Response to Reviewer Comments

Reviewer 1

Open Review

(x) I would not like to sign my review report
( ) I would like to sign my review report

English language and style

( ) Extensive editing of English language and style required
( ) Moderate English changes required
(x) English language and style are fine/minor spell check required
( ) I don't feel qualified to judge about the English language and style

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>Can be improved</th>
<th>Must be improved</th>
<th>Not applicable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Does the introduction provide sufficient background and include all relevant references?</td>
<td>(x)</td>
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<td>Is the research design appropriate?</td>
<td>(x)</td>
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<td>Are the methods adequately described?</td>
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<td>Are the results clearly presented?</td>
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<td>(x)</td>
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<td>Are the conclusions supported by the results?</td>
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<td>(x)</td>
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</table>

Comments and Suggestions for Authors

Grover et al., have described three cases of combining experimental data and computational modeling to understand the complex chemical reaction network occurring in the test tube. The data mostly from their previous work (ref 12,13,15) were used and the fitting was performed with the computational models. The computational methods used are the ones used widely in the field and not new. Nevertheless, the manuscript clearly describes what the authors have performed in the current study and their findings. However, I think the manuscript can be improved by adding more explanation about the results as listed below.

Response: We agree that the data and modeling results have been previously published and the methods used in the paper are not new. We have reclassified this paper as a Perspectives paper in the revision process, to avoid confusion about this point.

Changes made to manuscript: Manuscript is now a Perspective, not an Article.

For the first case.

Line 198 to 209: The authors describe the reaction dynamics observed with the simulation without no data. It is hard to follow, what they mean by “Analysis of the simulation results demonstrated that the phase behavior could be predicted in most cases based purely on the concentration of peptides in the system C, and its relationship to the two solubilities.” without plots. Similarly, “The modeling study inspired a new set of experiments”, “Simulation results further show that particle size will increase and particle number will decrease with increasing $\chi$”. These sentences also lack the data.
Response: We agree that the description could be more clear. We have revised these statements in the new version, for clarify, and to avoid confusion that there may be new data in this paper (which there is not).

Changes made to manuscript:

“Simulations with $SC$ less than $SC_{1*}$ and $SC_{2*}$ (‘undersaturated’) demonstrated behavior according to Figure 1(a). In the supersaturated regime ($SC$ greater than $SC_{1*}$ and/or $SC_{2*}$), simulations with $SC_{2*} < SC_{1*}$ usually demonstrated behavior as Figure 1(g). However, when $SC_{2*} > SC_{1*}$, then the system usually exhibited behavior such as Figure 1(b). Overall, the simulations showed that the phase behavior could be predicted in most cases based purely on $SC$, the initial concentration of peptides in the system, and its relationship to the two solubilities. The phase with the smaller solubility is the observed phase at long times. However, if both phases are undersaturated ($SC$ is less than $SC_{1*}$ and $SC_{2*}$), then all peptide remains free in solution. In some cases the kinetics were too slow to reach steady state during the simulation time, which may also be the case in the experiments.”

and

“As shown in Equation (5), the Flory-Huggins parameter $\chi$ impacts $SC_{1*}$, but not $SC_{2*}$. Simulation results showed that particle size increases and particle number decreases with increasing $\chi$ $\cite{Hsieh:17}$. Since $\chi$ describes the peptide-solvent interaction, it can thus be tuned experimentally by changing the solvent composition. This interpretation was supported by experiments with KLVFFAE, as shown in the TEM images in Figure 2. As the acetonitrile concentration is increased (i.e. $\chi$ is decreased), the number of particles increases and the size of particles decreases. Further experiments with circular dichroism demonstrated that increasing the acetonitrile concentration speeds up peptide assembly, reducing the formation of particles and driving the peptides more rapidly into the assembly phase. Together, the TEM and CD measurements supported the inverse relationship between acetonitrile concentration and Flory-Huggins constant, and the key role of $\chi$ in determining the system-level phase behavior.”

For the third case.

Line 275 to 276: “However, the new experimental data did not demonstrate this inversion, invalidating the hypothesis.” Which data shows the invalidation?

Response: We agree this was not clear. The revised version clarifies that Figure 5A and B show the original data, while the “new” data is Figure 5C and D. All of this data was previously published in Ref. 15.

Changes made to manuscript:

“The model consisted of ordinary differential equations for each species, and was simulated using a deterministic method. Rate constants were estimated by minimizing the sum-squared error. The preliminary results in Figure 5A and B indicated that binding favored (R)-methodol, while reaction favored (S)-methodol. Further experiments were conducted (Figure 5C and D) to demonstrate this intriguing idea---that the selectivity was invertible based on catalyst loading. However, the additional
experimental data did not demonstrate this inversion, invalidating the original hypothesis. Because there was not enough data initially, the parameter estimates were not unique (as indicated by large confidence intervals). The larger set of experimental data that was collected, as shown in Figure 5, ultimately led to an improved model with tighter confidence intervals on parameters and a wider range of applicability.”

Likewise, the manuscript lacks the details in many places and is more like a review. I personally enjoyed reading the paper, and what they performed are described clearly. However, because of the above reasons, I suggest the authors to add a bit more details before being published.

*Response:* Thank you, we agree this paper is more of a review, with the goal of highlighting numerical and statistical methods that could be included in a Systems Chemistry graduate curriculum.

*Changes made to manuscript:* The manuscript is now classified as a Perspective, and not an Article.