Electronic Supplementary Information

Temperature dependence of dynamic, tunnelling and kinetic isotope effects in Formate Dehydrogenase

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Table S1 Number of reactive (RP type) and non-reactive (RR and PP types) trajectories for protium (H) and tritium (T) at the three different temperatures. In brackets are shown the proportions (in %) of reactive and non-reactive trajectories.

	H			T		
T / K	**RP**	**RR**	**PP**	**RP**	**RR**	**PP**
278	53 (45)	25 (21)	40 (34)	57 (48)	28 (24)	33 (28)
300	47 (42)	28 (25)	37 (33)	54 (48)	27 (24)	31 (28)
318	47 (42)	41 (36)	25 (22)	55 (49)	35 (31)	23 (20)

Table S2 Weights of the coordinates of the atoms in the transition vector (TV).

Residue	Atom	TV
Ile122	N	0.00704915
	H	**0.02702425**
	CA	0.0042971
	HA	0.00402892
	CB	0.00170045
	HB	0.00561409
	CG2	0.0026112
	HG21	0.00163947
	HG22	0.00253326
	HG23	0.00238297
	CG1	0.00222501
	HG11	0.0016728
	HG12	0.00126028
	CD	0.00208957
	HD1	0.0018801
	HD2	0.0010046
	HD3	0.00166856
	C	0.01033023
	O	**0.03947655**
Asn146	N	0.00436096
	H	0.00639459
	CA	0.00176366
	HA	0.00179814
	CB	0.0036699
	HB1	0.00273447
	HB2	0.00335117
	CG	0.00837598
	OD1	**0.03329363**
	ND2	0.01553344
	HD21	**0.04128665**
	HD22	**0.02485483**
	C	0.00234761
	O	0.01368991
Thr282	N	0.0028248
	H	0.00453958
	CA	0.00107031
	HA	0.0010695
	CB	0.00107269
	HB	0.00166297
	CG2	0.0003813
	HG21	0.00076866
	HG22	0.00065209

	HG23	0.00013944
	OG1	0.00550336
	HG1	0.0043971
	C	0.00212678
	O	0.01058304
Arg284	N	0.00158347
	H	0.00331901
	CA	0.0003105
	HA	0.00073809
	CB	0.00028423
	HB1	0.00028142
	HB2	0.00068786
	CG	0.00074847
	HG1	0.00109306
	HG2	0.00094976
	CD	0.00069494
	HD1	0.00183061
	HD2	0.00238203
	NE	**0.01124523**
	HE	**0.01139259**
	CZ	0.00703944
	NH1	**0.03050248**
	HH11	**0.03073982**
	HH12	**0.08015914**
	NH2	**0.02308165**
	HH21	**0.02535607**
	HH22	**0.06395551**
	C	0.00078801
	O	0.00261991
Asp308	N	0.00065729
	H	0.00158912
	CA	0.00023823
	HA	6.7233E-05
	CB	0.00141078
	HB1	0.00061463
	HB2	0.00090629
	CG	0.0037543
	OD1	0.00975816
	OD2	0.01350109
	C	0.0004711
	O	0.00113483
Gln313	N	4.3561E-05
	H	8.2361E-05
	CA	4.2124E-05

	HA	1.0092E-05
	CB	0.00012285
	HB1	0.00028835
	HB2	0.00033007
	CG	0.00018107
	HG1	0.00032616
	HG2	0.00017335
	CD	0.0008021
	OE1	0.00404068
	NE2	0.00374634
	HE21	0.00586119
	HE22	0.00412497
	C	4.0326E-05
	O	0.00011273
His332	N	0.00045145
	H	0.00156033
	CA	0.00071149
	HA	0.0007719
	CB	0.00077129
	HB1	0.00153137
	HB2	0.00080805
	CG	0.00346697
	ND1	0.01088052
	CE1	0.00837556
	HE1	0.01280852
	NE2	**0.02241254**
	HE2	**0.07999732**
	CD2	0.00412771
	HD2	0.00509649
	C	0.00150372
	O	0.00759044
Ser334	N	0.00169754
	H	0.00318302
	CA	0.00040707
	HA	0.00021021
	CB	0.00267515
	HB1	0.00143703
	HB2	0.00047713
	OG	0.00614757
	HG	0.00578101
	C	0.00411598
	O	0.00529659
NIC	N1	0.04255691
	C2	0.05439442

	H2	0.05135826
	C3	0.09179438
	H3	0.03124282
	C4	**0.5682355**
	H41*	**0.60837253**
	H42	**0.1372176**
	C5	0.09554173
	C6	0.04420773
	H6	0.04724603
	C8	0.03028545
	O8	0.0562824
	N9	0.00962394
	H91	0.01596196
	H92	0.00997472
	C1'	0.03972636
	H1'	0.01074703
	C2'	0.01144311
	H2'	0.00815359
	O2'	0.00824575
	HT2'	0.00683679
	C3'	0.00687304
	H3'	0.00547614
	O3'	0.00273581
	HT3'	0.00591354
	C4'	0.01073881
	O4'	0.00744018
	H4'	0.00359979
	C	**0.40806505**
	O1	**0.10587222**
CO_2	**O2**	**0.21183413**

***H41** is the transferring particle labeled as H in Fig. 1 in the manuscript

Note: It is important to point out that the weight of the coordinates of each atom in the transition vector is calculated as the module of the Cartesian coordinates of each atom obtained from the Hessian. The sum of all the weights does not add up to unity, but the square root function of the sum of the square of all the coordinates of the atoms amounts to unity. The weight of the coordinates can be normalized by dividing each weight of the coordinates of each atom among the sum of all the weight of the coordinates.

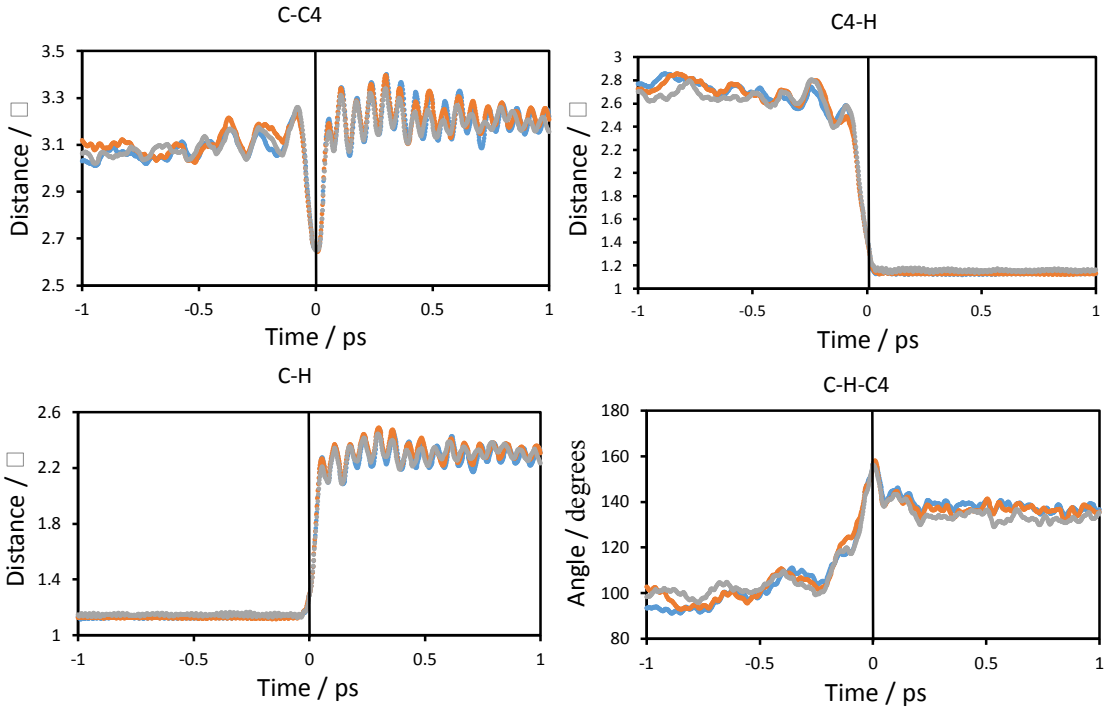

Figure S1. Time evolution of interatomic distances and angle of the atoms that participate in the definition of the antisymmetric reaction coordinate averaged over the reactive trajectories at different temperatures, at 278 K (blue), at 300 K (orange) and at 318 K (grey), for tritium as the particle to be transferred. The atoms that define the distances or angle depicted are shown at the top of each graphic. Distances are in Å and the angle in degrees.

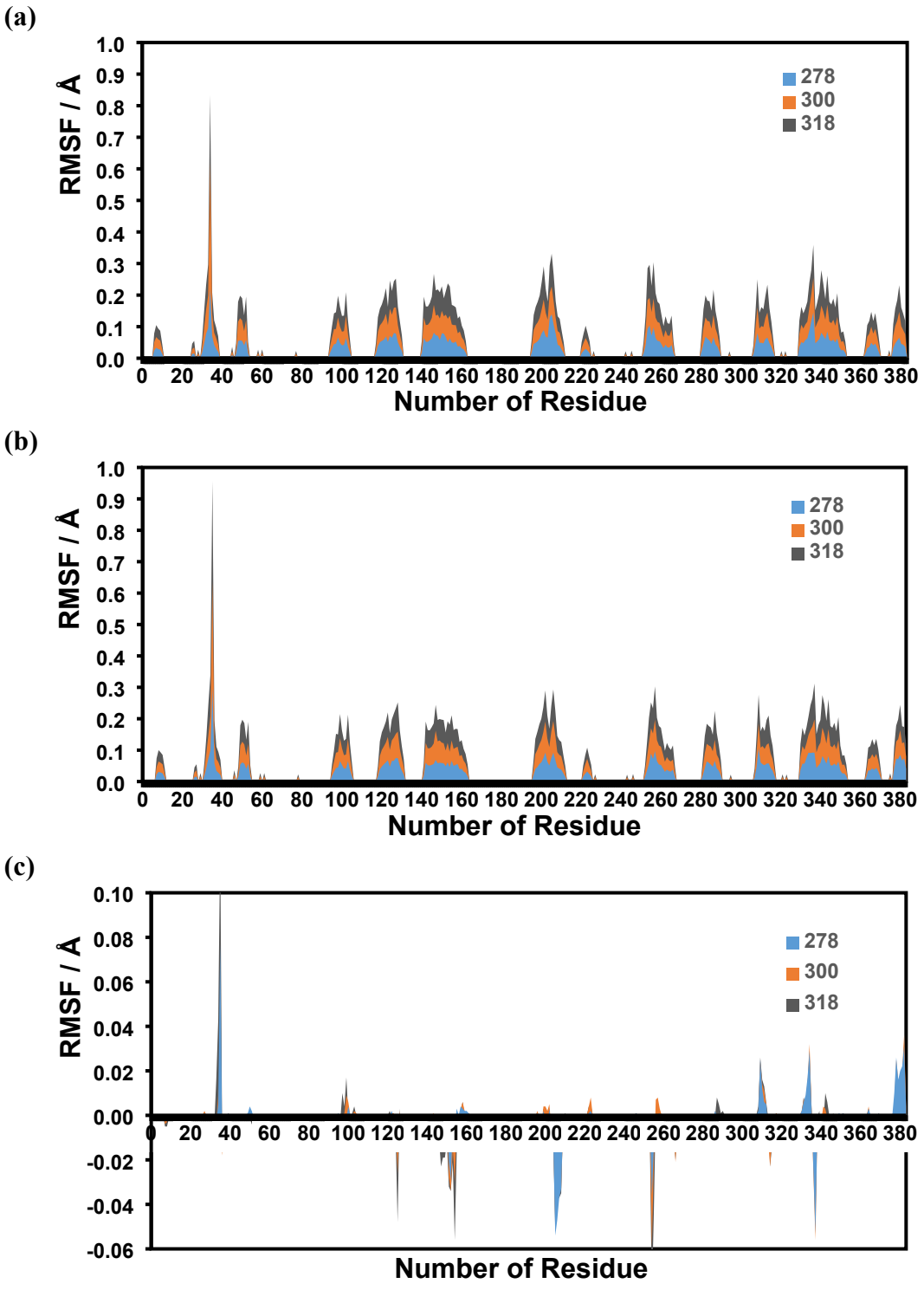

Figure S2. RMSF of the amino acid residues of chain B of *Ps*FDH along the QM/MM MD simulations restrained at the value of (a) reactant reaction coordinate and (b) TS

reaction coordinate and (c) the RMSF difference between the TS and reactants at different temperatures, 278 K (blue), 300 K (orange) and 318 K (gray).

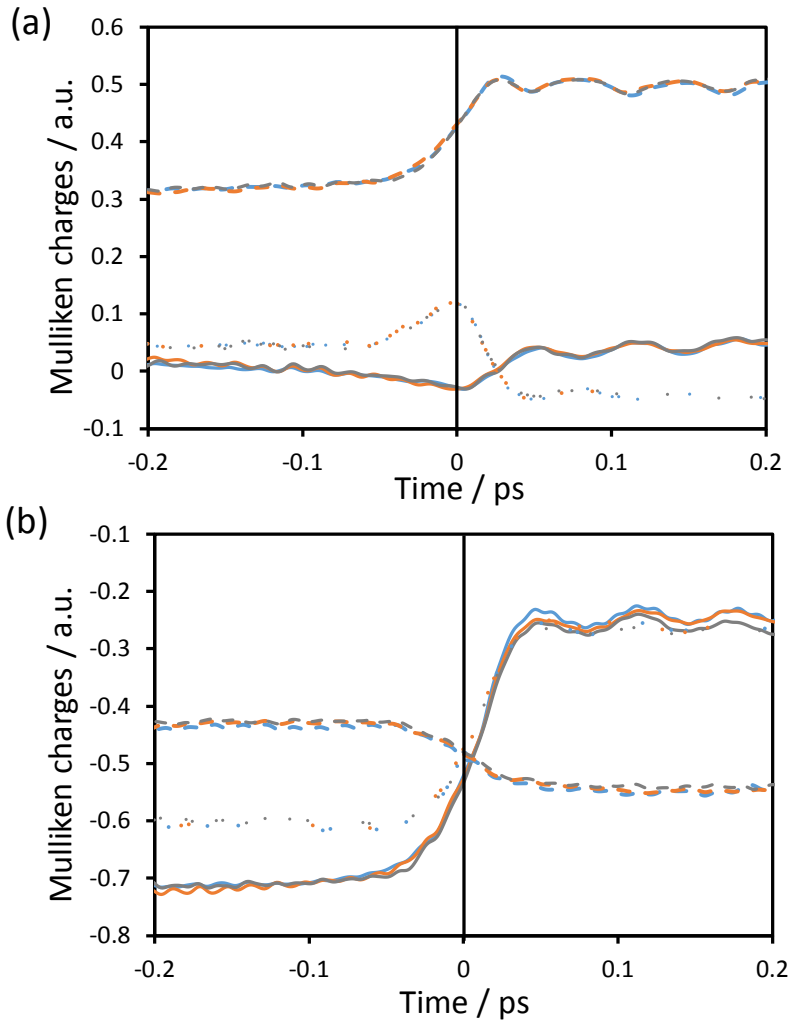

Figure S3. Time evolution of the Mulliken charges on some key atoms averaged over the reactive trajectories for tritium as transferring particle computed at 278 K (blue), 300 K (orange) and 318 K (grey) for (a) C (dashed line), H (solid line) and C4 (dot line); and (b) O8 (dashed line), O1 (solid line) and O2 (dot line). The vertical solid black line at t = 0 shows the position of the TS.

Table S3 Observed and intrinsic H/T KIEs obtained by Kohen and coworkers[1] (with the standard deviations).

Temperature (K)	Observed KIE	Intrinsic KIE
278	3.69±0.09	5.89±0.79
288	3.82±0.05	5.80±0.70
298	3.80±0.09	5.93±0.73
308	3.78±0.05	5.88±0.75
318	3.79±0.13	5.88±0.62

References

1 J. N. Bandaria, C. M. Cheatum and A. Kohen, *J. Am. Chem. Soc.*, 2009, **131**, 10151.