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Electronic Supplementary Information

Time-gated fluorescence signalling under dissipative conditions

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

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1. Materials and instrumentation

All commercially available reagents were purchased from Merck unless mentioned otherwise and used as received. ATP and adenosine stock solutions were prepared in MilliQ water by weight and the exact concentration was calculated by UV-Vis spectroscopy using the molar extinction coefficients: ε_{259} (ATP, adenosine) = 15400 M^{-1} cm^{-1}. Inorganic phosphate solution was prepared by weight in MilliQ water. $Zn(NO_3)_2$ was purchased as analytical grade product. The concentration of the metal ion stock solution was determined by ICP (inductively coupled plasma) analysis. The buffer 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was prepared as 100 mM solutions in MilliQ water and adjusted to a pH of 7.0 at room temperature using a pH-meter Metrohm-632 equipped with Ag/AgCl/KCl reference electrode.
The stock solution of the fluorophore laurdan dye, 6-dodecanoyl-N,N-dimethyl-2-naphthylamine was prepared in DMF (*N*,*N*-dimethylformamide) by weight. Coumarin 153 was prepared as a stock solution by weight in spectroscopic grade methanol. The synthesis and characterization of C_{12}TACN has been reported previously.[1]

Fluorescence Spectroscopy: Fluorescence measurements were recorded on a Varian Cary Eclipse fluorescence spectrophotometer equipped with a thermostatted multiple cell holder hosting a maximum of 4 cuvettes. Experiments were typically conducted in 1 mL fluorescence quartz cuvettes. Fluorescence spectra were obtained in either scan mode or kinetics mode in which the emission at a particular wavelength was monitored over time. After each addition of the respective component to the sample cuvette, the sample was allowed to equilibrate before measurement. Fluorescence measurements for gels were recorded on a TECAN M1000 PRO plate reader.

UV-Vis Measurements: UV-Vis measurements were recorded on a Varian Cary50 spectrophotometer holding a thermostatted multiple cuvette holder. Individual spectra were acquired in Scan Mode. The spectra were then exported and analysed using Microsoft Excel © and plotted using Origin ©. Analysis were conducted using 1 mL Quartz cuvettes.

DLS Analysis: DLS measurements were recorded on a Malvern Zetasizer Nano-S instrument. Samples were analysed in disposable low volume cuvettes.

TEM Analysis: TEM images were recorded on a Jeol 300 PX electron microscope. One drop of sample was placed on the sample grid for 1 minute. For staining purposes, it was then placed on a drop of uranyl acetate (2%) for 30 s. The solvent was evaporated before the stained grid was imaged. TEM images were elaborated using the software ImageJ.

LSCM Analysis: Confocal images were taken using a laser scanning confocal microscope (BX51WI-FV300-Olympus) coupled to a frequency doubled Ti:Sapphire femtosecond laser at 400 nm, 76 MHz (VerdiV5Mira900-F Coherent). The laser beam was scanned on a 40x40 µm sample area with a 512x512 resolution, using a 60x water immersion objective (UPLSAPO60xW-Olympus). Images were visualised by adding coumarin153 at a concentration of 0.5 µM.

Photographs and video: Photographs and video clips were taken with a Sony DSC-RX100M5. Video of the entire process was shot using a Subea Geye 500 with a resolution of 1080p and 30 FPS with a 130° wide angle lens. Video analysis was carried out using VSDC Video Editor©.

2. Fluorescence experiments

2.1 Enzymatic degradation of ATP in solution under different amounts of enzyme

ATP (50 µM) was added to a solution of $C_{12}TACN{\cdot}Zn^{2+}$ (100 µM), laurdan dye (2.0 µM) and AP (0-2.0 U) at a concentration at which vesicles are formed. An immediate increase in fluorescence at 472nm was observed. After reaching the maximum, the intensity decreased at a different rate in the presence of different amounts of alkaline phosphatase, as shown in Fig. S1a. The rate of decay was calculated from the gradient of the linear portion of the decay curve at the different enzyme concentrations. The values were plotted against enzyme concentrations, as shown in Fig. S1b.

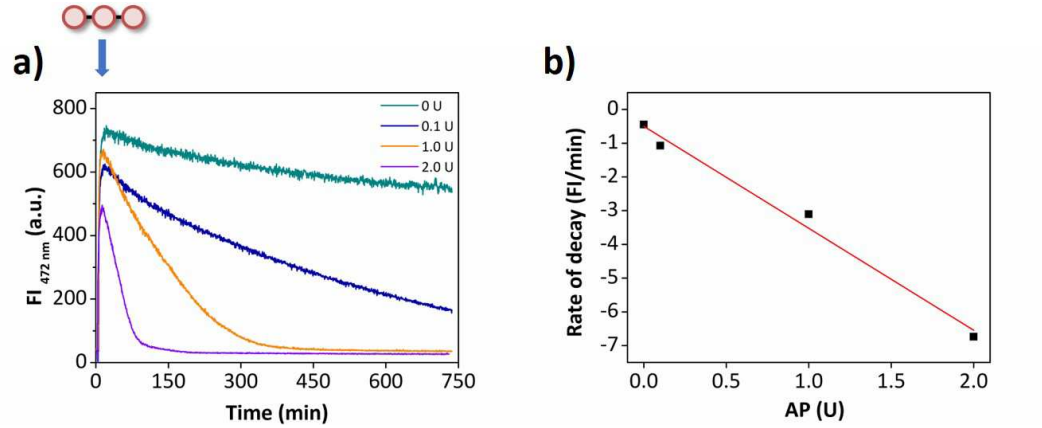

Fig. S1. a) The fluorescent intensity (FI) at 472 nm following additions of ATP (50 µM) to a solution of $C_{12}TACN{\cdot}Zn^{2+}$ (100 µM) and laurdan dye (2 µM) in the presence of different concentrations of alkaline phosphatase (0, 0.1, 1.0, 2.0 U). λ_{ex} = 370 nm, Slits = 5/10 nm (ex/em), T = 25 ^{o}C, [HEPES] = 5 mM, pH 7.0, [laurdan dye] = 2 µM. b) Decay rate of the linear decrease in fluorescence intensity recorded by the fluorimeter in a) as a function of the concentration of alkaline phosphatase (AP), y = -3.002x – 0.499, R^2 = -0.994.

2.2. Enzymatic degradation of different amounts of ATP in solution in the presence of the same amount of enzyme

The addition of 100 µM ATP to $C_{12}TACN{\cdot}Zn^{2+}$ (100 µM) resulted in an immediate increase in fluorescence intensity, and the fluorescence signal was stable in the absence of AP. This indicates the formation of intermediate state vesicles. The fluorescent signal gradually decayed in the presence of 2.0 U enzyme since the enzymatic degradation of ATP caused the dissociation of vesicles (Fig. S2a). However, when 200 µM ATP was added to the same surfactant concentration, the fluorescence intensity remained low in the absence of alkaline phosphatase (AP). However, when the same experiment was carried out in the presence of 2.0 U alkaline phosphatase (AP), the fluorescence intensity was initially low, but over time, it increased and then decayed. These observations show that 200 µM ATP is high enough to cause the formation of non-fluorescent aggregates, and the hydrolysis of ATP in the presence of enzyme reduces the amount of ATP to a concentration at which the transition from aggregates to vesicles takes place (Fig. S2b).

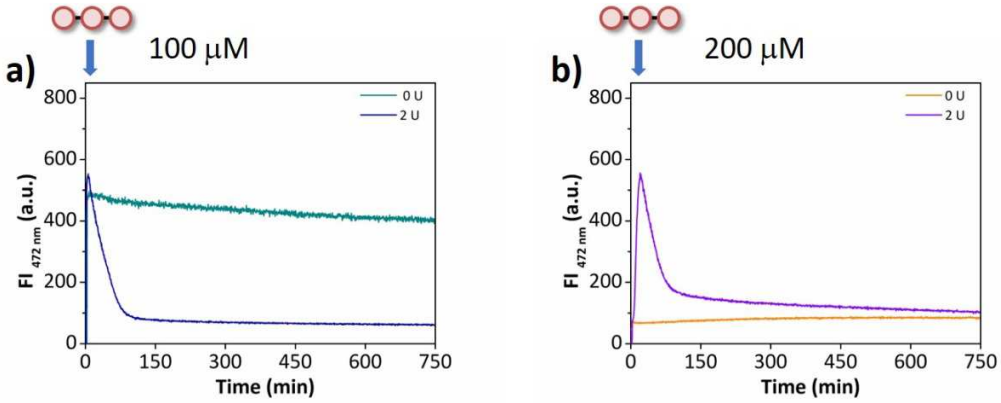

Fig. S2. a) Fluorescent intensity (FI) at 472 nm following additions of ATP (100 μM) to a solution of $C_{12}TACN{\cdot}Zn^{2+}$ (100 μM) and laurdan dye (2 μM) in the presence of different concentrations of alkaline phosphatase (AP). b) Fluorescent intensity (FI) at 472 nm following additions of ATP (200 μM) to a solution of $C_{12}TACN{\cdot}Zn^{2+}$ (100 μM) and laurdan dye (2 μM) in the presence of different concentrations of alkaline phosphatase (AP). λ_{ex} = 370 nm, Slits = 5/10 nm (ex/em), T = 25 ^{o}C, [HEPES] = 5 mM, pH 7.0.

3. Microscopy analysis

3.1. TEM images

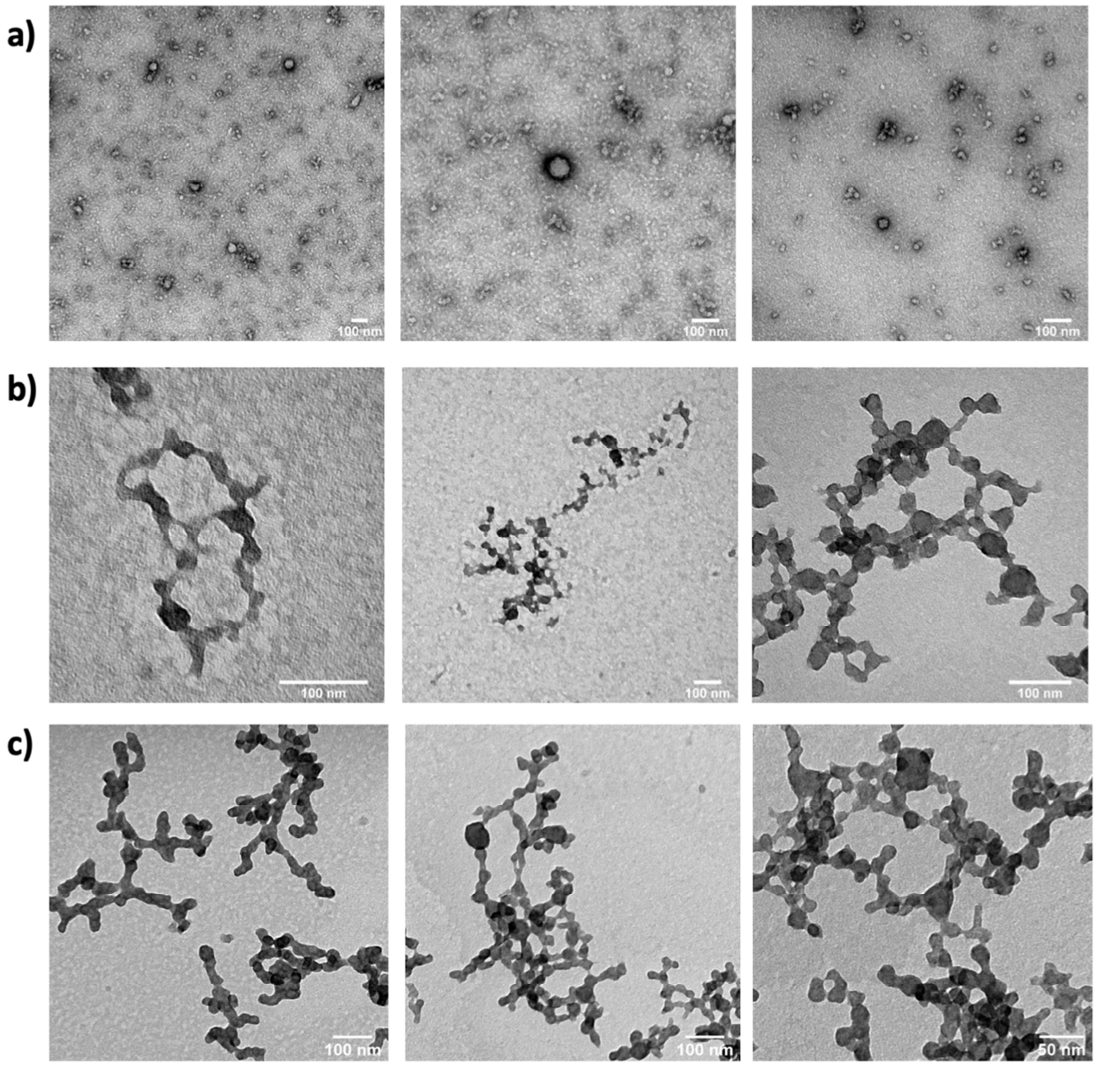

Fig. S3. More TEM images of aggregates. a) with [C_{12}TACN·Zn^{2+}] = 100 µM and [ATP] = 50 µM; b) and c) [C_{12}TACN·Zn^{2+}] = 100 µM and [ATP] = 200 µM. [HEPES] = 5 mM, pH 7.0, T = 25 ^{o}C.

3.2. Confocal images

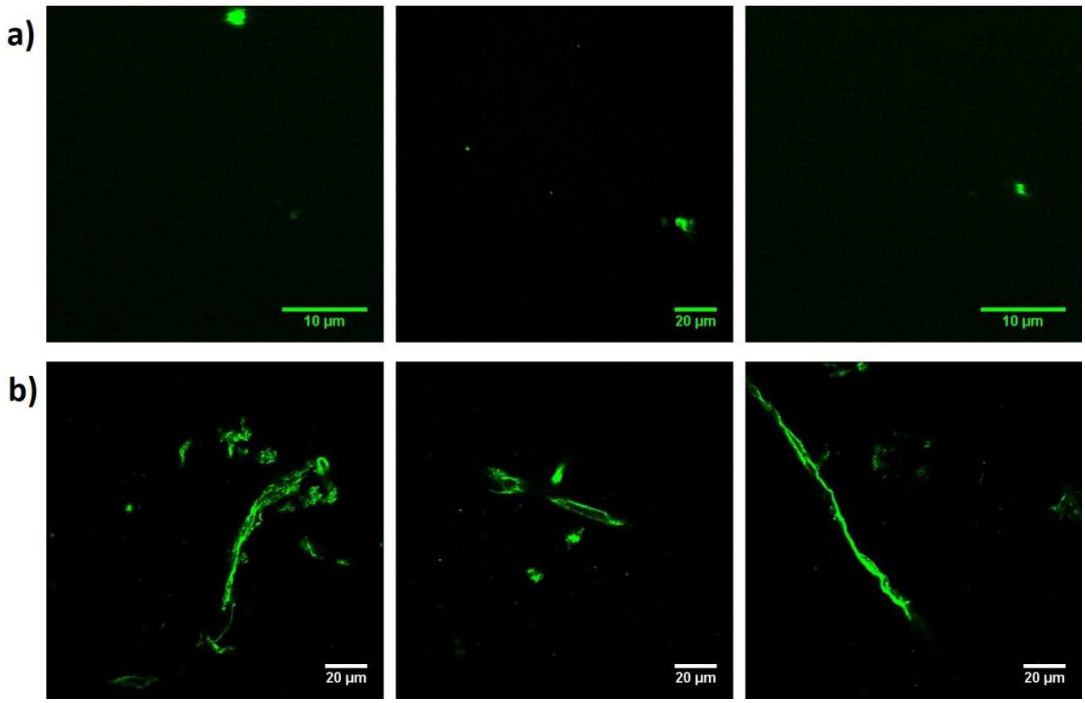

Fig. S4. More LSCM images of aggregates. a) with [C_{12}TACN·Zn^{2+}] = 100 µM and [ATP] = 50 µM, and b) [C_{12}TACN·Zn^{2+}] = 100 µM and [ATP] = 200 µM. [HEPES] = 5 mM, pH 7.0, [Coumarin 153] = 0.5 µM, T = 25 ^{o}C.

3.3. Visualisation of degradation of assemblies upon hydrolysis of ATP by alkaline phosphatase (AP)

The degradation of the vesicles was visualised by LSCM upon addition of ATP (10 µM) to a buffered solution of $C_{12}TACN{\cdot}Zn^{2+}$ (100 µM), alkaline phosphatase (5U) and Coumarin 153 (0.5 µM). Periodic images were taken at 10 minutes intervals. Under the conditions chosen (10 µM ATP and 5 U AP), the entire process could be visualized in 200 minutes.

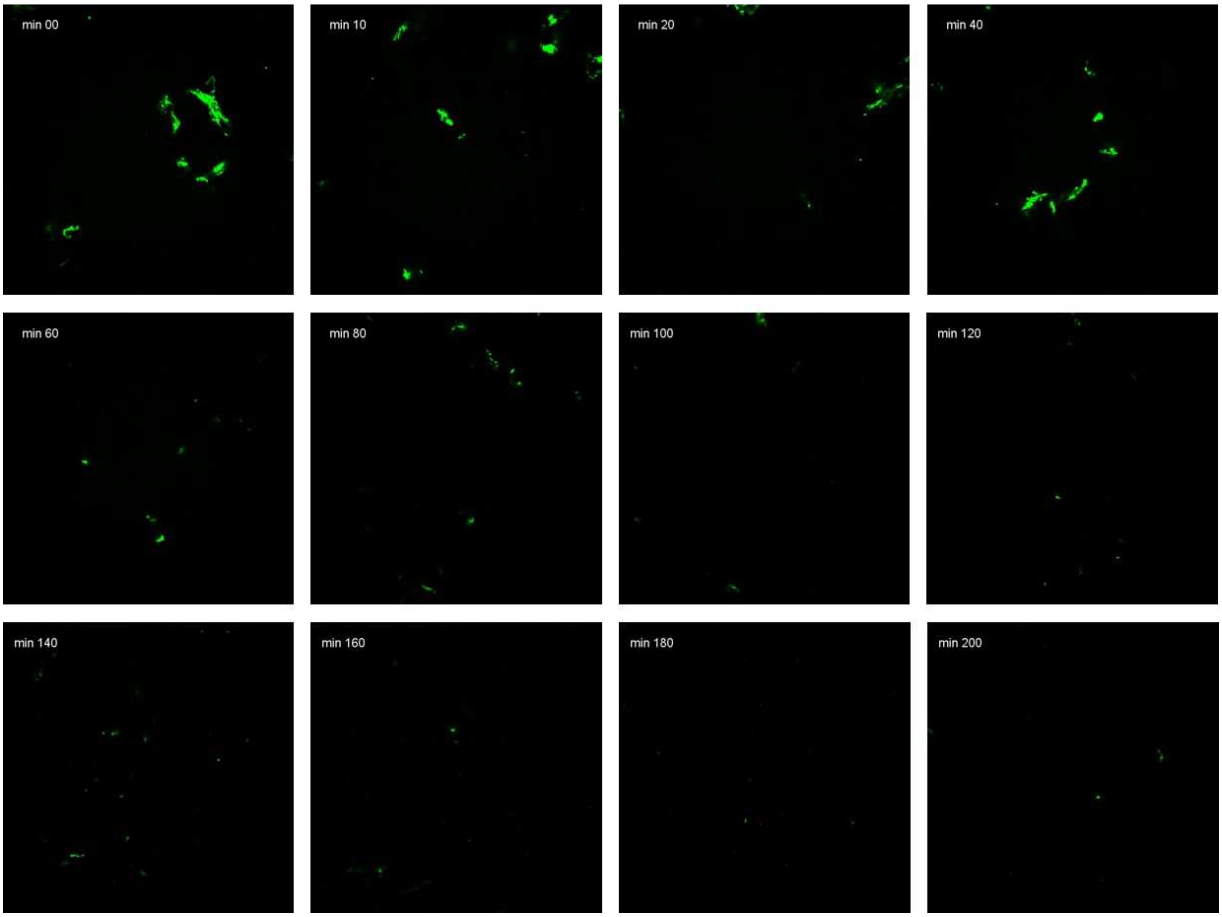

Fig. S5. LSCM images of assemblies with [$C_{12}TACN{\cdot}Zn^{2+}$] = 100 µM, [ATP] = 10 µM and [AP] = 5U over time. [HEPES] = 5 mM, pH 7.0, [Coumarin 153] = 0.5 µM, T = 25 ^{o}C.

4. Videos

Vials containing a 1 mL solution of C_{12}TACN·Zn^{2+} (100 µM), laurdan dye (3 µM) and the enzyme AP (10 U) in HEPES buffer (10 mM, pH 7.0) were prepared and irradiated with UV-light. The experiment was started by adding different concentrations of ATP to the different vials (Fig. 3b).

Video S1 shows a fast-tracked low-resolution video depicting the entire time-gated fluorescence process for three concentrations of ATP: A = 50 µM, B = 100 µM, C = 400 µM. The time for the entire process to be visualized was approximately 210 minutes (3.5 hours). The video was accelerated 16 times.

Video S2 shows a combined set of short clips shot with a high-resolution camera at intervals during the same process. The same conditions as Video S1 were used for four different ATP concentrations: A = 10 µM, B = 50 µM, C = 100 µM, D = 400 µM.

Video S3 shows the process in Fig. S5 over time.

5. References

1. M. A. Cardona, L. J. Prins, *Chem. Sci.* 2020**, 11**, 1518-1522.