

Peer Review File

Exploring the Programmability of Autocatalytic Chemical Reaction Networks



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REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

In the manuscript entitled "Exploring the Programmability of Autocatalytic Chemical Reaction Networks," the authors present a novel experimental study on the batch kinetics and CSTR dynamics trypsin-catalyzed autocatalytic reaction network and its capability to perform some logic operations. According to the presented results the addition of metal ions (La(III), Ca(II), Nd(III)) opens an effective way to control the kinetics of the autocatalysis. In CSTR experiments, Nd(III) has a nonlinear effect depending on the presence of the second cation. The capability of the system to perform logic operations is demonstrated. The interpretation of the experiments is valid and robust. The presented results remarkably contribute to the development of designing intelligent chemical systems. The methodology is appropriate and presentation of the data is clear. The manuscript references previous literature appropriately.

Questions and comments:

(1) Could the authors discuss the system's robustness, especially in the context of logic operations?

(2) The CSTR experiments were performed in two serial reactors. According to the theory of serial CSTRs reactions that produce bistability in a single CSTR (with two stable, one unstable steady states), in a series of CSTRs with N reactors, have up to $2^{N+1} - 1$ steady states, of which only $N+1$ are stable. (A. Varma, Ind. Eng. Chem. Fundam. 1980, 19, 316. ;G. Dangelmayr, I. Stewart, SIAM J. Appl. Math. 1985, 45, 895). It seems that this multiplicity of states may appear in the experiments presented in the manuscript. Could the author comment on it? Would this multiplicity affect the operation of the system?

(3) In the context of logic operations, it seems that an obvious question is how to connect two more logic gates based on the presented configurations to perform somewhat more examples of computations.

(4) Could the authors clarify the meaning of "Bistability in temporal logic operations"? What are the characteristics of the two stable states, and how the coexistence of these states can be observed?

(5) In the conclusions, the authors state, "We envision that this work will offer novel strategies to exploit the programmability of small and simple chemical reaction networks for building artificial systems with responsive, adaptive, and other life-like properties characteristic for intelligent systems." What are the main steps of this novel strategy, and in what type of systems can it be applied?

I suggest addressing the above questions and comments and revising the manuscript.

Reviewer #2 (Remarks to the Author):

The manuscript "Exploring the Programmability of Autocatalytic Chemical Reaction Networks," written by Dmitrii V. Kriukov, Jurriaan Huskens, and Albert S. Y. Wong, is concerned with the application of trypsin catalysis as a chemical logic gate. The subject is interesting but not new because the first chemical logic gates were described 40 years ago. According to my knowledge, the application of trypsin-catalyzed reaction for chemical information processing is a new element in the study. The logic gates are useful when concatenating them into more complex information processing devices. This does not seem easy within the presented approach because concentrations of metal ions are used as the gate input, but the output is extracted from time-dependent trypsin concentration $[Tr](t)$. This means that connecting two gates requires a device (presumably electronic) that analyzes $[Tr](t)$ and translates the result to the ion pump rates of another CSTR. Moreover, the results of Fig. 4 suggest that a gate can make a few operations per hour, which is rather slow to attract industrial attention. Therefore, considering the limited practical applicability of the presented medium and narrow field of study, I do not recommend publication in a journal focused on a wide audience. The results are better suited for a specialized journal focused on natural or unconventional computing.

In my opinion, the present version of the manuscript is poorly written and does not include

all the information needed to understand what was done.

Here are some remarks on manuscript fragments that must be improved before it is submitted to another journal.

The authors refer to a mathematical model that is not well described. The model variables should be listed. I also expected to find the kinetic equations with flow terms included. The article SI just gives the set of reactions and comments on the reactions.

The mathematical symbols are used without their precise definition in the text. What is $[A]_{\text{feed}}$ (Fig.3 caption)? According to what I read, the symbol $[A]_0$ denotes the concentration of reagent A at the beginning of the experiment ($t=0$). How shall I understand Fig. 3a, where $[Nd^{3+}]_0$ is plotted as a function of time, and its caption says that $[Nd]_0$ was changed?

In some places (for example, p.3, lines 13/14), the numerical values of variables are given without the corresponding units.

Reviewer #3 (Remarks to the Author):

This is an interesting paper, with some thought provoking ideas. The authors use metal ions to control the rate of autocatalysis with trypsin and this feature is used to program the output response of the autocatalyst in a flow reactor. They mention implications in terms of eg neuromorphic computing. Some of the manuscript could be more clearly written, and I am unclear on the interpretation of results in some parts.

In the abstract It is not immediately clear what is meant by “reconfigured in a programmable manner”; this could do with clearer explanation. What are you reconfiguring? There has been quite a lot done with computing in eg reaction diffusion systems; using light, chemical inputs etc to implement Boolean logic and image processing – can it be better pinpointed what is the difference here?

Some explanation of a neuromorphic system and the desired features of such a system in the introduction would be useful for the more general audience.

Figure 2. I'm not sure that fig 2d data can be interpreted as Nd is an "inhibitor" in the traditional sense, since without the metal, autocatalysis doesn't proceed. Technically, it results in a lower rate only as the concentration of Nd is increased above a threshold value. Possibly there is some initial binding of Nd to Tr to form an active state but further binding results in an inactive state – similar to binding of acid to enzymes. Nevertheless, the experimental results are still the same, it is just interpretation that I am unclear on.

Response on referees' comments—Manuscript ID (NCOMMS-24-31240-T)

Changes to the text of our manuscript, based on the below reviewers' comments, are highlighted in yellow and indicated with "Page XX, line YY", referring to the revised version.

Reviewer #1:

In the manuscript entitled "Exploring the Programmability of Autocatalytic Chemical Reaction Networks," the authors present a novel experimental study on the batch kinetics and CSTR dynamics trypsin-catalyzed autocatalytic reaction network and its capability to perform some logic operations. According to the presented results the addition of metal ions (La(III), Ca(II), Nd(III)) opens an effective way to control the kinetics of the autocatalysis. In CSTR experiments, Nd(III) has a nonlinear effect depending on the presence of the second cation. The capability of the system to perform logic operations is demonstrated. The interpretation of the experiments is valid and robust. The presented results remarkably contribute to the development of designing intelligent chemical systems. The methodology is appropriate and presentation of the data is clear. The manuscript references previous literature appropriately.

We thank the reviewer for their positive comments on our manuscript. Below, we indicate how we have used their constructive comments to improve the manuscript.

(1) Could the authors discuss the system's robustness, especially in the context of logic operations?

The reviewer correctly pointed out that a discussion on the robustness of the system was not included. Robustness is a network property which ensures that specific functions of the system are maintained [Kitano, H. *Nat. Rev. Gen.* (2004)]. In our work, robustness can be defined as the parameter window wherein the desired logic operation can be obtained. We did discuss that the various logic operations can be found in a "narrow range of parameters" (page 4, line 44) but we did not comment on the differences between the operations in terms of their robustness. We have incorporated the suggestion of the reviewer as follows:

- Page 4, line 47: The phase plot shows that a parameter window exists to maintain each logic operation, ensuring that the desired functions of the system are robust³⁹. The robustness, however, differs per operation.
- Additional reference: 39. Kitano, H. Biological Robustness *Nat. Rev. Gen.* 5, 826–837 (2004).

*(2) The CSTR experiments were performed in two serial reactors. According to the theory of serial CSTRs reactions that produce bistability in a single CSTR (with two stable, one unstable steady states), in a series of CSTRs with N reactors, have up to $2^{N+1} - 1$ steady states, of which only $N+1$ are stable. (A. Varma, *Ind. Eng. Chem. Fundam.* 1980, 19, 316. ;G. Dangelmayr; I. Stewart, *SIAM J. Appl. Math.* 1985, 45, 895). It seems that this multiplicity of states may appear in the experiments presented in the manuscript. Could the author comment on it? Would this multiplicity affect the operation of the system?*

The reviewer provided an interesting perspective and appealing strategy for constructing multiple states in our system. We gladly take this perspective on board for our future studies (see our answer to comment 5).

Here, to clarify our work, the experimental setup indeed comprises two CSTRs but we noted that the first reactor is the main reaction chamber and that the second reactor serves as a detection chamber (page 13, line 14-15). The residence time in the second reactor was deliberately made twice as small as the first reactor to ensure that we could detect the output signal as pseudo-online. In addition, mixture of DMSO and DMFA was used to dissolve the detection molecule (BAPNA) and reduce the rate of trypsinogen conversion. We checked experimentally that even small percentage of DMSO slows down Tg conversion dramatically. Hence, we did not actually perform our experiments in serial CSTRs but in one CSTR: The second reactor was engineered to allow for the fast detection of trypsin concentration by converting BAPNA

into pNA (detectable moiety at 405 nm) without any side processes that could potentially lead to multiplicity of steady states.

We extended the method section as follows:

- *Page 14, line 14:* First reactor served as a main reaction chamber whereas the second was receiving its outflow and reporting solution at the constant (2:1) ratio. The residence time in the second reactor was deliberately made twice as small as the first reactor to ensure that we could detect the output signal as pseudo-online. Reporting solution consisted of 4 mM BAPNA in mixture of MQ:DMSO:DMFA (0.2:0.08:0.72). The mixture of DMSO and DMFA was used to dissolve the detection molecule (BAPNA) and reduce the rate of trypsinogen conversion. Hence, the second reactor was engineered to allow for a fast detection of $[Tr]$ by converting BAPNA into pNA (detectable moiety at 405 nm) without any side processes.

(3) In the context of logic operations, it seems that an obvious question is how to connect two more logic gates based on the presented configurations to perform somewhat more examples of computations.

We can relate to the comment from the reviewer. Briefly, it is possible to have sequential logic gates when output from a reactor feeds the next reactor with $[Tr]$. Our method of CSTR fabrication, in fact, does not restrict to a sequence of two reactors but also allows for the design of spatially-coupled CSTRs of other configurations (serial, parallel, arrays). It will require two conditions: supply of the same reactants further down the chain (but without $[Tr]_0$), and elimination of significant portion of the liquid from previous reactors to avoid buildup of the flow and concentration catalytic ions. Our intentions, however, are not to connect logic gates. In our future studies, we will compare various configurations and examine the capacity of autocatalytic processes to perform (primitive) nonlinear operations in flow, including the introduction of spike-driven conditions (a method commonly used in neuromorphic processing). In this regard, the XOR-gate can be considered as a primitive nonlinear operation [Chen, T. *et al. Nature*, 577, 341-345 (2020)]. The latter sentence was added to the manuscript:

- *Page 5, line 48:* Additionally, a trypsin inhibitor was incorporated into the network to create a bistable system capable of long-term depression of the input and history dependency in the output (*i.e.*, characteristics for neuromorphic behavior). In this regard, the XOR-gate can be considered as a primitive nonlinear operation³⁶. Building on this foundation, future work could include the introduction of spike-driven conditions to perform other nonlinear operations under out-of-equilibrium conditions.⁹
- *Additional reference:* 36. Chen, T. *et al. Classification with a disordered dopant-atom network in silicon Nature*, 577, 341-345 (2020).

(4) Could the authors clarify the meaning of "Bistability in temporal logic operations"? What are the characteristics of the two stable states, and how the coexistence of these states can be observed?

We appreciate the questions of reviewer and apologize for lack of clarity with regards to the subheading "Bistability in temporal logic operations". We reported that "the response became bistable (page 5, line 26)" and this phenomenological observation is supported by our model but we didn't include the prediction of the parameter space wherein co-existence of such states can be observed. We are exploring the control over stability of steady states in the presence of metal ions in separate work.

In this section, we showed that the autocatalytic system can be maintained at a low trypsin concentration by introducing an inhibitor (Soybean Trypsin Inhibitor) in the network. The incorporation of the inhibitor enables a stable and low steady state, as Tr becomes inactive, but does not change the stability of the high steady state, which is supported by autocatalytic production of Tr . Without the inhibitor, the low steady state has to be stabilized by continuously depleting Tr using flow, which leads to an unstable steady state.

We made the following changes:

- Page 5, line 7: Increasing the control over Bistability in temporal logic operations
- Page 5, line 11: The use of an external component such as an inhibitor for thresholding provides further control over the network properties. Without the inhibitor, the low steady state was stabilized by continuously depleting *Tr* using flow, which leads to an unstable steady state. The incorporation of the inhibitor enables a stable and low steady state, as *Tr* becomes inactive, but does not change the stability of the high steady state, which is supported by autocatalytic production of *Tr*.³⁰
- Page 5, line 48: Additionally, a trypsin inhibitor was incorporated into the network to create a bistable system capable of long-term depression of the input and history dependency in the output (*i.e.*, characteristics for neuromorphic behavior).

(5) In the conclusions, the authors state, "We envision that this work will offer novel strategies to exploit the programmability of small and simple chemical reaction networks for building artificial systems with responsive, adaptive, and other life-like properties characteristic for intelligent systems." What are the main steps of this novel strategy, and in what type of systems can it be applied?

We appreciate the questions of reviewer, and gladly take this opportunity to elaborate how we foresee follow-up studies based on this work. This work can be extended with perturbation studies in a single CSTR but also extended to configurations comprising multiple bistable systems in series-, parallel- or array-coupled CSTRs. As mentioned above, in our answer to comment 3, such studies allow us to elucidate the capacity of autocatalytic reactions to perform (primitive) nonlinear operations in flow. The implementation of different configurations will be a crucial next step is to determine how exactly the states in such molecular systems can be changed, retained, and used to build responses that are more complex than those shown in the current manuscript. The strategy to systematically increase the number of collective feedback loops in the system is based on the concept that interconnected feedback loops could bestow CRNs with intelligent behavior (*i.e.*, the capacity to perceive and retain information, and apply it towards adaptive behavior).

We incorporated the following sentence in the conclusions of the manuscript:

- Page 6, line 2: The implementation of different configurations of multiple autocatalytic systems (based on series-, parallel- or array-coupled CSTRs) will be a crucial next step to systematically increase the number of collective feedback loops in the system. It will provide the potential to bestow CRNs with the capacity to perceive and retain information and apply it towards adaptive behavior. Hence, we envision that this work will offer novel strategies to exploit the programmability of small and simple chemical reaction networks for building artificial systems with responsive⁴¹, adaptive⁴², and other life-like properties² characteristic for intelligent systems.

Reviewer #2:

The manuscript "Exploring the Programmability of Autocatalytic Chemical Reaction Networks," written by Dmitrii V. Kriukov, Jurriaan Huskens, and Albert S. Y. Wong, is concerned with the application of trypsin catalysis as a chemical logic gate. The subject is interesting but not new because the first chemical logic gates were described 40 years ago. According to my knowledge, the application of trypsin-catalyzed reaction for chemical information processing is a new element in the study. The logic gates are useful when concatenating them into more complex information processing devices. This does not seem easy within the presented approach because concentrations of metal ions are used as the gate input, but the output is extracted from time-dependent trypsin concentration $[Tr](t)$. This means that connecting two gates requires a device (presumably electronic) that analyzes $[Tr](t)$ and translates the result to the ion pump rates of another CSTR. Moreover, the results of Fig. 4 suggest that a gate can make a few operations per hour, which is rather slow to attract industrial attention. Therefore, considering the limited practical applicability of the presented medium and narrow field of study, I do not recommend publication in a journal focused on a wide audience. The results are better suited for a specialized journal focused on natural or unconventional computing. In my opinion, the present version of the manuscript is poorly written and does not include all the information needed to understand what was done. Here are some remarks on manuscript fragments that must be improved before it is submitted to another journal.

The reviewer acknowledges that “the application of trypsin-catalyzed reaction for information processing is a new element in the study” but expresses their concerns about the novelty with regards to existing work based on chemical logic gates and the practical applicability of our approach. We gladly provide our view on these aspects in this rebuttal and have elaborated the conclusions as a result.

First, the reviewer correctly points out that our system, or any chemical system for that matter, cannot compete with the state-of-the-art technologies in computing. Among other reasons, the time scales for transitions between states based on molecular interactions (in our case hours) are nowhere near those that are based on electronic or photonic interactions (fractions of a second). To clarify, our intentions are not to connect logic gates as suggested by the reviewer’s comment nor to compete with, emulate, or mimic methods in traditional computing.

We have added new sections to the introduction to emphasize a connection between autocatalytic and neuromorphic computational systems as follows:

- Page 1, Line 30: That is, they have the ability to perceive information and retain it as knowledge to execute complex tasks¹³, highlighting how inherent analogue processes can enable biological computation. Opportunities in rethinking the design of neuromorphic systems lie in using analogue and approximate computing.^{4,17}
- Additional reference: 17. Valiant, L., Probably Approximately Correct: Nature's Algorithms for Learning and Prospering in a Complex World. 2013.

This manuscript aims to set up a practical experimental foundation to use autocatalytic reactions, and chemical reaction networks in general, to perform (primitive) nonlinear operations. In this regard, the XOR-gate can be considered as a primitive nonlinear operation [Chen, T. *et al. Nature*, 577, 341-345 (2020)].

- Page 5, line 48: Additionally, a trypsin inhibitor was incorporated into the network to create a system capable of long-term depression of the input and history dependency in the output (*i.e.*, characteristics for neuromorphic behaviour). In this regard, the XOR-gate can be considered as a primitive nonlinear operation³⁶. Building on this foundation, future work could include the introduction of spike-driven conditions to perform other nonlinear operations under out-of-equilibrium conditions.⁹
- Additional reference: 36. Chen, T. *et al.* Classification with a disordered dopant-atom network in silicon *Nature*, 577, 341-345 (2020).

While sequential logic gates is not within the scope of this work, we wish to state that it is possible to perform such experiments. Our method of CSTR fabrication, in fact, does not restrict to a sequence of two reactors but also allows for the design of spatially coupled CSTRs of other configurations (serial, parallel, arrays) as well. The emphasis in our future work, however, is not to connect reactors to combine gates. The implementation of different configurations will be a crucial next step is to determine how exactly the states in such molecular systems can be changed, retained, and used to build responses that are more complex than those shown in the current manuscript. The strategy to systematically increase the number of collective feedback loops in the system is based on the concept that interconnected feedback loops could bestow CRNs with intelligent behavior (*i.e.*, the capacity to perceive and retain information, and apply it towards adaptive behavior). We incorporated the following sentence in the conclusions of the manuscript:

- *Page 6, line 2:* The implementation of different configurations of multiple autocatalytic systems (based on series-, parallel- or array-coupled CSTRs) will be a crucial next step to systematically increase the number of collective feedback loops in the system. It will provide the potential to bestow CRNs with the capacity to perceive and retain information and apply it towards adaptive behavior. Hence, we envision that this work will offer novel strategies to exploit the programmability of small and simple chemical reaction networks for building artificial systems with responsive, adaptive, and other life-like properties characteristic for intelligent systems.

Second, the observation that the range of logics is controllable based on a kinetic parameter sets this work apart from “the first chemical logic gates described 40 years ago (reviewer comment line 3)”: Our system can accept instructions to perform a range of logic operations and demonstrates that autocatalytic reactions can be programmable. Furthermore, we demonstrated that *i)* the output signal can be reset, *ii)* the method of creating logic gates is not restricted to Nd^{3+} , and ions can be combined to change the phase space, and *iii)* Boolean functions can be readily tuned and modeled mathematically.

We incorporated the following section in the main text to clarify the novelty with regards to chemical logic gates as follows:

- *Page 5, line 40:* We ~~showed~~ demonstrated that trypsin autocatalysis can be controlled to emulate polynomial functions with different degrees and Boolean functions with various outcomes (*e.g.*, AND, OR, XOR, NAND, NOR), demonstrating that autocatalytic reactions can be programmable. Furthermore, we demonstrated that *i)* the output signal can be reset, *ii)* multiple ions can be combined to change the phase space, and *iii)* Boolean functions can be readily tuned and modelled mathematically, and *iv)* the history-dependent nature of autocatalysis in flow can retain information. That a range of logic are tunable and controllable based on a kinetic parameter sets this work apart from traditional molecular logic gates⁴⁰.
- *Additional reference:* 40. Andréasson, J. & Pischel, U. Molecules with a sense of logic: a progress report. *Chem. Soc. Rev.*, 44, 1053-1069 (2015).

Third, the comment “the present version of the manuscript is poorly written and does not include all the information needed to understand what was done” seems to relate to the description of the mathematical model (*i.e.*, the “manuscript fragments that must be improved”). We provided our answer below, wherein we clarified, and rectified, the possible confusion that came from our distribution of details on the model (supplementary information vs. supplementary materials).

The authors refer to a mathematical model that is not well described. The model variables should be listed. I also expected to find the kinetic equations with flow terms included. The article SI just gives the set of reactions and comments on the reactions.

We can relate to the reviewers’ requests (*i.e.*, to list the model variables, incorporate kinetic equations, and provide an SI that gives more than the set of reactions). However, we do wish to point out that this assessment is not entirely accurate. To clarify, the mathematical model was provided as a set of ready-to-

use scripts in supplementary materials. We described how the model is built and used in our work (with a reference to both supplementary information and supplementary information) in the methods section of the main text (page 13, line 32-43). Nevertheless, we extended the Supplementary Information per request of the reviewer:

- *Supplementary Information, Page 2:* We incorporated a process scheme (new Supplementary Scheme 1) to explain the modelling workflow.
- *Supplementary Information, Page 3:* New section (Equations for the model) has been added to elaborate on equations used for the model.
- *Supplementary Information, Page 6-7:* Supplementary table is updated and split in two tables to clarify the chemical meaning of the processes reflected in the new section 'Equations for the model'.

The mathematical symbols are used without their precise definition in the text. What is $[A]_{\text{feed}}$ (Fig.3 caption)? According to what I read, the symbol $[A]_0$ denotes the concentration of reagent A at the beginning of the experiment ($t=0$). How shall I understand Fig. 3a, where $[Nd^{3+}]_0$ is plotted as a function of time, and its caption says that $[Nd]_0$ was changed?

The reviewer correctly pointed out that that the assignment of symbols is inconsistent in the manuscript. There are two occasions in the main text where we did not explicitly write that the initial concentration of the ions can be abbreviated with brackets and the zero as subscript. We have corrected this as follows:

- *Page 2, line 41:* **Fig. 2b-c** show that $[Tr]$ increases until it saturates as **the initial concentration of Ca^{2+} and La^{3+} ($[Ca^{2+}]_0$ and $[La^{3+}]_0$)** are increased. *ii)* Nd^{3+} , in contrast, can accelerate and decelerate the rate of autocatalysis. **Fig. 2d** shows that the $[Tr]$ increases when **the initial concentration of Nd^{3+} ($[Nd^{3+}]_0$)** increases from 0 to 0.3 mM.

In another section, we corrected:

- *Page 7, line 11:* **$[Tr]$ denotes concentration of Tr ; t and τ denote time and residence time, respectively.**

Specifically for Fig. 3a, $[Nd^{3+}]_0$ is changed over time using our flow setup (Methods section, page 13, line 11). In our setup, we use stock solutions of the components of the system and store them in glass syringes, which content is connected by polytetrafluoroethylene tubing's to the CSTR. The low-pressure pumps are used to independently control the syringes and feed each stock solution into the CSTR. The feeding concentrations for the components (Tg , Tr , I , and X) are abbreviated as $[Tg]_{\text{feed}}$, $[Tr]_{\text{feed}}$, $[I]_{\text{feed}}$, $[X]_{\text{feed}}$, respectively. Initial concentrations ($[Tg]_0$, $[Tr]_0$, $[I]_0$, $[X]_0$) reported in the figure captions and legends are calculated based on the feeding concentration and flow rate of each component. Hence, when we changed the initial concentration of $[Nd]_0$, we meant that we increased the inflow rate of a stock solution with Nd^{3+} . At the same time, we decreased the supply of the buffer to balance the overall flowrate.

We have extended the method section with an explanation of the experimental setup:

- *Page 14, line 22:* **In our setup, we use stock solutions of the components of the system and store them in glass syringes, which content is connected by polytetrafluoroethylene tubing's to the CSTR. The low-pressure pumps are used to independently control the syringes and feed each stock solution into the CSTR. The feeding concentrations for the components (Tg , Tr , I , and X) are abbreviated as $[Tg]_{\text{feed}}$, $[Tr]_{\text{feed}}$, $[I]_{\text{feed}}$, $[X]_{\text{feed}}$, respectively. Initial concentration ($[Tg]_0$, $[Tr]_0$, $[I]_0$, $[X]_0$) reported in the figure captions and legends are calculated based on the feeding concentration and flow rate of each component. The total flow rate is kept constant throughout the experiments.**

In some places (for example, p.3, lines 13/14), the numerical values of variables are given without the corresponding units.

We found two instances and corrected them as follows:

- *Page 3, line 28:* In accordance with our batch experiments, a maximum in $[Tr]$ could indeed be reached when the input satisfied the condition $0.20 \leq [Nd^{3+}]_0 \leq 0.40$ mM.
- *Page 13, line 45:* Proteins were stained by Bradford technique, namely, 2g of Coomassie Blue R250 in 1 L of 10% acetic acid and 40% ethanol solution.

Reviewer #3:

This is an interesting paper, with some thought provoking ideas. The authors use metal ions to control the rate of autocatalysis with trypsin and this feature is used to program the output response of the autocatalyst in a flow reactor. They mention implications in terms of eg neuromorphic computing. Some of the manuscript could be more clearly written, and I am unclear on the interpretation of results in some parts.

We appreciate that the reviewer sees the manuscript as interesting and thought provoking. We agree that the clarity in the manuscript can be improved and thank the reviewer for their constructive comments.

In the abstract It is not immediately clear what is meant by “reconfigured in a programmable manner”; this could do with clearer explanation. What are you reconfiguring? There has been quite a lot done with computing in eg reaction diffusion systems; using light, chemical inputs etc to implement Boolean logic and image processing – can it be better pinpointed what is the difference here?

The reviewer is correct in their request for clarity with regards to reconfiguration and programmability, and the difference of our systems towards other existing work. Reconfigure refers to the rewiring of feedback loops [Kholodenko, B. N. *Nat. Rev. Mol. Cell Biol.* 7, 165–176 (2006)]. This reference was already provided and we have included the citation as follows:

- Page 1, Line 15: How this chemical analogue of a positive feedback mechanism can be controlled reconfigured in a programmable manner is, however, unknown.
- Page 1, Line 35: How feedback loops in CRNs can be tuned, scaled, and reconfigured (or, rewired)²⁸ in a programmable manner (i.e., accept instructions to perform a range of tasks, rather than just one), however, remains elusive.

To clarify our work in this context, we showed that the intrinsic rates in metal ion-induced autocatalysis (scheme 1) can be “reconfigured” when multiple ions are present in the same solution. The mapping of a chemical output onto polynomial functions as demonstrated in Fig. 3 underscores the flexibility of using metal ions in reconfiguring the autocatalytic network.

Accordingly, we have changed the manuscript as follows:

- Page 4, Line 9: The mapping of a chemical output onto polynomial functions as demonstrated here underscores the flexibility of using metal ions in controlling (or, reconfiguring) the autocatalytic network.

We also showed that “tuning” was achieved by changing an operation as a function of the rate of the autocatalysis reaction. More specifically, we changed the input concentration of Nd^{3+} , which affected the steady state concentration of *Tr*. The same input sequence ($[\text{Nd}^{3+}]_{0|0}$; $[\text{Nd}^{3+}]_{1|0}$; $[\text{Nd}^{3+}]_{1|1}$; $[\text{Nd}^{3+}]_{0|1}$) therefore allowed a range of different logic operations. That a diverse set of functions can be chosen by sets of initial conditions as presented in Fig. 4 demonstrates that autocatalytic reactions can be “programmable”. We incorporated the following section in the main text to clarify the novelty with regards to chemical logic gates:

- Page 5, line 35: We showed demonstrated that trypsin autocatalysis can be controlled to emulate polynomial functions with different degrees and Boolean functions with various outcomes (e.g., AND, OR, XOR, NAND, NOR), demonstrating that autocatalytic reactions can be programmable. Furthermore, we demonstrated that *i)* the output signal can be reset, *ii)* multiple ions can be combined to change the phase space, and *iii)* Boolean functions can be readily tuned and modelled mathematically, and *iv)* the history-dependent nature of autocatalysis in flow can retain information. That a range of logic are tuneable and controllable based on a kinetic parameter sets this work apart from traditional molecular logic gates⁴⁰.
- Additional reference: 40. Andréasson, J. & Pischel, U. Molecules with a sense of logic: a progress report. *Chem. Soc. Rev.*, 44, 1053-1069 (2015).

Some explanation of a neuromorphic system and the desired features of such a system in the introduction would be useful for the more general audience.

Biological neural computation is inherently analogue and stochastic. Most neuromorphic hardware design first define hardware and subsequently develop the algorithm and application. Despite tremendous progress in neuromorphic processors, opportunities in rethinking the design neuromorphic systems lie in using analogue, approximate and mixed-signal computing [Schuman, C. D. *et al. Nat. Comput. Sci.* 2, 10–19 (2022)]. We consider that the nonlinear kinetics of autocatalytic reactions could suggest chemical equivalents to electronic artificial neurons. [Arcadia, C. E. *et al. Chem. Sci.* 12, 5464–5472 (2021)].

We have added new sections to the introduction to emphasize a connection between autocatalytic and neuromorphic computational systems as follows:

- Page 1, Line 30: That is, they have the ability to perceive information and retain it as knowledge to execute complex tasks¹³, highlighting how inherent analogue processes can enable biological computation. Opportunities in rethinking the design of neuromorphic systems lie in using analogue and approximate computing.^{4,17}
- Additional reference: 17. Valiant, L., Probably Approximately Correct: Nature's Algorithms for Learning and Prospering in a Complex World. 2013.
- Page 1, Line 40: Autocatalysis — a chemical process in which the product acts as the catalyst for its own formation — is at the core of the mechanisms of the aforementioned examples of CRNs, facilitating a fast and nonlinear response to stimuli from the environment²⁸. The nonlinearity of artificial neurons can, essentially, be resembled by the kinetic feature of autocatalytic reactions.¹⁰

Figure 2. I'm not sure that fig 2d data can be interpreted as Nd is an "inhibitor" in the traditional sense, since without the metal, autocatalysis doesn't proceed. Technically, it results in a lower rate only as the concentration of Nd is increased above a threshold value. Possibly there is some initial binding of Nd to Tr to form an active state but further binding results in an inactive state – similar to binding of acid to enzymes. Nevertheless, the experimental results are still the same, it is just interpretation that I am unclear on.

The reviewer is correct. We agree that the term “inhibition” should not be used to describe the referred experimental results. Therefore, we change the term “inhibiting” to “slowing down” in the main text as suggested by the reviewer.

- Page 3, Line 5: Based on these batch experiments, we assume that *i*) autocatalysis cannot occur without any of the metal ions; *ii*) binding of the ions to *Tr* (depicted as $[TrX]$) can ‘activate’ autocatalysis, changing the apparent rate constant for the conversion of *Tg* into *Tr* (indicated by k_x); *iii*) binding of Nd^{3+} to *Tg* (depicted as $[Nd^{3+}Tg]$) can ‘de-activate’ autocatalysis, slowing down the conversion of *Tg* into *Tr*.

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

The authors answered my questions correctly and corrected the related parts in the modified version of the manuscript. In the revised version, it is more apparent that their intention is not to create a chemical analog of a digital computer with logic gates but to mimic biological computation by designing a neuromorphic like system. In the introduction, the authors should write a few more sentences about the basics of biological computation and neuromorphic systems to help readers become more familiar with these topics. After this minor revision, I suggest accepting the manuscript.

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We thank the reviewer for their positive comments on our revised manuscript and their suggestion to accept this version for publication. We acknowledge that few more sentences about the basics of biological computation and neuromorphic systems will further help readers become more familiar with these topics. Accordingly, we incorporated a section in the beginning of the introduction:

- *Page 1, line 25:* Living systems, from a simple slime mold to a more complex Venus flytrap to a highly sophisticated cephalopod, exhibit intelligent behavior¹. That is, they have the ability to perceive information and retain it as knowledge to execute complex tasks². The underlying processes by which decisions are made are fundamentally different from conventional (digital) computing, as they rely on complex, decentralized networks of biochemical reactions that operate in parallel². Artificial neuromorphic systems take inspiration from natural and biological processes³, particularly from how neurons process and transmit signals in the brain⁴. Rethinking the design of neuromorphic systems do not necessarily restrict to neural architectures⁵ and opportunities may lie in using analogue and approximate computing that arise from purely (bio)chemical processes⁶.
- *Additional reference:* 3. Castro, L. N. de. Fundamentals of natural computing: an overview. *Phys. Life Rev.* **4**, 1–36 (2007).