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Supporting information for article:

Fixed-target serial femtosecond crystallography using *in cellulo* grown microcrystals

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Table S1 Interactions of HEX-1 monomers forming the crystal lattice.

Group1	Monomer 1	Distance	Monomer 2	Type
I	ARG 41[NH1]	3.57	ASP 44[OD1]	Salt bridge
I	ARG 41[NH2]	2.46	ASP 44[OD1]	Salt bridge
I	ARG 41[NH1]	3.04	ASP 44[OD2]	Salt bridge
I	ARG 41[NH2]	3.50	ASP 44[OD2]	Salt bridge
I	HIS 39[NE2]	3.59	ASP 44[OD2]	Salt bridge
I	ASP 44[OD1]	3.57	ARG 41[NH1]	Salt bridge
I	ASP 44[OD1]	2.46	ARG 41[NH2]	Salt bridge
I	ASP 44[OD2]	3.04	ARG 41[NH1]	Salt bridge
I	ASP 44[OD2]	3.50	ARG 41[NH2]	Salt bridge
I	ASP 44[OD2]	3.59	HIS 39[NE2]	Salt bridge
I	ARG 41[HH12]	2.26	ASP 44[OD2]	H bond
I	GLN 105[H]	1.95	SER 92[O]	H bond
I	GLU 121[H]	2.28	SER 92[OG]	H bond
II	ARG 68[NE]	3.61	GLU 81[OE1]	Salt bridge
II	ARG 68[NH2]	2.87	GLU 81[OE1]	Salt bridge
II	ARG 68[NE]	2.61	GLU 81[OE2]	Salt bridge
II	ARG 68[NH2]	3.47	GLU 81[OE2]	Salt bridge
II	GLU 81[OE1]	3.61	ARG 68[NE]	Salt bridge
II	GLU 81[OE1]	2.87	ARG 68[NH2]	Salt bridge
II	GLU 81[OE2]	2.61	ARG 68[NE]	Salt bridge
II	GLU 81[OE2]	3.47	ARG 68[NH2]	Salt bridge
II	SER 84[OG]	2.92	GLN 49[OE1]	H bond
II	GLN 49[OE1]	2.92	SER 84[OG]	H bond
III	ILE 56[N]	2.56	GLN 127[OE1]	H bond
III	GLN 134[HE22]	2.28	VAL 125[O]	H bond

Interactions were identified using the PDBePISA server (Krissinel & Henrick, 2007 www.ebi.ac.uk/pdbe/pisa/);

¹ as defined by (Yuan *et al.*, 2003).

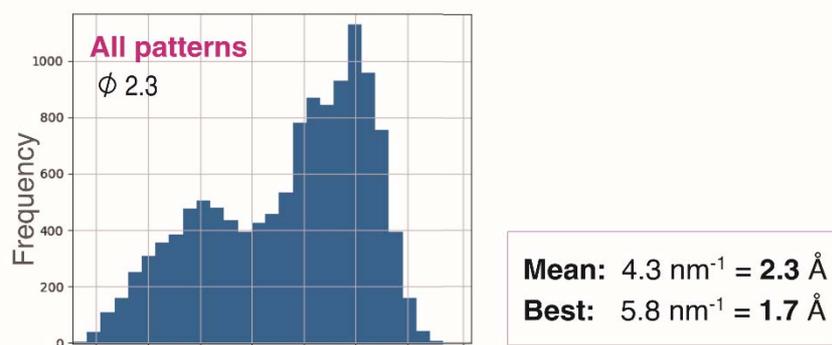
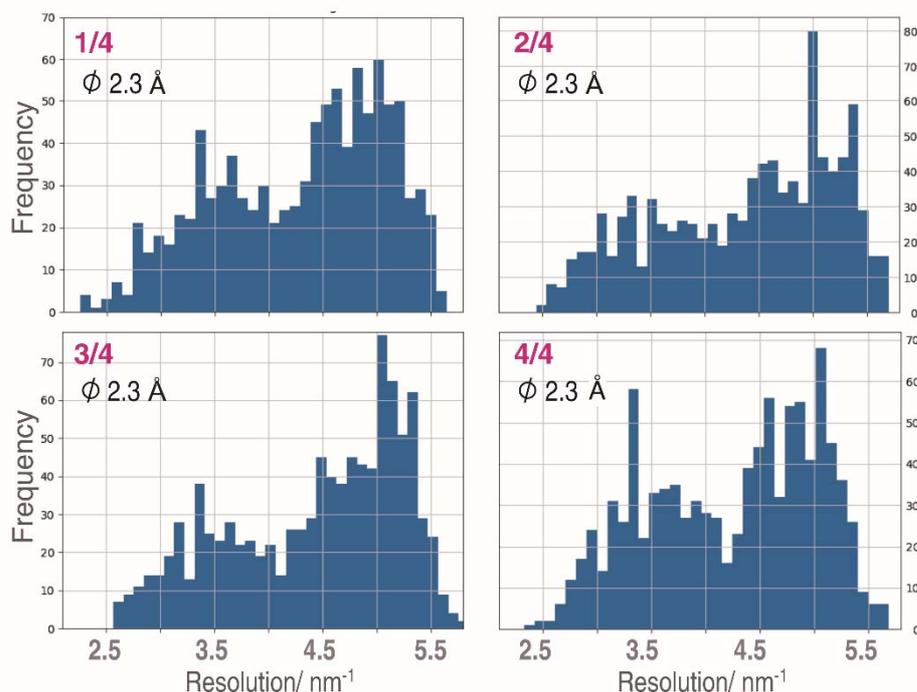
(a) Resolution based on all indexing results**(b) Resolution based on indexing results in quarters**

Figure S1 Histograms of the average resolution based on indexing results as determined by CrystFEL. Resolution in nm⁻¹ is shown on the x-axis, frequency of patterns with detected Bragg-peaks to this resolution-shell are presented on the y-axis. The distribution of resolution based on all indexing results were extracted from (a) the final streamfile including patterns from chip 1 and chip 2 after stream-grep. (b) Then, the streamfile with indexing results from diffraction patterns collected with chip 1 was ordered according to the sequence of data collection and sequentially cut in quarters with truncate-stream of the CrystFEL suite, mean and best resolutions were determined with ave-resolution as before. The average resolution of 2.3 Å and the best resolution of 1.7 Å remain the same throughout the quarters of diffraction data collection.

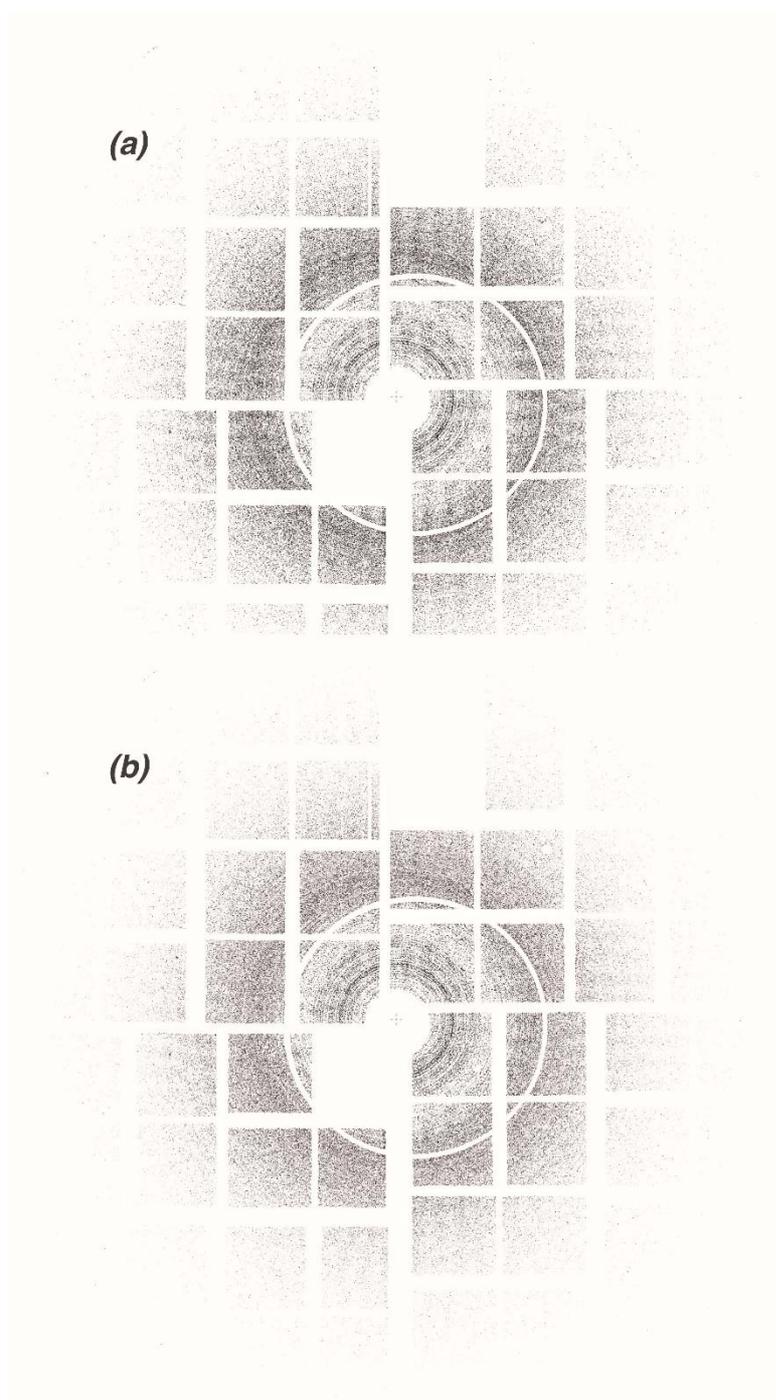


Figure S2 Virtual powder pattern of FT-SFX HEX-1 *in-cellulo* crystal diffraction does not show a preferred orientation of the crystals. Depicted is the sum of all Bragg peaks found by *indexamajig* of the CrystFEL suite, (a) of indexed diffraction patterns resulting from measuring one chip, as opposed to (b) of indexed diffraction patterns resulting from the measurements on two chips. CSPAD panels and the non-centrosymmetric shadow of the goniometer setup are masked.

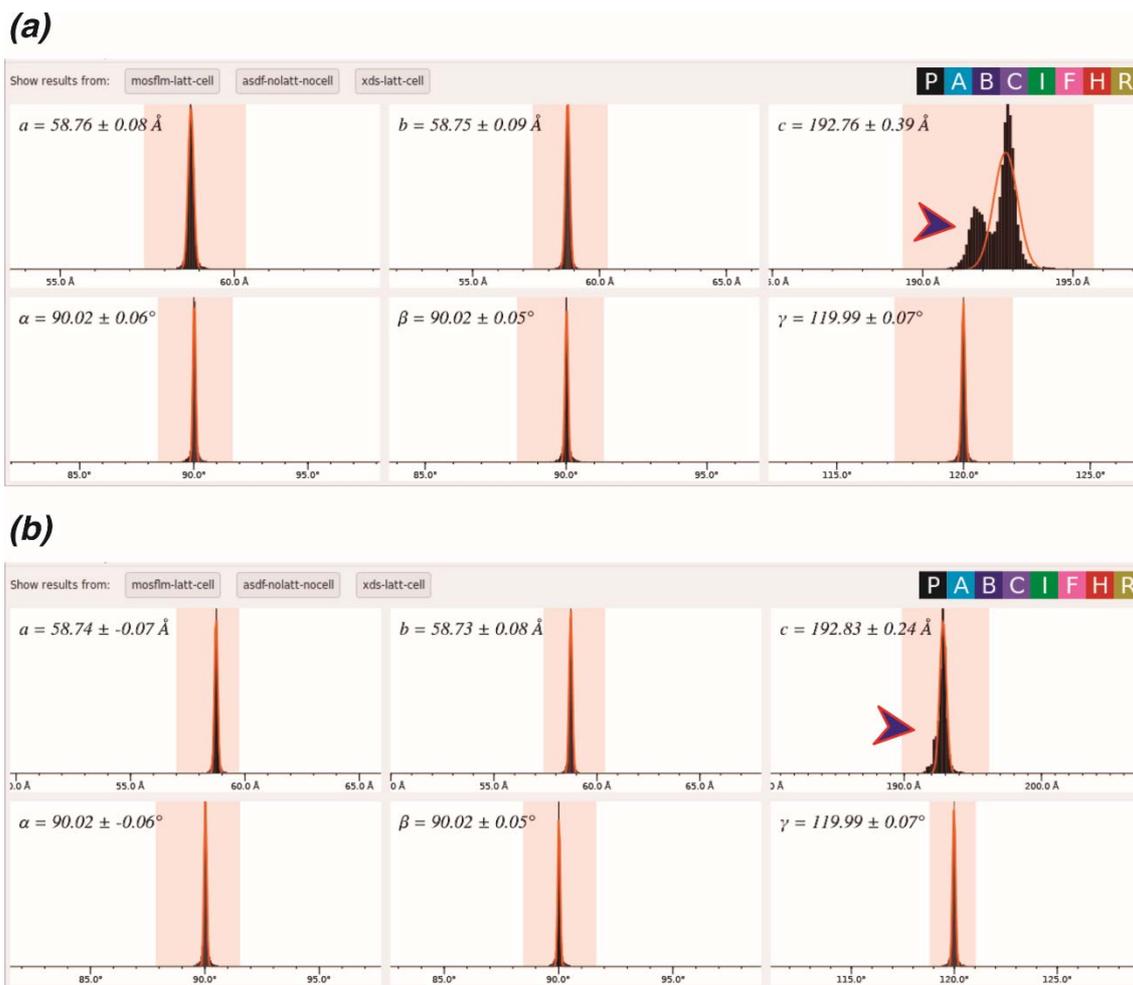


Figure S3 Cell parameter histogram of HEX-1 before (a) and after (b) stream-grep. Indexing algorithms mosflm, asdf and XDS were used, allowing the detection of multiple lattices in one pattern. Two distinct populations are visible with cell-parameter c of 192.76 \AA and a smaller one $c = 191.83 \text{ \AA}$, highlighted with arrows. The graphics were made with the *cell_explorer* script of the CrystFEL suite.

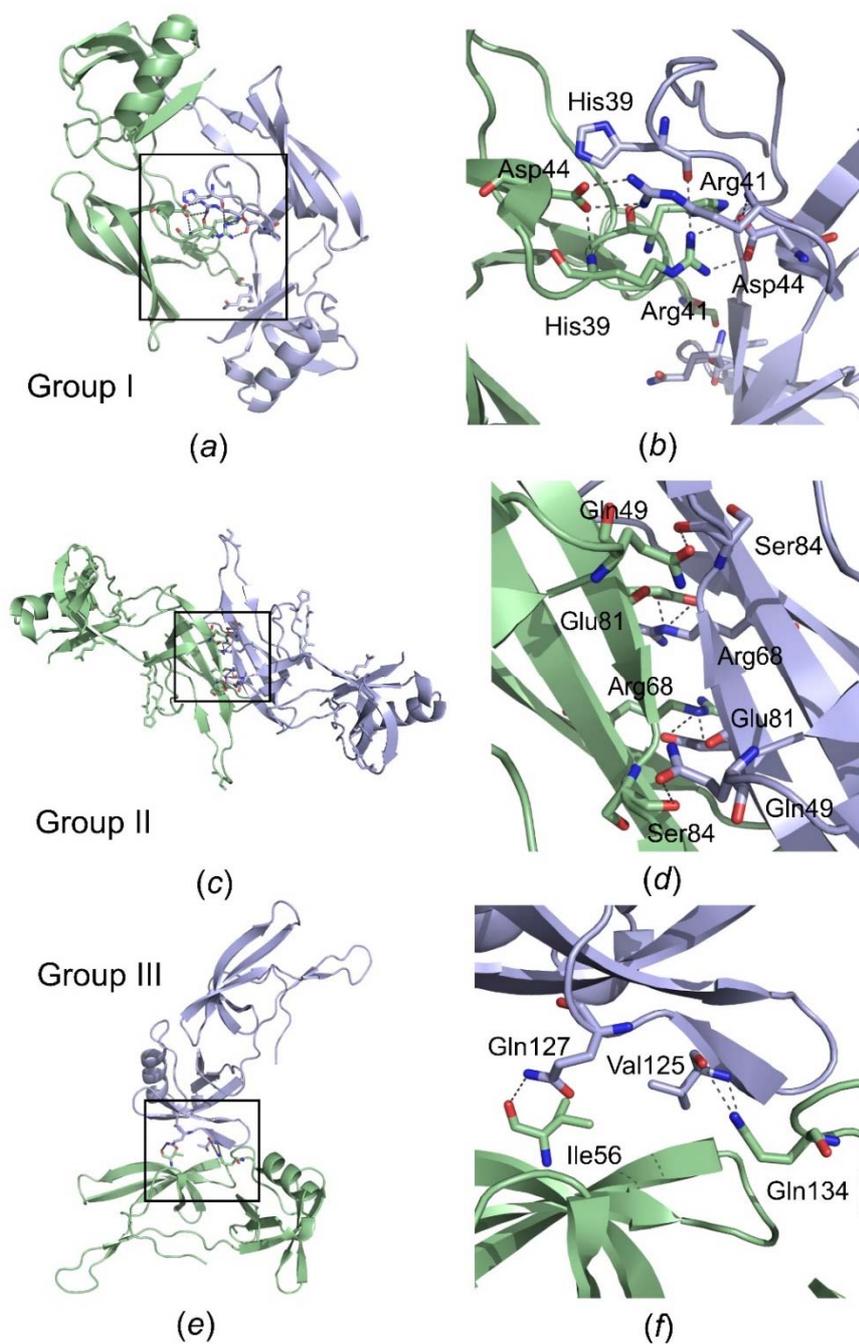


Figure S4 Intermolecular interactions between HEX-1 molecules. Overall views of interacting molecules in blue and green are shown in the left panels and magnified views in the right panels. (*a*, *b*) Group I interactions include the central N-terminal salt bridges between Arg41 and Asp44 stabilized by His39 and N- to C-terminal domain contacts. (*c*, *d*) Group II interactions link the N-terminal domains by salt bridges between Arg68 and Glu81 as well as flanking hydrogen bonds formed by Ser84/Gln49 and Ser61/Glu81. (*e*, *f*) Interactions of the C-terminal amino acids Gln127 and Val125 of one molecule with the N-terminal Ile56 and C-terminal Gln134 of the other molecule are classified as group III interactions. Definitions of the interaction classes as previously described in Yuan et al.(2003).

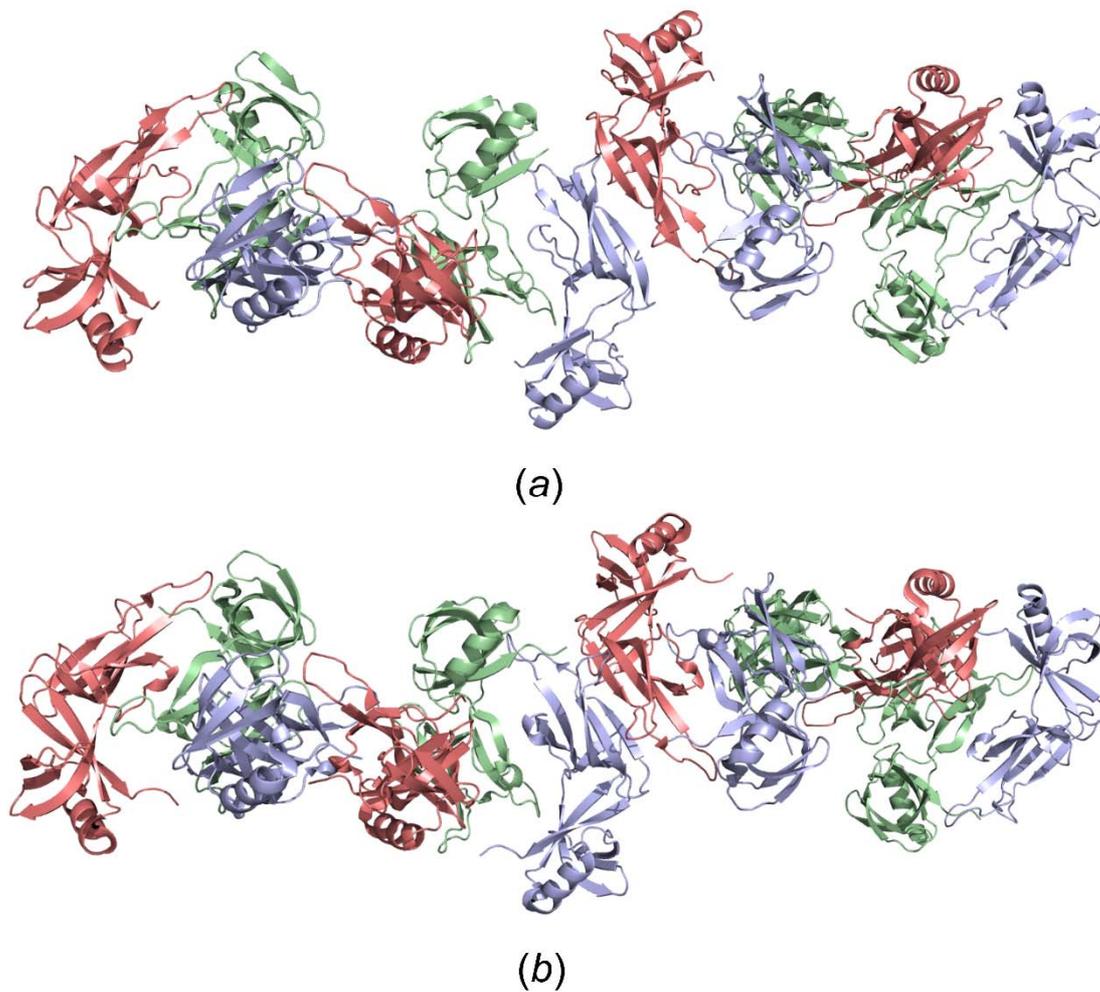


Figure S5 Molecular assembly of HEX-1 forming the crystal lattice. (a) A coiled filament characterized by 12 alternating HEX-1 molecules per turn (shown in blue, green, and red) is formed by group I and group II interactions, almost superimposable to that previously described by Yuan *et al.* (2003; PDB 1KHI) (b).