To the Editorial Board:

Please find enclosed our manuscript **"Genome-scale analysis of small molecule regulatory networks and the trade-off between regulation and enzymatic activity"** for consideration for publication in Cell Systems. Although transcriptional regulation of metabolism has been studied extensively, our understanding of the **regulatory interactions between endogenous small-molecule metabolites and enzymes** (*e.g.* allosteric inhibition) remains limited, and has been the subject of several prominent recent reports (*e.g.* Hackett, Rabinowitz, *et al* Science 2016; Gerosa, Sauer, *et al* Cell Systems 2015). While small molecule/enzyme regulatory interactions are critical to the rapid adaptation of metabolic flux, there are no experimental platforms for genome-scale discovery of such functional interactions.

In the submitted manuscript, we report a first-of-its-kind **computational framework for the reconstruction and analysis of genome-scale small molecule regulatory networks (SMRNs)** by leveraging the vast biochemical literature available for many organisms. Our results blend informatic, computational, and theoretical approaches together with experimental data, to analyze the architecture of small molecule regulation and its contribution to metabolic flux regulation.

The main results of our work are:

1. **An informatic pipeline for reconstruction of a network of regulatory (activating/inhibiting) interactions between endogenous metabolites and enzymes.** The code for this pipeline is publically available at http://github.com/eladnoor/small-molecule-regulation and can be applied to reconstruct a small molecule regulatory network (SMRN) of any organism for which a standard genome-scale metabolic model (*e.g.* those used in standard flux balance analysis) is available.
2. We apply this pipeline to **reconstruct the small molecule regulatory network (SMRN) for the model organism *E. coli*** and provide the result as a **resource for the metabolic research community.** We show that a relatively small number of metabolites (*e.g.* ATP) and reactions (*e.g.* nucleotide salvage reactions) are the focal points for small molecule regulation. We also identify a core set of regulatory interactions which are broadly conserved across many phylogenetic taxa. The recurrence of these interactions suggest that they may be essential to the proper functioning of metabolism across all organisms.
3. Using our SMRN in combination with metabolomics measurements, we address fundamental questions regarding the **design principles underlying small molecule regulation.** We evaluate the potential for thermodynamic and economic arguments to explain the patterns of regulation we observe in the SMRN.
4. **Control analysis of metabolic regulators** r**eveals a direct trade off between regulation and enzymatic activity**. We mathematically prove a relationship between the activity of an enzyme and the control potential of its regulators that holds true for substrates, activators and inhibitors. Thus, we reveal the general principle that regulating metabolic flux by small molecules always comes with the cost of effectively lowering the activity of the enzyme. Applying this theoretical result to our dataset, we show that although binding sites of inhibitors are significantly less saturated than those of substrates, their control potential is higher.

Since this work stands at the crossroads between theoretical, computational, and chemical biology, Cell Systems seems the most appropriate place for it. We believe that our study is of interest to a wide community of biochemists, systems biologists and metabolic engineers and hope that you will consider our manuscript for publication.

Sincerely,
Ed Reznik, Dimitris Christodoulou, and Elad Noor