

Explaining complex phenomena by discovering, validating, and falsifying in silico mechanistic hypotheses:

a demonstration focused on multiple features of acetaminophen hepatotoxicity

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Context: We focus on aspects of temporal and spatial liver pathology produced in mice following a single toxic dose acetaminophen (APAP) and its significant inhibition by a single dose of SP600125 (an anthranyrazolone inhibitor of Jun N-terminal kinase) given 2 h later.

Challenge: Discover and improve minimal in silico component interactions that stand as challengeable mechanistic hypotheses capable of explaining complex phenomena.

Approach: Achieve delineated near and long-term requirements by experimentally challenging and iteratively refining discrete event, agent-based analogs comprised of nested modular spaces and components assembled into biomimetic mechanisms.

Methods: Our validation targets are drawn from a diverse set of phenomena that we eventually wish to explain. Each phenomenon is a targeted attribute (TA). We cycle through an Iterative (mechanism and component) Refinement Protocol, which has five key stages. 1) Specify the subset of TAs to be validation targets during the current work cycle along and quantitative similarity criteria (SC). A validation target is achieved when an analog phenomenon attains the SC prespecified for a TA. 2) Formulate minimal computational mechanisms intended to mimic essential features of referent mechanisms. Analog phenomena generated during execution are products of component interactions. 3) Instantiate those mechanisms by refactoring and reparameterizing components and mechanisms from already validated in silico analogs. Design and execute experiments. Record and measure simulated phenomena. 4) Use SC to compare corresponding simulated and wet-lab measurements. When SC for several TAs are achieved, the analog has attained a degree of validation: it stands as a plausible, concrete, explanation of the targeted phenomena. 5) Challenge/falsify Stage 4 mechanisms in the next work cycle by including new TAs at Stage 1.

Goal for This Work: At stage 5 in the most recent cycle, our analog had achieved multiple degrees of validation. We added TAs characterizing SP600125 inhibition of hepatocyte necrosis following a single dose given two hours after a toxic APAP dose. Simulating inhibition required: 1) a separate set of SP600125 objects that would percolate simultaneous with APAP objects through and interact with analog components; 2) giving analog components the ability to distinguish between APAP and SP600125 and interact appropriately; and 3) achieving SP600125 absorption, distribution, and clearance validation targets.

Results: Earlier specifications and quantitative validation targets achieved include features of APAP pharmacokinetics, its hepatic clearance and metabolite ratios; lobule zonation of metabolism, glutathione depletion, dose dependent damage caused by the reactive metabolite, and repair of damage. We demonstrate achieving a key validation target: dose dependent hepatocyte necrosis occurs first adjacent to lobule central veins. Following SP600125 dosing, a coarse grain interaction mechanism enabled achieving time-dependent inhibition of necrosis TAs.

Conclusion: The evidence presented strengthens our case that biomimetic synthetic analogs using

concrete parsimonious mechanistic hypotheses provide an explanation for how APAP toxicity emerges within and across biological scales. The methods used facilitate in silico experimentation to falsify (or not) previously validated mechanisms. They also make it straightforward to iteratively reuse and refine components to establish new, more ex