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Supplementary Information

Controlled release and characterisation of photocaged molecules using in situ LED illumination in solution NMR spectroscopy

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Table of Contents:

Materials and Methods

Sample Preparation: Adenosine 5'-triphosphate, P3-(1-(2-nitrophenyl)ethyl) ester, disodium salt (NPE caged-ATP, ThermoFisher Scientific, A1048) was prepared as a 10 mM stock in $^{2}H_2O$ (99.9%, Sigma-Aldrich), aliquoted, and frozen at -20°C. For NMR samples, NPE caged-ATP aliquots were defrosted immediately before use, and added to a final concentration of 1 mM (and thus adding 10% $^{2}H_2O$ required for NMR field-frequency lock). All experiments were conducted in 100 mM Tris buffer, pH 7.6 (Tris base and HCl, both Sigma-Aldrich), with 200 mM sodium acetate (Sigma-Aldrich, S2889), 5 mM $MgCl_2$ (Fisher, 10647032), 1 mM NPE caged-ATP and 10% $^{2}H_2O$. Acetate kinase (lyophilised powder from *Escherichia coli*, Sigma-Aldrich, A7437) was prepared fresh in buffer as a 100 U/ml stock, and used at 2 U/ml final concentration in NMR experiments.

NMR spectroscopy: ^{1}H NMR experiments were performed using a Bruker 500 MHz Avance III spectrometer with a QCI-F cryoprobe with cooled ^{1}H channel and sample temperature control. NMR samples were placed in 5 mm quartz NMRtorch tubes prepared as described earlier,[1] and sealed with transparent caps made from quartz rods. *In situ* illumination was conducted using an NMRtorch equipped with a single array of 4x365 nm LEDs (LZ4-V4UV0R from LED Engin, nominal power 10W), with illumination duration controlled by the NMR console and transistor-transistor logic (TTL) triggers (see Bramham & Golovanov, 2022 [1] for more details about the NMRtorch approach). ^{1}H NMR experiments (2 dummy scans, 8 scans, 6 s effective relaxation delay) were recorded at 25°C using the excitation sculpting water suppression pulse sequence (zgesgp), modified as needed to be acquired in a pseudo-2D time-resolved fashion and with pulse sequence control of illumination triggering and duration. ^{1}H decoupled ^{31}P NMR spectra were acquired using a Bruker 400 MHz spectrometer with broadband probe and the zgpg30 pulse sequence (1k scans).

NPE caged-ATP photolysis: Initial calibration of the photorelease was performed by repeated cycles of 1 s illumination with a 1D ^{1}H NMR spectra recorded after each illumination. The resulting calibration curve was fit to the exponential equation:

$$[Free\ ATP]_T = [Free\ ATP]_{maximum} x(1 - e^{-kT})$$

where *k* is the rate constant, and *T* is total illumination time at given time point. Thus, the illumination time required to release a given amount of ATP from photocaged can be determined. For the titration experiments, illumination durations used were 0.85, 0.96, 1.08, 1.25, 1.47, 1.8, 2.31, 3.24, and 5.46 s in sequential order.

Data processing and analysis: NMR spectra were initially processed and analysed using Topspin 4.1 and Dynamics Centre 2.7 (both Bruker), with spectral deconvolution performed using SpinWorks (University of Manitoba). Graphs were plotted using Prism 9 (GraphPad), and figures prepared using CorelDRAW 2020 (Corel).

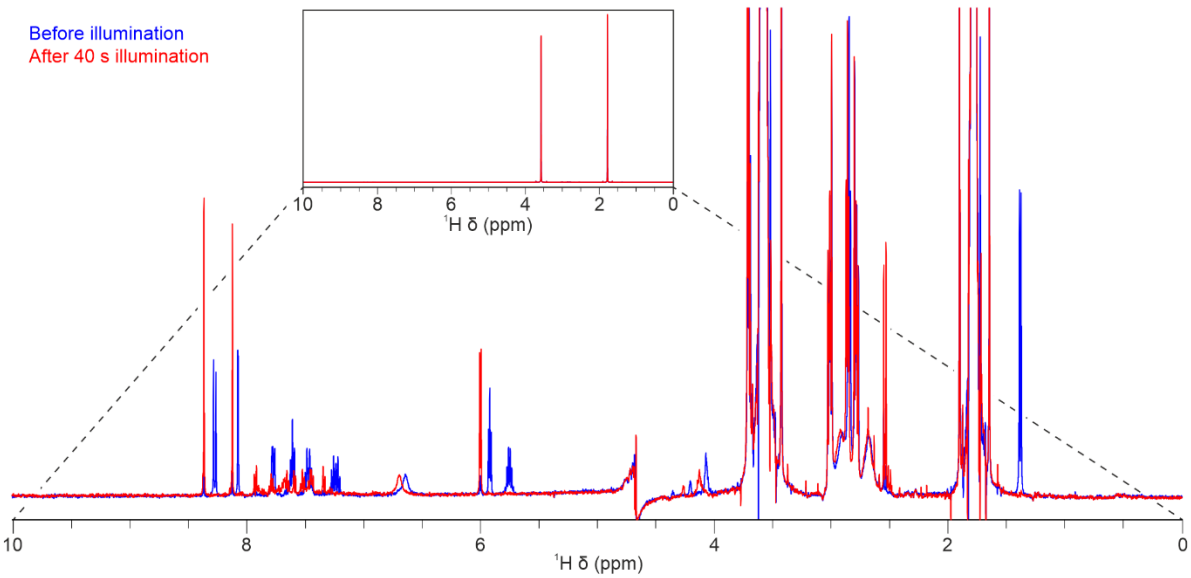

Figure S1. ^{1}H NMR spectra of NPE-ATP before and after 40 s illumination. The characteristic signals in the spectrum are strongly affected by illumination. The inset shows that the spectrum as a whole is dominated by high concentration Tris buffer (~3.6 ppm) and acetate (~1.8 ppm) signals.

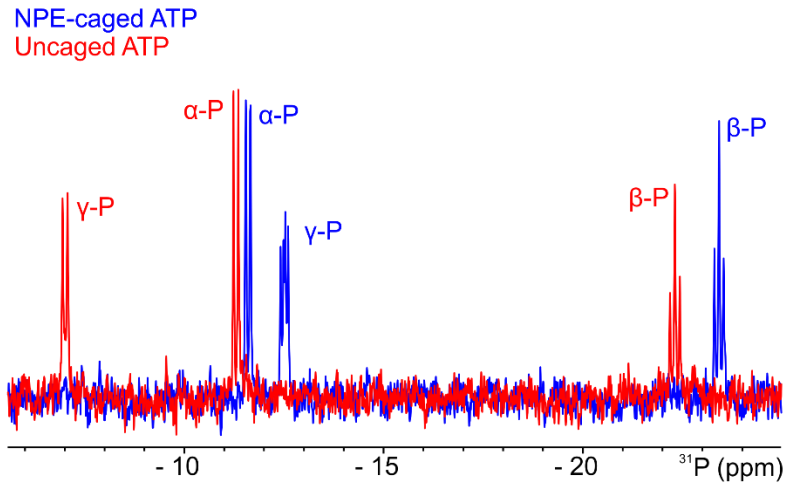

Figure S2. Assessment of the uncaging of ATP by ^{1}H-decoupled ^{31}P NMR spectroscopy. Complete uncaging observed (within the limits of detection) after 30 s illumination.

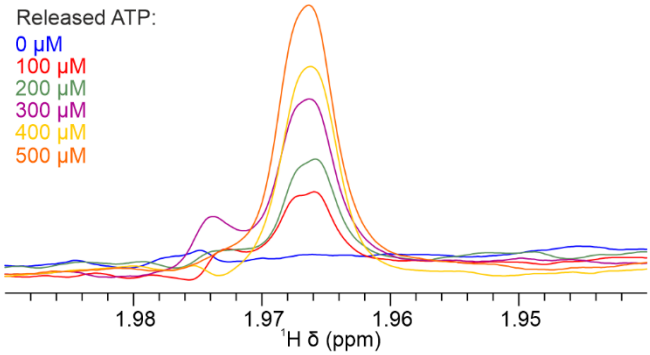

Figure S3. Growth of acetyl phosphate methyl signal in ^{1}H NMR following ATP release. Signal may suffer from some distortions due to proximity to very high intensity acetate methyl signal.

Supplementary References

1. J. E. Bramham and A. P. Golovanov, *Communications Chemistry*, 2022, **5**, 90. https://doi.org/10.1038/s42004-022-00704-5