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**Supporting information for article:**

**Three dimensional structure and ligand-binding site of carp fishellectin (FEL)**

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**Table S1** Secondary structure assignments.

<b>Blade number</b>	<b>Element of secondary structure</b>	<b>Start residue</b>	<b>End residue</b>	<b>Number of residues</b>	<b>Sequence</b>
1	Strand A	12	16	5	QIDAG
1	Strand B	19	23	5	SVVGV
1	Strand C	28	33	6	ETFVLI
1	Strand D	36	43	8	VFTKISGS
2	Strand A	46	50	5	HFSVG
2	Strand B	53	57	5	GQLGV
2	Strand C	62	67	6	NIFKYQ
2	Strand D	70	77	8	GFVQLAGL
3	Strand A	80	82	3	QVD
3	Strand B	89	92	4	IAGV
3	Strand C	98	101	4	IYCL
3	Helix	103	106	4	MDAN
3	Strand D	117	119	3	VQL
4	Strand A	125	128	4	YYSC
4	Strand B	133	136	4	CWGV
4	Strand C	141	145	5	QIFIM
4	Strand D	160	165	6	INPGL
5	Strand A	168	171	4	MIEV
5	Strand B	177	180	4	VFGV
5	Strand C	186	190	5	LYQRT
5	Strand D	201	205	5	DWISM
6	Strand A	212	218	7	HKHVSFD
6	Strand B	221	226	6	VLWLVC
6	Strand C	231	235	5	IRKCI
6	Strand D	2	6	5	DCTVI

**Table S2** Main contacts between the Ca<sup>2+</sup> ion and cFEL residues and water molecules in the crystals of the apoprotein.

<b>FEL residues</b>	<b>Atom</b>	<b>Distance (Å)</b>
<b>Asp 14 (A)</b>	<b>CG</b>	<b>2.89</b>
<b>Asp 14 (A)</b>	<b>OD1</b>	<b>2.51</b>
<b>Asp 14 (A)</b>	<b>OD2</b>	<b>2.54</b>
<b>Asp 82 (A)</b>	<b>CG</b>	<b>3.42</b>
<b>Asp 82 (A)</b>	<b>OD1</b>	<b>2.39</b>
<b>Asp 82 (A)</b>	<b>OD2</b>	<b>3.75</b>
<b>Glu 170 (A)</b>	<b>CD</b>	<b>3.36</b>
<b>Glu 170 (A)</b>	<b>OE1</b>	<b>3.86</b>
<b>Glu 170 (A)</b>	<b>OE2</b>	<b>2.38</b>
<b>Asp 14 (B)</b>	<b>CG</b>	<b>2.89</b>
<b>Asp 14 (B)</b>	<b>OD1</b>	<b>2.51</b>
<b>Asp 14 (B)</b>	<b>OD2</b>	<b>2.53</b>
<b>Asp 82 (B)</b>	<b>CG</b>	<b>3.39</b>
<b>Asp 82 (B)</b>	<b>OD1</b>	<b>2.35</b>
<b>Asp 82 (B)</b>	<b>OD2</b>	<b>3.75</b>
<b>Glu 170 (B)</b>	<b>CD</b>	<b>3.36</b>
<b>Glu 170 (B)</b>	<b>OE1</b>	<b>2.37</b>
<b>Glu 170 (B)</b>	<b>OE2</b>	<b>3.83</b>
<b>Solvent molecules</b>	<b>-</b>	<b>-</b>
<b>Water # 1 (A)</b>	<b>1W(O)</b>	<b>2.35</b>
<b>Water # 2 (A)</b>	<b>2W(O)</b>	<b>2.39</b>
<b>Water # 3 (A)</b>	<b>3W (O)</b>	<b>2.36</b>
<b>Water # 1 (B)</b>	<b>6W(O)</b>	<b>2.37</b>
<b>Water # 2 (B)</b>	<b>7W(O)</b>	<b>2.42</b>
<b>Water # 3 (B)</b>	<b>8W (O)</b>	<b>2.36</b>

**Table S3** Significant contacts between the two protomers of carp FEL in the dimer in the orthorhombic crystals.

<b>Amino acid residue Monomer A</b>	<b>Atom</b>	<b>Distance (Å)</b>	<b>Amino acid residue Monomer B</b>	<b>Atom</b>
Gly 18	O	3.20	Ser 68	N
Ser 19	OG	3.24	Gln 54	NE2
Leu 32	O	2.80	Tyr 66	OH
Asp 34	N	3.02	Tyr 66	OH
Asp 34	OD2	3.22	Ser 41	OG
Asp 34	OD2	2.83	Ser 41	N
Asp 34	N	3.30	Gly 69	O
Asn 35	N	2.98	Gly 69	O
Ser 41	N	2.85	Asp 34	OD2
Ser 41	OG	3.16	Asp 34	OD2
Gln 54	NE2	3.34	Ser 19	OG
Tyr 66	OH	2.86	Leu 32	O
Tyr 66	OH	2.99	Asp 34	N
Ser 68	N	3.16	Gly 18	O
Ser 68	O	3.02	Lys 233	NZ
Gly 69	O	3.20	Asp 34	N
Gly 69	O	2.91	Asn 35	N
Asp 86	OD1	2.43	Asp 86	OD1
Asp 86	OD2	2.99	Gln 87	NE2
Gln 87	NE2	3.02	Asp 86	OD1
Asp 104	OD1	2.89	Arg 194	NH2
Asp 104	OD2	2.89	Arg 194	NE
Trp 109	O	3.29	Gly 220	N
Arg 194	NH1	2.80	Asp 104	OD2
Arg 194	NH2	3.26	Asp 104	OD1
Gly 220	N	3.45	Trp 109	O
Lys 233	NZ	3.46	Ser 68	O

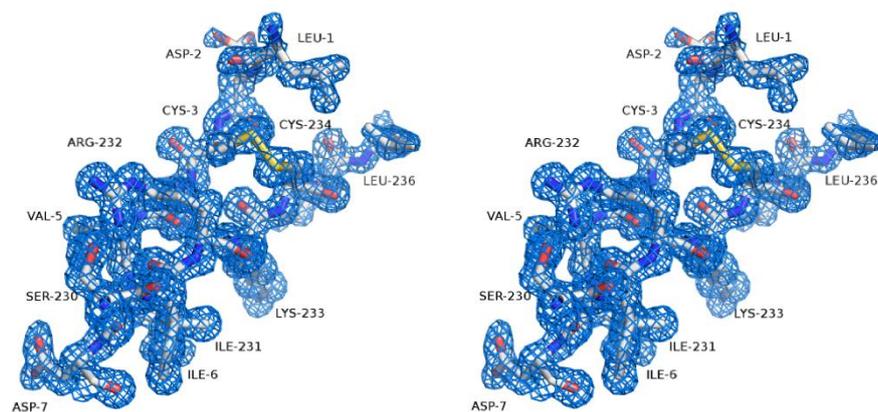
**Table S4** Ligand occupancy of the binding sites.

Site	$\alpha$ -A	$\beta$ -A	$\alpha$ -B	$\beta$ -B	$\alpha$ -C	$\beta$ -C	$\alpha$ -D	$\beta$ -D	$\alpha$ -E	$\beta$ -E	$\alpha$ -F	$\beta$ -F
Ligand	1PE	NDG	NDG	NDG	NAG	NAG	1PE	NDG	1PE	NAG	NDG	NDG
Number of water molecules in the binding site	-	2	2	1	3	1	-	1	-	1	3	2

**NAG = N-acetyl- $\beta$ -D-glucosamine, 2-(acetilamino)-2-deoxy-beta-D-glucopyranose**

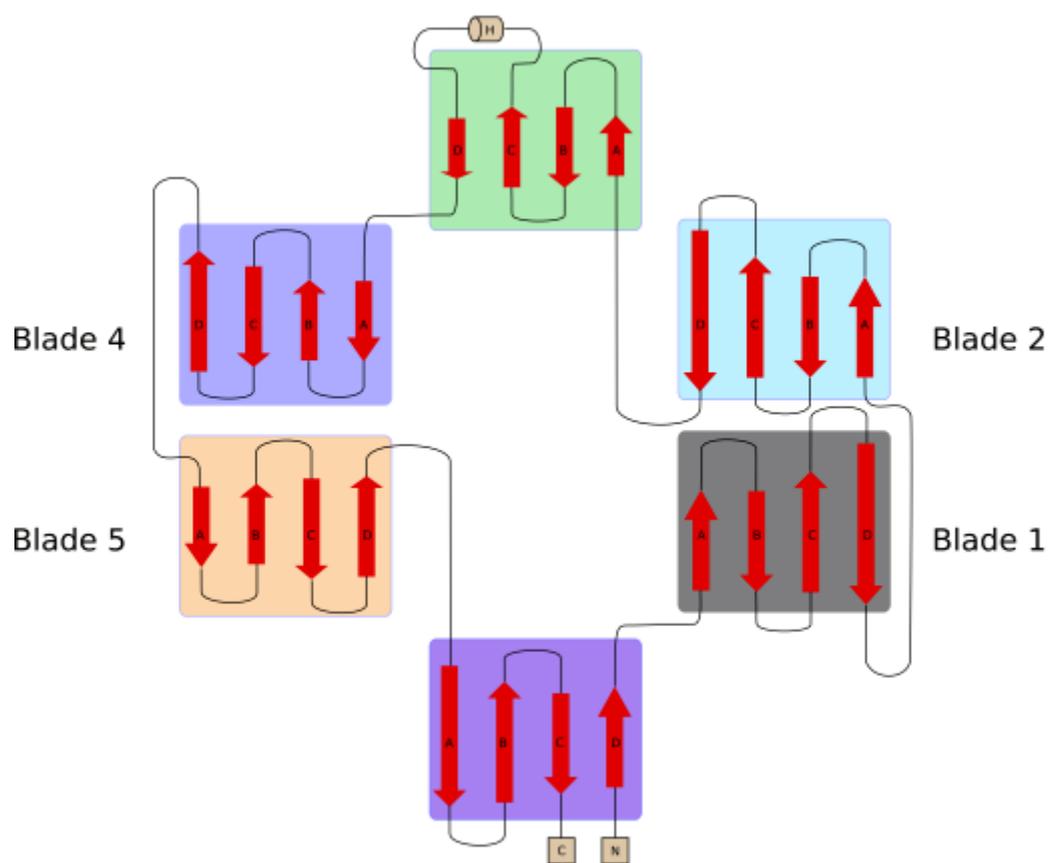
**NDG = N-acetyl- $\alpha$ -D-glucosamine, 2-(acetilamino)-2-deoxy-alpha-D-glucopyranose**

**1PE = PEG, Polyethylene glycol**



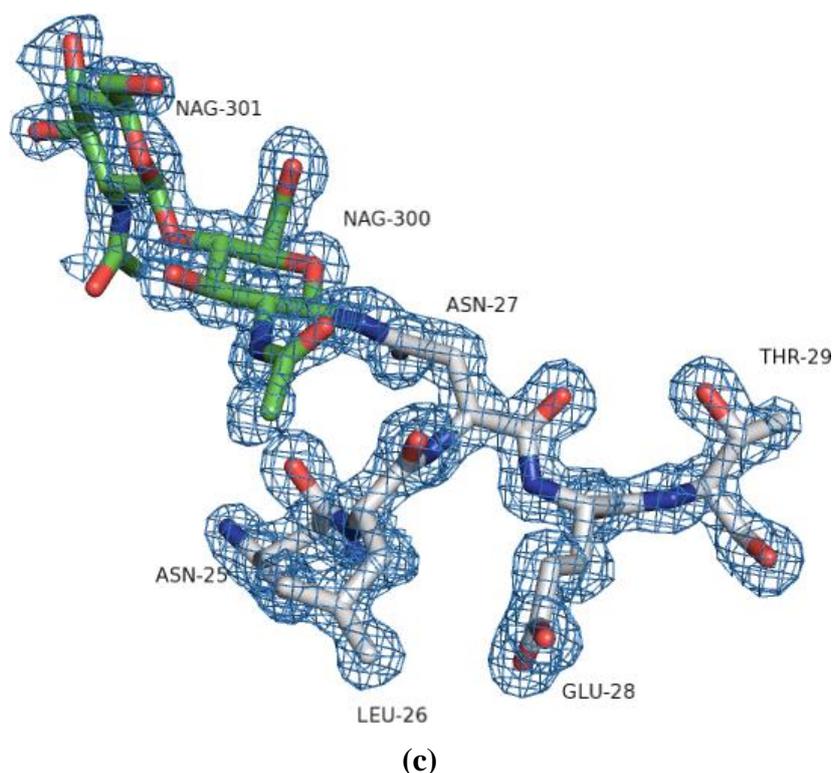
(a)

Blade 3

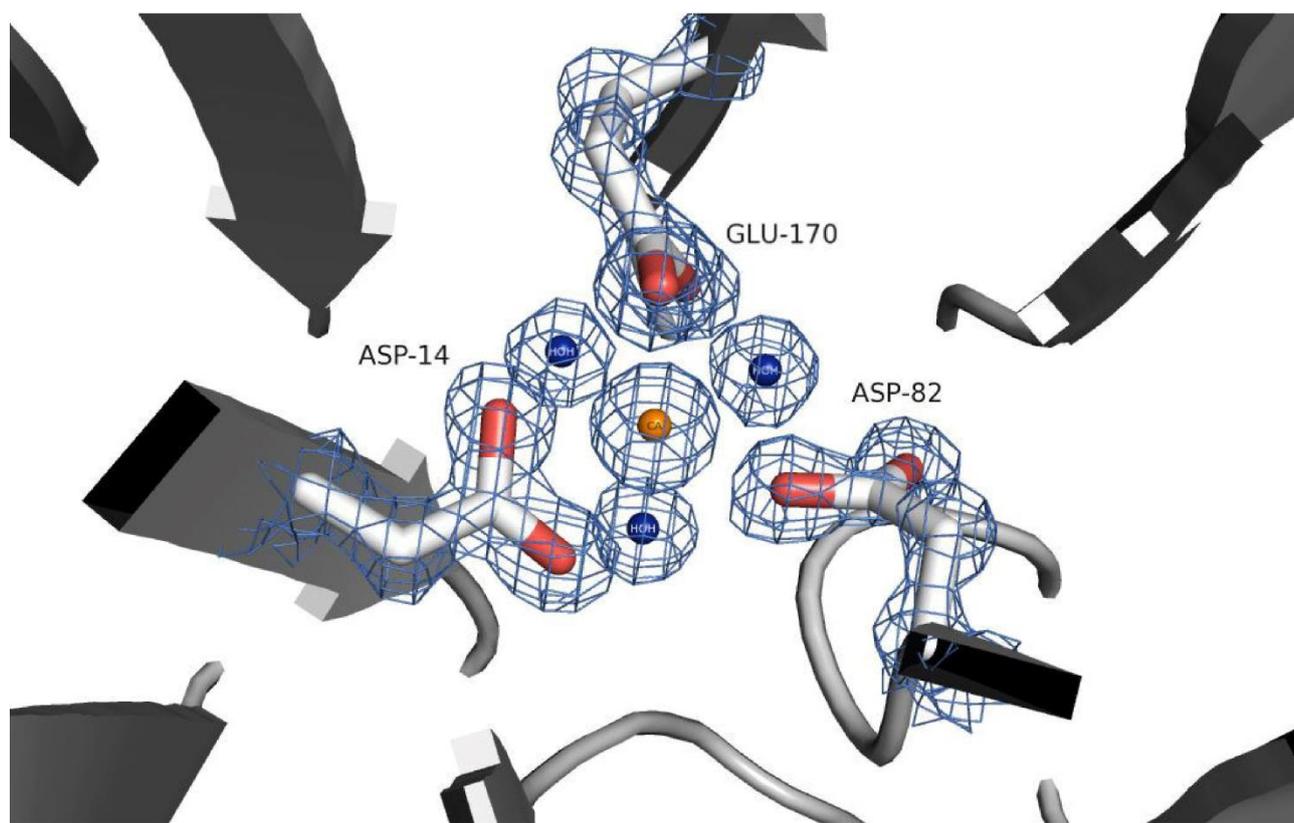


Blade 6

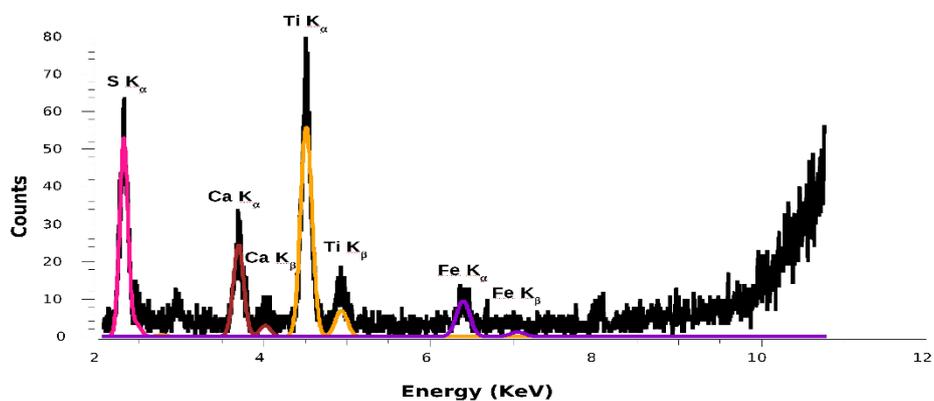
(b)



**Figure S1** (a) Stereo diagram of a  $2F_{obs} - F_{calc}$  map of a representative part of the crystal structure showing one of the four disulphide bridges present in the molecule, Cys 3- Cys 234, linking the N and C terminal ends of the protein molecule. The  $1.0 \sigma$  level electron density is represented blue and the bonds are colour coded according to their atom type. (b) Topology diagram in which the twenty four strands of beta sheet are identified with the letters A, B, C and D starting from the N terminus in each of the six blades that are highlighted with different colours. Note the position of the short helix linking strands C and D in the third blade. (c) Electron density of the polypeptide chain spanning residues Asn 25 –Thr 29 showing the carbohydrate bound to Asn 27. The  $2F_{obs} - F_{calc}$  map was contoured at a  $1.0 \sigma$  level.

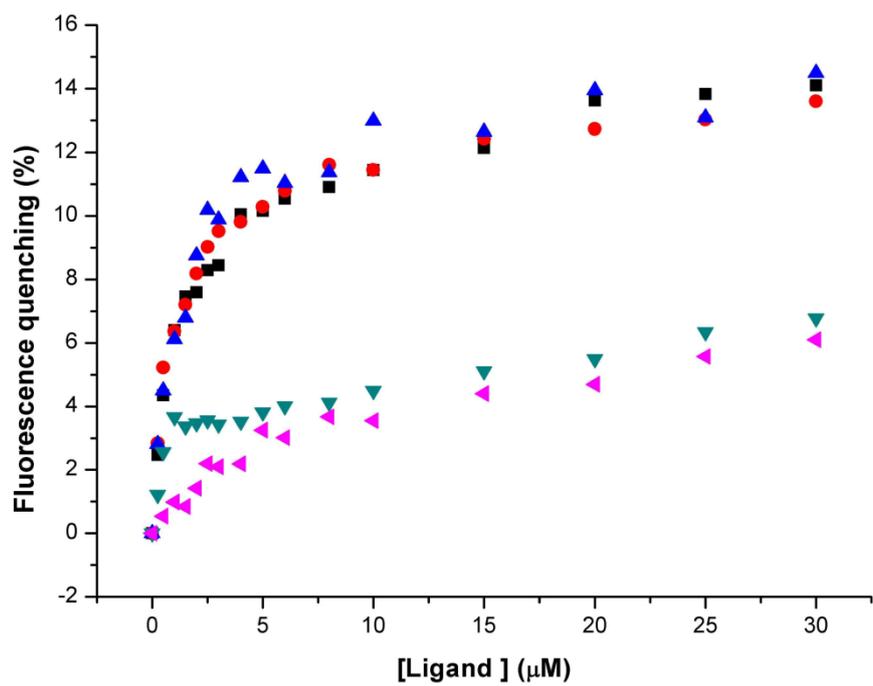


(a)

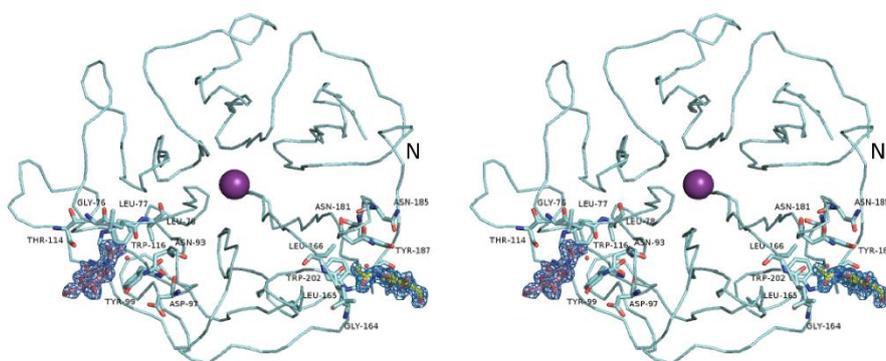


(b)

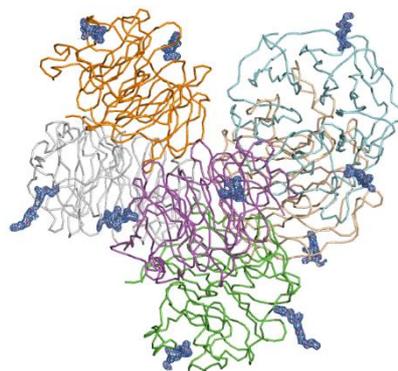
**Figure S2** (a) Electron density of the metal ion and the nearby amino acids. The six-fold axis is approximately perpendicular to the plane of the figure. The Ca<sup>2+</sup> ion is orange and the solvent molecules are blue. The 2F<sub>obs</sub> – F<sub>calc</sub> map was contoured at a 1.0  $\sigma$  level. (b) X-ray fluorescence emission spectrum collected from a crystal of apo carp FEL irradiated with monochromatic synchrotron radiation (12.7 keV).



(a)

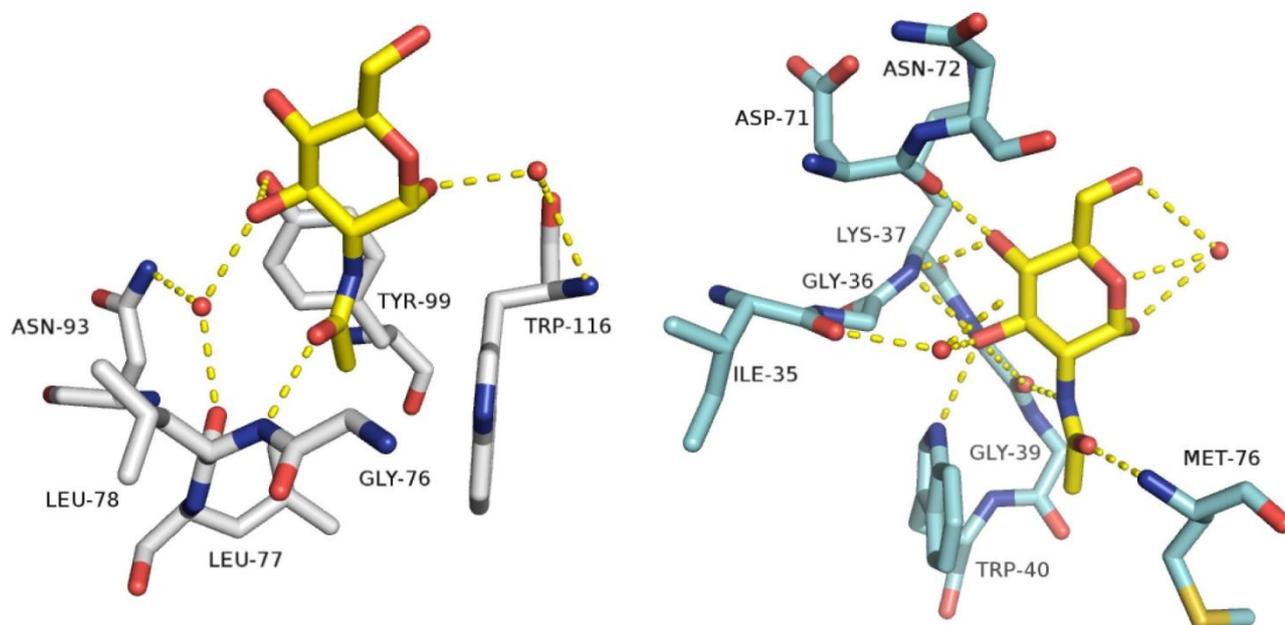


(b)



(c)

**Figure S3** (a) Fluorometric titration of carp FEL with different carbohydrates. Only the raw data are shown and correspond to black squares, N-acetyl glucosamine; blue triangles, N-acetyl galactosamine; red circles, N-N-diacetylchitobiose; cyan triangles, glucose and magenta triangles, mannose. The experimental conditions were as those described for Figure 4 (B). (b) Stereo line drawing with the C $\alpha$  chain trace of the C protomer of the monoclinic crystal form showing the relative position of the two ligand-binding sites and the main residues involved in the interaction with N-acetyl- $\beta$ -D-glucosamine. The magenta sphere in the center of the central channel is the calcium ion. (c) Electron density of the ligands bound to the six protomers present in the asymmetric unit of the co-crystals. The 2F<sub>obs</sub> - F<sub>calc</sub> map was contoured at a 1.0  $\sigma$  level.



**Figure S4** Details of ligand binding ( $\alpha$ GlcNAc) to cFEL (left) and Tachylectin 2 (right). The dotted lines join atoms at relevant short distances and solvent molecules are represented as red spheres.

