

## Supporting Information

**Title:** Activation of Two Sequential H-transfers in the Thymidylate Synthase Catalyzed Reaction

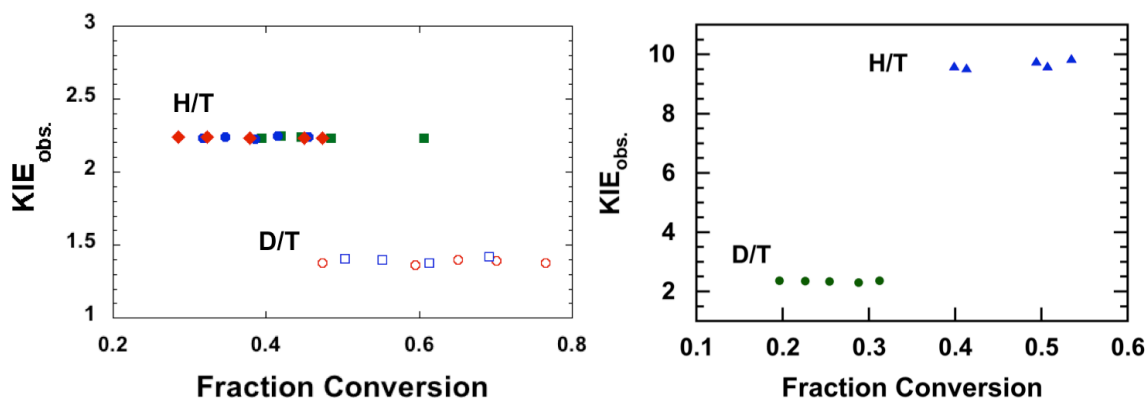
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**Figure S1:** Observed KIEs for the WT (left) and R166K (right). Independent experiments are presented by different colors for the WT. The left panel was reproduced from the ref 1 with the permission from ACS.

**Table S1:** Observed and Intrinsic KIEs on the proton abstraction catalyzed by R166K.

	H/T <sub>obs</sub>	D/T <sub>obs</sub>	H/T <sub>int</sub>
5 °C	10.99±0.08	2.63±0.08	41.15±1.86
15 °C	9.66±0.27	2.37±0.03	25.11±1.47
25 °C	5.23±0.07	1.90 ±0.03	13.38±1.2
35 °C	3.10±0.09	1.56±0.02	7.70±1.09

**Table S2:** Observed and Intrinsic KIEs on the hydride transfer catalyzed by R166K.<sup>2</sup>

	H/T <sub>obs</sub>	D/T <sub>obs</sub>	H/T <sub>int</sub>
5 °C	5.74±0.02	2.00±0.02	16.99±1.06
15 °C	6.43±0.10	1.97±0.01	13.50±0.58
25 °C	6.31±0.10	1.89±0.009	10.66±0.37
35 °C	6.09±0.02	1.80±0.04	9.19±1.22

**References:**

- (1) Wang, Z.; Kohen, A. *J. Am. Chem. Soc.* **2010**, *132*, 9820-9825.
- (2) Islam, Z.; Strutzenberg, T. S.; Gurevic, I.; Kohen, A. *J. Am. Chem. Soc.* **2014**, *136*, 9850-9853.