X-ray crystallographic analysis of the 6-aminohexanoate cyclic dimer hydrolase: catalytic mechanism and evolution of an enzyme responsible for nylon-6 byproduct degradation

SUPPLEMENTARY DATA ON-LINE

Legend for supplementary figures

Fig. S1. **Multiple 3D-alignment of 6-aminohexanoate cyclic dimer hydrolase (NylA), glutamyl-tRNA^{Gln} amidotransferase subunit A (GAT), malonamidase E2 (MAE2) and peptide amidase (PAM)**. α-Helices and β-strands of NylA shown in Fig. 2A are illustrated at the top with green cylinders (α-helix) and orange arrows (β-strands). Multiple 3D alignments were carried out using Secondary Structure Matching (SSM) (45), and the secondary structures are shown as *green* (helix) or *orange* (β-strand) letters. The numbering of GAT, MAE2 and PAM is the same as the numbering registered in PDB. The Ser-*cis*Ser-Lys catalytic triads conserved in AS family enzymes [Ser174-*cis*Ser150-Lys72 (NylA), (Ser178-*cis*Ser154-Lys79) (GAT), Ser155-*cis*Ser131-Lys62 (MAE2), Ser226-*cis*Ser202-Lys123 (PAM)] are shown as *red* letters marked with *yellow*. Acd-binding residues (Asn125 and Cys316) in NylA are marked with *light blue*.

Fig. S2. Stereoviews of overall structures of 6-aminohexanoate cyclic dimer hydrolase (NylA). Superimposition of NylA structure (*green*) with glutamyl-tRNA^{Gln} amidotransferase subunit A (PDB ID: 2DF4; *blue*) (**A**), malonamidase E2 (PDB ID: 1ocm; *purple*) (**B**), and peptide amidase (PDB ID code: 1m21; *olive*) (**C**). Superimposition was carried out on the transformation matrix generated by Secondary Structure Matching (SSM) (45).

Fig. S3. CD spectra at far UV for NylA and various mutants in 20 mM potassium phosphate buffer (pH 7.3) containing 10% glycerol. CD measurements were carried out in a J-720WI spectropolarimeter (Jasco). Cuvette with a pathlength of 1 mm was used for far UV CD. The results are expressed as the mean residue molar ellipticity, [θ], defined as $[\theta]= 100\ (\theta_{obs}-\theta_{back})/l\ c$, where θ_{obs} is the observed ellipticity in degrees, θ_{back} is the observed ellipticity in degrees without enzyme as background, c is the molar concentration of the residue, and l is the length of the light path (in centimeters). The temperature was controlled at 25 °C with a Jasco PTC-348WI peltier system. The protein concentration used (in far UV CD measurements) was 0.11 mg ml^{-1}. **A.** Wild-type NylA (*orange*), NylA-A^{150} (*green*); **B.** Wild-type NylA (*orange*), NylA-A^{125} (*blue*), NylA-S^{316} (*yellow green*), NylA-D^{316} (*pink*).

Fig. S4. Temperature factors for NylA and NylA-A^{174}/Acd complex. A. Average values for C_α, C, N in each amino acid residue are plotted as a function of residue number. **B.** Data for amino acid positions 300-450 are shown with the positions of α-helices (H14-H18), β-strands (β9-β11), and the loop region. Open circle, wild-type NylA; closed circle, NylA-A^{174}/Acd complex.

Supplementary Table

Table S1. Primer DNA used for site-directed mutagenesis of *nylA* gene

Primer	Mutation	Sequence
A		
FE1K72A	K72A	5`-TGCCCTATCTTCTG**GCG**GACCTCACC-3’
FE1S150A	S150A	5'-TCGGTTGGCGGA**GCG**AGCGGCGGCTCA-3'
FE1S174A	S174A	5'-GACGCGGCAGGT**GCT**GTGCGCATACCT-3'
RE1C316E	C316E	5'-GATCGCGACGTC**CTC**AATCGTCGAGTAG-3'
RE1C316X	C316D, C316G C316S,	5'-GATCGCGACGTC**NNN**AATCGTCGAGTAG-3'
B		
FE1N125A	N125A	5'-GAGATGGGC**GCT**CAGGTAACGACGGAGCCC-3'
RE1N125A		5'-CGTTACCTG**AGC**GCCCATCTCCGGTGTATT-3'
FE1C316-3	C316A	5'-ACGATT**NCC**GACGTCGCGATCGCGCGA-3'
RE1C316-3		5'-GACGTC**GGN**AATCGTCGAGTAGTCCTT-3'
FE1C316-2	C316K	5'-ACGATT**NAG**GACGTCGCGATCGCGCGA-3'
RE1C316-2		5'-GACGTC**CTN**AATCGTCGAGTAGTCCTT-3'
FE1C316-1	C316N	5'-ACGATT**NAC**GACGTCGCGATCGCGCGA-3'
RE1C316-1		5'-GACGTC**GTN**AATCGTCGAGTAGTCCTT-3')

A. Site-directed mutagenesis was carried by "modification of restriction-site (MR)" method (23).
B. Site-directed mutagenesis was performed using the PrimeSTAR mutagenesis kit (Takara Bio Inc., Otsu, Japan). Mutated sites in the primer sequences are underlined.


NylA 3 KVDLWQDATAQAELVRSGEISRTELLEATIAHVQAVNP---EINAVIIPLFEKARRESEL---------AS- 61
GAT 1 MSIRYESVENLLTLIKDKKIKPSDVVKDIYDAIEETDP---TIKSFLALDKENAIKKAQELDELQAKDQMD- 68
MAE2 1 ----MISLADLQRRIETGELSPNAAIAQSHAAIEAREK---EVHAFVRHDKSARA-QA-----------S- 51
PAM 43 FPYAETDVADLQARMTAGELDSTTLTQAYLQRIAALDRTGPRLRAVIELN-PDALKEAAERDRE-RRDGRLR 112
NylA 62 GPFAGVPYLLKDLTVVSQGDINTSSIKGMKESGYRADHDAYFVQRMRAAGFVLLGKTNTPEMGN-------Q 126
GAT 69 GKLFGIPMGIKDN-IITNGLETTCASKMLE--GFVPIYESTVMEKLHKENAVLIGKLNMDEFAM-------G 130
MAE2 52 GPLRGIAVGIKDI-IDTANMPTEMGSEIYR--GWQPRSDAPVVMMLKRAGATIIGKTTTAFAS-------R 113
PAM 113 GPLHGIPLLLKDN-INAAPMATSAGSLALQ--GFRP-DDAYLVRRLRDAGAVVLGKTNLSEWANFRGNDSIS 180
NylA 127 VTTEPEAWGATRNPWNLGRSVGGSSGGSGAAVAAALSPVAHGNDAAGSVRIPASVCGVVGLKPTRGRISPGP 198
GAT 131 GSTETSYFKKTVNPFDHKAVPGGSSGGSAAAVAAGLVPLSLGSDTGGSIRQPAAYCGVVGMKPTYGRVSRFG 202
MAE2 114 D------PTATLNPHNTGHSPGGSSSGSAAAVGAGMIPLALGTQTGGSVIRPAAYCGTAAIKPSFRMLPTVG 179
PAM 181 GWS--ARGGQTRNPYRISHSPCGSSSGSAVAVAANLASVAIGTETDGSIVCPAAINGVVGLKPTVGLVSRDG 250
NylA 199 LVTDSDNVAGAAHEGLFARSVRDIAALLDVVS-GHRPGDTF---CAPTASRPYAQGISENDPGSLRVGVLT- 264
GAT 203 L-VA--FASSLDQIGPLTRNVKDNAIVLEAIS-GADVNDST---SAPVDDVDFTSEIG-K-IKGLKVALPKE 266
MAE2 180 V-KC--YSWALDTVGLFGARAEDLARGLLAMTGRS----------------EFSGIVP-AKA--PRIGVVRQ 229
PAM 251 I-IP--ISFSQDTAGPMARSVADAAAVLTAIA-GRDDADPATATMPGRAVYDYTARLDPQGLRGKRIGLLQ- 317
NylA 265 --H--NPVGDFAL--DPECAAAARGAAAALAALGHDVNDAYP-EA-LG-DRSFLKDYSTICDVAIAREI--- 324
GAT 267 YLG--EG-----V--ADDVKEAVQNAVETLKSLGAVVEEVSL-PN-T---KFGIPSYYVIASSEASSNL--- 321
MAE2 230 --EFAGA-----V--EPAAEQGLQAAIKAAERAGASVQAIDLPEAVHEAWR----IHPIIQDFEAHRAL--- 285
PAM 318 --T--P------LLKYRGMPPLIEQAATELRRAGAVVVPVEL-PN-Q---GAWAEAERTLLLYEFKAGLERY 374
NylA 325 ----------ERNGELI-------------GRPL--TEDD------VE---WTSWEMVKRA--DQ-VTG--R 357
GAT 322 ----------SR-FDGIRYGYHSKEAHSLEELYKMSRSEG------FG---KEVKRRIFLG--TFALSS--G 369
MAE2 286 ----------A--WEF--------------SEH---HDE------IA---PMLRASLDAT--VGLTP---K 313
PAM 375 FNTHRAPLRSLADL-----------------I----AFNQAHSKQELGLFG--QELLVEADAT--AGLADP 420
NylA 358 A----FAAC-VDELRYYAGKVERW-WEA-GWDLLILPTVTRQTPEIG---E-LMLAKGTDLEGRQSAFISGS 418
GAT 370 YYDAYYK-KSQKVRTLIKNDFDKV-FE--NYDVVVGPTAPTTAFNLG---E-EIDDPL------------TM 421
MAE2 314 E----YD-EARRIGRRGRRELGEV-FE--GVDVLLTYSAPGTAPAKA---LAST---------------- 356
PAM 421 AY---IR-ARSDARRLAGPEGIDAALAAHQLDALVAPTT-GVAWPIRSEGD-DF---------------- 468
NylA 419 LQMLAFTVPFNVSGQPAISLPIGMSSDGMPIGVQIVAAYGREDLLLQVAAQLEGALPWVARRPQLLN 485
GAT 422 YANDLLTTPVNLAGLPGISVPCGQS-NGRPIGLQFIGKPFDEKTLYRVAYQYETQY-NLHDVYEKL 485
MAE2 357 -GDPRYNRLWTLMGNPCVNVPVLKV-GGLPIGVQVIARFGNDAHALATAWFLEDAL-AKRPPHF 529
PAM 469 --PGESYSAAAVAGYPSLTVPMGQI-DGLPVGLLFMGTAWSEPKLIEMAYAYEQRTRAR 524

Fig. S1

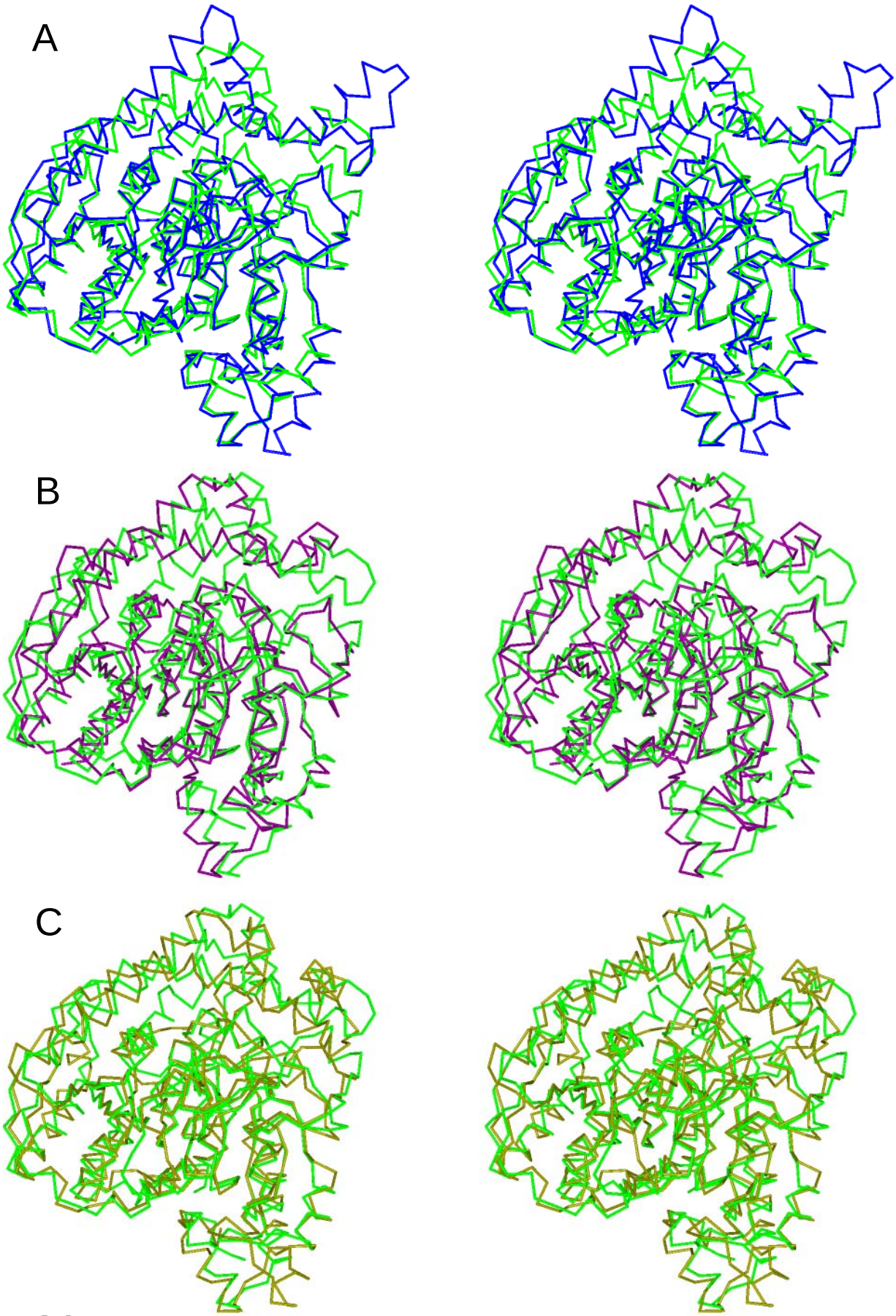
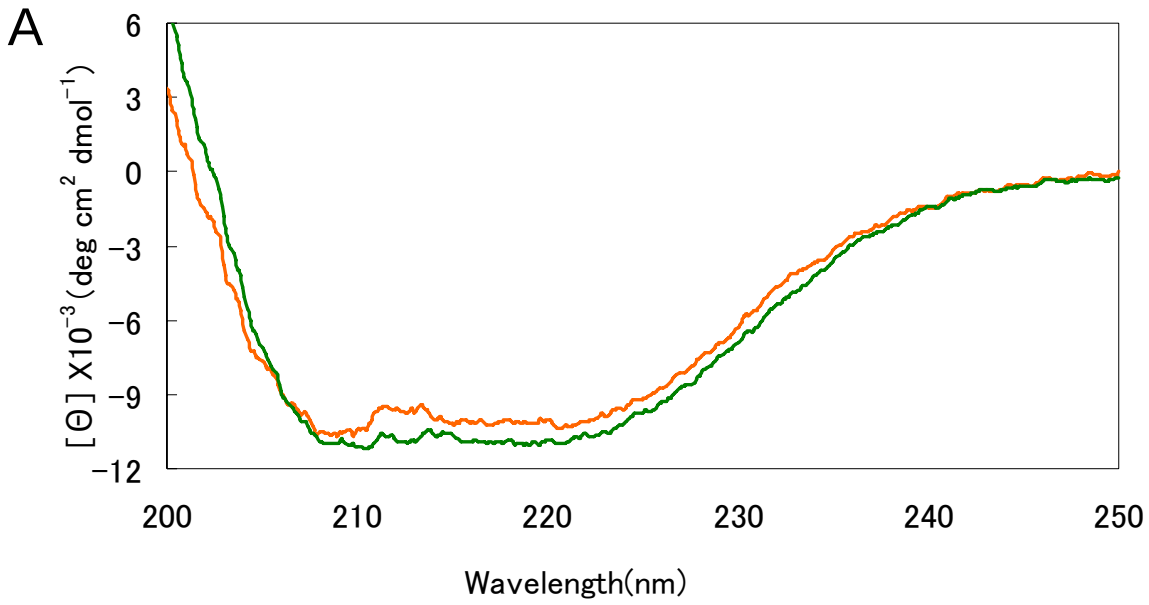


Fig. S2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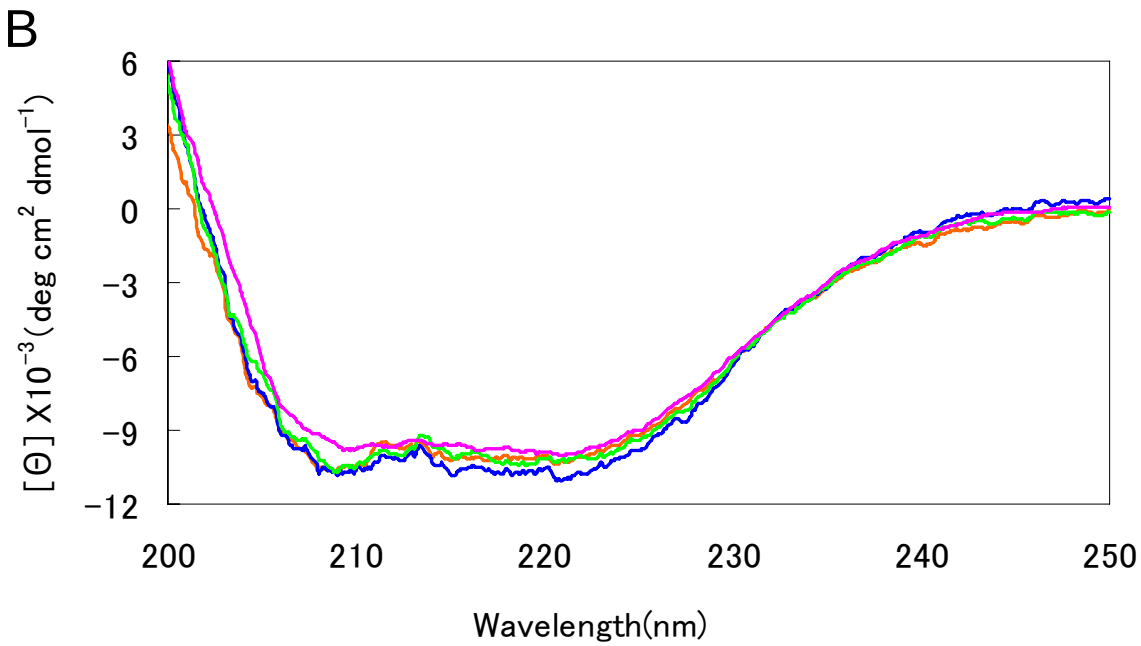
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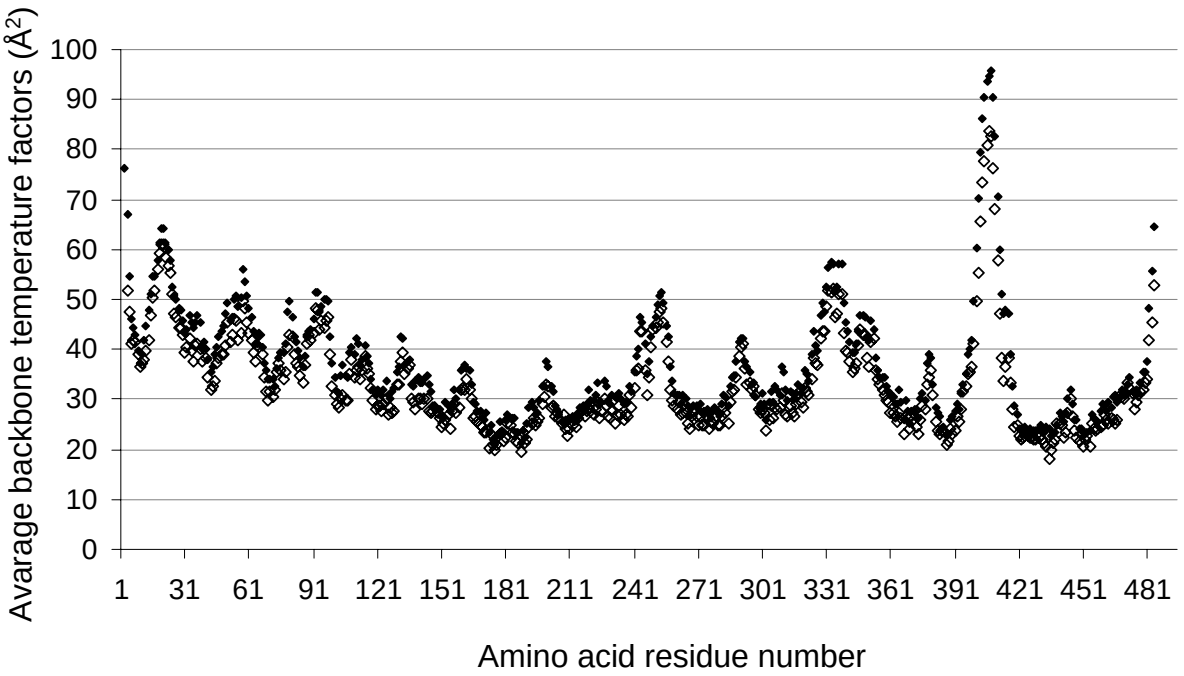
Fig. S3

A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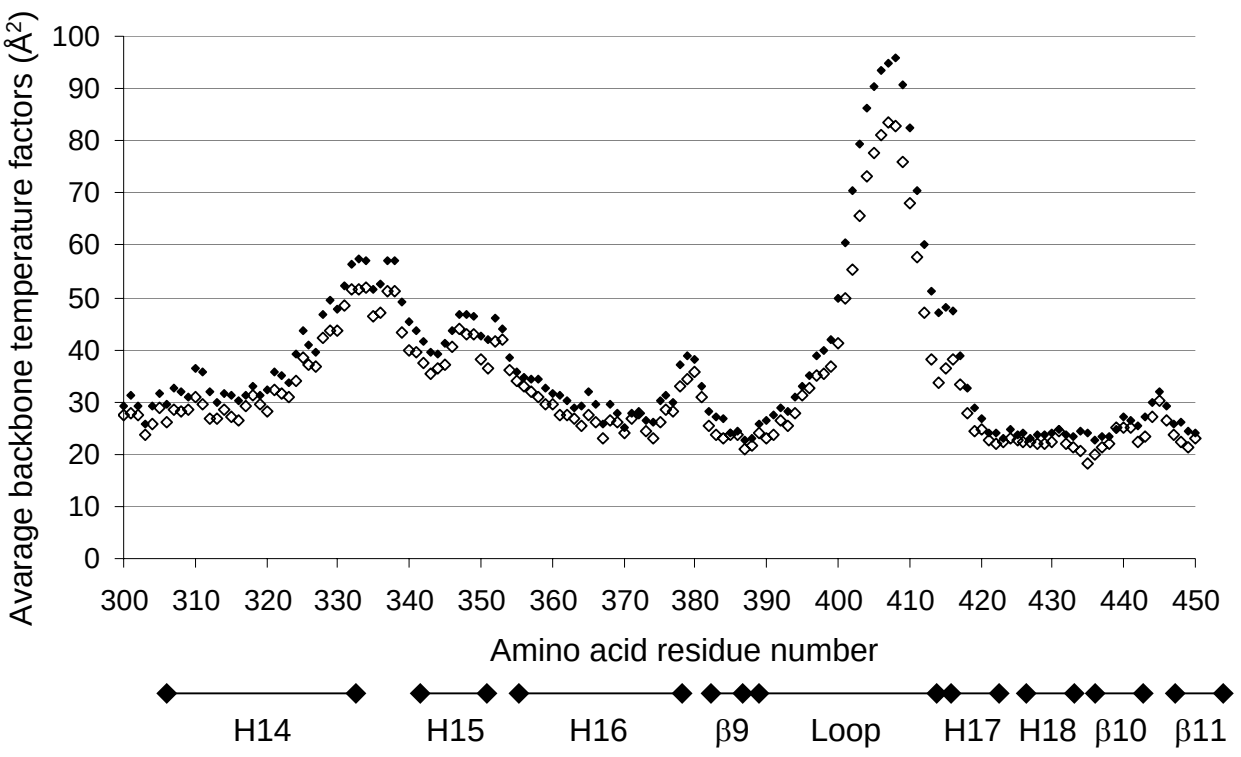
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Amino acid residue number
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H15
H16
β9
Loop
H17
H18
β10
β11

Fig. S4