The Stories Tryptophans Tell:

Exploring Protein Dynamics of Heptosyltransferase I from *Escherichia coli*[†]

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[†]This work was supported by NIH grants 1R15AI119907-01 (E. A. T.) and 2T32GM008271-24 (J. M. C.).

Table S1. Kinetics parameters determined for HepI and mutant enzymes using 100 μM ADPH, 100 mM KCl with varying ODLA concentration at 37^{o}C, unless otherwise indicated.

	Reaction Kinetics		
	k_{cat} (s^{-1})	ODLA K_m (μM)	k_{cat}/K_m ($M^{-1}s^{-1}$)
HepI	0.45 ±0.01	6.1 ±0.4	7.4± 0.8 x10^4
HepI 25 °C	0.196±0.008	6±1	3.3±0.6 x10^4
HepI in 2M KCl	0.71 ±0.07	59 ±10	1.2± 0.2 x10^4
W47F	0.33 ±0.02	11 ±2	2.9± 0.5 x10^4
W62F	0.32 ±0.01	6.9 ±0.6	4.6± 0.4 x10^4
W66F	0.36 ±0.02	13 ±2	2.8± 0.5 x10^4
W116F	0.36 ±0.01	12.4 ±0.1	2.9± 0.8 x10^4
W194F	0.40 ±0.02	19.2 ±0.1	2.1± 0.1 x10^4
W199F	0.35 ±0.01	16 ±2	2.2± 0.3 x10^4
W217F	0.32 ±0.03	6.6 ±2.1	4.8± 0.4 x10^4

Table S2. QuikChange Lightning PCR forward primers for mutations Trp to Phe in HepI, the red bolded letters corresponds to the mutation.

Mutant	Primer
W47F	5'-GCACAGATTCCT**TC**CTTCCACGCTGCCCTTGAGCG-3'
W62F	5'- CGACCTTATTCCTGTGGCAATACGTCGCT**TC**CGTAAAGCC-3'
W66F	5'- CGTAAAGCCT**TC**TTCTCGGCCCCCATAAAAGCGGAAC -3'
W116F	5'- GTAAAGCATGGCATGGACT**TC**CAAACCGCTCGCGAACC-3'
W194F	5'-CATGCGACGACCCGTGATGATAAACACT**TC**CCGGAAGAACAC-3'
W199F	5'-CCGGAAGAACACT**TC**CGAGAATTGATTGGTTTACTGGCTG-3'
W217F	5'-GAAATACGGATTAAACTTCCGT**TC**GGCGCGCCGCATGAG-3'

Figure S1. In triplicate WT HepI apo A) Thermal melts at 222 nm and B) Van't Hoff plot at 222nm.

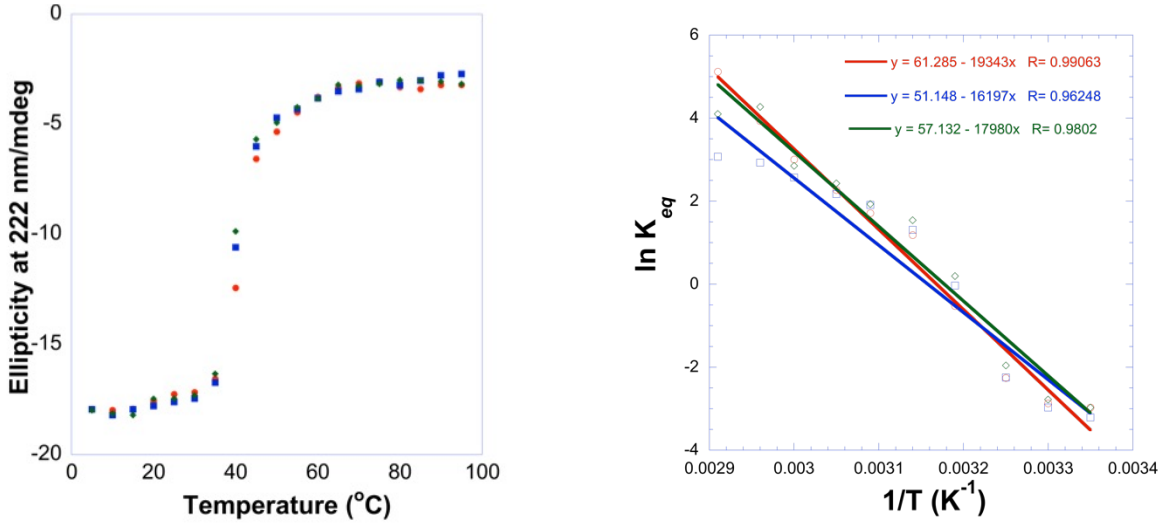
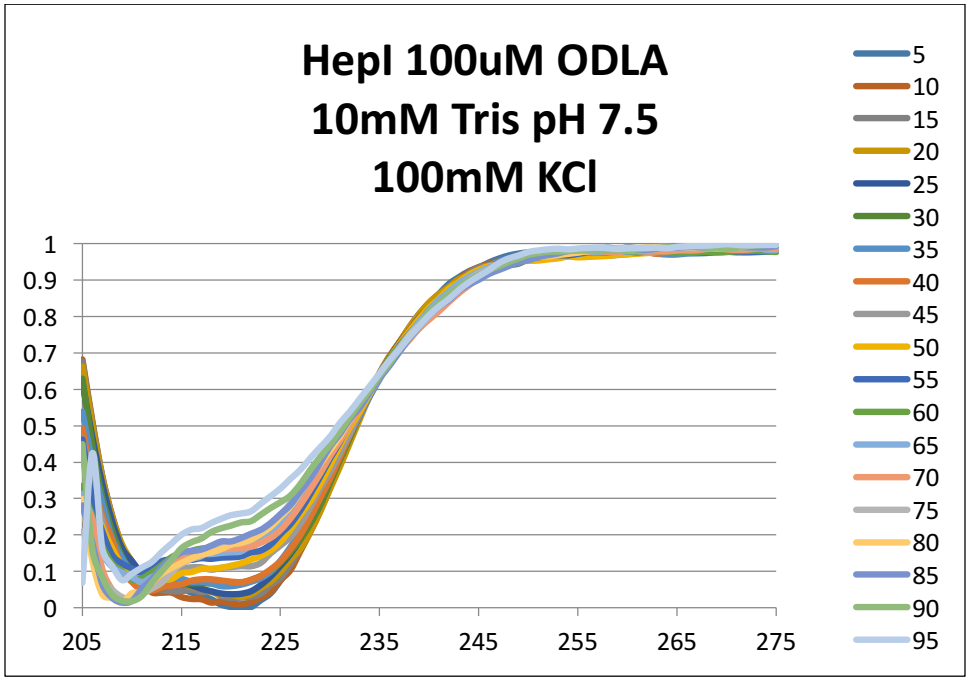


Figure S2. Representative CD thermal melts of HepI in low and high salt buffers: (A) 100 µM KCl, (B)

A.



B.

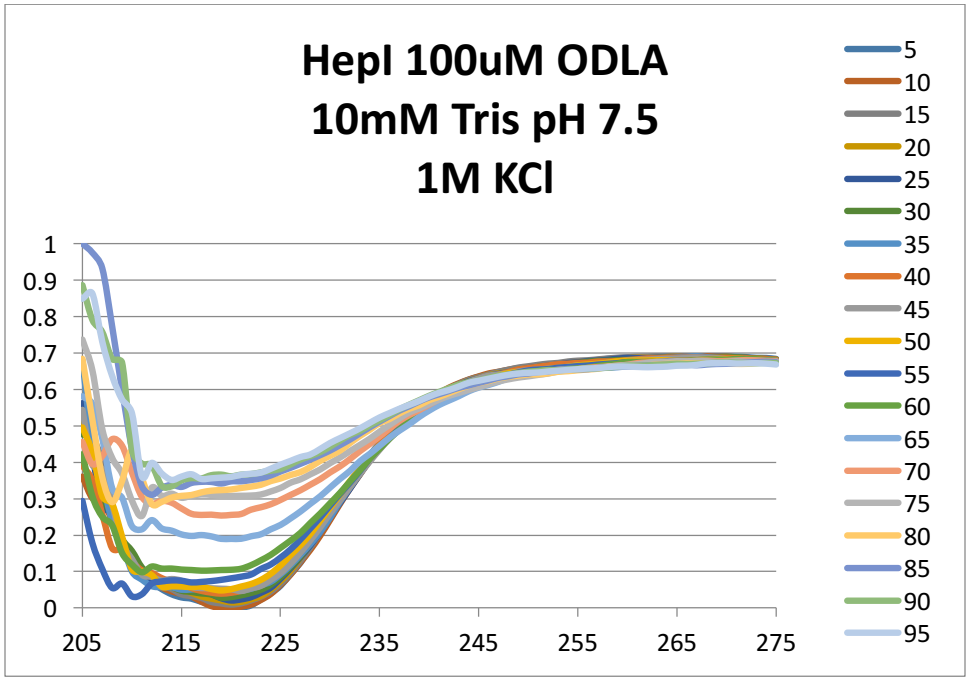


Table S3. Thermodynamic parameters calculated from CD melt experiments for HepI-WT and mutants in the absence of substrates.

	Tm (°C)	$\Delta_u S°$ (J /K)	$\Delta_u H°$ (kcal/mol)
HepI	39.7±0.3	0.112±0.003	35±1
HepI ADPH	44.1±0.5	0.0962±0.0004	30.5±0.1
HepI 2M KCl	56.9±0.3	0.065±0.003	21±1
W47F	36.6±0.6	0.090±0.004	28±4
W62F	41.5±0.2	0.0943±0.0008	29.8±0.2
W66F	43.2±0.1	0.102±0.002	32.3±0.5
W116F	47±1	0.095±0.004	30±1
W194F	36.5±0.7	0.111±0.003	34±1
W199F	52.3±0.4	0.0686±0.0009	22.3±0.3
W217F	48.8±0.5	0.0828±0.0005	26.7±0.2

Table S4. Trp residue SASA values determined from crystal structure (PDB 2GT1) and 100 ns MD simulation.

	Crystal (Å^2)	Mean (Å^2)	Standard Deviation (Å^2)	Max (Å^2)
W35	0	0.0976	0.3114	5.0720
W47	5.5142	12.1088	7.9629	74.1469
W62	13.5126	16.1062	10.7940	79.9459
W66	37.5801	128.2969	28.3375	207.2929
W116	137.6760	131.8050	14.1150	197.1310
W194	1.2858	28.8520	13.8658	138.3586
W199	0.4831	1.9377	1.8762	13.9760
W217	37.3442	45.9663	12.6857	109.0094

Figure S3: Normalized fluorescence spectra for HepI WT and all TRP Mutants.