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Propagation of oscillating chemical signals through reaction networks **

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Abstract. *Akin to electronic systems that can tune to and process signals of select frequencies, systems/networks of chemical reactions also „propagate" time-varying concentration inputs in a frequency-dependent manner. Whereas signals of low frequencies are transmitted, higher frequency inputs are dampened and converted into steady-concentration outputs. Such behavior is observed in both idealized reaction chains as well as realistic signaling cascades, in the latter case explaining the experimentally observed responses of such cascades to input calcium oscillations. These and other results are supported by numerical simulations within the freely available Kinetix web application we developed to study chemical systems of arbitrary architectures, reaction kinetics, and boundary conditions.*

One of the most exciting emerging areas of chemical research has been the study of chemical systems – that is, systems of individual chemical reactions connected into networks. Motivation for this effort often comes from biology which operates large reaction networks in massively parallel ways[1], using them skillfully as synthetic "assembly lines" [2], control elements[3], and information-processing units[4]. Although only in its infancy, systems' chemistry[5] has already produced some exciting examples of rationally designed synthetic networks enabling multistep one-pot syntheses of pharmaceuticals[6], rudimentary image recognition[7], self-replication[8], oscillations[9], control of supramolecular self-assembly[10] and chirality[11], or cascading of chemicals[12] down coupled reaction cycles[13]. At the same time, we are only beginning to understand how chemical systems "process" the incoming chemical signals, especially when the input concentrations vary with time. Here, we use our Kinetix systems-chemistry software to show that – akin to electronic circuits – networks of chemical reactions can respond to oscillatory signals in a frequency dependent manner with certain characteristics conserved over different system architectures. In particular, for systems ranging from idealized linear reaction chains to realistic biochemical signaling cascades comprising "nonlinear" elements (e.g., futile cycles and/or feedback loops) (1) signals only up to a certain threshold frequency are transmitted; (2) this threshold shifts to lower frequencies as reactions become more reversible and/or the length of the chain/cascade increases; and (3) the frequency of transition from transmissive to damped regimes can be estimated by dimensionless parameters that describe system's ability to "push mass" past certain points of the system. Remarkably, such theoretical considerations relate to and explain some counterintuitive experimental results (here for Ras/ERK signaling cascades) whose understanding has so far been elusive.

Concentrations of species within a chemical system/network change in time according to the underlying kinetic equations. Although analytical solutions can be derived for simple cases, more complex systems require numerical integration schemes. Since most chemists are not necessarily conversant with applied mathematics tools like Mathematica[14a] or Matlab[14b], and since system's biology platforms[15,16] are not easily customizable (in particular, to include time-varying input signals), we have developed an easy-to-use and general purpose Kinetix software (cf. Computational Methods, **Movies S1-S3**, and tutorial in the SI, Section S1) for modelling chemical systems having arbitrary topology and reaction kinetics, as well as arbitrary functional forms of boundary conditions. Kinetix is made freely available to the academic community at http://kinetixapp.grzybowskigroup.pl/ and provides the basis for the analyses described below (in some cases, accompanied by analytical arguments).

Let us begin by considering the simplest system of several irreversible, first-order reactions, each with rate constant k, arranged into a straight chain, and supplied with an oscillatory "signal" of frequency f (this system can be construed as analogous to a chain of low-pass filters with k corresponding to $1/RC$, where R is resistance and C capacitance). **Figure 1a** shows this chain drawn in Kinetix as a bipartite graph[17,18] with two types of nodes: larger circles corresponding to the chemicals and smaller diamonds corresponding to reaction operations. Mathematically, the system is described by ordinary differential equations, ODEs, of the form $dN_{i+1}(t)/dt = -kN_i(t)$ with boundary condition at the signal-input node, $N_0(t) = A_0 \sin(2\pi f t) + const$, and a constant-concentration sink at the terminal node. This system of ordinary differential equations can be

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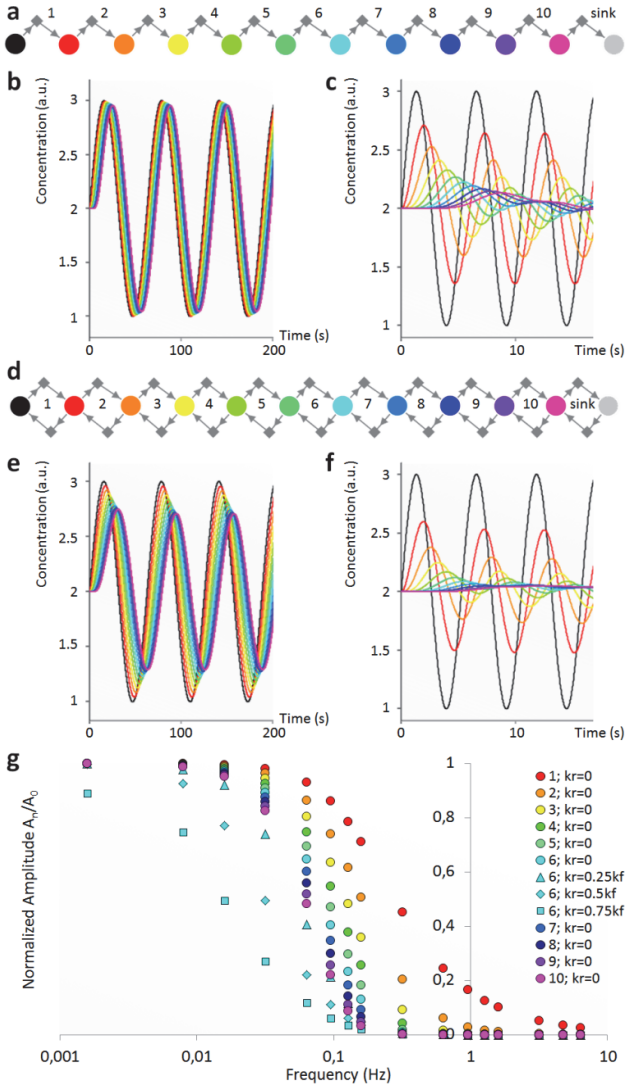

Figure 1. Signal propagation along simple reaction chains. **a)** The chain of irreversible, first order reactions as drawn in Kinetix in the bipartite representation. Circular nodes = molecules; smaller, gray diamonds = reaction operations. While in this particular example inclusion of the diamond reaction nodes might appear spurious, they are generally important to account for all causal relationships in systems in which more than one substrate/product are made (see [17,18]). Here, all reaction rates are the same, k = 1 s^{-1}. See also **Movie S2** illustrating chain's dynamics (i.e., sizes of the nodes proportional to time-changing concentrations). Numerically calculated concentration profiles at different nodes for **b)** f = 0.016 Hz (i.e., $2\pi f/k < 1$) and **c)** f = 0.19 Hz ($2\pi f/k > 1$). Colors of the curves correspond to the colors of substance nodes in (a). **d)** A same-length, same input reaction chain but with all reactions reversible. Concentration profiles of such reversible chain at different nodes for **e)** f = 0.016 Hz and **f)** f = 0.19 Hz. Colors of the curves correspond to the colors of substance nodes in (d). **g)** The amplitudes of the signal at chain's different nodes plotted as a function of frequency. For all curves, the forward rate is the same (k_f = 1 s^{-1}) but reverse rates can vary from k_r = 0 (irreversible reactions) to k_r = 0.75 k_f.

solved analytically to give $N_n(t) = A_0/(1+(2\pi f/k)^2)^{n/2}\sin(2\pi f t + n\varphi) + const$, where the phase is given by $\sin\varphi = -2\pi f/\sqrt{(2\pi f)^2 + k^2}$. The oscillatory part of the signal propagates without substantial damping for $2\pi f \ll k$, for which the amplitude at n-th node in the chain changes as $A_n/A_0 = 1/(1+(2\pi f/k)^2)^{n/2}$. In other words, for frequencies lower than reaction rates, all nodes of the system gradually develop oscillations of almost the same amplitude A_0 (see **Figure 1b** for concentration profiles calculated in Kinetix). For higher input frequencies, however, the amplitude decays rapidly along the chain (**Figure 1c**) and the signal is dampened. We note that the threshold/transition frequency gradually shifts to lower values for the nodes along the chain (cf. **Figure 1g**).

Figures 1e and **1f** evidence that similar trends are observed for chains comprised of reversible (or both reversible and irreversible) reactions characterized by different rate constants. Amplitude vs. frequency curves in **Figure 1g** (derived from numerical simulations in Kinetix[19]) evidence that transition from the "transmissive" to "damped" regimes remains relatively sharp while the frequency of this transition shifts to lower frequencies as the reactions become more reversible (i.e., when k_f/k_r decreases).

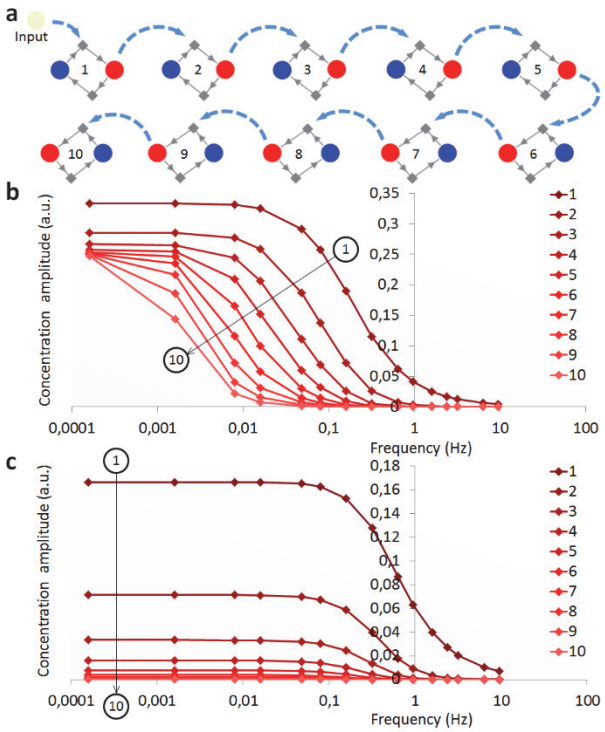

Figure 2. Signal propagation along basic "signaling" cascades. **a)** Representation of the cascade in Kinetix. For each loop, k_f=1, k_r= 2, and C =1. Blue dotted arrows mark connections between upstream activators and reactions they regulate (see SI, Section S1 for details how to represent such regulatory/catalytic steps in Kinetix). Blue nodes correspond to inactive enzymes and red, to their activated forms. Plots of concentration amplitudes at different nodes in two distinct regimes: **b)** $k_r < k_f C$ and **c)** for $k_r > k_f C$. In both cases, input oscillates as $(1+\sin(\omega t))/2$.

Next, we investigated frequency response of more complex and biologically relevant systems. Of particular interest to us were cascades comprising layers of futile cycles interconverting active and inactive forms of enzymes and effectively transmitting chemical signals "down" the cascade (e.g., phosphorylation signals in MAPK [SI, Section S4] or Ras/ERK cascades [later in the text]). Even for the simplest cascade in **Figure 2a** – in which total amounts C of active and inactive enzymes in each loop are conserved and activation steps are second-order (first-order in inactive enzyme and first-order in the upstream "activator") – analytical solution are prohibitively complex, mandating the use of software like Kinetix. Most importantly, plots of amplitude at different levels of the cascade again feature transitions from transmissive-to-dampening regimes – that is, they are qualitatively similar to those we have previously seen in reaction chains. Interestingly, this particular cascade is rather inefficient in transmitting oscillations as even in the low-frequency regime, the amplitude of transmitted signals decreases – slightly for $k_r < k_f C$ (**Figure 2b**) but drastically for $k_r > k_f C$ (**Figure 2c** , see also SI,

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Section 4 and later in the text). In the subsequent, example, we will see how this inefficiency can be remedied by inclusion of global feedback loops.

Figure 3a shows complete and physiologically relevant wiring diagram of the Ras/ERK signaling cascade which is one of mammalian kinase cascades responsible for cell growth, proliferation and differentiation, and apoptosis[20]. The equations describing this system are listed in the SI, Section S6 and comprise (i) the main segment taken from Kholodenko[21a], with enzyme interconversion steps modelled by Michaelis-Menten-type kinetics and (ii) the segment describing modulation of the upstream Ras by calcium waves taken from Yi[21b] and described by Hill kinetics. Cyclical changes in the cellular concentration of calcium are prescribed by $[Ca^{2+}] = 0.45\sin(2\pi f t) + 0.55$ [μM] to remain within typical cellular Ca^{2+} levels[21c, 22c]. Total concentration of active and inactive Ras is constant at 100 nM, while total concentrations Raf, MEK and ERK in all forms are each equal to 300 nM[21]. We note that in addition to two connected loops (to account for double phosphorylation) at all three layers, the cascade features a negative/inhibitory feedback connection between ERK-PP and phosphorylation of Raf. In kinetic equations 3 and 4 in Table S2, the "strength" of this connection is quantified by parameter $F < 1$ (if $F = 1$, there is no feedback).

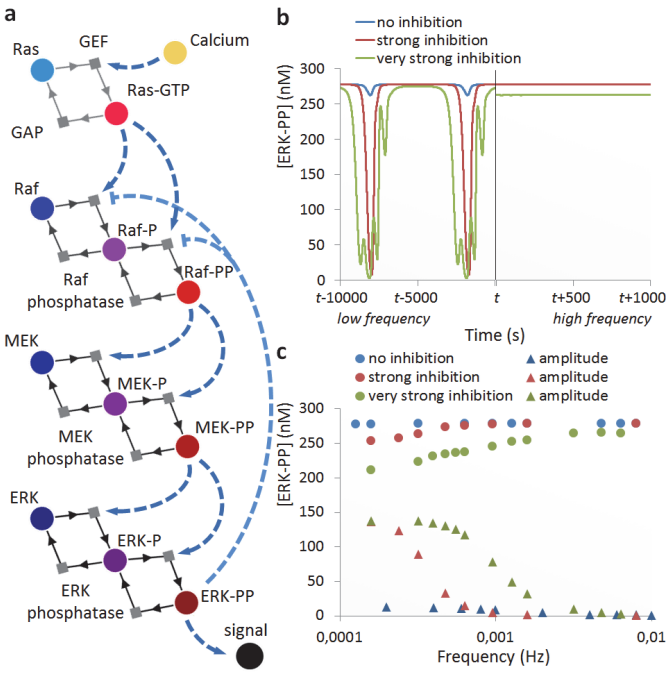

Figure 3. Signal transmission along the Ras/ERK signaling pathway, from input calcium oscillations to the output node of doubly-phosphorylated ERK-PP. **a)** Wiring diagram of the pathway with regulatory connections marked by dashed, pointed arrows and the inhibitory feedback of Raf phosphorylation by ERK-PP, by the dashed, flat-tipped arrows. **b)** Typical concentration profiles of ERK-PP for low- (left part, $f = 1.59\cdot10^{-4}$ Hz) and high-frequency ($f = 1.59\cdot10^{-2}$ Hz) calcium inputs without feedback ($F = 1$, blue curve) and with feedback ($F = 0.34$, red curve, and $F = 0.01$, green curve). **c)** Full frequency dependence of ERK-PP amplitude (solid triangles) and average concentration (filled circles) for $F = 1$, 0.34, 0.01.

As seen from the plots in **Figures 3b** and **3c**, the cascade without a feedback would be a poor transmitter of oscillations (similar to simpler cascades in Figure 2) and the concentration of terminal ERK-PP would be close to maximal value for all input frequencies. With the inhibitory feedback present, however, the cascade passes efficiently low frequency signals but dampens oscillations with frequencies above ~0.001-0.04 Hz threshold. This threshold regime is near the „center" of the physiologically observed range of calcium oscillations (from 10^{-5} Hz in neurons up to 100 Hz in skeletal muscle cells[22b]) and, remarkably, is close to a transmission-dampening transition recorded experimentally[23] at ~ 0.0083-0.016 Hz for the Ras/ERK pathway. We note that neither experimental nor theoretical findings of this transition in ref [21b, 23] were accompanied by any analysis or interpretation – our results, for various systems we have described, indicate that such frequency-dependent transitions are more of a norm rather than an exception in (bio)chemical systems, although their biological significance remains to be explained.

Given the apparent generality of frequency transitions, we pondered whether it might be possible to estimate the ability of a system (or its part) to transmit oscillations without performing full-fledged numerical simulations and, instead, based only on its steady-state at constant (not oscillatory) input. In this spirit, we define an "effective forward rate constant," k_{eff}, that approximates system's propensity to "push" mass past a certain node n. First, drawing upon the analytical expression for the propagation of amplitude, A_n/A_0, along a chain of irreversible reactions (cf. Figure 1) and noting that for $2\pi f \ll k$ and for large n, the amplitude of the oscillatory signal can be approximated as $1/(1+(2\pi f/k)^2)^{n/2} \approx 1/(1+0.5n(2\pi f/k)^2$, we replace reactions leading to the n-th mode by a single "effective" reaction and define effective rate constant as $0.5n(2\pi f/k)^2 = 0.5n(2\pi f/k_{eff,n})^2$. Because, in general, each node has incoming/outgoing reactions with different reaction rates, we extend this definition to $1/k_{eff,n}^2 = 0.5\sum_1^n 1/k_{eff,i}^2$, where $k_{eff,i}$ is the effective forward reaction at individual node i between system's input and node n. Next, taking a reaction chain as an example, we impose a steady-state in the system (with fixed arbitrary concentration in the first node), obtain all concentrations, N_i, and then consider the "inflow" of mass into node i from "preceding" node(s) i-1 (at steady-state, kept at constant concentration). The effective rate constants $k_{eff,i}$ at any node i of the chain are then calculated from $dN_{i,inflow}/dt = k_{eff,i}N_{i-1,const}$ such that $k_{eff,i} = (\Delta N_{i,inflow}/\Delta t)/N_{i-1,const}$). Plots in **Figure 4a** evidence that this simple heuristic measure of $k_{eff,i}$ rescales the frequency responses at different nodes of the reaction chains we studied to approximately a single master curve. With this result, our ansatz principle stipulates that for $2\pi f/k_{eff,n}$ smaller than ca. 1, the system will propagate an oscillatory signal past node n, but will dampen it for higher values.

With some additional examples provided in the SI, Section S3, we note that in order to apply similar reasoning to cascades such as those in **Figure 2**, one must take into account the fact that the total concentration of inactive and active enzymes at each layer is fixed (at C), while their individual concentrations change rhythmically under incoming oscillatory signals. Accordingly, for the cascade to "push mass" effectively, the oscillations in its different layers should be synchronized. While analytical solution for the underlying nonlinear kinetic equations is not possible, extensive simulations in Kinetix allowed us to estimate that oscillations can propagate only for $k_r < k_f C$. The effective rate constant for the n-th step in the cascade can then be approximated as $k_{eff} = k_r \cdot \left(\frac{k_r}{k_f C}\right)^{n/2}$ (see also SI, Section S4) such that, akin for the simpler chain systems, the rescaled curves describing signal transmission nearly overlap (**Figures 4b,c** and SI Section 5 for the MAPK cascade). The cascade is then expected to transmit oscillatory signals of frequencies, f, past the n-th loop unit only if $2\pi f < k_{eff}$. Finally, for the cascade with global feedback from **Figure 3** (and for other systems with "nested" motifs), the algebra is more involved and to estimate the overall k_{eff} (**Figure 4d**), one has to first calculate effective rates for individual loops and then iteratively combine them into larger modules. Some examples are discussed in the SI, Section S6. In general, cascade of cycles and linear reactions constitute basic motifs in all known reaction networks.

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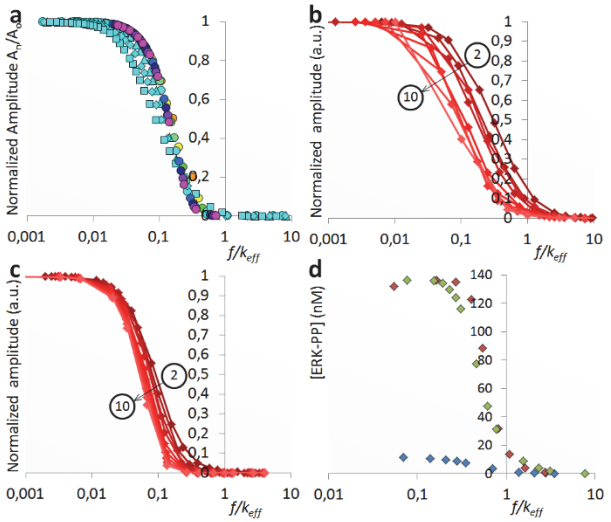

Figure 4. Normalized signal amplitude plotted against f/k_{eff} ratio for **a)** linear chains of reactions from Figure 1, **b)** cascade from Figure 2 when $k_r < k_f C$ and **c)** same cascade but for $k_r > k_f C$; and **d)** the Ras/ERK cascade from Figure 3.

In summary, the above results point to several regularities in the behavior of chemical networks transmitting oscillatory signals – at the same time, they certainly do not capture the full richness of the phenomena one might wish to consider. For instance, while we have quantified here the conditions for the dampening of oscillations, the opposite is also possible and oscillations can be amplified at well-defined locations within a system. For instance, **Figure 5** and the accompanying **Movie S3** illustrate signal propagation along a chain of irreversible reactions in which first 15 steps are faster (k_1 = 2 s^{-1}) than the last 15 steps (k_2 = 0.5 s^{-1}). For low input frequency, the amplitude of oscillations gradually decreases over the first half of the chain, but then spikes (by over 300%) at the node at which the k's change. This result generalizes to all types of chains we tested, and for a linear chain of first-order reactions, the analytical solution prescribes $A_{n+1}/A_n = k_n/(k_{n+1}^2 + (2\pi f)^2)^{1/2}$ – thus, for $2\pi f \ll k_n, k_{n+1}$ and $k_{n+1} \ll k_n$, the amplitude can be amplified by a factor of up to k_n/k_{n+1}. Localized amplification is also observed in chains of multiple fast reactions interrupted by a single slow one (see SI, Section S7) – this is potentially interesting because the slow step could represent, for instance, a transport process across a cellular membrane. In general, we surmise such localized oscillation "hot spots" could be used quite productively in spatially distributed reaction systems (i.e., reaction-diffusion systems) to "turn on" desired chemical events at distant but precisely defined locations. Another class of interesting systems to study would be those with multiple oscillatory inputs for which we anticipate a wealth of interference-like phenomena and system-wide synchronization. We hope that in all such studies and in the development of systems chemistry at large, Kinetix will prove useful to practicing organic chemists and biochemists, enabling them to independently – without the help from physicists or mathematicians – model even the most complex networked systems.

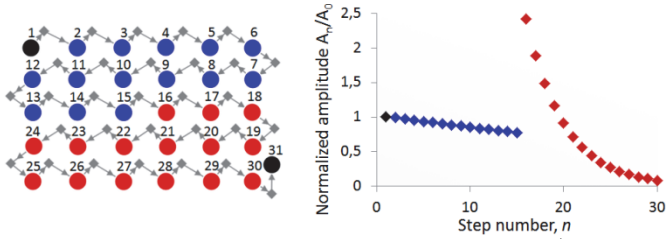

Figure 5. Kinetix scheme of a chain of 15 fast (k_1 = 2 s^{-1}) reactions followed by 15 slow ones (k_2 = 0.5 s^{-1}). The graph plots the amplitude of oscillations at different nodes for the input frequency of f = 0.064 Hz. Note a sharp discontinuity at node #16 where the rate constants change from fast to slow. See also **Movie S3**.

Computational Methods.

Kinetics software allows the user to (i) draw an arbitrary chemical network of interest; (ii) specify in an intuitive manner arbitrary stoichiometry and kinetics for all constituent reactions as well as the concentrations of inputs, including time-varying profiles; (iii) visualize systems' dynamics as both "movies" and traditional graphs; and (iv) create output files that could then be reloaded, analyzed and modified by other users. These features are illustrated in **Movies S1-S3** and a short "tutorial" in the SI, Section S1.

Down to technical details, Kinetix is a web-application that works under all modern browsers (e.g. Google Chrome, Mozilla Firefox, Opera, Safari). The web-app is written in JavaScript using meteor framework[24a] which is based on node.js server. Meteor is chosen since it allows reactive programing, distributed data protocol for data exchange between client and server, as well as the ability to use just one programming language (JavaScript) on client and server sides and share the code between them. To visualize the graphs and plots, the d3.js library[24b] is used. This library is one of the most comprehensive and mature solutions for web-applications supporting low-level manipulation of data and various modes of visualization (as SVG graphics). All data created by users (e.g., account information, reaction networks, calculated reactants' concentrations) are stored in a MongoDB database[24c]. The web-app follows the so-called "database everywhere" principle, which means that all data is stored in the Mongo database and is exchanged between client and server when needed. In contrast to standard object-oriented programming, data is independent from the application logic and the entire application is a set of functions falling under four categories: (i) data presentation (animation, plots, exporting data), (ii) interaction with the user (menu events), (iii) verification of correctness of the user-provided data, and (iv) differential-equation calculations. The first two sets of operations, (i) and (ii), are performed only on the client side; calculations (iv) are performed on the server side. Code for checking correctness, (iii), is shared between client and server.

Since the software accepts arbitrary kinetic expressions (linear, non-linear, any algebraic expression), it is important to ensure that the scheme for numerical integration can handle stiff systems[24a] of ODE's. While "stiffness" is not rigorously defined, it is known that for stiff systems of ODE's, standard explicit methods (i.e., those in which concentrations at time $t + \Delta t$ are calculated based on derivatives at time t) are numerically unstable. Since publicly available libraries of numerical integrators use standard explicit Runge-Kutta methods and also do not allow to modify the integration scheme (e.g., to maintain constant concentration of selected reagents), we wrote our own integrator based on the Crank-Nicolson method[24b, 24c] with adaptive time step. The Crank-Nicholson scheme is a combination of implicit (backward) and explicit (forward) Euler methods with the value of a function at time $t + \Delta t$ expressed as: $f(t + \Delta t) = f(t) + (f'(t) + f'(t + \Delta t))\Delta t/2$, where the primes denote first derivatives. Importantly, we verified the stability and correctness of the integrated numerical solutions by comparing them to known analytical solutions for highly non-linear systems of various chemical oscillators, cycles, and systems with rare kinetic behavior, including third- and fourth-order reactions with different initial settings (e.g., constant or time-varying concentration of some species, different proportions of reactants).

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Chemical Systems

Propagation of time-varying chemical signals through reaction networks

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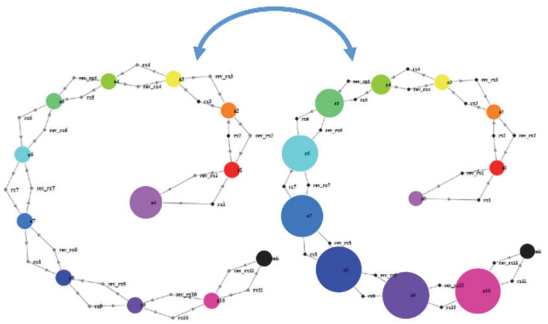

TOC TEXT: Reaction networks do not like high frequencies. Similar to electronic systems that can tune to and process signals of select frequencies, systems/networks of chemical reactions also "propagate" oscillatory concentration inputs in a frequency-dependent manner. In particular, analytical considerations and simulations in the Kinetix software reveal that for diverse system architectures – including examples of biologically relevant signalling cascades – oscillations are transmitetd only up to a certain threshold value and are dampended for higher frequencies.

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