

A Chemical Tool for Guiding Li Therapy




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In vitro selected DNazymes afford a molecular beacon sensor for tracking Li⁺ ion uptake in living neurons derived from bipolar disorder patients.

School science textbooks classify metal ions like calcium, sodium, potassium, iron, copper, zinc, and manganese as minerals, one of the main classes of seven essential nutrients. Indeed, these metal ions sustain life as signaling, catalytic, and structural entities. Another important yet oft underappreciated aspect of metals in biology is the use of nonessential metal ions in medicine.¹ Outside the chemistry community, “organic” is associated with natural biology, and hence, organic molecules are considered safe. Conversely, metal ions or “inorganic” is commonly associated with toxicity. In many instances, perhaps, this is right, since metal ion-based drugs or metallo drugs, although life-saving treatments for many severe diseases including cancer and neurological disorders, cause acute side effects. Why is this the case? The answer is that biological metal ion regulation involves intricate uptake, assimilation, and efflux pathways. These pathways are strictly controlled for essential metal ions. Metallo drugs often use these paths to enter living systems and can treat disorders when they reach a diseased cell, but they can also be easily mistargeted thus displacing essential metal ions and causing side effects. Hence, the fate of a metallo drug inside a living biological system has critical teleological consequences. Tracking metallo drugs in living cells, albeit challenging, can provide a significant handle to guide their therapeutic applications. In this issue of ACS Central Science, McGhee et al. image the cellular uptake of Lithium ions (Li⁺), a prophylactic metallo drug for bipolar disorder (BD),¹ by strategically developing a fluorescent chemical tool that can selectively detect low mM levels of Li⁺.²

Notably, the authors have designed and implemented a DNzyme molecular beacon that overcomes the challenge of detecting the therapeutic range of Li⁺ over the relatively much higher mM, physiological levels, of monovalent cations like sodium and potassium.²

In this issue of *ACS Central Science*, McGhee et al. image the cellular uptake of Lithium ions (Li⁺), a prophylactic metallo drug for bipolar disorder (BD), by strategically developing a fluorescent chemical tool that can selectively detect low mM levels of Li⁺.

It is important to obtain accurate information on the uptake and in vivo speciation for any therapeutic. Assessments of bioavailability—the circulation time within living systems before the removal of a drug—and any possible sites of mistargeting are necessary to minimize side effects. Several factors make the evaluation of these pharmacological parameters extremely tricky in metallo drugs: (1) Counterions associated with therapeutic metal ions often get exchanged with endogenous anions leading to complex speciation in vivo. (2) Metallo drugs can use multiple pathways to enter cells. (3) The active therapeutic is frequently different from the original metallo drug. In addition to these complications associated with most metallo drugs, Li⁺ therapy has complex genetic nuances in the context of BD treatment.³

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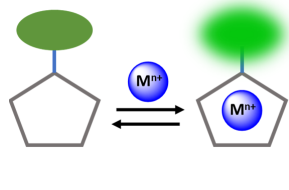
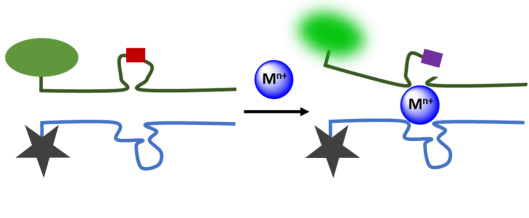
Fluorescent Metal Ion Sensors		
	Rationally-designed binding based	In vitro selected molecular beacons
		
Design	Coordination chemistry based Metal-selectivity predictable	In vitro selection based Metal-selectivity may be unexpected but high
Reversibility	Sensor response reversible allowing imaging of metal ion dynamics in vivo	Sensor response irreversible allowing imaging of metal ion localization
Sensing schemes	Turn-on Ratiometric allowing quantification	Turn-on Ratiometric detection can be incorporated in future
Solubility & In vivo stability	Aqueous solubility often limited Stability high	Aqueous solubility high Stability often low
Cell-permeability	Mostly cell-permeable	Cell-impermeable , needs delivery agents
Modularity	Low	High , modifiable via in vitro selection

Figure 1. Comparison of rationally designed binding-based versus in-vitro-selected DNAzyme-based metal ion sensors. Favorable properties of each detection strategy can be incorporated into the other to achieve flexible chemical tools for in vivo metal ion tracking in the future. Refer to the legend in Figure 2 for interpreting the molecular beacon representation.

For example, only one-third of BD patients respond to Li^+ therapy, and some reports indicate that this response might be inherited.³ Further, the bioavailability of Li^+ seems to depend on the counterion used in the therapeutic formulation, which could be either carbonate or citrate.¹ Finally, while Li^+ therapy is an extremely effective treatment for a subset of BD patients, it is currently on the decline due to severe side effects including renal dysfunction and hypothyroidism.⁴ A significant challenge from the medical practitioner's point of view is the decision on Li^+ ion dosage based on a patient's symptomatic history. In this backdrop, a fluorescent sensor that can report Li^+ uptake in patient cells can be a critical therapy-guiding chemical tool. Such a sensor could be used, for example, to delineate entry pathways by mutating putative metaldrug transporters or to tease apart Li^+ uptake differences between responder and nonresponder patients. Similarly, cellular uptake differences between different therapeutic formulations could be quantified, thereby guiding effective treatment dosages.

Since, Li^+ ions are administered as salts of small anions,¹ a fluorescent sensor that can detect Li^+ would be a first-level chemical tool to track Li^+ uptake in cells. Label-free imaging of Li uptake in fixed cells using techniques like synchrotron

X-ray fluorescence imaging is not possible since this element does not have a characteristic X-ray line, further necessitating Li^+ sensors. At a first glance, this goal seems trivial given the significant advances in the development and successful application of rationally designed metal ion sensors (Figure 1) that rely on metal ion selective binding scaffolds.^{5–9} However, it is extremely challenging to design selective binding-based sensors for metal ions that either (1) have similar coordination preferences as other physiologically relevant metal ions; (2) are weak binders; or (3) are present in considerably lower levels in vivo in comparison to competing ions. For Li^+ detection, the presence of significantly higher levels of sodium and potassium ions in vivo poses a key challenge. Most Li^+ sensors reported to date do not have the requisite selectivity and sensitivity to detect the therapeutically administered concentration window (low mM) of Li^+ ions.¹⁰ Further, sensors based on organic chelators are often water-insoluble.¹⁰ An attractive alternative to sensors that use coordination chemistry principles to detect metal ions are in-vitro-selected DNAzyme sensors.¹¹ A DNAzyme is a single-stranded short DNA sequence that can catalytically cleave a substrate strand that hybridizes with it. Often, the substrate strand has a single RNA base at the cleavage site. The

2'-hydroxy group in the RNA base assists hydrolysis. Since DNA and RNA are negatively charged, they frequently bind to metal ions which can in-turn catalyze the hydrolysis reaction. An in vitro selection setup can be used to pick out DNAzymes that can cleave a substrate only in the presence of a particular metal ion.¹¹ Since the method is not based on differentiating metal ions only on the basis of coordination preferences, often counterintuitive metal ion selectivity can be achieved (Figure 1).¹¹ To realize a Li⁺ selective DNAzyme, the authors first designed a DNA strand that was linked covalently to the substrate strand. The successful DNAzymes that could cleave the substrate in the presence of Li⁺ were selected on the basis that cleaved strands would have low molecular weights and would move faster on a PAGE gel. The first generation of selected DNAzymes was subjected to mutations to generate a DNAzyme with 7–10 times higher Li⁺ responsive cleaving activity. Finally, the substrate and DNAzyme strands were separated such that the two entities would be noncovalently linked. The selected DNAzyme showed high specificity toward Li⁺ ions and notably demonstrated 100-fold selectivity over most other essential metal ions.

The DNAzyme was converted to a fluorescent sensor for Li⁺ by employing a molecular-beacon scheme (Figure 2). Importantly, the limit of detection of the sensor for Li⁺ was 1 mM in a biomimetic environment. This concentration was similar to the levels of Li⁺ used in BD treatment. A key advantage of the sensor was aqueous solubility. Although the sensor was cell-impermeable, it could be introduced into living cells via transfection. An application of the sensor to BD patient derived neurons showed an increased accumulation of Li⁺ ions in disease cells when compared to healthy controls indicating that the sensor could be applied to decipher Li⁺ uptake differences in living cells, thus making it a strategic chemical tool to decipher molecular mechanisms of Li⁺ therapy.

The successful application of DNAzymes to develop a sensor for detecting therapeutic levels of Li⁺ in living cells highlights the promise of the molecular beacon approach. The in vitro selection strategy has the potential to provide chemical tools for metal ions that are difficult to track using rationally designed sensors based on metal coordination preferences.

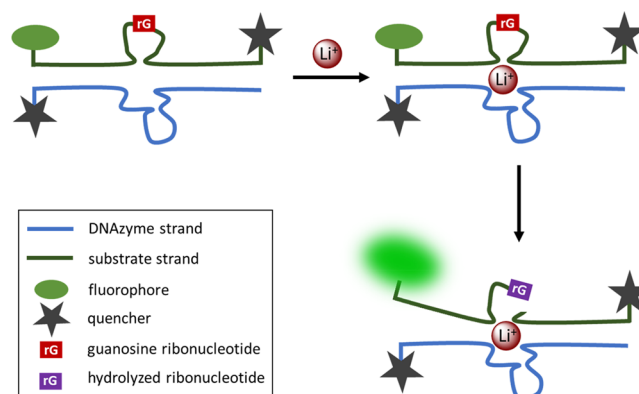


Figure 2. DNAzyme-based molecular beacon sensing strategy for detecting Li⁺ ions employed by McGhee et al.

The successful application of DNAzymes to develop a sensor for detecting therapeutic levels of Li⁺ in living cells highlights the promise of the molecular beacon approach. The in vitro selection strategy has the potential to provide chemical tools for metal ions that are difficult to track using rationally designed sensors based on metal coordination preferences. Importantly, in vitro selection can potentially afford sensors with varying metal ion concentration response ranges covering therapeutic to toxic levels. The ease of implementation should allow rapid generation of such a sensor library. Looking to the future, key developments required to track therapeutic metal ions in vivo using DNAzymes will include quantitative, ratiometric detection schemes along with easily accessible platforms to homogeneously deliver the currently cell-impermeable DNAzymes into multicellular organisms.

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