Extended data

Exploring the Programmability of Autocatalytic Chemical Reaction Networks

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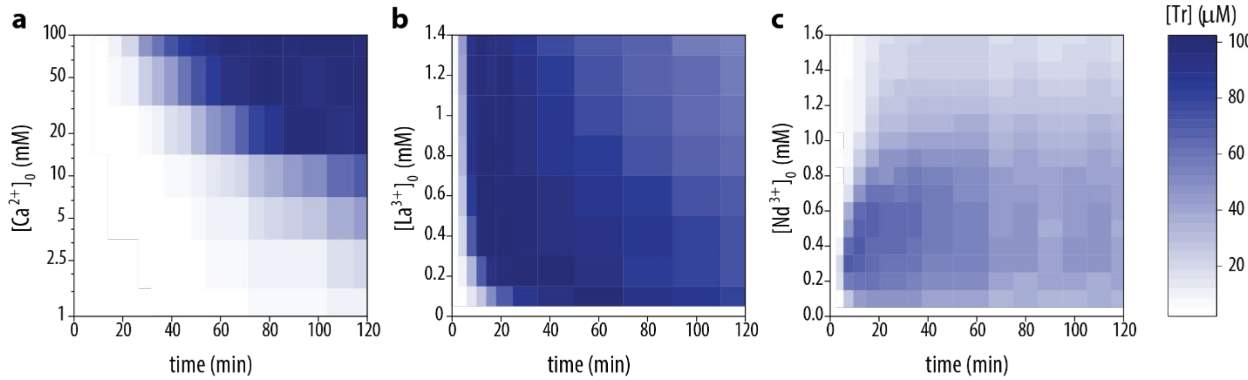
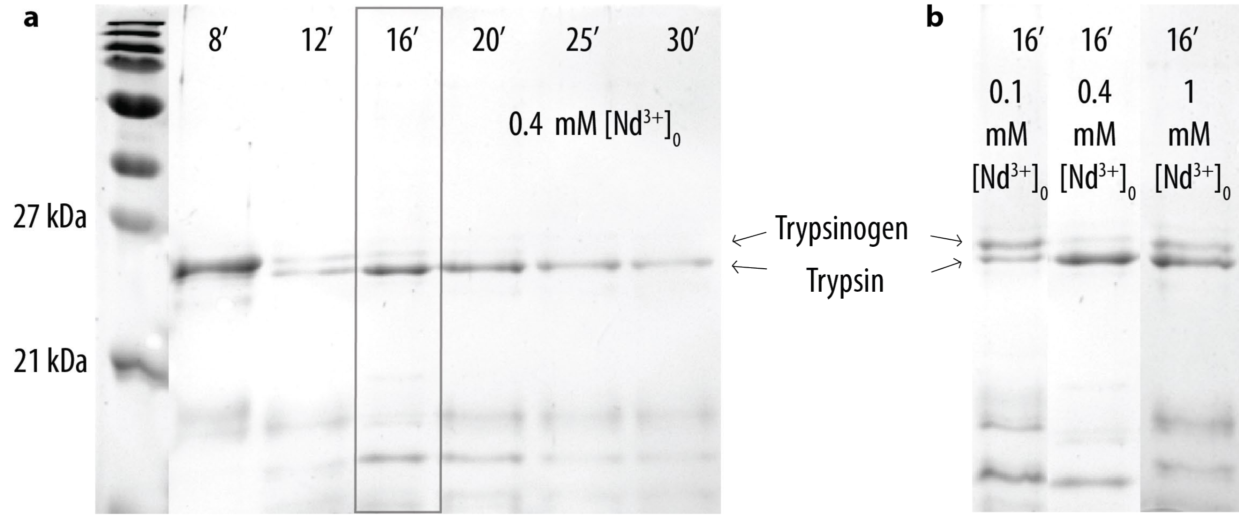
The PDF file includes:

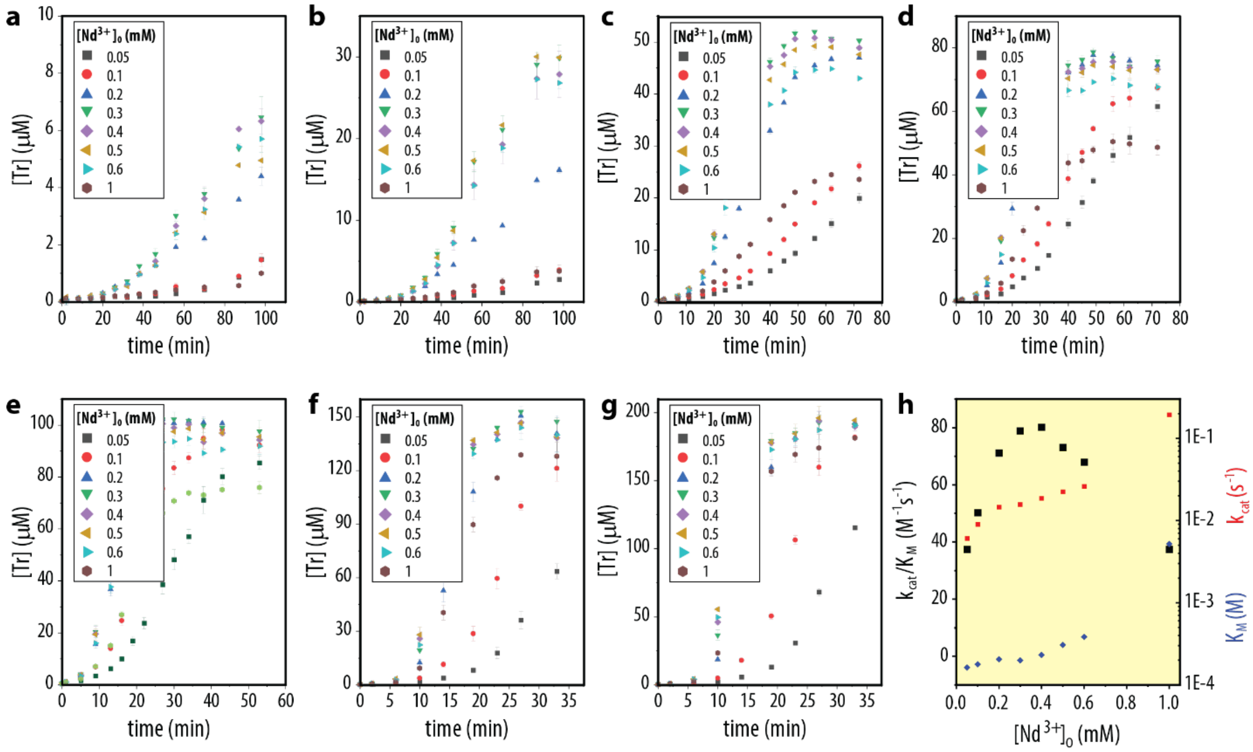
- **Extended Data Fig. 1-10**



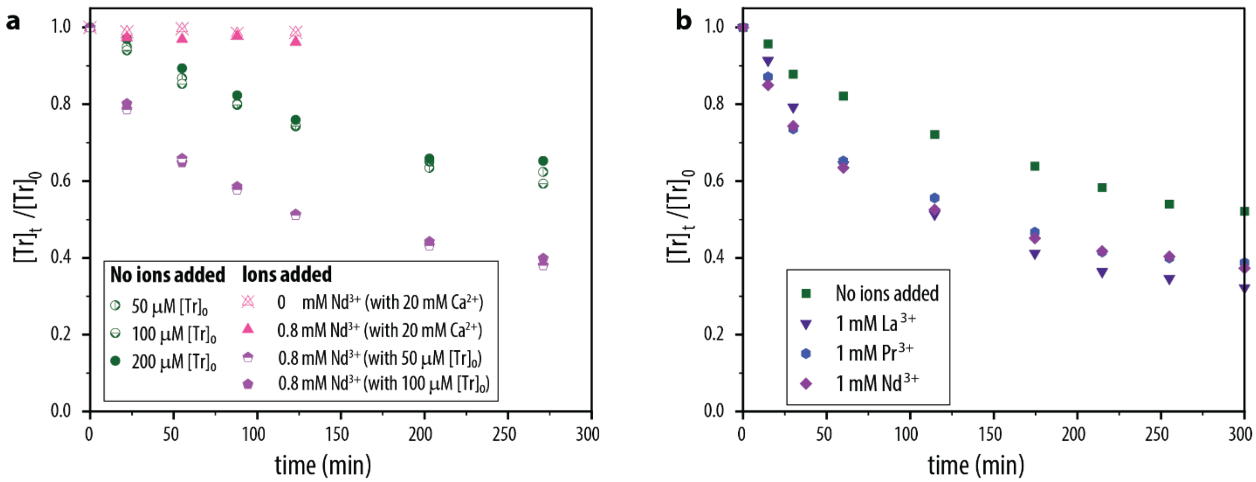
Extended Data Fig. 1: Influence of individual metal ions on the rate of *Tr* autocatalysis under batch conditions. Reaction mixtures consisted of 100 mM $[Tg]_0$, 1 mM $[Tr]_0$, 100 mM TRIS-HCl pH=7.8 and respective metal ions (**a**, Ca^{2+}, **b**, La^{3+}, **c**, Nd^{3+}) at indicated concentrations. Data collected according to *Trypsin assay* section of **Methods**.



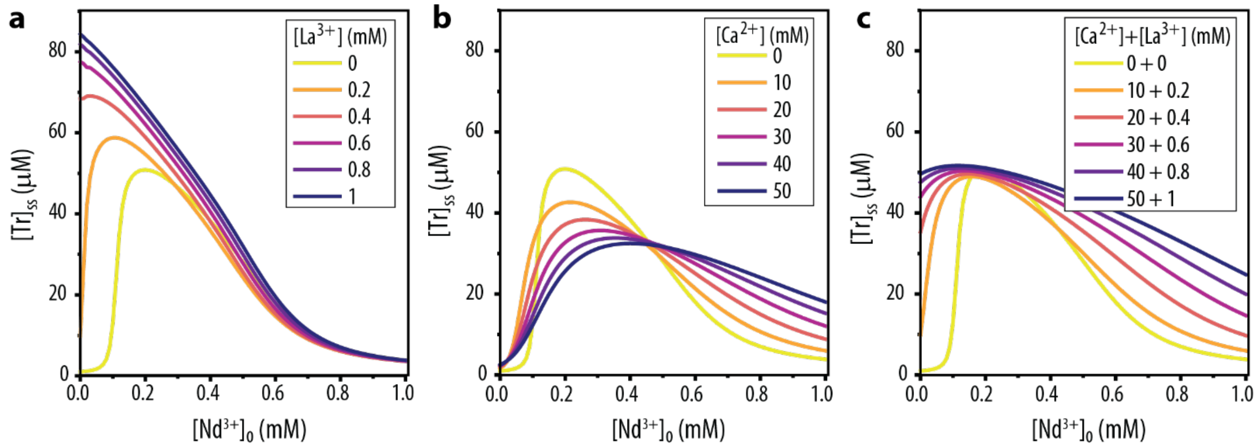
Extended Data Fig. 2: Conversion of *Tg* at high concentrations of [Nd^{3+}]$_0$. a, SDS-PAGE time course for 0.4 mM Nd^{3+} in the absence of calcium. **b**, Comparison of effect of [Nd^{3+}]$_0$ on degradation and autocatalysis at a fixed time. Reaction mixtures consisted of 100 mM [*Tg*]$_0$, 1 mM [*Tr*]$_0$, 100 mM TRIS-HCl pH=7.8 and [Nd^{3+}]$_0$ at indicated concentrations. Data collected according to *SDS PAGE protocol* section of **Methods**.



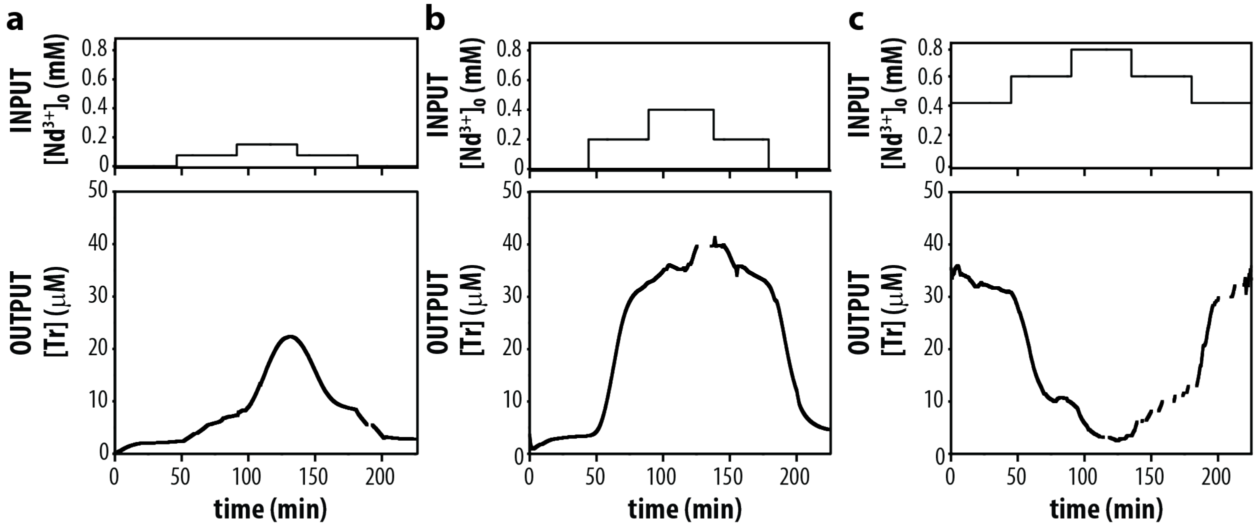
Extended Data Fig. 3: Determination of Michaelis-Menten parameters in *Tr* autocatalysis mediated by Nd^{3+}. **a-g**, Autocatalysis of *Tr* wherein the initial concentration of *Tg* was varied. Initial concentrations: **a**, 0.5 mM $[Tr]_0$ and 10 mM $[Tg]_0$, **b**, 0.5 mM $[Tr]_0$ and 30 mM $[Tg]_0$, **c**, 1 mM $[Tr]_0$ and 50 mM $[Tg]_0$, **d**, 1 mM $[Tr]_0$ and 75 mM $[Tg]_0$, **e**, 0.5 mM $[Tr]_0$ and 100 mM $[Tg]_0$, **f**, 0.25 mM $[Tr]_0$ and 150 mM $[\mathrm{Tg}]_0$, **g**, 0.25 mM $[Tr]_0$ and 200 mM $[Tg]_0$. Reaction mixtures consisted of $[Tg]_0$, $[Tr]_0$, 100 mM TRIS-HCl pH=7.8, 20 mM $CaCl_2$, and $[Nd^{3+}]_0$ at indicated concentrations. Data collected according to *Trypsin assay* section of **Methods**. **h**, determination of the value k_{cat}/K_M from a Michaelis-Menten approximation. Kinetic analysis for this analysis and acquiring k_{cat}/K_M value is based on reference[33].



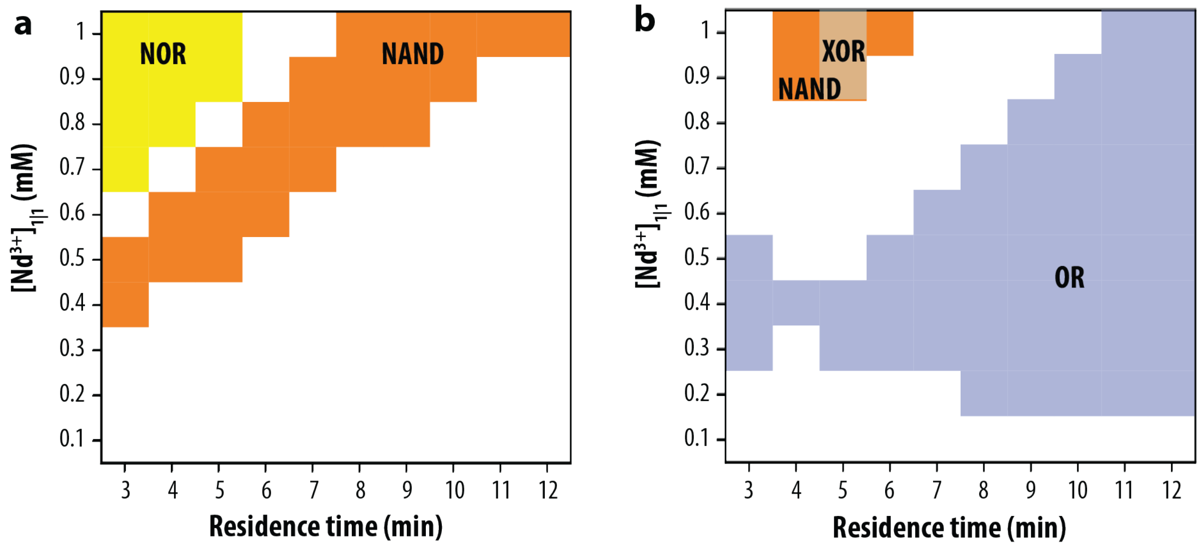
Extended Data Fig. 4: Degradation of *Tr* mediated by Nd^{3+}. **a**, Decrease in *Tr* activity towards BAPNA over time, and influence of 0.8 mM $[Nd^{3+}]_0$ and 20 mM $[Ca^{2+}]_0$. The existing degradation is promoted by Nd^{3+} and inhibited by Ca^{2+}. The degradation rate does not depend on the initial concentration of Tr. **b**, Comparison of effects of 1 mM $[La^{3+}]_0$, $[Pr^{3+}]_0$ and $[Nd^{3+}]_0$. The effect of Nd^{3+} is not unique. Reaction mixtures consisted of $[Tr]_0$, 100 mM TRIS-HCl pH=7.8, metal ions at indicated concentrations. Data collected according to *Trypsin assay* section of **Methods**.



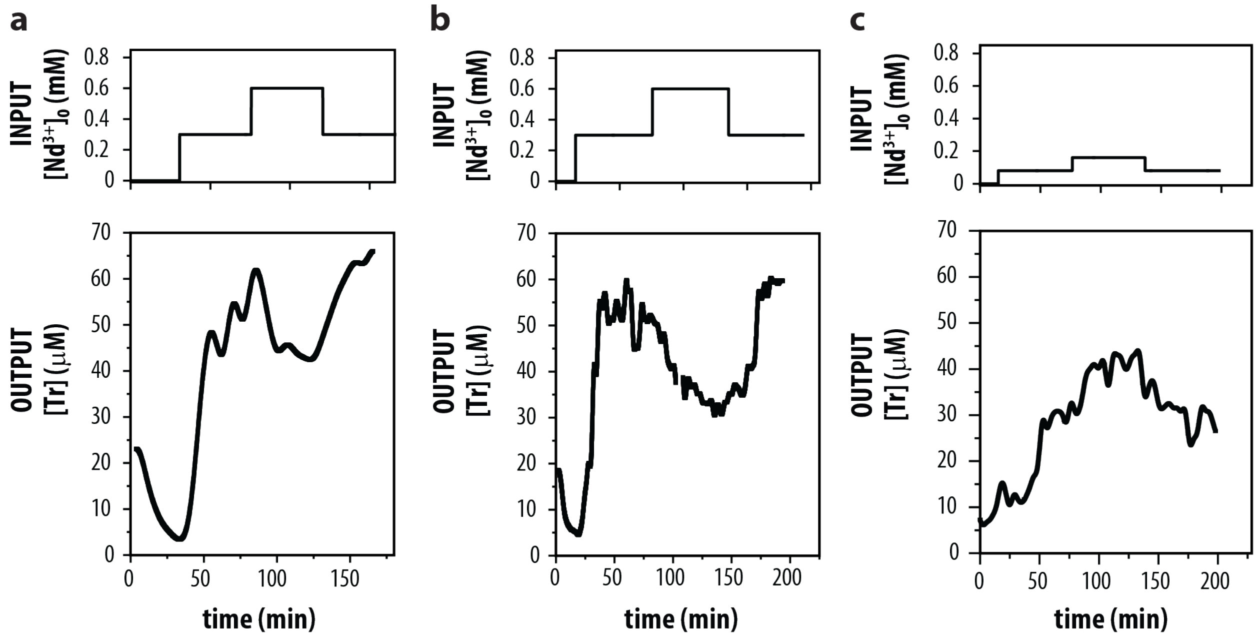
Extended Data Fig. 5: Modelled effect of mixtures of ions on the rate of trypsin autocatalysis in flow. Dependence of the steady state of [*Tr*] on $[Nd^{3+}]_0$ in the absence and presence of other ions. **a**, The Fig. shows how different the system can response on $[Nd^{3+}]_0$ in the presence of different $[La^{3+}]_0$. **b**, The Fig. shows how different the system can response on $[Nd^{3+}]_0$ in the presence of different $[Ca^{2+}]_0$. **c**, The response of the system based on $[Nd^{3+}]_0$ in the presence of different combinations of $[La^{3+}]_0$ and $[Ca^{2+}]_0$. Parameters: $[Tg]_0$=100 µM, $[Tr]_0$=1 µM, 6 minutes of residence time. Data is simulated using **Supplementary Data Scripts** according to *Mathematical model* section of **Supplementary information**.



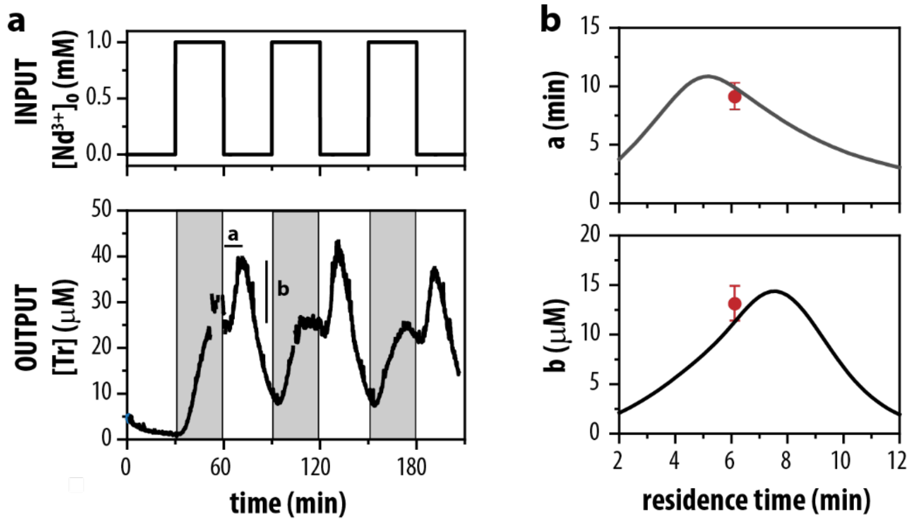
Extended Data Fig. 6: Experimental validation of AND, OR and NOR gates. Experimental time series with an output sequence that corresponds to **a**, AND, **b**, OR, **c**, NOR Boolean function. Initial conditions are same as on **Fig. 4b**: $[\text{TRIS-HCl}]_0$=60-100 mM (pH 7.8), T=21-23ºC, $[Tg]_0$=100 µM, $[Tr]_0$=1 µM, 5 minutes of residence time. Data collected according to *Flow experiments* section of **Methods**.



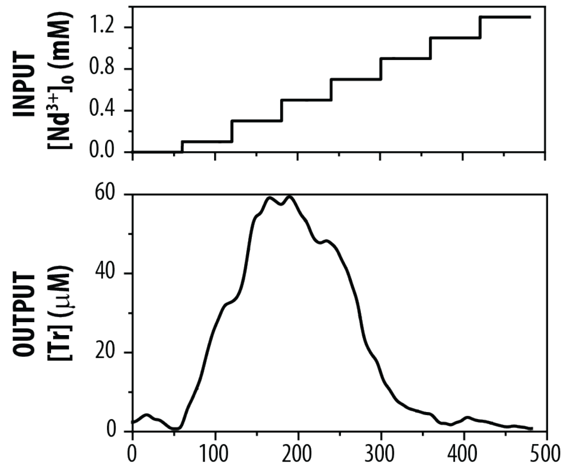
Extended Data Fig. 7: Modelled phase plots for logic operations in the presence of La^{3+} and Ca^{2+}. A phase plot similar to **Fig. 4d** but in the presence of **a**, 1 mM La^{3+}, and **b**, 20 mM Ca^{2+}. Data is simulated using **Supplementary Data Scripts** according to *Mathematical model* section of **Supplementary information**.



Extended Data Fig. 8: Changing a logic operation. **a**, OR gate obtained from XOR gate (**Fig. 4d**) by addition of 20 mM $[Ca^{2+}]_0$. **b**, OR gate obtained from XOR gate (**Fig. 4d**) by increasing the residence time from 5 minutes to 10 minutes. **x**, OR gate obtained from AND gate by increasing the residence time from 5 minutes to 10 minutes. Initial conditions are same as on **Fig. 4D**: $[\text{TRIS-HCl}]_0$=60-100 mM (pH 7.8), T=21-23°C, $[Tg]_0$=100 µM, $[Tr]_0$=1 µM. Data collected according to *Flow experiments* section of **Methods**.



Extended Data Fig. 9: Nd^{3+}-driven hysteresis. a, A sudden decreased of $[Nd^{3+}]_0$ causes the existence of a pulse in [*Tr*], which can be characterized by the duration, a, and intensity, b, thereof. Experimental conditions: $[\text{TRIS-HCl}]_0$=75-100 mM (pH 7.8), T=21-23°C, $[Tg]_0$=200 μM, $[Tr]_0$=2 μM, t =6 min. Data collected according to *Flow experiments* section of the **Methods**. **b**, The appearance of the pulse is dependent on the residence time. The data points (in red) depict the average of the three pulses, and the black line depicts the simulated response based on our model. Error bars represent standard deviation. Data is simulated using **Supplementary Data Scripts** according to *Mathematical model* section of **Supplementary information**. See the discussion of the results in *Validation of history-dependency* section of **Supplementary information**.



Extended Data Fig. 10: $[Nd^{3+}]_0$ profile (Fig. 3b) at longer residence time. Change in [*Tr*] output from the CSTR according to change in $[Nd^{3+}]_0$. Initial conditions are same as on Fig. 3A: $[TRIS\text{-}HCl]_0$=60-100 mM (pH 7.8), T=21-23ºC, $[Tg]_0$=100 µM, $[Tr]_0$=1 µM, 10 minutes of residence time. Data collected according to *Flow experiments* section of **Methods**.