“Supporting Information”

The Effect of the Protein Mass Modulation on Human Dihydrofolate Reductase

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Figures and Tables

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human       MVGSLNCIVAVSQNMGIGKNGDLPWPPL--RNEFRYFQRMTTTSSVEGKQNLVIMGKKTW
E. coli     ---MISLIAALAVDRVIGMENAMPW---NLPADLAWFKRNTLNKPV-------IMGRHTW

human       FSIPEKNRPLKGRINLVLSRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVWIVGGSS
E. coli     ESI---GRPLPGRKNIILSSQPGTDDRVTWV—KSVDEAIAA------CGDVPEIMVIGGG

human       VYKEAMNHPGHLKLFVTRIMQDFESDTFFPEIDLEKYKLLPEYPGVLSDVQEEKGIKYKF
E. coli     RVYEQFL—PKAQKLYLTHIDAEVEGDTHFPDYEPDDWESVFSE---FHDADAQNSHSYCF

human       EVYEKND
E. coli     EILERR-
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Figure S1. Sequence alignment of Human and *E. coli* DHFR. The alignment was performed using the UniProtKB sequences P00374 and P0ABQ4 for human and *E. coli* DHFRs, respectively. Highlighted in yellow are those also highlighted in Figure 1 of the main text.

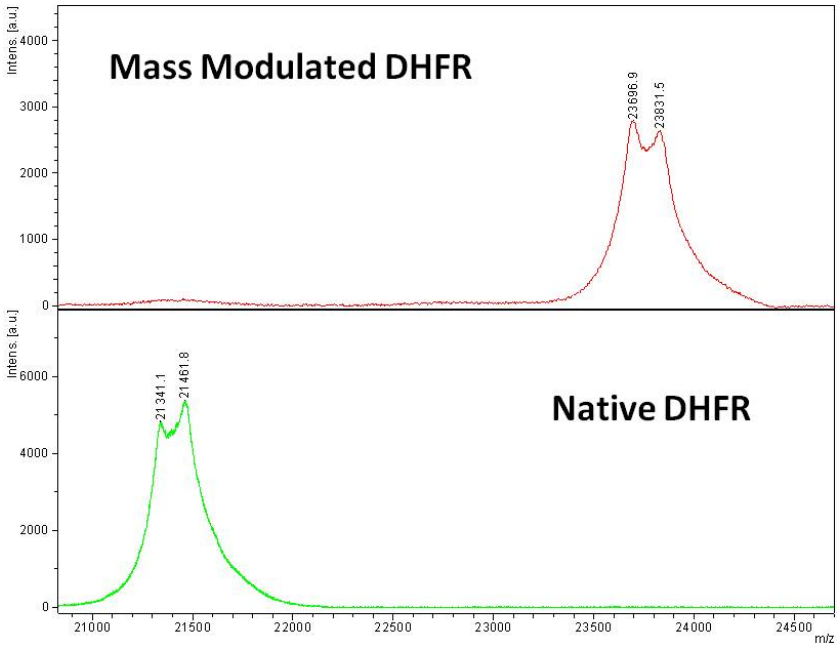

Figure S2. MALDI-TOF of native and mass modulated hsDHFR. The mass modulated enzyme was 11% heavier than native hsDHFR. The two MS ions observed for both the heavy and light hsDHFs could be due to the partial removal of amino acids during ionization, but since the higher mass in the bottom panel matches the sequence-predicted mass of l-hsDHFR the lighter mass was not explored further.

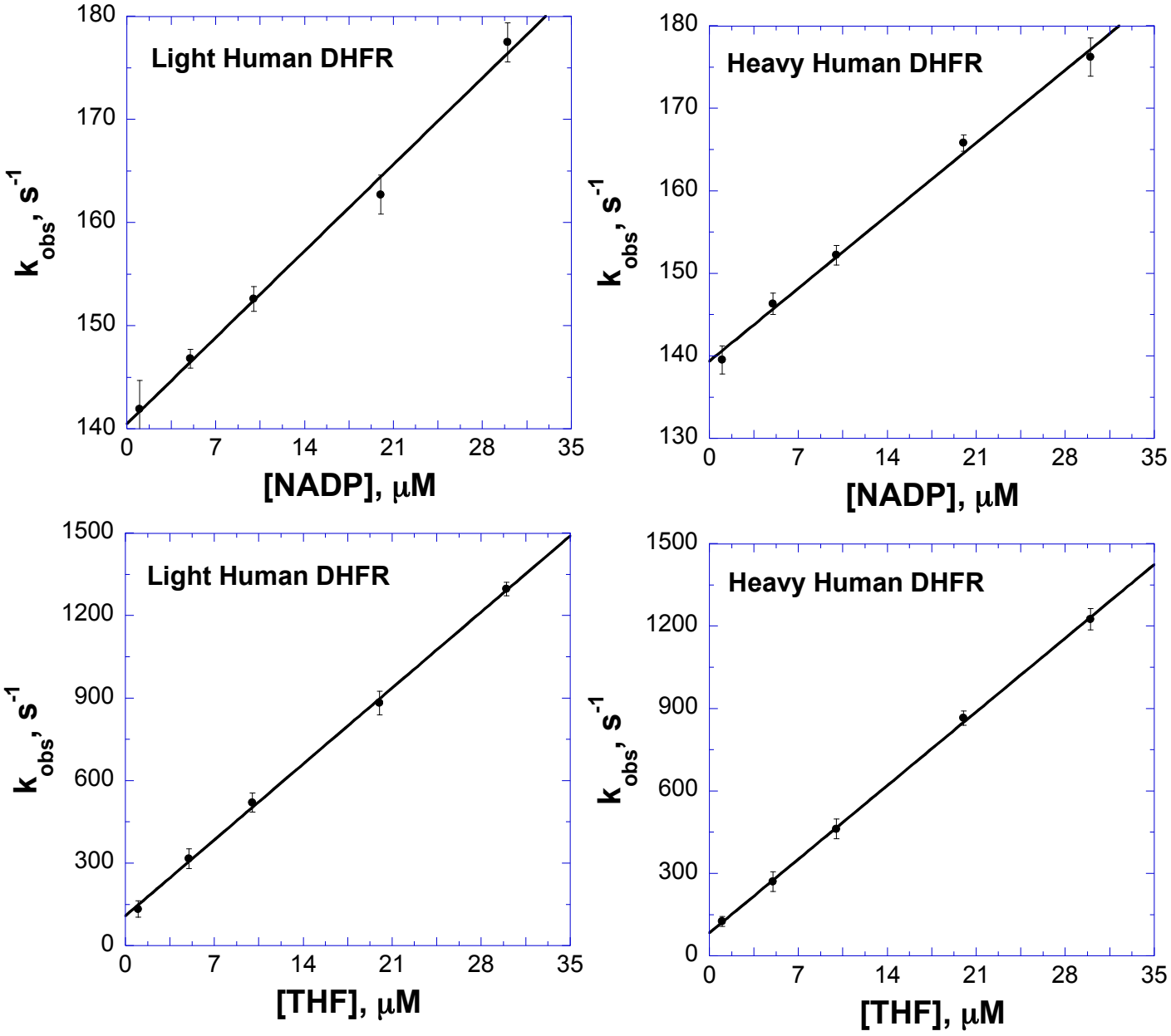

Figure S3. Product binding kinetics of light and heavy hsDHFR in 50 mM MTEN pH 7.65 and 25 ˚C.

Table S1: Intrinsic and Observed KIEs of Wild-Type hDHFR at pH 9.0[a]

Temp. ˚C	Observed H/T KIE	Observed D/T KIE	Intrinsic H/T KIE
5	2.86 ± 0.01	1.45 ± 0.02	4.92 ± 0.16
15	2.80 ± 0.09	1.44 ± 0.03	4.84 ± 0.12
25	2.94 ± 0.05	1.46 ± 0.04	4.93 ± 0.17
35	2.83 ± 0.04	1.45 ± 0.08	5.04 ± 0.25
45	2.79 ± 0.03	1.45 ± 0.05	5.07 ± 0.30

[a] Observed KIEs were measured in 50 mM METN buffer (50 mM MES, 25 mM Tris, 25 mM ethanolamine, and 100 mM NaCl). The values represent at least 5 independent measurements with their standard deviation.

Table S2: Intrinsic and Observed KIEs of Mass-Modulated hDHFR at pH 9.0[a]

Temp. ˚C	Observed H/T KIE	Observed D/T KIE	Intrinsic H/T KIE
5	2.98 ± 0.02	1.47± 0.03	5.15 ± 0.13
15	2.76 ± 0.05	1.44 ± 0.02	5.06 ± 0.32
25	2.86 ± 0.04	1.46 ± 0.03	5.30 ± 0.11
35	2.81 ± 0.04	1.45 ± 0.03	5.17 ± 0.29
45	2.78 ± 0.03	1.45 ± 0.04	5.31 ± 0.36

[a] Observed KIEs were measured in 50 mM METN buffer (50 mM MES, 25 mM Tris, 25 mM ethanolamine, and 100 mM NaCl). The values represent at least 5 independent measurements with their standard deviation.