

Nucleic Acid Footprinting of HIV Reverse Transcriptase

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As preliminary studies prior to time-resolved nucleic acid and protein footprinting, we are biochemically establish the contacts between thumb and the template-primer. This will be done by comparing the hydroxyl radical footprinting of HIV-1 RT or HIV-1 RT-nevirapine complex on synthetic DNA template-primer duplexes. The thumb moves away from the template-cleft when bound to nevirapine. Thus, sites protected by apo-RT should be de-protected in the presence of nevirapine during hydroxyl-radical footprinting. Once we have established the portions of the template-primer that are protected specifically by the thumb sub-domain, we will perform x-ray footprinting during controlled polymerization. Omitting one or more dNTPs in the reaction will allow controlled polymerization. Since RT is known to catalyze the polymerization at a rate of many milliseconds per monomer, it will be possible to measure the time-dependent changes in thumb position via detection of protection of template-primer and/or the thumb or lack of it.