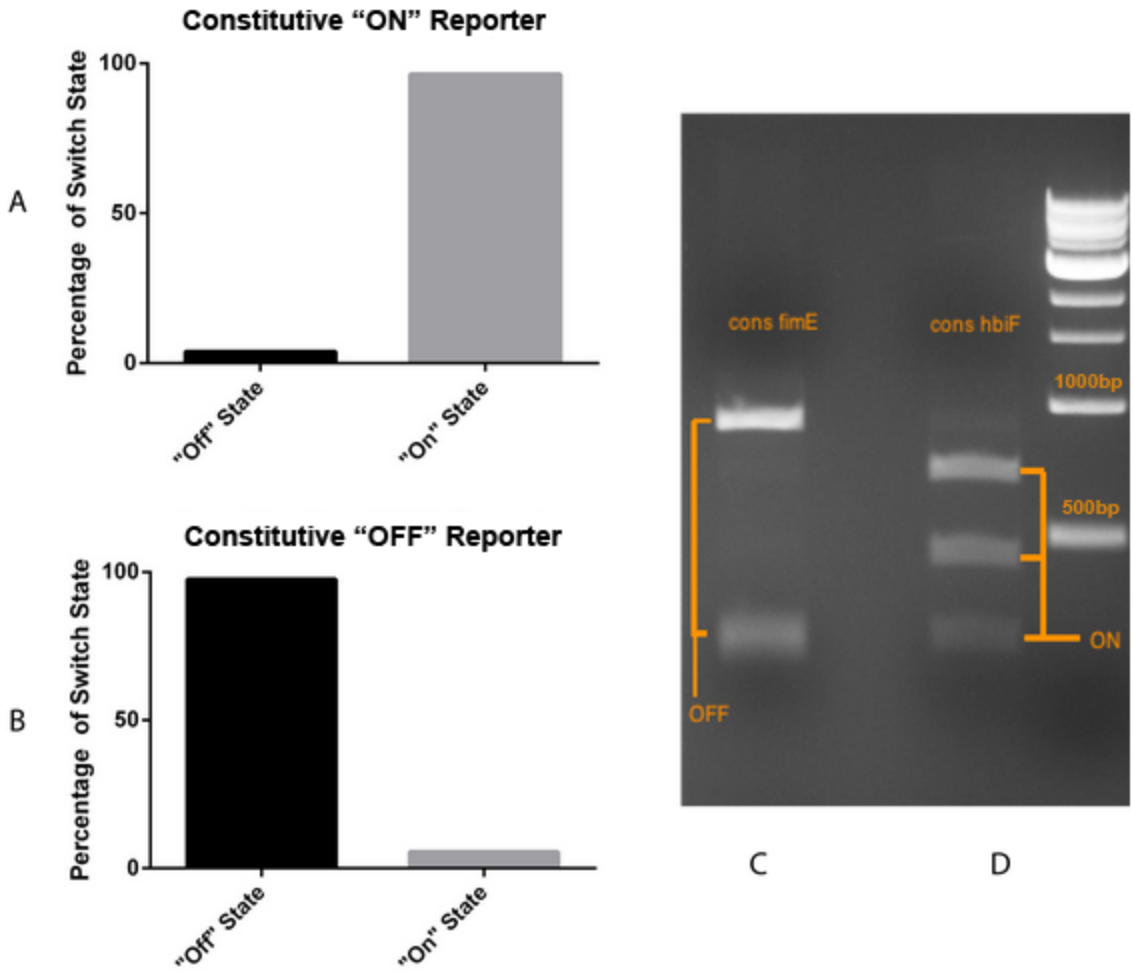

Fig. 1 - **The fim transcriptor is capable of changing states completely and unidirectionally**

NEB 10-beta E. coli, which lack the native fim switch (fimS) and known fim recombinases, were co-transformed with either constitutive hbiF or fimE and BBa_K1077007 (J23100 fim switch, ON orientation), plated on LB plates and grown overnight for ~16 hours. The state of the switch was assayed by using an asymmetric digest assay on PCR amplified switch. There are two hincII sites located within the K1077007 switch, one of which changes position depending on the state of the switch. The result is that when the switch is in the ON position, a 870bp, 273bp, and 248bp band is produced. When the switch is in the OFF position, a 680bp, 473bp, and 273bp band is produced. **A** and **B**)The digest assay was quantified using densitometry and showed greater than 95% of the switch in the desired state (ON when co transformed with constitutive hbiF and OFF when co transformed with constitutive fimE). This is consistent with hbiF's previously observed functionality of catalyzing the inversion of fimS from the OFF to ON orientation and fime's previously observed functionality of catalyzing the inversion of fimS from the ON to OFF orientation. **C** and **D)** A gel of the digest assay fragments quantified in A and B.

The faint ~870bp band in 1D**,** seemingly indicating residual OFF state, may correspond to the constitutive recombinase generator which is 858bp. We did not have time to cure the

bacteria of the constitutive recombinase generator plasmid. Additionally, the switch is on the high copy pSB1C3 plasmid and so it could be that some switch plasmids are escaping recombination. We did not have time to move the switch to a low copy plasmid or the chromosome.

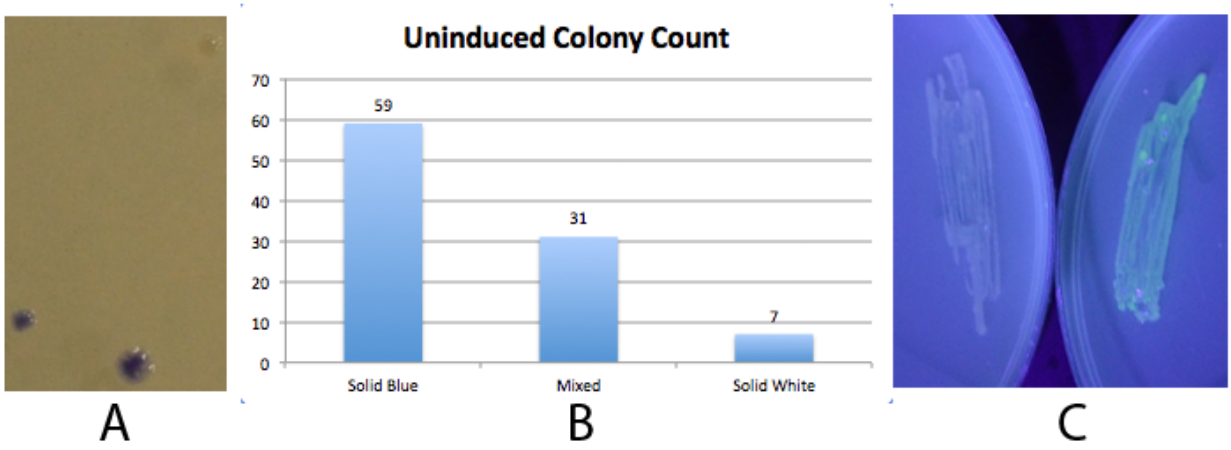

Fig 2. - **An inducible fim transcriptor system changes states and produces protein output**

NEB 10-beta E. coli, which lack the native fim switch (fimS) and known fim recombinases, were co-transformed with K1077007(amilCP j23100 fim switch) or K1077003(GFP j23100 fim switch), and K1077002 (aTc inducible fimE, HSL inducible hbiF) and plated on to LB plates with or without inducer. **A)** Close up of 3 colonies on a plate containing K1077007 and K1077002 co transformants. Three distinct phenotypes were observed: Solid Blue colonies(bottom left), Mixed colonies that had distinct white and blue regions(bottom middle), and Solid White colonies(top right). Therefore, the uninduced fim transcriptor in the context of K1077002 is subject to leaky fimE and/or hbiF activity. **B)** Quantification of the phenotypes observed on the plate in A. **C)** When induced with 4.32μM aTc and grown overnight (left) , no GFP is produced as expected due to induced fimE turning the switch OFF. When induced with 1μM HSL and grown overnight (right), GFP is produced as expected due to induced hbiF turning the switch ON.

Given the absence of GFP in the aTc induced switch shown in 2C, leaky hbiF expression is overcome by the induced fimE expression. Nothing can be said about induced hbiF overcoming leaky fimE since the green may still indicate only some of the plasmids are flipped. Whether or not the phenotypes of the colonies observed in 2A are due to variable leakage rates of each recombinase or variable enzymatic activity of each of the recombinases, or both, can not be determined. Since these are co transformations, the white and mixed colonies observed could simply be due to chance production of fimE only or first in the first generation, flipping the whole population of switch plasmids in the cell (~1). Subsequent generations might inherit the switch mostly in the initial state.

We did generate only inducible hbiF and fimE plasmids, but did not have time to co

transform them with the switch or submit them to the registry. These would allow us to test the effects of leakage of each recombinase on the switch. Other assays, such as time course data of recombinase activity under the same inducible system, would be needed to determine relative rates of recombinase activity. Moving the inducible recombinase generator to a low copy plasmid, may reduce leakage effects. Ideally, a more tightly regulated inducible system should be coupled to the recombinases. Promoter and/or rbs optimization should be performed to minimize recombinase leakage above the threshold needed for significant flipping activity.

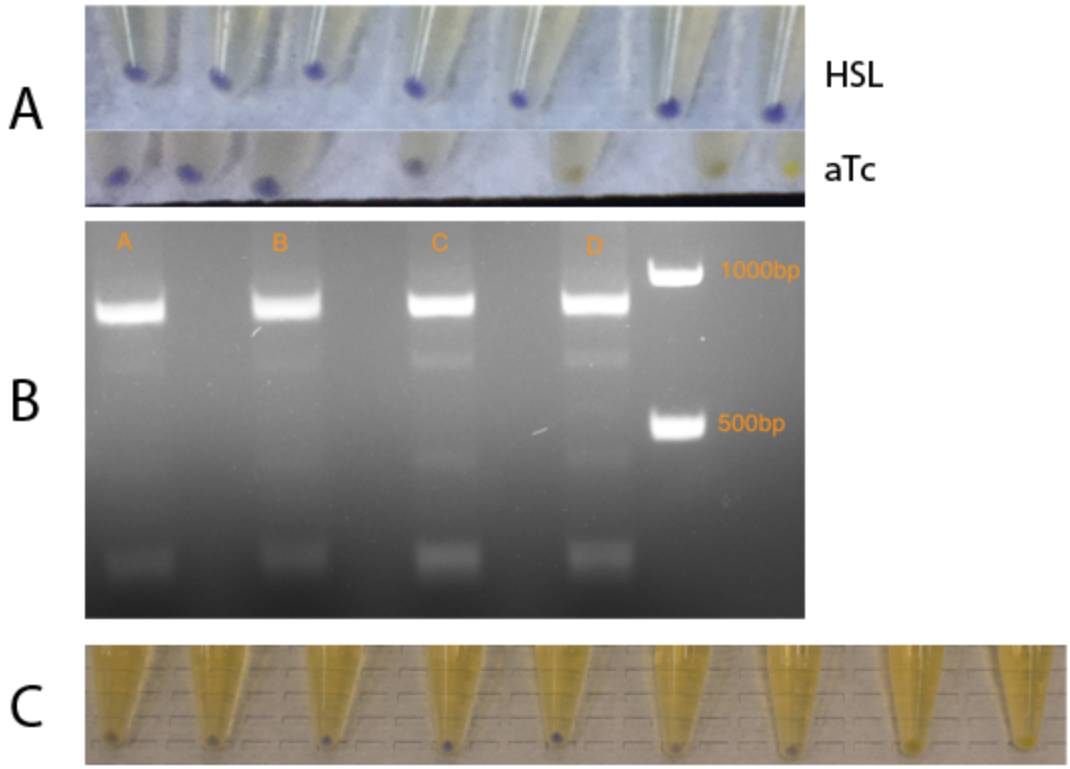

Fig 3. - **Determination of inducer concentrations necessary to flip the fim transcriptor completely**

Mixed state colonies of NEB 10-beta containing K1077007(amilCP j23100 fim switch) and K1077002(aTc inducible fimE, HSL inducible hbiF) were inoculated with varying concentrations of either aTc or HSL in LB and grown for 24 hours in A and B, and 19 hours in C. 1mL of cultures were pelleted. **A)** The top row shows HSL induced cultures and the bottom row shows aTc induced colonies. Top row (HSL) concentrations from left to right are: 0, .2nM, 1nM, 2nM, 2.5nM, 5nM, and 10nM. Bottom row (aTc) concentrations from left to right are: 0, 432nM, 1.29μM, 2.16μM, 3.02μM, 4.32μM, and 8.64μM. **B)** Digest assay of 4 induced switches separate from those in shown in A and C, but containing the same plasmids. Lane A - 4.32μM aTc, Lane B - 6.04μM aTc, Lane C - 5nM HSL, Lane D - 10nM HSL. **C)** A mix of aTc or HSL induced cultures. From left to right, concentrations are: 0, 100nM HSL, 1μM HSL, 3μM HSL, 7μM HSL, 12μM HSL, 20μM HSL, 6.04μM aTc, and 7.78μM aTc.

We were able to determine the concentration of aTc necessary to flip the switch nearly completely to the OFF state, as shown in 3A and lanes A and B of 3B. Adding a few μM more aTc ended up killing the cells or severely slowing down their growth. As we increased HSL concentration in 3A, we saw a slight increase in the amount of amilCP expression, as indicated by the deeper blue pellets. After performing the digest assay, shown in lanes C and D of 3B however; we found that only a small population of the plasmids are flipping to the ON state when induced by 5-10nM HSL. Thus, we realized that examining fluorescent protein output is not a reliable way to assay the state of a transcriptor when using multicopy plasmids. The digest assay is necessary to accurately measure the percentage of the switch in each state. In 3C we tried increased concentrations of HSL and saw a corresponding increase in amilCP expression; however, somewhere between 7-12μM HSL the amount of HSL seems to slow down cell growth as indicated by the sudden decrease in amilCP expression. This may be due to the increased metabolic burden of having more plasmids in the ON state, producing protein. Unfortunately, we ran out of time before we could perform the digest assay on the cultures. The reason for the difficulty in turning the switch ON inducibly may not be solvable by adding more HSL. The lux induced transcription level of hbiF may not be high enough and/or hbiF may just have a lower enzymatic activity. One way to fix this problem, would be to decrease the copy number of the switch per cell.

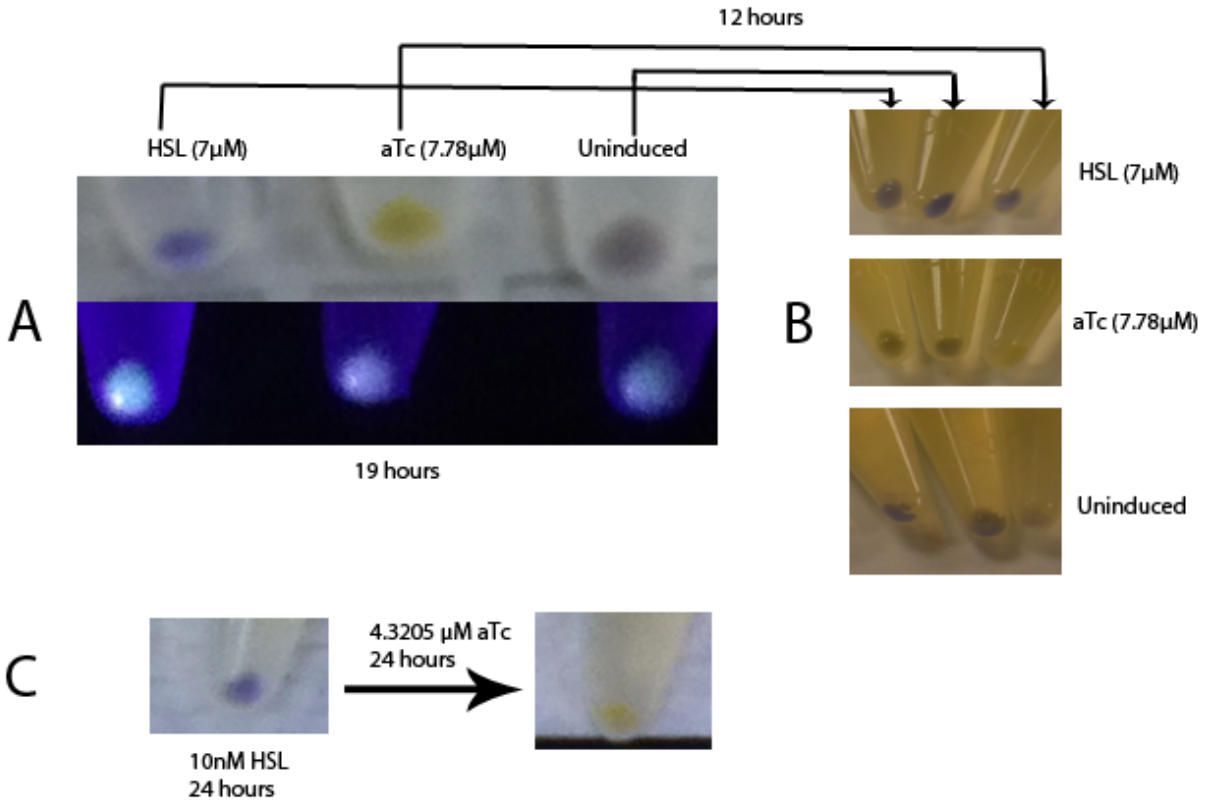

Fig 4. **- An inducible recombinase generator is capable of cycling the state of the fim transcriptor**

A) 1mL of the 3 of the cultures from fig. 3C and 3 cultures containing K1077003(GFP j23100 fim switch) and K1077002 (aTc inducible fimE, HSL inducible hbiF) with varying concentrations of inducers were spun down and washed three times with PBS. All cultures were grown for 19hours. On the top row are the amilCP cultures from 3C and on bottom are the GFP cultures. For both rows, from left to right, the concentration of inducer is: 7μM HSL, 7.78μM aTc, and 0 (uninduced). **B)** 100uL of 1mL pellted and resuspended amilCP cultures from A was added to 5mL of LB and grown for 12 hours in either 7μM HSL, 7.78μM aTc, or 0 (uninduced). The arrows indicate which cultures from A are represented in the column of B. The row indicates which inducer was added to the culture from A. **C)** Although, going from ON to OFF in B yielded mixed colored colonies, this could be due to the short incubation time or the fact that a lot of culture was used to inoculate. C shows K1077007 (amilCP switch) flipping from ON to OFF in a previous experiment in which both cultures were grown for 24 hours in different amounts of inducer. It was also able to flip from uninduced to OFF (data not shown). The 4.32μM aTc culture in C was subcultured by taking taking only 1uL of the 10nM HSL culture and adding it to 5mL of LB.

In 4A, we see high protein expression when switch is turned ON by adding HSL as expected, no protein expression when the switch is turned OFF by adding aTc, and an in between level of expression when the switch is uninduced. The inducible transcriptor is working; though to what degree it is in the ON state is unknown until we do the digest assay. In 4B, we flipped the ON, OFF, and mixed states each to either the ON, OFF, or mixed states. When induced with HSL, all switches produce a significant amount of amilCP. When induced with aTc, there is no or significantly less production of amilCP. The remaining amilCP in the aTc induced ON and OFF/ON cultures is likely due to not allowing enough time for the reporter protein to degrade. 4C shows successful switching of aTc induced ON culture to an OFF state. The inducer levels and growth time was very different from that in 4A and 4B. Degradation tags should be added to both the recombinases and reporter proteins to facilitate faster switching of the transcriptor state. The uninduced OFF culture shows a surprising amount of stability as indicated by the lack of amilCP. Again, we ran out of time to perform the digest assay on the cultures.

Discussion:

We engineered the fim switch by replacing the native promoter with another promoter, namely J23100, and showed that it can flip completely in both directions and function as

expected. The transcriptor is the last, most basic component, needed in order to enable functional and useful biological, computational circuits. Therefore, customizabilty of the fim transcriptor will be extremely important. We submitted the switch halves separately so that other teams can flip whatever part they desire, resulting in a complete fim transcriptor system. We improved upon several natural and engineered biobrick parts. We demonstrated that the fim switch can function as a reliable and efficient biological transistor, or "transcriptor". Future engineers can utilize this switch for tight control of output or to store a state within DNA itself.

The prospect of combining this transcriptor with the ones previously described ([6],[9],[10]) is tantalizing in that it should enable larger rewritable DNA storage than is currently possible, at least doubling the current limit. Additionally, having more switches that respond to independent sets of recombinases allows for more complex circuits to be engineered as described in [9],[10].

There are still a few optimization experiments to be performed to prevent leakage and increase response time. Altering copy number, adjusting rbs strengths, and creating a positive feedback loop all come to mind. Even without the optimizations though, the fact that the fim transcriptor flipped unidirectionally to both states, as predicted, the first time we tried it, even on a high copy plasmid, is a testament to its robustness. Its predictability throughout multiple rounds of testing demonstrates its reliability. It will be interesting to see if it can be ported to other species. The fim transcriptor is the first transcriptor to demonstrate complete unidirectional 2-way state switching and thus provides a solid foundation for the future of transcriptor based biological computing, state storage, and state switching.

Moore's law states that the number of transistors on integrated circuits doubles approximately every two years. With the addition of the fim transcriptor, we have poised the nascent field of transcriptor based biological computing to beat Moore's law! Notably, the 2013 Toulouse iGEM team, whom we were in touch with, has already planned and began constructing a circuit utilizing the Int/Ex and fim transcriptors.

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