Ultrasensitivity in "anti-zero-order" regimes

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***Short Abstract* — Zero-order ultrasensitivity is a well-known model for generating sharp switch-like responses. However, it requires enzyme saturation, which is not always the case *in vivo*. Here we describe a simple and novel mechanism that generates ultrasensitive responses in phosphorylation cycles under conditions that are the opposite of the zero-order case. We were able to analytically quantify the level of ultrasensitivity for a few simple cases, capture some experimental observations, and find response curves that need not be monotonic, but have a peak for intermediate levels of input.**

***Keywords* — Ultrasensitivity, phosphorylation cycles, two-stage binding.**

I. Background

ULTRASENSITIVITY is a response that describes a sigmoidal switch, i.e., a switch with a Hill coefficient higher than one. The classical mechanism that details how simple phosphorylation cycles generate such switches is zero-order ultrasensitivity [1]. The critical assumption of that model, without which it would not produce ultrasensitive responses, is that the enzymes are saturated, i.e., the ratio of the number of enzymes to that of substrate is much smaller than one.

However, in endogenous conditions, the situation is often the opposite - there are more enzymes than substrate [2]. Yet, ultrasensitive switches have also been reported in this regime [3]. Previous modelling efforts have investigated this phenomenon [3-5], but either lacked a general scope or required *ad hoc* constraints and assumptions.

Here we introduce a simple mechanism that produces ultrasensitivity when enzymes are in excess relative to their substrate, and identify where the origin of this ultrasensitivity lies.

II. Model and methods

Our model is a simple multi-step phosphorylation cycle with any number n of phosphosites. The only impositions are that the enzymes must bind to a docking motif in the substrate before modifying its phosphorylation state (hence it is a two-stage binding model) [3], and unbind after each modification (such that the multiple phosphosites cannot be treated as, effectively, a single site with slower kinetics).

We used analytical and numerical methods to calculate the Hill coefficient, quantify its dependence on n, the number of phosphosites, and on the equilibrium constants, and evaluate other interesting features of the response such as basal levels, peaks of activity or occupancy states.

III. Results

Our model is able to generate ultrasensitive responses (Hill coefficient greater than one) for systems where the concentration of enzymes exceeds that of their substrate. This behavior is accessible to many combinations of parameters and different initial concentrations. We determined that the higher limit of the Hill coefficient is n+1, thus even systems with a single phosphosite can exhibit some level of ultrasensitivity. Introducing a modification in the model (explicit steric hindrance), we find rare cases where it is possible to achieve Hill coefficients significantly larger than that limit, indeed as high as those predicted by classic zero-order saturation.

More importantly we can provide a simple intuitive explanation for why the excess of enzymes generates ultrasensitivity: when the kinase, for example, is more abundant than the phosphatase, it sequesters the substrate at its end of the cycle (the fully phosphorylated state), either by steric hindrance or an allosteric effect, thus rendering the system insensitive for a large variation in the concentration of phosphatase.

Perhaps surprisingly we find cases for which the response is not monotonic, whether ultrasensitive or not, but shows instead a peak for intermediate levels of input. This effect could offer organisms an option for more interesting and complex regulatory control.

Finally, we can reproduce some experimental observations that previously required a set of phenomenological *ad hoc* assumptions [3].

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