Poster Presentation

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Initial phases from phosphate SAD in the direct methods determination of RNA

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Atomic resolution diffraction data from crystals of double-stranded RNAs can often resist automated structure determination by ab initio methods including charge flipping and traditional direct methods. Often it is possible to obtain quick success at direct methods structure determination by supplying the positions of one or more heavier atoms, which are used to calculate a starting set of phase angles. Long wavelength radiation such as that near the iron K absorption edge can be used to measure the weak anomalous diffraction data from phosphorous atoms in the RNA backbones. These anomalous diffraction data can be used to locate the positions of some of the phosphorous atoms. Next, the phosphorous positions can be used to provide initial phases for direct methods structure determination with atomic resolution diffraction data collected with shorter wavelength radiation. We tested this hybrid approach with two double-stranded RNAs, one with 31 unique phosphates and a second with 44 unique phosphates. We used a combination of programs including those in the CCP4, SHELX, and Sir program suites. We varied the number of sweeps of images collected at the iron edge to find the minimum number (and hence minimum exposure) required to find enough of the phosphate substructure for success at direct methods with the native data before collecting atomic resolution diffraction data with the short wavelength radiation. Our results suggest that diffraction data could be collected at these two wavelengths from a single crystal to avoid problems with non-isomorphism.

Keywords: phosphate SAD, direct methods, RNA structure