Science Signaling
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Science Signaling **5** (205), lc2. [DOI: 10.1126/scisignal.2002716]

Science Signaling (ISSN 1937-9145) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue, NW, Washington, DC 20005.

THEORETICAL BIOLOGY

Response to Comment on "'Load-Induced Modulation of Signal Transduction Networks': Reconciling Ultrasensitivity with Bifunctionality?"

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Straube suggests that a model that reflects the bifunctional nature of the cycle enzyme uridylyltransferase/uridylyl-removing enzyme (UTase/UR) should be used, in which the UT and UR activities are distinct and reciprocally regulated activity states of the enzyme, and notes that if such a model is used, the effects of retroactivity at intermediate stimulation will be different. However, such a model does not accurately match the observed enzyme regulatory properties and fails to predict the ultrasensitive response obtained in the experiments. Here, we argue that modeling the UTase/UR enzyme as a bifunctional enzyme with reciprocally regulated activity states misses important aspects of the system.

We suggest that signal transduction enzymes, like enzymes in general, are likely to present a diversity of structure and regulatory properties—and that this extends to bifunctional signaling enzymes. In the simplest case, as examined by Ortega *et al*. (*1*) and now by Straube (*2*), the two activities are considered mutually exclusive states of the enzyme, with the stimulatory effector serving to switch the enzyme between states. Theory suggests that zero-order ultrasensitivity is not possible in such an enzyme (*1*). However, in principle, other forms of ultrasensitivity are possible, including multistep ultrasensitivity (*3*), resulting from a stimulatory effector acting at distinct steps in an overall process, and ultrasensitivity that derives from substrate-mediated inhibition of one of the enzyme activities (*4*).

The uridylyltransferase/uridylyl-removing enzyme (UTase/UR) of *Escherichia coli*, which was used in our experiments (*5*), is a bifunctional enzyme. However, the regulation of its two antagonistic activities is not strictly reciprocal, and thus, the UT and UR activities cannot be considered to be distinct activity states of the enzyme. Compared with the UT activity, the UR activity of the enzyme is fairly weak and exhibits substantial basal activity that is only increased about threefold upon saturation of the enzyme with glutamine (*6*). Conversely, the UT activity of the enzyme is very strong (~40-fold stronger than the UR activity), yet it can be completely inhibited by glutamine (*6*). Thus, although the two activities of the UTase/UR are regulated by glutamine binding to a common site on the enzyme, their regulation is neither tightly coupled in a reciprocal fashion nor completely independent, but rather something in between these two extremes. Specifically, multiple forms of the enzyme catalyze the UR activity.

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We imagine that the regulation of the antagonistic activities of bifunctional enzymes may range from tight reciprocal coupling, which is captured in the models of Ortega *et al*. (*1*) and Straube (*2*), to being completely independent. Our prior work on the bifunctional adenylyltransferase (ATase) enzyme that catalyzes the reversible adenylylation of *Escherichia coli* glutamine synthetase showed that multiple enzyme forms were able to catalyze each of the two antagonistic activities (*7*). Yet, a third bifunctional enzyme that we have studied, the two-component system transmitter protein NRII, also showed that multiple enzyme forms were responsible for one of its two functions (*8*). An important challenge for the future will be developing mathematical models that capture the intricacies of such enzymes. Modeling the UTase/UR-PII system as containing two independent monofunctional enzymes accurately predicted the steady-state characteristics of the cycle (*9*). As a consequence, this model was also used for the dynamic analysis (*5*).

The analytical results of the dynamic analysis of (*5*) are independent of whether the cycle enzyme is bifunctional for many conditions, as Straube noted. In particular, in the case in which the Michaelis-Menten constants K_1, K_2 are much larger than the total amount of cycle protein W_T, a model assuming two different enzymes and a model assuming a bifunctional enzyme become the same. Hence, all the analytical results obtained in our paper (*5*) for the case in which the isolated system has a hyperbolic response (K_1, $K_2 >> W_T$) are unchanged for a model with bifunctional enzyme: Load increases the response time to extreme stimuli and decreases the bandwidth of the cycle. Additionally, increasing the enzyme amounts leads to insulation from loading effects. The prediction that load decreases the response time to intermediate stimuli relies on the assumption that the isolated system operates in the ultrasensitive regime [see the Supplementary Materials of (*5*)]. As a consequence, this prediction does not necessarily hold for a model with a bifunctional enzyme that displays tight reciprocal regulation, unless the system has an ultrasensitive response in which the sensitivity is diminished by loading.

How could an ultrasensitive response be converted to a hyperbolic response by loading if zero-order ultrasensitivity is not involved? As Straube notes, to understand this it is necessary to learn the source of ultrasensitivity in the UTase/UR-PII system. In our published work,

we showed that the cycle displays a moderately ultrasensitive response to glutamine, corresponding to an apparent Hill coefficient of $n_H \sim 2$ (*9*). These results were observed both with the wild-type homotrimeric substrate protein PII and with a heterotrimeric form of PII in which only one of the three subunits can be modified. Hence, the ultrasensitivity of the system cannot be attributed to multiple successive modifications of the PII protein, as pointed out by Straube (*2*). In our ongoing work, we have found that the sensitivity of the glutamine response depends on the PII concentration and displays a biphasic response to change in the PII concentration, with a maximal sensitivity corresponding to $n_H \sim 2.6$ when PII was slightly higher than in our published work (*5*, *9*) and a minimal sensitivity corresponding to $n_H \sim 1.5$ when PII was at low concentration. Furthermore, we have found that the midpoint of the glutamine response ($S_{0.5}$) also displayed a biphasic response to change in the PII concentration. Furthermore, one of the activities of the enzyme exhibited strong substrate inhibition, which is likely the main source of ultrasensitivity in the system. Thus, the UTase/UR has proven to be surprisingly complex, with different K_m for each activity, product inhibition of both activities, substrate inhibition of one activity, and only partially coordinated regulation of the two activities. In light of our current understanding of the enzyme, it seems that loading diminished the substrate inhibition by PII—and by so doing decreased the sensitivity of the system. This may explain both the ultrasensitivity of the system (*5*, *9*) and how response times became faster upon presenting a load at intermediate stimulation (*5*). If these ideas prove to be correct, then it may be the case that a load can increase the response times for any ultrasensitive system at intermediate stimulation, regardless of the mechanism of ultrasensitivity, if the load can directly reduce the sensitivity.

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10.1126/scisignal.2002716

Citation: P. Jiang, A. C. Ventura, E. D. Sontag, S. D. Merajver, A. J. Ninfa, D. Del Vecchio, Response to comment on "'Load-induced modulation of signal transduction networks': Reconciling ultrasensitivity with bifunctionality?" *Sci. Signal* **5**, lc2 (2012).