



Figure S2. (A) Binding of hPifHD to probe PST55 in the presence of nucleotide cofactors. The same binding patterns and relative binding affinities were observed in the presence of nucleotide cofactors (5mM cofactor and $MgCl_2$) on all probes tested (*e.g.* probes with different 5' ssDNA tail lengths), except that binding affinities were all reduced significantly. Binding to probes is measurable under these conditions in the presence of ATP/ Mg^{2+} since the relatively high salt use (75 mM final) inhibits unwinding activity.

(B) ATPase activity of hPifHD (50 nM) was determined in the absence of DNA, or in the presence of a 60 base pair dsDNA sequence (ds60, see Supplementary Figure 1) or the 55 base T55 oligonucleotide at three concentrations (25, 50 and 200 nM). Product release (5 μ l of reaction) was determined after 10 minute's incubation as described in materials and methods.