

SUPPLEMENTARY MATERIAL

Protein conformational dynamics underlay selective recognition of thermophilic over mesophilic Enzyme I by a substrate analogue

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X: active site **X**: conserved residues — EIN — EIC --- active site loops *: mutations

eEI	1	MISGILASPGIAFGKALLKEDEIVIDRKKISADQVDQEVEERFLSGRAKASAQLETIKTK	60
tEI	1	MLKGVAASPGIAGKAFLYTKEKVITINVEKIEESKVEEEIAKFRKALEVTQEEIEKIKEK	60
eEI	61	AGETFGEKEAIFEGHIMLLEDEELEQEIIALIKDKHMTADAAAEVIEGQASALEELDD	120
tEI	61	ALKEFGKEKAEIFEAHLMLASDPELIEGVENMIKTELVTADNAVNVIEQNASVMESLND	120
eEI	121	EYLKERAADVRDIGKRLLRNILGLKIIDLSAIQDEVILVAADLTTPSETAQNLNKKVLGFI	180
tEI	121	EYLKERAVIDLRDVGNRIENLLGVKS VNLS DLEEVVVIARDLTTPSDTATMKKEMVLGFA	180
eEI	181	TDAGGRTSHTSIMARSLELPAIVGTGSVTSQVKND DY LILD AVNNQV YVNPTNEVIDKMR	240
tEI	181	TDVGGRTSHTAIMARSLEIPAVVGLGNVTSQVKAGDLVIVDGLEGI VIVNPDEKTVEDYK	240
eEI	241	AVQEQVAS EKAE LAKL KDL PAITLDGHQVEVCANIGTV [*] *RDVEGAERN GAEGVGLYRTTEFL	300
tEI	241	SKKESYEKKVEGLKQLKDLPAETPDGKKVMLAANIGTPKDVASALANGAEGVGLFRTTEFL	300
eEI	301	*FMDRD [*] ALP [*] T [*] EEEEQAAYKAVAACGSQAVIVRTM [*] DIGGD [*] KELPYM [*] N [*] F [*] PKE [*] ENPF LGW [*] RAI	360
tEI	301	YMDRNSLPSEEEEQFEAYKEVVEKMGRPVTIR [*] TLDIGGD [*] KELPYLDMPKEMNPF LGYRAI	360
		(res. 296-309) β2a2 loop (res. 332-360)	
eEI	361	RIAMDRKEIILRDQLRAILRASAFGKLRIMFPMIISVEEVRALRKEIEIYKQELRDEGKAF	420
tEI	361	RLCLDRPDIFKTQLRAILRASAYGNVQIMYPMIS SVEEVRKAN SIL EEVKAELDREGVKY	420
eEI	421	DESIEIGVMVETPAAATIARHLAKEVDFFSIGTNDLTQYT LAVDR [*] GND [*] M [*] I [*] SH [*] L [*] YQP [*] M [*] S [*] PS	480
tEI	421	DKEIKVGIMVEIPSAAVTADILAKEVDFFSIGTNDLTQYT LAVDR [*] MNEHVKEYYQPFHPA	480
		β6a6 loop (res. 454-477)	
eEI	481	VLNLIKQVIDASHAEGKW TGMCGELAGDERATLLLLMGGLDEF SMSAISIPRIKKIIRNT	540
tEI	481	ILRLVKMVIDAAHKEGKFAAMCGEMAGDPLAAVILLGLGLDEF SMSATS IPEIKNIIRNV	540
eEI	541	NFEDAKVLAEQALAQP TTDELMTLVNK FIEEKTIC	575
tEI	541	EYEKAKEIAEKALNMSEAREIEKMMKDVI--KDIG	573

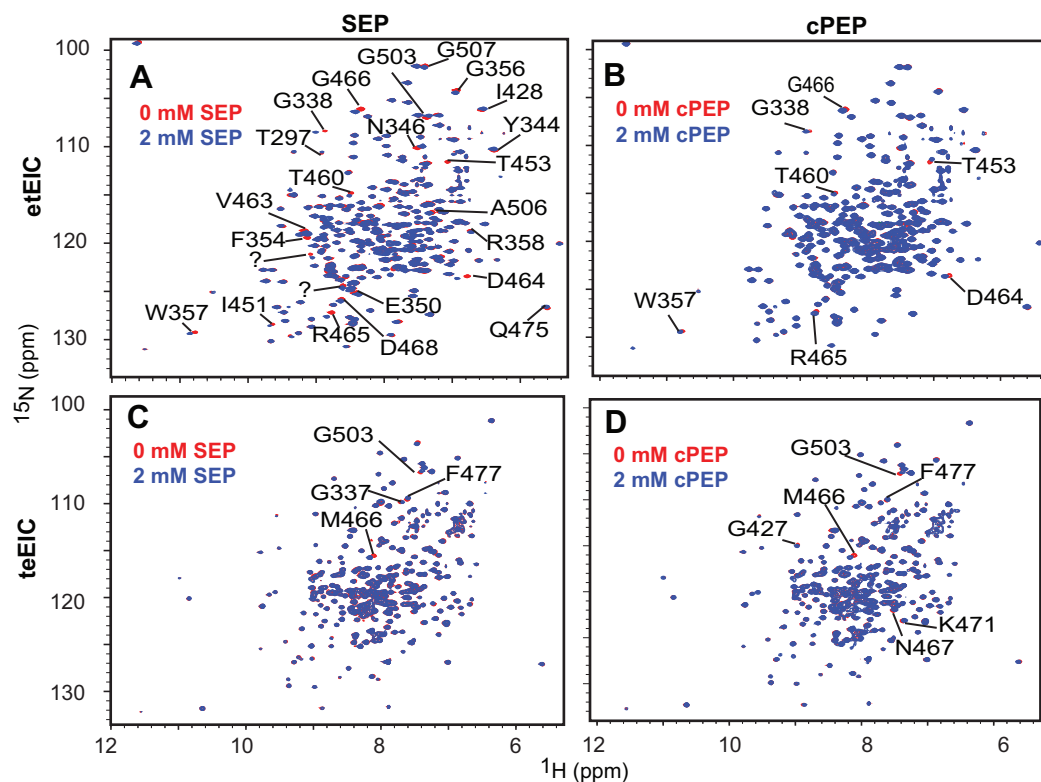


Figure S2. 800 MHz ^1H - ^{15}N TROSY spectra of etEIC and teEIC measured in the absence (red) and in the presence (blue) of 2 mM SEP or cPEP. Panels (A), (B), (C), and (D) display the data measured for the etEIC-SEP, etEIC-cPEP, teEIC-SEP, and teEIC-cPEP systems, respectively. Peaks that shift upon addition of ligand are assigned in the spectra. “?” indicates peaks with unknown assignment.

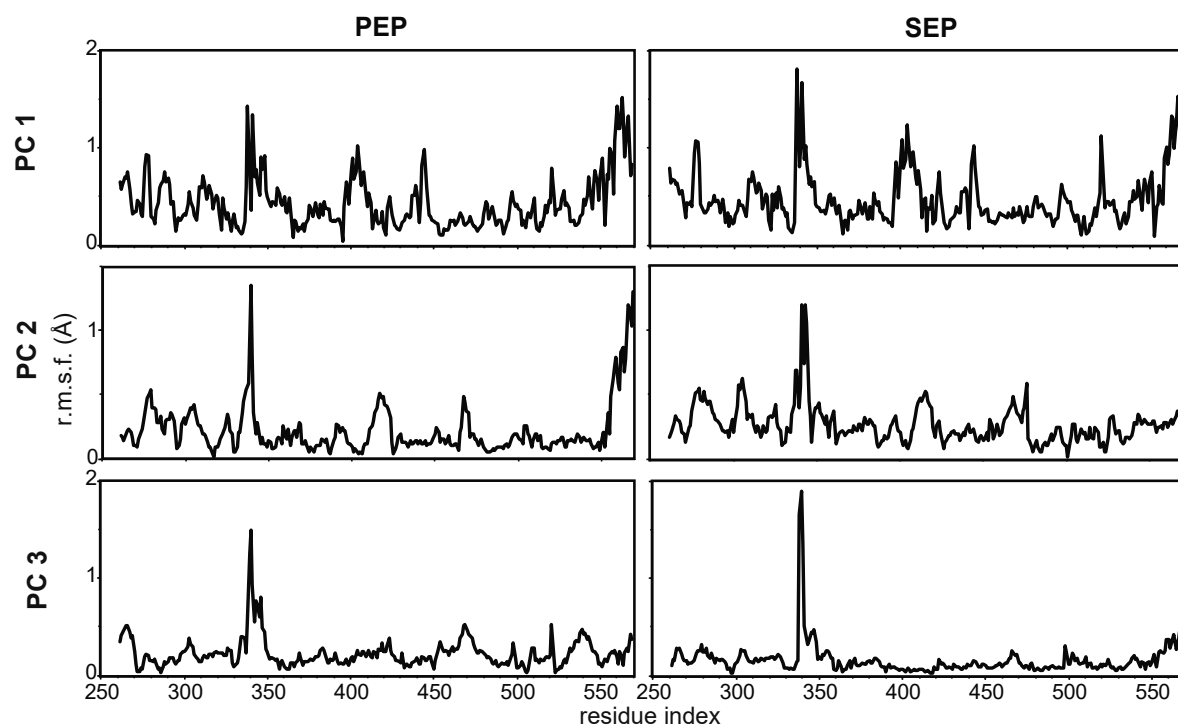


Figure S3. Residue-specific r.m.s.f. values (relative to the average structure) in PC 1 (top), PC 2 (middle), and PC 3 (bottom) calculated over the concatenated trajectory of the EIC-PEP (left) and EIC-SEP (right) complexes are plotted versus the residue index to emphasize the specific contribution of different EIC regions to each PC.