Supplementary Information

Yeast CAF-1 assembles histone $(H3\text{-}H4)_2$ tetramers prior to DNA deposition

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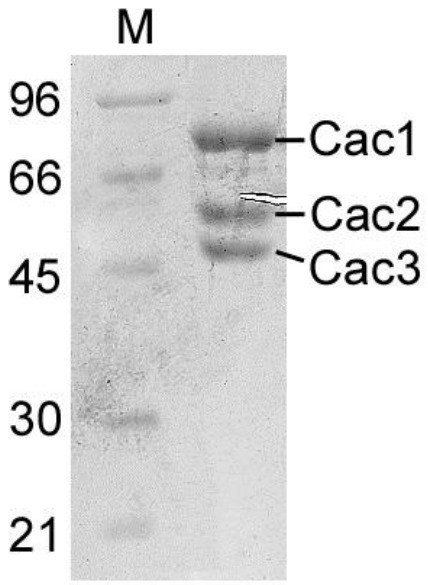
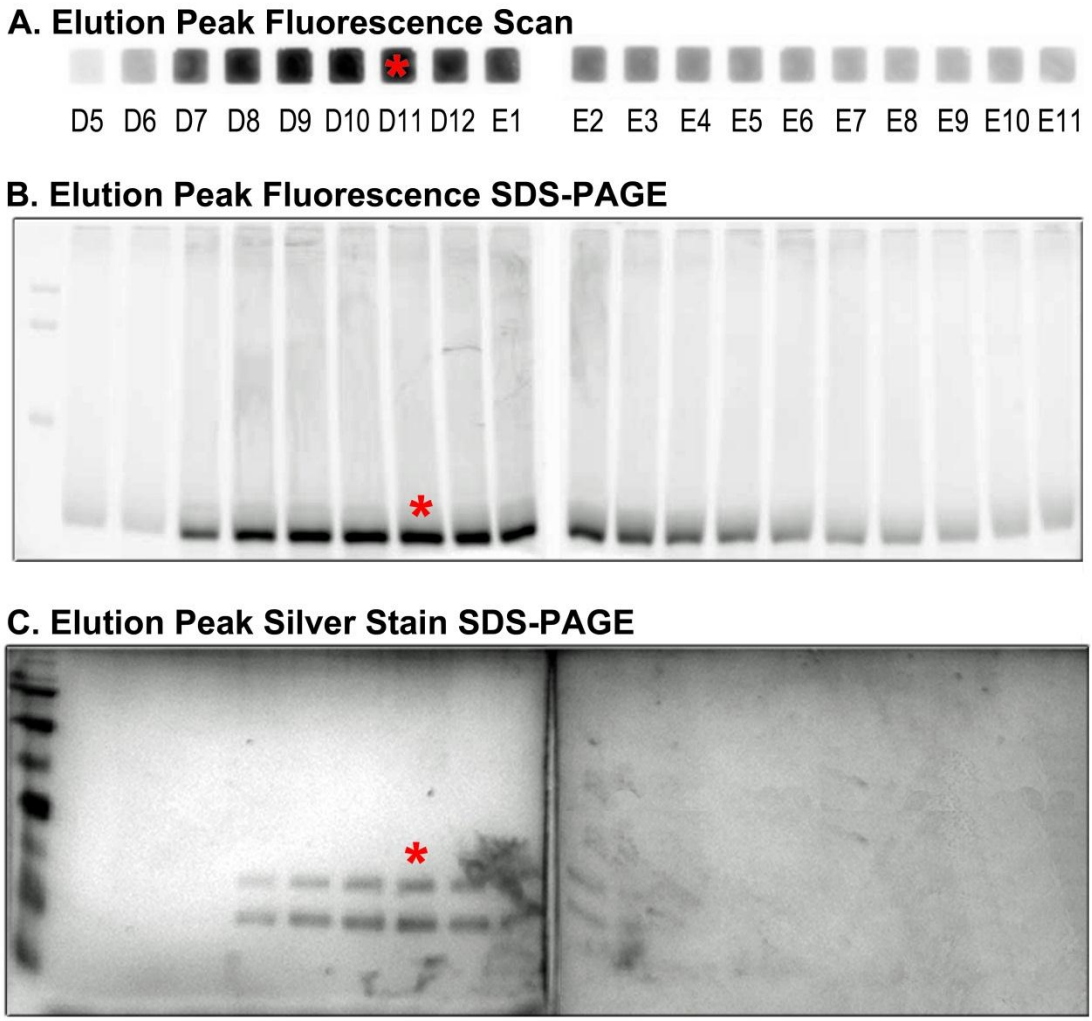
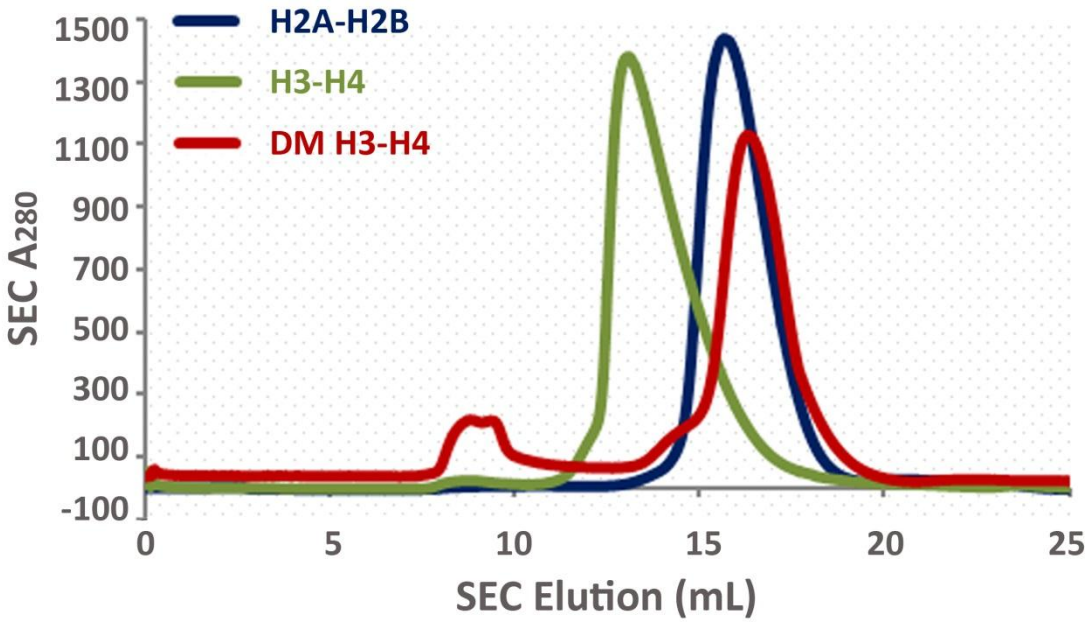


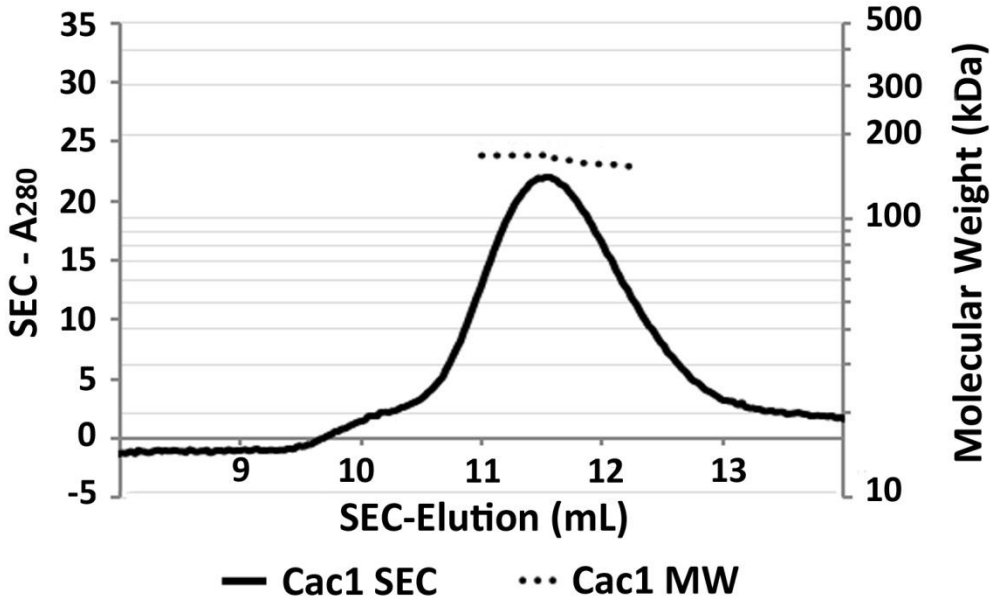
S. Figure 1 – SDS-PAGE analysis of recombinantly purified CAF-1. The three yeast CAF-1 subunits are shown in the right lane and correspond to Cac1 (70 kDa), Cac2 (51 kDa), and Cac3 (47 kDa). A low molecular weight marker (M) is shown the the left lane to provide a frame of reference.



S. Figure 2 – Analysis of the oligomeric state of H3-H4 using SEC. The elution fractions for each concentration of H3-H4 tested are aliquoted into a prepared 384-well clear-bottom microplate. The low concentration H3-H4 samples prohibit monitoring by 280 nm absorbance. The plate is then scanned for fluorescence of the H3-H4 conjugated fluorophore (panel A). The elution fractions are then run out on a 12% SDS-PAGE, scanned for fluorescence (panel B), and silver stained (panel C) to confirm overlapping of the fluorescence signal and H3-H4 polypeptides. The asterisks enable same sample (SEC elution fraction D11; highest fluorescence signal) comparison between panels A, B, and C.



S. Figure 3 – Size-exclusion chromatography peaks for high concentration (100 µM) histone complexes. Recombinantly expressed histones H2A and H2B; H3 and H4 are combined, refolded and then run over a Superdex 200 10/300 size exclusion chromatography column to ensure homogeneity and oligomeric state. Consistently, wild-type $(H3\text{-}H4)_2$ tetramers elute at a 14 mls (green); wild-type H2A-H2B dimer elution is delayed to 16 mls (blue). The elution profile of the H3 (L126R, I130R)-H4 variant (16.5 mls, red) is comparable to the H2A-H2B dimer which supports the inability of this histone complex to form tetramers even at high concentrations. The void volume for this column is near 8 mls.



S. Figure 4 – Determination of the apparent molecular weight of the Cac1 subunit using SEC-MALS. The Cac1 subunit elutes from the Superdex 200 GL column between 11 and 12 mls, which is slightly earlier than the complete CAF-1 complex. Light scattering confirms a molecular weight consistent with a Cac1 dimer (dotted line, right axis and Table 2). The asterisks denote same sample (SEC fraction D11; highest fluorescence signal fraction) for comparison across panels.