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Supplemental Information

A Molecular Model for Lithium's Bioactive Form

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Supporting Material

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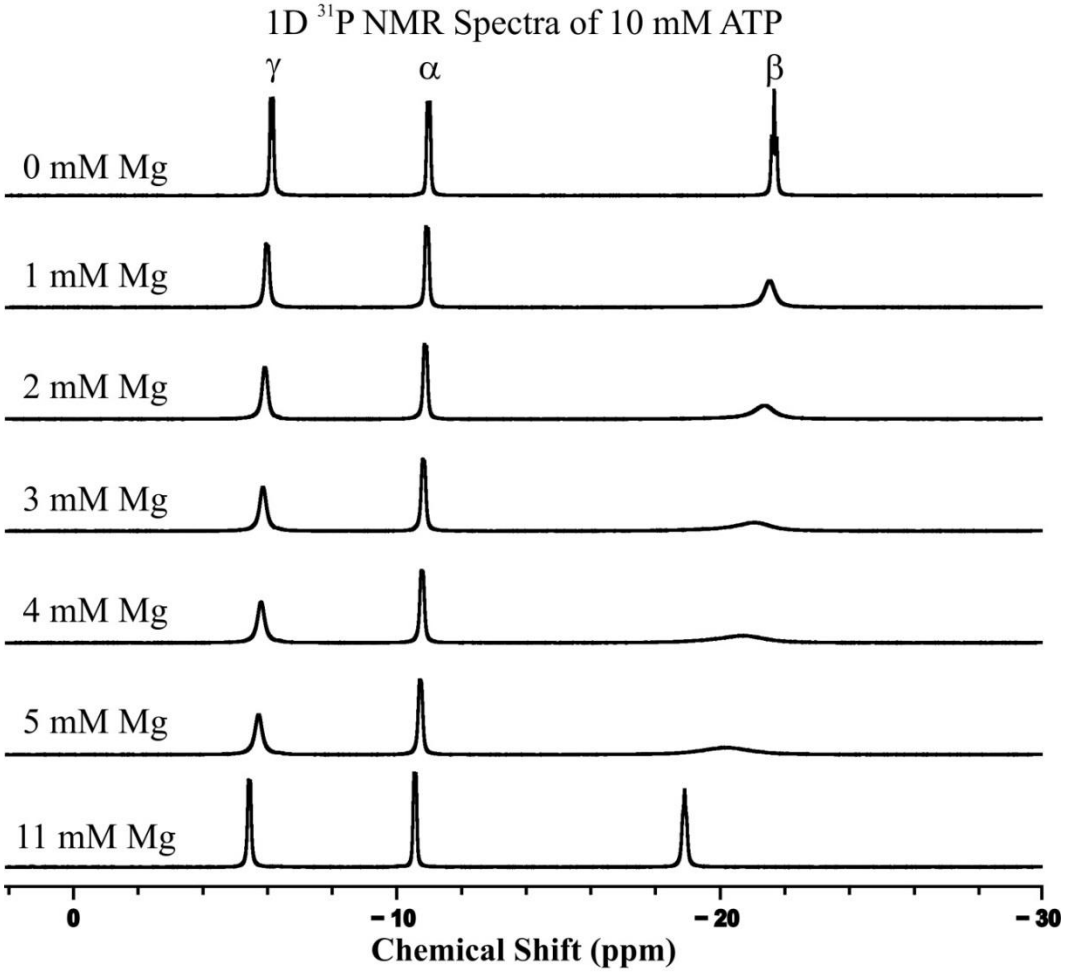

Figure S1. Saturation of ATP with $MgCl_2$. 1D ^{31}P NMR spectra of a sample of 10 mM ATP with various concentrations of Mg^{2+}, as indicated at the left of each spectrum. Each phosphate peak in the presence of Mg^{2+} represents an averaging of the free and bound states because Mg^{2+} is in fast exchange with ATP. Resonances of the α, β, and γ phosphates of ATP are labeled in the top NMR spectrum; these shift slightly with the addition of $MgCl_2$, particularly for the β and γ phosphates, which chelate Mg^{2+}. Significant line broadening is evident in the intermediate exchange regime (e.g., at $MgCl_2$ concentrations of 1-5 mM), where the free phosphate population is shifting to Mg^{2+}-bound. Saturation of the ATP phosphates by Mg^{2+} is shown by the shifting and sharpening of the peaks with increasing Mg^{2+} concentration, which reflects a shift of the population of phosphates to the fully bound state. At 11 mM $MgCl_2$, the 10 mM ATP was fully saturated as seen in the bottom spectrum. All samples were in a buffer of 25 mM sodium chloride and 1 mM sodium cacodylate, at 37 °C and pH 6.5. Data were collected on a 600 MHz NMR spectrometer.

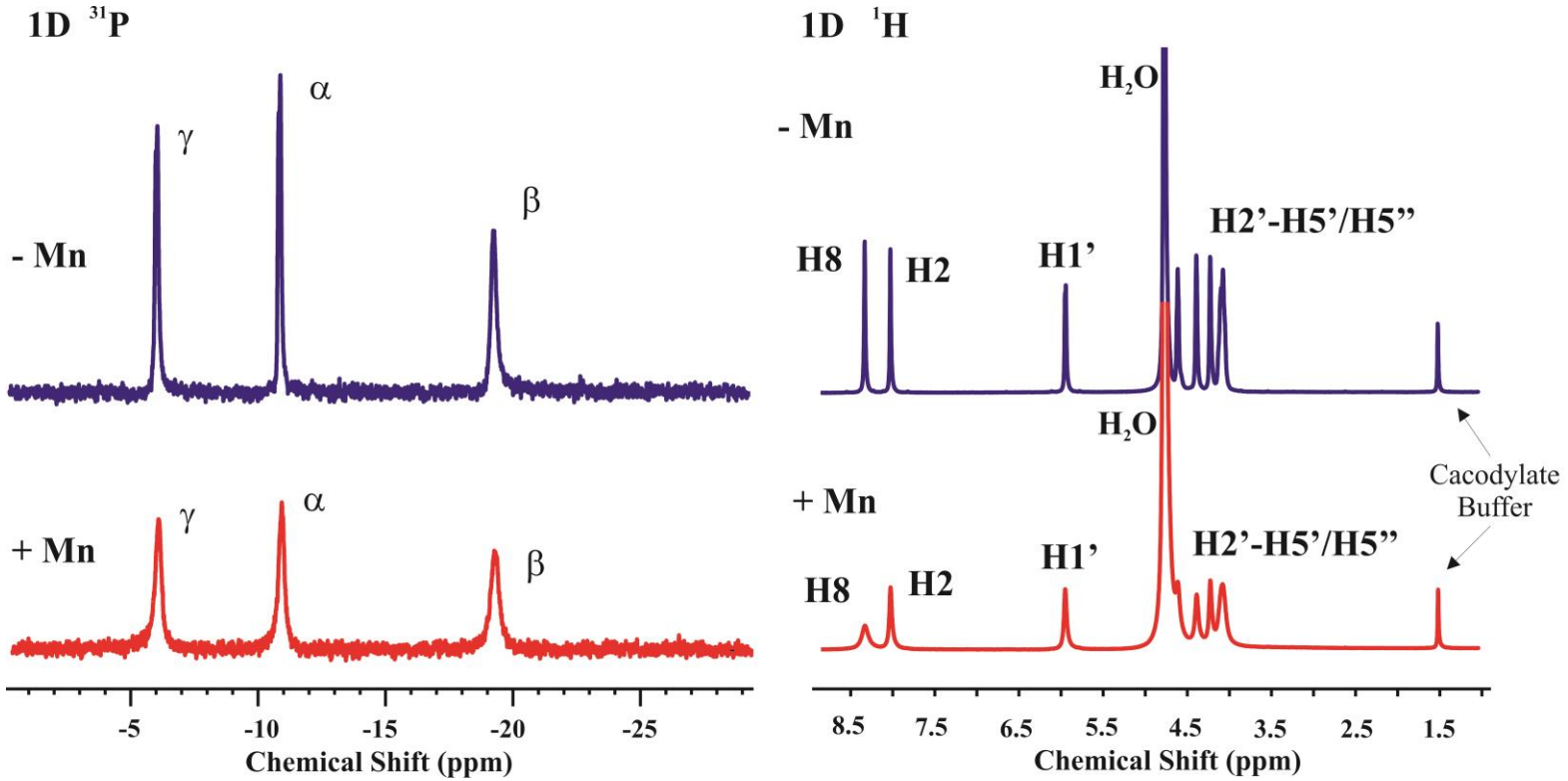

Figure S2. Manganese Sampling of Magnesium Binding Sites Observed by ^{31}P and ^{1}H NMR. 1D NMR spectra of ATP are compared with (red) and without (blue) 50 μM $MnCl_2$. The phosphates of ATP are observed in the ^{31}P spectra (left) and the hydrogens of ATP in the ^{1}H spectra (right). The distance dependence of relaxation by the Mn^{2+} electron magnetic moment can be seen in the non-uniform broadening. For example, the H8 resonance is broader than the H2 resonance due its closer proximity to the Mn^{2+} and also indicates the adenosine and phosphate tail of ATP are in the 'anti' conformation. Data were collected on a 500 MHz NMR spectrometer at 10 °C.

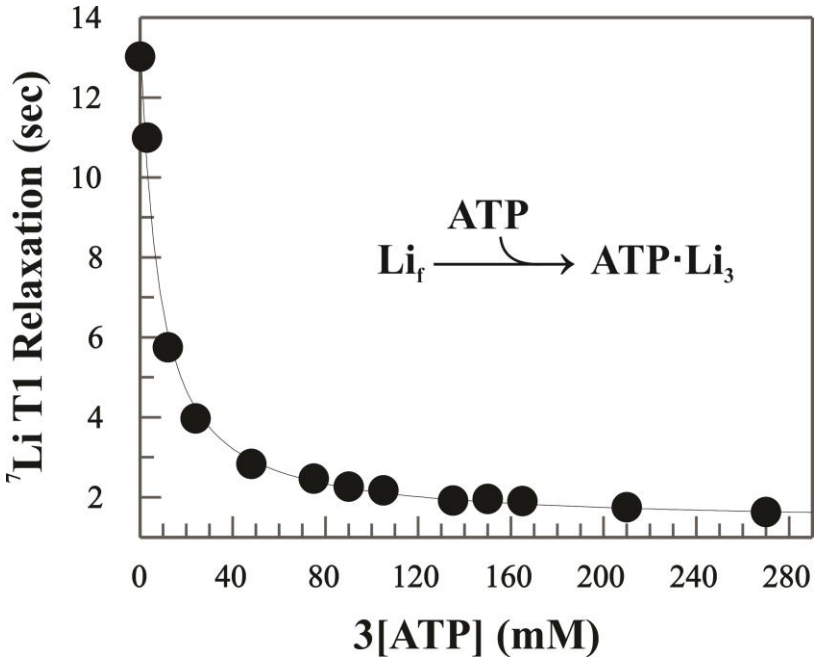

Figure S3. Measured Affinity of Li^+ Binding to ATP. Plot of the ^{7}Li T1 relaxation times (black circles) measured for 2 mM LiCl at 10 °C as a function of the concentration of ATP. An estimated stoichiometry of 3 Li^+ to 1 ATP is assumed in the fitting based on simple charge balance and to allow a comparison with the Mg·Li data in which Mg^{2+} and Li^+ together contribute three positive charges in their complex with ATP. Quadratic equation fit (curve shown) yielded a Li^+ equilibrium dissociation constant, K_d: 7.1 ± 0.8 mM, for ATP (reported uncertainty is the standard error calculated from least-squares fitting).

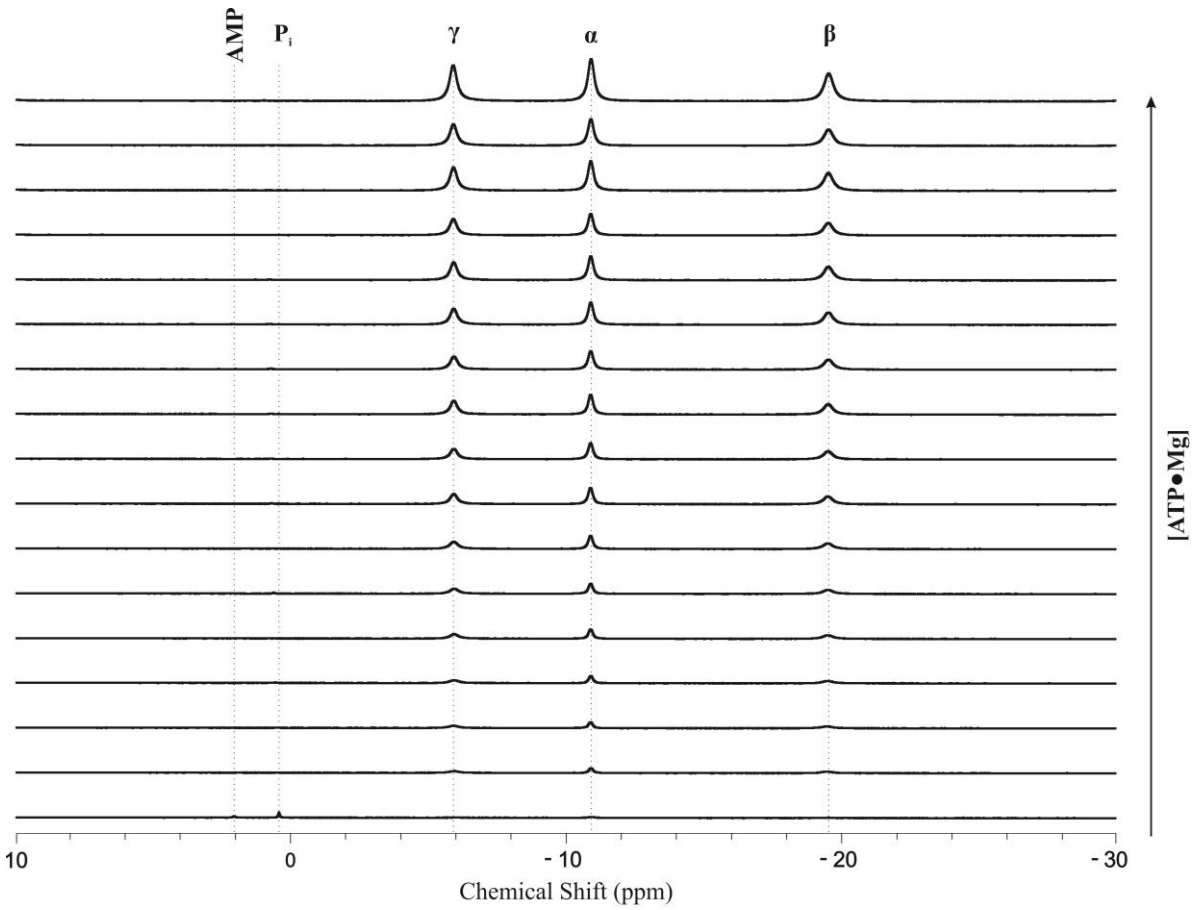

Figure S4. Detection of ATP hydrolysis using ^{31}P NMR over the course of an experimental titration. The integrity of ATP in the 17 NMR samples that were used to calculate Li^+ binding affinity was measured upon completion of those experiments. 1D ^{31}P NMR spectra show the phosphates of ATP, labeled α, β, and γ. The ATP·Mg concentration increases from the bottom to top spectrum. Of the 17 samples, only one, the sample with the lowest concentration of ATP·Mg (bottom spectrum), showed any hydrolysis of ATP to AMP and inorganic phosphate (P_i), at the completion of the several days of ^{7}Li T1 experiments. Data were collected on a 600 MHz NMR spectrometer at 10°C.

Table S1. T1 Relaxation of Lithium with ATP, ADP, TP, GTP, GDP. ^{7}Li T1 relaxation (in seconds) are tabulated for the samples containing: 10 mM ATP, ADP, triphosphate (TP), guanosine triphosphate (GTP), guanosine diphosphate (GDP), or 2,3-diphosphoglycerate (DPG), and 11 mM $MgCl_2$, 10 mM LiCl, and 50 μM $MnCl_2$; presence and absence of a species in the sample are indicated by plus and minus signs, respectively. The tabulated ^{7}Li T1 relaxation times are the average of triplicate experiments with standard deviation, except for DPG, indicated by an asterisk, where the standard errors are estimated from the fit to single experiments. Relaxation Enhancement (%) was calculated by subtracting the PRE T1 value (+$MnCl_2$) from the initial T1 value (–$MnCl_2$), dividing the difference by the initial T1, and multiplying by 100. Experiments were performed on a 600 MHz NMR spectrometer at 10°C.

ATP	ADP	TP	GTP	GDP	DPG	$MnCl_2$	^{7}Li T1 (s)	Relaxation Enhancement (%)
-	-	-	-	-	-	-	12.96 ± 0.26	
-	-	-	-	-	-	+	12.24 ± 0.10	5.5
+	-	-	-	-	-	-	7.16 ± 0.63	
+	-	-	-	-	-	+	2.30 ± 0.43	67.8
-	+	-	-	-	-	-	9.75 ± 0.23	
-	+	-	-	-	-	+	2.17 ± 0.09	77.7
-	-	+	-	-	-	-	7.59 ± 0.04	
-	-	+	-	-	-	+	1.76 ± 0.04	76.8
-	-	-	+	-	-	-	10.54 ± 0.10	
-	-	-	+	-	-	+	4.13 ± 0.04	60.8
-	-	-	-	+	-	-	7.65 ± 0.22	
-	-	-	-	+	-	+	3.69 ± 1.36	51.8
-	-	-	-	-	+	-	6.86 ± 0.02*	
-	-	-	-	-	+	+	2.81 ± 0.02*	59.0

Table S2. Dissociation Constants of Lithium with Phosphate-containing Molecules. Li^+ equilibrium dissociation constants for ATP•Mg, GTP•Mg, TP•Mg, ADP•Mg, GDP•Mg, and DPG•Mg were determined. The dissociation constant (K_d) was determined by exponential fitting of the ^{7}Li T1 relaxation times as a function of the concentration of ATP•Mg, GTP•Mg, TP•Mg, ADP•Mg, GDP•Mg, or DPG•Mg. Li^+ K_d values were similar, i.e., within ~1.5-2-fold, regardless of whether there are two or three phosphates on the nucleotide, or which purine base, adenine or guanine, is present. This emphasizes that the key aspect of this molecular interaction is the Mg^{2+} and Li^+ coordination. The Li^+ K_ds observed, especially for the nucleotides and DPG, are physiologically relevant because they are comparable to the serum Li^+ concentration safely allowed for patients with bipolar disorder (0.8-1.1 mM). The Li^+ K_ds are reported with errors from the fitting. The asterisk denotes the K_d reported for ATP is the average of triplicate experiments, with the standard deviation. All other reported errors are from the fitting of single experiments. Data were collected on 600 MHz NMR spectrometers. (n.d., not determined)

Molecule	K_d (mM) at 10°C	K_d (mM) at 37°C
ATP·Mg	1.60 ± 0.21*	1.45 ± 0.18
GTP·Mg	4.81 ± 0.55	2.50 ± 0.71
TP·Mg	0.71 ± 0.23	0.31 ± 0.16
ADP·Mg	3.24 ± 0.56	n.d.
GDP·Mg	6.76 ± 1.21	n.d.
DPG·Mg	0.89 ± 0.13	0.71 ± 0.11

Table S3. Potential formation of the ATP•Mg•Li complex under normal plasma and cytoplasmic concentrations of ATP and magnesium at clinical dosing levels of lithium. The concentration of ATP and the dose levels of lithium were taken from published literature. The estimated concentration of the ATP•Mg•Li complex under each condition was estimated using simple equilibrium binding equations and binding constants determined in this study for Li^+ binding to ATP•Mg (K_d ~ 1.6 mM). For the purpose of estimating the concentration of the ATP•Mg•Li that may form in the plasma and cytoplasm, ATP is assumed to be in the ATP•Mg form. Note that at elevated ATP concentrations and assuming the upper dosage concentration of Li^+ in the cytoplasm, binding of Li^+ is saturating and all of the Li^+ would be expected to be bound by ATP•Mg absent other competing binding interactions.

Component	Estimated Plasma Concentration (1-3)	Estimated Cytoplasmic Concentration (1, 4)
Lithium Dosing Level	0.8 mM - 1.1 mM	0.1 mM - 0.4 mM
ATP•Mg*	0.028 µM - 0.24 µM	1 mM - 2 mM
ATP•Mg•Li	0.014 µM - 0.16 µM	63 µM – 400 µM
% Complex (ATP•Mg•Li/ ATP•Mg)	50 – 66	6.3 – 20
% Lithium Bound (ATP•Mg•Li /Lithium Dose)	0.002 – 0.01	63 – 100

*ATP•Mg concentrations can reach higher levels locally, such as at replication sites, within certain organelles (e.g, in mitochondria), and in the extracellular matrix (4-6).

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