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Supporting information for article:

Structural analysis of ibuprofen binding to human adipocyte fatty-acid binding protein (FABP4)

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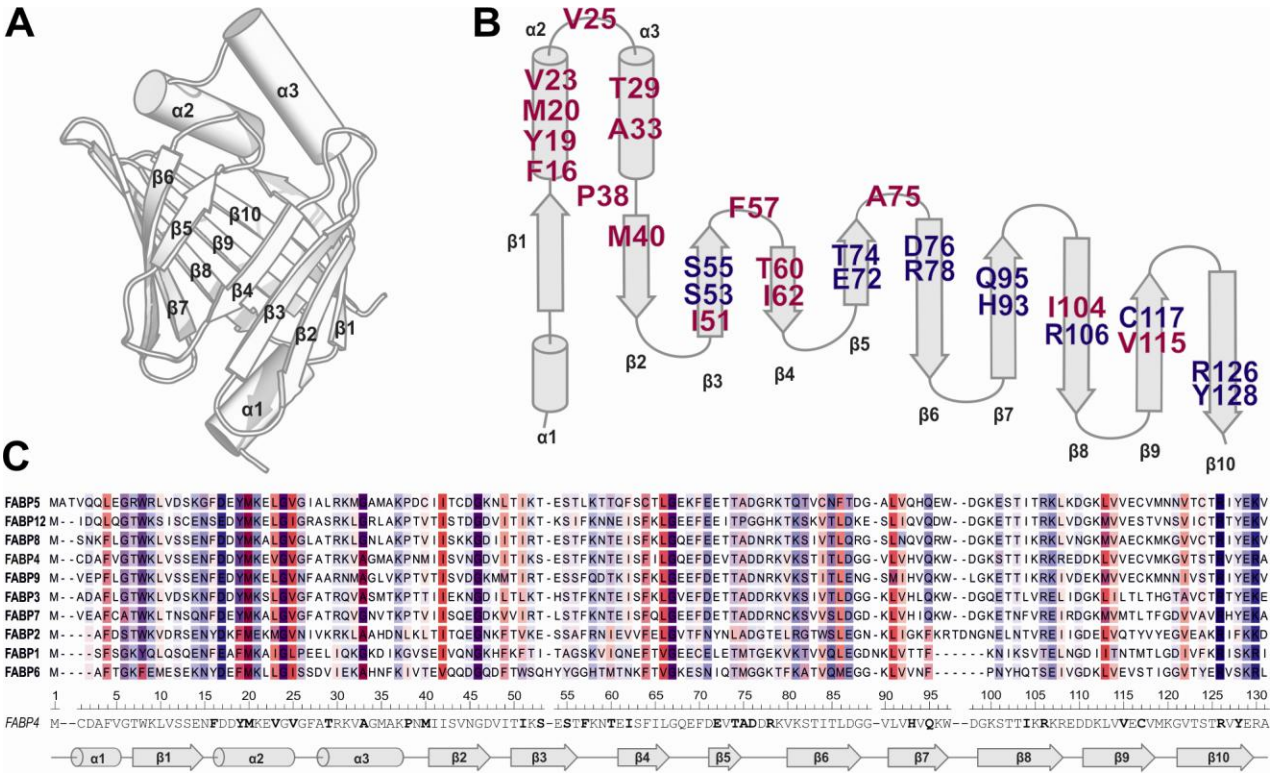

Figure S1 Distribution of amino acid residues lining the FABP4 ligand-binding cavity. Three-dimensional fold (**A**), and topology (**B**), depicting the localization of amino acid residues facing the protein ligand-binding cavity. Ligands L2 to L6 interact primarily with FABP4 hydrophobic residues located in helices α2 and α3, and sheets β2 and β3. (**C**) Structure-guided sequence alignment comparing FABP4 with the other human FABP isoforms (UniProt entries Q6FGL7, P12104, Q6IBD7, E7DVW4, Q01469, P51161, O15540, P02689, Q0Z7S8, and A6NFH5, for FABPs 1 to 9, and 12, respectively). Amino acid numbering for FABP4 (*lower scale*) is provided for comparison. Coloring from *blue* to *red* indicate increasing hydrophobicity, whereas color shading intensity is proportional to the degree of conservation of each amino acid position in the alignment (30 % conservation threshold).

Ligand	K_L (M)	$-\Delta G_B$ (kcal/mol)	Volume (Å^3)
L4	1.48×10^{-4}	5.22	161.2
L3	7.30×10^{-5}	5.64	170.2
L2	7.37×10^{-7}	8.36	186.7
L5	1.33×10^{-7}	9.37	211.2
L6	9.50×10^{-8}	9.57	211.2

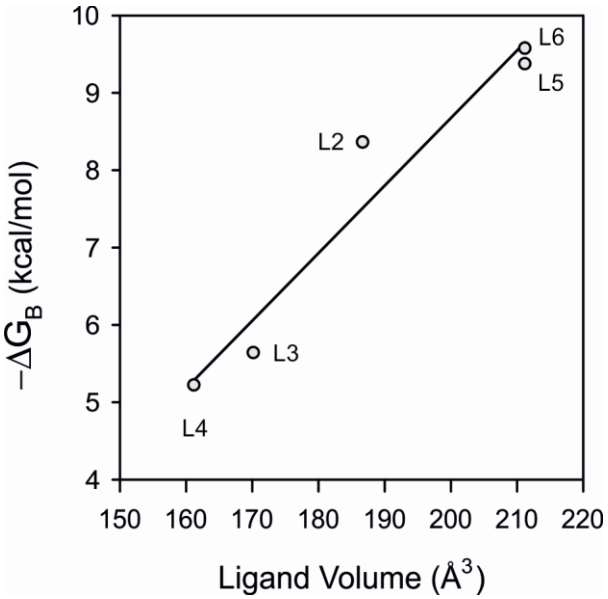

Figure S2 Correlation of binding free energies with ligand size. Free energy of binding is calculated as $\Delta G_B = RT \ln(K_L)$, where $R = 1.987 \times 10^{-3}$ kcal K^{-1} mol^{-1} (gas constant), T = 298 K (temperature), and K_L is the dissociation constant for each ligand. Ligand volumes were calculated with MIPC (Molinspiration Cheminformatics).

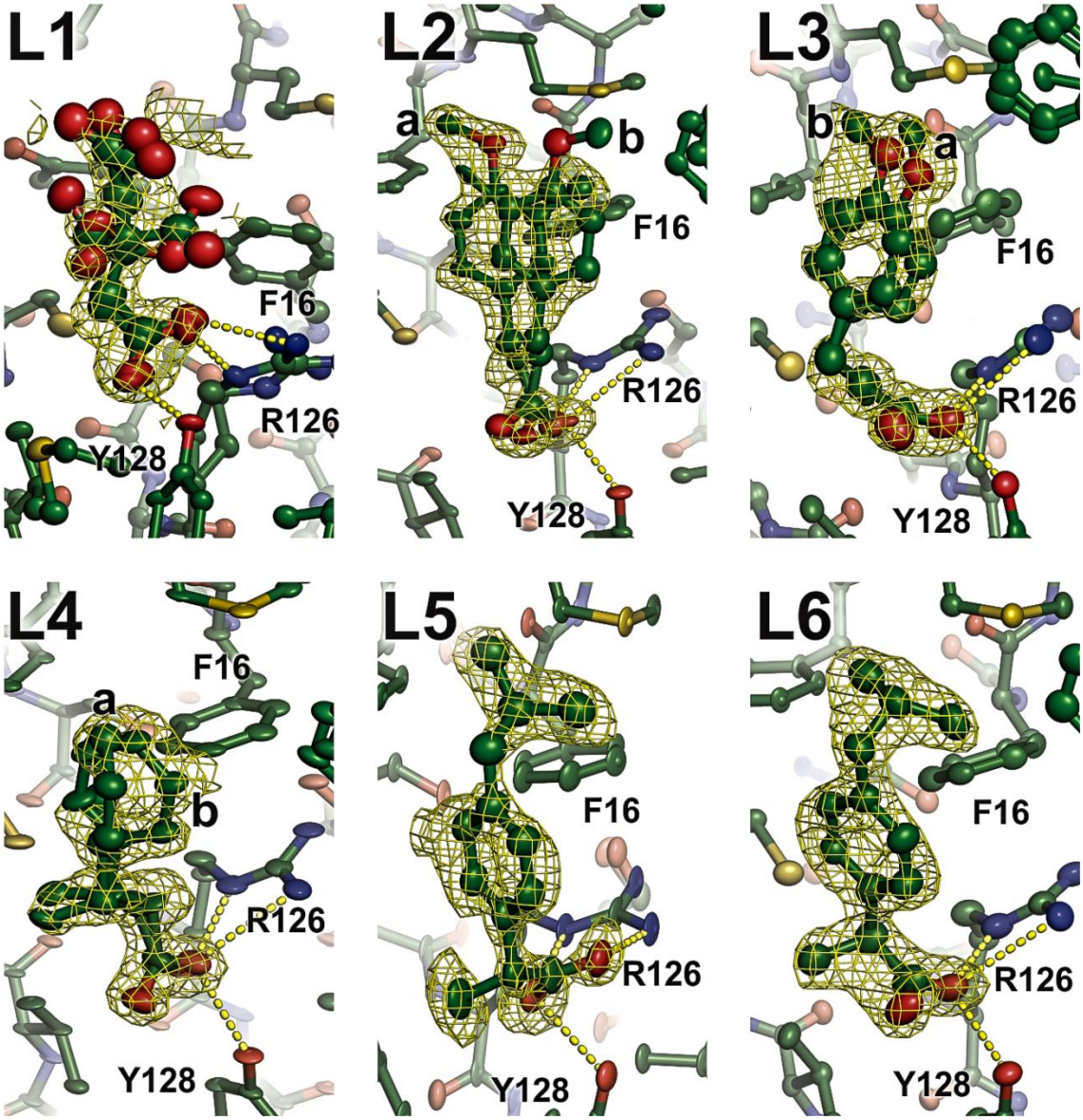

Figure S3 Electron density distribution around ligands L1 to L6. Each panel corresponds to the indicated ligand, depicted as a ball-stick representation (*green*). Wireframe surfaces (*yellow*) indicate electron density contoured at 1 σ. Ellipsoids indicate 25 % probability anisotropic B factors. Water molecules have been omitted for clarity. Alternate conformers are indicated by lowercase letters. Residues R126 and Y128 are involved in the main electrostatic interactions (*yellow dotted lines*), whereas residue F16 interacts with ligands L2-L6 through edge-to-face aromatic interactions.

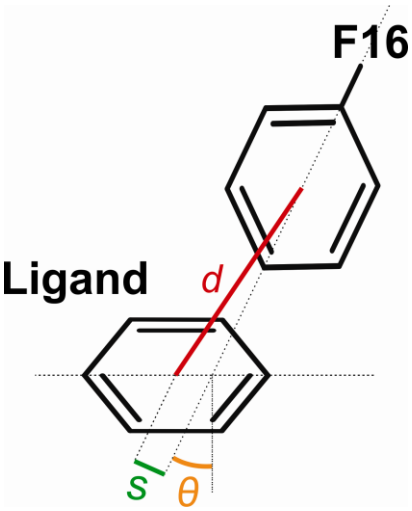

Figure S4 Schematic representation of parameters describing edge-to-face aromatic interactions. Parameters are: *d* (*red*, distance between aromatic centers of ligand and F16 sidechain), *s* (*green*, shift of the ligand aromatic ring with respect to the ideal direction of interaction), and θ (*orange*, angle of interaction, measured as the angle between the ideal direction of interaction, and a direction perpendicular to the plane of the aromatic ring in the ligand).

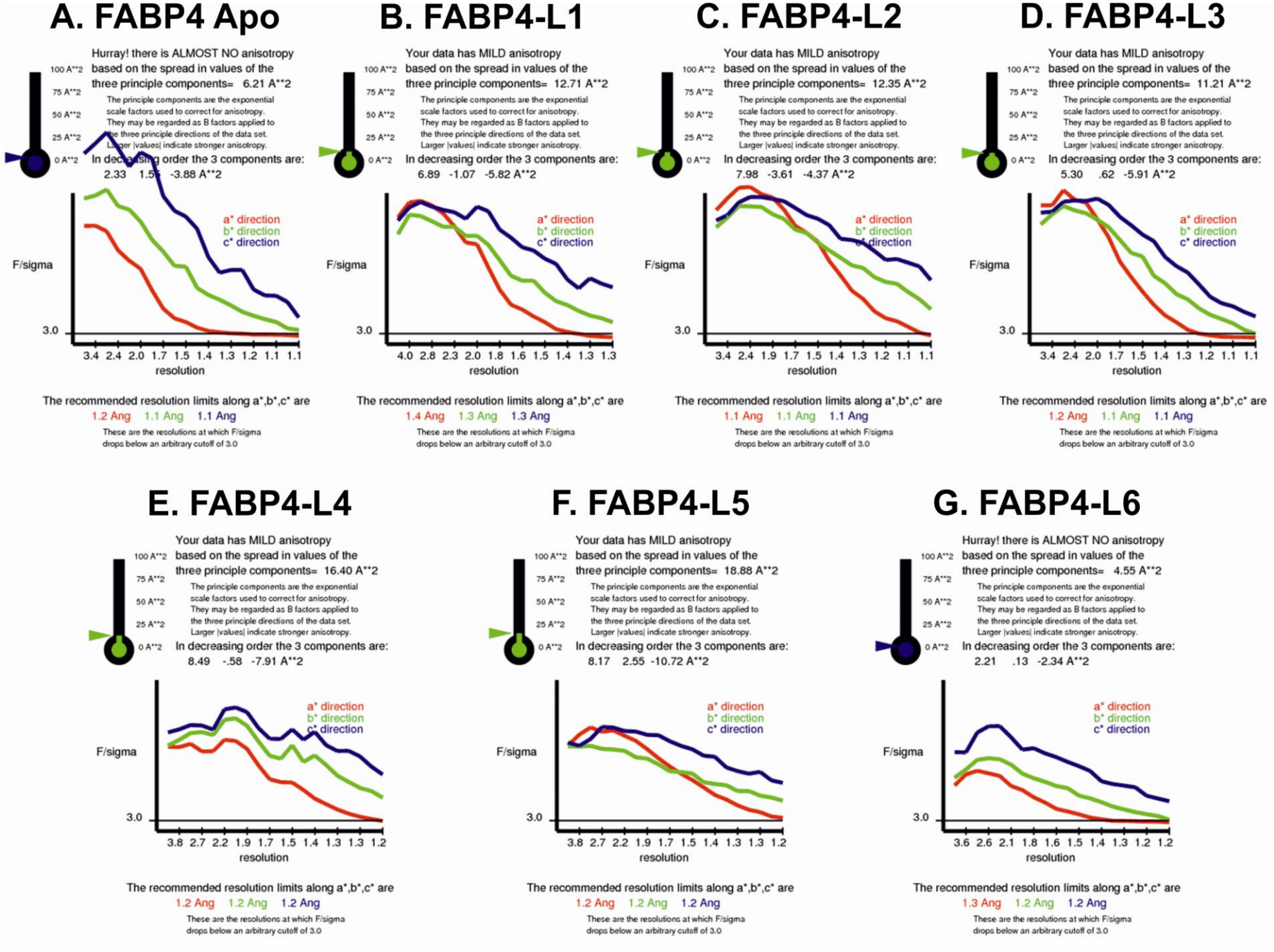
A. FABP4 Apo
Hurray! there is ALMOST NO anisotropy
based on the spread in values of the
three principle components= 6.21 A**2
The principle components are the exponential
scale factors used to correct for anisotropy.
They may be regarded as B factors applied to
the three principle directions of the data set.
Larger |values| indicate stronger anisotropy.
In decreasing order the 3 components are:
2.33 1.5 -3.88 A**2
100 A**2
75 A**2
50 A**2
25 A**2
0 A**2
a* direction
b* direction
c* direction
F/sigma
3.0
3.4 2.4 2.0 1.7 1.5 1.4 1.3 1.2 1.1 1.1
resolution
The recommended resolution limits along a*,b*,c* are
1.2 Ang 1.1 Ang 1.1 Ang
These are the resolutions at which F/sigma
drops below an arbitrary cutoff of 3.0
B. FABP4-L1
Your data has MILD anisotropy
based on the spread in values of the
three principle components= 12.71 A**2
The principle components are the exponential
scale factors used to correct for anisotropy.
They may be regarded as B factors applied to
the three principle directions of the data set.
Larger |values| indicate stronger anisotropy.
In decreasing order the 3 components are:
6.89 -1.07 -5.82 A**2
100 A**2
75 A**2
50 A**2
25 A**2
0 A**2
a* direction
b* direction
c* direction
F/sigma
3.0
4.0 2.8 2.3 2.0 1.8 1.6 1.5 1.4 1.3 1.3
resolution
The recommended resolution limits along a*,b*,c* are
1.4 Ang 1.3 Ang 1.3 Ang
These are the resolutions at which F/sigma
drops below an arbitrary cutoff of 3.0
C. FABP4-L2
Your data has MILD anisotropy
based on the spread in values of the
three principle components= 12.35 A**2
The principle components are the exponential
scale factors used to correct for anisotropy.
They may be regarded as B factors applied to
the three principle directions of the data set.
Larger |values| indicate stronger anisotropy.
In decreasing order the 3 components are:
7.98 -3.61 -4.37 A**2
100 A**2
75 A**2
50 A**2
25 A**2
0 A**2
a* direction
b* direction
c* direction
F/sigma
3.0
3.4 2.4 1.9 1.7 1.5 1.4 1.3 1.2 1.1 1.1
resolution
The recommended resolution limits along a*,b*,c* are
1.1 Ang 1.1 Ang 1.1 Ang
These are the resolutions at which F/sigma
drops below an arbitrary cutoff of 3.0
D. FABP4-L3
Your data has MILD anisotropy
based on the spread in values of the
three principle components= 11.21 A**2
The principle components are the exponential
scale factors used to correct for anisotropy.
They may be regarded as B factors applied to
the three principle directions of the data set.
Larger |values| indicate stronger anisotropy.
In decreasing order the 3 components are:
5.30 .62 -5.91 A**2
100 A**2
75 A**2
50 A**2
25 A**2
0 A**2
a* direction
b* direction
c* direction
F/sigma
3.0
3.4 2.4 2.0 1.7 1.5 1.4 1.3 1.2 1.1 1.1
resolution
The recommended resolution limits along a*,b*,c* are
1.2 Ang 1.1 Ang 1.1 Ang
These are the resolutions at which F/sigma
drops below an arbitrary cutoff of 3.0
E. FABP4-L4
Your data has MILD anisotropy
based on the spread in values of the
three principle components= 16.40 A**2
The principle components are the exponential
scale factors used to correct for anisotropy.
They may be regarded as B factors applied to
the three principle directions of the data set.
Larger |values| indicate stronger anisotropy.
In decreasing order the 3 components are:
8.49 -.58 -7.91 A**2
100 A**2
75 A**2
50 A**2
25 A**2
0 A**2
a* direction
b* direction
c* direction
F/sigma
3.0
3.8 2.7 2.2 1.9 1.7 1.5 1.4 1.3 1.3 1.2
resolution
The recommended resolution limits along a*,b*,c* are
1.2 Ang 1.2 Ang 1.2 Ang
These are the resolutions at which F/sigma
drops below an arbitrary cutoff of 3.0
F. FABP4-L5
Your data has MILD anisotropy
based on the spread in values of the
three principle components= 18.88 A**2
The principle components are the exponential
scale factors used to correct for anisotropy.
They may be regarded as B factors applied to
the three principle directions of the data set.
Larger |values| indicate stronger anisotropy.
In decreasing order the 3 components are:
8.17 2.55 -10.72 A**2
100 A**2
75 A**2
50 A**2
25 A**2
0 A**2
a* direction
b* direction
c* direction
F/sigma
3.0
3.8 2.7 2.2 1.9 1.7 1.5 1.4 1.3 1.3 1.2
resolution
The recommended resolution limits along a*,b*,c* are
1.2 Ang 1.2 Ang 1.2 Ang
These are the resolutions at which F/sigma
drops below an arbitrary cutoff of 3.0
G. FABP4-L6
Hurray! there is ALMOST NO anisotropy
based on the spread in values of the
three principle components= 4.55 A**2
The principle components are the exponential
scale factors used to correct for anisotropy.
They may be regarded as B factors applied to
the three principle directions of the data set.
Larger |values| indicate stronger anisotropy.
In decreasing order the 3 components are:
2.21 .13 -2.34 A**2
100 A**2
75 A**2
50 A**2
25 A**2
0 A**2
a* direction
b* direction
c* direction
F/sigma
3.0
3.6 2.6 2.1 1.8 1.6 1.5 1.4 1.3 1.2 1.2
resolution
The recommended resolution limits along a*,b*,c* are
1.3 Ang 1.2 Ang 1.2 Ang
These are the resolutions at which F/sigma
drops below an arbitrary cutoff of 3.0

Figure S5 Diffraction Anisotropy Reports. The figures (**A** to **G**) display the raw outputs obtained with the UCLA-MBI Diffraction Anisotropy Server (http://services.mbi.ucla.edu/anisoscale/), applied to each of the structures obtained in this work. Each figure shows the structure factors falloff along each reciprocal space direction, as a function of resolution. As described in the text, the ellipsoidal truncation and anisotropic scaling were not applied, given the low to mild anisotropy at the chosen resolution cutoff values. (**A)** FABP4-Apo (PDB 3RZY), (**B)** FABP4-L1 (PDB 3P6C), (**C**). FABP4-L2 (PDB 3P6D), (**D**) FABP4-L3 (PDB 3P6E), (**E**) FABP4-L4 (PDB 3P6F), (**F**) FABP4-L5 (PDB 3P6G), and (**G)** FABP4-L6 (PDB 3P6H).