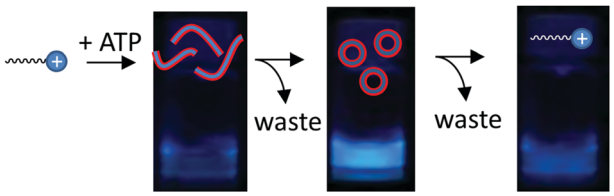
Chemical Communications d0cc05993e

We have presented the graphical abstract image and text for your article below. This briefly summarises your work, and will be presented with your article online.




**Time-gated fluorescence signalling under dissipative conditions**

Maria A. Cardona, Rui Chen, Subhabrata Maiti, Ilaria Fortunati, Camilla Ferrante, Luca Gabrielli, Krishnendu Das and Leonard J. Prins*

A lag time between addition of a trigger and the emergence of a fluorescence signal is observed in an ATP-fueled self-assembly process under dissipative conditions.

Q1

Please check this proof carefully. Our staff will not read it in detail after you have returned it.

Please send your corrections either as a copy of the proof PDF with electronic notes attached or as a list of corrections. **Do not edit the text within the PDF or send a revised manuscript** as we will not be able to apply your corrections. Corrections at this stage should be minor and not involve extensive changes.

**Proof corrections must be returned as a single set of corrections, approved by all co-authors. No further corrections can be made after you have submitted your proof corrections as we will publish your article online as soon as possible after they are received.**

Please ensure that:

- The spelling and format of all author names and affiliations are checked carefully. You can check how we have identified the authors' first and last names in the researcher information table on the next page. **Names will be indexed and cited as shown on the proof, so these must be correct.**
- Any funding bodies have been acknowledged appropriately and included both in the paper and in the funder information table on the next page.
- All of the editor's queries are answered.
- Any necessary attachments, such as updated images or ESI files, are provided.

Translation errors can occur during conversion to typesetting systems so you need to read the whole proof. In particular please check tables, equations, numerical data, figures and graphics, and references carefully.

Please return your **final** corrections, where possible within **48 hours** of receipt following the instructions in the proof notification email. If you require more time, please notify us by email to chemcomm@rsc.org.

# Funding information

Providing accurate funding information will enable us to help you comply with your funders' reporting mandates. Clear acknowledgement of funder support is an important consideration in funding evaluation and can increase your chances of securing funding in the future.

We work closely with Crossref to make your research discoverable through the Funding Data search tool (http://search.crossref.org/funding). Funding Data provides a reliable way to track the impact of the work that funders support. Accurate funder information will also help us (i) identify articles that are mandated to be deposited in **PubMed Central (PMC)** and deposit these on your behalf, and (ii) identify articles funded as part of the **CHORUS** initiative and display the Accepted Manuscript on our web site after an embargo period of 12 months.

Further information can be found on our webpage (http://rsc.li/funding-info).

### What we do with funding information

We have combined the information you gave us on submission with the information in your acknowledgements. This will help ensure the funding information is as complete as possible and matches funders listed in the Crossref Funder Registry.

If a funding organisation you included in your acknowledgements or on submission of your article is not currently listed in the registry it will not appear in the table on this page. We can only deposit data if funders are already listed in the Crossref Funder Registry, but we will pass all funding information on to Crossref so that additional funders can be included in future.

### Please check your funding information

The table below contains the information we will share with Crossref so that your article can be found *via* the Funding Data search tool. **Please check that the funder names and grant numbers in the table are correct and indicate if any changes are necessary to the Acknowledgements text.**

| Funder name | Funder's main country of origin | Funder ID (for RSC use only) | Award/grant number |
|---|---|---|---|
| H2020 Marie Skłodowska-Curie Actions | European Union | 100010665 | 642793 |
| Università degli Studi di Padova | Italy | 501100003500 | MSCA Seal of Excellence |

# Researcher information

Please check that the researcher information in the table below is correct, including the spelling and formatting of all author names, and that the authors' first, middle and last names have been correctly identified. **Names will be indexed and cited as shown on the proof, so these must be correct.**

If any authors have ORCID or ResearcherID details that are not listed below, please provide these with your proof corrections. Please ensure that the ORCID and ResearcherID details listed below have been assigned to the correct author. Authors should have their own unique ORCID iD and should not use another researcher's, as errors will delay publication.

Please also update your account on our online manuscript submission system to add your ORCID details, which will then be automatically included in all future submissions. See here for step-by-step instructions and more information on author identifiers.

| First (given) and middle name(s) | Last (family) name(s) | ResearcherID | ORCID iD |
|---|---|---|---|
| Maria A. | Cardona | X-9208-2019 | 0000-0002-6320-1585 |
| Rui | Chen | | |
| Subhabrata | Maiti | | 0000-0002-2554-0762 |
| Ilaria | Fortunati | | |
| Camilla | Ferrante | | 0000-0002-4869-449X |
| Luca | Gabrielli | | |
| Krishnendu | Das | | 0000-0002-2210-7007 |
| Leonard J. | Prins | | 0000-0001-6664-822X |

# Queries for the attention of the authors

Journal: **ChemComm**

Paper: **d0cc05993e**

Title: **Time-gated fluorescence signalling under dissipative conditions**

For your information: You can cite this article before you receive notification of the page numbers by using the following format: (authors), Chem. Commun., (year), DOI: 10.1039/d0cc05993e.

Editor's queries are marked on your proof like this Q1, Q2, etc. and for your convenience line numbers are indicated like this 5, 10, 15, ...

Please ensure that all queries are answered when returning your proof corrections so that publication of your article is not delayed.

| Query reference | Query | Remarks |
|---|---|---|
| Q1 | Please confirm that the spelling and format of all author names is correct. Names will be indexed and cited as shown on the proof, so these must be correct. No late corrections can be made. | |

ChemComm

COMMUNICATION

# Time-gated fluorescence signalling under dissipative conditions†

Cite this: DOI: 10.1039/d0cc05993e

Received 5th September 2020,
Accepted 12th October 2020

DOI: 10.1039/d0cc05993e

rsc.li/chemcomm

Maria A. Cardona,[a] Rui Chen,[a] Subhabrata Maiti,[b] Ilaria Fortunati,[a] Camilla Ferrante,[a] Luca Gabrielli,[a] Krishnendu Das[a] and Leonard J. Prins*[a]

**Precise control over specific functions in the time domain is ubiquitous in biological systems. Here, we demonstrate time-gated fluorescence signalling under dissipative conditions exploiting an ATP-fueled self-assembly process. A temporal ATP-concentration gradient allows the system to pass through three states, among which only the intermediate state generates a fluorescent signal from a hydrophobic dye entrapped in the assemblies. The system can be reactivated by adding a new batch of ATP. The results indicate a strategy to rationally programme the temporal emergence of functions in complex chemical systems.**

Self-assembly provides an attractive tool to create supramolecular architectures through the spontaneous organization of molecular building blocks.[1–3] The resulting architecture is the direct consequence of the self-assembly pathway during which reversible non-covalent interactions develop between the building blocks.[4,5] Even though supramolecular chemistry has traditionally mainly dealt with thermodynamically controlled self-assembly processes, recent studies have shown that additional kinetic control over the self-assembly pathway provides access to a much wider range of complex structures.[6,7] Most current efforts in this direction rely on the exploration of intermediate kinetic structures that form *en route* to the thermodynamic end product upon mixing the building blocks.[2,8,9] On the other hand, kinetic control in nature is obtained using high energy molecules, such as ATP or GTP, which can trigger self-assembly processes. Temporal control over the formed structures is obtained by a simultaneous catalytic process which converts the high energy molecules in waste molecules unable to sustain the self-assembled structure.[10] One of the most notable examples is the GTP-driven self-assembly of tubulin dimer in polymeric microtubules, which spontaneously disassemble upon GTP hydrolysis by the assembly.[11] The unique behaviour of biological systems has inspired chemists to design chemically fuelled self-assembly processes in which a forward reaction activates the building blocks for self-assembly and a concurrent backward reaction brings the system back to the basal non-assembled state. Although we have recently shown that the way in which chemical energy is exploited can be very different, a common treat of all chemically fuelled self-assembly processes is the transient formation of the assembly – and the associated function – upon the batch wise addition of fuel.[12] This approach has now been amply used to transiently activate materials,[13–17] receptors,[19] catalysts[20–23] and more.[24–32]

It is relevant to stress the difference with the kinetic states of conventional self-assembled systems which progress towards the thermodynamic end state; once such systems have reached the thermodynamic state, they become unresponsive to external stimuli.[30] In contrast, chemically fuelled systems which have reached the final state after fuel depletion can be reactivated by providing a new batch of fuel.[12–32] Hence, the development of chemically fuelled self-assembly processes that display complex self-assembly pathways offers a new direction to control structure and function in a similar way as happens in Nature.[32]

Here, we show an ATP-templated self-assembly process that involves three states (unassembled → vesicle-like assemblies → aggregates), which get populated depending on the ATP-concentration (low-intermediate-high, respectively) (Fig. 1a). We couple this fuelled self-assembly process with an enzymatic fuel depletion process to create a temporal concentration gradient of ATP in the system. We show that this system displays time-gated signal generation, which implies the presence of a lag time between the addition of the chemical trigger and the generation of the output signal. The lag time originates from the fact that only the metastable intermediate state – formed at intermediate ATP concentrations – is fluorescently active (Fig. 1b). We demonstrate that the lag time is determined by the amount of ATP added to the system and that the system can be reactivated by adding a new batch of ATP.

[a] Department of Chemical Sciences, University of Padova, Via Marzolo 1, 35131 Padova, Italy. E-mail: leonard.prins@unipd.it
[b] Department of Chemical Sciences, Indian Institute of Science Education and Research (IISER) Mohali, Knowledge City, Manauli 140306, India

† Electronic supplementary information (ESI) available. See DOI: 10.1039/d0cc05993e

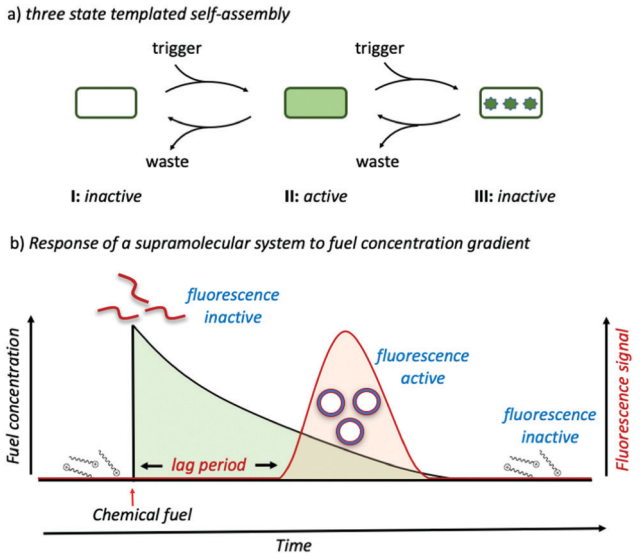



Fig. 1 (a) Three-state templated self-assembly under dissipative conditions. Addition of a chemical trigger activates building blocks for self-assembly, but at higher concentrations a second transition occurs to an inactive state. Dissipative conditions cause a gradual decrease to the original unassembled state. (b) Schematic illustration of the complex pathway towards temporal fluorescent signal generation in response to the fuel concentration gradient under dissipative condition.

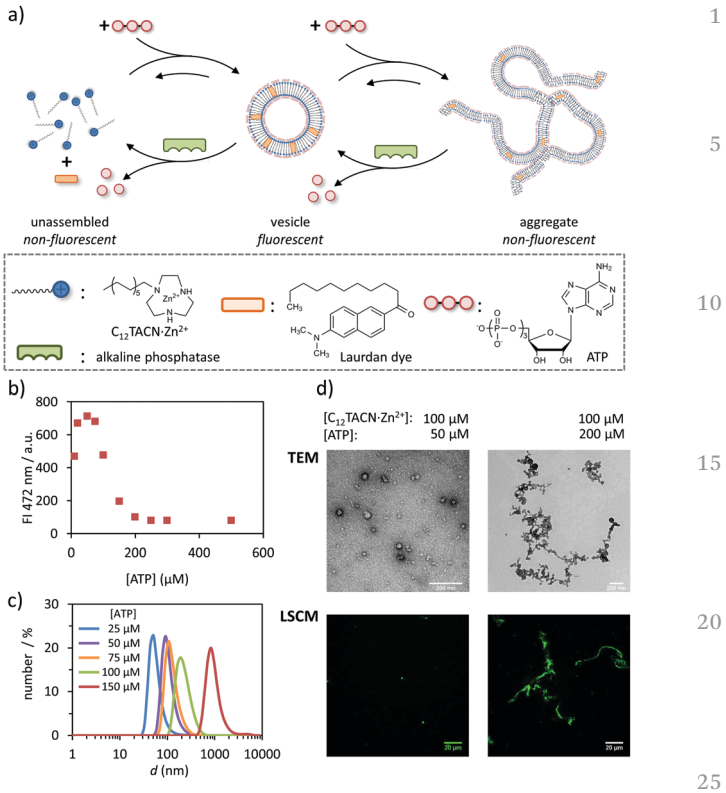



Fig. 2 Formation and characterization of ATP-templated assemblies. (a) Schematic representation of the ATP-templated assembly of $C_{12}$TACN·$Zn^{2+}$ into spherical assemblies and, at higher ATP concentrations, aggregates. A disassembly pathway is installed in the presence of the ATP-cleaving enzyme alkaline phosphatase. (b) Fluorescence intensity (FI) at 472 nm as a function of the ATP concentration added to solution of $C_{12}$TACN·$Zn^{2+}$ (100 μM) and laurdan dye (2 μM). (c) Hydrodynamic diameter of assemblies in the presence of ATP at different concentrations measured by DLS ([$C_{12}$TACN·$Zn^{2+}$] = 500 μM). (d) TEM (above) and LSCM (below) images of aggregates with [$C_{12}$TACN·$Zn^{2+}$] = 100 μM and [ATP] = 50 μM (left) and [$C_{12}$TACN·$Zn^{2+}$] = 100 μM and [ATP] = 200 μM (right). For LSCM measurements Coumarin 153 was used as a hydrophobic fluorescent probe. TEM images are stained with 2% uranyl acetate solution. The TEM and LSCM-images at [ATP] = 50 μM have been previously reported.[21] General experimental conditions: [HEPES] = 5 mM, pH 7.0, $T$ = 25 °C, $\lambda_{ex}$ = 370 nm, slits = 5/10 nm (ex/em) for laurdan dye and $\lambda_{ex}$ = 400 nm for Coumarin 153.

In recent years, we have extensively studied the ATP-templated self-assembly of amphiphiles composed of a Zn(II)-TACN (1,4,7-triazacyclonane) complex attached to hydrophobic saturated carbon chains of different length (C12–C20) (Fig. 2a).[15,23,27] In the presence of ATP the critical aggregation concentration (CAC) of the amphiphile is lowered, which is attributed to a combination of favourable entropic (multivalent counterion) and enthalpic (interactions between phosphate and Zn(II)-complex) contributions. The length of the carbon chain plays an important role in defining the structure of the templated assembly – ranging from flat disks for C20[27] to spherical vesicle-like structures for C16[15] and C12[23] – and the kinetic stability. An important feature of these templated assemblies is that they have a limited lifetime under dissipative conditions installed by the presence of a phosphatase enzyme, because the waste products of ATP hydrolysis have a much lower templating ability.

In the present study we use C12TACN·$Zn^{2+}$ as the amphiphile which is known to form vesicle-like aggregate far below its CAC (>1 mM) in the presence of low ATP concentration (0–50 μM).[23] Here, our objective was to explore the effect of ATP concentration on the assembly behaviour of the amphiphile. The formation of amphiphile-based assemblies can be conveniently followed by fluorescence spectroscopy owing to the capacity of the assemblies to up take a hydrophobic fluorophore in the apolar domains of the assemblies. Towards this objective we first titrated a fixed concentration (100 μM, far below its CAC) of C12TACN·$Zn^{2+}$ with increasing concentration of ATP. Laurdan dye (2 μM), a fluorophore which is widely used to study membrane properties, was present as a fluorescent probe to report on assembly formation.[33,34] Laurdan dye is hardly soluble in water and its fluorescence quantum yield is much higher in membranes compared to aqueous environments, resulting in a turn-on of the fluorescence intensity when the dye partitions into hydrophobic membranes. As expected, the fluorescence intensity increased upon the addition of ATP, indicating templated self-assembly (Fig. 2b). However, to our surprise the fluorescence intensity reached a maximum at around 50 μM of ATP after which it gradually decreased and completely disappeared at higher concentrations indicating a structural change in the self-assembly product (Fig. 2b).

This ATP concentration dependent fluorescence signalling intrigued us to perform morphological studies of the system. Previously reported studies on C12TACN·$Zn^{2+}$ amphiphiles had already shown the formation of vesicle-like structures at low

ATP concentrations.[23] Hence we first employed DLS measurements to confirm the formation of assemblies upon the addition of ATP (25–150 μM) to C12TACN·$Zn^{2+}$ (500 μM) (Fig. 2c). At low ATP concentrations structures with a hydrodynamic radius of around 40 nm were detected, which is indeed in line with those reported before.[23] Yet, an increase in hydrodynamic radius up to 1000 nm was observed when the ATP concentration was gradually increased to 150 μM. Slow precipitation over night was observed when the ATP concentration exceeded 100 μM. Further structural information on the assemblies was obtained from transmission electron microscopy (TEM) and laser scanning confocal microscopy (LSCM) (Fig. 2d and Fig. S3, S4, ESI†). TEM images obtained from a solution of C12TACN·$Zn^{2+}$ (100 μM) and low ATP (50 μM) showed spherical assemblies with dimensions in line with those measured using DLS. Yet, at higher ATP concentrations (200 μM), the assemblies were much less defined and appeared as elongated aggregates (Fig. 2d). Similar observations were made by LSCM (Fig. 2d).

Altogether, the data confirm that ATP templates the self-assembly of C12TACN·$Zn^{2+}$ in spherical vesicle-like assemblies, but at higher ATP:C12TACN·$Zn^{2+}$ ratios these assemblies further aggregate into larger sized objects with a rather undefined structure. This effectively means that the system can be present in three different states depending on the concentration of ATP; an unassembled state in the absence of ATP, a vesicular state in the presence of low amounts of ATP and a condensed aggregated state at higher ATP concentrations. The three different states present three different chemical environments with different properties. Indeed, the fluorescence titrations demonstrate that only the intermediate vesicular state generates a fluorescent signal. From biophysical studies of membranes it is known that the increased amount of water in distorted membranes causes a significant quenching of the fluorescence emission of laurdan dye.[35] The observation of fused, irregularly shaped structures in TEM images of the aggregates (Fig. 2d and Fig. S3, ESI†) leads us to postulate that the lower structural order of the aggregates leads to a quenching of the fluorescence emission of laurdan dye by water.

The presence of an ATP-concentration dependent three state assembly process and the observation of fluorescent signalling exclusively for the intermediate assembly state prompted us to investigate whether we could generate time-gated fluorescent signalling by creating a temporal high-to-low ATP concentration gradient in the presence of an enzyme. Time-gated signalling implies the presence of a lag time between the addition of the trigger and the emergence of the signal and would generate a temporal signal profile that is markedly different from those typically observed in equilibrium systems. In equilibrium systems, a trigger changes the equilibrium position according to Le Chatelier's principle leading to a new equilibrium state accompanied with the generation of a signal that remains constant over time (Fig. 3a). Recently, we have shown that under dissipative conditions – in which the chemical trigger is gradually deactivated in the system – the profile is characterized by a (rapid) increase in signal followed by a spontaneous gradual decrease (Fig. 3a).[15,36,37] As opposed

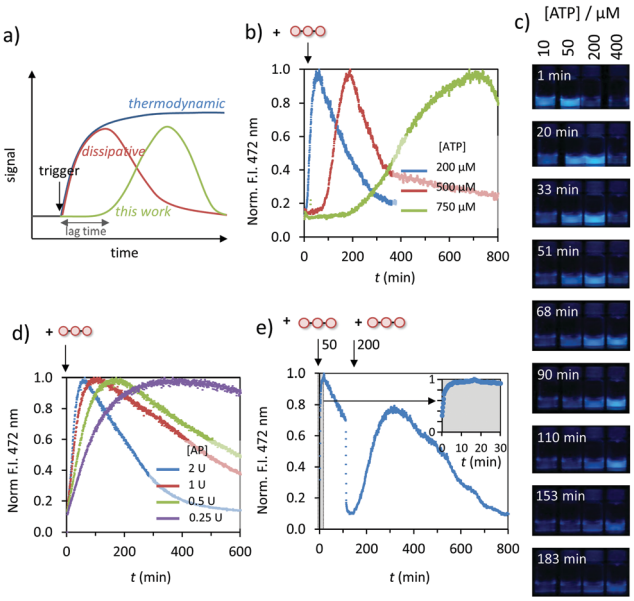



Fig. 3 Time-gated signal generation. (a) Signal output as a function of time in a system operating under thermodynamic (blue) and dissipative (red) conditions and the current system (green). (b) Normalized fluorescent intensity (FI) at 472 nm as a function of time following the addition of different concentrations of ATP to $C_{12}$TACN·$Zn^{2+}$ (100 μM) and laurdan dye (2 μM) in the presence of alkaline phosphatase (0.5 U). (c) Periodic photos taken when different ATP concentrations were added to solution of $C_{12}$TACN·$Zn^{2+}$ (100 μM), laurdan dye (3 μM) and alkaline phosphatase (10 U). Numbers indicate the time interval between the addition of ATP and the moment the image was taken. (d) Normalized fluorescent intensity (FI) at 472 nm following additions of ATP (200 μM) to a solution of $C_{12}$TACN·$Zn^{2+}$ (100 μM) and laurdan dye (2 μM) in the presence of different concentrations of alkaline phosphatase. (e) Normalized fluorescent intensity (FI) at 472 nm following sequential additions of ATP (50 μM, 200 μM) to a solution of $C_{12}$TACN·$Zn^{2+}$ (100 μM) and laurdan dye (2 μM) in the presence of alkaline phosphatase (0.3 U). General experimental conditions: $\lambda_{ex}$ = 370 nm, slits = 5/10 nm (ex/em), [HEPES] = 5 mM, pH 7.0, $T$ = 25 °C.

to an equilibrium assay, such a system can respond to the arrival of a new trigger. Yet, the potential introduction of a lag time in the current system is fascinating as it would indicate the possibility of temporal programming of events in a complex system regulated by chemical triggers.[15,36–39]

In a first experiment, ATP was added to a solution of $C_{12}$TACN·$Zn^{2+}$ (100 μM), laurdan dye (2.5 μM) and alkaline phosphatase (0.5 U) at a concentration (200 μM) at which aggregates are formed. As we have shown before, the presence of alkaline phosphatase causes the gradual conversion of ATP in the waste products adenosine (A) and phosphate ($P_i$) which are unable to template assembly formation at these concentrations.[23] After addition of ATP, no fluorescence was initially observed, but within minutes the fluorescence intensity started to increase and reached a maximum after around 40 minutes after which the signal gradually decreased in intensity (Fig. 3c). This observation can be explained by the enzymatic degradation of ATP, which causes the transition from non-fluorescent aggregates to fluorescent vesicles and finally to a non-fluorescent disassembled state. This delayed increase in fluorescence intensity was even more pronounced for samples

in which ATP was added at higher concentrations of 500 and 750 μM, respectively. Delay times of 70 and 200 minutes, respectively, were observed between the addition of the enzyme and the onset of the transient fluorescent signal (Fig. 3c). The lag times are determined by the time required for the enzyme to reduce the ATP concentration down to around 150 μM, which is the concentration around which the transition from aggregates to vesicles takes place. In a separate experiment, ATP-regulated time-gated fluorescence could be directly visualized by adding four different concentrations of ATP (10, 50, 200 and 400 μM) to a solution containing $C_{12}$TACN·$Zn^{2+}$, laurdan dye and the enzyme AP and taking photographs at regular time intervals under irradiation with UV-light. It can be clearly seen that the time required to observe the maximum in fluorescence intensity increases as a function of ATP. The dependence on enzyme activity emerged from an experiment in which different amounts of enzyme (0.25–2 U) were added to a solution containing a constant concentration of ATP (200 μM) and $C_{12}$TACN·$Zn^{2+}$ (100 μM) (Fig. 3e). The time required to reach the maximum of FI increased from 61 minutes to 365 minutes (which is nearly 6 hours!) when the enzyme concentration decreased from 2 U to 0.25 U. The unique response of this system to ATP was illustrated in an experiment in which the system was initially activated with 50 μM of ATP (Fig. 3f). During the decay phase of the signal, a new batch of ATP (200 μM) was added which provoked an immediate drop in fluorescence intensity illustrating that the transition into non-fluorescent aggregates occurred instantaneously. Yet, after a short lag time the fluorescence intensity increased again to reach a maximum before it decreased again until complete disappearance. It is important to note that the ATP-templated assemblies at these concentrations were stable over night in the absence of enzyme, indicating that the decrease in FI is not simply a result of precipitation. This is also evident from the homogeneous emergence of fluorescence in the unstirred cuvettes shown in the images of Fig. 3d and from the observation that shaking cuvettes after fluorescent decay did not result in an increase in fluorescence intensity.

In conclusion, we have shown that the sequential population of different assembly states in a chemically fuelled self-assembly process under dissipative conditions offers an attractive possibility to control systems' properties in the time domain. By installing an ATP-concentration gradient in time it is possible to temporally activate the fluorescence signalling of an assembly that is only present in a certain ATP-concentration regime. Importantly, the ATP-concentration not only determines the lifetime of the signal, but also regulates at which future moment after addition of the trigger the signal starts to appear. We envisage that this strategy will become an important tool to rationally programme the temporal emergence of functions in complex chemical systems.

Funding from the European Union Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement 642793, the Italian Ministry of Education and Research (grant 2017E44A9P), the Department of Excellence programme 'NExuS' and the MSCA SealofExcellence@UNIPD programme is acknowledged.

## Conflicts of interest

There are no conflicts to declare.

## Notes and references

1 J.-M. Lehn, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 4763–4768.
2 P. A. Korevaar, C. J. Newcomb, E. W. Meijer and S. I. Stupp, *J. Am. Chem. Soc.*, 2014, **136**, 8540–8543.
3 G. M. Whitesides, J. P. Mathias and C. T. Seto, *Science*, 1991, **254**, 1312–1319.
4 J.-M. Lehn, *Angew. Chem., Int. Ed. Engl.*, 1990, **29**, 1304–1319.
5 T. Aida, E. W. Meijer and S. I. Stupp, *Science*, 2012, **335**, 813–817.
6 Y. Mai and A. Eisenberg, *Chem. Soc. Rev.*, 2012, **41**, 5969–5985.
7 E. Mattia and S. Otto, *Nat. Nanotechnol.*, 2015, **10**, 111–119.
8 P. A. Korevaar, S. J. George, A. J. Markvoort, M. M. J. Smulders, P. A. J. Hilbers, A. P. H. J. Schenning, T. F. A. De Greef and E. W. Meijer, *Nature*, 2012, **481**, 492–497.
9 A. Aliprandi, M. Mauro and L. De Cola, *Nat. Chem.*, 2016, **8**, 10–15.
10 C. T. Walsh, B. P. Tu and Y. Tang, *Chem. Rev.*, 2018, **118**, 1460–1494.
11 A. Akhmanova and M. O. Steinmetz, *Nat. Rev. Mol. Cell Biol.*, 2015, **16**, 711–726.
12 G. Ragazzon and L. J. Prins, *Nat. Nanotechnol.*, 2018, **13**, 882–889.
13 S. De and R. Klajn, *Adv. Mater.*, 2018, **30**, 1706750.
14 J. Boekhoven, W. E. Hendriksen, G. J. M. Koper, R. Eelkema and J. H. van Esch, *Science*, 2015, **349**, 1075–1079.
15 S. Maiti, I. Fortunati, C. Ferrante, P. Scrimin and L. J. Prins, *Nat. Chem.*, 2016, **8**, 725–731.
16 S. Debnath, S. Roy and R. V. Ulijn, *J. Am. Chem. Soc.*, 2013, **135**, 16789–16792.
17 M. Tena-Solsona, B. Riess, R. K. Grotsch, F. C. Lohrer, C. Wanzke, B. Kasdorf, A. R. Bausch, P. Muller-Buschbaum, O. Lieleg and J. Boekhoven, *Nat. Commun.*, 2017, **8**, 15895.
18 A. Jain, S. Dhiman, A. Dhayani, P. K. Vemula and S. J. George, *Nat. Commun.*, 2019, **10**, 450.
19 E. Del Grosso, A. Amodio, G. Ragazzon, L. J. Prins and F. Ricci, *Angew. Chem., Int. Ed.*, 2018, **57**, 10489–10493.
20 P. Solís Muñana, G. Ragazzon, J. Dupont, C. Z.-J. Ren, L. J. Prins and J. L.-Y. Chen, *Angew. Chem., Int. Ed.*, 2018, **57**, 16469–16474.
21 H. L. Che, S. P. Cao and J. C. M. van Hest, *J. Am. Chem. Soc.*, 2018, **140**, 5356–5359.
22 S. Bal, K. Das, S. Ahmed and D. Das, *Angew. Chem., Int. Ed.*, 2019, **131**, 250–253.
23 M. A. Cardona and L. J. Prins, *Chem. Sci.*, 2020, **11**, 1518–1522.
24 S. M. Morrow, I. Colomer and S. P. Fletcher, *Nat. Commun.*, 2019, **10**, 1011.
25 L. Heinen and A. Walther, *Sci. Adv.*, 2019, **5**, eaaw0590.
26 S. Dhiman, A. Jain and S. J. George, *Angew. Chem., Int. Ed.*, 2017, **56**, 1329–1333.
27 F. della Sala, W. Verbeet, S. Silvestrini, I. Fortunati, C. Ferrante and L. J. Prins, *ChemNanoMat*, 2018, **4**, 821–830.
28 S. Chandrabhas, M. Olivo and L. J. Prins, *ChemSystemsChem*, 2020, **2**, e1900063.
29 C. Wanzke, A. Jussupow, F. Kohler, H. Dietz, V. R. I. Kaila and J. Boekhoven, *ChemSystemsChem*, 2020, **2**, e1900044.
30 A. Sorrenti, J. Leira-Iglesias, A. J. Markvoort, T. F. A. de Greef and T. M. Hermans, *Chem. Soc. Rev.*, 2017, **46**, 5476.
31 J. Leira-Iglesias, A. Sorrenti, A. Sato, P. A. Dunne and T. M. Hermans, *Chem. Commun.*, 2016, **52**, 9009.
32 S. Dhiman, A. Jain, M. Kumar and S. J. George, *J. Am. Chem. Soc.*, 2017, **139**, 16568–16575.
33 T. Parasassi, G. De Stasio, A. Dubaldo and E. Gratton, *Biophys. J.*, 1990, **57**, 1179–1186.
34 F. M. Harris, K. B. Best and J. D. Bell, *Biochim. Biophys. Acta, Biomembr.*, 2002, **1565**, 123–128.
35 Y. Ma, A. Benda, J. Kwiatek, D. M. Owen and K. Gaus, *Biophys. J.*, 2018, **115**, 1498–1508.
36 C. Pezzato and L. J. Prins, *Nat. Commun.*, 2015, **6**, 7790.
37 E. Shandilya and S. Maiti, *ChemSystemsChem*, 2020, **2**, e1900040.
38 S. Dhiman, A. Jain, M. Kumar and S. J. George, *J. Am. Chem. Soc.*, 2017, **139**, 16568–16575.
39 T. Heuser, E. Weyandt and A. Walther, *Angew. Chem., Int. Ed.*, 2015, **54**, 13258–13262.