Supporting Information

# Temperature Dependence of the Kinetic Isotope Effects in Thymidylate Synthase. A Theoretical Study

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and

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Table S1. Averaged distances corresponding to interactions between the substrate and protein at 278 K.

| T= 278 K | Reactants | TS | Products |
|---|---|---|---|
| S-Wat | 11.0 ± 0.5 | 11.1 ± 0.7 | 11.4 ± 0.5 |
| O2(dUMP)-Asp169 | 2.00 ± 0.13 | 2.03 ± 0.15 | 2.00 ± 0.14 |
| P(dUMP)-Arg166 | 2.50 ± 0.12 | 2.49 ± 0.11 | 2.60 ± 0.13 |
| O1(dUMP)-Arg126’ | 4.1 ± 0.3 | 4.1 ± 0.3 | 4.1 ± 0.3 |
| O1(dUMP)-Arg21 | 3.17 ± 0.21 | 3.14 ± 0.20 | 3.14 ± 0.18 |
| O2(dUMP)-Arg126’ | 2.49 ± 0.17 | 2.49 ± 0.19 | 2.52 ± 0.17 |
| O4(dUMP)-Arg166 | 1.85 ± 0.19 | 1.95 ± 0.25 | 1.90 ± 0.17 |
| O4(dUMP)-Arg166 | 2.5 ± 0.3 | 2.3 ± 0.4 | 2.9 ± 0.4 |
| O3(dUMP)-Arg166 | 3.6 ± 0.3 | 3.6 ± 0.4 | 4.4 ± 0.5 |
| O3(dUMP)-Arg166 | 2.34 ± 0.24 | 2.2 ± 0.3 | 2.49 ± 0.23 |
| O3(dUMP)-Arg127’ | 3.85 ± 0.10 | 3.78 ± 0.12 | 3.80 ± 0.12 |

Table S2. Averaged distances corresponding to interactions between the substrate and protein at 293 K.

| T= 293 K | Reactants | TS | Products |
|---|---|---|---|
| S-Wat | 4.5 ± 0.8 | 3.2 ± 0.8 | 2.31 ± 0.16 |
| O2(dUMP)-Asp169 | 2.13 ± 0.19 | 2.10 ± 0.17 | 2.09 ± 0.18 |
| P(dUMP)-Arg166 | 2.51 ± 0.13 | 2.49 ± 0.12 | 2.52 ± 0.13 |
| O1(dUMP)-Arg126’ | 3.86 ± 0.25 | 3.86 ± 0.25 | 4.1 ± 0.4 |
| O1(dUMP)-Arg21 | 3.15 ± 0.19 | 3.24 ± 0.20 | 3.5 ± 0.5 |
| O2(dUMP)-Arg126’ | 2.71 ± 0.20 | 2.62 ± 0.20 | 2.73 ± 0.18 |
| O4(dUMP)-Arg166 | 2.2 ± 0.3 | 2.0 ± 0.3 | 1.87 ± 0.20 |
| O4(dUMP)-Arg166 | 1.88 ± 0.21 | 2.2 ± 0.4 | 2.3 ± 0.3 |
| O3(dUMP)-Arg166 | 3.4 ± 0.3 | 3.7 ± 0.4 | 3.7 ± 0.4 |
| O3(dUMP)-Arg166 | 2.05 ± 0.25 | 2.2 ± 0.3 | 2.4 ± 0.3 |
| O3(dUMP)-Arg127’ | 3.82 ± 0.13 | 3.79 ± 0.15 | 3.72 ± 0.18 |

Table S3. Averaged distances corresponding to interactions between the substrate and protein at 303 K.

| T= 303 K | Reactants | TS | Products |
|---|---|---|---|
| S-Wat | 3.25 ± 0.7 | 3.3 ± 0.7 | 2.9 ± 0.4 |
| O2(dUMP)-Asp169 | 2.07 ± 0.15 | 2.08 ± 0.15 | 2.56 ± 0.15 |
| P(dUMP)-Arg166 | 2.50 ± 0.12 | 2.50 ± 0.13 | 4.60 ± 0.24 |
| O1(dUMP)-Arg126’ | 4.62 ± 0.22 | 4.56 ± 0.24 | 3.74 ± 0.24 |
| O1(dUMP)-Arg21 | 4.0 ± 0.3 | 3.9 ± 0.4 | 2.62 ± 0.21 |
| O2(dUMP)-Arg126’ | 2.70 ± 0.18 | 2.64 ± 0.18 | 3.74 ± 0.24 |
| O4(dUMP)-Arg166 | 2.2 ± 0.3 | 2.10 ± 0.24 | 2.1 ± 0.3 |
| O4(dUMP)-Arg166 | 2.4 ± 0.3 | 2.4 ± 0.3 | 2.6 ± 0.4 |
| O3(dUMP)-Arg166 | 3.2 ± 0.3 | 3.24 ± 0.23 | 3.0 ± 0.3 |
| O3(dUMP)-Arg166 | 2.05 ± 0.24 | 2.11 ± 0.24 | 2.2 ± 0.3 |
| O3(dUMP)-Arg127’ | 4.08 ± 0.11 | 4.08 ± 0.12 | 4.2 ± 0.24 |

Table S4. Averaged distances corresponding to interactions between the substrate and protein at 313 K.

| T= 313 K | Reactants | TS | Products |
|---|---|---|---|
| S-Wat | 3.8 ± 0.8 | 3.3 ± 0.7 | 3.1 ± 0.6 |
| O2(dUMP)-Asp169 | 2.09 ± 0.17 | 2.08 ± 0.18 | 1.98 ± 0.15 |
| P(dUMP)-Arg166 | 2.53 ± 0.13 | 2.54 ± 0.14 | 2.65 ± 0.18 |
| O1(dUMP)-Arg126’ | 4.7 ± 0.4 | 4.8 ± 0.4 | 5.0 ± 0.3 |
| O1(dUMP)-Arg21 | 3.5 ± 0.4 | 3.56 ± 0.25 | 3.6 ± 0.3 |
| O2(dUMP)-Arg126’ | 2.82 ± 0.25 | 2.72 ± 0.20 | 2.78 ± 0.21 |
| O4(dUMP)-Arg166 | 1.89 ± 0.20 | 1.97 ± 0.21 | 2.0 ± 0.3 |
| O4(dUMP)-Arg166 | 2.3 ± 0.4 | 2.5 ± 0.3 | 2.7 ± 0.4 |
| O3(dUMP)-Arg166 | 3.4 ± 0.3 | 3.22 ± 0.25 | 3.3 ± 0.3 |
| O3(dUMP)-Arg166 | 2.34 ± 0.25 | 2.3 ± 0.3 | 2.5 ± 0.3 |
| O3(dUMP)-Arg127’ | 4.07 ± 0.14 | 4.11 ± 0.16 | 4.21 ± 0.25 |

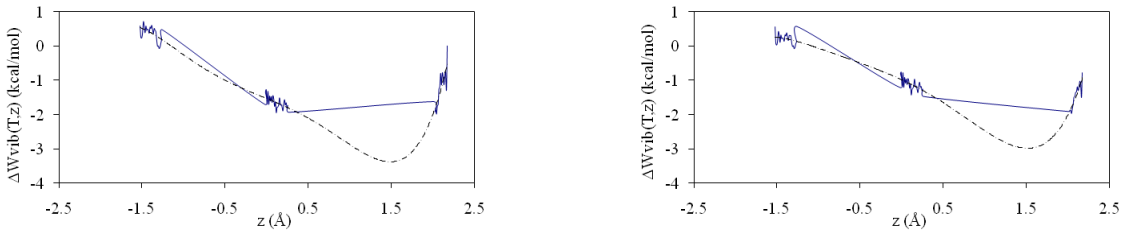



Figure S1. Quatum vibracional free energy correction for protim (a) and tritium (b) computed at 278 K.

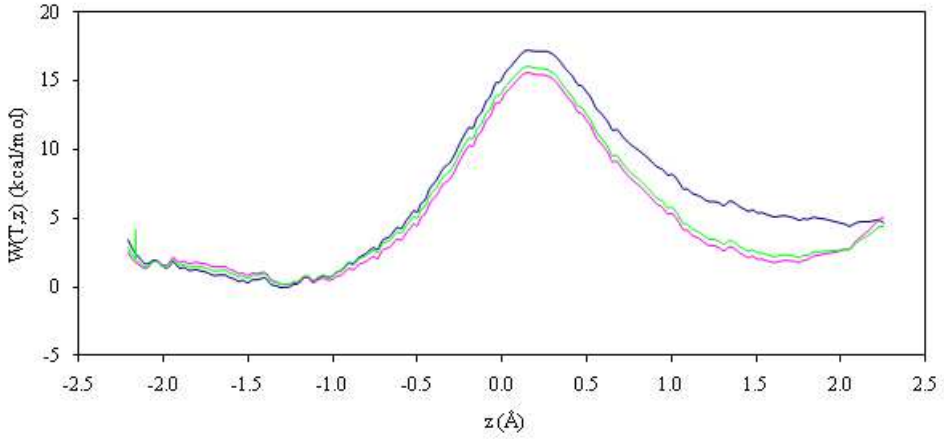



Figure S2. Classical mechanical PMF for hydride transfer (blue line), quasiclassical PMF including quantum vibrational free energy correction for protium (magenta line) and tritium (green line). Results obtained at 278 K.

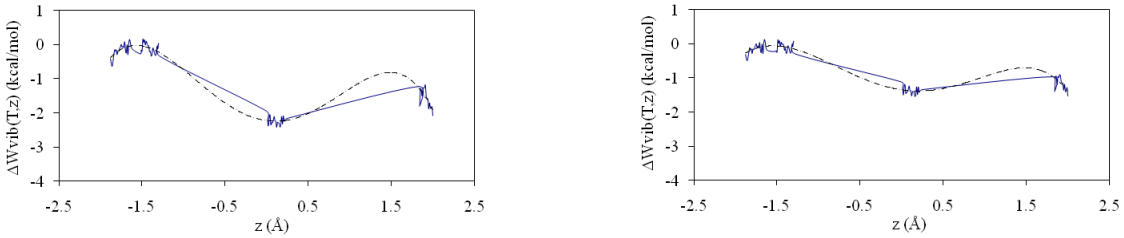



Figure S3. Quatum vibracional free energy correction for protim (a) and tritium (b) computed at 303 K.

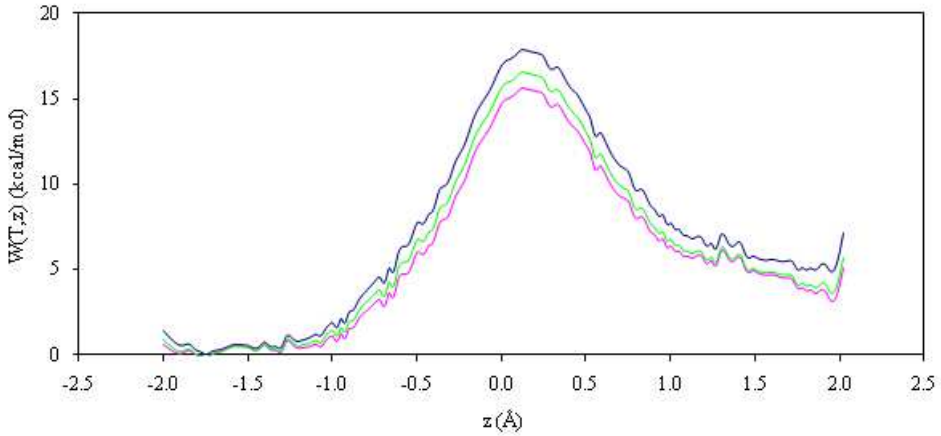
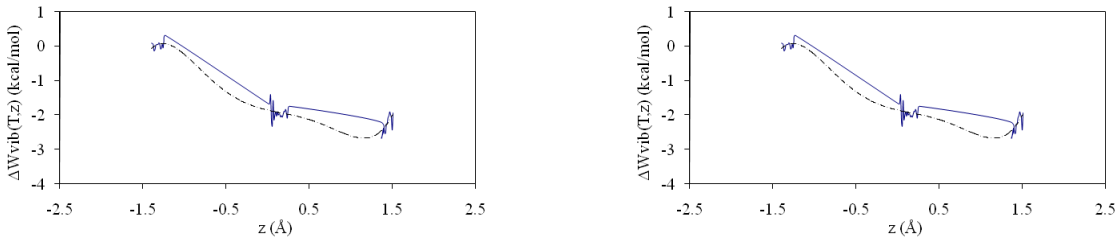



Figure S4. Classical mechanical PMF for hydride transfer (blue line), quasiclassical PMF including quantum vibrational free energy correction for protium (magenta line) and tritium (green line). Results obtained at 303 K.




(a) (b)

Figure S5. Quatum vibracional free energy correction for protim (a) and tritium (b) computed at 313 K.

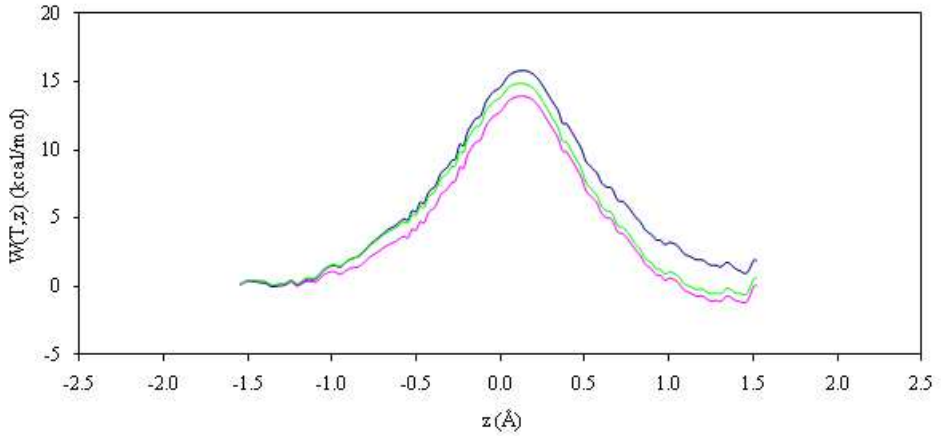
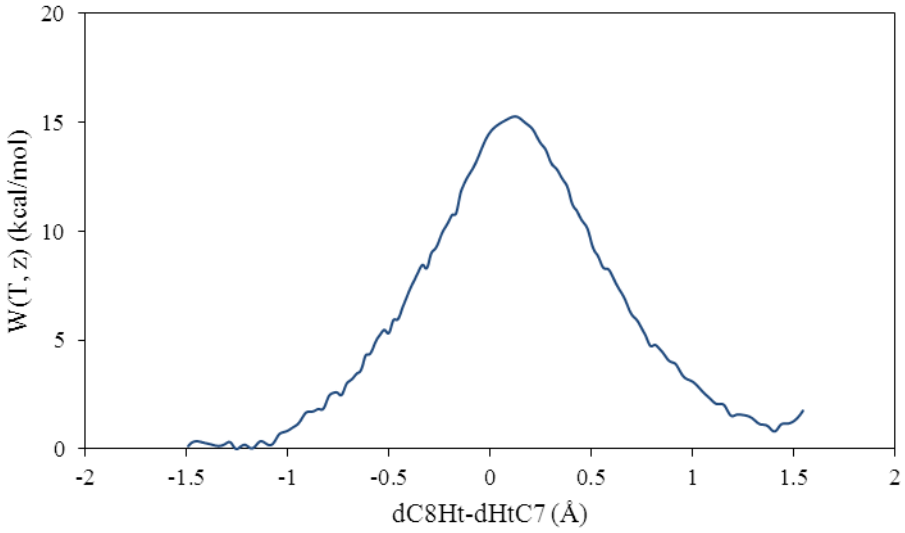



Figure S6. Classical mechanical PMF for hydride transfer (blue line), quasiclassical PMF including quantum vibrational free energy correction for protium (magenta line) and tritium (green line). Results obtained at 313 K.




S7. Classical mechanical PMF obtained at 303 K using as the initial structures those previously employed to get the classical mechanical PMF at 313 K.

Complete reference 50:
MacKerell, A. D.; Bashford, D.; Bellott, M.; Dunbrack, R. L.; Evanseck, J. D.; Field, M. J.; Fischer, S.; Gao, J.; Guo, H.; Ha, S.; Joseph-McCarthy, D.; Kuchnir, L.; Kuczera, K.; Lau, F. T. K.; Mattos, C.; Michnick, S.; Ngo, T.; Nguyen, D. T.; Prodhom, B.; Reiher, W. E.; Roux, B.; Schlenkrich, M.; Smith, J. C.; Stote, R.; Straub, J.; Watanabe, M.; Wiorkiewicz-Kuczera, J.; Yin, D.; Karplus, M. *Journal of Physical Chemistry B* **1998**, *102*, 3586.