We thank the reviewers for their additional suggestions to improve the manuscript, "Dynamic Modeling of Cell-Free Biochemical Networks using Effective Kinetic Models." Below, we outline the changes we've made to address these suggestions.

Reviewer 1 (Changes highlighted in Yellow on MARKED manuscript)

1. Regarding the identification, it would be useful (if possible) to report the computational time needed to perform the identification in Figure 7, and to discuss briefly how the computational complexity would scale with the network size.

> The following sentence was added to the last paragraph of the Materials and Methods section specifying the computation time required for the simulations in Figure 7:
>
> " … The optimization simulations shown in Figure 7 required several hours to complete on a single CPU Apple workstation (Apple, Cupertino, CA; OS X v10.10). "
>
> We also address issues of computational complexity and network size in the second to last paragraph of the Discussion section. The following sentences were added:
>
> " … Though we expect computational complexity will scale poorly with network size, we are optimistic that large-scale, predictive models of metabolism are possible. There is evidence to suggest that achieving a quantitative understanding of complex biological systems should not require complete parametric knowledge. "

2. Since the authors are presenting a new biochemical network modeling framework, a brief comparison of the new framework with existing frameworks such as power-law models from Biochemical Systems Theory would be very useful.

> We thank the reviewer for this suggestion. We agree that a comparison of our modeling framework with previous approaches would be insightful. We've added the following paragraph in the Discussion section highlighting differences between our framework and previous ones:
>
> " … The proposed modeling framework also differs appreciably from previously established kinetic approximations of complex biochemical network behavior. Such frameworks replace parameter dense mechanistic kinetic expressions with heuristics quantifying the relationship between metabolic rate and metabolite effectors. A review of approximative kinetic formats can be found in (Heijnen 2005). These approaches arose in response to uncertainties associated with obtaining correct mechanistic kinetic expressions and parameters of *in vivo* systems. Similarly, available kinetic parameters measured *in vitro* may differ in a specialized cell-free *in vitro* environments. Factors affecting kinetics, such as enzyme channeling, macromolecular crowding, and pH, are likely dramatically different in cell-free environments than in both *in vivo* systems as well as typical *in vitro* conditions used for parameter measurements. Thus, a more generalized, approximate biochemical reaction network formulation may be desirable in the case of cell-free systems. Our approach is similar to generalized mass action-based power law formulations of Savageau and colleagues (Savageau 1991) and linlog kinetics of Visser *et al.* (Visser 2003) in that metabolic rates are proportional to corresponding enzyme levels modified by metabolite effectors. Power law and linlog approaches suffer from several known limitations (Heijnen 2005). Power law reaction rates do not capture saturation effects and become infinite for small concentrations of inhibitory regulators. Linlog kinetics also become ill-defined when

effector concentrations go to zero. Also, models employing linlog kinetics typically rely on an experimentally determined reference state to describe dynamics taking place after a perturbation to a steady-state. Our framework does not suffer from such drawbacks. Moreover, cell-free systems are unlikely to satisfy such a steady-state approximation after extract preparation and prior to culture initiation. Our framework is similar to the generic kinetic formulation from Hadlich *et al* (Hadlich 2009), but differs in its inclusion of cooperative effects as well as proposes a simplified integration of competition amongst allosteric effectors using max/min rules. In summary, our proposed framework offers an effective kinetic approximation that captures saturation effects and allosteric competition within cell-free systems that may also be extensible to *in vivo* metabolic and gene regulatory networks. “

New References:

Savageau, M.A. Biochemical systems theory: operational differences among variant representations and their significance. J Theor Biol **1991**, 151, 509–30.

Visser, D.; Heijnen, J.J. Dynamic simulation and metabolic re-design of a branched pathway using linlog kinetics. Metab Eng **2003**, 5, 164–76.

Hadlich, F.; Noack, S.; Wiechert, W. Translating biochemical network models between different kinetic formats. Metab. Eng. 2009, 11, 87–100.

Heijnen, J.J. Approximative kinetic formats used in metabolic network modeling. Biotechnol Bioeng **2005**, 91, 534–45.

Reviewer 3 (Changes highlighted in Green on MARKED manuscript)

1. One of the questions raised by the reviewer (#5) was whether cybernetic control laws are heuristic or mechanistic. The reviewer is glad at the authors' recognition that cybernetic control laws have a solid basis in optimal control theory, meaning that it is inappropriate to say that cybernetic laws are heuristic - thus, the term "heuristic" should be avoided anycase even if they disagreed to consider them biologically mechanistic. All other issues have properly been taken care of in the revision. Thus, with this correction, the manuscript would be publishable.

We have removed all descriptions of cybernetic control laws as being heuristics. The last sentence of first paragraph of the Introduction has been altered to reflect this:

“ …. However, recent cybernetic formulations from Ramkrishna and colleagues have successfully treated this identifiability challenge through elementary mode reduction [11,12]. “

Also, two instances of the phrase “metabolic control heuristics” have been altered in the second paragraph of the Discussion to read “metabolic control strategies.”