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Supporting information for article:

Expression, purification and crystallization of a protein resulting from the inversion of the amino-acid sequence of a helical bundle

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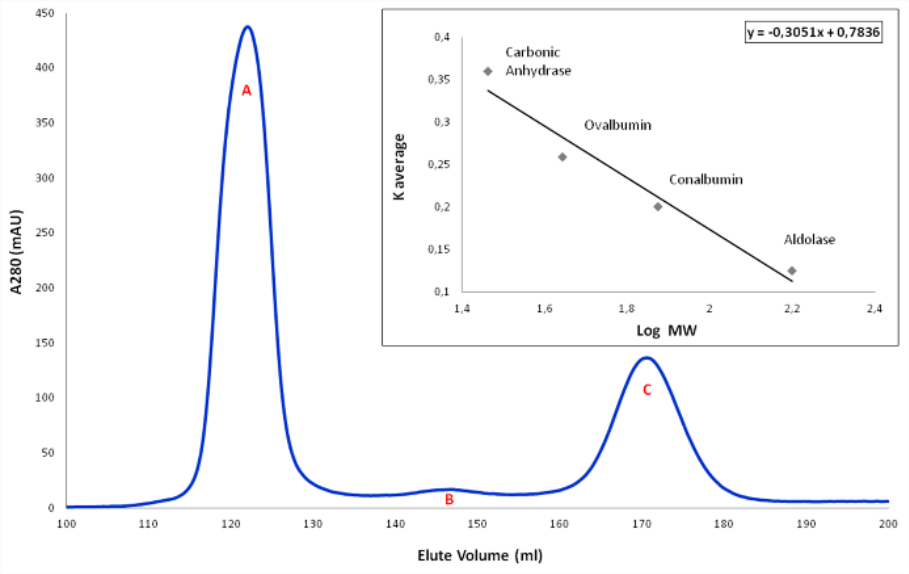

Figure S1 Chromatographic purification of revRM6 using a Sephacryl S-200 gel filtration column. The column was run at 1.0 ml/min in 25 mM Tris-HCl pH 8.0, 100 mM NaCl and 15 mM β-mercaptoethanol. Gel filtration was preceded by a purification step of His-tag affinity chromatography using a Ni–NTA column. The standard curve of the Sephacryl S-200 column is given in the inset, with $k_{average}=(V_e-V_o)/(V_t-V_o)$, where V_e is the elute volume, V_o=121.2 ml and V_t= 314.1 ml. The revRM6 protein elutes with peak C as shown in Fig. S2. Deviations of the molecular weight of the tetrameric (shown in Fig. S3) molecule as estimated by the standard curve, from the value calculated on the basis of its amino acid sequence probably reflect a non-globular structure adopted by revRM6 (Fig.S3).

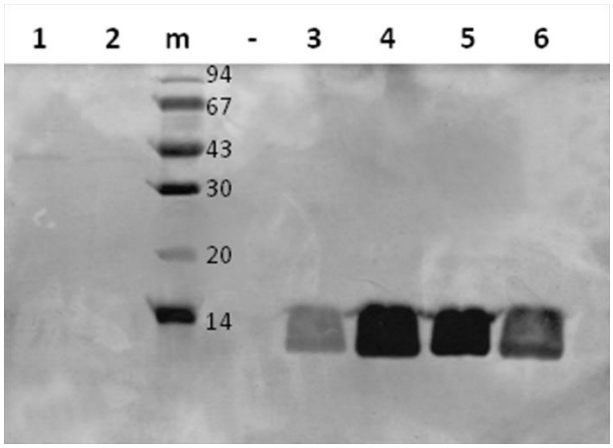

Figure S2 Analysis of the eluates of the three gel filtration peaks (A, B, C) shown in Fig. S1 using a 12% SDS-PAGE gel. Lanes 1, 2 correspond to peaks A, B respectively, lanes 3-6 to peak C and contain the revRM6 protein. The molecular weight markers (m) used are from GE Healthcare.

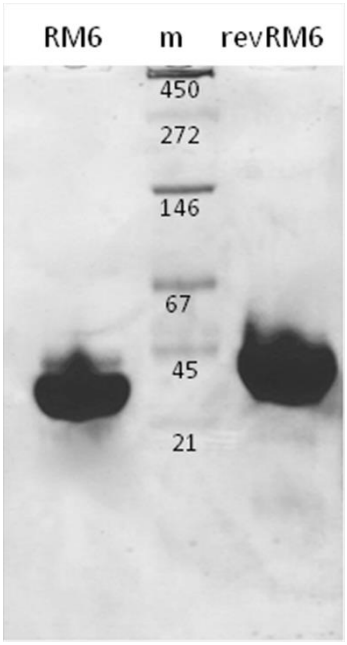

Figure S3 Native gel of purified RM6 and revRM6. For the molecular weight markers (m) the SERVA Native Marker Liquid Mix was used. Both proteins migrate similarly through the gel. Since RM6 is a homotetramer, this suggests that revRM6 also migrates as a tetramer. Deviations of the native gel-based molecular weight estimations for the His-tagged revRM6 and RM6 proteins from the values calculated from their amino acid sequences (7.5 kDa for monomer/ 30 kDa for tetramer), probably arise from a non-globular conformation, comparable to the one determined for RM6 by X-ray crystallography (Glykos *et al*., 2006).

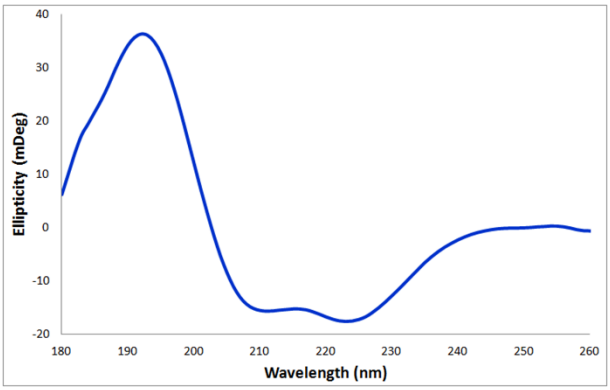

Figure S4 Circular dichroism spectrum for revRM6 recorded for a protein concentration of 14 mg ml^{-1}, in 25 mM Tris buffer, pH 8.0, 100 mM NaCl and 15 mM β-mercaptoethanol, at 293 K. The CD spectrum is characteristic of a highly α-helical protein. The measurement was performed using the synchrotron radiation beamline DISCO at the SOLEIL Synchrotron (Paris).

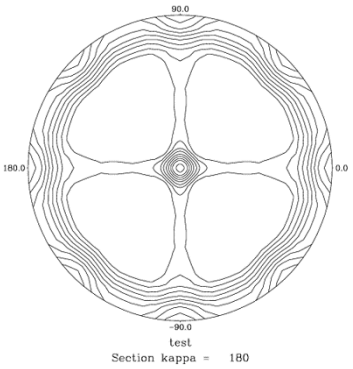

Figure S5 κ= 180° section of the self-rotation function of the revRM6 dataset. The calculation was performed with POLARRFN program (Kabsch *et al.*, 1976) from the CCP4 package, with a contour level of 10% and an increment of 10%.

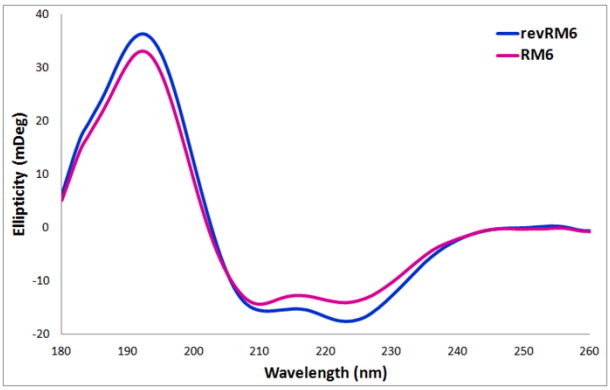

Figure S6 Comparison of the CD spectra for RM6 and revRM6 at 293 K. For both proteins the spectra were recorded at a concentration of 14 mg ml^{-1}, 25 mM Tris buffer, pH 8.0, 100 mM NaCl and 15 mM β-mercaptoethanol. The similarity of the spectra which are characteristic of highly α-helical proteins, suggests similar secondary structures for RM6 and revRM6. The measurements were performed using the synchrotron radiation beamline DISCO at the SOLEIL Synchrotron (Paris).