of models, but use of Moleculizer unavoidably involves SSA-based Monte Carlo simulation.

Notably, the method of first generating a network and then simulating it has proven useful. For example, it has been used in model-based studies of early events in immunoreceptor signaling[2,8,9]. This approach has also been used to generate a model that is closely related to models discussed by Lok and Brent for yeast α-factor signaling. This model, available at our web site (http://cellsignaling.lanl.gov), was first mentioned in ref. 3 (as an example of BioNetGen capabilities) and is based on a scheme illustrated in ref. 1. It demonstrates that on-the-fly network generation is not always necessary. More work is needed to better understand the advantages and disadvantages of the two approaches, which we think are complementary.

Lok and Brent suggest a formula (on p. 135) for assigning rate constants to reactions. This formula is applied inappropriately, as we will discuss below, but its introduction is meant to account for the diffusivities of reactants, which depend on their molecular weights. Modification of rate constants based on molecular weights, which is an optional feature of Moleculizer, is an example of a context-sensitive model refinement, one that predicts how reactions of the same essential type are affected by varying molecular context. Here, context is variable because the molecular weights (and diffusivities) of reactants depend on association of binding partners. However, model modifications for this type of contextual variability, even with the use of applicable formulas, is unjustified or unnecessary in many cases.

Rates of reactions depend on the molecular weights (or equivalently, diffusivities) of reactants only when reactions are diffusion-limited; no corrections are needed or justified in reaction-limited cases. Furthermore, when diffusion is limiting (that is, much slower than chemical transformation), modifications of rate constants are expected to be minor in many typical situations[4]. For example, binding of a cytosolic protein to a membrane protein cannot be expected to significantly affect the diffusivity of the complex, because the viscosity of the cell membrane is far greater than that of the cytosol.

Finally, as mentioned earlier, the formula given by Lok and Brent is inapplicable for the types of reactions under consideration. The equation from which it is derived depends on the assumption of an ideal gas[10] (also see ref. 7 of the paper). In fact, the underlying basis for the formula is the kinetic theory for an ideal gas. Applicable formulas can be derived from diffusion theory and used if refinements of the kind suggested by Lok and Brent are needed[4]. BioNetGen now implements two methods of on-the-fly network generation, the method described by Lok and Brent and a closely related method described in ref. 4.

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Lok and Brent respond:

Our simulation program, Moleculizer, calculates cellular reaction networks by allowing protein complexes to form *in silico*. From the correspondence of Hlavacek and colleagues, we take two main points. They argue that there are advantages to generating reaction networks beforehand, followed by running the simulation to solve them. They also point out that the formulation we use to estimate new intracellular reaction rates is likely to be an oversimplification.

We agree with these points, and we discussed them both extensively in our published paper. Using Moleculizer or other rule-based programs to generate reaction networks that are then fed into the other kinds of simulators has, in some cases, the advantage of decreasing computational cost. We envisioned that the use of Moleculizer to generate networks solved by other simulators would be perhaps its best use in the future. We discussed this explicitly in the article; among other things, it is why we placed so much work and emphasis on enabling export of reaction networks via systems biology markup language (SBML). Similarly, we recognized that our formula for calculating intracellular diffusion is at best a simplification—in the paper, we refer to it as a "placeholder"—and stressed in the discussion that the modular nature of the existing code makes it easy for users to experiment with other, perhaps more complex, formulae that might give better results. To the extent that Hlavacek and colleagues suggest we did not consider or discuss these points in our published work, we believe that these authors are attacking a straw man, perhaps to focus attention on their own forthcoming work on simulation of cellular reaction networks.

We wonder if another trigger for their correspondence may have been a difference in scientific cultures. Moleculizer and other ongoing simulation work arise from a biological research effort, the Alpha project, whose ultimate goal is to predict the behavior of a single, extremely well-characterized, signal transduction pathway in yeast. Just like the biological experimentation to which it is coupled, Moleculizer is a work in progress; at the end of the day, we view it as a tool. This view enables us to write modular code to solve problems simply and replace those solutions with more sophisticated ones as the need arises. When we wish to compute a reaction network, we are comfortable beginning with a computationally inefficient route until it becomes too burdensome to follow further. When we wish to accommodate molecular diffusion, we are comfortable beginning with a simple formula until such time as the results from using it diverge unacceptably from those obtained by measurements of the living system. In contrast, Hlavacek and colleagues are physicists in a theoretical department. In some areas of physics, 'theory' is relatively more important, and there may be a tendency to try to get the theoretical basis right from first principles, rather than being resigned to introducing, modifying and discarding ideas and formulas as one goes along. Neither stance is more 'correct' than the other. However, we believe that, for a good deal of the work that needs to be done to compute the behavior of biological systems, concentration on building a 'perfect' simulator may not be as important as production of supple (and complex!) code that can handle the significant challenges, including the myriads of different protein complexes, posed by living systems, and that can be continually modified as tight coupling to ongoing experimentation produces new challenges and results. For at least some applications of simulation to biology, the perfect may be the enemy of the good.

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