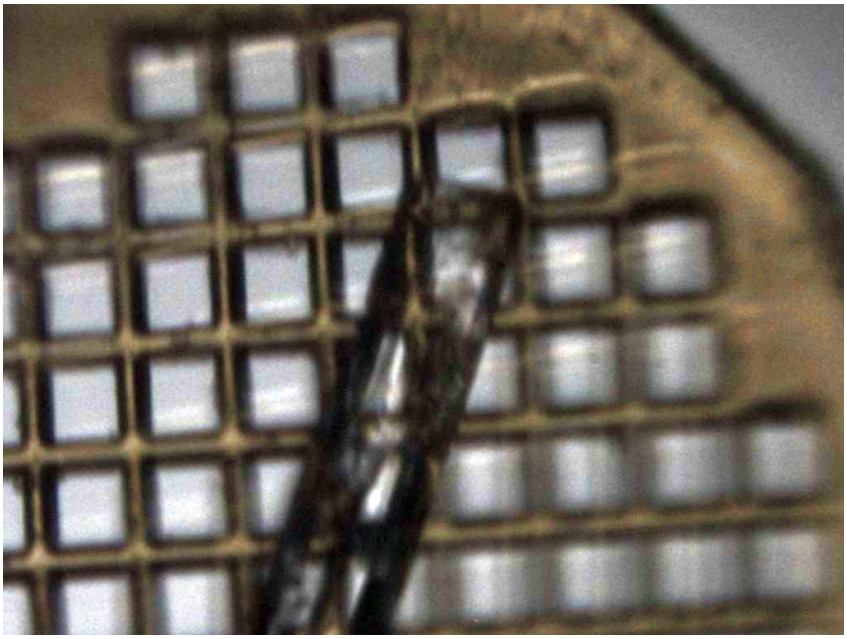
Four complete turns of a curved 3_{10}-helix at atomic resolution: The crystal structure of the peptaibol trichovirin I-4A in polar environment suggests a transition to α-helix for membrane function

Renate Gessmann,[a] Danny Axford,[b] Robin L. Owen,[b] Hans Brückner[c,d] and Kyriacos Petratos[a]*

[a]IMBB-FORTH, N. Plastira 100, Heraklion, 70013, Greece, [b]Diamond Light Source Ltd, Harwell Science and Innovation Campus, Didcot OX11 0DE, England, [c]Research Center for BioSystems, Land Use and Nutrition (IFZ), Institute of Nutritional Science, Department of Food Science, University of Giessen, Heinrich-Buff-Ring 26-32, 65392 Giessen Germany, [d]Institute of Food Science and Nutrition, King Saud University, P.O. Box 2460, Riyadh 11450, Kingdom of Saudi Arabia

Correspondence email: petratos@imbb.forth.gr

Supplementary material



Supplementary Figure 1. Trichovirin I-4A crystal mounted on a micromesh support for data collection. The mesh size is 25μm.

Crystallization. Evaporation and heating were employed for crystallization of trichovirin I-4A. Several trials were performed every 3 months using the same material by adding aliquots of acetonitrile, methanol and water in a small vial. An approximate composition of the final crystallization solution would be 5-10% (v/v) methanol and acetonitrile in water. We saw crystals about 7 years after the first crystallization trial.

Details of refinement. The refinement was performed essentially unrestrained. As the side-chain geometry of three out of the four disordered residues became poor, the side chains of Val7, Val27 and Leu31 have been restrained, where, for example, the Cβ - Cγ1_a and the Cβ - Cγ1_b distance counts as 2 restraints. Together with the side chain restraints for Lol34 this makes 24 distance (=1, 2) restraints and 12 angle (=1, 3 distance) restraints for the peptaibols. The methanols, one disordered, add 3 distance restraints and 4 additional were applied for the acetonitriles. The total rms deviations are given in Table 1 and are taken from the pdb validation report. MolProbity lists a rotamer score of zero and for the clash score there is one disordered solvent molecule (MeOH71) close (2.36 Å) to HD21 of Asn22(B). The Mogul program of the Cambridge Structure Database was employed to check the deviations of the bond lengths and angles from mean values of deposited structures of small molecules. The result was, 2 bond lengths of mol-A and 2 bond angles of mol-B exhibit a z-score > 4.0.

Supplementary Table 1. Backbone and side chain torsion angles for trichovirin I-4A

mol-A	φ	ψ	ω	χ^1	χ^2	χ^3	χ^4	θ^4
Ac0								
Aib1	-58	-32	-179					
Asn2	-62	-18	-179	-52	73/-102			
Leu3	-92	4	179	-53	-57/-179			
Aib4	-53	-32	177					
Pro5	-54	-22	180	-29	41	-36	18	8
Ala6	-57	-27	177					
Val7	-90	-10	-174	93/-156				
Aib8	-52	-37	-177					
Pro9	-59	-24	179	-29	38	-32	13	11
Aib10	-58	-23	180					
Leu11	-84	-4	180	-58	-55/180			
Aib12	-47	-43	-179					
Pro13	-73	-8	-175	-12	23	-24	17	-3
Lol14	-112			-55	-75/162			
mol-B								
Ac0								
Aib1	-56	-44	-173					
Asn2	-73	-18	177	-55	-48/141			
Leu3	-103	6	180	-53	-56/180			
Aib4	-51	-36	180					
Pro5	-55	-25	-177	-31	40	-33	13	11
Ala6	-57	-28	177					
Val7	-90	-8	-174	-62/57				
Aib8	-53	-38	-177					
Pro9	-58	-25	-178	-29	37	-32	14	10
Aib10	-62	-23	178					
Leu11	-82	-5	-176	-63	-57/177			
Aib12	-50	-42	-176					
Pro13	-77	-3	-176	-5	11	-12	9	-2
Lol14	-117			-70	-84/153			

Supplementary Table 2. Hydrogen bonds

Table S2a. Intramolecular hydrogen bonds

		mol-A		mol-B	
donor N	acc^ O	length (Å)	angle (°) C=O-N	length (Å)	angle (°) C=O-N
Asn2	Sc* Asn2	2.98	80.9	3.25	64.9
Leu3	Ac	2.89	134.6	2.95	123.8
Aib4	Ac			3.23	166.1
Aib4	Aib1	2.95	122.9	3.40	106.7
Ala6	Leu3	2.94	141.4	2.92	140.2
Val7	Aib4	2.94	135.6	3.18	133.9
Aib8	Pro5	2.92	117.9	2.89	119.8
Aib10	Val7	2.99	137.2	2.99	137.7
Leu11	Aib8	2.94	133.2	2.95	132.4
Aib12	Pro9	2.91	123.3	2.92	122.7
Lol14	Leu11	2.84	136.1	2.89	135.0

Table S2b. Head-to-tail Hydrogen bonds

mol-A: direct

d[#] N	acc O	length (Å)	angle (°) C=O-N	symm
Aib(1A)	Aib(12A)	2.94	130.8	x+1,y-1,z

mol-A: via water to x+1,y-1,z

d	acc O	length (Å)	angle (°) C=O-O
54	Aib(12A)	2.82	133.6
54	Lol(14A)	2.72	107.2

mol-B: via water to x-1,y-1,z

57	Aib(12B)	2.63	137.8
57	Lol(14B)	2.47	116.4

mol-A: H-bonds via water to x+1,y-2,z

d/acc	length (Å)	O	length (Å) OPro13	angle (°) O-O=C
OScAsn(2A)	2.65	53	2.75	128.7
OScAsn(2A)	2.72	60	2.99	151.2
OScAsn(2A)	3.10	64	2.78	129.9
NScAsn(2A)	2.94	64		

mol-B: H-bonds via water to x-1,y-2,z

d/acc	length (Å)	O	length (Å) to OPro13	angle (°) O-O=C
NScAsn(2B)	3.12	65	2.80	142.0

A to B direct

d/acc	d/acc	length (Å)	angle (°) C=O-N	symm
NScAsn(2A)	OScAsn(2B)	2.86	123.5	x+1,y,z

Table S2c. Between mol-A and mol-B via water to x+1,y,z

d/acc	length (Å)	O	length (Å)	acc	angle (°) O-O=C
NScAsn(2A)	2.95	52	2.61	OAsn(2B)	128.9
OAsn(2A)	2.73	55	2.87	OScAsn(2B)	112.0
OScAsn(2A)	3.16	59	2.83	OAib(1B)	145.8

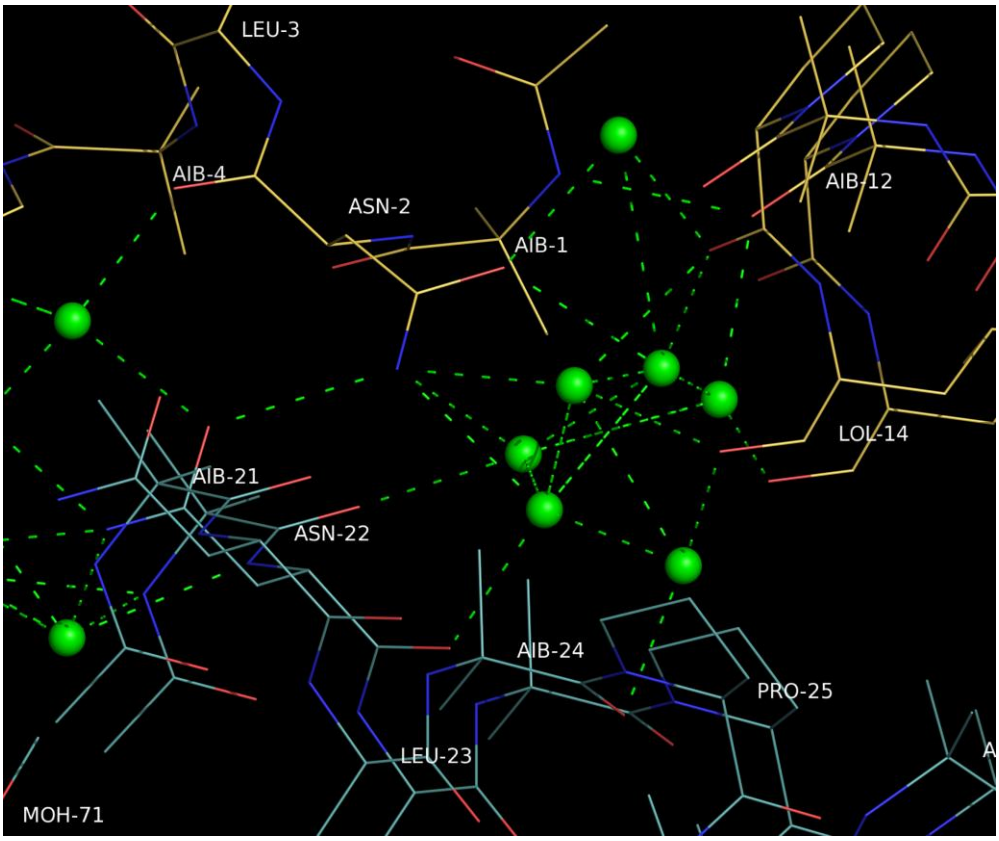
Table S2d. Between mol-A or B and solvent

d/acc	d/acc	length (Å)	symm
OAib(4A)	56	2.85	x,y+1,z
OHLol(14A)	51	2.87	x-1,y+2,z
OAc(0B)	62	2.98	-x,y+1/2,-z+1
NAib(1B)	63	3.15	
NAsn(2B)	63	2.88	
NScAsn(2B)	58	2.90	
NScAsn(2B)	63	3.17	
OAib(4B)	51	2.82	x-1,y+1,z
OPro(9B)	70	3.10	x+1,y+1,z
OHLol(14B)	58	2.57	x+1,y+2,z

Table S2e. Between solvent (I,II: cluster I,II)

d/acc	d/acc	length (Å)	symm	
51	52	2.67		I
52	59	2.77		I
52	60	2.79		I
53	60	3.48		I
54	59	3.13		I
54	60	2.99		I
55	56	2.82		II
55	61	2.94		II
56	58	2.73	x+1,y,z	II
57	61	2.44	x-1,y,z	II
57	63	2.98		II
58	65	3.20		II
61	63	3.14	x+1,y,z	II
61	65	2.23	x+1,y,z	II
63	65	2.95		II
71	72a	2.45		none
62	70	2.70		none
51	64	3.05		I
52	64	2.30		I
60	64	2.57		I

*Sc is side chain
^acc is acceptor
#d is donor



Supplementary Figure 2. Environment of water cluster I viewed approximately down the *b*-axis. C-atoms of mol-A are in orange and C-atoms of mol-B in light blue. The residue numbering of mol-B here is as in the deposited coordinates 3SBN (residue of mol-A plus 20).This figure is a snapshot of the attached video s.avi.

The simple numbers 51-70 above and in the following text denote ordered water molecules. In the crystal head-to-tail helical planes are formed in the following way.
Mol-A is hydrogen bonded head-to-tail (N1 - O12, symmetry operator x+1, y-1, z) and this O12 of the symmetry related molecule is in addition hydrogen bonded to the first water cluster (54 - O12). The same water forms with the identical molecule a second hydrogen bond (54-O14) (angle O12-54-O14 = 42°).

Three waters of the same cluster mediate indirect head-to-tail hydrogen bonds to another symmetry related molecule A (OD1(Asn2,A) - 53 - O13(A); OD1(Asn2,A) - 60 - O13(A); OD1(Asn2,A) - 64 - O13(A); ND2(Asn2,A) - 64 - O13(A), symmetry operator x+1, y-2, z). By this hydrogen bond network molecules A are linked via water molecules to form layers parallel to the crystal ab-plane, linking molecules head-to-tail by the symmetry operators (x+1, y-1,z) and (x+1,y-2,z).
The head-to-tail hydrogen bonding in Mol-B is mediated by a water molecule from the second water cluster. This forms a bifurcated bond to C-terminal oxygens (N1(B) - 57 - O12(B) and N1(B) - 57 - O14(B), symmetry operator x-1, y-1, z). Similar to the head-to-tail hydrogen bonding of molecules A, a second mediated hydrogen bond connects to the identical mol-B translated along the b-axis (ND2(Asn2,B) - 65 - O13(B), symmetry operator x-1, y-2, z). The layer of molecules B stacks parallel to the layer of molecules A, while the direction of the curved helical axis in one layer adopts about a right angle with respect to the other layer.
One mol-A is hydrogen bonded to one mol-B at the N-terminal. There is one direct hydrogen bond between the two Asn2 side chains (ND2(Asn2,A) - OD1(Asn2,B) and three mediated by waters (ND2(Asn2,A) - 52 - O2(B); O2(A) - 55 - OD1(Asn2,B); OD1(Asn2,A) - 59 - O1(B), all 4 with symmetry operator x+1, y, z). This leads to a pairwise arrangement of the head-to-tail hydrogen bonded layers of one layer A to one layer B in z-direction.
The space group symmetry related layers A and A and the symmetry related layers B and B form the closest Van der Waals contact (3.2Å) with the convex middle part of the molecules. In this convex part lie also the two carbonyl groups of each chain which do not participate in hydrogen bonding, namely the carbonyl group of Ala6 and Aib10.

Supplementary Table 3. Valence geometry around the C_α-atom in Aib residues

residues	N - C_α - $C_{\beta L}$	C - C_α - $C_{\beta L}$	N - C_α - $C_{\beta R}$	C - C_α - $C_{\beta R}$
Aib1(A)	106.99	106.33	109.28	108.59
Aib4(A)	109.01	105.82	109.34	109.35
Aib8(A)	108.41	107.81	107.54	111.34
Aib10(A)	107.82	106.99	110.63	108.66
Aib12(A)	110.92	108.79	102.12	107.05
Aib1(B)	106.77	110.06	112.13	108.54
Aib4(B)	107.43	107.13	109.33	110.70
Aib8(B)	109.32	107.44	108.76	110.87
Aib10(B)	108.34	107.42	110.19	109.28
Aib12(B)	111.61	109.60	104.58	105.84

Valence geometry in Aib residues

Experimental and theoretical studies have shown that the valence geometry around the C_α-atom for Aib residues in 3_{10}-helical structures is asymmetric (Paterson *et al.*, 1981). If one designates as $C_{\beta L}$ and $C_{\beta R}$ the atoms that occupy the same position as the C_β and the hydrogen atom bonded to the C_α-atom in L-amino acids, respectively, the angles N - C_α - $C_{\beta L}$ and C - C_α - $C_{\beta L}$ in right-handed 3_{10}-helices are usually significantly smaller than the ideal tetrahedral value (109.45°); the opposite behavior is observed for the angles N - C_α - $C_{\beta R}$ and C - C_α - $C_{\beta R}$. Based on the observed values shown in Supplement Table 3 no such correlation could be found for both molecules A and B. Interestingly, also the C-terminal 4, 8 and 12 peptide fragments of trichovirin do not show the usual asymmetry in the valence geometry.

VMD images

A. For Windows users:
To see the cell packing as cartoons or van der Waals (space filling) presentations:
- download the attached files: topc_c.pdb
  packing_spider.zip
  packing_VDW.zip
  to a folder whose address has no spaces.
- download the program vmd from http://www.ks.uiuc.edu/Research/vmd/
  (Version 1.8.6 surely works on Windows). Note that you need to register before downloading.
- install it (very simple)

- Unzip the *.zip files to *.vmd
- Edit the packing_spider.vmd and packing_VDW.vmd files with WordPad and change the line starting with 'mol new' (about at the middle of the files) to address the location of the downloaded files on your computer. Please, TAKE CARE to avoid spaces in the address and the slashes "/ /" have to be in the correct direction!
- start vmd by clicking the right mouse button on any of the .vmd files
- in the vmd main window file go to 'Load state', select either packing_spider.vmd or packing_VDW.vmd and in order to observe the packing from all directions:

- rotate (type r in the display window, use either mouse button to move)
- translate (type t, etc.)
- scale the molecules (type s, etc.)

all solvent molecules are omitted for clarity.

B. For Linux users:

To see the cell packing as cartoons or van der Waals (space filling) presentations:
- download the attached files: topc_c.pdb
  packing_spider.zip
  packing_VDW.zip
  to a folder whose address has no spaces.
- download a tar compressed archive of a vmd version suitable for your computer from the web-site: http://www.ks.uiuc.edu/Research/vmd/. Note that you need to register before downloading.
- Uncompress the tar file
- Extract the files in the archive
- Follow the instructions for Quick Installation described in the README file sitting in the created 'vmd-' directory.
- Unzip the *.zip files to *.vmd
- Edit the packing_spider.vmd and packing_VDW.vmd files with a text editor and change the line starting with 'mol new ' (about at the middle of the files) to address the location of the downloaded files on your computer.
- start vmd by activating the 'vmd' startup script sitting in the /bin directory.
- in the vmd main window file, under the 'File' tab select the 'Load State' option to load either packing_spider.vmd or packing_VDW.vmd and in order to observe the packing from all directions:

- rotate (type r in the display window, use either mouse button to move)
- translate (type t, etc.)
- scale the molecules (type s, etc.)

all solvent molecules are omitted for clarity.

Movies

The two attached files, a.avi (superposed mol-A and mol-B with intramolecular H-bonds) and s.avi (molecular packing with solvent viewed down the *b*-axis), can be downloaded and played with any movie player.

Reference

Paterson, Y., Rumsey, S. M., Benedetti, E., Némethy, G., Scheraga, H. A. (1981) *J. Am. Chem. Soc.* **103**, 2947–2955.