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for

Driving Force Analysis of Proton Tunnelling Across a Reactivity Series for an Enzyme-Substrate Complex

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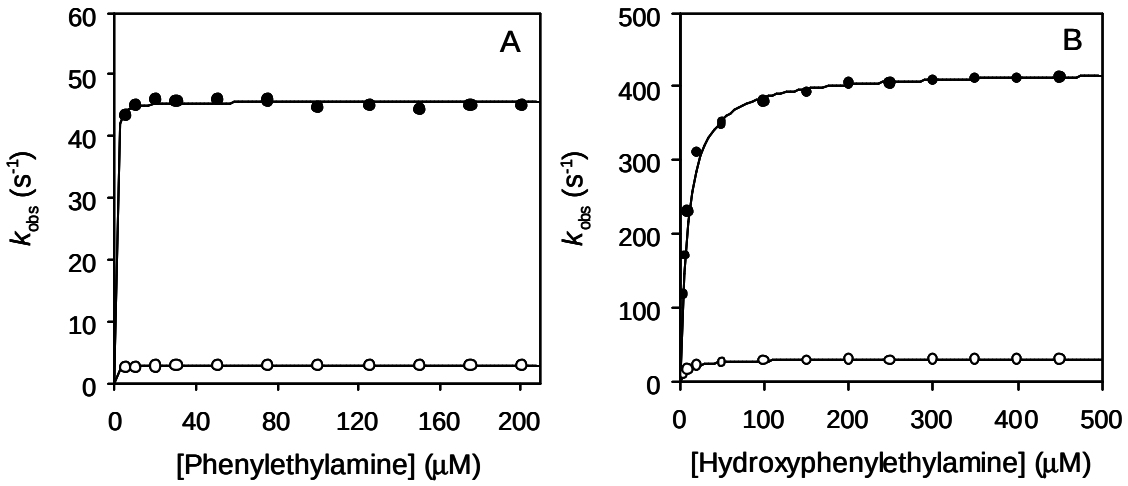

Figure S1. Stopped-flow kinetic data for the reaction of AADH with *p*-substituted phenylethylamines. *Panel A*, kinetic data for reactions with phenylethylamine as substrate. Filled circles, protiated phenylethylamine; open circles, dideuterated phenylethylamine. Observed rate constants were obtained by fitting to the standard single exponential. Reactions were performed using 1 μM enzyme (reaction cell concentration) in 10 mM BisTris propane buffer, pH 7.5, at 25 °C. *Panel B*, as for panel A, but for reactions with hydroxyphenylethylamine. The fits shown are to the standard hyperbolic expression. Similar data were obtained with other *p*-substituted phenylethylamines (Table 1).

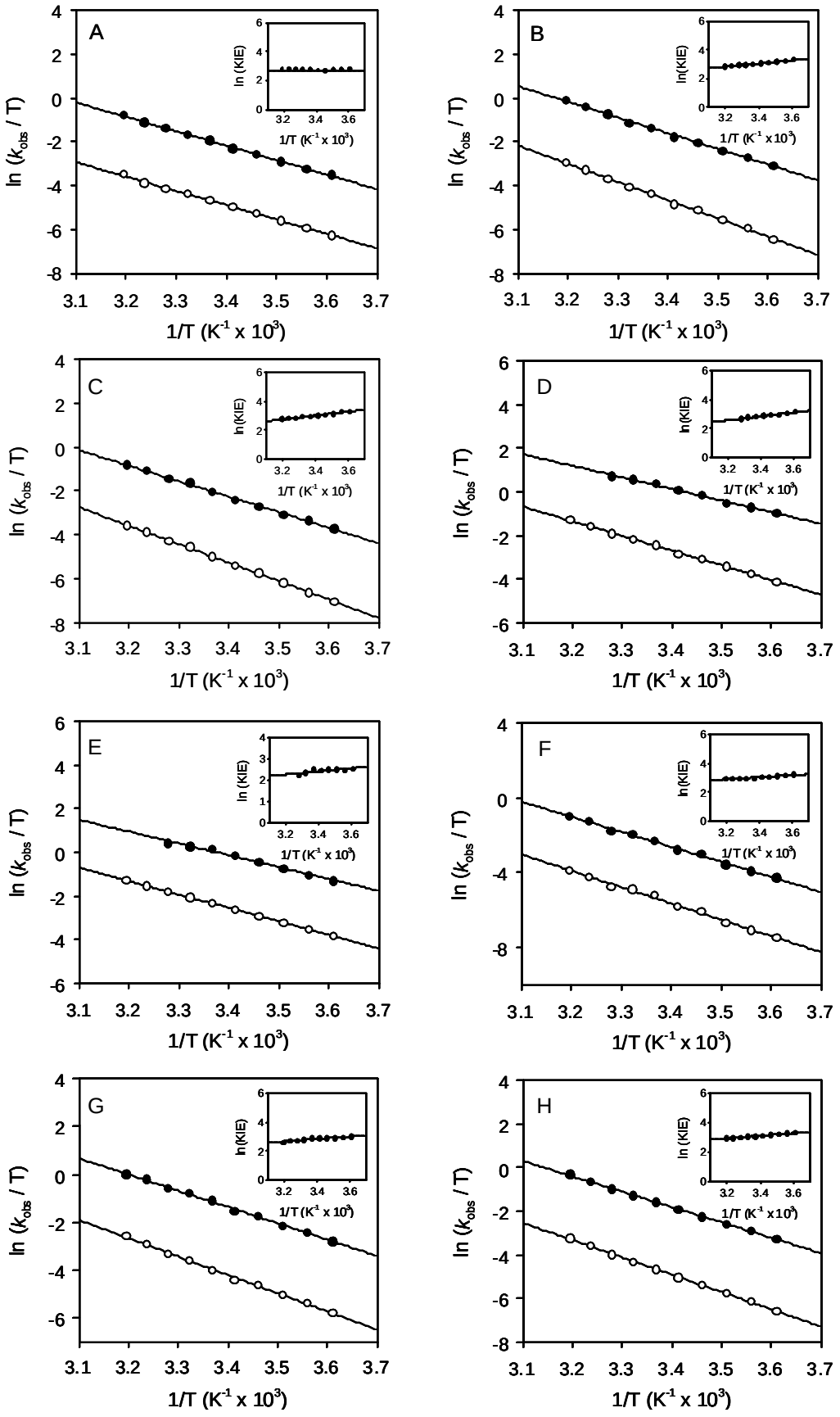

Figure S2. Eyring plots for reactions of AADH with *p*-substituted phenylethylamines. *Panel A*, plot of ln (k_{obs} /T) versus 1/T for reactions with phenylethylamine. Filled circles, protiated phe-

nylethylamine; open circles, dideuterated phenylethylamine. *Inset*, plot of ln (KIE) versus 1/T for the kinetic data presented in the main panel. Conditions: 1 µM AADH, 10 mM BisTris propane buffer, pH 7.5, 4 – 40 °C. Rate constants are observed rate constants measured with 200 µM phenylethylamine and were obtained by fitting to the standard single exponential expression. For each reaction at least four replicate measurements were collected and averaged. *Panel B-H*, as for panel A, but for bromo, methyl, methoxy, hydroxy, nitro, fluoro and chlorophenylethylamines, respectively. One standard deviation in each activity measurement (n=4) at a defined temperature is <7 % of the determined value. Parameters were obtained from fitting data to the Eyring equation (Table 2 in the main text).

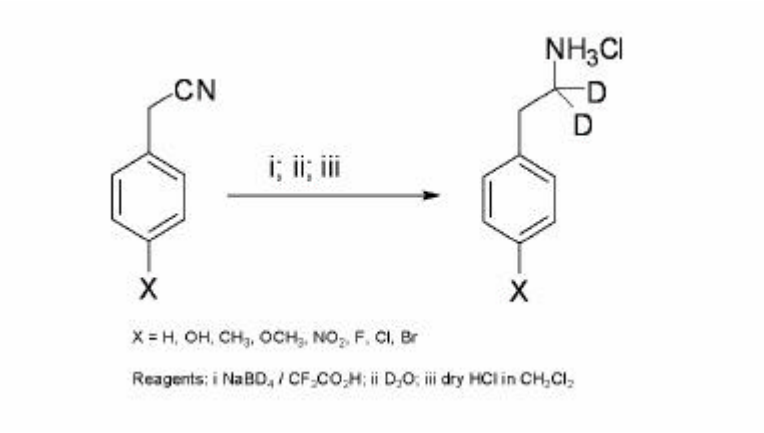

Figure S3. Scheme for the synthesis of dideuterated phenylethylamines. Experimental details are provided in the main text of the paper.

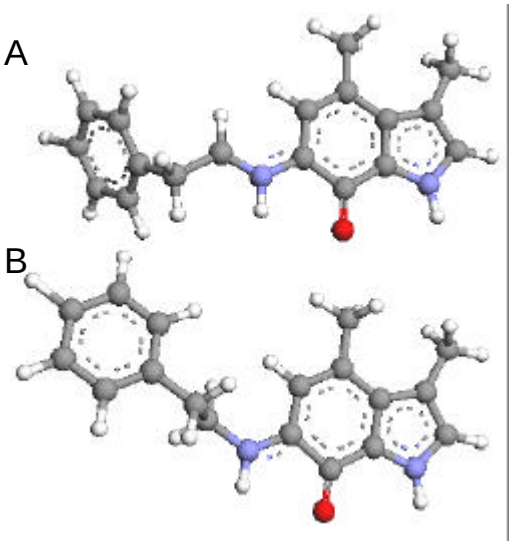

Figure S4. The structures of the energy-minimized A) deprotonated and B) protonated iminoquinone species formed between substrate and the TTQ cofactor calculated using PM3 in the gas phase.

Table S1. Calculated values for the changes in bond dissociation energies (BDEs) across the *p*-substituted phenyleyhylamine reactivity series.

p substituent	ΔBDE[a] (kJ mol^{-1})	*r*(C-H$_1$) Å	k_{lim}^{H} 298 K (s^{-1})	KIE 298 K
H	0	1.112	45.6 ± 0.3	15.3 ± 0.3
OH	-3.64	1.112	412.7 ± 7.0	13.4 ± 0.3
CH_3	13.06	1.128	41.3 ± 0.3	19.0 ± 0.9
OCH_3	-4.36	1.112	417.6 ± 10.7	15.8 ± 0.5
NO_2	19.39	1.128	29.4 ± 0.2	16.6 ± 0.2
F	2.53	1.112	93.1 ± 0.7	17.3 ± 0.3
Cl	1.45	1.112	66.1 ± 0.3	19.5 ± 0.7
Br	1.88	1.112	73.8 ± 0.3	19.6 ± 0.2
NH_2	-4.67	1.128	30.1 ± 0.3	N.D.
tryptamine[b]	-13.10	1.114	3500 ± 1000	55 ± 6
benzylamine[c]	-0.18	1.112	1.47 ± 0.01	4.6 ± 0.2

[a] BDE relative to phenylethylamine and calculated in the gas phase using PM3. Additional kinetic data from [b] ref [1] (N.B. this value is extrapolated); [c] ref [2].

Table S2. Data collection and final refinement statistics for crystal structures of AADH soaked with phenylethylamines.

	Phenylethylamine soaked AADH	*p*-Methoxyphenylethylamine soaked AADH
PDB code	2HKM	2HKR
spacegroup	$P2_1$	$P2_1$
resolution (Å)	20-1.50	20-1.40
completeness	98.5	100.0
R_{merge}	6.7	5.9
I/? I	10.2	11.4
R/R_{free}	15.9/19.1	14.9/17.1
$B_{average}$ (Å^2)	17.1	13.5
rmsd bond lengths (Å)	0.012	0.011
rmsd bond angles (º)	1.3	1.3

References

[1] L. Masgrau, A. Roujeinikova, L. O. Johannissen, P. Hothi, J. Basran, K. E. Ranaghan, A. J. Mulholland, M. J. Sutcliffe, N. S. Scrutton, D. Leys, *Science* **2006**, *312*, 237.

[2] P. Hothi, A. Roujeinikova, K. Abu Khadra, M. Lee, P. Cullis, D. Leys, N. S. Scrutton, *Biochemistry* **2007**, *46*, 9250.