Engineering a synthetic protein network that functions like a D-latch

Using synthetic proteins composed of WW domains [S1], maltose binding protein (MBP) [S2], calmodulin [S3], adenylate cyclase [S4, S5], guanylate cyclase [S6], cAMP binding domain from protein kinase A (PKA) [S7-S9], and cGMP binding domain from protein kinase G (PKG) [S9, S10], a biomolecular reaction network was simulated that behaved similar to a digital memory element (i.e D-latch), showing that a network of proteins can remember state (Figure A). D-latches are simple digital memory units that synchronize the setting of a data bit (value that can be 0 or 1) to a clock signal (also can be 0 or 1). When the clock is 1, the D-latch maintains the previously set value; when the clock is 0, the D-latch output is changed to the input D. D-latches are often incorporated in digital systems to remember the state of a machine and the machine responds specifically to inputs depending on its state. Similarly, cells have the notion of a state as it responds differently to the same stimuli such as in stem cell differentiation. We showed that a network of proteins with particular interactions can create a D-latch and therefore a protein network can be used to encode states. Since a D-latch is made fundamentally by switches in a particular configuration, proteins engineered to perform switching can theoretically create similar memory units. A synthetic binding or catalytic protein can be engineered to have two regulatory sites, where each site serves as an input signal and the binding or catalytic activity of the protein serves as the output signal. Furthermore, this protein can be engineered such that as long as one site is occupied (representing a 1 input), the binding activity is inhibited (representing a 0 output). This switching behaviour is known as a NOR logic gate. From digital logic theory, we can create a D-latch using 4 NOR logic gates (Figure S1A).

Specifically, Ca^{2+} and maltose were chosen as inputs to Data and Clock, respectively. The output of the two NOR gates, N_{data} and N_{clock}, were the binding activity of the WW_1 and WW_2 domain, respectively (Figure S1B). The synthetic protein N_{data} can be engineered by fusing WW_1 domain with both CaM and MBP such that when either Ca^{2+} or maltose was present, the activity of N_{data} was inhibited (Figure S1B). Similarly, N_{clock} was created using WBP_1 (a binding partner for WW_1) and MBP to receive input that inhibited the function of WW_2 (Figure S1B). Since N_{data} had two inhibitory sites responding to input signals whereas N_{clock} only had one, when both Ca^{2+} and maltose signals were present, N_{data} was inhibited much higher than N_{clock}. To correct for the imbalance, a lower affinity MBP* was used on N_{data}. The output of memory module $N_{output1}$ and $N_{output2}$ were the activities of adenylate cyclase (AC) and guanylate cyclase (GC) that produced cAMP and cGMP, which were constantly degraded by background phosophodiesterases. The synthetic protein $N_{output1}$ and $N_{output2}$ were also designed with cGMP and cAMP binding domains from PKG and PKA such that when bound to cGMP and cAMP, they inhibited the catalytic activity of $N_{output1}$ and $N_{output2}$, respectively (Figure B). Thus, in any steady equilibrium state, either $N_{output2}$ or $N_{output1}$ was active but not both. Furthermore, $N_{output1}$ and $N_{output2}$ were connected to the logic module by the activity of N_{data} and N_{clock}, respectively. When N_{data} or N_{clock} was active, its WW_1 or WW_2 domain bound and inhibited $N_{output1}$ or $N_{output2}$, respectively. This was described by the following biomolecular reactions (Figure S1B):

$$N_{data} + N_{output1} \rightarrow inactive\_N_{output1}$$

$$N_{clock} + N_{output2} \rightarrow inactive\_N_{output2}$$

$$cAMP + N_{output1} \rightarrow inactive\_N_{output1}$$

$$cGMP + N_{output2} \rightarrow inactive\_ N_{output2}$$

$$N_{output2} \rightarrow N_{output2} + cAMP$$

$$N_{output1} \rightarrow N_{output1} + cGMP$$

$$cAMP \rightarrow degraded$$

$$cGMP \rightarrow degraded$$

After fine tuning the kinetics parameters and stoichiometry, our protein network functions like a D-latch (Figure S1C). The concentration of the maltose and Ca^{2+} were controlled, while the changes in concentration of $N_{output1}$ and $N_{output2}$ were tracked over time. When the maltose concentration (representing the Clock) was set to low, the adenylate cyclase activity followed the concentration of Ca^{2+} (representing Data). When the maltose concentration was high, both the adenylate cyclase and guanylate cyclase activity maintained previous levels despite the changes in Ca^{2+} concentration. The unevenness of the output was due to molecular fluctuations as there were only 500 molecules of $N_{output1}$ and $N_{output2}$ in the simulation.

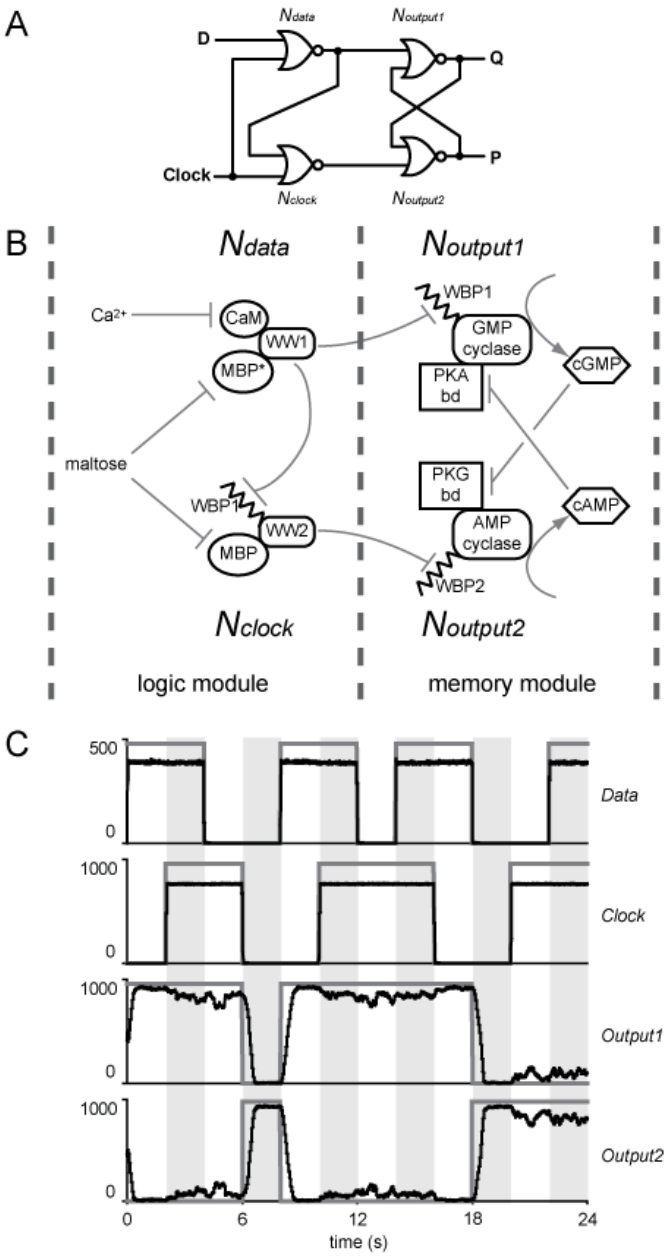

Figure S1 - The synthetic protein D-latch.

A, Schematic of a D-latch in electronics representation, the four NOR gates are names N_{data}, N_{clock}, $N_{output1}$, and $N_{output2}$. B, Protein circuit implementation of the D-latch. C, the concentration over time of electronic D-latch in comparison with protein D-latch. Grey lines indicated the response expected for digital circuit, black lines were the response from the simulated protein circuit.

References:

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Notes:

The association rate constant of maltose to N_{data} (MBP*) and N_{clock} (MBP) were 10^6 and 5×10^6 $M^{-1}s^{-1}$, respectively. The dissociation rate constant of maltose from both MBP* and MBP were 10^{-2} s^{-1}. All protein-protein interaction had an association rate constant of 10^7 $M^{-1}s^{-1}$ and a dissociation rate constant of 10^{-2} s^{-1}. The association and dissociation rate constants of Q and P to $N_{output2}$ and $N_{output1}$ were 10^6 $M^{-1}s^{-1}$ and 10^{-2} s^{-1}. The catalytic rate of Q and P creation was 5×10^{-1} s^{-1}, while the degradation rate of Q and P was 10^{-1} s^{-1}.