We have found that consideration of this additional complexity is necessary if the model is to make accurate predictions about network dynamics and the role of specific components, such as individual sites of tyrosine phosphorylation[11,13].

Drawing a process diagram with 356 species to represent the interactions of only five proteins[11] would be inefficient and difficult to accomplish or read. Moreover, there are no obvious modules that could be introduced to simplify the process diagram, because the reaction network is highly branched[11]. In any case, a module has the drawback that protein-protein interactions are either altogether hidden (when the module is closed) or obscured by the possibly large number of species and reactions that can arise from the interactions (when the module is open).

Given that protein-protein interactions can generate myriad species and reactions for combinatorial reasons, what can be done to capture the essence of these interactions without ignoring their combinatorial complexity? To address this problem, we have proposed that protein-protein interactions and their effects be represented in the form of reaction rules that are generators of species and reactions[14,15]. More recently, we have introduced graphical reaction rules[6,7], in which graphs similar to the pictograms of process diagrams are used to represent features of proteins and protein complexes. Graphical rules were introduced to allow the connectivity of proteins in a complex to be explicitly represented, and they also provide a means to comprehensibly visualize protein–protein interactions, as illustrated in **Figure 1**.

In summary, process diagrams are useful for representing the individual species and reactions that can arise in a signaling system. However, representation at this microscopic level of detail may not be practical. In the face of combinatorial complexity, diagrams can be overly complicated or hide information about protein-protein interactions. An alternative approach is to represent not the species and reactions resulting from the interactions of proteins in a system but rather the interactions themselves. This task can be accomplished relatively easily using graphical reaction rules. A set of rules can be interpreted to obtain a mathematical model that accounts comprehensively for the species and reactions logically consistent with the rules, even when large numbers of species and reactions are possible[7,14,15]. We are currently extending the BioNetGen software package[14,15] to provide tools for drawing and interpreting graphical reaction rules (http://cellsignaling.lanl.gov/). In the future, we believe such model-generation tools will play an important role in obtaining a mechanistic understanding of cellular information processing and in manipulating signaling systems for therapeutic and biotechnological purposes.

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Kitano *et al.* respond:

The first issue raised by Blinov *et al.* suggests that pathway maps are too simplistic to represent the protein combinatorial explosion in signal cascades. They detail Figure 3e in our article to illustrate their point; however, this figure was used solely to demonstrate the look-and-feel of how to represent pathways as process diagrams. Therefore, we used part of the diagram in a Hanahan and Weinberg paper[1], which is also a pathway extensively used in simulation studies[2,3]. It was not argued that this was a comprehensive representation of the EGFR pathway. Our recent interaction map published in *Molecular Systems Biology*[4] was intended to be a comprehensive EFGR map of experimentally validated interactions. We did not enumerate all possible interactions and molecular states and recognize that there are interactions not listed in the map due to lack of experimental validation, despite theoretical and intuitive possibilities. The process diagram is neutral on what should be described in the map. It defines the graphical representation of an interaction map; thus, the oversimplification critique does not apply to the process diagram itself as construction of these maps relies on experimental evidence.

The second issue raised was that describing all combinatorial states of molecules and resulting complexes would result in a combinatorial explosion making a rule-based approach more appropriate for modeling. We would argue that this depends on the intended use of the map. The process diagram was motivated by an experimentalist's need partly to represent detailed interactions, including residue modification state, to improve experimental design, and partly to visualize their data in the context of a pathway map where each combinatorial state has been explicitly described, regardless of the level of complexity. It is imperative that software tools make such complex and large-scale maps accessible to users.

Although the rule-based approach has attracted much attention as a viable approach for dynamical simulation[5,6], it may not allow users to project experimental data on to each combinatorial state without expansion. As illustrated by Blinov *et al.* wherever the rule-based approach is shown to be effective, the process diagram can then be used to expand graphical notation to represent rules and the network generated from the rule. We would like to incorporate such features into the process diagram and are receptive to constructive critiques to create standard graphical notations; to this end, we have formed an international alliance to standardize graphical notation called Systems Biology Graphical Notation (http://www.sbgn.org/).

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