Poster Presentation

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Dehydration and cation replacement dramatically improve crystals of large RNAs

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Preparation of well-ordered crystals of large RNAs remains a daunting experimental challenge. This probably reflects the relatively undifferentiated molecular surface of folded RNAs (dominated by a regular array of phosphates), the comparatively low free energy of RNA tertiary structure stabilization, and the resulting tendency of RNAs to be conformationally polydisperse. We have found empirically that dehydration and exchange of counterions, can dramatically improve the diffraction properties of some RNA crystals. Two examples from our work are crystals of the glmS ribozyme-riboswitch [1] and of the ternary complex of a T-box riboswitch, its cognate tRNA and an RNA binding protein [2]. Untreated, flash-frozen crystals of the glmS ribozyme diffracted synchrotron X-rays to 3.3 Å resolution. Upon controlled dehydration by soaking into solutions with higher osmolarity, the unit cell contracted by approximately 10%, and diffraction data could be collected that extended to 1.7 Å resolution. Untreated crystals of the T-box ternary complex diffracted X-rays only to 8 Å resolution. A combination of controlled dehydration and exchange of the magnesium an lithium ions needed for crystal growth with strontium (a soft divalent cation), dramatically extended the diffraction limit, allowing the structure to be solved by SAD at 3.2 Å resolution. Because it is a polyanion, RNA is heavily hydrated and surrounded by a diffuse cloud of counterions. It can also site-specifically bind to partially or wholly desolvated metal ions. Hydration and ion binding not only control RNA folding, but also modulate crystallogenesis. Therefore, controlled dehydration and cation exchange are post-crystallization treatments that should be routinely explored for RNA. This work was supported in part by the Intramural Program of the National Heart, Lung and Blood Institute.

[1] *Klein, D.J. & Ferré-D'Amaré, A.R., Science 313:1752 (2006),* ***[2]*** *Zhang, J. & Ferré-D'Amaré, A.R., Nature 500:363 (2013).*

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