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Circular dichroism

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MATERIALS

Synchrotron radiation circular dichroism (SRCD) experiments were carried out at 15 °C on the DISCO beamline (SOLEIL synchrotron). D-10-camphorsulfonic acid was used to calibrate the SRCD signal using the CDtool software. Spectra were obtained using calcium fluoride circular cells (Hellma) of 50 mm pathlength. They were loaded with ZitR_{MG} protein (8 µg) in 20 mM Tris-HCl (pH 7.0), 50 mM NaCl and 100 µM ZnSO₄ buffer. Acquisitions at 1 nm step per second between 170 to 305 nm were recorded in triplicates. Averaged spectra were corrected with respect to the baseline by buffer subtraction and set to zero in the 300 - 305 nm region. Standard circular dichroism (CD) measurements were carried out at 20 °C on a JASCO J-810 spectropolarimeter. Temperature was controlled by a Peltier (Jasco PFD423S/L) (biophysics platform, LEBS/IMAGIF). Spectra from 185 to 260 nm were obtained using a 100 mm pathlength suprasil quartz cell (Hellma) containing ZitR_{MG} protein (400 mg) in 20 mM Tris-HCl (pH 7.0), 50 mM NaCl and 100 µM ZnSO₄ buffer. All data processing was performed using CDtool software and secondary structure prediction of ZitR_{MG} protein was carried out on Dichroweb server (<http://dichroweb.cryst.bbk.ac.uk>) using all available algorithms (CONTINLL, SELCON3, CDSSTR and K2D3) and all sets of proteins (database 1-7, SP175, SMP180).

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