

# The Brownian flip-flop, a 5°-level design for high-fidelity, high-speed biochemical signal transduction

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Allosteric signaling networks (ASN)—stable assemblies of proteins that communicate using localized information in the form of coupled domain movements—are the principal devices used by the cell to process intracellular and extracellular chemical information [1]. The regulatory apparatus of cardiac muscle is a prototype ASN. It consists of the Ca<sup>2+</sup>-sensitive troponin C (TnC), troponin I (TnI), troponin T, tropomyosin (Tm), seven polymerized actin and myosin (collectively called actomyosin, AM). The molecular components can be parsed into discrete modules, or 4°-level protein complexes. With this division, the regulatory apparatus can be viewed as a 5°-level structure consisting of interacting 4°-level protein modules.

The regulatory apparatus is a reusable sensing device that modulates the strength of AM association according to the Ca<sup>2+</sup>-binding status of the associated TnC. Strong AM generate force of contraction; weak AM and unassociated AM enable relaxation. Like any reusable (cyclic) measuring device, the system must reset itself (deactivate) after taking its measurement (activation). An ASN can be formulated as a Markov network or non-Hamiltonian 1D spin system comprised of components that isomerize through jump-like events [2]. The free energies in of the lowest energy system-states form the “metastable-state free energy-phase landscape” (MEPL) of the network. The MEPL offers critical insight into the function of an ASN because using the Boltzmann equation, the MEPL determines the equilibrium distribution of system-states at any [Ca<sup>2+</sup>]. As illustrated in Fig. 1, an ASN, with components that *passively* isomerize through nearest-neighbor contacts, faces a design trade-off between signaling fidelity and the rate at which the system can reset. Two model types are shown; each is optimized for a different design emphasis. The Type I model in (a) has a low energy species (red sphere) that functions as a network attractor. Its presence ensures that the system will switch-on (terminal component is active) upon Ca<sup>2+</sup> binding. Deactivation is slow because after Ca<sup>2+</sup> release (blue arrow), the system must make a slow uphill transition before fully deactivating (blue sphere). In the Type II model (b), deactivation is rapid since the uphill transition has been eliminated. The probability to switch-on, given bound Ca<sup>2+</sup>, is only 1/2 because the system-states (1\10) and (1\11) are iso-ergonic.

The MEPL of the cardiac regulatory apparatus (Fig. 2). The architecture, which we call the Brownian flip-flop (BFF), clarifies the role of AM in signaling. The BFF consists of two Type II modules connected back-to-back—a Type II module (the Ca<sup>2+</sup> regulatory switch, or CRS) connected to an inverted Type II module (the AM switch, or AMS). The MEPL of the CRS module, recently obtained from time-resolved FRET, stopped flow FRET, and FRET-Ca<sup>2+</sup> titration experiments [3], is shown in panel a. The MEPL of the AMS module is

constructed from previously reported measurements. The BFF has distinctive architecture features that enable its function. The landscape features a plateau flanked by two receptors, TnC (left) and AM (right) that, according to their ligand binding status, function either as attractors (dips) or repellers (hills) for the network. ADP release followed by ATP binding causes AM to dissociate. AM dissociation eliminates the attractor for activation (red sphere) and creates the repeller for deactivation (yellow sphere). This attractor-repeller flip-flopping is mirrored by TnC, which alternates between an attractor for the deactivation (blue sphere) and repeller for activation (green sphere) upon binding  $\text{Ca}^{2+}$ . Both activation and deactivation involve non-uphill movement from a repeller to an attractor. The plateau supports rapid bi-directional movement; relaxation rates are fast for both TnI ( $305 \text{ s}^{-1}$ ) [3] and Tm ( $> 500 \text{ s}^{-1}$ ) [4].

The MEPL architecture reveals how the BFF functions as Brownian computer that executes a binary logical “equivalence” operation ( $\Leftrightarrow$ ) at the rate of  $\sim 10 \text{ s}^{-1}$ . The equivalence operation is the intersection of an “implication” and a “reverse implication”,  $(s_0 \Leftrightarrow s_n) = (s_0 \Leftarrow s_n) \cap (s_0 \Rightarrow s_n)$ , appreciated from the truth table

$s_0$	$s_n$	$s_0 \Leftarrow s_n$	$s_0 \Rightarrow s_n$	$s_0 \Leftrightarrow s_n$
1	1	1	1	1
1	0	1	0	0
0	1	0	1	0
0	0	1	1	1

Representing a Type II module with ( $\Leftarrow$ ), both the architecture and the logical operation of the BFF are expressed

$$\text{BFF} : \Leftrightarrow = (\Leftarrow \cap \Leftarrow') = \Leftarrow \Rightarrow .$$

## References

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Figure 1: MEPL illustrating the design trade-off in ASN. The landscape is shown under desaturating and saturating  $\text{Ca}^{2+}$ . System: receptor protein (left box) associated with a passively isomerizing reporter protein (right box)  $\pm$  ligand ( $\blacktriangleright$ ). Each protein can assume either an inactive (0, white) or active (1, grey) isomerization state. An increase in  $[\text{Ca}^{2+}]$  (pump) jointly translates of all  $\text{Ca}^{2+}$ -unbound species upward (shaded). (a) Type I module with high sensitivity, moderate specificity, and low deactivation speed. (b) Type II module with low sensitivity, high specificity, and high deactivation speed.

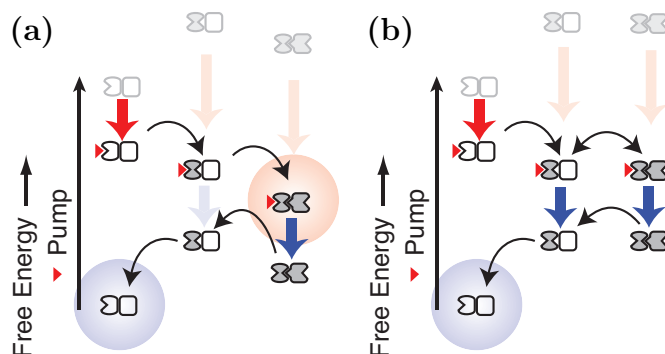


Figure 2: MEPL of the cardiac regulatory apparatus. The landscape is shown under desaturating and saturating  $\text{Ca}^{2+}$ . (a) The CRS module (left-most portion of the ASN), 15 C [3]. System-states are represented by  $(s_0 \setminus s_1 s_2)$ .  $s_1$ , isomerization state of TnC;  $s_2$ , isomerization state of TnI;  $s_0$ , liganded status of TnC (bound = 0, unbound = 1).  $p\text{Ca} = -\log[\text{Ca}^{2+}]$ . Ball radius represents equilibrium probability density. (b) Semi-quantitative MEPL of the complete ASN. System (boxes, left to right) consists of TnC, the receptor for  $\text{Ca}^{2+}$  ( $\blacktriangleright$ ); TnI; Tm; and AM, the receptor for ATP ( $\blacktriangleleft\bullet$ ), ADP ( $\blacktriangleleft$ ), and  $\text{P}_i$  ( $\bullet$ ). Functional states of TnC: closed (C),  $\text{Ca}^{2+}$ -primed (P), and open (O). Functional states of Tm: blocked (B),  $\text{Ca}^{2+}$ -induced (I), and myosin-induced (M). Functional states of AM: unassociated (U), weak (W), and strong (S).

