

# Evolution of Optimized Hydride Transfer Reaction and Overall Enzyme Turnover in Human Dihydrofolate Reductase

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**Table S1: Primers Used in This Study**

mutant	primer sequence
F32M	5'-GGCCACCGCTCAGGAATGAAATGAGATATTTCCAGAGAATGAC-3' 5'-GTCATTCTCTGGAAATATCTCATTTTCATTCCTGAGCGGTGGCC-3'
F32L	5'-CCACCGCTCAGGAATGAATTGAGATATTTCCAGAGAATGAC-3' 5'-GTCATTCTCTGGAAATATCTCAATTCATTCCTGAGCGGTGG-3'
PP26N	5'-GACCTGCCCTGGCTCAGGAATGAATTC-3' 5'-GAATTCATTCCTGAGCCAGGGCAGGTC-3'
PEKN62G	5' CTGGTTCTCCATTCGAAGAATTAATTTAGTTC-3' 5' GAACTAAATTAATTCTTCGAATGGAGAACCAG 3'
$\Delta$ 62-PEKN	5' GGTTCTCCATTCGACCTTTAAAGGG 3' 5' CCCTTTAAAGGTCGAATGGAGAACC-3'
$\Delta$ 26-PPLR- $\Delta$ 62-PEKN	5' ACCTGCCCTGGCCAGAATTCAGATA 3' 5' TATCTGAATTCTGGCCAGGGCAGGT 3'

**Table S2: Intrinsic and Observed KIEs of F32M hsDHFR at pH 9.0<sup>a</sup>**

Temp. °C	Observed H/T KIE	Observed D/T KIE	Intrinsic H/T KIE
5	1.89 ± 0.04	1.48 ± 0.01	36.38 ± 1.41
15	2.29 ± 0.04	1.60 ± 0.01	31.06 ± 0.90
25	1.85 ± 0.03	1.43 ± 0.01	24.78 ± 0.90
35	2.66 ± 0.06	1.70 ± 0.02	22.29 ± 0.41

<sup>a</sup> 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 S3: Intrinsic and Observed KIEs of F32L hsDHFR at pH 9.0<sup>a</sup>**

Temp. °C	Observed H/T KIE	Observed D/T KIE	Intrinsic H/T KIE
5	1.91 ± 0.03	1.41 ± 0.04	19.06 ± 1.05
15	2.00 ± 0.05	1.39 ± 0.01	11.24 ± 0.82
25	1.93 ± 0.14	1.36 ± 0.06	8.44 ± 1.91
35	1.90 ± 0.01	1.31 ± 0.01	5.93 ± 0.19

<sup>a</sup> 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 S4: Intrinsic and Observed KIEs of F32L-PP26N-PEKN62G hDHFR at pH 9.0<sup>a</sup>**

Temp. °C	Observed H/T KIE	Observed D/T KIE	Intrinsic H/T KIE
5	1.97 ± 0.02	1.44 ± 0.01	19.31 ± 0.37
15	3.01 ± 0.05	1.70 ± 0.01	18.09 ± 0.93
25	3.10 ± 0.02	1.75 ± 0.01	20.29 ± 0.26
35	3.42 ± 0.02	1.78 ± 0.01	18.38 ± 0.21

<sup>a</sup> 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 S5: Intrinsic and Observed KIEs of  $\Delta 62$ -PEKN hDHFR at pH 9.0<sup>a</sup>**

Temp. °C	Observed H/T KIE	Observed D/T KIE	Intrinsic H/T KIE
5	4.13 ± 0.02	1.64 ± 0.02	6.92 ± 0.18
15	3.97 ± 0.04	1.62 ± 0.01	6.71 ± 0.14
25	3.77 ± 0.03	1.60 ± 0.03	6.63 ± 0.20
35	3.53 ± 0.02	1.57 ± 0.01	6.37 ± 0.16
45	3.40 ± 0.03	1.55 ± 0.02	6.24 ± 0.21

<sup>a</sup> 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 S6: Intrinsic and Observed KIEs of  $\Delta 26$ -PPLR- $\Delta 62$ -PEKN hDHFR at pH 9.0<sup>a</sup>**

Temp. °C	Observed H/T KIE	Observed D/T KIE	Intrinsic H/T KIE
5	3.96 ± 0.02	1.57 ± 0.01	5.36 ± 0.14
15	3.60 ± 0.02	1.54 ± 0.01	5.43 ± 0.17
25	3.39 ± 0.01	1.52 ± 0.01	5.35 ± 0.15
35	3.17 ± 0.03	1.50 ± 0.02	5.43 ± 0.21
45	3.02 ± 0.01	1.48 ± 0.01	5.33 ± 0.12

<sup>a</sup> 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.