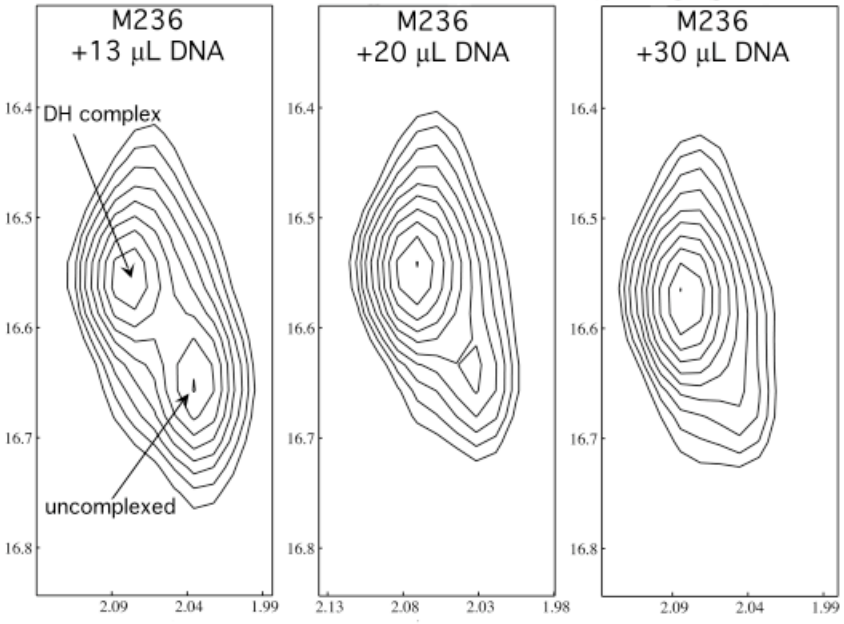
Supplementary Material - Hairpin MS

In both the previous and the present studies of [methyl-^{13}C]methionine Pol β, we found that the addition of a gapped DNA substrate - either serially, as described by Bose-Basu et al. (2004), or as a double hairpin in the present studies, resulted in a shift for most but not all of the resonance intensity corresponding to Met236. This observation has several potential interpretations, e.g. multiple DNA-Pol β complexes that are in slow exchange on the shift time scale, enzyme heterogeneity, or DNA heterogeneity. The latter can result from actual differences in the DNA composition, or from differences in the DNA secondary structure. In order to evaluate the possibility that DNA heterogeneity is the basis for this observation, we increased the nominal DNA T-hairpin:Pol β ratio and obtained the series of spectra for Met236 methyl resonance shown below:



As is apparent from these spectra, increasing the DNA:Pol β ratio does, in fact, increase the intensity ratio of the shifted/unshifted Met236 methyl resonance. The addition of 30µl of DNA stock solution caused the maximal amount of shifted resonance intensity (addition of 35µl caused no further change – not shown).

If the shift heterogeneity described above resulted from insufficient DNA, an increase in the DNA:Pol β ratio of ~45% should have completely removed the unshifted resonance but the center panel of the figure shows that 10% of the unshifted resonance intensity remains after an increase in the DNA:Pol β ratio of 54%. This result apparently indicates that there are impurities in the DNA preparation that compete for the Pol β binding site. The fact that this observation is only made for Met236 probably results from the high intensity of this resonance, which corresponds to the only methionine residue

located on the surface of the enzyme. Thus, a small fractional impurity still has measurable intensity. In principle, similar observations should be possible for other resonances which shift significantly in the presence of the DNA. We have also found that in the presence of the abortive Pol β-gapped DNA-dNTP ternary complex, we can see a small resonance at the unshifted value for Met282 if we take a sufficiently low contour. In summary, it appears that there are probably some minor DNA impurities that contribute to the apparent Met236 resonance shift heterogeneity.

Reference:

Bose-Basu, B., DeRose, EF, Kirby, TW, Mueller, G. A., Beard, W. A., Wilson, S. H., and London, R. E. (2004) Dynamic characterization of a DNA repair enzyme: NMR studies of [methyl-13C]methionine-labeled DNA polymerase beta. Biochemistry 43: 8911-8922.