

# ***In Silico* Robustness Analysis of Cellular Systems: A Molecular Perturbation Approach**

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Robustness has been recognized as a fundamental organizational principle in the evolution of cellular functions (1–2). The term *biological robustness* describes the maintenance of specific functionalities (phenotypes) against perturbations in the cellular internal and external conditions (2). The term does not necessarily imply that the system is static. On the contrary, a wide array of cellular processes, from signaling to gene expression, is orchestrated in response to a perturbation. Despite its obvious benefits, robustness property can turn into an Achilles heel if the cellular mechanisms that confer this property are hijacked, such as in cancer (3). Thus, the understanding of robustness and its tradeoffs in cellular systems can greatly benefit the drug discovery efforts for human diseases (4).

The analysis of biological robustness has become an active area of research within systems biology (5–8). Most of the published analyses relate the property of robustness to output sensitivity. An output is called robust to a perturbation when it is relatively insensitive to such change. On the other hand, there also exist perturbations that can cause a large output modification, pointing to a system *fragility*. Most of the existing robustness analyses share a common feature; the perturbations are introduced in the system parameters. These parameters typically consist of kinetic rate constants, transport coefficients, binding energies, *etc.* that correspond to the many cellular processes involved. Unfortunately, the experimental validation of parametric sensitivities is often impractical. This motivates the development of a new robustness analysis which can give experimentally relevant sensitivities that are easy to validate.

The novelty of the new analysis proposed here is that the sensitivities are evaluated for perturbation of the system states rather than the usual parametric change. The proposed sensitivity coefficient is mathematically defined as:

$$S_{i,j}^x(t, \tau) = \frac{\partial x_i(t)}{\partial x_j(\tau)} \frac{x_j(\tau)}{x_i(t)} \text{ for } t \geq \tau, \quad (1)$$

which describes the relative change in the state  $x_i$  at time  $t$  due to the perturbation in the state  $x_j$  at some previous time  $\tau$ . As the sensitivities are computed for perturbations in the states, the result can be validated in relatively simple experiments involving over-expression or knock-out of genes or RNA interference.

We have applied the proposed analysis to a model of the cell death regulation in Figure 1 (9). The activation of caspase-3 follows a switch-like response as shown in Figure 1 (see inset) by way of caspase-8 and caspase-6-dependent pathway (type-I) or mitochondria-dependent pathway

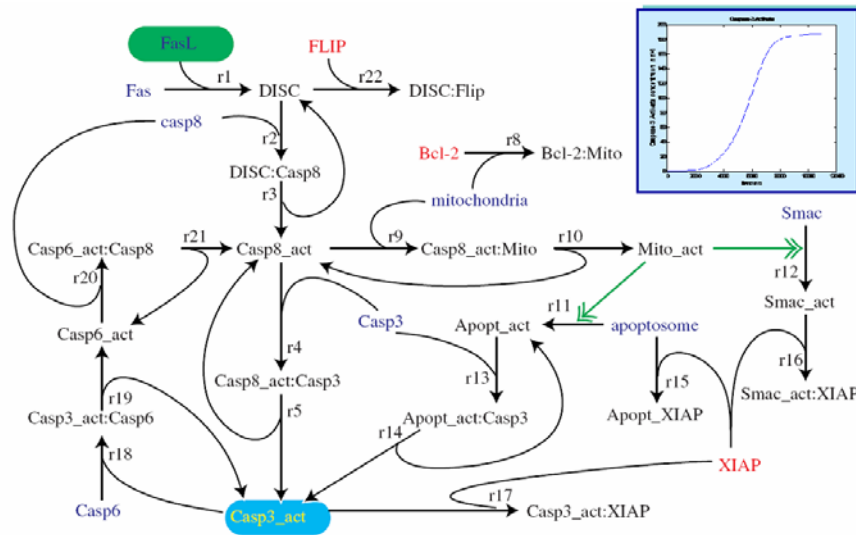


Figure 1. A Model of FasL-induced Cell Death Signaling.

(type-II). The result is shown in Figure 2, which illustrates the sensitivities of caspase-3 activation to the levels of the death signal FasL, caspase-8, caspase-6, and “activated” mitochondria (mitochondria after permeabilization by Bcl-2).

The analysis indicated that the cell death mainly depends on the type-II pathway as indicated by the high sensitivity (darker regions) to activated mitochondria, and the lack thereof to caspase-6. In addition, the analysis also illustrated the timing of the key molecules in activating caspase-3: FasL is early, followed by caspase-8 and finally by mitochondria permeabilization. These findings implied that the FasL induction of cell death in this cell line primarily depends on the type-II pathway, in agreement with experiments (10).

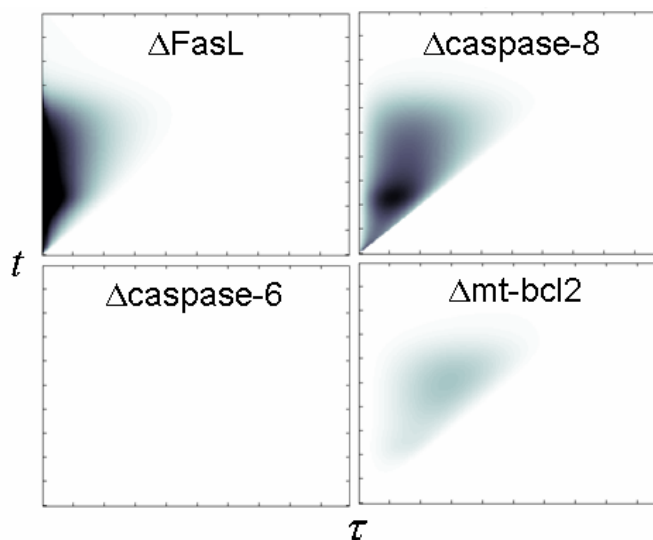


Figure 2. Two-time Sensitivity Analysis of the Cell Death Signaling Model.

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