HECW2

PDB:2LFE
Entry Clone Accession:hecw2.BC117194.OBS.MHS1768-98081163.pCR-XL-TOPO
Entry Clone Source: OpenBiosystems
SGC Clone Accession:hecw2.0043-0162.192A09 (SDC192A09)
Tag:MHHHHHHSSGRENLYFQG
Host: Competent BL21 (DE3) cells (Invitrogen, C6000-03)
Vector:pET28MHL vector (GenBank, EF456735)

Sequence:

LQRANSDTDLVTSESRSSLTASMYEYTLGQAQNLIIFWDIKEEVDPSDWIGLYHIDENSPANFWDSKNRGVTGTQKGQIVWRIEPGP
YFMEPEIKICFKYYHGISGALRATTPCITVKNP

Growth

Medium: M9 minimal media (100 uM $ZnSO_4$, 8.55 mM NaCl, 47.6 mM Na_2HPO_4, 22 mM KH_2PO_4, 100 mM $MgSO_4$, 2 mM biotin, 1.5 mM thiamine.HCl, 10 mM $ZnSO_4$, and 0.1 M $CaCl_2$) supplemented with $^{15}NH_4Cl$, $^{13}C_6$-D-glucose, and 50 µg/ml kanamycin

Procedure: Competent BL21 (DE3) cells (Invitrogen, C6000-03) were transformed and incubated overnight (18 hours) at 220 rpm at 37 °C in a 125 ml flask containing 50 ml of growth medium. The overnight starter culture was transferred to a 2 L flask containing 1 L of growth medium, and incubated at 37 °C. When the OD_{600} reached a value of 1.0, protein expression was induced with 100 µM isopropyl-thio-ß-D-galactopyranoside and the cells were incubated overnight (15.5 hours) at 220rpm at 15 °C. Cell pellets were collected by centrifugation (7000 rpm, 20 mins) and frozen in 50 mL Falcon tubes at -80 °C for storage.

Purification

Procedure:
The lysate was clarified by centrifugation for 20 min at 4 oC. The supernatant was mixed with 2 mL of Ni^{2+} affinity beads per 40 mL lysate. The mixture was incubated with mixing for 20 minutes at 4 oC. The lysate was spun at 2000 rpm for 6 minutes, and the supernatant was decanted. The remaining resin was resuspended and washed twice with lysis buffer, followed by two washes with with 5 mL of cold wash buffer. The washed resin was transferred to a gravity filter column and further washed with 2 mL of wash buffer. Samples were eluted from the resin by exposure to 5mL of elution buffer.

The purified protein was exchange from elution buffer into MOPS-based NMR buffer by ultracentrifugation using 5 mL concentrators with a 5,000 molecular weight cut-off (VivaSpin 2

MES) at 3000 rpm, resulting in a final volume of 300ul (final protein concentration of 0.9 mM). The concentrated protein was transferred to a 5mm Shigemi NMR tube.

Extraction

Procedure:
The frozen cell pellet stored in a 50 ml Falcon tube obtained from 1L of culture was thawed by soaking in warm water, and resuspended in 40 mL lysis buffer. The cell pellet was lysed by sonication (Branson Sonicator, probe catalog #) on ice for 10 minutes total sonication time (10 sec pulses at half-maximal frequency with 10 second rest)].
Concentration:0.9 mM

Structure Determination

NMR Spectroscopy:A series of spectra (3D 1H-13C NOESY, 3D 1H-15N NOESY, 2D 1H-13C Constant Time HSQC, 3D HNCO, 3D HNCA, 3D CBCA(CO)NH, 3D HBHA(CO)NH, 3D 1H-13C Aromatic NOESY, 3D (H)CCH-TOCSY, and 3D H(C)CH-TOCSY) were generated using a 600MHz Bruker AVANCE spectrometer and a 800MHz Bruker AVANCE spectrometer. NMR data was processed and analyzed using Topspin, NMRPipe, NMRDraw, Sparky, FMC-GUI, CYANA, CNS, TALOS, and PSVS.