

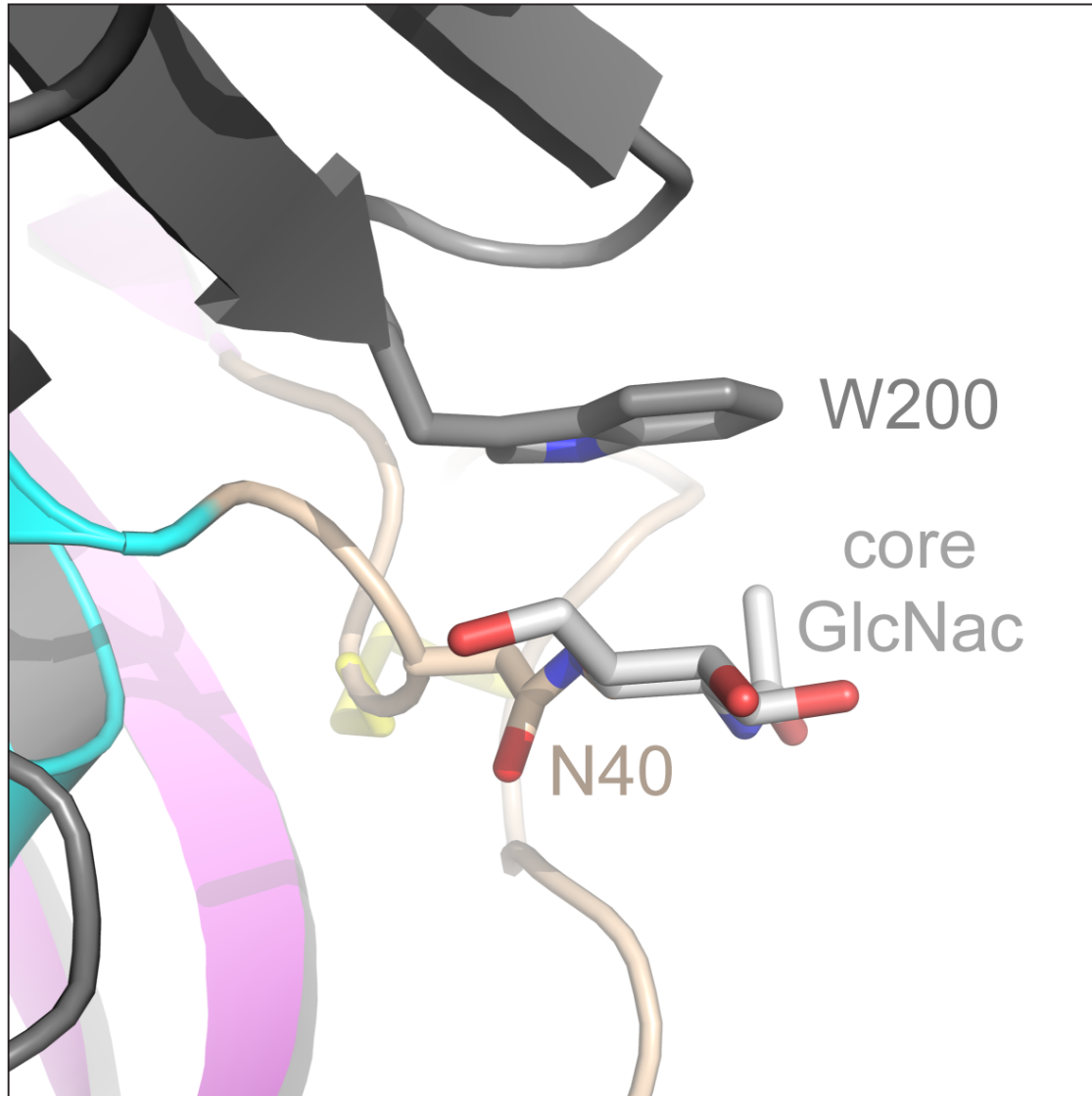
# IUCrJ

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**Supporting information for article:**

**Structure of mammalian plasma fetuin-B and its mechanism of selective metallopeptidase inhibition**

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**Supplementary Figure S1 — Crystal packing detail of unbound human fetuin-B.** Examination of the crystal packing of intact mouse fetuin-B from HEK293S cells explains why it crystallized in its unbound form, whereas the material produced in insect cells did not: endoglycosidase H-processing of the high-mannose N-glycans of the HEK293S-derived protein (a step performed during purification) left a single N-acetylglucosamine moiety (GlcNac) attached to N<sup>40</sup> that stacks against W<sup>200</sup> of a symmetry-related molecule. This introduces a crucial crystal packing contact that would most likely be hindered by the presence of additional sugar residues attached to the core N-acetylglucosamine, such as those normally found in proteins expressed in insect cells.



hFA1	YRQPN	CDDPETE	-E--AALVAIDYINQNL	PW-GYKH-TLNQIDEVKVWPQQPSGE-LFE-IEIDTLET	
hFA2	PLLAPLNDTRVV-H--AAKAALAAFNAQNNGS--NF-QLEEISRAQLVPL-PP-S-TYVEFTVSGTD-				
mFB1	34	LHPLG	CNDSEVL	-A--VAGFALQINIRDQKD-GYML-SLNRVHDVREHYQEDMGS-LFY-LTLDVLET	94
mFB2	158	PSPIDLSNPSAL-E--AATESLAKFNSKSPSK--KY-ELVKVTKAMN--QWVSGP-AYY-VEYLIKEA			215
hKN1	SEEID	CNDKDLF	-K--AVDAALKKYNSQNQS-NNQFVLYRITATEK-----TVGSDTFYSFKYEIKEG		
hKN2	VHPISTQSPDLEPILRHGIQYFNNNTQHSSLFMLNEVKRAQR-----QVVAGL-NFR-ITYSIVQT				
hKN3	PRDIPTNSPELEETLHTITIKLNAENNATFYFKIDNVKKARV-----QVVAGK-KYF-IDFVARET				
hCYC	PMDAS--VEE-EGVRRALDFAVGEYNKASN-DMYHS-RALQVVRARK--QIVAGV-NYFLDVELGRT-				
hCYB	PSATQPATAETQ-H--IADQVRSQLEEKEN---KKFPVFKAVS-FKS--QVVAGT-NYFIKVHVGDED				

hairpin 1



mFA1	T	CHVLDPTPVA-R	CSVRQLKEHAVEGD	CDFQLLKLDGK-----FSVVYAK--CDSS		
hFA2	-	CVAKEATEAA-K	CNLLAEKQ---YGF	CKATLSEKLG-----AEVAVT---CMVF		
mFB1	95	D	CHVLSRKAQK-D	CKPRMFYE-SVYGQ	CKAMFHINKPR-----RVLYLPAYN	143
mFB2	216	P	CTKSQA-----S	CSLQHSDS-EPVGI	CQGSTVQSSLRHVPLIQPVEKSVTVT-CEFF	266
hKN1	D	CPVQSGKTWQ-D	CEYKDAAK--ATGE	CTATVGKRSST-----KFSVATQT-CQIT		
hKN2	N	CSKENFLFLTPD	CKSLWNGD---TGE	CTDNAYIDIQL-----RIASFSQL-CDIY		
hKN3	T	CSKESNEELTES	CETKKLQ---SLD	CNAEVYVVPWE-----KKIYPTVN-CQPL		
hCYC	T	CTKTQPNLD--N	CPFHDQPHLKRKAF	CSFQIYAVPWQ-----GTMTLSKST	CQDA	
hCYB	FVHLRVFQSLPHENKPL	TLSN-----YQTNKAKHD-----ELTYF-----				

hairpin 2

**Supplementary Figure S2 — Sequence alignment of cystatin-like domains.** Alignment of the sequences of cystatin-like modules spanning from the first residue of the first  $\beta$ -strand to the last residue of the last strand, i.e. without N- or C-terminal extensions. hFA1, hFA2: domains 1 and 2 from human fetuin-A (UP P02765); mFA1, mFA2: domains from mouse fetuin-B (UP Q9QXC1); hKN1, hKN2, hKN3: domains from human kininogen-1 (UP P01042); hCYC: human cystatin-C (UP P01034); and hCYB: human cystatin-B *alias* stefin B (UP P04080). The residue numbers correspond to the sequence of mouse fetuin-B. Secondary-structure elements above the alignment are for the CY2 domain of mouse fetuin-B.

## CY1



## CY2



**Supplemental Figure S3 — Amino-acid sequence alignment of selected vertebrate fetuins.** FB\_mouse: fetuin-B from *Mus musculus* (UP Q9QXC1); FB-duck: *Anas platyrhynchos* (UP U3IFE7); DB\_anole: *Anolis carolinensis* (UP H9GD35); FB\_human: *Homo sapiens* (UP Q9UGM5); FB\_chimp: *Pan troglodytes* (UP A0A2J8M5X2); FB\_bat: *Pteropus alecto* (UP L5JPD4); FB\_elephant: *Loxodonta africana* (UP G3SVW6); FB\_cow: *Bos taurus* (UP Q58D62); FB\_whale: *Physeter catodon* (UP A0A2Y9F7I5); FB\_hhog: *Erinaceus europaeus* (UP A0A1S2ZIC8); FB\_dog: *Canis familiaris* (UP E2R9B6); FB\_molerat: *Heterocephalus glaber* (UP G5BT88); FB\_horse: *Equus caballus* (UP F6RRV1); FB\_shark: *Callorhynchus milii* (UP V9KTH3); FB\_fish: *Danio rerio* (UP E7FE90); FA\_mouse: fetuin-A from *Mus musculus* (UP P29699); FA\_coe: *Bos taurus* (UP P12763); and FA\_human: *Homo sapiens sapiens* (UP P02765). Residue numbers and secondary structure elements correspond to mouse fetuin-B (UP Q9QXC1), see also Fig. 1A. Cysteine residues are in red, the first one (C<sup>39</sup> in mouse fetuin-B) is linked to a cysteine in the CTR (C<sup>374</sup>). LNK indicates the localization of the CPDC-trunk in the linker between CY1 and CY2. The hairpin-loops of each domain are indicated.