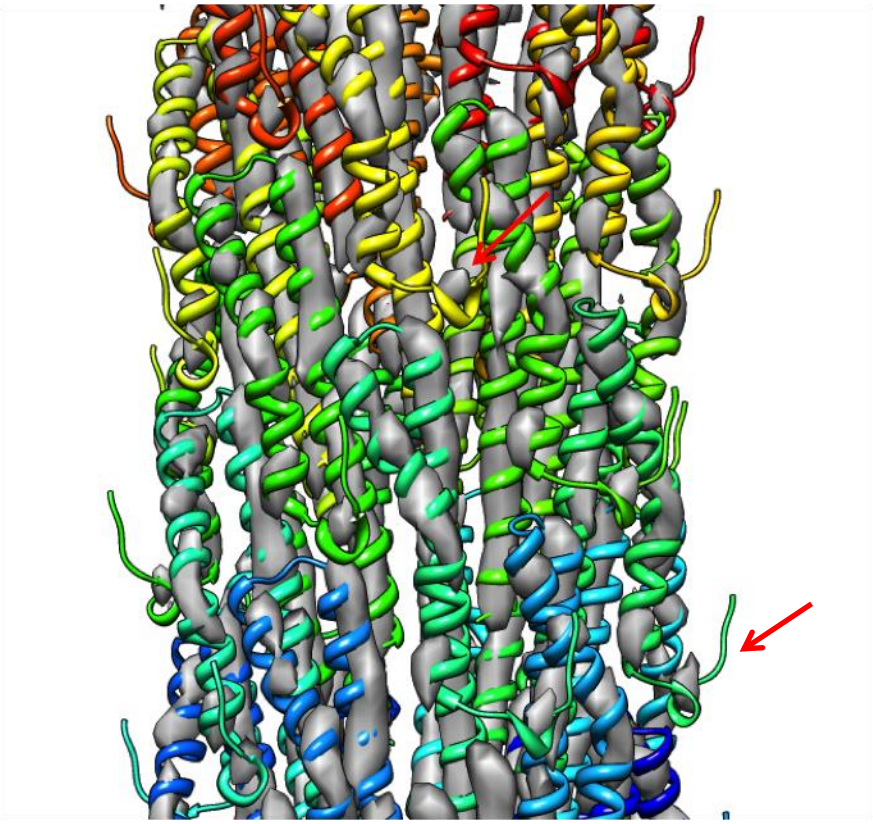
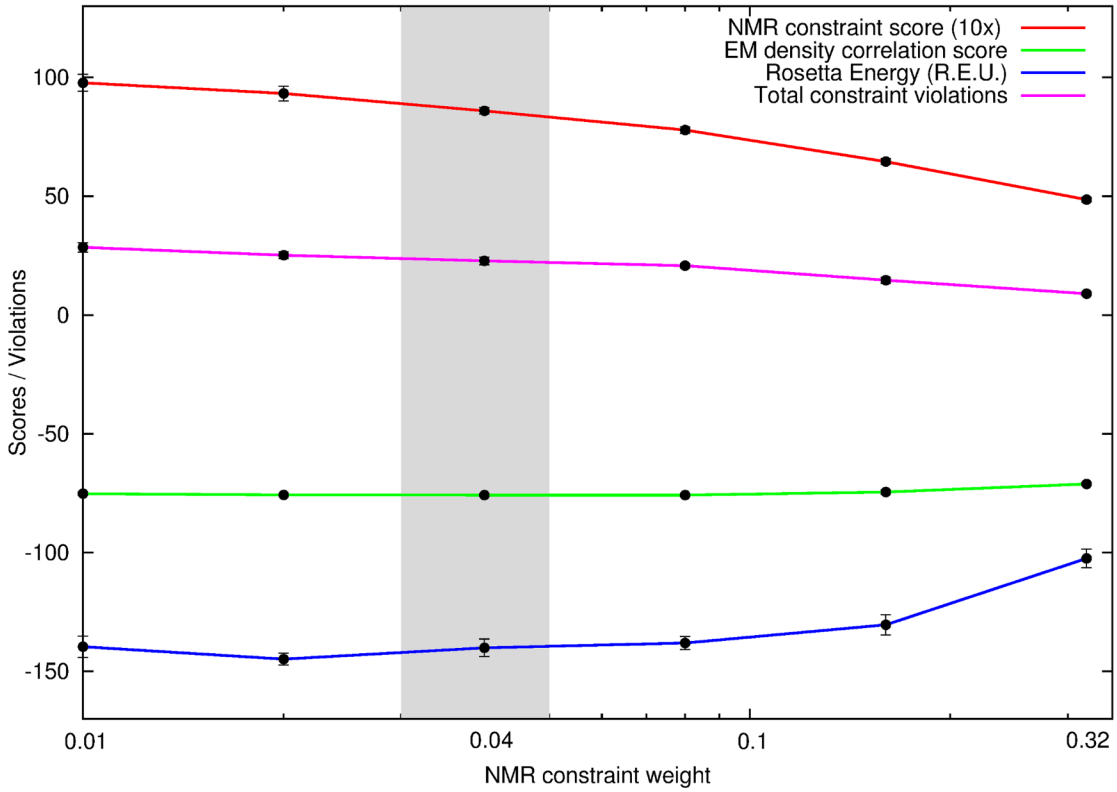
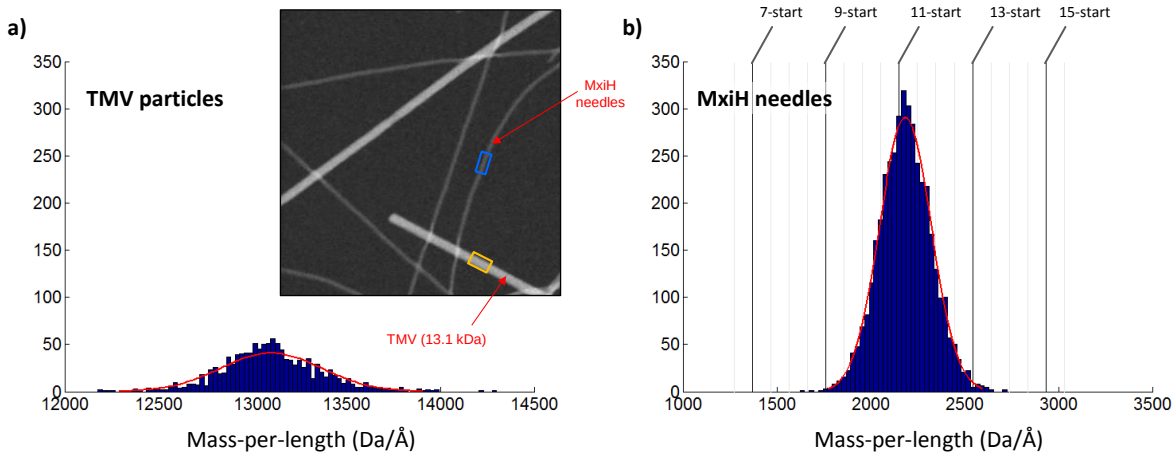
Supplementary Figures and Tables



Supplementary Figure 1. Fit to the high-resolution cryo-EM density (reference to Figure 2).
Rigid-body fit of the final MxiH model (PDB ID 2MME, model #1) on the 7.7 Å cryo-EM density map [1]. A good fit to the high-resolution EM density is obtained (correlation of 0.67), with the individual map features overlapping with structural features of the model. The "protrusion" region of the map is occupied by a short α-helical segment of the MxiH subunit N-terminus (red arrow). The rigid-body fit to the EM density and final figure rendering was performed using the program CHIMERA[2,3].



Supplementary Figure 2. Calibration of ssNMR constraint weights (reference to online methods). Individual score terms and total ssNMR constraint violations as a function of increasing weight of the NMR constraint term (relative to the Rosetta force field[4]) used in independent structure refinement calculations (x-axis, in logarithmic scale). The EM score (green) measures agreement with the 7.7 Å cryo-EM density map[1] in negative units, as described previously[5]. Error bars represent 1 standard deviation observed in 10 calculated structures for each weight value. The EM correlation term is reported in the same scale as the Rosetta Energy (blue), while the plotted NMR constraint penalty score (red) is scaled up by a factor of 10. A favorable range of weights (0.03-0.05) used in the final calculations is indicated with the shaded area. A constant EM score weight of 0.05 was used in all refinement calculations, optimized using a similar grid-search procedure. The NMR constraint score uses a flat-bottom potential with an upper limit of 9 Å and an exponential penalty function, as outlined in online methods. R.E.U: Rosetta Energy Units.



Supplementary Figure 3. Scanning Transmission Electron Microscopy data.
Distributions of mass-per-length of **(a)** Tobacco mosaic virus particles and **(b)** *in vitro* polymerized MxiH needles observed in scanning transmission electron microscopy (STEM) images. Expected mass-per-length values for representative needle helical geometries from 7- to 15-start (assuming a 24 Å helical pitch) are indicated on the x-axis. The peak at a mass-per-length of 2184±2 Da/Å is consistent with an 11-start arrangement and axial displacement of 4.3 Å/subunit, in good agreement with the final hybrid structural models. Image processing and final model fit parameters are outlined in the methods section. (middle panel) Dark-field STEM image of MxiH needles. The integration region for a MxiH needle is indicated (blue) as well as the region for a TMV particle (yellow).

Supplementary Table 1: List of NMR experiments used in obtaining long-range constraints (reference to online methods)

Nuclei	Mixing	Labeling	^{1}H freq. (MHz)	AQ_1; AQ_2 (ms)	TD_1; TD_2	SW_1; SW_2 (ppm)	Recycling delay (s)	No. of scans	Total time
^{13}C-^{13}C	PDSD 300 ms	1-Glc	600 MHz	14 ; 16	1120; 1274	265; 265	3.0	64	2d 19h
^{13}C-^{13}C	PDSD 850 ms	1-Glc	600 MHz	15 ; 17	900; 1354	199; 265	3.0	128	5d 16h
^{13}C-^{13}C	PDSD 700 ms	1-Glc	800 MHz	8.5; 13.7	520; 1600	153; 292	2.2	448	7d 22h
^{13}C-^{13}C	PDSD 400 ms	1-Glc	850 MHz	17 ; 21	1722; 2992	237; 334	2.5	80	4d 17h
^{13}C-^{13}C	PDSD 850 ms	1-Glc	850 MHz	18 ; 22	1824; 3134	237; 334	2.4	96	6d 16h
^{13}C-^{13}C	PDSD 850 ms	1-Glc	850 MHz	8.5; 17	530; 2420	146; 334	2.0	720	12d 17h
^{13}C-^{13}C	PDSD 300 ms	2-Glc	600 MHz	17 ; 21	1360; 1674	265; 265	3.1	80	4d 20h
^{13}C-^{13}C	PDSD 850 ms	2-Glc	600 MHz	15.5; 17	930; 1354	199; 265	2.6	176	6d 12h
^{13}C-^{13}C	PDSD 850 ms	2-Glc	800 MHz	8.7; 13.7	512; 1600	146; 292	2.2	496	9d 1h
^{13}C-^{13}C	PDSD 400 ms	2-Glc	850 MHz	15 ; 21	1520; 2992	237; 334	2.2	64	2d 23h
^{13}C-^{13}C	PDSD 850 ms	2-Glc	850 MHz	16 ; 20	1620; 2846	237; 334	2.6	96	6d 12h
^{15}N-^{13}C	NhhC 250 µs	Uniform	800 MHz	11.5; 15	80; 2076	43; 346	2.0	7168	13d 15h
^{13}C-^{13}C	ChhC 250 µs[a)]	Uniform	800 MHz	9 ; 15	432; 2076	120; 346	2.0	1344	13d 15h
^{13}C-^{13}C	ChhC 250 µs[b)]	Uniform	800 MHz	9 ; 15	432; 2076	120; 346	2.0	928	9d 15h

a) 87.5 µs for all CP contact times, b) 300 µs for initial CP contact time, 255 µs for bracketing CP contact times

Supplementary Methods

I) Description of the iterative assignment/structure determination steps (as outlined in Figure 2):

1) Initialization of the interface assignments from raw NMR constraints according to a preliminary structure model:
perl toolbox/assign_from_scratch.pl [pdb filename] [raw constraint table] |grep -v "##" > [fullatom Rosetta constraint file]

2) Mapping full-atom constraints to low-resolution (CB only sidechain) mode:
cat [fullatom Rosetta constraint file] | perl toolbox/map_csts_to_centroid_simple.pl > [centroid Rosetta constraint file]

3) Computing violations for a set of preliminary models:
for i in [list of pdb files]; do echo $i; perl toolbox/violation_analysis.pl $i [raw constraint table] >$i.viol; done;

4) Compile list of interface assignments and violation statistics:
*cat *.viol | grep -v "#" |awk '{print $17, $19}' | perl toolbox/interface_analysis.pl >[interface assignments file]*

,that produces the following output columns:
entry, # models evaluated, interface assignment, fraction models assigned to the dominant interface, fraction models satisfying the distance upper limit, average distance

Y60CD2-K72CA	10	6	0.9	0.7	6.85
Y60CD2-K72CA	10	6	0.9	0.4	12.72
Y60CG-I79CD1	10	6	1	1	8.99

5) Filter restraints according to chosen assignment criteria (i.e. Satisfied in more than 30% of the models and consistently assigned to the same interface in more than 70% of the models):

cat *[interface assignments file]* | perl -ne \ 'if(/(\S+)\s+(\d+)\s+(\d+)\s+(\S+)\s+(\S+)\s+(\S+)/){print if($5>=0.3 && $4>=0.7);}' | awk '{print $1, $3}' > *[filtered interface assignments file]*

6) Create final Rosetta constraints files according to the established interface assignments

a) fullatom constraints
perl toolbox/assign_from_known_interface.pl [pdb file] [filtered interface assignments file] | grep -v "#" > *[fullatom Rosetta constraint file]*

b) centroid constraints
cat [fullatom Rosetta constraint file] | perl toolbox/map_csts_to_centroid_simple.pl > [centroid Rosetta constraint file]

To further simplify the centroid constraints (not necessary step):
cat [centroid Rosetta constraint file] | perl toolbox/remove_below_master.pl | perl toolbox/simplify_centroid_csts.pl > [simplified centroid Rosetta constraint file]

All scripts within the folder “toolbox” are provided in Supplementary Software 1.

Steps (1) and (2) are executed only when an initial homology model of the system is available. Otherwise, the assignments are initialized manually using the “anchor points” described in the main text, and the iterative procedure stars from step (3).

The scripts can be adapted for use with any given symmetry type and number of subunits (more details available in the file headers).

Examples of input and output file formats:

[raw constraint table] – also available as supporting data
A33CA-K53CD
A33CA-L37CA
A33CA-L37CG
A33CA-S52CA
A33CB-Y50CB
A36CA-L46CB

[Rosetta constraint file] – also available as supporting data

```
AtomPair    CD    1215   CA    1195   BOUNDED 1.550 9.000 0.300 NOE; A33CA-K53CD 0
AtomPair    CA    1199   CA    1195   BOUNDED 1.550 9.000 0.300 NOE; A33CA-L37CA 0
AtomPair    CG    1199   CA    1195   BOUNDED 1.550 9.000 0.300 NOE; A33CA-L37CG 0
AtomPair    CA    1214   CA    1195   BOUNDED 1.550 9.000 0.300 NOE; A33CA-S52CA 0
AtomPair    CB    1212   CB    1195   BOUNDED 1.550 9.000 0.300 NOE; A33CB-Y50CB 0
AtomPair    CB    1208   CA    1198   BOUNDED 1.550 9.000 0.300 NOE; A36CA-L46CB 0
```

II) Rosetta3[1] steps and flags for running the structure calculations:

1) Compute CS-derived 3mer and 9mer backbone fragments from the PDB, as outlined in detail previously[2].

2) Obtain a high-resolution EM density map form the EMDB.

3) Create a symmetry definition file containing the symmetry type and degrees of freedom[3].

4) Run the Rosetta fold-and-dock protocol[4] using EM[5] and NMR constraints:

minirosetta.static.linuxgccrelease @[flag file] -out:file:silent decoys.out

The flag file is an ASCII file containing the calculation parameters and input files:

-run:protocol broker
-broker:setup setup_init.tpb
-database [path of Rosetta database folder]
-nstruct 100
-in:file:fasta [fasta sequence of the monomeric subunit]
-symmetry_definition **[symmetry definition file]**
-file:frag3 **[3mer fragment file]**
-file:frag9 **[9mer fragment file]**
-out:file:silent_struct_type binary
-fold_and_dock::rotate_anchor_to_x
-rg_reweight 0.001
-abinitio:increase_cycles 0.02
-rigid_body_cycles 1
-abinitio::recover_low_in_stages 0
-rigid_body_disable_mc
-run:reinitialize_mover_for_each_job
-use_incorrect_hbond_deriv false
-fail_on_bad_hbond false
-ignore_unrecognized_res
-rigid_body_frequency 0.2
-residues:patch_selectors CENTROID_HA
-constraints:cst_weight 3.0
-constraints:cst_file **[centroid Rosetta constraint file]**
-relax:fast
-default_max_cycles 200
-relax:default_repeats 2
-relax:jump_move true
-constraints:cst_fa_file **[fullatom Rosetta constraint file]**
-constraints:cst_fa_weight 0.1
-score:patch patch_relax
-edensity:mapfile **[EM density map, in standard EMDB format]**
-edensity:mapreso 10
-edensity:grid_spacing 5
-edensity:whole_structure_ca_wt 0.1
-edensity:score_symm_complex true

,where the file setup_init.tpb is an ASCII file containing the statements:

CLAIMER FoldandDockClaimer
END_CLAIMER

Information on downloading and compiling ROSETTA3 can be found at: www.rosettacommons.org

III) Sparky extension module to display the resonance frequency of corresponding cross-peaks for intra-residue, sequential or all correlations:

A suitable set of scripts to perform this task is provided in Supplementary Software 1.
To use, copy the three SPARKY files to your %SPARKY_HOME%\python\sparky\ directory and follow the instructions provided in the header of each file.

Supplementary References

1. Leaver-Fay, A., Tyka, M., Lewis, S. M., Lange, O. F., Thompson, J., Jacak, R., Kaufman, K., Renfrew, P. D., Smith, C. A., Sheffler, W., Davis, I. W., Cooper, S., Treuille, A., Mandell, D. J., Richter, F., Ban, Y. E., Fleishman, S. J., Corn, J. E., Kim, D. E., Lyskov, S., Berrondo, M., Mentzer, S., Popovic, Z., Havranek, J. J., Karanicolas, J., Das, R., Meiler, J., Kortemme, T., Gray, J. J., Kuhlman, B., Baker, D., Bradley, P. ROSETTA3: an object-oriented software suite for the simulation and design of macromolecules. *Methods Enzymol* **487**, 545-74 (2011).
2. Vernon, R., Shen, Y., Baker, D., Lange, O. F. Improved chemical shift based fragment selection for CS-Rosetta using Rosetta3 fragment picker. *Journal of Biomolecular Nmr* **57**, 117-27 (2013).
3. DiMaio, F., Leaver-Fay, A., Bradley, P., Baker, D., Andre, I. Modeling symmetric macromolecular structures in Rosetta3. *PLOS One* **6**, e20450 (2011).
4. Das, R., Andre, I., Shen, Y., Wu, Y., Lemak, A., Bansal, S., Arrowsmith, C. H., Szyperski, T., Baker, D. Simultaneous prediction of protein folding and docking at high resolution. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 18978-83 (2009).
5. DiMaio, F., Tyka, M. D., Baker, M. L., Chiu, W., Baker, D. Refinement of Protein Structures into Low-Resolution Density Maps Using Rosetta. *J. Mol. Biol.* **392**, 181-90 (2009).