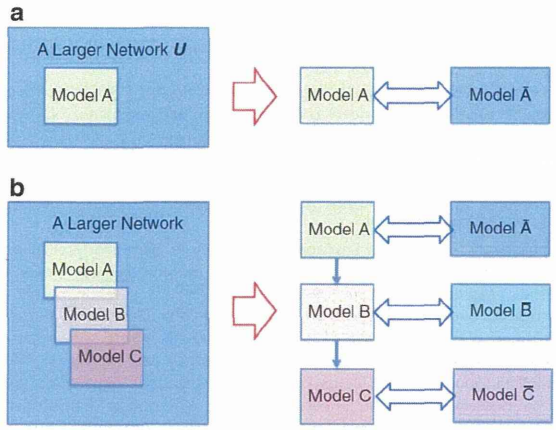

Figure 7. A Tandem model approach. (**a**) In general, a model represents a subset of the network (Model A) within an entire network (Model U) (Left). In this case, what is happening in practice is to implicitly consider Model A is interfacing Model $\bar{A}$ which is a black box of the rest of the network. With proper parameter tuning and value range restrictions, the model can provide reasonably accurate prediction of behaviors of the subset of the network. (**b**) Three models represent overlapping part of the network, and validated for their accuracy. This implies that these models are tuned for their counter-part black box. Within predefined range of values, a tandem application of three models is expected to deliver qualitatively useful predictions on behaviors of the network.

often results in inconsistency in the kinetic parameter values and therefore, these values have to be re-tuned after model integration. The constraint here is that fine-tuning the kinetic parameters in an integrated model may not work owing to different levels of abstraction among models. Even if the level of abstraction and methods of model description have been made consistent, it is a computationally intensive and time-consuming task that often requires additional experimental data for accurate estimates of kinetic parameter values.

In this study, we applied an alternative approach that involves a tandem use of multiple models, each of which is validated to be consistent with the experiments. In general, computational models of biological networks are developed focusing on a subset of networks such as the MAPK and insulin receptor pathways and a model of the entire network is not created. Nevertheless, many of these models achieve a practically sufficient accuracy that can be used for further scientific studies and drug discovery.[51] This degree of accuracy may be attributable to the modularity and robustness of the biological network. If the entire network is so tightly connected, perturbation of any part of the network affects the behaviors of all other parts. This results in an extremely unstable system, leading to an evolutionary dead-end.[52] Thus, in practice, we can define a subset of the interaction network and consider the rest to be a 'black box' from which effects can be implicitly represented within a specific parameter setting of the model (Figure 7a). The success of this approach largely depends on appropriately defining the boundary of a focused network, so that strong interactions with a black box do not affect the dynamical behaviors of the model beyond the level adjustable by parameter settings. In this study, we used three models each representing a distinct functional pathway in the metabolic network, and they were validated experimentally (Figure 7b). Furthermore, the models were interfaced by the dose change of specific molecules. The output of the first model (glycogen metabolism and glycolysis) is a dose change of glucose and G6P that in turn became inputs for the second model (pentose–phosphate pathway). The interface between the second model and the third model (glutathione metabolism) is $NADP^+$ and NADPH. Fortunately, no obvious feedback loop exists between the models that could seriously alter the behaviors of an upstream model depending on the outcome of the downstream models. It is noteworthy that the purpose of the simulation was to understand the qualitative propensity of this part of the metabolic network following sunitinib administration, rather than computing the exact dynamics with quantitative precision. The results of this tandem simulation correctly predicted physiological responses to the administration of sunitinib. Further studies may reveal conditions for this tandem approach to be practically applicable, which would lay the foundation for scaling-up computational studies efficiently for larger networks. It should be noted that we used the model to understand how sunitinib administration may qualitatively influence the metabolic system, rather than to obtain precise numerical results. To this end, the models were only required to deliver the proper degree of change in output and therefore, could have different baseline variables. In other words, the dose of the interface molecule could be 100 units in one model, and the dose level of the same molecule could be represented as 1,000 units in the other model. This is because the only information required is the degree of change and not the absolute number in each model. In such cases, a combination of simulations using separate models is expected to provide reasonable predictions.

The results reported herein demonstrate that *in silico* prediction (with appropriately designed experimental validation) is an effective strategy to examine toxicological issues associated with kinase inhibitors. Results of this study have major implications for the use of sunitinib in clinical practice, as it is essential to overcome adverse reactions associated with sunitinib tolerability.[13] Suppression of sunitinib-mediated thyroid dysfunction may improve patients' quality of life by ameliorating subjective symptoms, such as fatigue. More importantly, since the sunitinib-mediated reduction in platelet counts is a dose-limiting toxicity, an improvement in thrombocytopenia will allow the continued use of higher doses, which is desirable in treating the primary disease. Indeed, the therapeutic efficacy of sunitinib in RCC correlates positively with the area under the plasma concentration curve for the drug.[53] The experiments conducted in the present study strongly indicate that the multi-organ toxicity suffered by patients receiving sunitinib may be alleviated by the concomitant administration of α-TN.

In conclusion, we demonstrated that a systems toxicological approach could be successfully utilized to elucidate molecular mechanisms underlying adverse reactions associated with kinase inhibitors. This approach may be applicable to other kinase inhibitors to maximize their clinical benefits.

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COMPETING INTERESTS

The University of Tokyo has filed a patent application about concomitant use of sunitinib and anti-oxidants (PCT/JP2013/081210, inventors: Masashi Honma, Hiroshi Suzuki, Takahiro Amemiya and Haruki Kume).

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