# SUPPLEMENTARY MATERIAL

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

Aayushi Singh[1], Daniel Burns[2], Sergey L. Sedinkin[1], Brett VanVeller[1], Davit A. Potoyan[1,2,*] and Vincenzo Venditti[1,2,*]

*[1] Department of Chemistry, Iowa State University, Ames, Iowa 50011, USA.*

*[2] Roy J. Carver Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames, Iowa 50011, USA.*

*Address correspondence to:

Vincenzo Venditti, Department of Chemistry, Iowa State University, Hach Hall, 2438 Pammel Drive, Ames, IA 50011, USA. Email: venditti@iastate.edu; Tel: 515-294-1044; Fax: 515-294-7550; ORCID 0000-0001-8734-0400.

Davit A. Potoyan, Department of Chemistry, Iowa State University, Gilman Hall, 2415 Osborn Drive, Ames, IA 50011, USA. Email: potoyan@iastate.edu; Tel: 515-294-9971; Fax: 515-294-7550.


**Figure S1.** Sequence alignment of eEI and tEI. The amino acid sequences of eEI and tEI were aligned in BLAST. Active site residues are colored red. Conserved residues are colored green. The EIN and EIC domains are indicated with a blue and red line underneath the amino acid sequence, respectively. The location of the active site loops is shown with dashed black lines underneath the amino acid sequence. Positions of the 21 single-point mutations performed to hybridize the EIC domain are indicated by asterisks over the amino acid sequence. Note that two mutations (at positions 278 and 279) lay outside of the β2α2, β3α3, and β6α6 loops. These residues were mutated because facing the β2α2 loop in the 3D structures of EIC (see reference 16 in the main text).

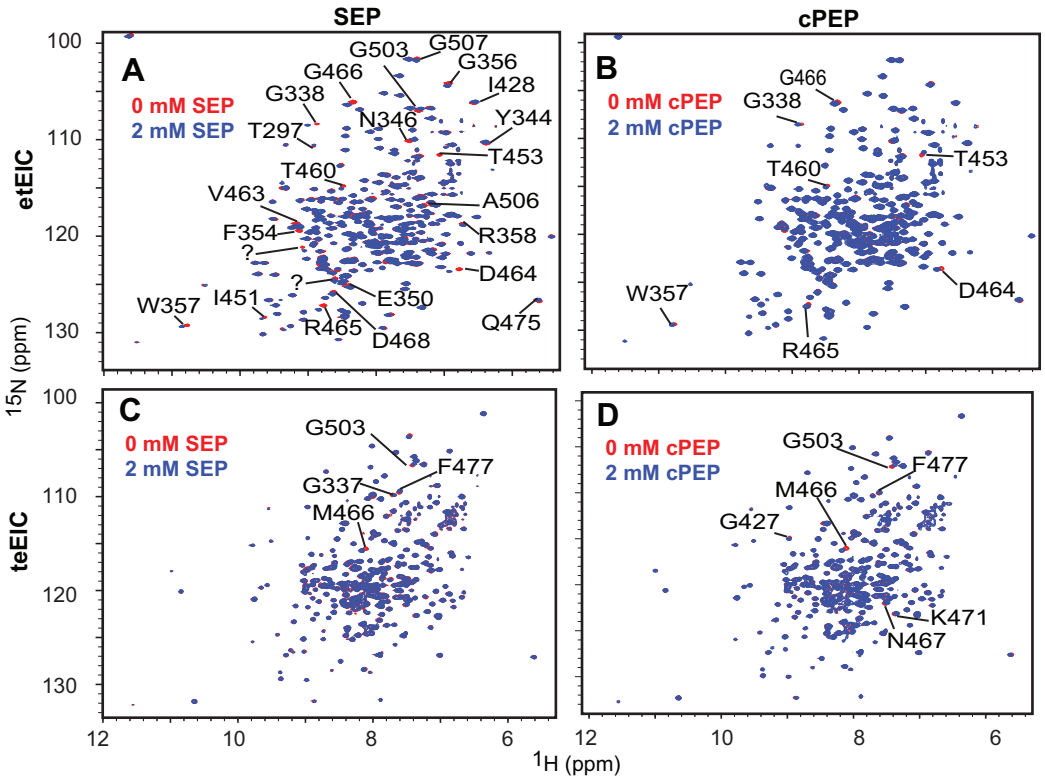


**Figure S2.** 800 MHz $^{1}$H-$^{15}$N TROSY spectra of etEIC and teEIC measured in the absence (red) and in the presence (blue) of 2 mM SEP or cPEP. Panels **(A)**, **(B)**, **(C)**, and **(D)** display the data measured for the etEIC-SEP, etEIC-cPEP, teEIC-SEP, and teEIC-cPEP systems, respectively.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

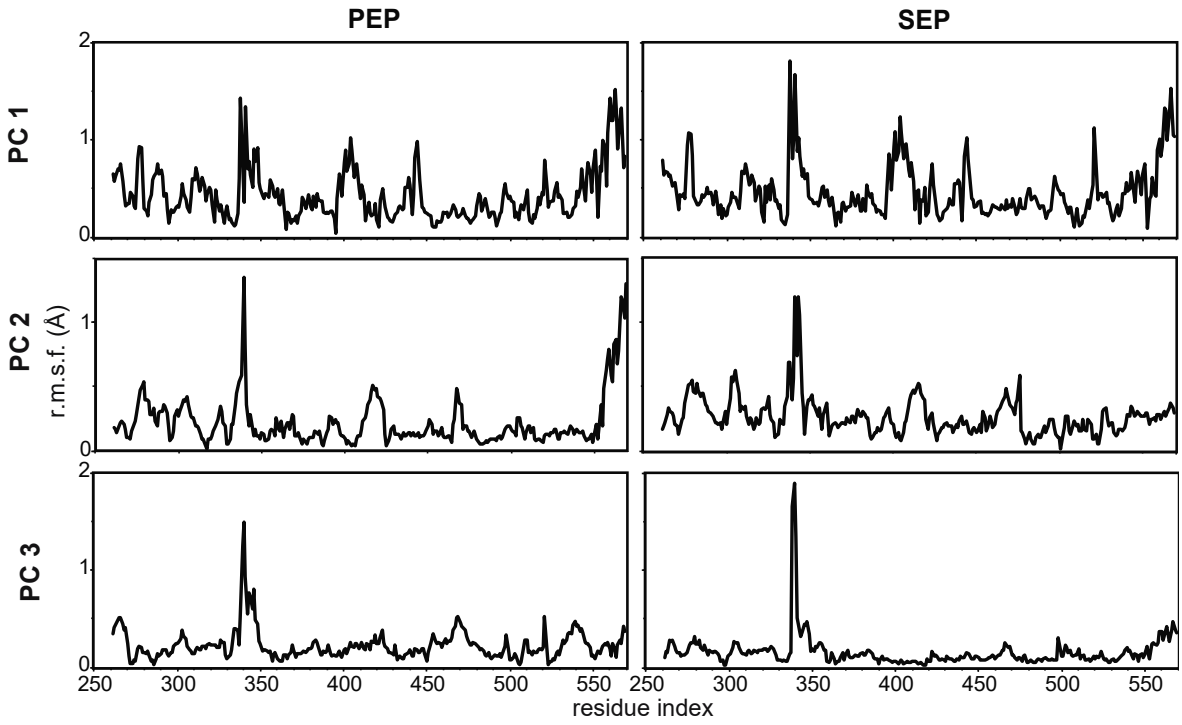


**Figure S3.** Residue-specific r.m.s.f. values (relative to the average structure) in PC 1 (top), PC 2 (middle), and PC 3 (bottom) calculated over the concatenated trajectory of the EIC-PEP (left) and EIC-SEP (right) complexes are plotted versus the residue index to emphasize the specific contribution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