

Supplemental Information

Structures of apo and product-bound human L-asparaginase:
Insights into the mechanism of autoproteolysis
and substrate hydrolysis

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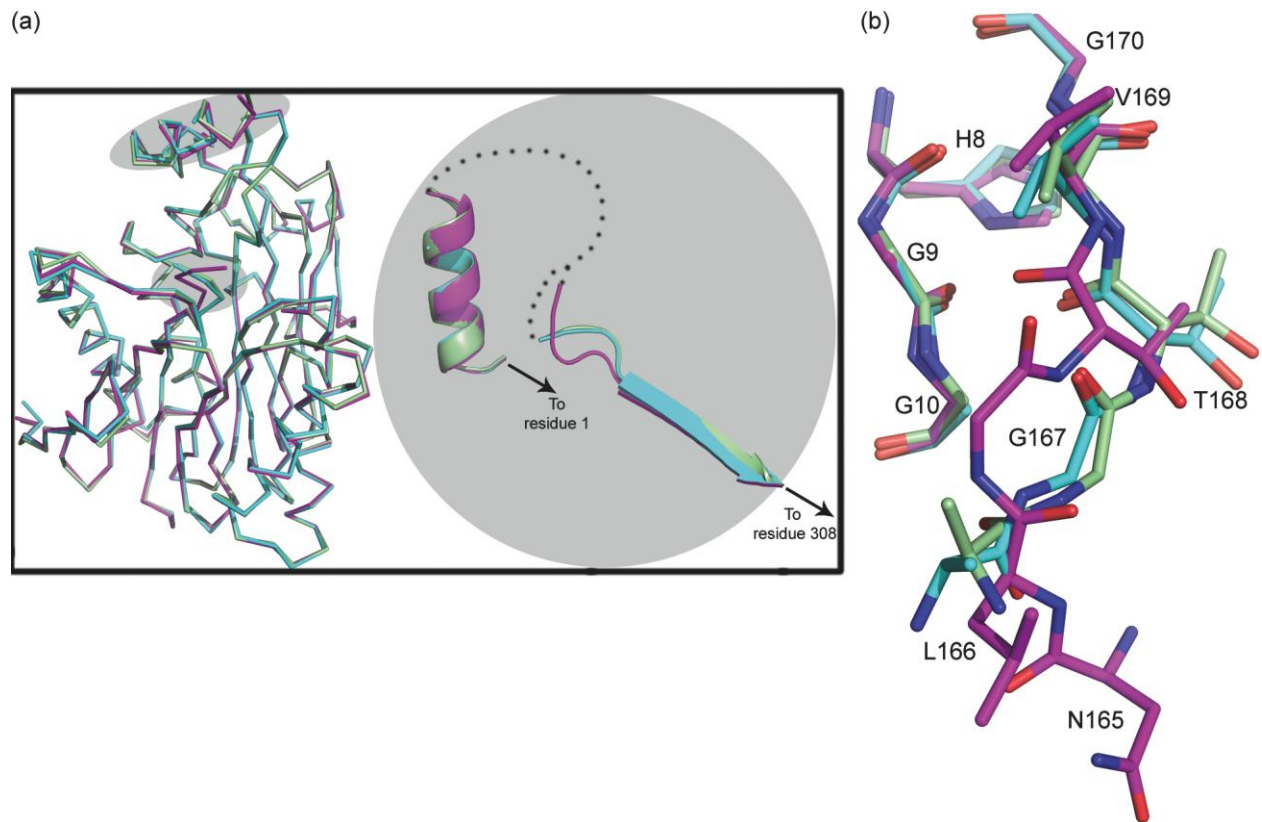


Figure S1. hASNase3 uncleaved structures. The uncleaved state of hASNase3 was observed in both protomers of the sulfate-complex structure (only protA shown, in cyan), and in protAs of the malonate-complex (green) and partially-cleaved ASP-complex (magenta) structures. (a) Overlay of the uncleaved protomers shows an overall identical structure. We could not model beyond the C-terminal helix of the a-chain until 2-3 residues prior to the scissile residue T168. While the secondary structural elements that span this region are identical, the residues preceding V169 occupy slightly differing conformations. This demonstrates the inherent flexibility of this region. (b) Detailed overlay around T168. Note that the carbonyl of G9 points towards the side chain of H8 in these uncleaved structures. G9 flips in the cleaved state.

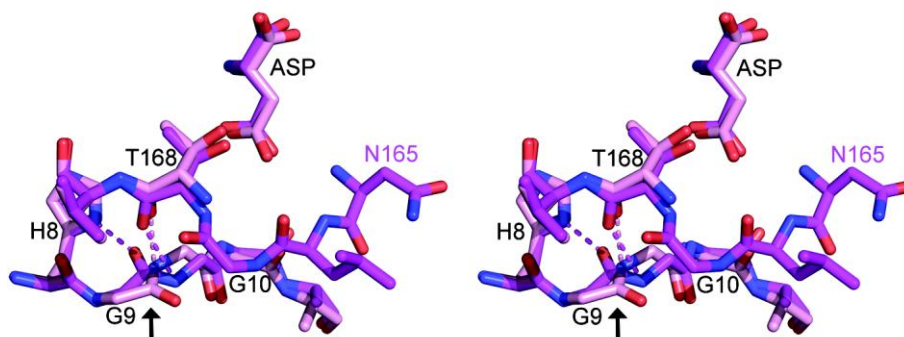


Figure S2. Glycine 9 flips conformation between the cleaved and uncleaved states. Stereo view of the ASP-complex hASNase3 structure, where the uncleaved protA (magenta) is overlaid on the cleaved protB (pink). The Gly9 carbonyl interacts with the His8 side chain in the uncleaved state but is flipped in the cleaved state (black arrow).

Figure S3.

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Q7L266-ASGL1_HUMAN -----MN--PIVVVHGGGAGPIS----- 16
Q4R7U8-ASGL1_MACFA -----MN--PIVVVHGGGAGPIS----- 16
Q32LE5-ASGL1_BOVIN -----MN--PVVVVHGGGASNIS----- 16
Q8C0M9-ASGL1_MOUSE -----MACARGTVAPPVRASIDVS--LVVVVHGGGASNIS----- 33
Q8VI04-ASGL1_RAT -----MATARPSSCGRDSVPATPRASIDVS--LVVVVHGGGASNIS----- 39
Q6GM78-ASGL1_XENLA -----MK--PVIIVHGGGAGK-IV----- 15
Q5BKW9-ASGL1_DANRE -----ML--PVVVVHGGGAGH-IP----- 15
P37595-IAAA_ECOLI -----MG-KAVIAIHGGAGA-ISR----- 17
Q7CQV5-IAAA_SALTY -----MN-KAVIAIHGGAGA-IAR----- 17
Q9ZSD6-ASPG_LUPLU -----MG-GWSIALHGGAGD-IP----- 16
P30364-ASPG_LUPAN -----MG-GWSIALHGGAGD-IP----- 16
P50288-ASPG_LUPAL -----MG-GWSIALHGGAGD-IP----- 16
Q54WW4-ASGX_DICDI -----MKNKSVLVIHGGAGV-ISK----- 18
P50287-ASPGA_ARATH -----MV-GWAIALHGGAGD-IP----- 16
P20933-ASPG_HUMAN -----MAR-KSNLPVLLVPLLCQALVRC--SSPLPLVNTW----- 34
B4NWI1-ASPG1_DROYA -----MKRHLKACLWVLCFASTALSSLD-TTSP-----KPTLASA 35
Q8MR45-ASPG1_DROME -----MRRHLRASLWILCLATMAFSILA AVNTSP-----KPTL TSA 36
B4QHB1-ASPG1_DROSI -----MRTHLRASLWVLCASMAFSILA AVNTSP-----KPTLASA 36
Q9H6P5-TASP1_HUMAN MTMEKGMSSGEGLPSRSSQVSAGKI TAKELETKQSYKEKRGGFVLVHAG-AGYHS----- 54
Q8R1G1-TASP1_MOUSE MIMKGMNSGEGLPSRSSQASAAKVTVKELETQQPCKEKRGGFVLVHAG-AGYHS----- 54
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Q7L266-ASGL1_HUMAN -----KDRKERVHQGMVRAATVGYGILRE--GGS--AVDAVEG 50
Q4R7U8-ASGL1_MACFA -----KDRKERMHQGIVRAATVGYGILRE--GGS--AVDAVEG 50
Q32LE5-ASGL1_BOVIN -----KDRKERVQGILRAATVGYNILKQ--GGS--AVDAVEG 50
Q8C0M9-ASGL1_MOUSE -----ANRKELVREGIARAATEGYKILKA--GGS--AVDAVEG 67
Q8VI04-ASGL1_RAT -----PGRKELVSEGIAKAATEGYNILKA--GGS--AVDAVEG 73
Q6GM78-ASGL1_XENLA -----EELDATYRAGVKRAVLKGYDVL SQ--GGS--ALTAVEE 49
Q5BKW9-ASGL1_DANRE -----KERTEESTIGVKEAARTGYAILQR--GGS--AVDAVVE 49
P37595-IAAA_ECOLI -----AQMSLQQLRYIEALS AIVETGQKMLEA--GES--ALD VVTE 55
Q7CQV5-IAAA_SALTY -----AQMSHEQELRYIQALSEIVESGQKMLEA--GDS--ALD VVTE 55
Q9ZSD6-ASPG_LUPLU -----FSLPPERRKPREEGLRHCLQIGVEALKA--QKP--PLD VVEL 54
P30364-ASPG_LUPAN -----FSLPPERRQPREEGLRHCLQIGVEALKS--QKP--PLD VVEL 54
P50288-ASPG_LUPAL -----FSLPPERRKPREEGLRHCLQIGIEALKA--QNP--PLD VVEL 54
Q54WW4-ASGX_DICDI -----STISKEREEIFLNSLKNILLAGKII LKQ--GGT--SLD VVQE 56
P50287-ASPGA_ARATH -----IDLDPERRIPRESALRHCLDLGISALKS--GKP--PLDVAEL 54
P20933-ASPG_HUMAN -----PFKNATEAAWRALAS--GGS--ALDAVES 59
B4NWI1-ASPG1_DROYA FSGKSKTTAVSTALKANKTASELLPMVINTWNFTAANVLAWRI LKQSKGGLRQTRNAVVE 95
Q8MR45-ASPG1_DROME FSGKAGTTAV---KANKTTGELLPMVINTWNFTAANVLAWRI LKQSKGGLRQTRNAVVE 92
B4QHB1-ASPG1_DROSI FSGKAGT PAV---KANKTTGELLPMVINTWNFTAANVLAWRI LKQSKGGLRQTRNAVVE 92
Q9H6P5-TASP1_HUMAN -----ESKAKEYKHVCKRACQKAIEKLQA--GAL--ATDAVTA 88
Q8R1G1-TASP1_MOUSE -----ESKAKEYKHVCKRACQKAIEKLQA--GAL--ATDAVAA 88
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Q7L266-ASGL1_HUMAN AVVALED-DPEFNAGCGSVLNTNGEVEMDASIMDGKDL SAGAVSAVQCIANPIKLAR--- 106
Q4R7U8-ASGL1_MACFA AVVALED-DPEFNAGCGSVLNTDGEVEMDASIMDGKDL SVGAVSAVRCIANPIKLAR--- 106
Q32LE5-ASGL1_BOVIN AVTVLED-DPDFNAGCGSVLNENGEVEMDASIMNGKDL SAGAVSAVRCIANPIKLAR--- 106
Q8C0M9-ASGL1_MOUSE AVTVLEN-DPEFNAGYGSVLNVNGDIEMDASIMDGKDL SAGAVSAVRCIANPVKLAR--- 123
Q8VI04-ASGL1_RAT AVTMLEN-DPEFNAGYGSVLNADGDIEMDASIMDGKDL SAGAVSAVRCIANPVKLAR--- 129
Q6GM78-ASGL1_XENLA AVIVLED-EQIFNAGHGSVLNEKGDIEMDA IIMDGKNLDSGAVSAIRNIANPIKLAR--- 105
Q5BKW9-ASGL1_DANRE AVALMET-NPRFNAGRGSVLNIKEGEVEMDALVMDGR TLD S GAVSAVRRIANPVQLAR--- 105
P37595-IAAA_ECOLI AVRLL EE-CPLFNAGIGAVFTRDETHELDACVMDGNTL KAGAVAGVSHLRNPVLAAR--- 111
Q7CQV5-IAAA_SALTY AVRLL EA-CPLFNAGIGAVYTRDGTHELDACVMDGNTL KAGAVAGVSHVRHPVLAAR--- 111
Q9ZSD6-ASPG_LUPLU VVRELEN-IEHFNAGIGSVLTNSGTVEMEASIMDGNTM KCGAVSGLSTVLNPI SLAR--- 110
P30364-ASPG_LUPAN VVRELEN-IQHFNAGIGSVLTNSGTVEMEASIMDGNTM KCGAVSGLSTVLNPI SLAR--- 110
P50288-ASPG_LUPAL VVRELEN-IQHFNAGIGSVLTNSGTVEMEASIMDGNTM KCGAVSGLNTVMNPI SLAR--- 110
Q54WW4-ASGX_DICDI AVRLL EE-DPIYNAGKGSVFTELGTNEMDAAIMDGNTL KAGAVGGVSIIRNPI I AAR--- 112
P50287-ASPGA_ARATH VVRELEN-HPDFNAGKGSVLT AQGT VEMEASIMDGKTKRCGAVSGLTTVVNPI SLAR--- 110
P20933-ASPG_HUMAN GCAMCEREQCDGSGVFGGSPDELGETTLDAMIMDGT TMDV GAVGDLRRIKNAIGVAR--- 116
B4NWI1-ASPG1_DROYA GCSKCEKLQCDRTVGYGGSPDELGETTLDAMVMDGATMEV GAVAGLRRIKDAIKVAR--- 152
Q8MR45-ASPG1_DROME GCSKCEKLQCDRTVGYGGSPDELGETTLDAMVMDGATMDV GAVAGLRRIKDAIKVAR--- 149
B4QHB1-ASPG1_DROSI GCSKCEKLQCDRTVGYGGSPDELGETTLDAMVMDGATMDV GAVAGLRRIKDAIKVAR--- 149
Q9H6P5-TASP1_HUMAN ALVELED-SPFTNAGMGSNLNLLGIECDASIMDGKSLNFGAVGALSGIKNPNVSVANRLL 147
Q8R1G1-TASP1_MOUSE ALVELED-SPFTNAGISNLNLLGIECDASIMDGKSLNFGAVGALSGIKNPNVSAHRLL 147
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Q7L266-ASGL1_HUMAN -----LVMEKTPHCFLTDQGAQFAAAMGVPEIPGEKLVTERNKKRLEKEKHEKGAQ- 158
 Q4R7U8-ASGL1_MACFA -----LVMEKTPHCFLTDQGAQFAAAMGVPEIPGEKLVTEKNKKRLEKEKHEKGAQ- 158
 Q32LE5-ASGL1_BOVIN -----LVMDKTPHCFLTDQGAARFAAANGIPTIPGQQLVTERSRRKLEKEKLEKDAQ- 158
 Q8C0M9-ASGL1_MOUSE -----LVMEKTPHCFLTGHGAEKFAEDMGIPQVPVEKLITERTKKHLEKEKLEKGAQ- 175
 Q8VI04-ASGL1_RAT -----LVMEKTPHCFLTGRGAEKFAADMGIPTPAEKLITERTKKHLEKEKLEKGAQ- 181
 Q6GM78-ASGL1_XENLA -----LVMEKTDHMLLTCEGATLFAKAQGIPEVPNESLVTERSRRKRWMMKN-LKENS- 156
 Q5BKW9-ASGL1_DANRE -----LVMEKTKHLCLTAEGASKFARSMGVPEVPEESLITDYAKMRWKKN-LEPDAN- 156
 P37595-IAAA_ECOLI -----LVMEQSPHVMMIGEGAENFAFARGMERVSPEIFSTSLRYEQLLAARKEGATVL 164
 Q7CQV5-IAAA_SALTY -----LVMERSPHVLMVGEAENFAFSQGMARVSPDIFSTPARYEQLLAARAAGEMAL 164
 Q9ZSD6-ASPG_LUPLU -----LVMDKTPHIYLAFOGAQDFAKQQGVETVDSHSLITAENVERLKLAI EANRVQV 163
 P30364-ASPG_LUPAN -----LVMDKTPHIYLAFOGAQDFAKQQGVETVDSHSLITAENVERLKLAI EANRVQV 163
 P50288-ASPG_LUPAL -----QVMDKTPHIFLAFQGAQDLGKQQGVETVDSHSLITEENVERLKLAI EANRVQV 163
 Q54WW4-ASGX_DICDI -----AVMEHTNHCLLVGKGAEFAKSKNLEIVEPSFFFTQNRVYDQLLRAKDEKKLIL 165
 P20933-ASPG_ARATH -----LVMEKTPHIYLAFAEDAEAFARAHGVEVTVDSHSLITPENIARLQAEKFNRVQL 163
 P20933-ASPG_HUMAN -----KVLEHTHTLLVGESATTFQAQSMGFINE DLSTASQALHSDWLARNQCQPNYWR 169
 B4NWI1-ASPG1_DROYA -----HVLEHTQHTMLVGDAASAFANAMGFESLVTPE SKDMWLQWTAENCQPNFWK 205
 Q8MR45-ASPG1_DROME -----HVLEHTQHTMLVGDAASAFANAMGFESLVTPE SKDMWLQWTAENCQPNFWK 202
 B4QHB1-ASPG1_DROSI -----HVLEHTQHTMLVGDAASAFANAMGFESLVTPE SKDMWLQWTAENCQPNFWK 202
 Q9H6P5-TASP1_HUMAN CEGQKGLSAGRI PPCFLVGE GAYRWA VDHGIPSCPPNIMTTRFSLAAFRRNKRKLELAE 207
 Q8R1G1-TASP1_MOUSE CEGQKGLSAGRI PPCFLVGE GAYRWA VDHGIPSCPPSTMTTRFSLAAFRRNKRKLELAE 207

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Q7L266-ASGL1_HUMAN -----KTDCQKN-LGTVGAVALDCKGNVAYATST 186
 Q4R7U8-ASGL1_MACFA -----KTDCQKN- GTVGAVALDFKGNVAYATST 186
 Q32LE5-ASGL1_BOVIN -----KPDQCKN-LGTVGAVALDCQGNLAYATST 186
 Q8C0M9-ASGL1_MOUSE -----NADCPKN-SGTVGAVALDCRGNLAYATST 203
 Q8VI04-ASGL1_RAT -----KADCPKN-SGTVGAVALDCKGNLAYATST 209
 Q6GM78-ASGL1_XENLA -----PVADQIG-LGTVGAVAIDCEGNVACATST 184
 Q5BKW9-ASGL1_DANRE -----PVECQMGKMGTVGAVAVMDGNIACATST 185
 P37595-IAAA_ECOLI DHSG-----APLDEKQKMGTVGAVALDL DGNLAAATST 197
 Q7CQV5-IAAA_SALTY DHSG-----APLDEKQKMGTVGAVALDL DGNLAAATST 197
 Q9ZSD6-ASPG_LUPLU DYSQYNYPEPVKDDA-----EKELPLTNGDSQIGTVGCVAVD SHGNLASATST 211
 P30364-ASPG_LUPAN DYSQYNYPQPAQDDA-----EKELPLANGDSQIGTVGCVAVD SHGNLASATST 211
 P50288-ASPG_LUPAL DYSQYNYTQPVQDDA-----EKELPLANGDSQIGTVGCVAVD SHGNLASATST 211
 Q54WW4-ASGX_DICDI DHGDNLEKEKEKEKNNETSTTTTTISVGVDPIDPKYKMGTVGAVCLDSFGNLAATST 225
 P50287-ASPGA_ARATH DYTVP-----SPKVPDNCGDSQIGTVGCVAVD SAGNLASATST 201
 P20933-ASPG_HUMAN NVIPDPKSKYCGPYKPP----GILKQD-IPHKETEDDRGHDTIGMVIHKTGHIAAGTST 224
 B4NWI1-ASPG1_DROYA NVHPDPKVS CGPYKPRPTPLTRWKED-RARNEYEIGRKNHDTIGMIAIDVESNIHAGTST 264
 Q8MR45-ASPG1_DROME NVHPDPKVS CGPYKPRPTPLTRWKED-RARNEYEIGRKNHDTIGMIAIDVESNIHAGTST 261
 B4QHB1-ASPG1_DROSI NVHPDPKVS CGPYKPRPTPLTRWKED-RARNEYEIGRKNHDTIGMIAIDVESNIHAGTST 261
 Q9H6P5-TASP1_HUMAN RVDTFDFMQLKRRRQS-----SEKENDSGTLDTVGAVVVDHEGNVAAAVSS 252
 Q8R1G1-TASP1_MOUSE RVETDFIQKRRRQS-----SAKENDSGTLDTVGAVVVDHEGNVAAAVSS 252

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Q7L266-ASGL1_HUMAN GGI VNKMGVGRVGDSPCLGAGGYADN-----DIGAVSTTGHGESILKVNLARLTLFHIEQ 240
 Q4R7U8-ASGL1_MACFA GGI VNKMGVGRVGDTPCVGAGGYADN-----DIGAISTTGHGESILKVNLARLTLFHIEQ 240
 Q32LE5-ASGL1_BOVIN GGI VNKMPGRVGDTPCVSGGYADN-----DIGAVSTTGHGESILKVNLARLALFHVEQ 240
 Q8C0M9-ASGL1_MOUSE GGI VNKMGVGRVGDSPCIGAGGYADN-----NLGAVSTTGHGESILKVNLARLALFHVEQ 257
 Q8VI04-ASGL1_RAT GGI VNKMGVGRVGDSPCIGAGGYADN-----NLGAVSTTGHGESILKVNLARLALFHVEQ 263
 Q6GM78-ASGL1_XENLA GGLTNKMGVGRVGDPTACIGSGGYADN-----NVGAVSTTGHGESIMKVILARLILHHMEQ 238
 Q5BKW9-ASGL1_DANRE GGMINKMEGRVGDTPCVGCGGYADN-----KIGAVSPTGHGEAIMKVTL SRLVLFHMEQ 239
 P37595-IAAA_ECOLI GGMTNKLPGRVGDSPVVGAGCYANN-----ASVAVSCTGTGEV FIRALAA YDIAALMDY 251
 Q7CQV5-IAAA_SALTY GGMTNKLPGRVGDSPVVGAGCYANN-----ASVAVSCTGTGEV FIRTLAA YDIAALMEY 251
 Q9ZSD6-ASPG_LUPLU GGLVKNKMGVGRIGDTPPLIGAGTYAN-----ELCAVSATGKGEA IIRATVARDVAALMEF 264
 P30364-ASPG_LUPAN GGLVKNKMGVGRIGDTPPLIGAGTYAN-----ELCAVSATGKGEA IISATVARDVAALMEF 264
 P50288-ASPG_LUPAL GGLVKNKMGVGRIGDTPPLIGAGTYAN-----ELCAVSATGKGEA IIRATVARDVAALMEF 264
 Q54WW4-ASGX_DICDI GGMTNKMHGRVGDTPPIIGAGVYAN-----KNVAVSSTGTGEAFMRTVA AFDIAAMMEY 278
 P50287-ASPGA_ARATH GGYVKNKMGVGRIGDTPVIGAGTYAN-----HLC A ISATGKGEDIIRGTVARDVAALMEY 254
 P20933-ASPG_HUMAN NGIKFKI HGRVGDSPIPGAGAYADD-----TAGAAAATGNGDILMRFLPSYQAVEYMR 278
 B4NWI1-ASPG1_DROYA NGARHKIPGRVGDSPIPGAGAYADN-----EVGAAVATGDGDVMMRFLPSLLAVEAMRA 318
 Q8MR45-ASPG1_DROME NGARHKIPGRVGDSPIPGAGAYADN-----EVGAAVATGDGDVMMRFLPSLLAVEAMRA 315
 B4QHB1-ASPG1_DROSI NGARHKIPGRVGDSPIPGAGAYADN-----EVGAAVATGDGDVMMRFLPSLLAVEAMRA 315
 Q9H6P5-TASP1_HUMAN GGLALKHPRVGRVQAALYGCCGWAENTGAHNPYSTAVSTSGCGEHLVRTILARECSHALQA 312
 Q8R1G1-TASP1_MOUSE GGLALKHPRVGRVQAALYGCCGWAENTGAQNYPYSTAVSTSGCGEHLVRTILARECSHALQA 312

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Q7L266-ASGL1_HUMAN      -GKTVEEAADLSLGYMKS-----RVKGLGGLIVVSKT-----GDWVAKW 278
Q4R7U8-ASGL1_MACFA     -GKTVEEAADLSLGYMKS-----RVKGLGGLIVVSKT-----GDWVAKW 278
Q32LE5-ASGL1_BOVIN     -GKSLLEEANASLGHMKS-----KVKGVGGIIMVKA-----GEWAVKW 278
Q8C0M9-ASGL1_MOUSE     -GKTVEEAQALALDYMKS-----KLKGLGGLILVNKT-----GDWVAKW 295
Q8VI04-ASGL1_RAT       -GKTVDEAATLALDYMKS-----KLKGLGGLILINKT-----GDWVAKW 301
Q6GM78-ASGL1_XENLA     -GKSPPEEAADAGLNYMKS-----RVGGIGGVIIVNSS-----GDWTAKF 276
Q5BKW9-ASGL1_DANRE     -GKTPEEASDLALAYMKE-----RVDGLGGVVVVVDHN-----GTWAARF 277
P37595-IAAA_ECOLI      -GGLSLAEACERVVMEKLP-----ALGGSGGLIADHE-----GNVALPF 290
Q7CQV5-IAAA_SALTY      GGLSLADACERVVMEKLP-----ALGGSGGLIADHE-----GNVALPF 290
Q9ZSD6-ASPG_LUPLU      KGLSLKEAADFVIHER-----TPKGTVGLIAVSAA-----GEIAMPF 301
P30364-ASPG_LUPAN      KGLSLKEAADYVVER-----TPKGTVGLIAVSAA-----GEIAMPF 301
P50288-ASPG_LUPAL      KGLSLKEAADCVVHER-----TPKGTVGLIAVSAA-----GEIAMPF 301
G54WW4-ASGX_DICDI      GGLSLKDASNKVVMEKLI-----TVG-DGGVICVDKY-----GNVEMPF 316
P50287-ASPGA_ARATH     KGLSLTEAAAYVVVQSS-----VPRGSCGLVAVSAN-----GEVTPMF 291
P20933-ASPG_HUMAN      -GEDPTIACQKVISRIQK---HFPEFFGAVICANVTG-----SYGAACNKL 320
B4NWI1-ASPG1_DROYA     -GKPPADAAEESLRRIIR---HHKDFMGALIAVDRLG-----RYGAACYGL 360
Q8MR45-ASPG1_DROME     -GKPPAEAAQEGRLRILK---HHKDFMGALIAVDRLG-----NYGAACYGL 357
B4QHB1-ASPG1_DROSI     -GKPPAEAAQGGRLRILK---HHKDFMGALIAVDRLG-----NYGAACYGL 357
Q9H6P5-TASP1_HUMAN     EDHQALLETMQNKFISSPFLASEDGVLGIVLVRSCRCSAEPDSSQNKQTLLEFLWSH 372
Q8R1G1-TASP1_MOUSE     EDHQALLETMQNKFISSPFLACEDGVLGIVLVRSCRCSSSESQDKQTLLEFLWSH 372
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Q7L266-ASGL1_HUMAN      TSTSMPWAAK--DGKLFHFGIDPDDTTITDLP----- 308
Q4R7U8-ASGL1_MACFA     TSTSMPWAAK--DGKLFHFGIDPDDTAITDLP----- 308
Q32LE5-ASGL1_BOVIN     TSTSMPWAAK--DGKLFHSGIDFGDTSIIDLS----- 308
Q8C0M9-ASGL1_MOUSE     TSASMPWAAVK--NGKLQAGIDLCESTRTRDLPC----- 326
Q8VI04-ASGL1_RAT       TSASMPWAAVK--NGKLQAGIDLCESTRTRNLPTC----- 333
Q6GM78-ASGL1_XENLA     STNQMSWAAVK--DDQLHIGIYHGENNVTPEKAL----- 309
Q5BKW9-ASGL1_DANRE     SSLQMSWAAQ--QGKLFHFLHFGDHFTEPVEEHT----- 310
P37595-IAAA_ECOLI      NTEGMYRAWGYA-GDTPTTGIYREKGDVTATQ----- 321
Q7CQV5-IAAA_SALTY      NSEGMYRAWGYA-GDTPTTGIYRE----- 313
Q9ZSD6-ASPG_LUPLU      NTTGMFRACATE-DGYSEIAIWPTT----- 325
P30364-ASPG_LUPAN      NTTGMFRASATE-DGYSEIAIWPTT----- 325
P50288-ASPG_LUPAL      NTTGMFRACATE-DGYSEIAIWPTA----- 325
Q54WW4-ASGX_DICDI      NTEGMYRGYVII-DNNCENDQNDIINVSIIK----- 346
P50287-ASPGA_ARATH     NTTGMFRACASE-DGYSEIAIWPNN----- 315
P20933-ASPG_HUMAN      STFTQFSFMVYNSE-KNQPT-EEKVDCI----- 346
B4NWI1-ASPG1_DROYA     D---EFPFMVSSPAGRDGPTRLETVKCIAGQDKVNIVSF----- 396
Q8MR45-ASPG1_DROME     A---EFPFMVSSPAGADGPTRLETVKCIAGQDKVNIVAL----- 393
B4QHB1-ASPG1_DROSI     E---EFPFMVSSPAGADRPTRLETVKCIAGQDKVNIVAL----- 393
Q9H6P5-TASP1_HUMAN     TTESMCVGYMSAQDGKAKTHISRLPPGAVAGQSVAIEGGVCRLESPVN 420
Q8R1G1-TASP1_MOUSE     TTESMCVGYMSAQDGKAKTHISRLPPGAVAGQSVAIEGGVCRLESPVN 420

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Figure S3. Comprehensive sequence alignment of Ntn-enzymes most similar to hASNase3. Asparaginases from different organisms are aligned using the Clustal W program. N-terminal residues in a black bold font correspond to signal peptides. Strictly conserved residues as well as H8-G10 (hASNase3 numbering) are shaded in gray. Cyan color indicates residues that bind the substrate. The cleavage site between G167 (yellow) and T168 (highlighted in red) is indicated by an arrowhead. In green are the glycines orienting the N62 side chain. See text for details.

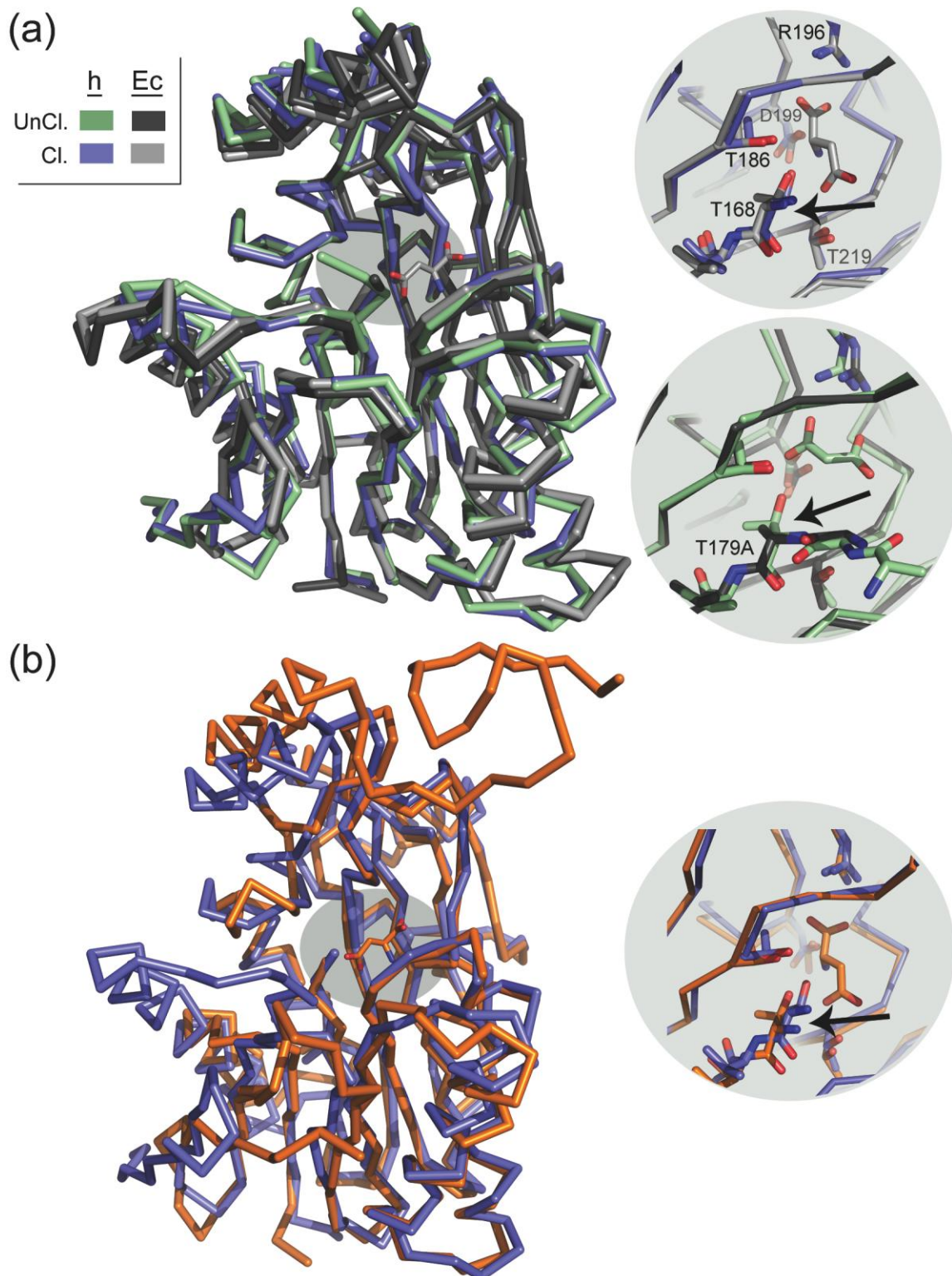


Figure S4. Structure alignment of hASNase3 with hAGA and *E. coli* Type III asparaginase. *E. coli* Type III (pdb codes: 2ZAK, 2ZAL, panel (a)) and hAGA (pdb code: 1APZ, panel (b)) are superimposed on the hASNase3 substrate-free structure. (a) Left panel: α -trace representation of hASNase3 uncleaved protA (green) / cleaved protB (blue) and *E. coli* Type III

uncleaved (dark gray) / cleaved (light gray). Right zoom panels focus on the active site. Top right zoom: hASNase3 and *E. coli* Type III cleaved structures. Bottom right zoom: hASNase3 and *E. coli* Type III uncleaved structures. Conserved active side residues are shown as sticks, with hASNase3 numbering. A black arrow indicates the scissile residue. The *E. coli* Type III T179A mutation, required for obtaining the uncleaved structure, is labeled. (b) Left panel: C α -trace representation of hASNase3 cleaved in protB (blue), and hAGA cleaved (orange). Right zoom panel highlights the active site region.

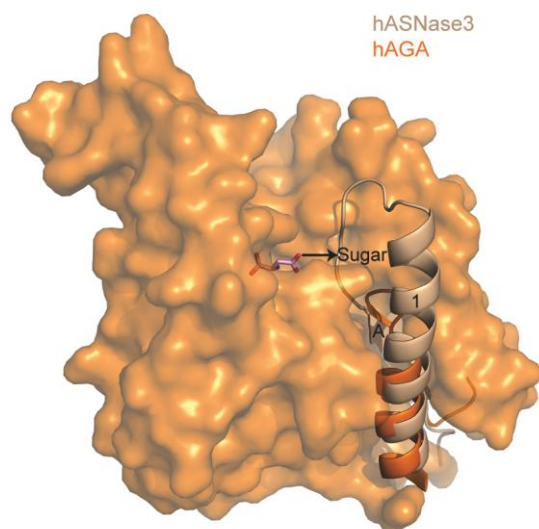


Figure S5. Rationalizing the different substrate specificities of hASNase3 and hAGA.

hAGA surface (pdb code: 1APZ; shown is a single protomer comprising the α - and β -chains) is represented in transparency and its bound ASP in sticks. hASNase3 and hAGA were overlaid, and the region encompassing β -strand A to α -helix 1 (see labels) from both structures are shown as a ribbon representation (hAGA in orange, hASNase3 in tan). The linker between these secondary structural elements is 16 residues longer in hASNase3 compared to hAGA. The consequence of the longer linker in hASNase3 is a block in accessibility to substrate-linked oligosaccharides. See also Fig. 2 for the sequence alignment that shows the specific residues of the linkers.