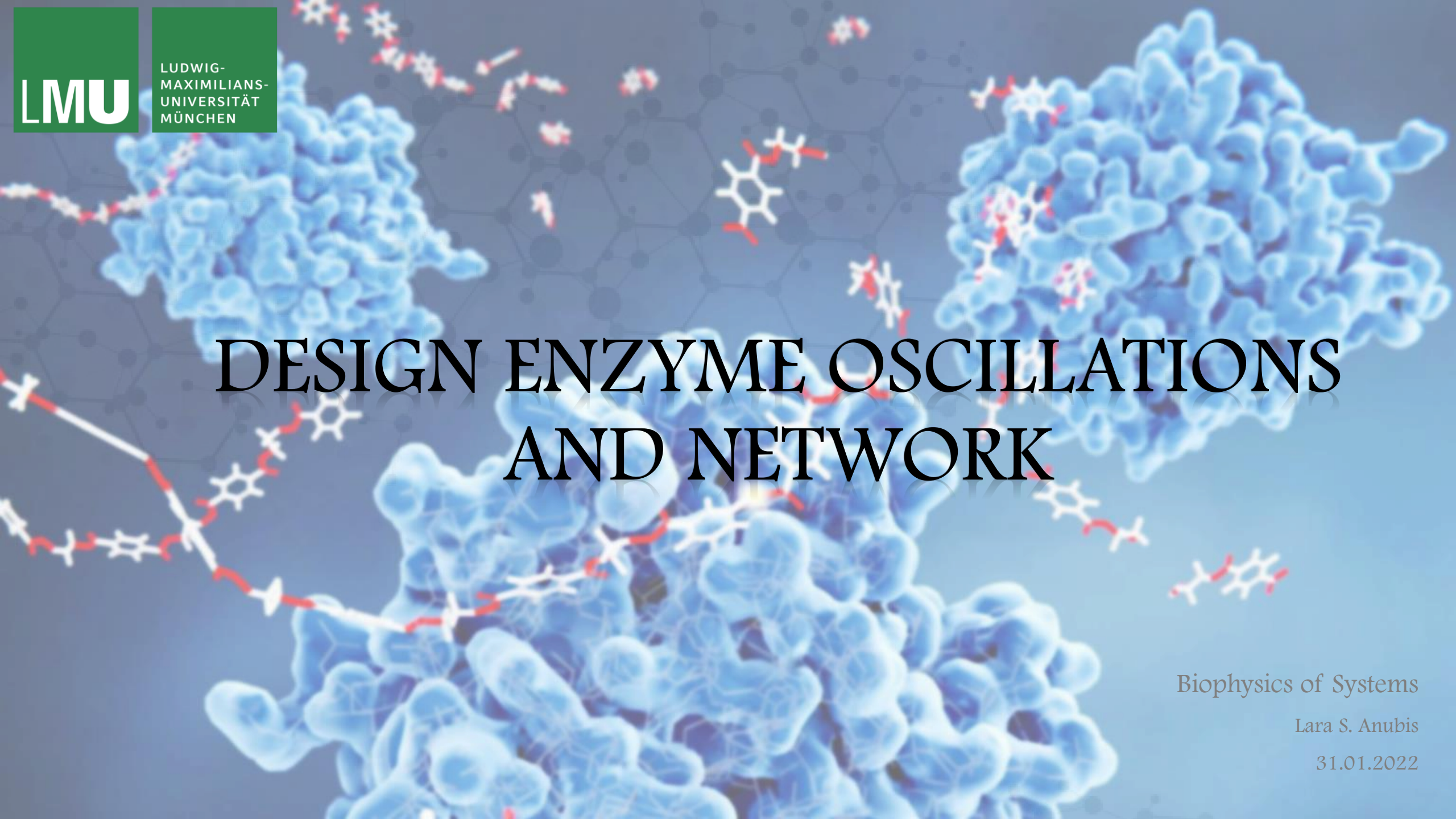


LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

DESIGN ENZYME OSCILLATIONS AND NETWORK

Biophysics of Systems

Lara S. Anubis

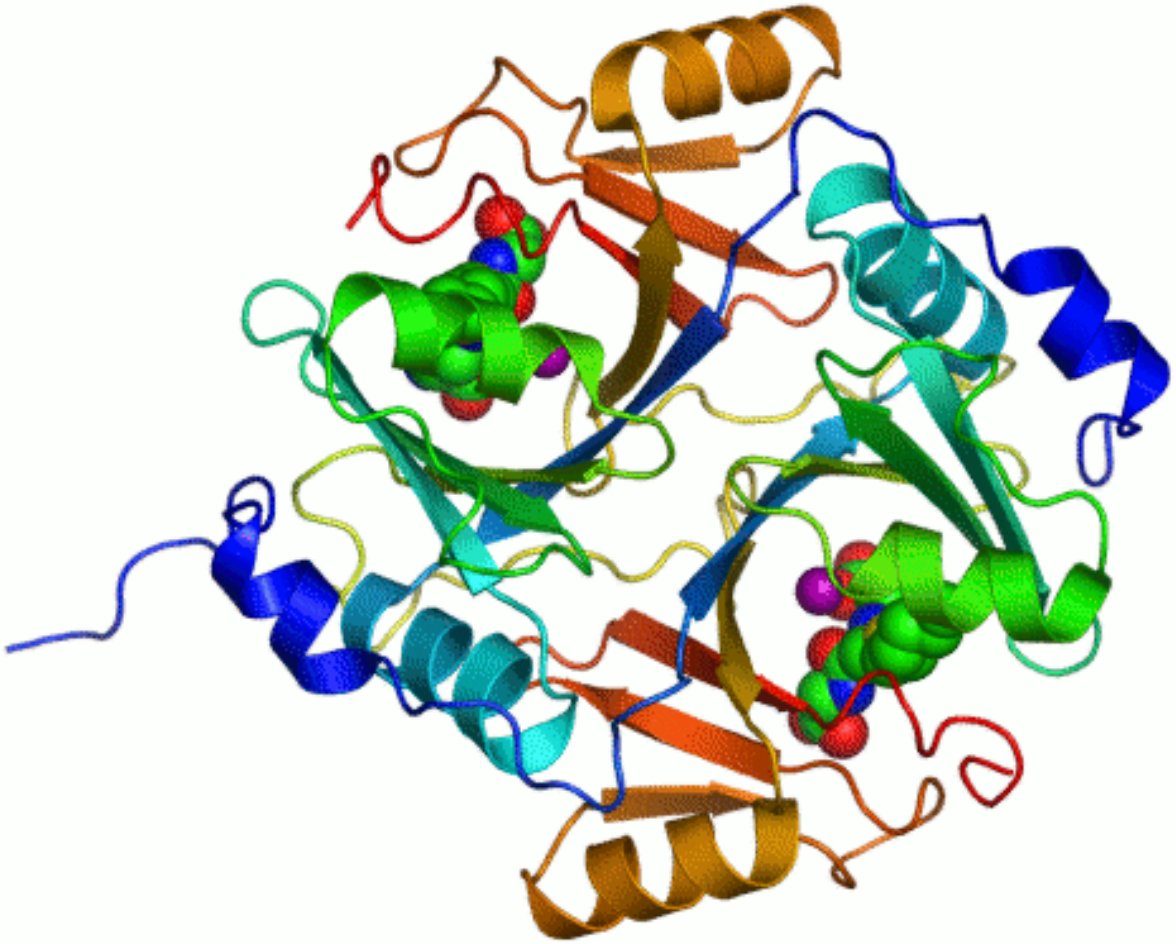
31.01.2022

Content

- Enzyme
- Chemical reaction networks (CRNs)
- Belousov–Zhabotinsky (BZ) oscillations
- PAPER 1: Rational design of functional and tunable oscillating enzymatic networks
- PAPER 2: Programmable chemical reaction networks: emulating regulatory functions in living cells using a bottom-up approach

Enzyme

= protein that catalyzes a chemical reaction

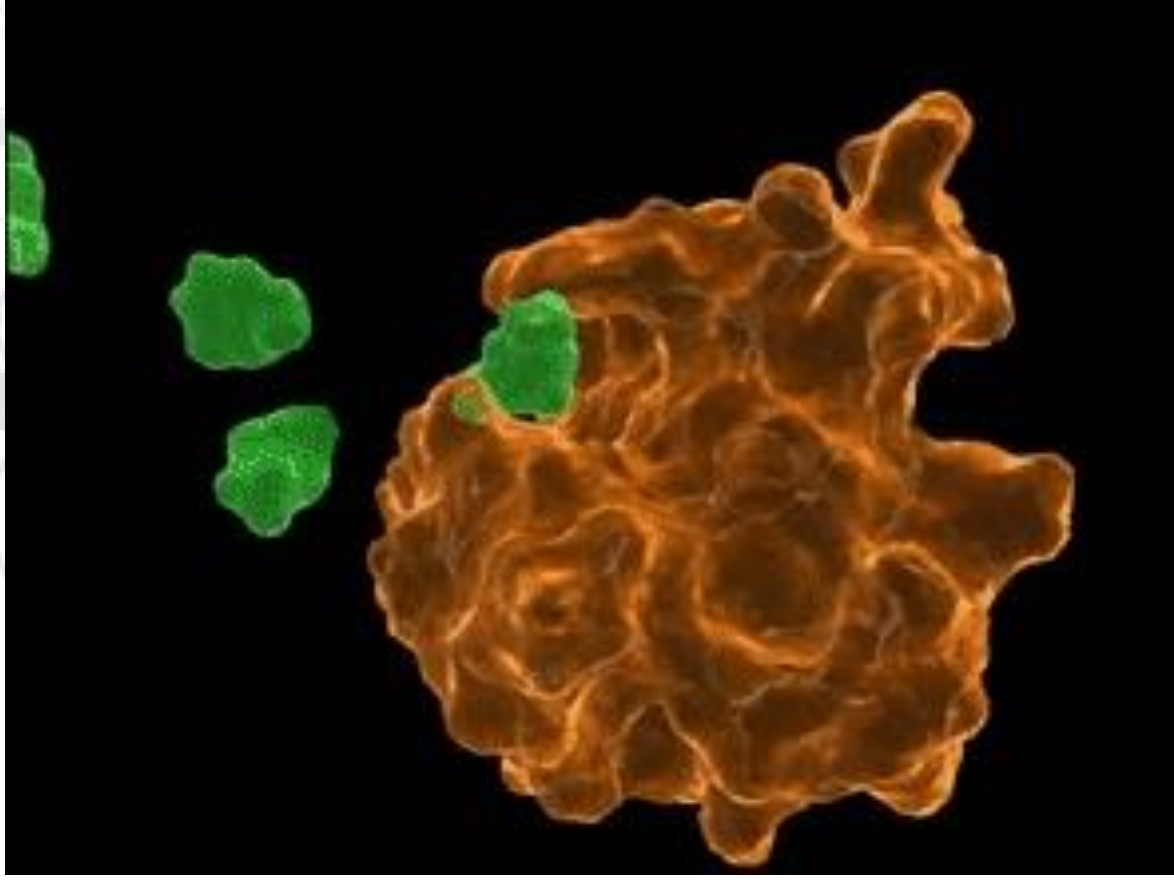


GIF Source: https://commons.wikimedia.org/wiki/File:GLO1_Homo_sapiens.gif

Enzyme

Tasks:

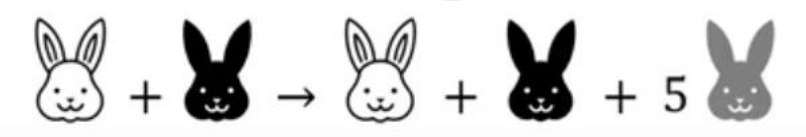
- Metabolism
- Protein synthesis
- Cell renewal
- Cell growth



GIF Source: https://www.mrdubuque.com/home/biodub-my-gifs-to-you-enzyme-reactions

Chemical reaction networks (CRNs)

- Chemical reaction networks are a widely used mathematical model in synthetic and system biology.
- set of species $\{X_1, X_2, \dots, X_N\}$
- Set of reactions: $\{Input\ Species\} \xrightarrow{\rho_r(x)} \{Output\ Species\}$
- $\rho_r(x)$: $propensity\ or\ rate\ at\ which\ raction\ r\ occurs$

- Species and reaction can take many forms:
- Traditional chemistry: $O_2 + 2H_2 \rightarrow 2H_2O$ Burning of Hydrogen
- Molecular biology: $G \rightarrow G + T$ A gene G produces a transcript T
- Abstract species and reactions: 

- Species: $\{X_1, X_2, \dots, X_N\}$
- Reactions: $\sum_i I_i^r X_i \xrightarrow{\rho_r(X)} \sum_i O_i^r X_i$
  - I_i^r: $Number\ of\ inputs\ of\ Species\ i\ in\ reaction\ r$
  - O_i^r: $Number\ of\ outputs\ of\ Species\ i\ in\ reaction\ r$
  - $Propensity\ \rho_r(X)$: $rate\ at\ which\ reaction\ r\ occurs$

- Deterministic Dynamics: $\frac{dX_i}{dt} = \sum_r (O_i^r - I_i^r)\rho_r(X)$

Belousov–Zhabotinsky (**BZ**) oscillations

- Sodium bromate
- Sulfuric acid
- Melonic acid

$$3\ CH_2(CO_2H)_2 + 4\ BrO^-_3 \rightarrow 4\ Br^- + 9\ CO_2 + 6\ H_2O$$

Source: https://www.youtube.com/watch?v=PpyKSRo8Iec

The BZ reaction was the first example of a class of inorganic chemical reactions that shows such out-of-equilibrium phenomena, e.g. bistability, and also oscillations and synchronization that are visible to the naked eye.

- Belousov was a Russian chemist who in 1956 discovered that some chemical reactions can oscillate. They can change from one state to another and back again repeatedly. He couldn't publish his paper because the journal said that that can't happened it's impossible and that he must have his experiment wrong. About 10 years later another Russian called Zhabotinsky refined the experiment to the point it was absolutely clear it really did work.

- To make Belousov–Zhabotinsky reaction we have four chemicals already mixed: sodium bromate, sulphuric acid and melonic acid. Mix them all together. Amazing pattern appears. That is called symmetry breaking.

ARTICLES
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nature chemistry

Rational design of functional and tunable oscillating enzymatic networks

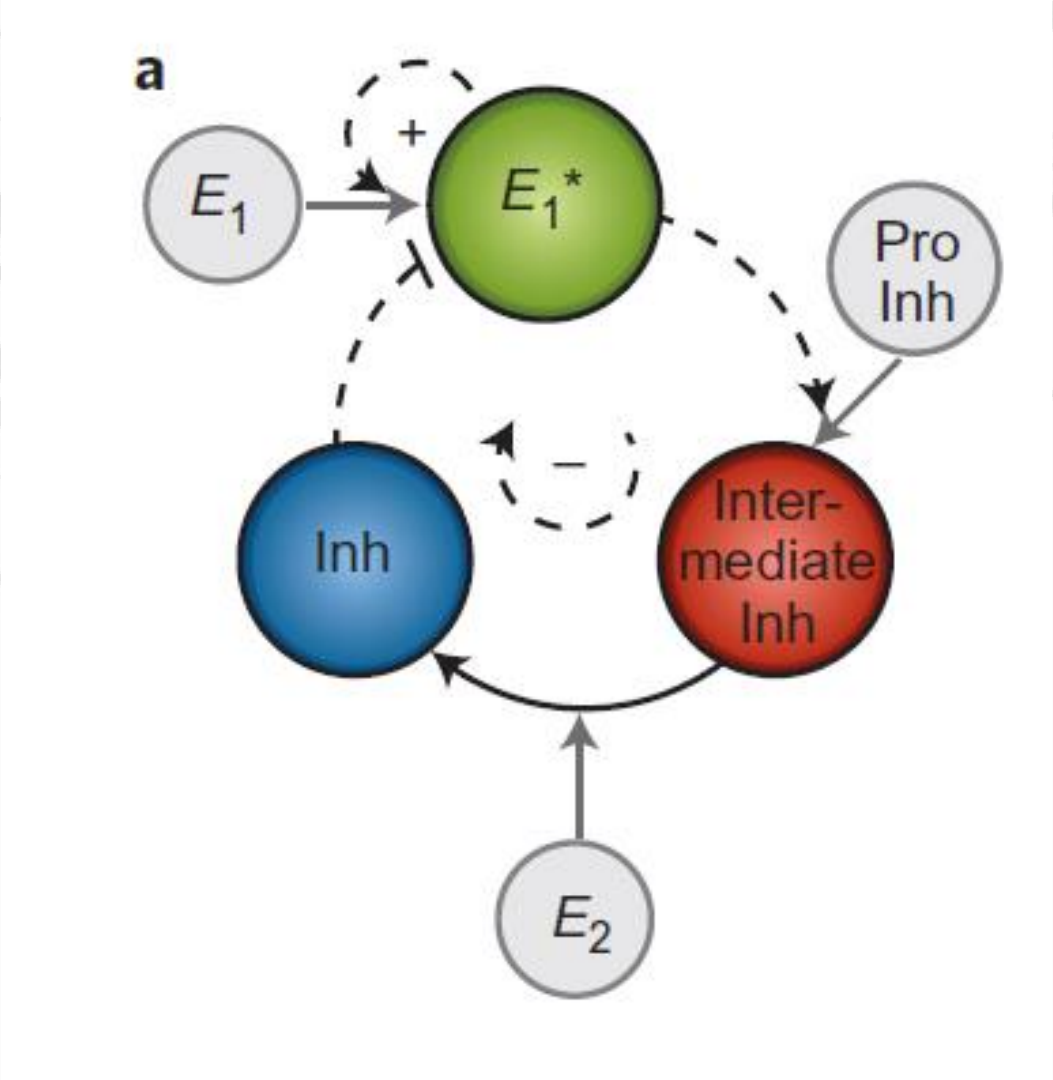
Sergey N. Semenov[1†], Albert S. Y. Wong[1†], R. Martijn van der Made[1], Sjoerd G. J. Postma[1], Joost Groen[1], Hendrik W. H. van Roekel[2], Tom F. A. de Greef[2] and Wilhelm T. S. Huck[1*]

Key points of the paper

- Spatiotemporal pattern formation
- Self-organizing systems
- IDEA: exploit the full power of chemical synthesis to construct CRNs tuned by small molecules approaching the tunability and functionality of living systems.
- Methodology based on enzymatic conversions of small molecules

Spatiotemporal = relating to both space and time
CRNs = Chemical Reaction Networks

Experimental assembly of a flow-based enzymatic oscillator



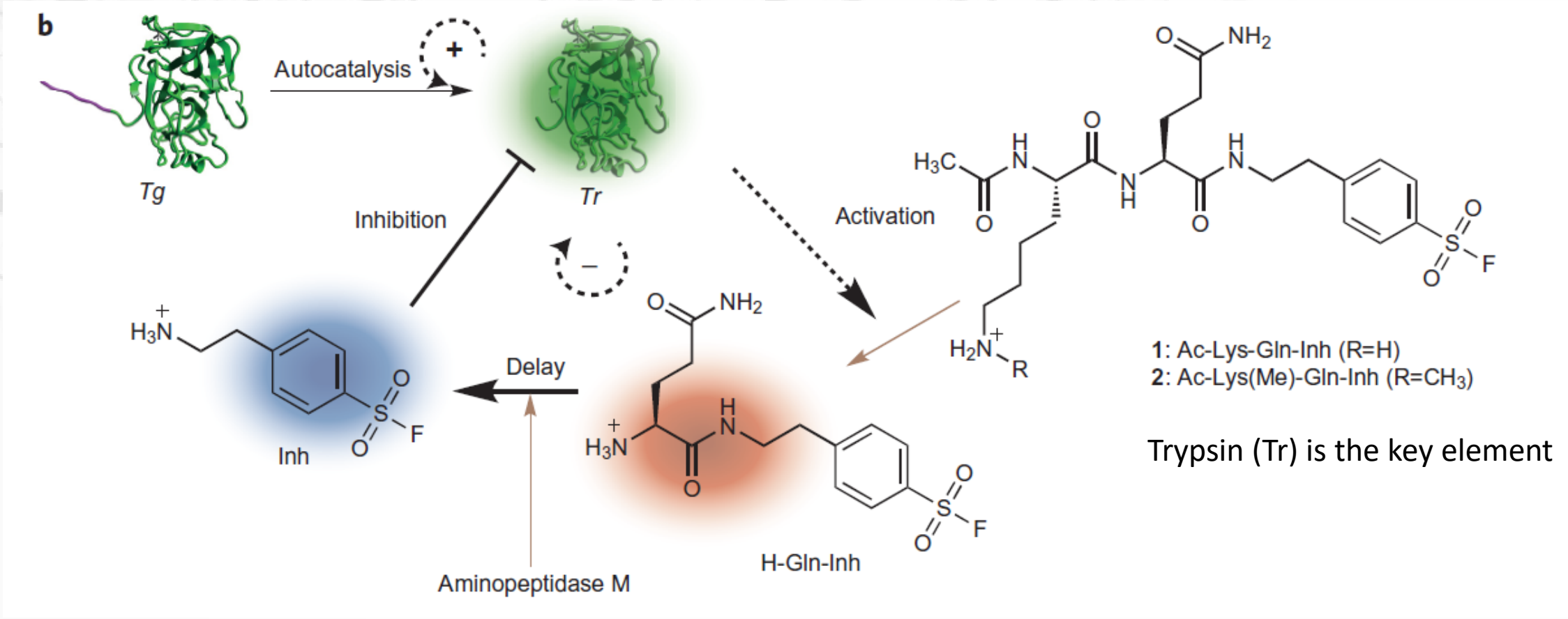
E_1 – enzymatically inactive
E_1^*- enzymatically active
E_2 – second enzyme

Source: Rational design of functional and tunable oscillating enzymatic networks, Sergey N. Semenov1, Albert S. Y. Wong, et al

This design of CRN is based on a time-delayed negative feedback topology, combined with a short positive feedback loop.

- **How it works:** Enzymatically inactive E1 is converted to active E1*. In a positive feedback loop, E1* catalyses its own formation. In addition, E1* catalyses the first sequence that unmasks an inhibitor of itself, with the second step being catalysed by a second enzyme E2. This two-step process constitutes a negative feedback loop. The combination of positive and negative feedbacks results in an oscillating system.

Detailed reaction diagram of the CRN

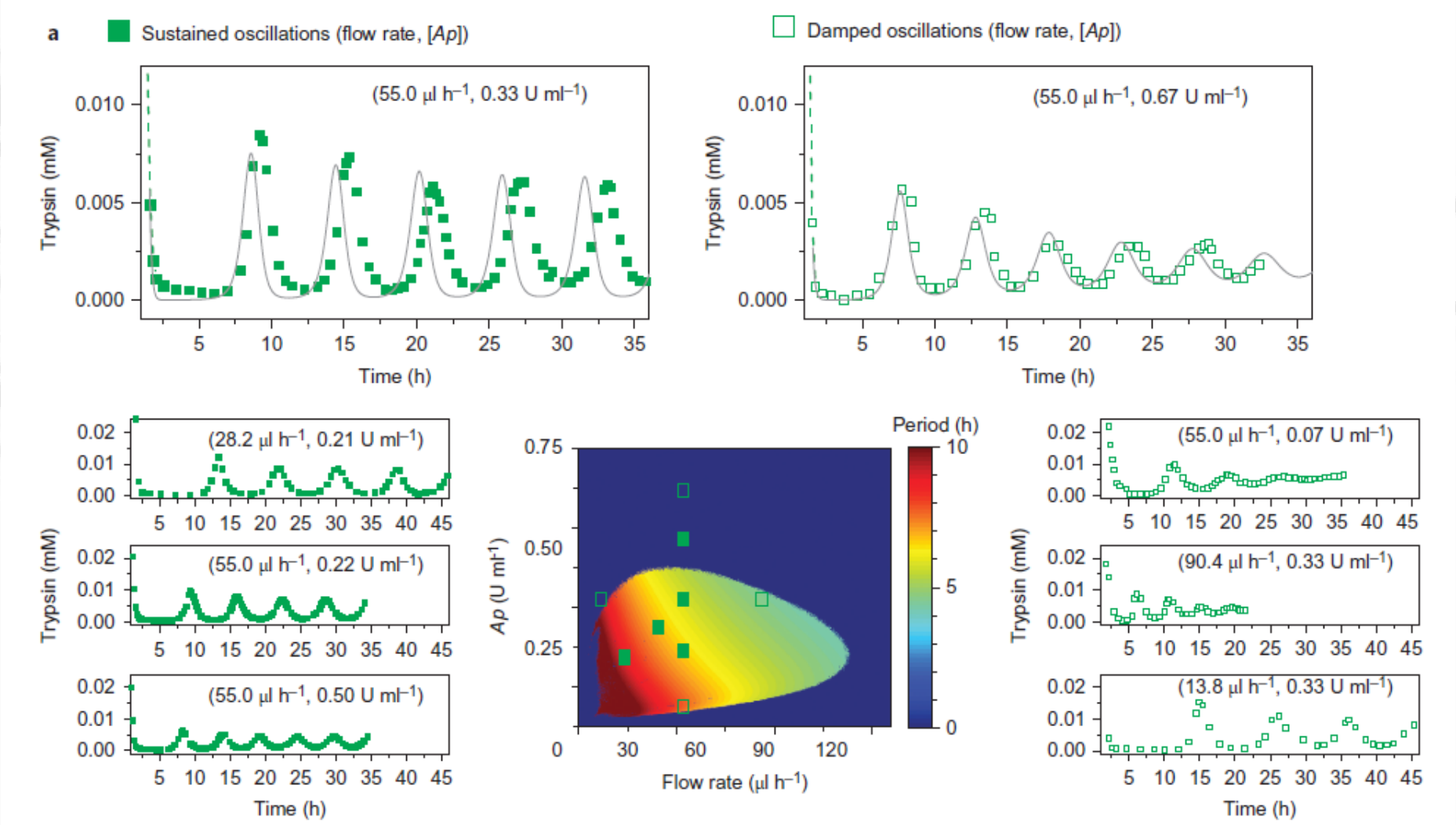


Trypsin (Tr) is the key element

Source: Rational design of functional and tunable oscillating enzymatic networks, Sergey N. Semenov1, Albert S. Y. Wong, et al

- To reduce design to practice, enzymes were selected whose activities can be modulated by small molecules.
- Trypsin (Tr) is the key element in this CRN, and positive feedback arises from the autocatalytic conversion of the enzymatically inactive trypsinogen (Tg) into Tr. To create the negative feedback loop, an active inhibitor must be formed as a result of the enzymatic activity of Tr.
- It is essential that the negative feedback resulting in Tr inhibition is delayed with respect to Tr production. We therefore split the negative feedback loop into two orthogonal steps, each amenable to rate-tuning. First, Tr cleaves the Lys residue of the proinhibitor, the N-terminus of which was acetylated to yield a well-soluble molecule and an endopeptidase substrate. Second, aminopeptidase M (Ap) cleaves an amino acid residue from the intermediate inhibitor, thereby activating the inhibitor.

Tunability and robustness of the enzymatic oscillator



[Ap]- aminopeptidase concentration

Source: Rational design of functional and tunable oscillating enzymatic networks, Sergey N. Semenov1, Albert S. Y. Wong, et al

Network for robustness—the persistence of sustained oscillations under external perturbations—by changing global parameters such as the overall flow rate or reaction temperature.

- The experiments in show the behaviour of the system as a function of [Ap] and flow rate for experiments carried out at 23 °C. The heat map shows the broad range of concentrations and flow rates for which sustained oscillations are obtained; outside this range the oscillations are damped and the system reaches a steady state. A further exploration of robustness was carried out by probing the temperature dependence of the CRN.

- Time courses in [Tr] corresponding to sustained and damped oscillatory behaviour for various values of the flow rate and [Ap] (in brackets). Solid lines represent model predictions using optimized parameters. Sustained and damped responses are mapped onto the (flow rate, [Ap]) plane.

Key takeaways

- Sustained oscillations provide a new, modular retrosynthetic approach
- Complex network topology by the chemical structures of small molecules
- The CRNs obtained are robust and maintain sustained oscillations
- Synthetic biology approach to the development of complex synthetic systems that operate according to the principles of life
- The CRNs are robust and maintain sustained oscillations within a certain range of global parameters

TUTORIAL REVIEW

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Cite this: *Chem. Soc. Rev.*, 2015, 44, 7465

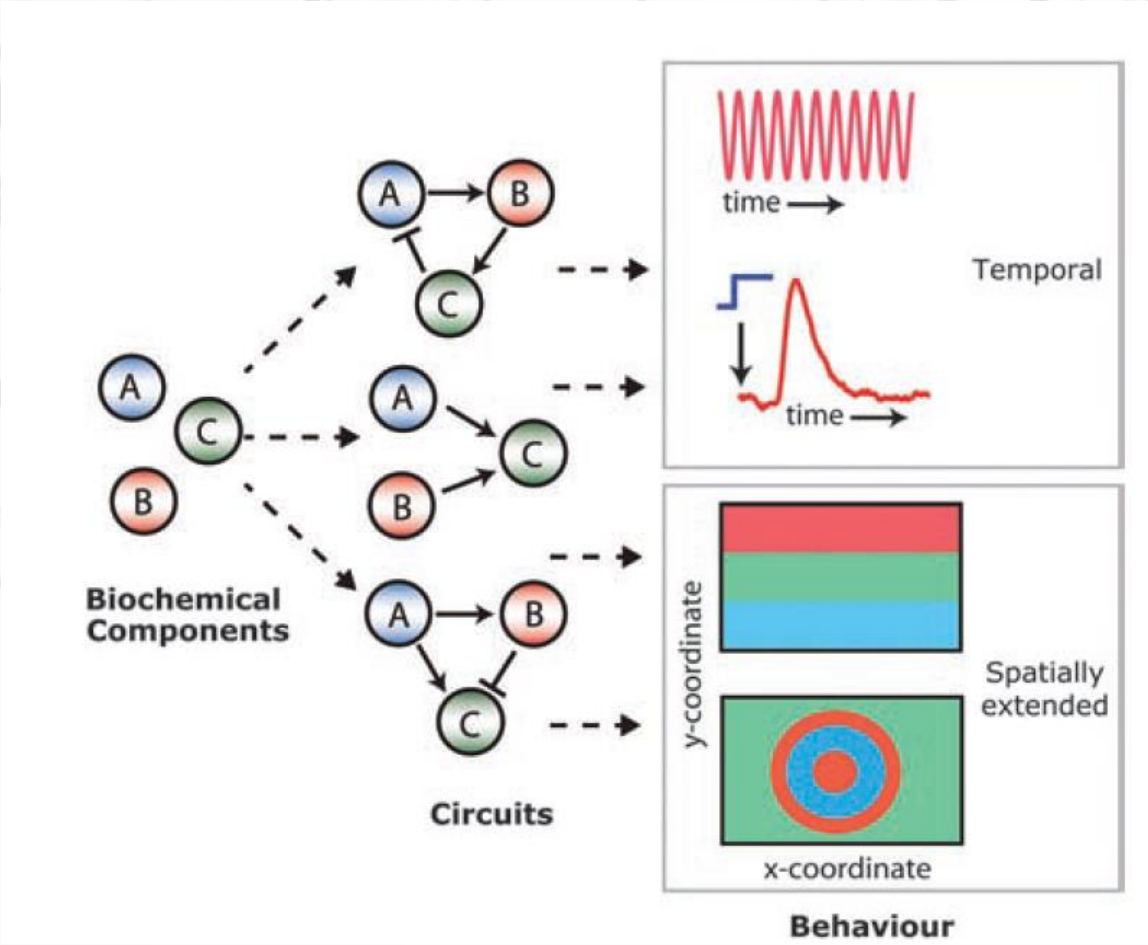
Programmable chemical reaction networks: emulating regulatory functions in living cells using a bottom-up approach

Hendrik W. H. van Roekel,†[ab] Bas J. H. M. Rosier,†[abc] Lenny H. H. Meijer,†[abc] Peter A. J. Hilbers,[ab] Albert J. Markvoort,*[ab] Wilhelm T. S. Huck*[d] and Tom F. A. de Greef*[abc]

Key points of the paper

- Biological responses are regulated by complex chemical reaction networks
- Key studies on inorganic CRNs
- Purified biochemical components
- Versatility of programmable biochemical reaction networks (BRNs) in analytical and diagnostic applications

Programmable cell-free biomolecular circuits



Cell-free circuits display self-organizing behaviors:

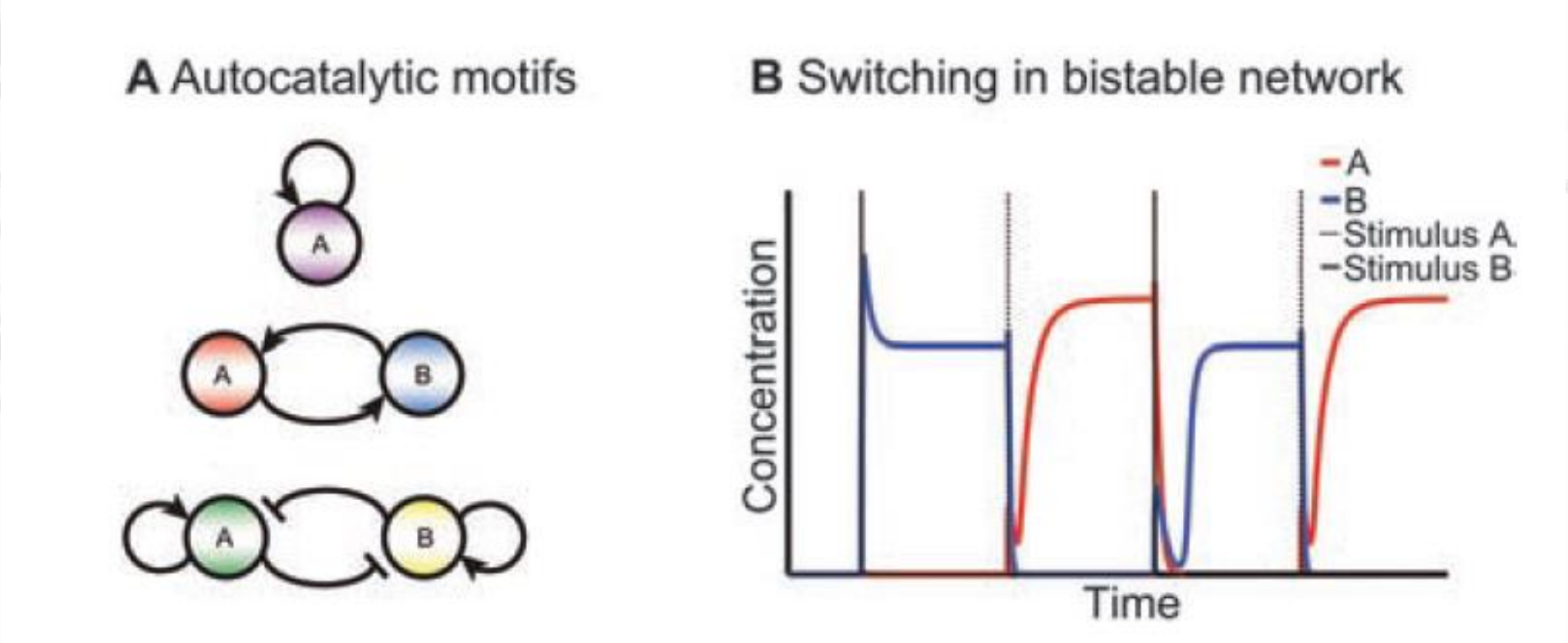
- signal transduction
- oscillations
- self-replication
- entrainment
- programmed pattern formation

Source: Programmable chemical reaction networks: emulating regulatory functions in living cells using a bottom-up approach, H.W.H. van Roekel, et al.

Programmable cell-free biomolecular circuits present a unique platform to systematically explore the molecular logic and physical design principles of regulatory networks in the living cell.

- One viable strategy to disentangle the complexity of cellular signalling networks is by applying a bottom-up approach, consisting of the construction of cell-free circuits that are able to display self-organising behaviours such as signal transduction, oscillations, self-replication, entrainment and programmed pattern formation.
- A **bottom-up** approach is the piecing together of systems to give rise to more complex systems, thus making the original systems sub-systems of the emergent system.

Autocatalysis and bistability

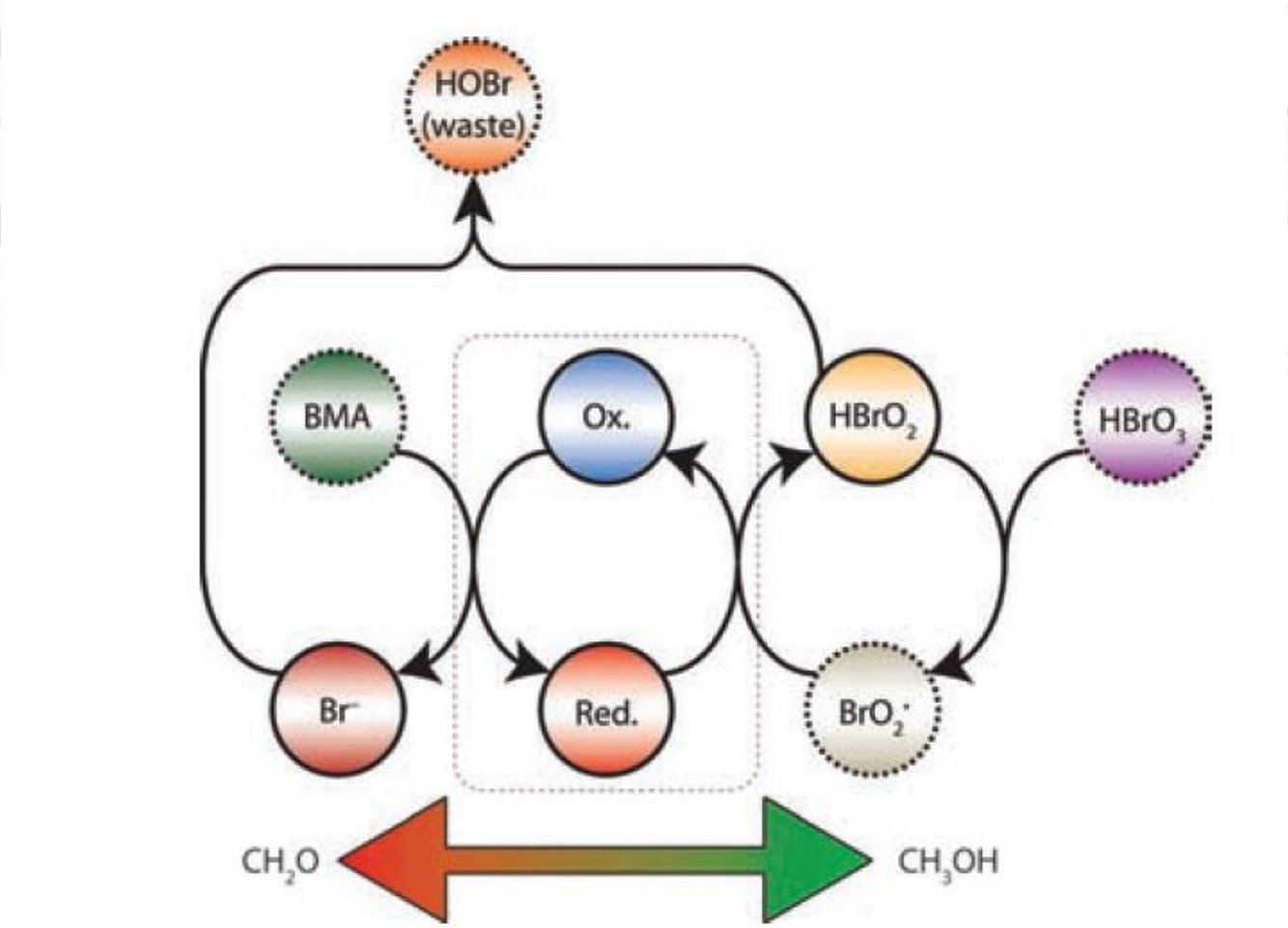


Motif - a nucleotide or amino-acid sequence pattern that is widespread and usually assumed to be related to biological function of the macromolecule

Source: Programmable chemical reaction networks: emulating regulatory functions in living cells using a bottom-up approach, H.W.H. van Roekel, et al.

- The BZ reaction was the first example of a class of inorganic chemical reactions that shows such out-of-equilibrium phenomena that are visible to the naked eye. Oscillations are signals in which temporal variations about a central value occur with well-defined frequencies.
- When appropriate negative feedback is introduced, the well stirred setup becomes either excitable (i.e. an input above a certain threshold leads to a spike in the output before returning to the initial steady state) or oscillatory.
- In a spatially extended system, this leads to a single propagating pulse or a wave train of constant velocity, respectively.
- (A) Figure A displays several topologies of autocatalytic networks
- Common autocatalytic motifs, with (top) direct autocatalysis, (middle) indirect autocatalysis and (bottom) mutual inhibition with autocatalysis.
- (B) Figure B displays bistability which can be triggered into one of the two possible steady-states using a stimulus. An autocatalytic network that can exist in two steady states, i.e. one where A is dominant (red) and one where B is dominant (blue). Switching between these states is inducible by external stimuli.

Key steps in the BZ reaction

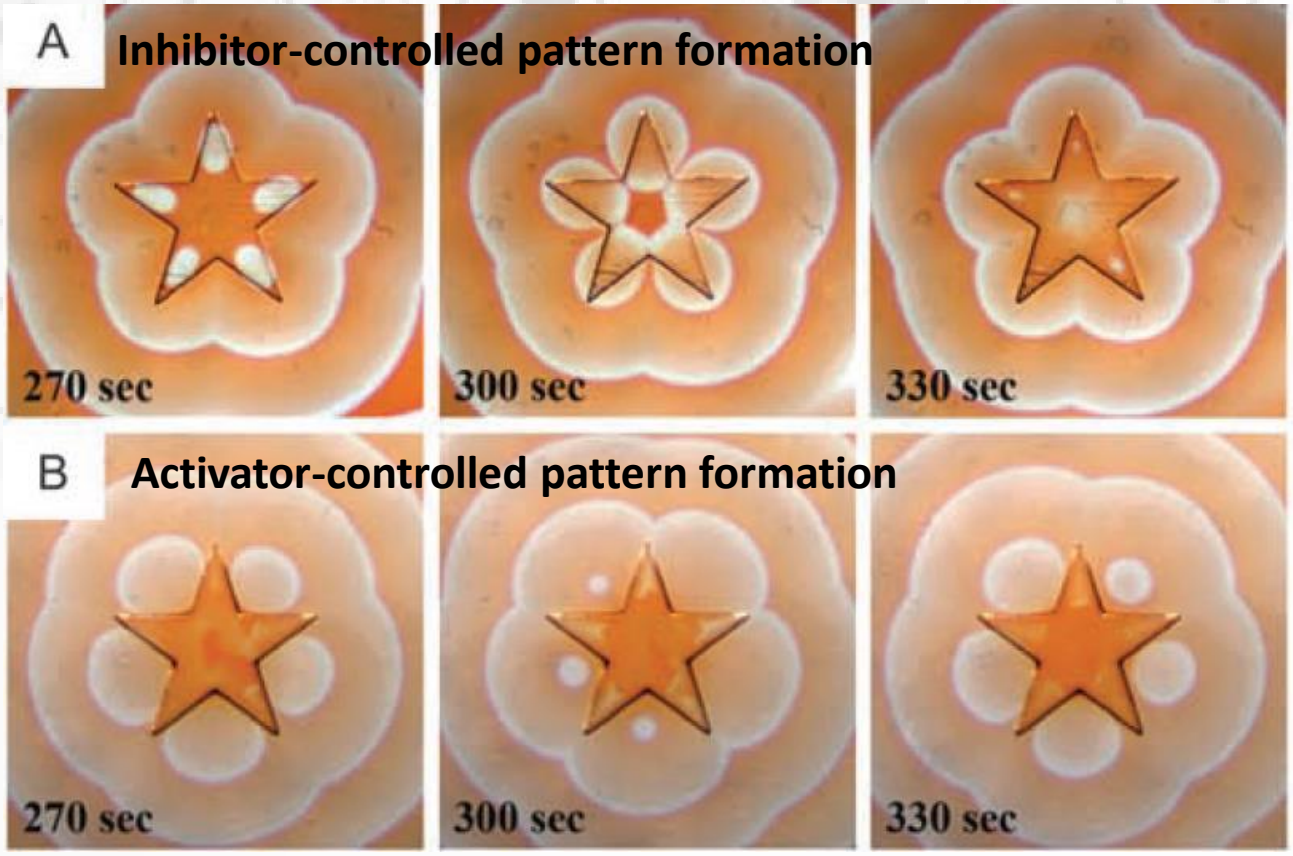


BZ = Belousov–Zhabotinsky

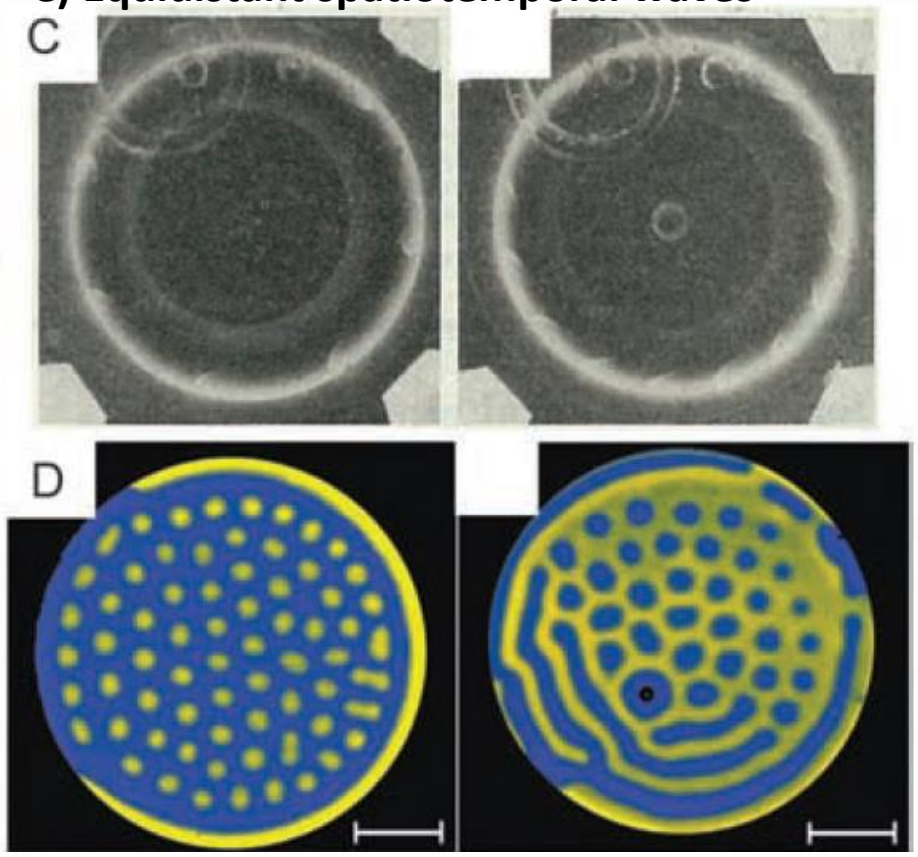
Source: Programmable chemical reaction networks: emulating regulatory functions in living cells using a bottom-up approach, H.W.H. van Roekel, et al.

- The BZ reaction can be excited in a so-called activator-controlled mode by methanol, or in an inhibitor-controlled mode by formaldehyde, as these species slightly shift the balance to autocatalysis of bromous acid H(BrO2) or bromide Br- production, respectively.
- The oxidation of the metal-ion catalyst (middle, enclosed with dashed box) is accompanied by conversion of BrO_2* to bromous acid ($HBrO_2$) resulting in an autocatalytic formation of $HBrO_3$ (right).
- The basic mechanism responsible for oscillations in the BZ reaction is the autocatalytic production of **bromous acid ($HBrO_2$)** that is inhibited by **bromide (Br)** via a delayed negative feedback mechanism. Moreover, the BZ reaction and equivalent inorganic CRNs are excitable by external stimuli.

Excitability and spatiotemporal pattern formation in inorganic CRNs



C) Equidistant spatiotemporal waves



D) Turing pattern formation in uncomplexed protons

Source: Programmable chemical reaction networks: emulating regulatory functions in living cells using a bottom-up approach, H.W.H. van Roekel, et al.

- This study presents one of the first realizations of an open reactor in which the BZ reaction is excited into generating stable chemical patterns in time. Here is used wet stamping to deliver chemical stimuli from complex geometries to a gel containing the BZ reagents. This setup resulted in excitation of the system in a so-called activator-controlled mode, or in-hibitor-controlled mode.

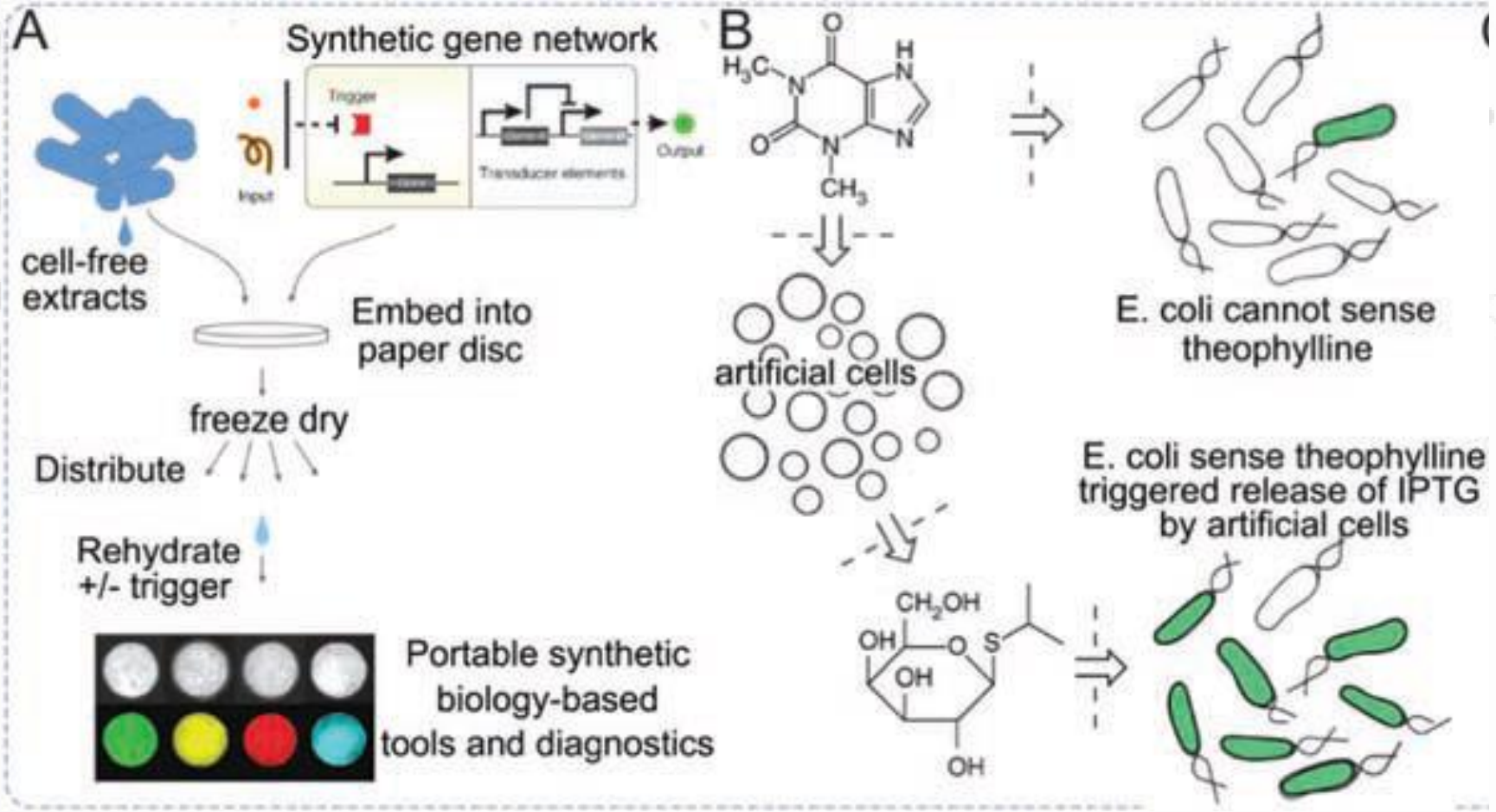
(A)Inhibitor-controlled pattern formation: triggering agent present in the star-shaped stamp, is formaldehyde for inhibitor-controlled mode

(B) **activator-controlled pattern formation** Triggering agent is methanol for activator-controlled mode - the autocatalytic step is initiated either in the star's tips or between the star's arms, resulting in focused wave emission

(C) **Equidistant spatiotemporal waves** resembling chemical pinwheels on an annular gel in which the BZ reaction takes place. Waves with a larger gap between them travel faster (left) than waves that are closer together (right).

(D) **Turing pattern formation in uncomplexed protons** (yellow represents : low pH, blue represents: high pH) --- high inhibitor concentration (left) and low inhibitor concentration (right).

Selected emerging applications of engineered biomolecular circuits



Source: Programmable chemical reaction networks: emulating regulatory functions in living cells using a bottom-up approach, H.W.H. van Roekel, et al.

(A) Usage of **synthetic-biology-based technologies** outside the laboratory is facilitated by paper-based technology where cell-free genetic networks are freeze-dried and, after distribution, reactivated by rehydration. The technology was used to trigger communication between two bacterial populations that otherwise are noncommunicative.

(B) **The idea of targeting existing cell signalling pathways**: They expanded the senses of E. coli by adding liposomes containing a genetic network that converts a chemical message that E. coli cannot sense to a molecule that activates a natural cellular response. This approach may allow for new opportunities in engineering cellular behaviour without exploiting genetically modified organisms.

Key Takeaways

- Studies on chemical self-organization
- Central element: understand and emulate complex kinetic networks of regulatory circuits in the living cell
- Inorganic CRNs can serve as useful model systems or even emulators for biological systems in several aspects
- Emerging application comprises cell-free genetic biosensors