

Supplementary information for manuscript

“Atomic structure of Lanreotide nanotubes revealed by cryo-EM”

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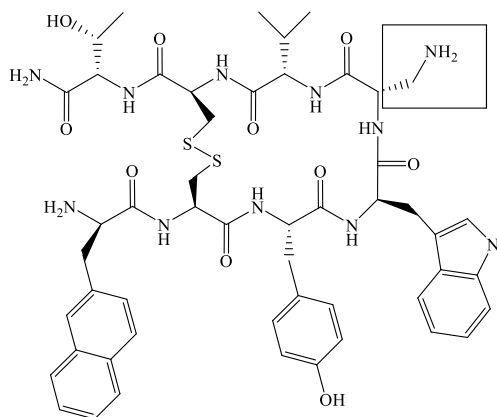
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LAN-DAP5 DERIVATIVE SYNTHESIS.



The lanreotide derivative was synthesized by solid-phase Fmoc/tBu chemistry. The L-lysine in position 5 in the lanreotide sequence was replaced by L-1,3-diamino propionic acid. The peptide chain was first assembled and then cyclized on resin. For this purpose we had to choose a low grafting density to avoid the formation of intermolecular disulfide bridge. Cleavage and side chain deprotection were performed simultaneously, followed by purification and counter-ion exchange^{1,2}. Purity of the final products was checked by HPLC/MS (see below and Supporting Information). The peptide sequence is: H-D-

2-Nal1-cyclo(Cys2-Tyr3-D-Trp4-Dap5-Val6-Cys7)-Thr8-NH2. The self-assembling properties of this derivative in pure water were first assessed in reference³.

Figure S1: Lan-Dap5 derivative

LC/MS ANALYSIS AND PURIFICATION.

HPLC was performed using a Waters system (2525 binary gradient module, in-line degasser, 2767 sample manager, 2996 Photodiode Array Detector). The eluent was a gradient of A (99.9% water/0.1% HCOOH) and B (99.9% ACN/0.1% HCOOH). Either analytical or preparative X-bridge C18 columns were used. The retention time (rt) was 8.65 minutes.

The mass spectrometer was a Waters Micromass ZQ system with a ZQ2000 quadrupole analyser. The ionization was performed by electrospray, and the other parameters were as follows: source temperature 120 °C, cone voltage 20 V and continuous sample injection at 0.3 ml/min flow rate. Mass spectra were recorded in positive ion mode in the m/z 100–2000 range and treated with the Mass Lynx 4.0 software. The experimental m/z of 1054.42780 [M+H]⁺ is the one expected for the cyclized molecule of Lan-Dap5 derivative (1054.42736).

NMR ANALYSIS.

The synthesized peptide was analyzed by ¹H NMR on a Bruker Avance 400 Ultrashield. Spectrum was recorded at room temperature at 400 MHz, and sample was dissolved in D₂O at a concentration of approximately 5 mM. The D₂O singlet signal was set up at 4.79 ppm. Chemical shifts are given in ppm and the coupling constants in Hz.

¹H NMR (D₂O): δ 0.74 (d, J = 6.5, 6H); 1.05 (d, J = 6.4, 3H); 1.94-2.03 (m, 1H); 2.23 (dd, J = 7.5, J = 13.5, 1H); 2.41-3.01 (m, 9H); 3.19 (dd, J = 8.8, J = 13.8, 1H); 3.29 (dd, J = 5.8, J = 13.7, 1H); 3.92 (d, J = 9.2, 1H); 4.01-4.1 (m, 2H); 4.14 (d, J = 4, 1H); 4.14-4.26 (m, 2H); 4.43 (t, J = 7.2, 1H); 4.63-4.83 (m, 3H); 6.61 (d, J = 8.5, 2H); 6.89 (d, J = 8.5, 2H); 6.99-7.05 (m, 2H); 7.11 (t, J = 7.3, 1H); 7.33 (dd, J = 1.4, J = 8.5, 1H); 7.37-7.42 (m, 3H); 7.66 (bs, 1H); 7.71-7.8 (m, 3H).

- (1) Roux, S.; Zekri, E.; Rousseau, B.; Paternostre, M.; Cintrat, J.-C.; Fay, N. Elimination and Exchange of Trifluoroacetate Counter-Ion from Cationic Peptides: A Critical Evaluation of Different Approaches. *Journal of Peptide Science* **2008**, *14* (3), 354–359. <https://doi.org/10.1002/psc.951>.
- (2) Gobeaux, F.; Fay, N.; Tarabout, C.; Meriadec, C.; Meneau, F.; Ligeti, M.; Buisson, D.-A.; Cintrat, J.-C.; Nguyen, K. M. H.; Perrin, L.; Valery, C.; Artzner, F.; Paternostre, M. Structural Role of Counterions Adsorbed

on Self-Assembled Peptide Nanotubes. *Journal of the American Chemical Society* **2012**, *134* (1), 723–733. <https://doi.org/10.1021/ja210299g>.

- (3) Tarabout, C.; Roux, S.; Gobeaux, F.; Fay, N.; Pouget, E.; Meriadec, C.; Ligeti, M.; Thomas, D.; Ijsselstijn, M.; Besselièvre, F.; Buisson, D.-A.; Verbavatz, J.-M.; Petitjean, M.; Valery, C.; Perrin, L.; Rousseau, B.; Artzner, F.; Paternostre, M.; Cintrat, J.-C. Control of Peptide Nanotube Diameter by Chemical Modifications of an Aromatic Residue Involved in a Single Close Contact. *Proceedings of the National Academy of Sciences of the United States of America* **2011**, *108* (19), 7679–7684. <https://doi.org/10.1073/pnas.1017343108>.

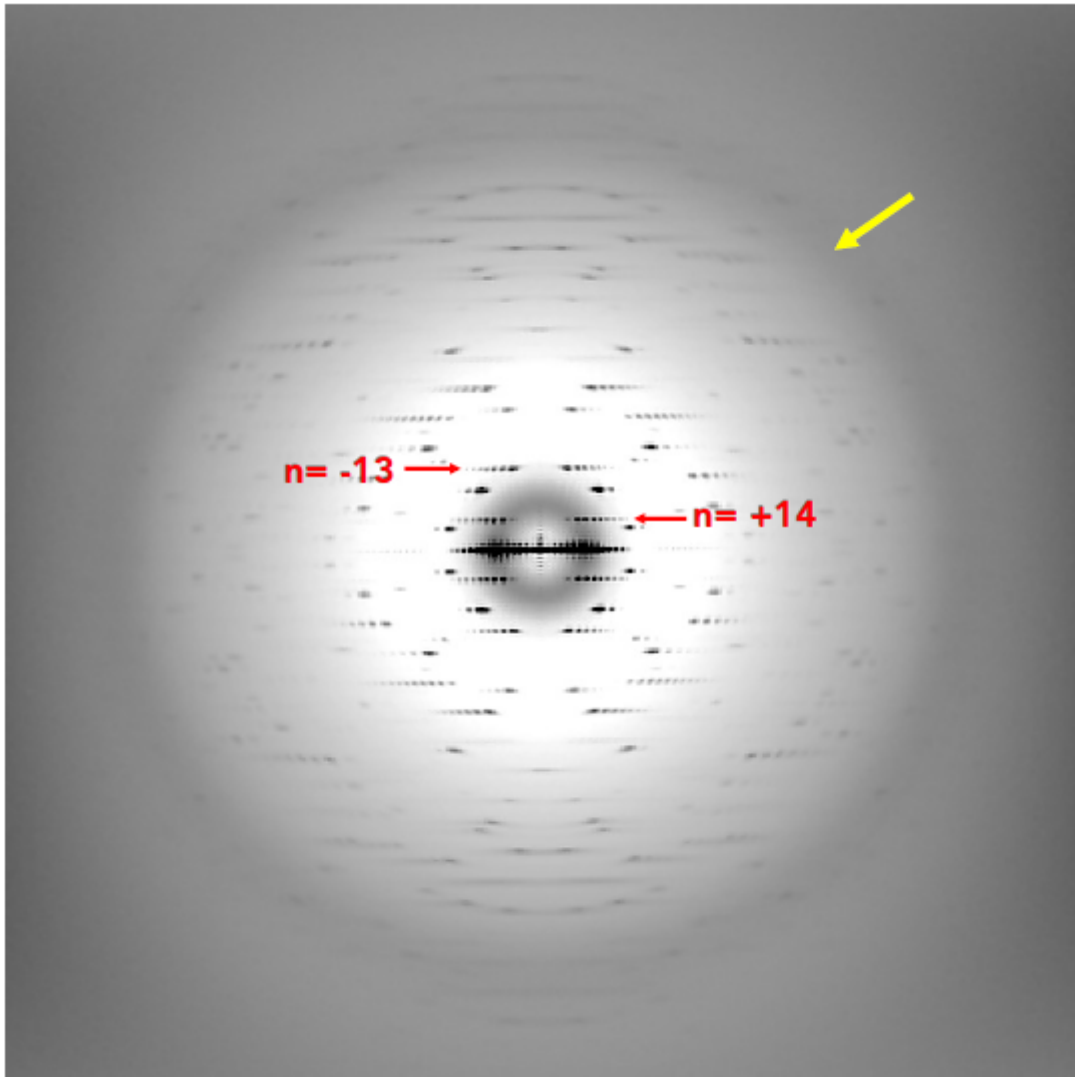


Figure S2: Power spectrum. An averaged power spectrum generated from 100,000 initial segments. Two layer lines are indexed with their Bessel orders: the $n=+14$ is at $1/(52.8 \text{ \AA})$, and the $n=-13$ is at $1/(19.6 \text{ \AA})$. The water ring at $\sim 1/(3.8 \text{ \AA})$ is indicated by the yellow arrow. It can be seen that layer lines extend beyond the water ring.

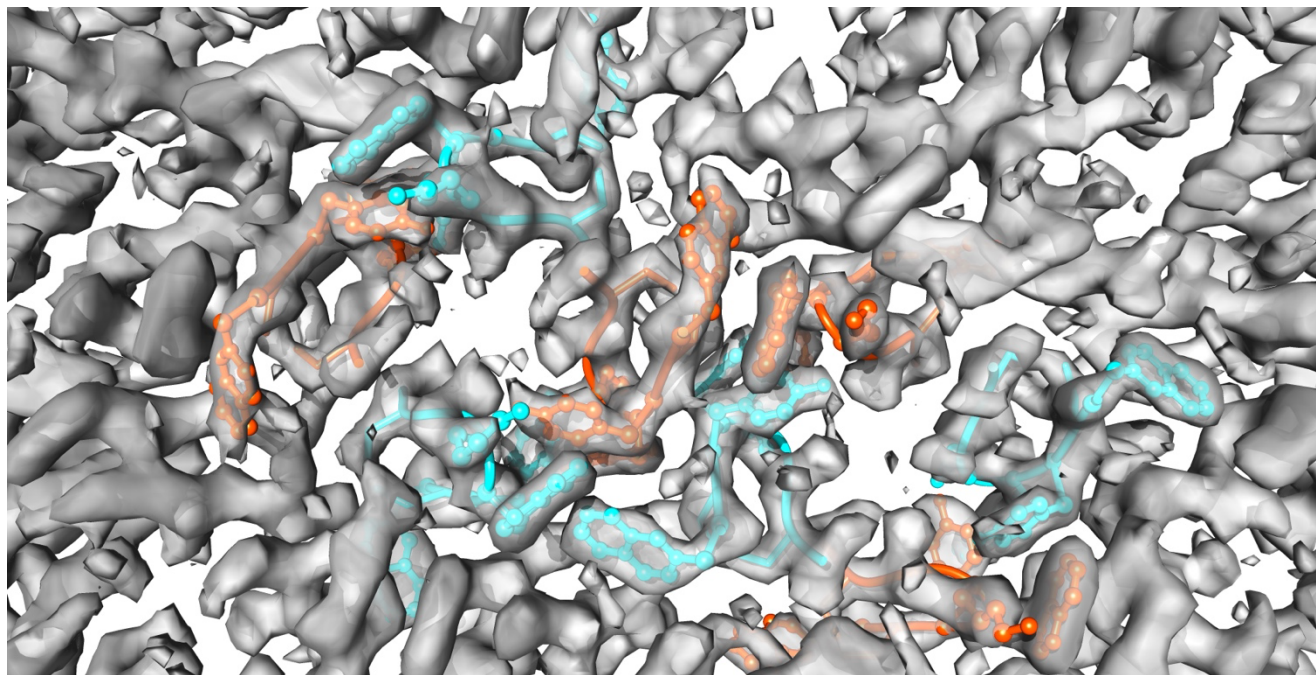


Figure S3: Details of the density map around the asymmetric unit. Molecules a, b, g & h are in orange and molecules c, d, e & f in cyan

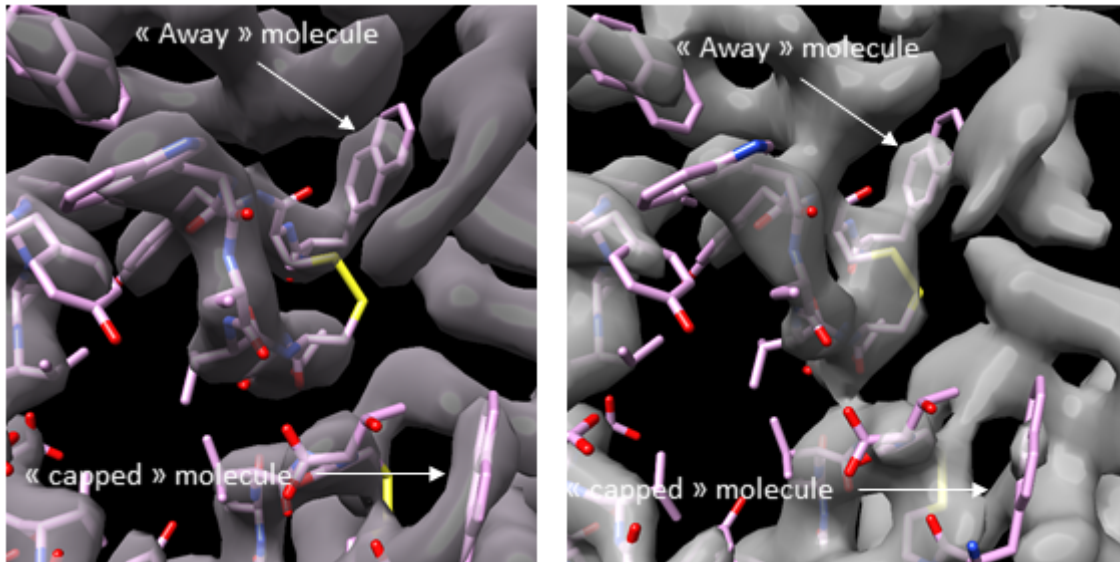


Figure S4: sensitivity of the S-S bridge to electron damage. Comparison of the density maps obtained at $20 \text{ e}^-/\text{\AA}^2$ (with dose weighting, the regular conditions) and at $2 \text{ e}^-/\text{\AA}^2$ by using a small subset of images in the legacy software SPIDER, so the resolution is $\sim 3.6\text{-}4 \text{ \AA}$). We applied the alignment parameters obtained in the $20 \text{ e}^-/\text{\AA}^2$ images to the $2 \text{ e}^-/\text{\AA}^2$ images. The disappearance of the density associated with the disulfide bridge can be seen at the higher dose. Interestingly, the density of the disulfide bridge associated with the “capped” molecule is less sensitive to electron radiation than the one associated with the “away” one.

		ALL ATOMS							
		a _c	b _c	c _a	d _a	e _a	f _a	g _c	h _c
BACKBONE	a _c	0.0	0.1	2.1	2.1	2.1	2.1	0.6	0.6
	b _c	0.1	0.0	2.2	2.2	2.2	2.1	0.6	0.6
	c _a	0.5	0.5	0.0	0.1	0.5	0.4	2.1	2.0
	d _a	0.4	0.4	0.1	0.0	0.5	0.4	2.1	2.1
	e _a	0.4	0.4	0.4	0.4	0.0	0.3	2.1	2.1
	f _a	0.4	0.4	0.4	0.4	0.1	0.0	2.0	2.0
	g _c	0.3	0.3	0.5	0.5	0.6	0.5	0.0	0.2
	h _c	0.3	0.2	0.5	0.4	0.5	0.5	0.1	0.0

Table S1: Comparison of the eight conformations of Lanreotide forming the asymmetric unit. Root-mean-square deviation in Å calculated between either: all atoms excluding hydrogens, or only the backbone atoms of the eight peptides, a to h, belonging to the asymmetric unit of the Lanreotide nanotube. These data show that the backbones are very similar among the eight molecules, whereas the side chains adopt different orientations, especially between the capped (molecules a_c, b_c, g_c & h_c) and away (molecules c_a, d_a, e_a & f_a) molecules

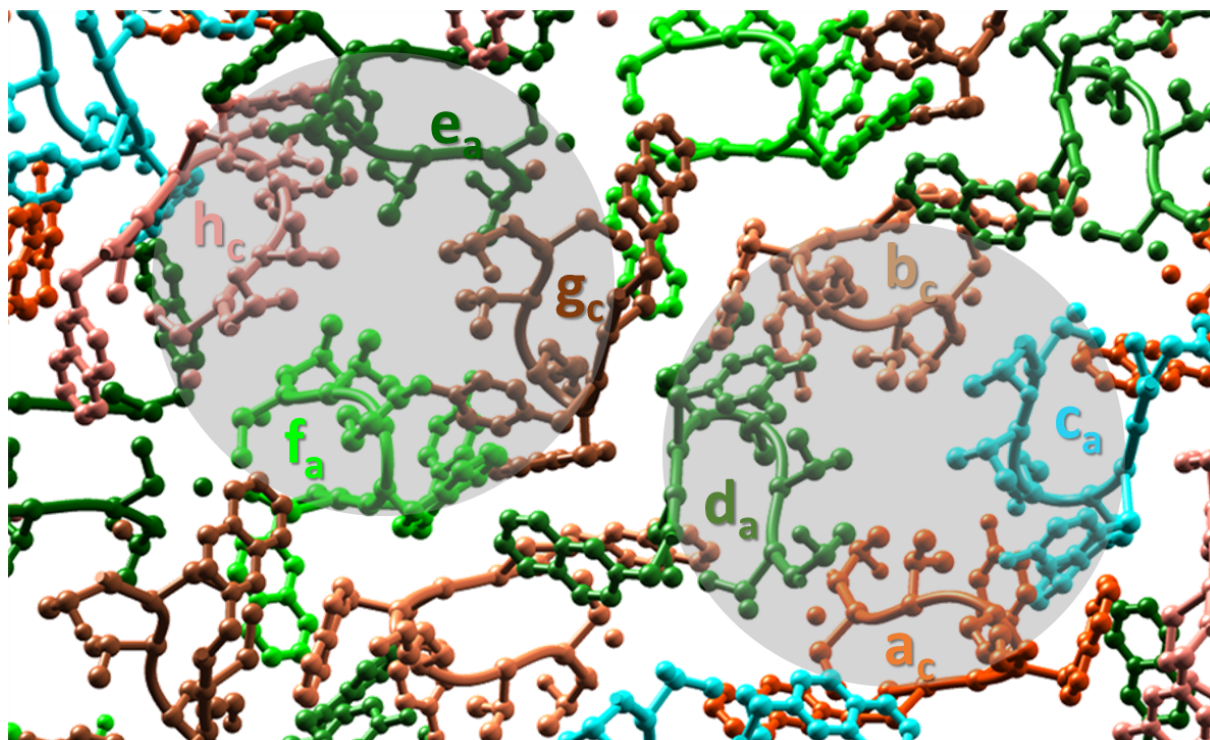


Figure S5: An asymmetric unit (highlighted by the two gray circles) and its direct environment

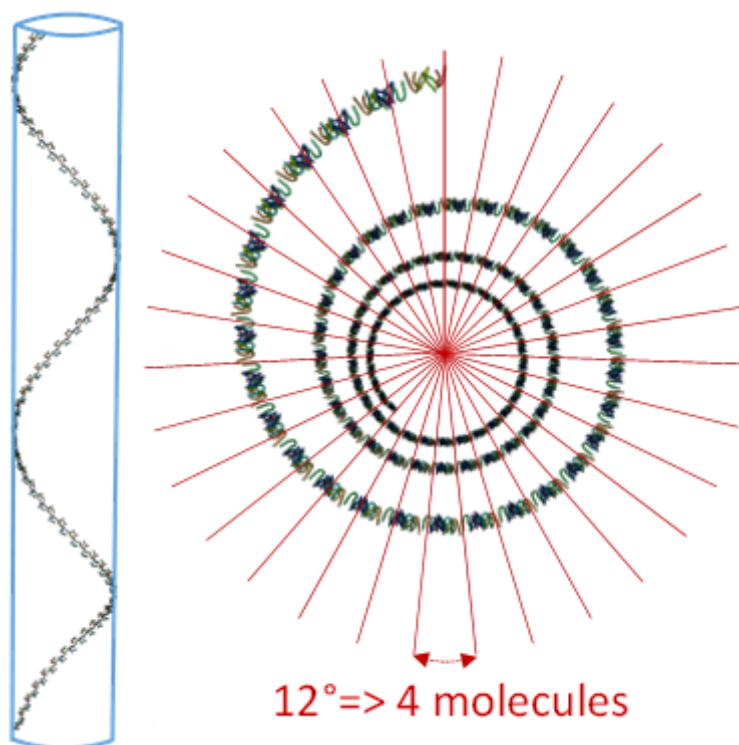


Figure S6: Depiction of every 27th asymmetric unit of the nanotube. This generates a continuous $a_c-d_a-g_c-f_a-a_c$... β -sheet along the nanotube forming a left-handed helix. Since the helical twist of the overall assembly is a rotation of 26.219° , the twist angle between two successive $a_c-d_a-g_c-f_a$ segments in the sheet is $27 \times 26.219^\circ = 707.913^\circ$, or -12.1° , and the rise between these two segments is $27 \times 1.04 \text{ \AA} = 28.1 \text{ \AA}$. The red radial lines show the rotation of $\sim 12^\circ$ for every four peptides (a_c, d_a, g_c, f_a) forming this sheet.

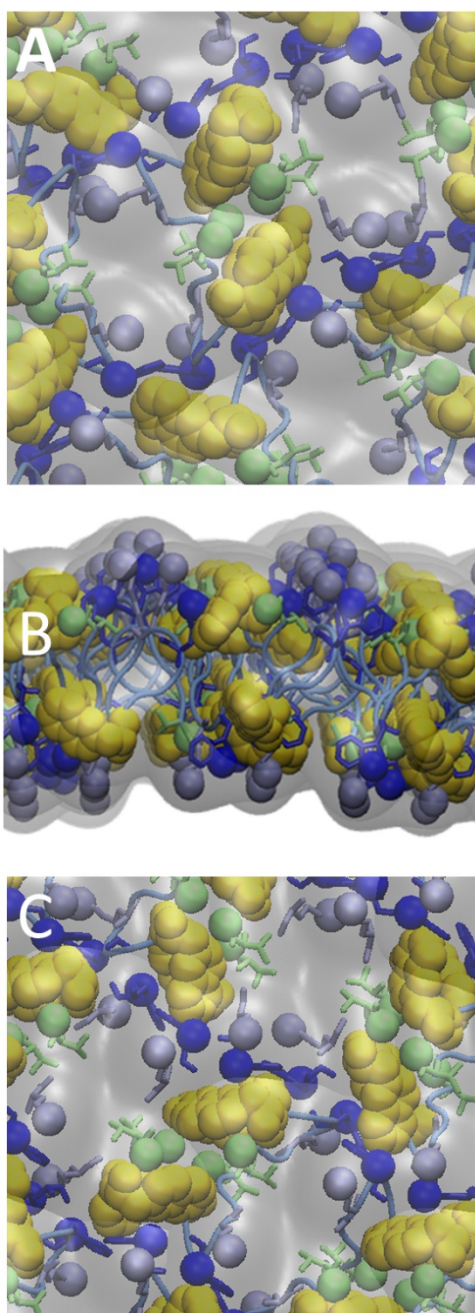


Figure S7: Surface accessible to the solvent. The nanotube wall viewed from the top (B) and from the side (A for the external surface and C for the luminal one), with the side chains colored as defined before: NaH is in Van der Waals sphere and in yellow, Thr, Lys and DTrp are respectively in green, steel and blue. Moreover, the nitrogens of the three previous residues are shown as Van der Waals spheres. As expected, the nitrogens are exposed to the water and surround the NaH, shielding it from water. The two surfaces are not equivalent as visible by the relative distribution of nitrogens and Nah on the external and luminal surfaces.

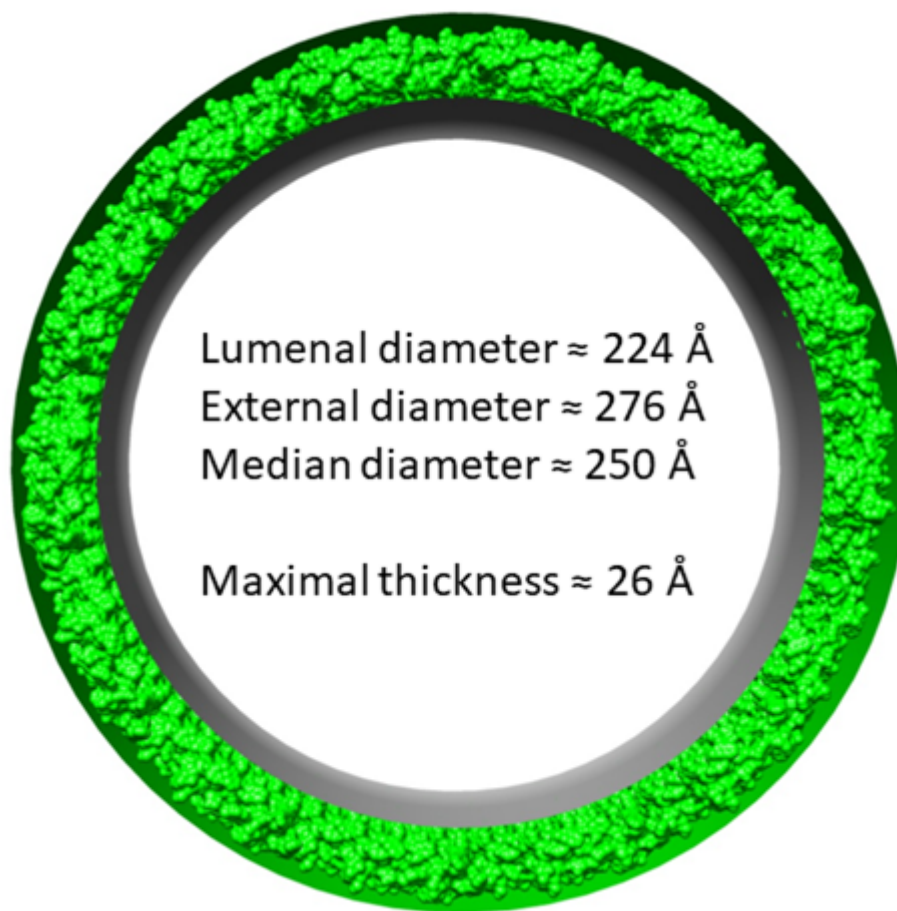


Figure S8: Estimation of the external and internal diameters and of the thickness of the nanotubes wall. Considering the two diameters, the external surface is 1.23 fold larger than the luminal one. This implies there are differences in the side chain packing. As the two external and luminal surfaces are very rough, the thickness of 26 \AA is the maximal possible one. The internal and external diameters of the tube were measured with Chimera version 1.15 (Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. (2004) UCSF Chimera – A visualization system for exploratory research and analysis. *J. Comput. Chem* 25 : 1605-1612 – DOI:10.1002/jcc.20084). The values of the diameters are determined when an atom of the tube begins to pass through the wall of the cylinder of a defined diameter.

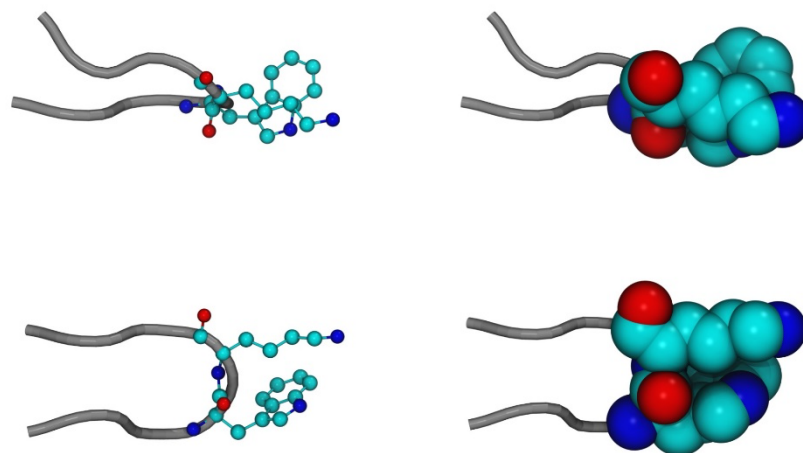


Figure S9: Details of the alignment between DTrp and Lys for molecule a_c . On the left, ball-and-stick representation of the DTrp and Lys and on the right, same view but in van der Waals representation. Top views of Lys and bottom side view of Lys and Dtrp. The backbone is in gray and the colors of atoms are: carbons in cyan, nitrogen in blue and oxygen in red.

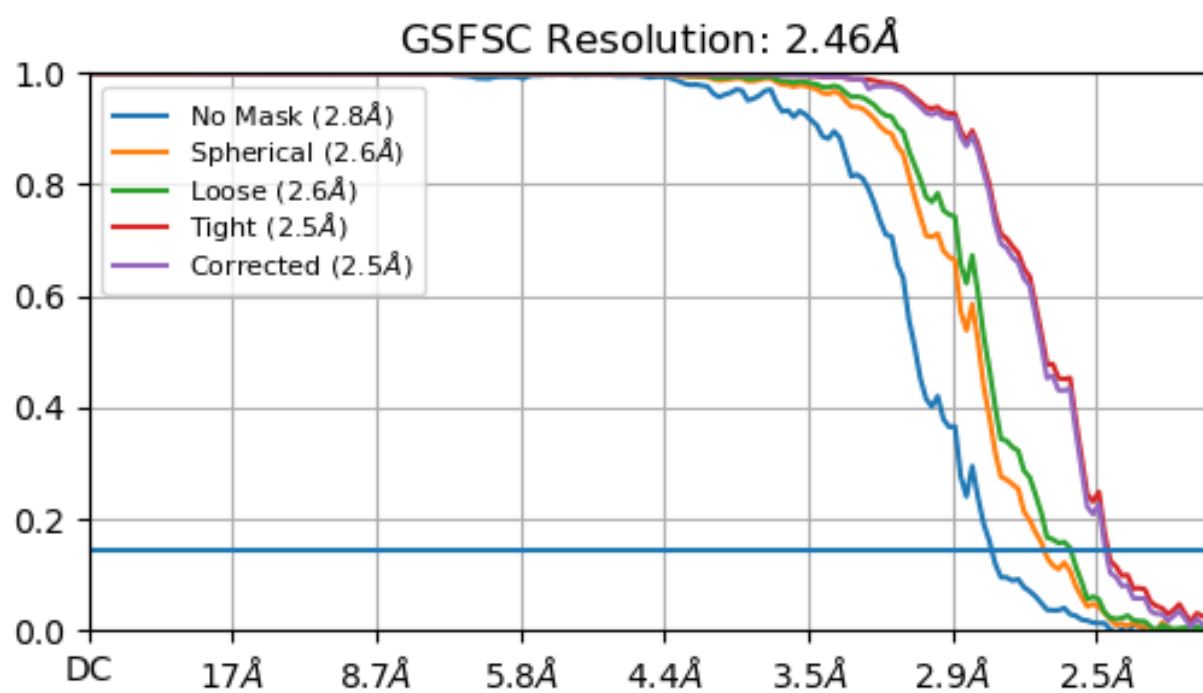


Figure S10 resolution of nanotube density map estimated by map:map “Gold Standard” FSC plot (FSC = 0.143).