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Supporting information for article:

Structural basis for the catalytic mechanism of homoserine dehydrogenase

Vikas Navratna, Govardhan Reddy and Balasubramanian Gopal

Table S1 List of primers used in this study

Target	Expression vector	Direction of primer	Primer sequence (5'- 3')
HSD	pET15b and pET28b	Forward Reverse	CACGGCTAGCATGAAAAAATTAAATATA CGACCTCGAGAACTCCTTCTACTGGGTA
K105A HSD	pET15b	Forward Reverse	GCAGATTTATTAGCAGTACATCTTAAACTTTTAGAAGATT ATTTGCGGTAATAACATGTTTTTTATTTTTAAG
K105R HSD	pET15b	Forward Reverse	AGAGATTTATTAGCAGTACATCTTAAACTTTTAGAAGATT ATTTGCGGTAATAACATGTTTTTTATTTTTAAG

Table S2 Crystallization conditions for *S. aureus* HSD

pH	Condition	Cryo-protectant	Method of crystallization
8.5	0.2M Magnesium acetate, 14% (w/v) PEG8000, 0.2M Bicine, 5% Glycerol (200µl of (1:1 ratio of paraffin and silicone) oil was overlaid on the mother liquor in reservoir well)	15% DMSO	Hanging drop vapour diffusion
7.5	0.2M Magnesium acetate, 16% (w/v) PEG8000, 0.1M Tris-HCl, 3% Glycerol (Crystal soaked with 5mM Lysine and 4mM NADP for 2min)	15% DMSO	Hanging drop vapour diffusion
7.0	0.2M Magnesium acetate, 16% (w/v) PEG3350, 0.1M HEPES, 20% Isopropanol, 5% Glycerol (Crystal soaked with 5mM Serine for 2min)	15% DMSO	Sitting drop vapour diffusion
6.5	0.2M Magnesium acetate, 16% (w/v) PEG3350, 0.1M Sodium cacodylate, 5% Glycerol (Crystal soaked with 5mM Serine and 2mM NADP for 2min)	15% DMSO	Sitting drop vapour diffusion
6.0	0.2M Magnesium acetate, 22% (w/v) PEG8000, 0.1M Bis-Tris, 1mM Serine, 5% Glycerol	15% DMSO	Microbatch

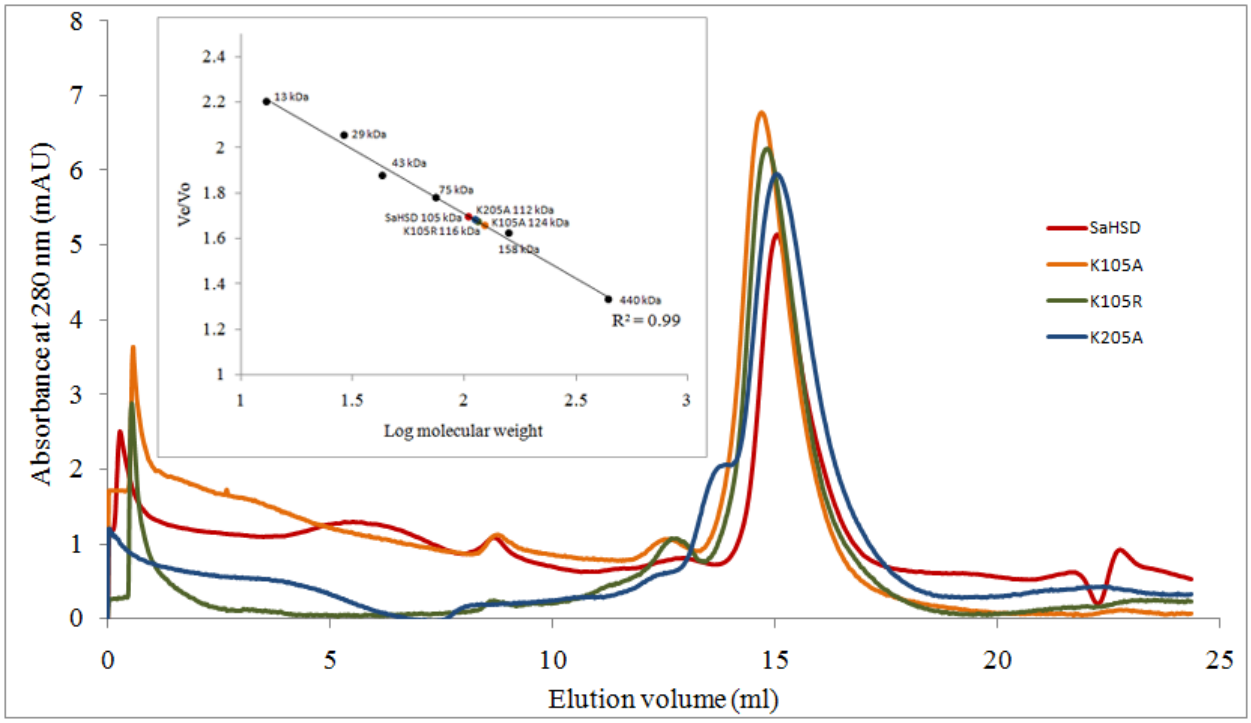


Figure S1 Characterization of K205A, K105A and K105R mutants. Quaternary structure analysis using size exclusion chromatography suggests that the mutation of either Lys105 or Lys205 did not alter the integrity and oligomeric association of HSD.

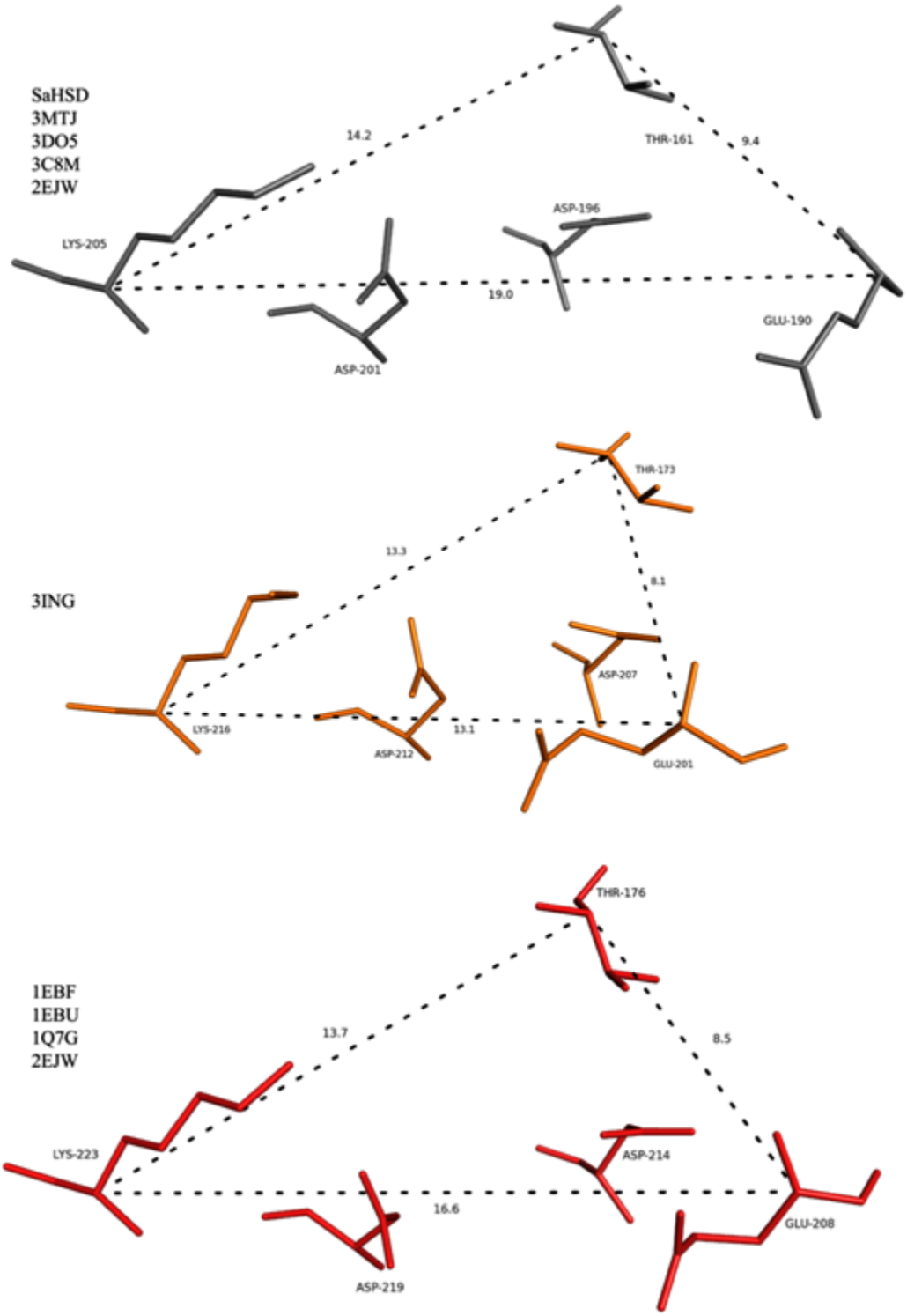
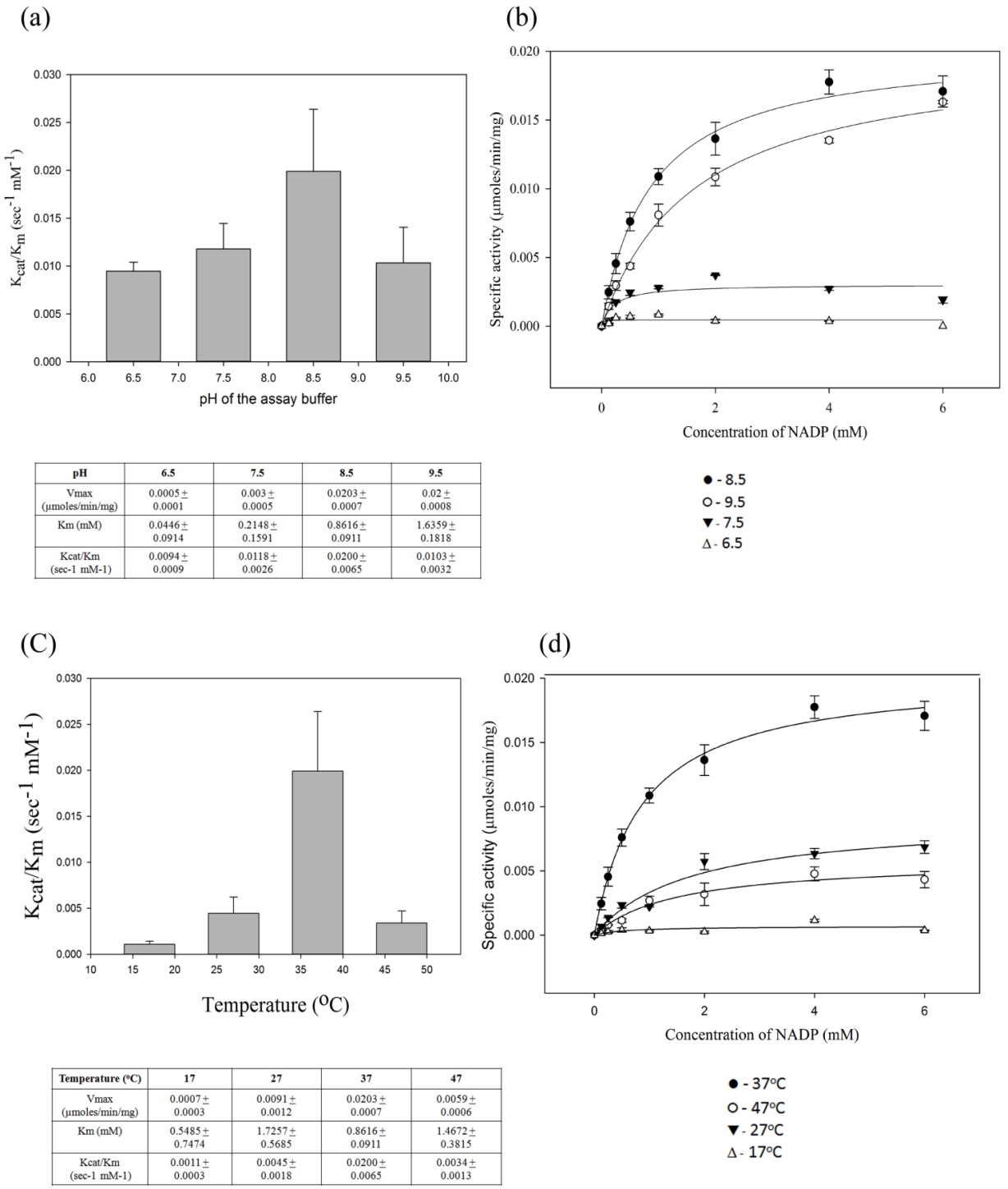


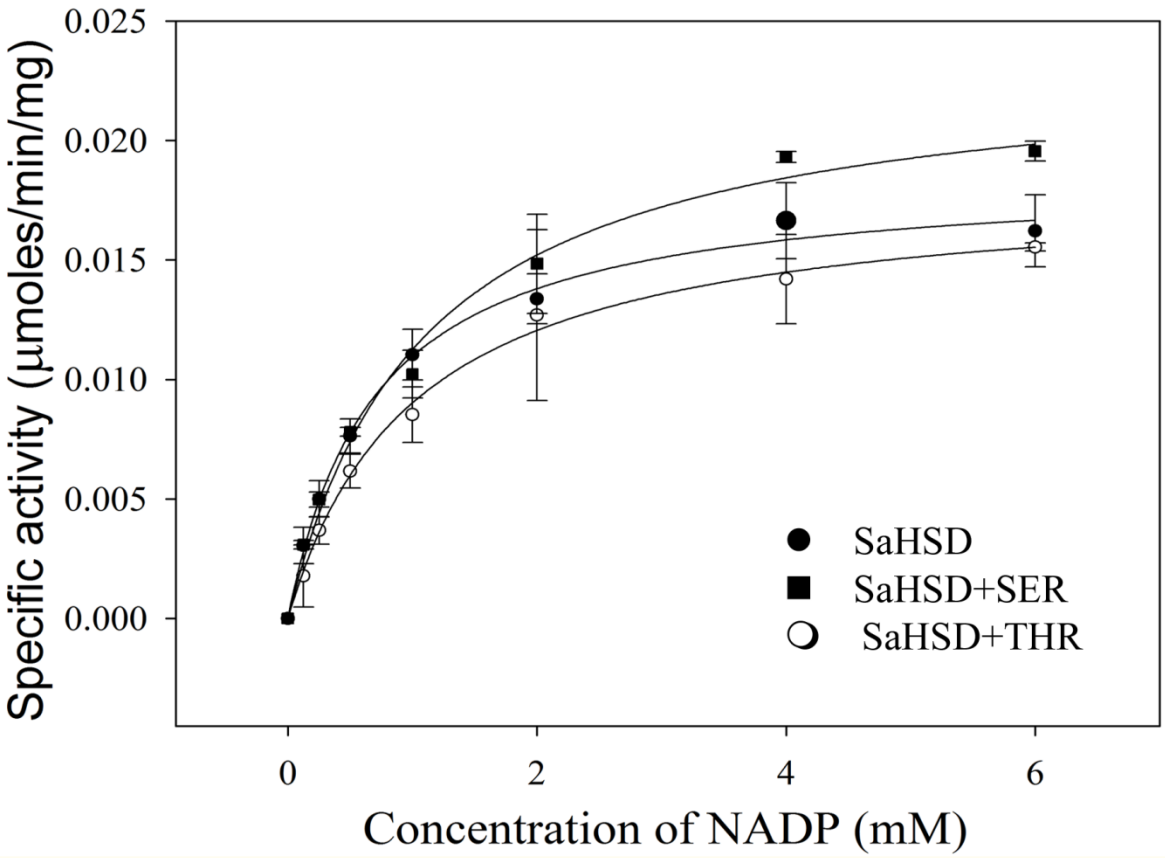
Figure S2 The variation in the distances between the catalytic residues in HSD across homologues. This analysis suggests that conformational changes, either by co-factor or activator binding, could trigger catalytic activity.



pH	6.5	7.5	8.5	9.5
Vmax (µmoles/min/mg)	0.0005 ± 0.0001	0.003 ± 0.0005	0.0203 ± 0.0007	0.02 ± 0.0008
Km (mM)	0.0446 ± 0.0914	0.2148 ± 0.1591	0.8616 ± 0.0911	1.6359 ± 0.1818
Kcat/Km (sec-1 mM-1)	0.0094 ± 0.0009	0.0118 ± 0.0026	0.0200 ± 0.0065	0.0103 ± 0.0032

Temperature (°C)	17	27	37	47
Vmax (µmoles/min/mg)	0.0007 ± 0.0003	0.0091 ± 0.0012	0.0203 ± 0.0007	0.0059 ± 0.0006
Km (mM)	0.5485 ± 0.7474	1.7257 ± 0.5685	0.8616 ± 0.0911	1.4672 ± 0.3815
Kcat/Km (sec-1 mM-1)	0.0011 ± 0.0003	0.0045 ± 0.0018	0.0200 ± 0.0065	0.0034 ± 0.0013

Figure S3 Specific activity measurements under various conditions. The specific activity (panels b and d) suggest that HSD is most active at basic pH (optima pH 8.5) and 37°C. The effect of pH and temperature variations on catalytic activity are represented as K_{cat}/K_m plots in panels (a) and (c) respectively.



Regulators	Thr (30mM)	Ser (30mM)	-
V_{max} (μmole/min/mg)	0.0234 ± 0.0010	0.0182 ± 0.0005	0.0186 ± 0.0005
Km (mM)	1.0807 ± 0.1324	1.0122 ± 0.0822	0.6942 ± 0.0574

Figure S4 L-Ser and L-Thr do not influence the catalytic activity of *S. aureus* HSD.

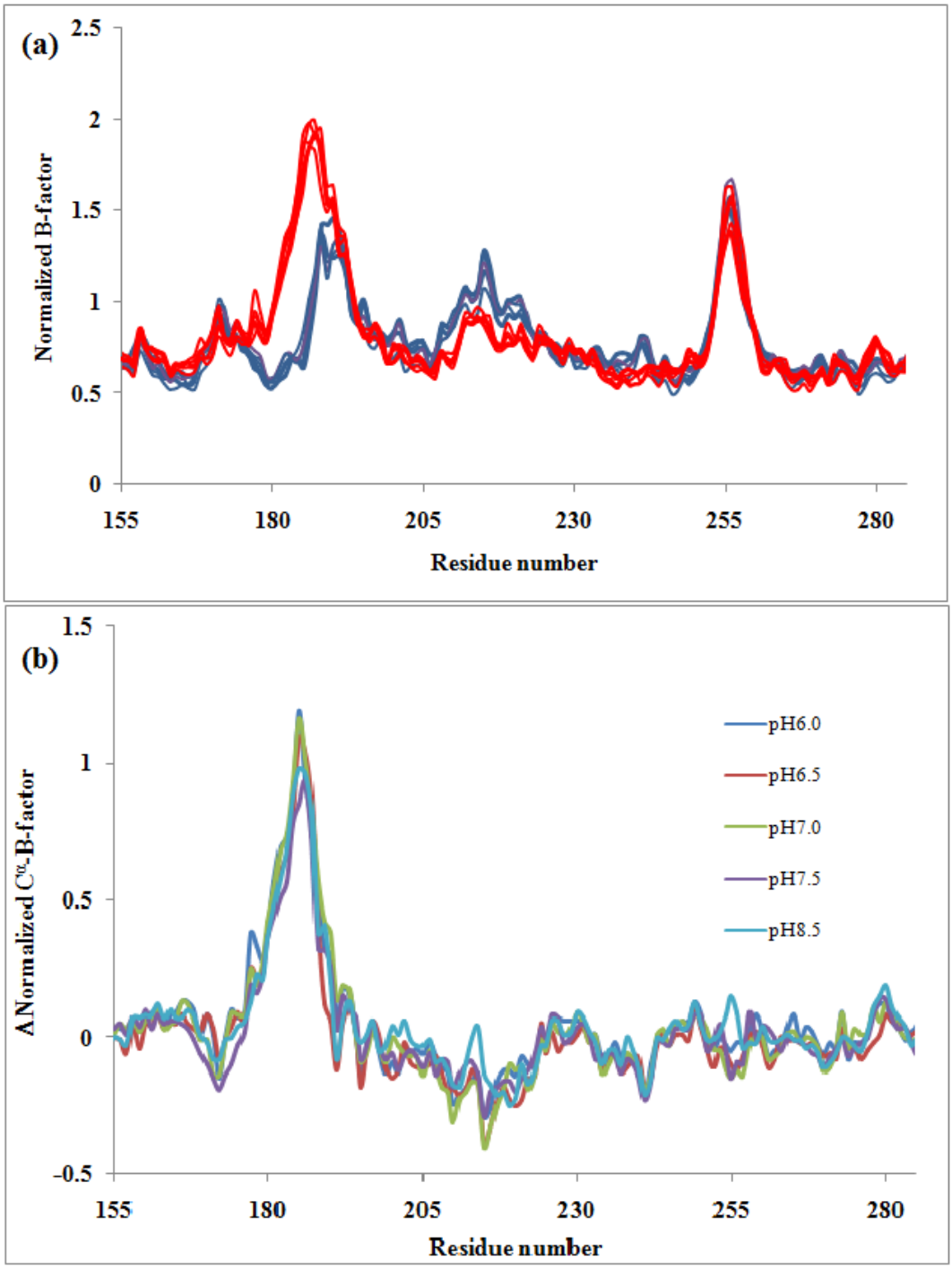


Figure S5 Normalized B-factor plots comparing the two monomers in *S. aureus* HSD. (a) A superposition of normalized Cα-B-factors for the residues comprising the active site (155-285) shows chain B (red) has higher B-factor than chain A (blue) in all the pH conditions that were examined. (b) A plot of difference in the normalized Cα-B-factors between chain B and chain A reiterates the observation that this difference is independent of pH.

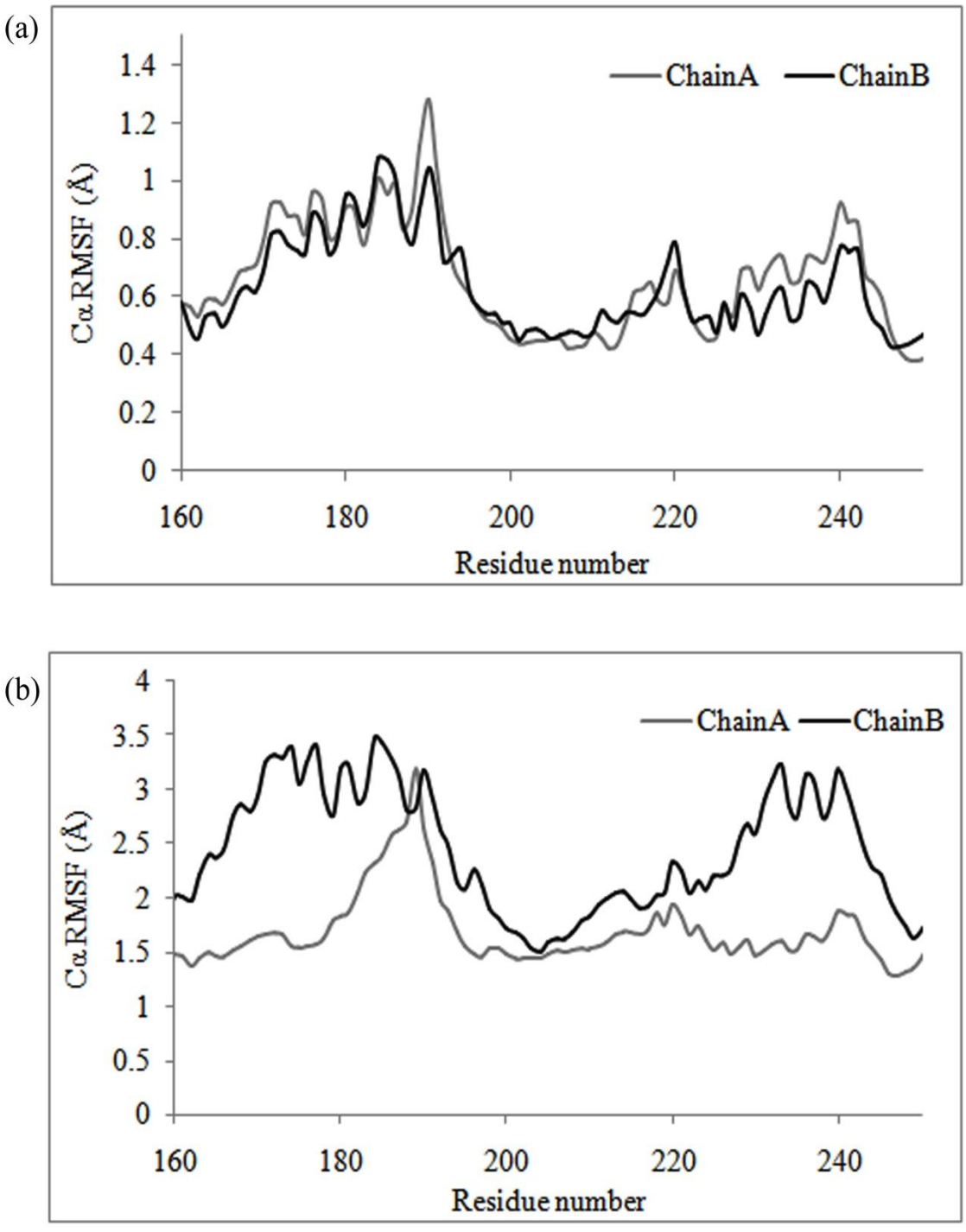


Figure S6 The Cα Root Mean Square Position Fluctuations (RMSF) of *S. aureus* HSD. The Cα RMSF analysis of an isolated dimer (a) and the dimer surrounded by three other dimers in the $P2_12_12_1$ unit cell (b suggests that apparent higher flexibility of one chain than the other can be ascribed to crystal packing.

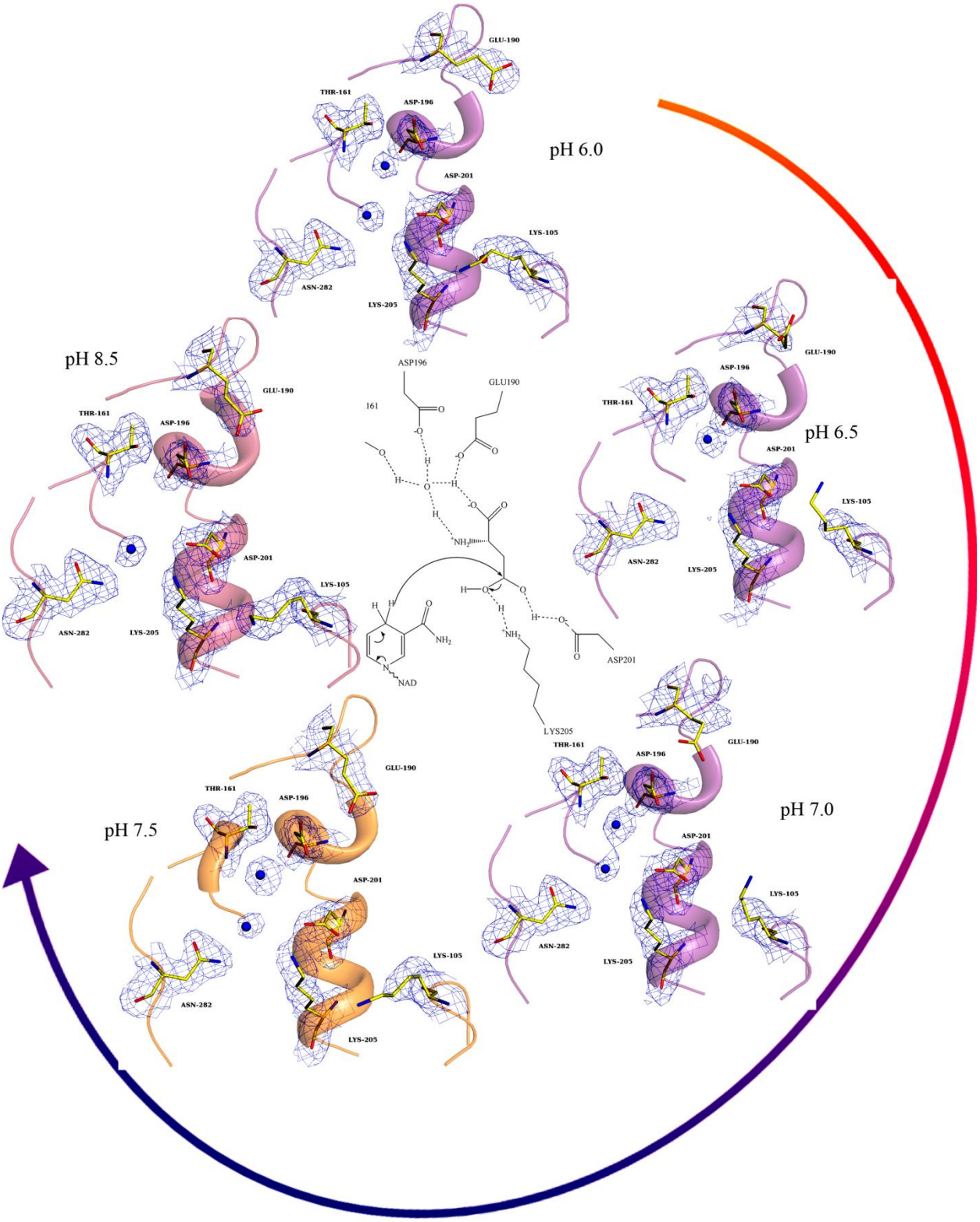


Figure S7 The hydration at the active site of the 'inert' monomer (Chain A). These snapshots (experimental maps contoured at 1σ) can be compared with figure 4.