

Supplemental Materials

cDNA fragments encoding human MCM2 (aa 63-154) and ASF1a (aa 1-157) were cloned into a pET-28a-SUMO and a pRSFDuet-1 vector (Novagen), respectively. Both proteins were expressed in the BL21 CodonPlus (DE3) RIL strain of *E. coli* as fusion proteins with an N-terminal poly(His)-SUMO or C-terminal poly(His) tag. Protein expressions were induced with 0.25 mM β -D-1-thiogalactopyranoside (IPTG) at 16 °C overnight when cell density reached OD₆₀₀ ~0.8. Cells were harvested, resuspended in buffer A (20 mM Tris-HCl, pH 8.0, 500 mM NaCl, 20 mM imidazole and 1mM PMSF) and lysed by sonication. Cell lysate was separated by centrifugation and the supernatant was loaded onto a Ni-IDA column (GE Healthcare) and the target protein was eluted by buffer B (20 mM Tris-HCl, pH 8.0, 100 mM NaCl, 250 mM imidazole). Eluted fractions were pooled and incubated with SUMO protease at 4°C for 16 hours to cut the His-SUMO tag off the MCM2 fusion protein. The cleaved tag was removed by chromatography using a Hitrap Q column (GE Healthcare) with a linear gradient of 0.1-1.0 M NaCl. The same step was used to purify ASF1-His. Full-length human histones H3.1 and H4 were co-expressed using a bicistronic plasmid using a pRSFDuet-1 vector, and the H3-H4 complex was purified under native conditions as previously described (Liu et al., 2012).

The purified proteins, MCM2, ASF1 and the histone H3-H4 complex were mixed at an approximate molar ratio of 1.5:1.5:1 and dialyzed against buffer C (20 mM Tris-HCl, pH 8.0, 500 mM NaCl, 1 mM EDTA, 1 mM DTT) at 4°C for 4 hours. Then, the sample was concentrated and applied to a Superdex HiLoad 200 16/60 column (GE Healthcare) pre-equilibrated with buffer C. The eluted fractions were analyzed by SDS-PAGE, and the target quaternary complex was pooled and concentrated to ~25 mg/ml for crystallization. Crystallization was carried out at 16°C by the hanging drop vapor diffusion method. Crystals were obtained with the mixture of 1.0 μ l protein solution and 1.0 μ l reservoir solution in a condition containing 0.1 M Tris-HCl pH 8.0, 20% PEG3350 and 2% Tacsimate. For cryogenic data collection, crystals were flash-frozen in liquid nitrogen using a cryo-protectant solution prepared from the reservoir solution supplemented with 20% Glycerol. Diffraction data were collected at beamline BL17U of Shanghai Synchrotron Radiation Facility (SSRF) using a Quantum 315r CCD detector (ADSC). A 3.5Å native data set was collected at a wavelength of 0.9792 Å and processed using HKL2000 software (Otwinowski and Minor, 1997).

The crystal belongs to the $P3_121$ space group, and there are six MCM2-ASF1-H3-H4-tetramers in one asymmetric unit. The structure was solved by molecular replacement (MR) using PHASER software in the CCP4 program suite (McCoy, 2007), using the ternary structure of human ASF1-H3-H4 as the search model (PDB code 2IO5). The electron density map was of high quality after several rounds of refinement with REFMAC (Murshudov et al., 1997; Natsume et al., 2007). Model of MCM2 molecule was built by docking the human MCM2-H3-H4 structure (PDB code 4UUZ) to the MR model by superposition of H3-H4 heterodimers in the two structures (Fig. S2). The structure was further refined with REFMAC and COOT (Emsley and Cowtan, 2004), and the refined model has R_{work} and R_{free} values of 0.230 and 0.275, respectively. Further data analyses using PHENIX (Adams et al., 2010) indicated merohedral twinning with a twinning fraction of 0.21. Refinement with intensity-based twin correction using REFMAC, the R_{work} and R_{free} of the final model are 0.216 and 0.253, respectively. Detailed statistics for data collection and refinement are shown in Table S1.

Table S1. Statistics for crystallographic analysis

| | |
|---------------------------------|---------------------|
| Data collection | |
| Wave length (Å) | 0.9792 |
| Space group | P3 ₁ 21 |
| Cell dimensions | |
| a, b, c (Å) | 147.7, 147.7, 261.8 |
| α, β, γ (°) | 90, 90, 120 |
| Resolution (Å) ^a | 50-3.50 (3.63-3.50) |
| R _{merge} ^b | 0.161 (0.690) |
| I/ σ I | 12.8 (2.8) |
| Completeness (%) | 99.8 (100.0) |
| Total/Unique reflections | 388727/42834 |
| Redundancy | 9.1 (9.2) |
| Refinement Statistics | |
| Rwork/Rfree ^c | 0.216/0.253 |
| No. atoms | |
| Protein | 17655 |
| B factor (Å ²) | |
| Protein | 47.4 |
| rmsd bonds (Å) | 0.005 |
| rmsd angles (°) | 0.915 |
| Ramachandran plots | |
| favored | 98.4% |
| allowed | 1.6% |
| outlier | 0.0% |

^a Values in the parentheses are that for the highest resolution range.

^b $R_{\text{merge}} = \frac{\sum |I - \langle I \rangle|}{\sum \langle I \rangle}$, where I and $\langle I \rangle$ are the averaged intensity of multiple measurements of the same reflection. The summation is over all the observed reflections.

^c R-factor = $\frac{\sum |F_o| - |F_c|}{\sum |F_o|}$, where F_o denotes the observed structure factor amplitude and F_c denotes the structure factor calculated from the model. R-work was calculated using the diffraction data used throughout the refinement, and R-free was calculated using the 5% of the data set aside during refinement.

Supplemental Figure S1

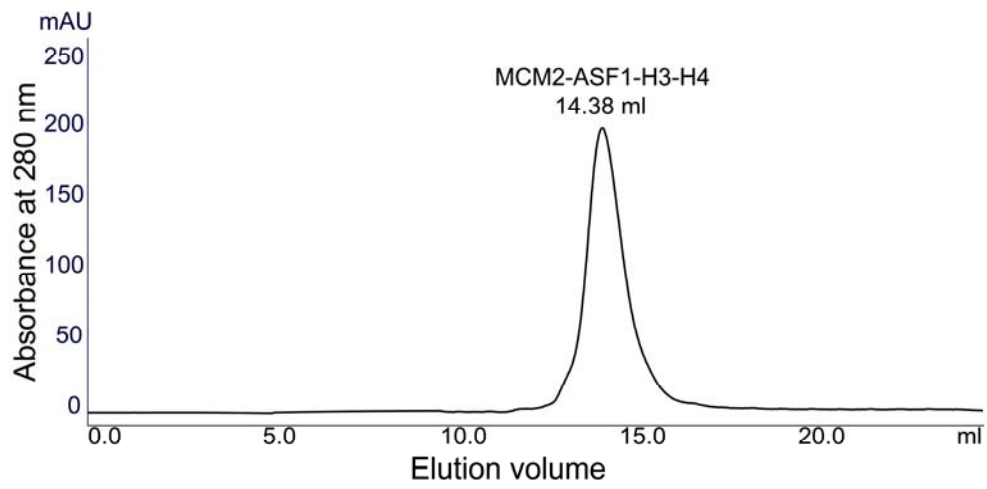


Fig. S1 Elution profile of the MCM2-ASF1-H3-H4 quaternary complex from a Superdex 200 10/300 GL size exclusion column (GE Healthcare).

Supplemental Figure S2

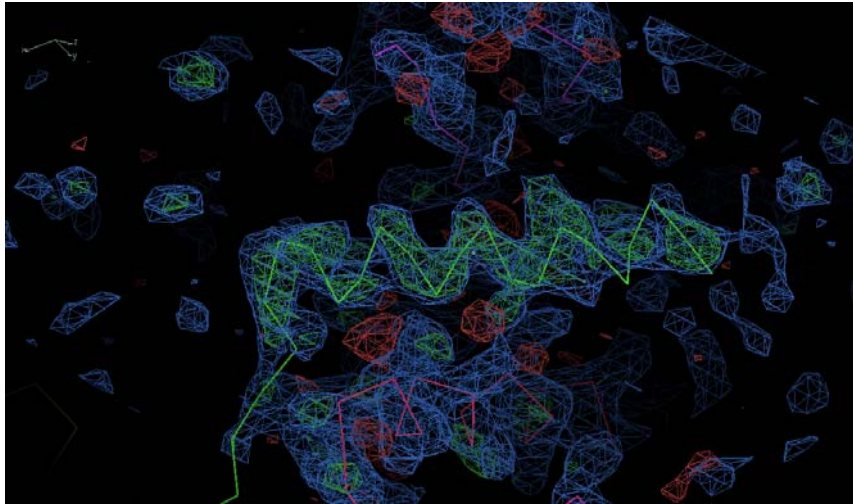


Fig. S2 Electron density map of the MCM2-ASF1-H3-H4 structure at 3.5 Å resolution. 2Fo-Fc map at 1.0 sigma (blue color) and Fo-Fc map at 2.5 sigma (green color) of the $\alpha 2$ helix of MCM2.

Supplemental References

Adams, P.D., Afonine, P.V., Bunkoczi, G., Chen, V.B., Davis, I.W., Echols, N., Headd, J.J., Hung, L.W., Kapral, G.J., Grosse-Kunstleve, R.W., *et al.* (2010). PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta crystallographica Section D, Biological crystallography* 66, 213-221.

Emsley, P., and Cowtan, K. (2004). Coot: model-building tools for molecular graphics. *Acta crystallographica Section D, Biological crystallography* 60, 2126-2132.

Liu, C.P., Xiong, C., Wang, M., Yu, Z., Yang, N., Chen, P., Zhang, Z., Li, G., and Xu, R.M. (2012). Structure of the variant histone H3.3-H4 heterodimer in complex with its chaperone DAXX. *Nature structural & molecular biology* 19, 1287-1292.

McCoy, A.J. (2007). Solving structures of protein complexes by molecular replacement with Phaser. *Acta crystallographica Section D, Biological crystallography* 63, 32-41.

Murshudov, G.N., Vagin, A.A., and Dodson, E.J. (1997). Refinement of macromolecular structures by the maximum-likelihood method. *Acta crystallographica Section D, Biological crystallography* 53, 240-255.

Otwinowski, Z., and Minor, W. (1997). Processing of X-ray diffraction data collected in oscillation mode. *Method Enzymol* 276, 307-326.