Chemical Science

Supplementary

Using a biomimetic membrane surface experiment to investigate the activity of magnetite biomineralisation protein Mms6.

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Supplementary information.

Mms6 protein sequence (cys-Mms6)

The amino acid sequence of the mature form of Mms6 (Mms6 sequence available at Uniprot with identifier Q2W8R5) protein produced for these experiments:

MGSHHHHHHHHGSTENLYFQGCPRMGGTIWTGKGLGLGLGLGLGAWGPIILGVVGAGAVYAYMKSRDIESAQSDEEVELRDALA

The *N*-terminus of the protein features the additional sequence MGSHHHHHHHHGSTENLYFQGCPRM which comprises an octa-histidine tag for purification, followed by a TEV cleavage site and single cysteine residue for Au attachment. The glycine leucine repeat motif is highlighted.

Peptide based on the acidic region of Mms6 sequence (cys-pep)

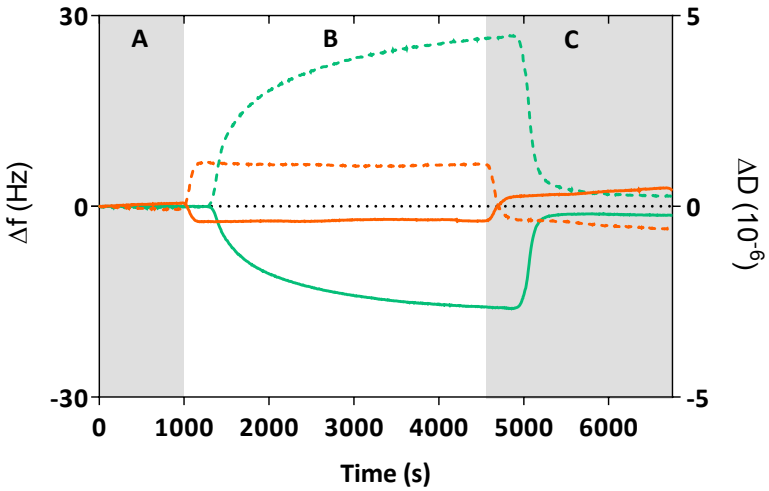
The amino acid sequence included an *N*-terminal cysteine residue and a flexible glycine serine linker:

C-GGS-KSRDIESAQSDEEVELRDAL

Sequences that are present in both cys-Mms6 and cys-pep are highlighted in yellow.

Table 1. Visoelastic properties measured with QCM-D of cys-Mms6 and cys-pep adsorbed onto clean gold crystals.[a]

Voigt Values	Cys-Mms6	Cys-pep
Viscosity (kg m^{-1} s^{-1})	0.0015	0.0012
Shear (MPa)	2.20	2.56
Thickness (nm)	2.8	0.9

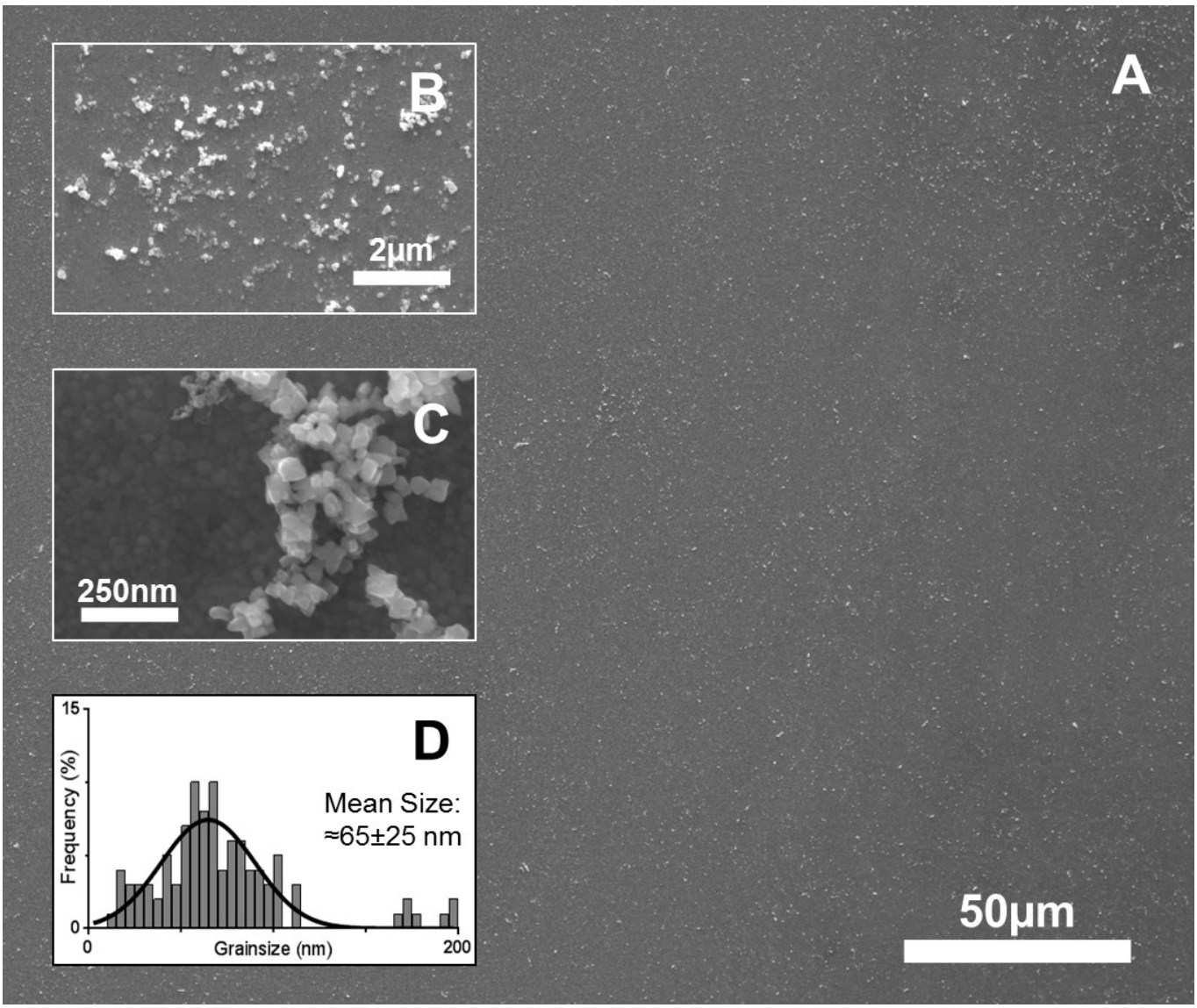


Supplementary fig. 1: Frequency (Δf, solid lines) and dissipation (ΔD, dashed lines) changes of the 7th overtone recorded with QCM-D during adsorption of cys-Mms6 (green) or cys-pep (orange) onto PEG coated gold quartz crystals. Grey regions (A and C) show when a Milli-Q water buffer was applied, and the white region (B) show when a PBS buffer containing cys-Mms6 or cys-pep at a concentration of 10 µg mL^{-1} was applied (flow rate 50 µL min^{-1}). This analysis finds that there was only limited binding to the protein resistant PEG surfaces, and that the mass of the PEG coated gold crystal reduced when it was exposed to cys-pep (this is probably an artefact introduced by residual frequency drift during the experiment).

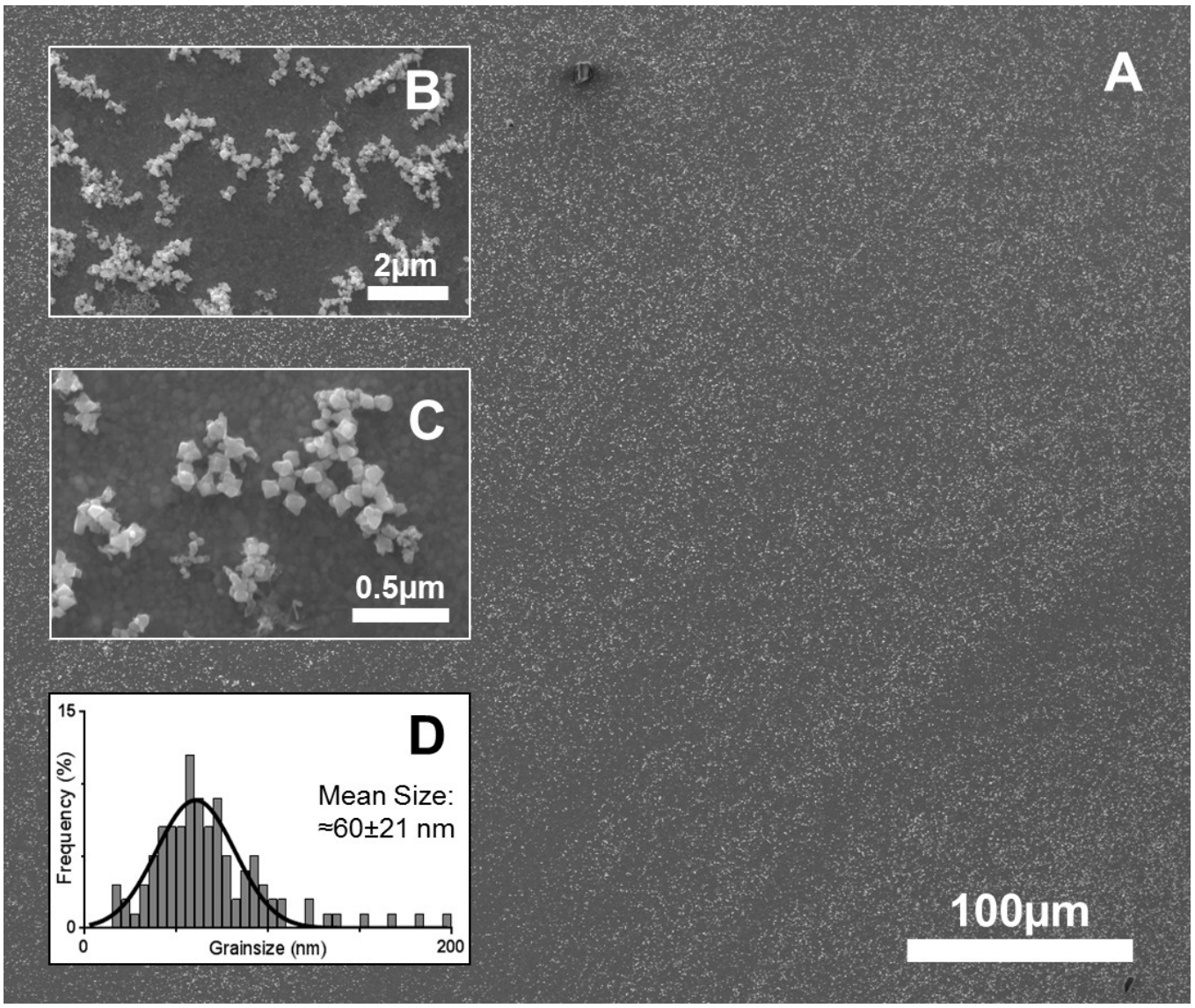
Table 2. The mass coverage measured with QCM-D of cys-pep adsorbed onto PEG coated gold crystals.[a]

Sauerbrey Values	Cys-Mms6	Cys-pep
Mass (ng cm^{-2})	30	-19.63
Coverage (pmol cm^{-2})	3	-7.55
Complete Monolayer (pmol cm^{-2})	≈24	≈83
Coverage (%)	≈13	≈-9

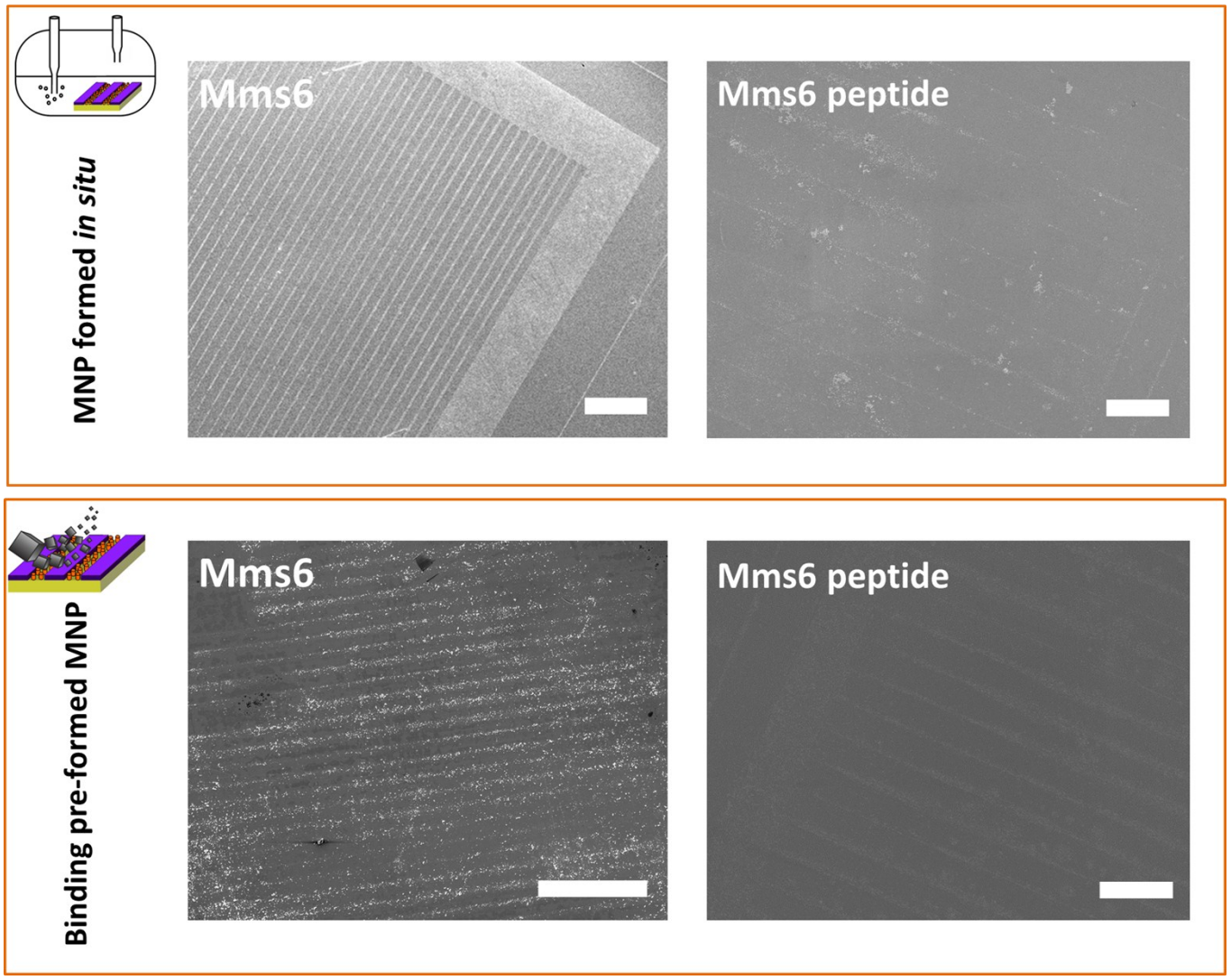
[a] All modelling was performed with QTools 2 Qsense software. Sauerbrey values were calculated from the 7th overtone, and Voigt values were calculated using all the recorded overtones (3rd, 5th, 7th, 9th, 11th, and 13th).



Supplementary fig. 2: SEM images of a gold surface with a complete surface of cys-pep (with no PEG SAM or surface patterning) after a POFHK reaction (A, B and C). A low density coverage of MNPs were found to form on the surface, with grainsize analysis (D) finding these particles had a mean size of ≈65±25 nm. Particle sizing histograms are shown with Gaussian fitting performed in GraphPad Prism.



Supplementary fig. 3: SEM images of a gold surface with a complete surface of cys-pep (with no PEG SAM or surface patterning) after being supplied with preformed magnetite nanoparticles (A, B and C). A low density coverage of MNPs were found to form on the surface, with grainsize analysis (D) finding these particles had a mean size of ≈60±21 nm. Particle sizing histograms are shown with Gaussian fitting performed in GraphPad Prism.



Supplementary fig. 4: Lower magnification SEM images of the surfaces displayed in Fig. 5. Scale bars are 100 µm.

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                      b   d   f
Assembly motif     |-------------|
Q2W8R5 mms6                                  -  --
Q9GUB5(fibroin)
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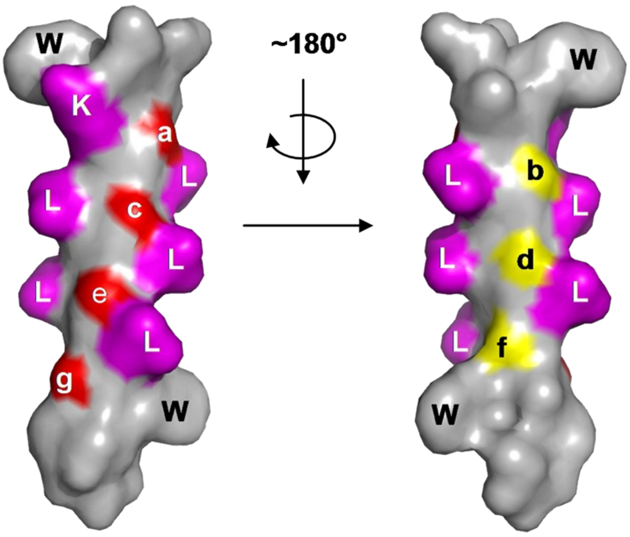
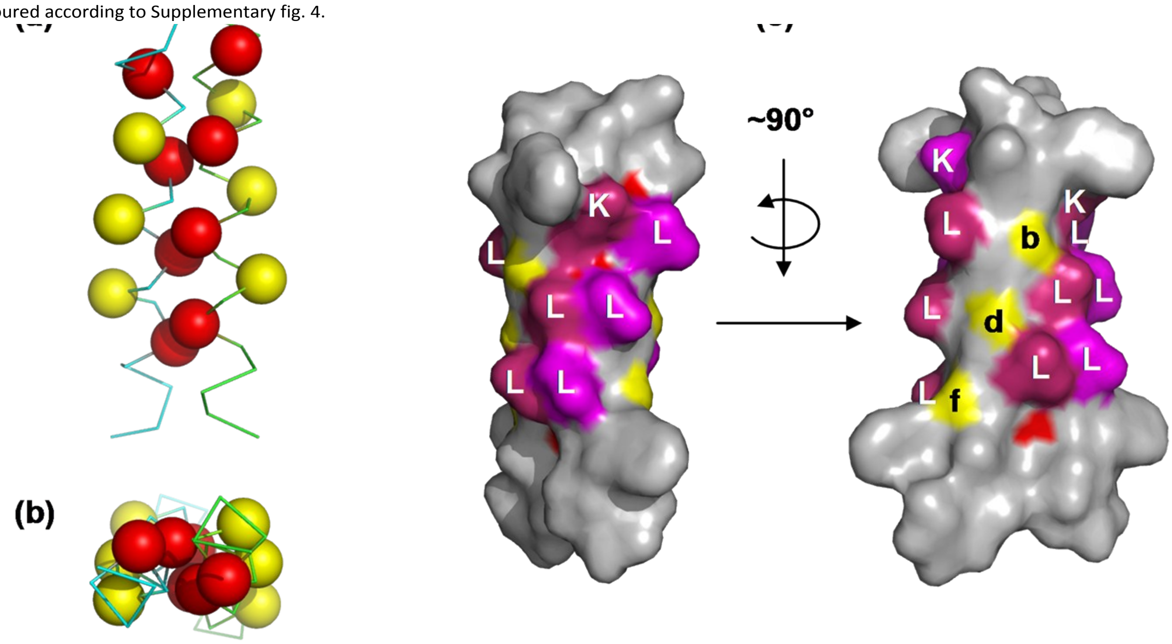
Supplementary fig. 5: Alignment of the sequence region containing the “GL repeat” motif of Mms6 with a similar sequence from fibroin. Conserved residues are indicated with an asterisk.



Supplementary fig. 6: Views of the two faces of the putative assembly motif of Mms6 shown as a molecular surface representation of an ideal α-helix. Residues a-g coloured according to Supplementary fig. 4.



Supplementary fig. 7: Model of parallel dimer of GL motif of Mms6. (a and b) side and end-on views respective of the dimer, represented as a ribbon, with the alpha carbon atoms of the conserved glycine residues shown in solid molecular representation, coloured as detailed in Supplementary fig. 4. (c) Views of two faces of the dimer represented as a molecular surface, with colouring as in Supplementary fig. 5 except that the bulky hydrophobic side-chains of the two protomers are shown in magenta and deep pink respectively.

Supplementary methods.

The Mms6 "assembly motif" (Supplementary fig. 5) was chosen for further analysis. It was made into an ideal α-helix using Swiss-PdbViewer[1] then examined in Pymol[2]. As shown in the molecular surface representation (Supplementary fig. 5), if the sequence adopts a helical conformation, then the glycine residues shown in red and yellow would form holes on opposite sides of the helix, with leucine sidechains forming knobs. Two or more Mms6 proteins might associate via a knobs-into-holes packing with a parallel arrangement of the helices. The HexServer (http://hexserver.loria.fr/)[3] was used to dock two monomers together, which yielded 100 dimer models all of which had a parallel arrangement. The model which was ranked highest is shown in supplementary fig. 6 and displays close packing of the glycine residues of the motif.

Supplementary references.

1. N. Guex and M. C. Peitsch, *electrophoresis*, 1997, 18, 2714-2723.
2. W. DeLano, The PyMOL Molecular Graphics System; DeLano Scientific: San Carlos, CA, 2002.
3. G. Macindoe, L. Mavridis, V. Venkatraman, M.-D. Devignes and D. W. Ritchie, *Nucleic Acids Research*, 2010, 38, W445-W449.