Electronic Supplementary Information (ESI) for Chemical Communications

Monitoring ATP hydrolysis and ATPase inhibitor screening using ^{1}H NMR†

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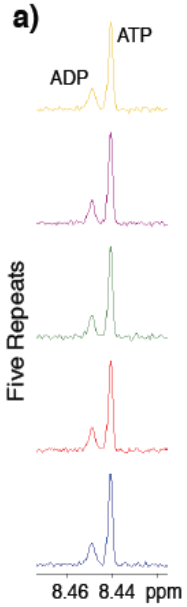
Supplementary Figures



b)

Repeats	1	2	3	4	5
ATP%	73.98	74.89	76.26	74.54	73.75
Average ATP%	74.68±0.45				
ATP concentration/μM	23.90±0.15				

Supplementary Figure 1. ATP/ADP (75/25) standard tested on a 500 MHz magnet without cryogenic probe. (a) ^{1}H 1D NMR Spectra of five independent repeats acquired on the same sample. (b) Integration of the peaks and standard error associated with the determination of ATP percentage and ATP concentration.

Experimental

Protein purification

In this study, rabbit skeletal muscle actin was purified from acetone powder, as described previously.[S2] The cloning, expression and purification of INF2-FFC and profilin utilized previously described protocols.[S1,S3]

ATP/ADP control experiment

No protein was used for the control runs, experiments were carried out with different ATP/ADP ratios in 50 mM KCl, 1 mM $MgCl_2$, 1 mM EGTA, 10 mM imidazole-HCl, 1.6 mM Tris-HCl, pH 7.4, 0.4 mM DTT and 1.25 mM sodium azide. Total ATP/ADP concentrations were kept the same as in the actin experiments, at 160 μM. Samples were boiled and diluted 5-fold in water as in the actin experiments. Experiments were run in the automation mode on a Bruker 700 MHz Avance III spectrometer at 298K using 600 μL of sample (with 5% D_2O) in a 5 mm NMR tube and excitation sculpting pulse sequences for water suppression.[S4] 256 scans were acquired in each experiment. Data were processed using a squared sine-bell window function with a shift factor of 4.5 before Fourier transform. Peaks corresponding to ATP, ADP and AMP were integrated to calculate the ATP percentage. R^2 of calculated samples from the theoretical ones were obtained using the following equation.

$$R^2 = 1 - \sum(y_{cal}-y_{theo})^2 / \sum(y_{cal}-y_{mean})^2$$

Where the y_{cal} is the calculated ATP percentage, y_{theo} is theoretical percentage and y_{mean} is the mean value between the two for each sample.

ATP hydrolysis experiment

Experiments were carried out at 23 °C in the same buffer as the controls. The actin concentration was 4.9 μM. In the "actin filament formation" condition, 2 μM IFN2-FFC was added, and in the "accelerated filament formation" condition, 2 μM INF2-FFC and 10 μM profilin were added. Reactions were terminated at different time points by boiling for 5 minutes, then cooled for 1 minute, centrifuged at 13,000 x g for 5 minutes in a fixed angle rotor (microfuge), and the supernatant diluted 5-fold in water. The boiling step

stops the reaction by denaturing the actin, which aggregates and sediments upon centrifugation. Samples were tested the same way as the control samples.

Inhibitor screening

Inhibitor screening experiments were based on the "accelerated filament formation" conditions with INF2-FFC and profilin. Seven random small molecules were selected and one known actin inhibitor latrunculin was used to make the small pool of molecules for inhibitor screening. Small molecules were added at 20 μM concentration and a reaction time of 30 min was used, followed by the procedure to terminate the reactions. To demonstrate the feasibility of screening in a low volume format, experiments were run in the automation mode on a Bruker 600 MHz Avance III spectrometer using a 1.7 mm NMR tube with only 35 μL of sample (10% D_2O), at 298 K. The pulse sequence used was the same as above, with 1024 scans acquired for each sample. Data were processed using a squared sine-bell window function with a shift factor of 5 before Fourier transform. Peaks corresponding to ATP, ADP and AMP were integrated to calculate the ATP percentage.

GTP/GDP control experiments

As a proof of concept that the method can be used to monitor GTP hydrolysis, samples containing different ratios of GTP/GDP was prepared in the same manner as the ATP/ADP standard,s in TBS buffer (20 mM Tris-HCl, pH 7.5, 137 mM NaCl). Samples were also boiled and diluted 5-fold in water, and tested in the automation mode on a Bruker 700 MHz Avance III spectrometer using 160 μL of sample (5% D_2O) in a 3 mm NMR tube, at 298 K. Data were processed using a squared sine-bell window function with a shift factor of 4.5 before Fourier transform. Peaks corresponding to GTP and GDP were integrated to calculate the GTP percentage.

ATP/ADP control experiments on 500MHz magnet with a room temterature probe

In this study, a 500 MHz spectrometer equipped with a room temperature TBI probe was used to test whether the same method can be applied utilizing commonly available NMR instrumentation (i.e. operating at a lower magnetic field and in absence of cryogenic

probe). The ATP/ADP sample at a ratio of 75/25 was used and prepared as described above in TBS buffer. 600 μL of sample with 5% D_2O was tested in a 5 mm NMR tube at 297 K, with the same pulse sequence and 128 scans. Data processing was the same except using a squared sine-bell window function with a shift factor of 6.5. Peaks corresponding to ATP and ADP were integrated to calculate the ATP percentage and concentration.

Supporting References

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S3. Harris, E. S., Li, F. & Higgs, H. N. The Mouse Formin, FRLα, Slows Actin Filament Barbed End Elongation, Competes with Capping Protein, Accelerates Polymerization from Monomers, and Severs Filaments. *J. Biol. Chem.* **279,** 20076–20087 (2004).

S4. Hwang, T. L. & Shaka, A. J. Water Suppression That Works. Excitation Sculpting Using Arbitrary Wave-Forms and Pulsed-Field Gradients. *J. Magn. Reson.* **A 112,** 275–279 (1995).