

Structure	MeCP2
PDB Code	6OGK
Entry clone accession	BC011612.1
Entry clone source	AU51-B9
Tag	C-terminal hexahistidine tag: AAHHHHHH
Construct sequence	ASASPKQRRIIIRDGRGPMYDDPTLPEGWTRKLKQRKSGRSAGKYDVYLINPQGKAFRSKVELI AYFEKVGDTSLDPNDFDFTVTGRGSPSRAAHHHHHH
Vector	pNIC-CH
Expression host	<i>Escherichia coli</i> BL21 (DE3)-RIL strain
Growth medium	TB A fresh transformation was used to inoculate 50 mL LB media containing 50 µg/mL kanamycin and 30 µg/mL Chloramphenicol. The culture was grown overnight at 37°C with shaking. The next day this starter culture was used to inoculate 2L of TB growth medium. The culture was grown in LEX at 37°C to OD <sub>600</sub> of 1.0.
Growth method	IPTG-based induction was carried out according to the manufacturer's protocol (final concentration for IPTG is 0.35 mM). The temperature was reduced to 16°C and the culture was incubated for a further 18 hours before harvesting the cells.
Extraction buffers	<b>Lysis buffer:</b> 20 mM Tris-HCl, pH 7.5, 500 mM NaCl, 0.5 mM PMSF and 5% glycerol Cells were harvested by centrifugation and pellets were stored in -80°C. Prior to purification,
Extraction procedure	the cell pellet was resuspended in lysis buffer. Cells were disrupted by sonication (10 minutes) and samples were centrifuged for 60 min at 70000 g.
Purification buffers	<b>NiNTA Elution buffer (EB):</b> 20 mM Tris-HCl, pH 7.5, 500 mM NaCl and 300 mM imidazole <b>Gel Filtration buffer:</b> 20 mM Tris-HCl, pH 7.5 and 150 mM NaCl, 1mM DTT. Column 1: Affinity purification, open Ni-NTA column Procedure: The supernatant was incubated with 4mL of 50% slurry Ni-NTA beads by rocking. After 30 min incubation at 4°C, the beads were
Purification procedure	washed with 50 mL of lysis buffer with additional 10 mM imidazole. The protein was eluted using ~20 mL EB. The Column 2: Gel Filtration (Superdex S75 16/60 Hi-Load, GE Healthcare). Then the fractions containing protein were identified on a SDS-PAGE gel.
Protein stock concentration	10mg/ml.
Crystallization	50% precipitant mix 4, 0.1M buffer system 1, 0.06M divalents (morpheus A4) (from screen MD1-46)
Data collection	