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SECONDARY STRUCTURES FOR AN EXPANDED SET OF RNA APTAMERS TARGETED TO HIV-1 REVERSE TRANSCRIPTASE

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Aptamers are structured nucleic acids that are selected from random-sequence libraries to bind directly to molecular targets. RNA aptamers that bind the reverse transcriptase of HIV-1 compete with primer-template substrates for access to RT to inhibit RT's polymerase and RNaseH activities, and their intracellular expression interferes with viral replication (see Lange et al poster). Some aptamers inhibit phylogenetically diverse RT's while others are much more sensitive to RT amino acid sequence variation.

Most of the 154 unique aptamer sequences (including 46 not previously reported) include segments capable of folding into pseudoknot structures, some of which conform to well-defined sequence motifs ("Class 1") while others do not ("Class 2"). While a small handful of aptamers have been carefully analyzed—including solution probing and a low-resolution crystal structure of bound complexes—extrapolation to the remaining aptamers is tenuous. We therefore sought to establish the RNA secondary structures responsible for RT binding and inhibition.

Putative pseudoknot cores were transcribed from DNA templates. The "class 1" motif accurately identified the minimal inhibitory segment in all cases, establishing the predictive value of this signature sequence. In contrast, only about half of the putative "class 2" pseudoknots were inhibitory when transcribed as per the originally proposed structures. Measuring RT binding and inhibition for an extensive set of aptamer truncations identified several new pseudoknot and non-pseudoknot structures. Finally, three class 1 pseudoknot aptamers with differing cross-clade specificities were subjected to in-line probing. In each case, nucleotides in the two loops of the pseudoknot became protected upon binding to RT from subtype B, implying increased order in these loop in the bound complex relative to the free RNA. Differences in protections patterns were evident when these same aptamers were probed in the presence of RT from subtype A. These results confirm that aptamer inhibition of phylogenetically diverse RT derives from a combination of aptamer structure and details of the molecular contacts in the bound complex.