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Supporting information for article:

**Crystal structure of a β -aminopeptidase from an Australian
Burkholderia sp.**

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Nyssa Drinkwater and Sheena McGowan**

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BcA5-bapA -----MRTRDLGIRIGLGTGPRFNAITDVPGVVVG 30
BapF -----MRARQLGITLGLGTGPFNAITDVPGVVVG 30
Ps_BapA -----MRIRELGITIGSGIPGVFNAITDVPGVVVG 30
DmpA -----MTSQTPTRKPRARDLGLPF-TGVTGPYNAITDVDGVVVG 38
3-2W4 MTSTQRLWSGALPLLTALIVSIAATASLAGPRARDLGVPF-EGTPGALNAITDVAGVEVG 59
PH0078 -----MKAQELGIKIGVFKPGKRNKITDVKGVKVG 30
          : : : * : : * * * * * * * *

BcA5-bapA HCTLNEEN-----GDASIRTVTVIEPRAGAAHDSPCFAGVHVLNNGDATGLEWIREAG 85
BapF HSTLNQRI-----DGRQVRTGVTLVQPAGAAARLQPCFAGCHVLNNGDATGLEWIREAG 85
Ps_BapA HHTLVNVE-----GDVSIHSGVTVIEPRAGATHLQPCFAGVHVLNNGDATGLEWIRER 85
DmpA FQTLIENEPR-PGRKRPARSGVTAI LPHMQSETPVVYAGVHRFNGNGEMTGTHWIEDGG 97
3-2W4 HTTVISGDGAMVIGKGPYRTGVTIIHPL-GKTSLDGVAAGRAVINGTGEWTMHLLVDEVG 118
PH0078 HVTLIKGGKGLIPGKGPVRTGVTAILPHEGNIYKEKVLGAFVMNGYSKPVGLIQLWELG 90
          . * : : : * : * * * * * * * * * *

BcA5-bapA LLTTPAIYTNTHSVGAVRDALVAN-----ER-AAAGRVYWCMPVVMTYDGLLNDIWGQH 140
BapF LLTTPAIITNTHSVGAVRDALIAE-----ERAELGDSGLYWCMPVVMTFDGLLNDIWGQH 141
Ps_BapA LLTSPIAYTNTHSVGVVRDALVAA-----ER-EMGKQHTYWCMPVVLTYDGLTNDIWGQH 140
DmpA YFLGPVVTINTHIGIMAHATVRWMVDRYASTYQTDDFLWIMPVVATYDGALNDINGFP 157
3-2W4 QFLGPIALTGTGNVGLVHQSMMDWSVGVKPE---EALFSRLLPVVATLDNNRLNDVFGHG 175
PH0078 TIETPIILNTLSIGTAVEGLLDYILEENED--IGVTTGSVNPLVLECNDSYLNDRGRH 148
          : * : * . * . : * . . . : * * * * * * . * * * * *

BcA5-bapA VSAAHVQRALAAQTGPVAEGGVGGGTGMICHEFKGGIGTASRVLAADAGGWTVGALVQA 200
BapF VGARQVGEALACAESGPVREGSVGGGTGMICHEFKGGIGSASRRLPAEQGGWTVGALVQA 201
Ps_BapA VTAEHVQLALQDARSQVQEGNVGGGTGMICHEFKGGIGTSSRVLGPEQGGWTVGLVQA 200
DmpA VTEADVRLKALDNVAGSPVQEGNCGGGTGMITYGFKGGTGTASRVVEFGGRSFTIGALVQA 217
3-2W4 LTRDHVFAALDGAAGKGPVAEGNVGGGTGMIAYTFKGGIGTSSRVSAGDTRYTVGLVQA 235
PH0078 VKREHVVEAIKRAD-EDFEEGAVGAGTGMSAFEFKGGIGSASRIVEIEGKKYTVGALVLS 207
          : . * * : . . * * * . * * * * * * * * * * * * * * * *

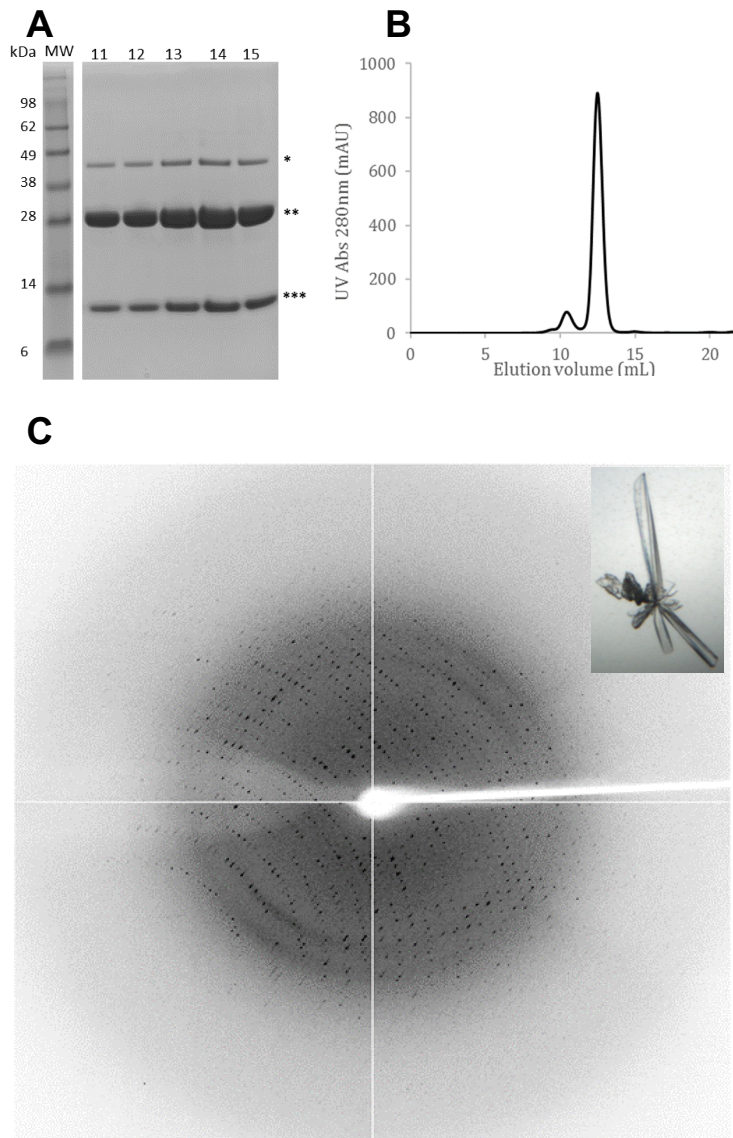
BcA5-bapA NYGVREMLRVAGYPVGEVLRHVSPFR-----APDAQGEAGMGIVVVTIATDAPLLPHQC 255
BapF NHGQRRELVDGYPVGRRLGDISPFS-----E---EGTPGMGSIVVILATDAPLLPHQC 253
Ps_BapA NYGVREALRVGGYPVGTVLGDVSPFK-----SEKKVGVPGMGSIVITATDAPLLPHQC 255
DmpA NHGQRDWLTIAGVPVQHMRDGTPQ-----SQLQERGSIIVVLATDPLMPHQL 266
3-2W4 NHGDRNDLRIAGVQIGKEIKGAWPEVNGIVAAGPDAGKPQDKNSLIVIATDAPLMPHQL 295
PH0078 NFGRRDLTIAGVPVGLLKNWPG-----RGGEGKGSIIMIATDAPLTGRQL 255
          * . * * * : * : * : * * * * * * * * * * * * * * * *

BcA5-bapA TRLAQRASVGLARVGGGTEDSGIFLAFATGNDGLPAANYGSKGAPT-TGVKMVNNDHI 314
BapF QRLAQRASIGIARTGGGTEDSGVFLAFATGNQDLPPADYARKDLPQSTPLRMLNNDHI 313
Ps_BapA TRLAQRASVGLARVGGGTEDSGDIFIAFVAGNSNLPAANFGHPGEPT-TALKMVNDYI 314
DmpA KRLARRASIGIGRNGTPGGNSGIFIAFSTANQRPMQ---HRSAPF-LDVEMVNDEPL 321
3-2W4 ERMARRAALGVGRNGSTAGALSGFALAFSTSHVIPLG---KPRLPA--IINDTSETM 349
PH0078 NRVAKRAIVGLARTGGYAYNGSGIAVAFSTANRIKHY-----EKEVIEIKALPDSVI 308
          * : * * * * : * : * * * * * * * * * * * * * * * *

BcA5-bapA SALFVAAAEAVEEAIVNALVAGGDVESR---GARVEGLQARLLDALREVGWRPGRGA 369
BapF SPLFAAAAEAVEEAIVNVLLAGEDMRTED--GRLVPALKGERLLAALRETGWPGR--- 366
Ps_BapA SPLFVAAADAVEEAILNAMLGADLTGC---GNTVLALKPERLLAALQVGWKAP--- 366
DmpA DTVYLAAVDSVEEAVNAMIAAEDMGTPFDRLLVQAIDHERLRAVLRQYGRLA---- 375
3-2W4 NALFRGVVQATEEALVNQLVASETMTGAN--NAKVYGIPHDQLARIMKARF--PRR-- 401
PH0078 SPLFKATAEAVEEAIINSLLEARTMDGRD--NHVRYALPKEELLRIMRRYGRLEE--- 361
          . : : . . . . . * * * * * * * * * * * * * * * *

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Supplementary Figure S1: Multiple sequence alignment of characterised β -aminopeptidases. The protein name is shown in the left column, amino acid sequence alignment and then amino acid number on the right. The *Burkholderia* sp. BcA5-bapA is in red text. Protein names as follows BapF, *P. aeruginosa* PAO1; Ps BapA *P. aeruginosa* sp. MCI3434; DmpA *O. anthropi*; 3-2W4 BapA *S. xenopeptidilytica* and PH0078 *P. horikoshii*. Residues identified to be important for catalysis in bold and highlighted in green. The nucleophilic serine is further underlined. The 15-20 residues that are important in the formation of the substrate-binding pocket are highlighted in yellow. The multiple sequence alignment was generated using Clustal Omega (McWilliam *et al.*, 2013; Sievers *et al.*, 2011).



Supplementary Figure S2. Purification and crystallisation of BcA5-BapA. (A) SDS-PAGE analysis of purified recombinant BcA5 BapA. Samples from fractions off size-exclusion chromatography are indicated at the top of the main panel. The uncleaved, α - and β -sub-units of BcA5-bapA are indicated by *, ** and *** respectively. Molecular weight markers are indicated on the left-hand panel. (B) Size exclusion chromatogram of BcA5-BapA. (C) X-ray diffraction image from BcA5-BapA crystal. A photo of the BcA5 BapA crystal is inset.