

Expanded View Figures

Figure EV1. CAL1 is predicted to be predominantly unstructured.

- A Disordered regions of CAL1 as predicted by Disopred (<http://bioinf.cs.ucl.ac.uk/psipred>). Residue number on x-axis and probability of disorder on y-axis.
- B Secondary structure composition and multiple sequence alignment of CAL1 N-terminus as performed using Psipred (<http://bioinf.cs.ucl.ac.uk/psipred>) and MUSCLE (Madeira et al, 2019). Numbers correspond to *Drosophila melanogaster* (*D. mel*), *Drosophila grimshawi* (*D. gri*), *Drosophila mojavensis* (*D. moj*), *Drosophila virilis* (*D. vir*), *Drosophila persimilis* (*D. per*), *Drosophila pseudoobscura pseudoobscura* (*D. pse*), *Drosophila ananassae* (*D. ana*), *Drosophila erecta* (*D. ere*), *Drosophila yakuba* (*D. yak*) and *Drosophila simulans* (*D. sim*).
- C SEC-MALS analysis of His-CAL1₁₋₁₆₀-CENP-A₁₀₁₋₂₂₅-H4 and His-CAL1₁₋₁₆₀-CENP-A₁₄₄₋₂₂₅-H4. Absorption at 280 nm (mAU, left y-axis) and molecular mass (kDa, right y-axis) are plotted against elution volume (ml, x-axis). Measured MW and the calculated subunit stoichiometry based on the predicted MW of different subunit compositions. Samples were analysed using Superdex 200 increase 10/300 in 50 mM HEPES pH 8.0, 2 M NaCl and 1 mM TCEP.

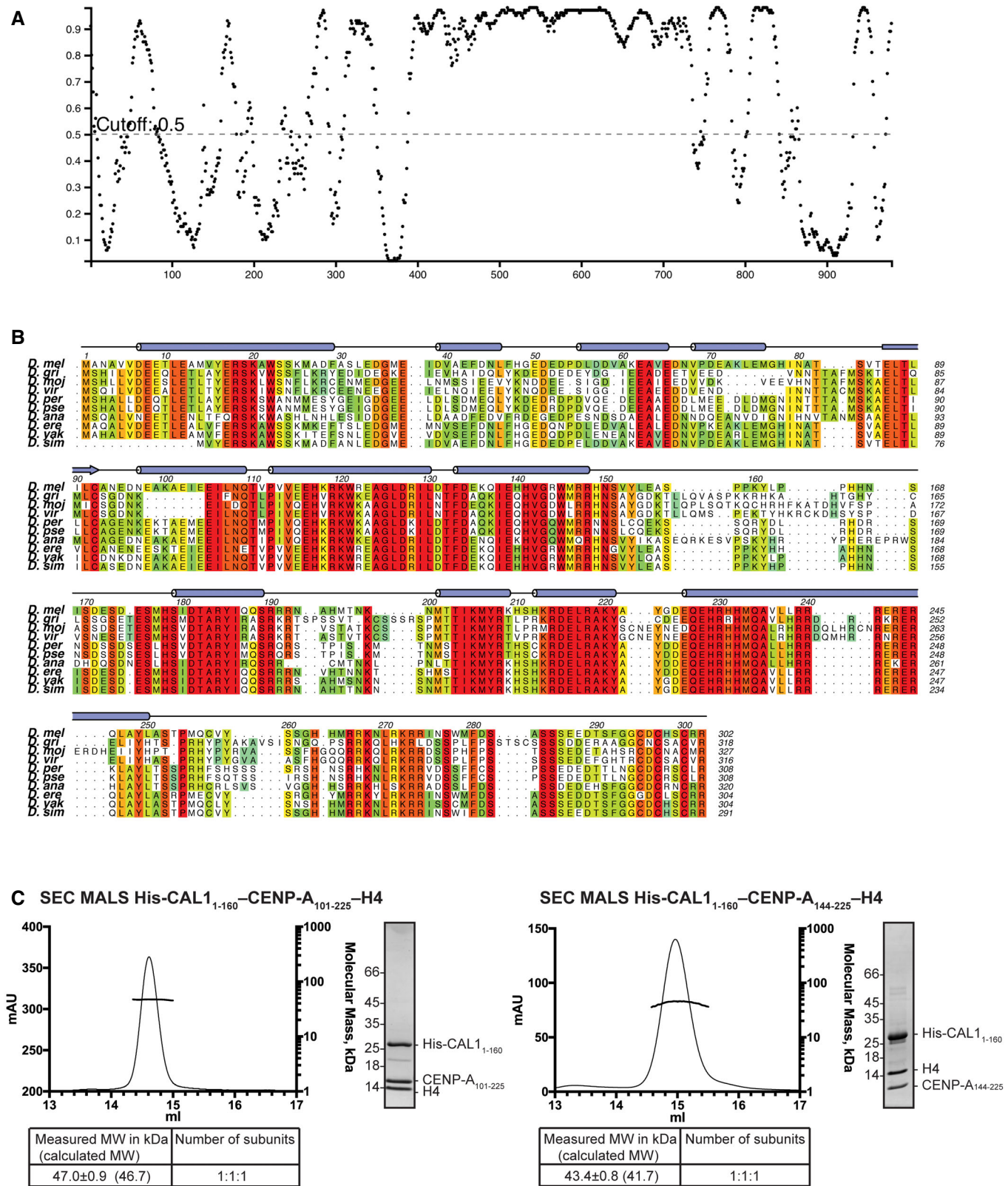


Figure EV1.

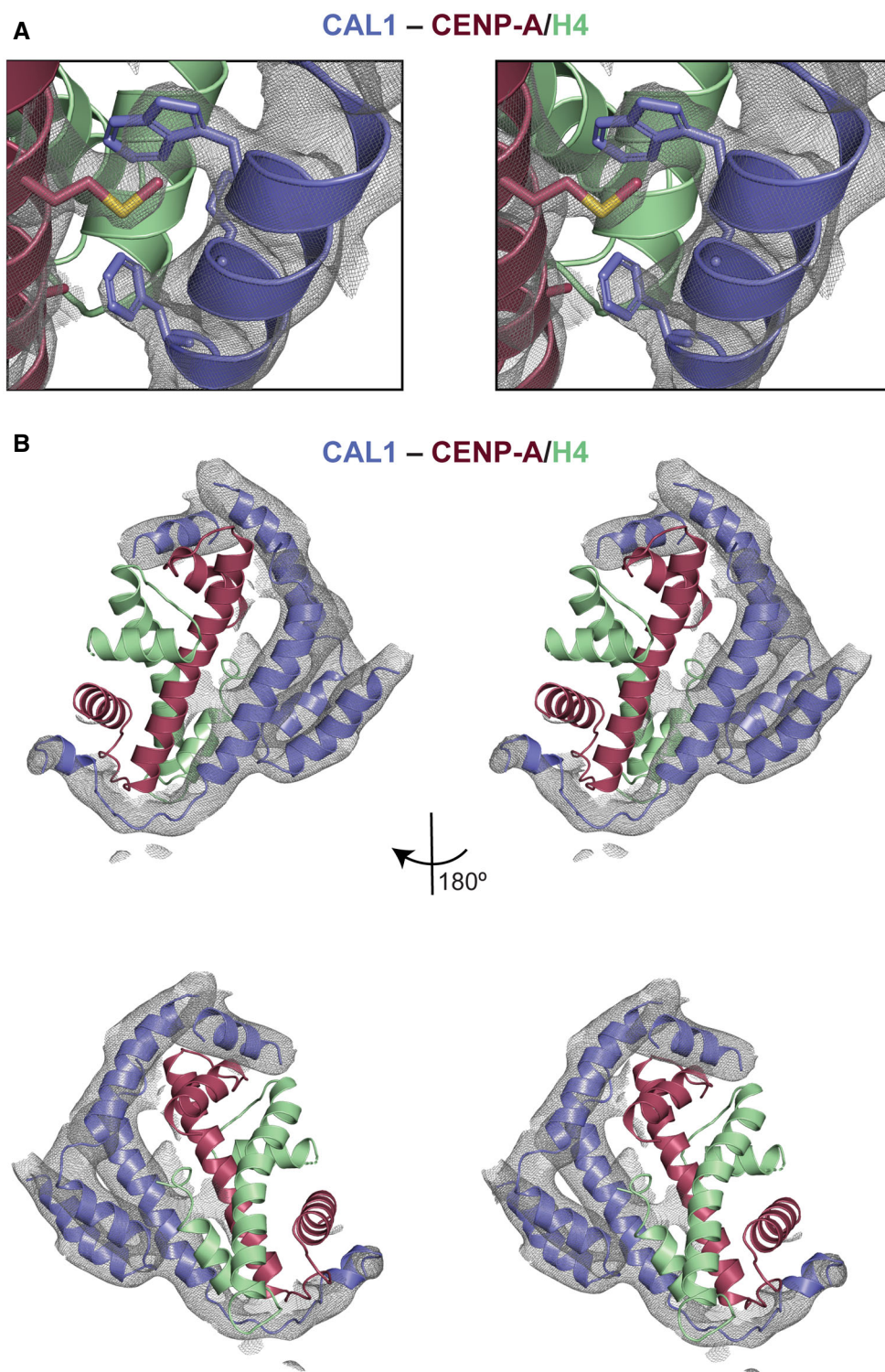


Figure EV2. Stereo images of the electron density maps and corresponding final models of CAL1–CENP-A/H4 from crystal form I and II.

A $2F_o - F_c$ electron density map contoured at 1 sigma for the vicinity of CAL1 residues W22 and F29 for crystal form I. CENP-A in maroon and H4 in green.

B $2F_o - F_c$ electron density map contoured at 1 sigma for CAL1 bound to CENP-A/H4 for crystal form II. CAL1 is shown in blue, CENP-A in maroon and H4 in green.

Figure EV3. Intra- and intermolecular contacts identified between CAL1 and CENP-A/H4 using cross-linking mass spectrometry (CLMS).

- A SDS-PAGE analysis of His-CAL1₁₋₁₆₀-CENP-A₁₀₁₋₂₂₅-H4 cross-linked with both EDC (left) and BS³ (right) cross-linker.
- B Linkage map showing the sequence position and cross-linked residue pairs between His-CAL1₁₋₁₆₀, CENP-A₁₀₁₋₂₂₅ and H4. Cross-linked samples were resolved with SDS-PAGE and then analysed by MS. CAL1 is shown in blue, CENP-A in maroon and H4 in green. Solid lines represent EDC cross-links, while dashed lines show BS³ cross-links.
- C High-resolution representative fragmentation spectra displayed using XiSpec (Ref: PMID: 29741719) for cross-linked peptides seen between CAL1₁₋₁₆₀ and CAL1₁₋₁₆₀, CAL1₁₋₁₆₀ and CENP-A₁₀₁₋₂₂₅ and CAL1₁₋₁₆₀ and H4.



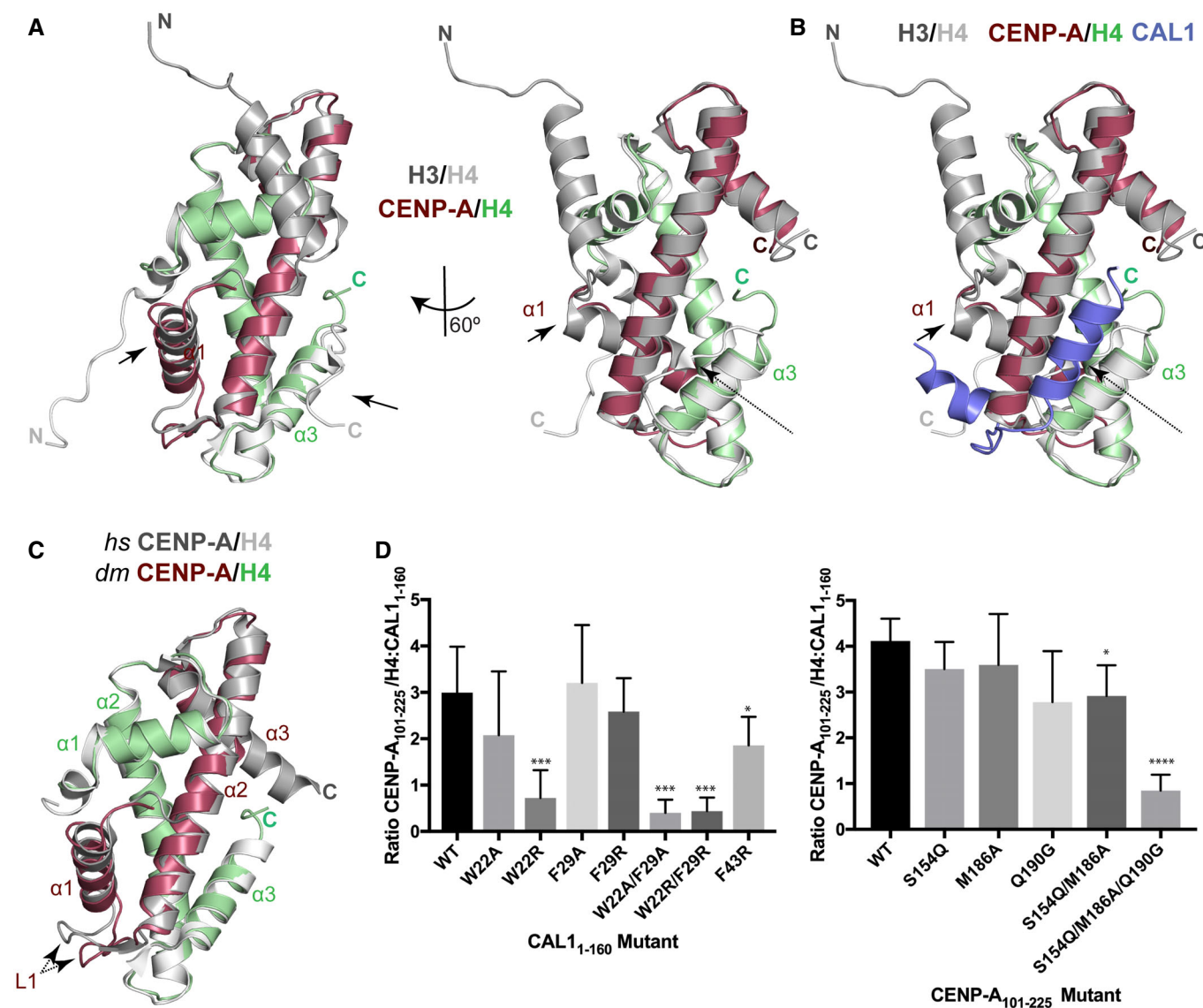


Figure EV4. Mode of H4 recognition by CENP-A by CAL1.

A Structural superposition of CENP-A/H4 with H3/H4 (Clapier et al, 2008). CENP-A is shown in maroon, H4 in green and H3/H4 in silver. Arrows indicate conformational changes.

B Structural superposition of CAL1-CENP-A/H4 (form I) with H3/H4 (Clapier et al, 2008). CAL1 is shown in blue, CENP-A in maroon, H4 in green and H3/H4 in silver. Arrows indicate conformational changes; dotted arrow highlights conformational changes in the loop regions.

C Structural superposition of *hs* CENP-A/H4 (Sekulic et al, 2010) with *dm* CENP-A/H4. *dm* CENP-A in maroon, *dm* H4 in green and *hs* CENP-A/H4 in silver. Dotted arrow highlights conformational changes in the loop regions.

D (left panel) Quantifications of Ni-NTA pull-down of His-CAL1₁₋₁₆₀ WT and indicated mutants with CENP-A₁₀₁₋₂₂₅-H4. Bar graph shows average ratio of the band intensities between CENP-A₁₀₁₋₂₂₅/H4 and His-CAL1₁₋₁₆₀ ($n = 7$ experiments). (right panel) Quantifications of Ni-NTA pull-down of His-CAL1₁₋₁₆₀ WT with CENP-A₁₀₁₋₂₂₅-H4 and indicated mutants. Bar graph shows average ratio of the band intensities between CENP-A₁₀₁₋₂₂₅/H4 and His-CAL1₁₋₁₆₀ ($n = 4$ experiments).

Data information: In (D), (left panel) data presented as mean $\pm$ SD of 7 experiments, P -values were calculated using a Mann-Whitney test. (right panel) Data presented as mean $\pm$ SD of 4 experiments, P -values were calculated using unpaired two-tailed t -test. (* $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$).

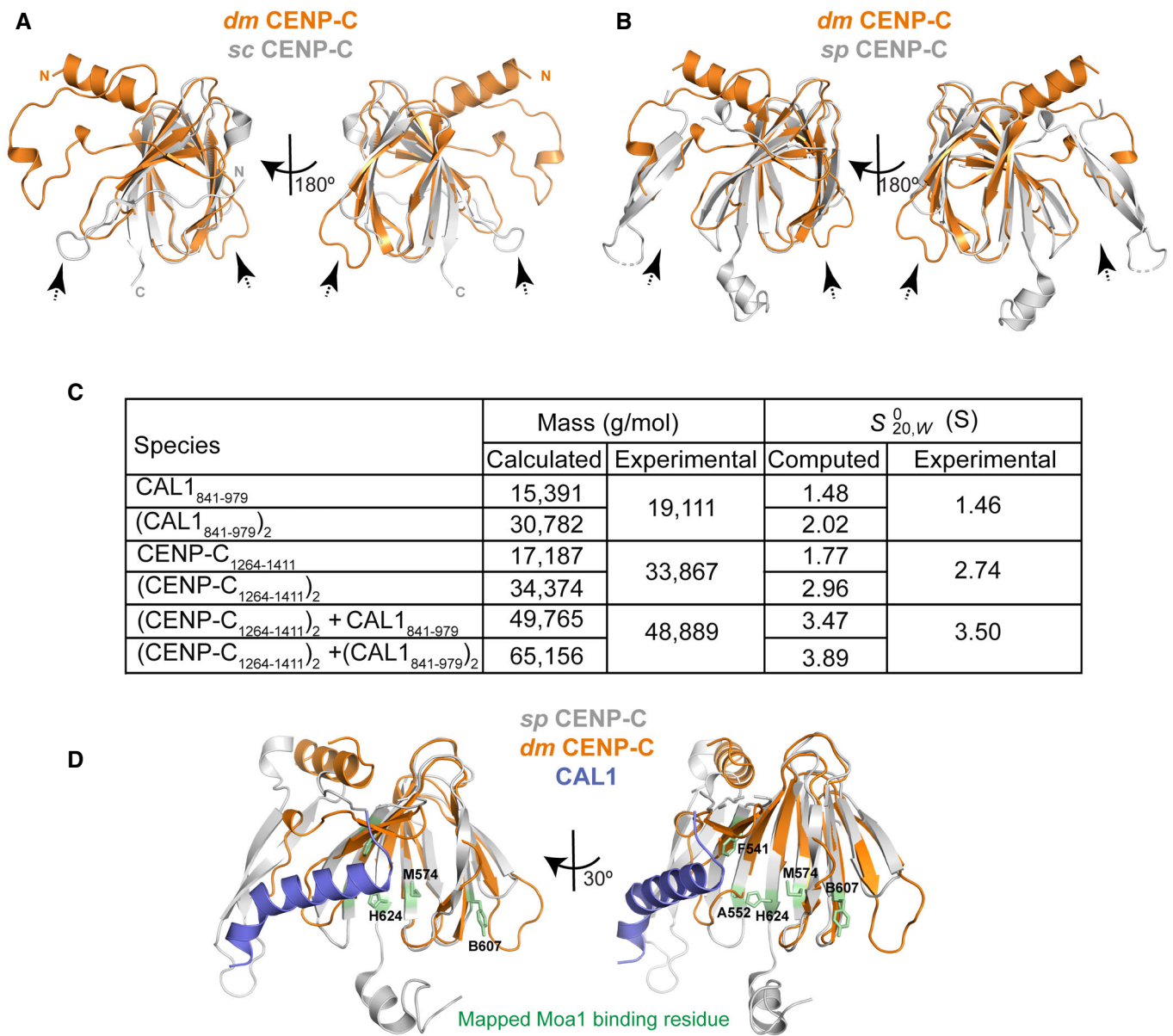


Figure EV5. CENP-C structure and binding to CAL1.

A Structural superposition of cupin domains of *dm* CENP-C and *sc* CENP-C (Mif2p) (Cohen et al, 2008). CENP-C is shown orange and Mif2p in silver. Dotted arrow highlights conformational changes in the loop regions.

B Structural superposition of cupin domains of *dm* CENP-C and *sp* CENP-C (Cnp3) (Chik et al, 2019). CENP-C is shown orange and Cnp3 in silver. Dotted arrow highlights conformational changes in the loop regions.

C Calculated and experimentally determined molecular masses and sedimentation coefficients for plausible solution states of CAL1₈₄₁₋₉₇₉, CENP-C₁₂₆₄₋₁₄₁₁ and complexes thereof.

D Structural superposition of CAL1 bound *dm* CENP-C cupin domain onto *sp* CENP-C cupin domain. *dm* CENP-C is shown orange, *sp* CENP-C in silver and CAL1 in blue. Amino acid residues identified to be crucial for Moa1 binding (by Chik et al, 2019) are shown in stick representation in green.