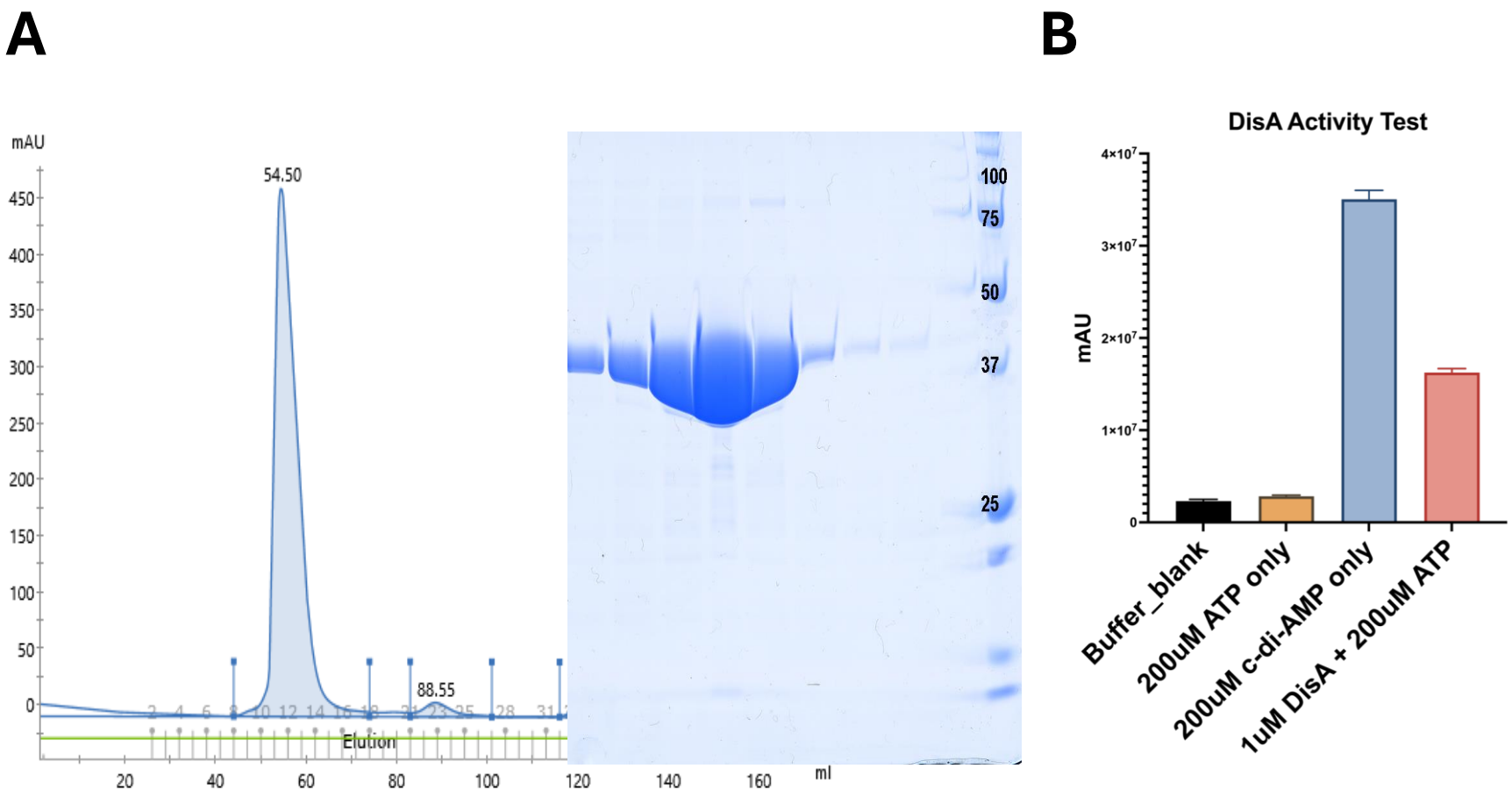


Figure 1: Purification of Mtb DisA and its activity assay. A: Size exclusion chromatography profile of Mtb DisA (left) along with the SDS-PAGE of the peak fractions (right). **B**: Results from the fluorescence-based assay that specifically detects c-di-AMP.

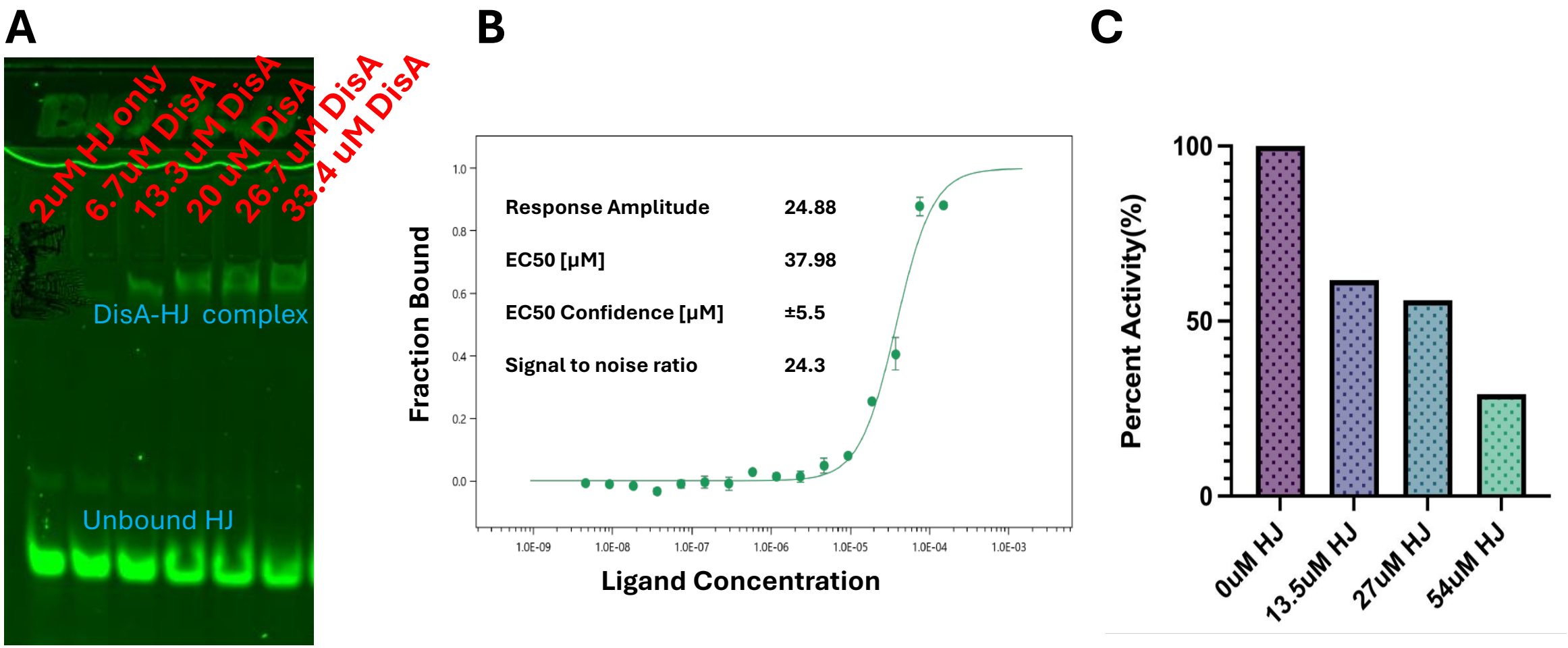
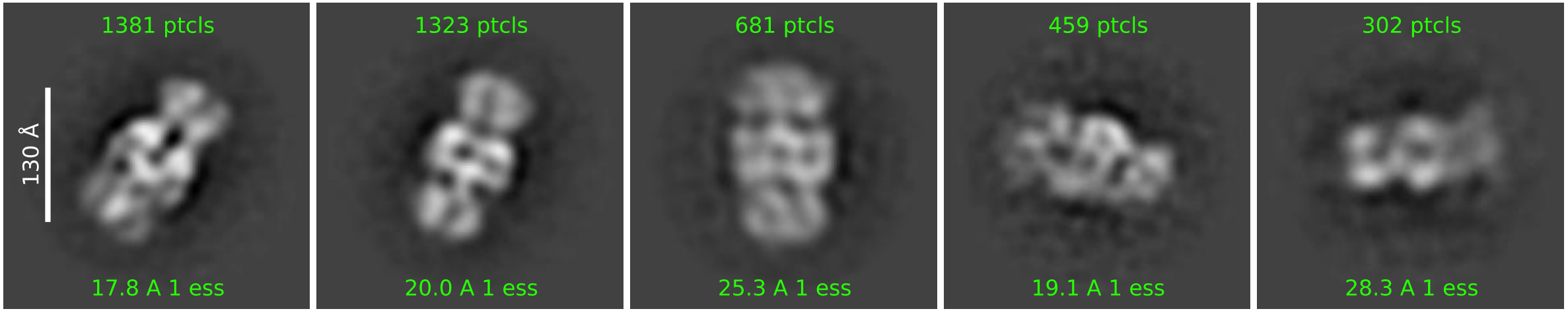


Figure 2: DisA-HJ binding and inhibition of DisA's catalytic activity. A: Electrophoretic mobility shift assay of DisA against HJ. HJ concentration is kept at 2µM at all lanes whereas DisA concentration is increased. **B**: Microscale thermophoresis binding curve of DisA-HJ interaction. **C**: Relative activity of DisA (10µM) in the presence of increasing concentrations of HJ.

0.5uM DisA



0.5uM DisA + 20uM HJ

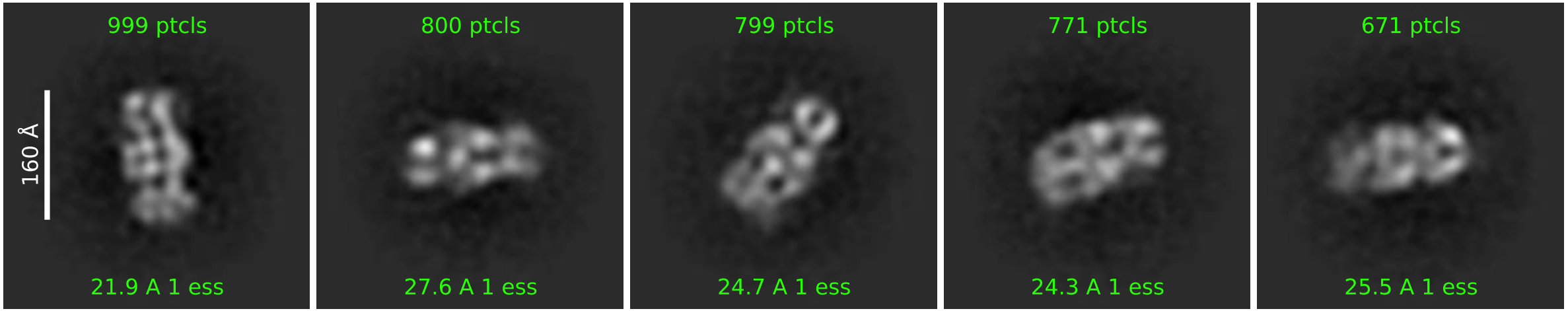
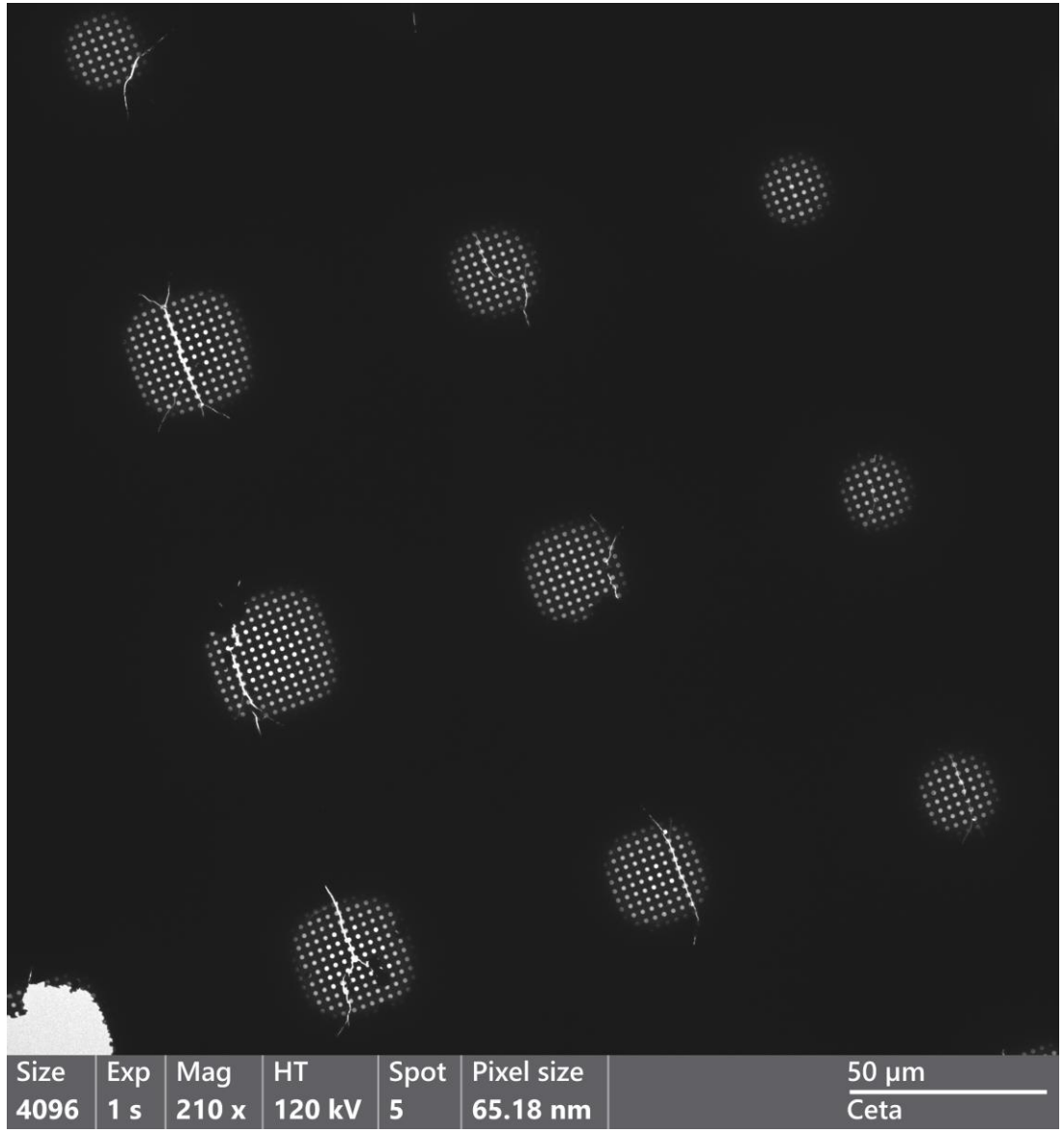


Figure 3: 2D class averages of DisA and DisA-HJ from negative staining. Top: 0.5μM DisA alone stained with 2% uranyl acetate. **Bottom**: 0.5μM DisA in the presence of 20μM HJ stained with 2% uranyl acetate. Class averages were generated using Cryosparc package. (not that the scale bars are different in two graphs).



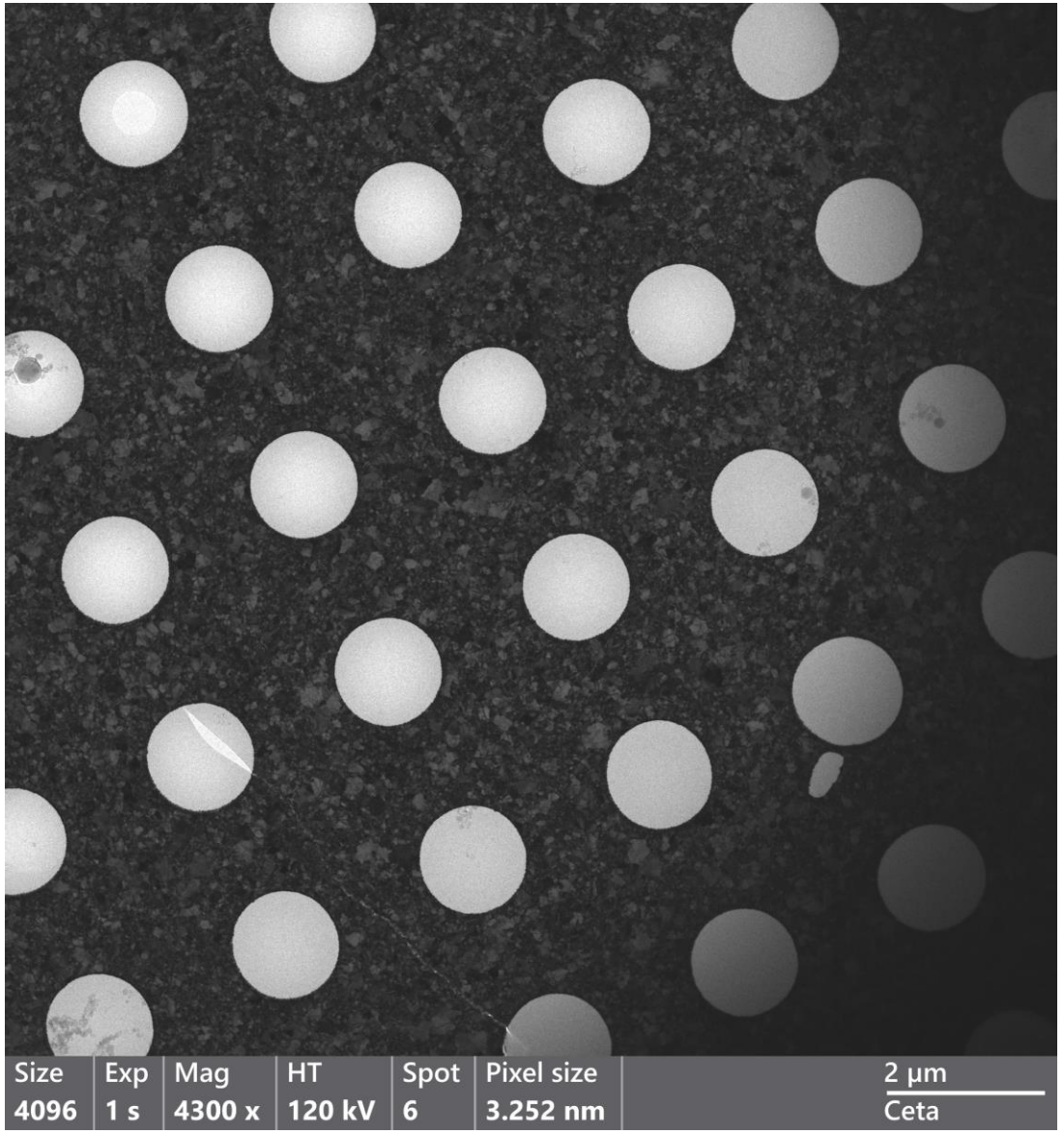


Figure 4: Grid squares from the frozen Cryo-grids. Holes from the grid squares are shown on the right. Ultra AU Foil R 1.2/1.3 grids were used.

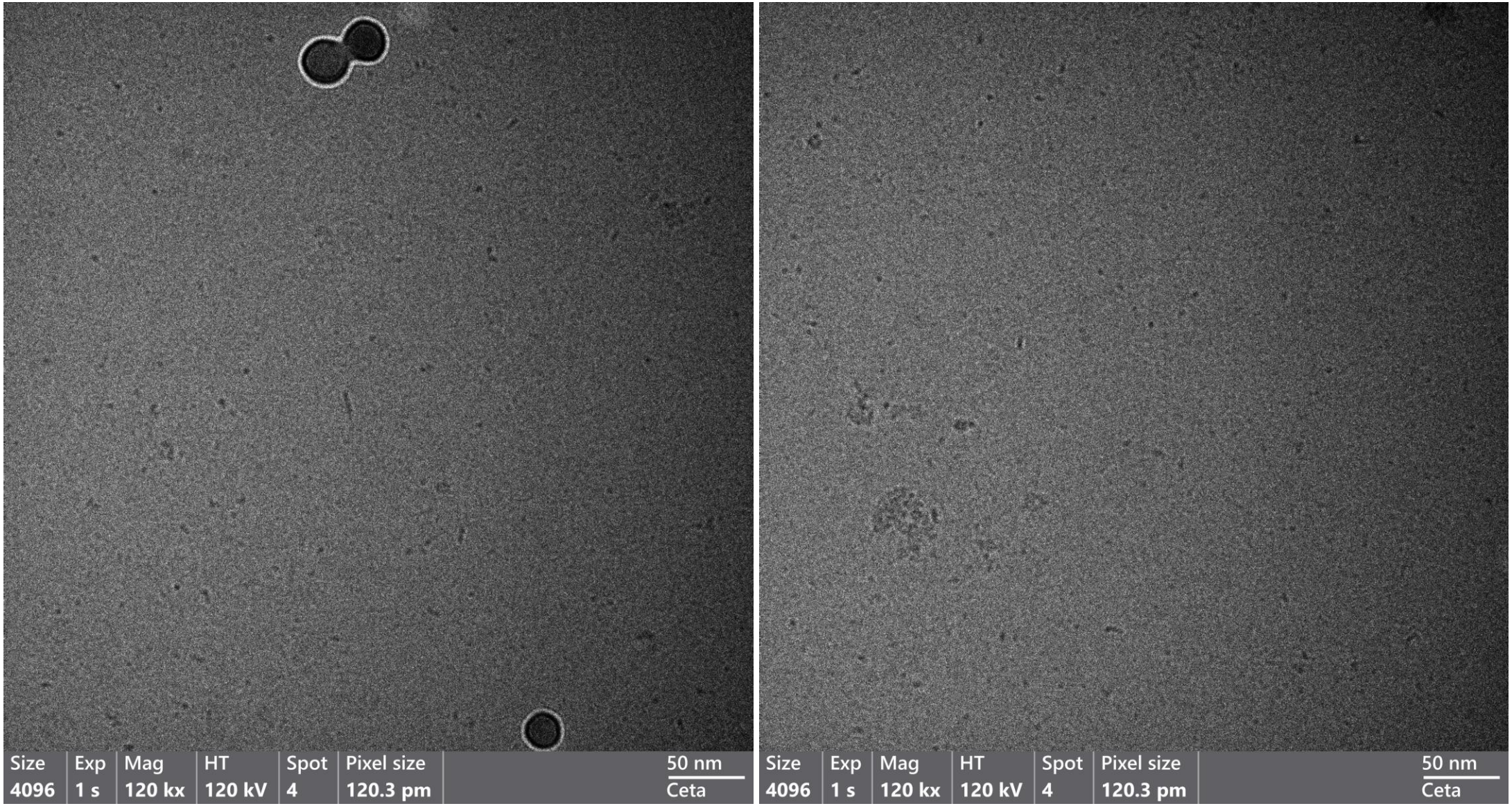


Figure 5: Cryo-micrographs from the frozen grid.

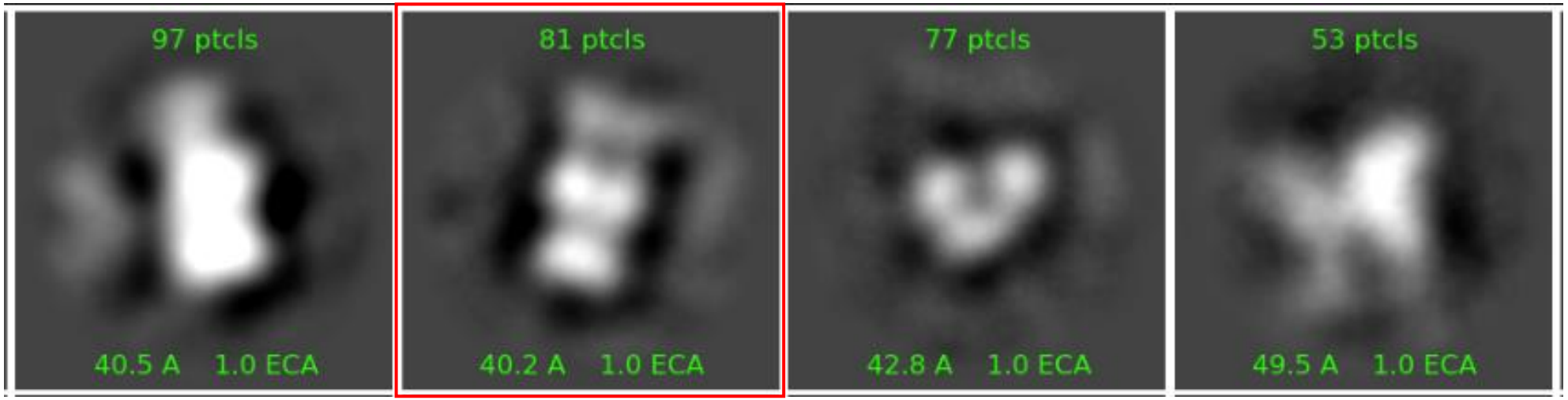


Figure 6: 2D class averages from the processing of the Cryo-micrographs. The class averages that shows the most resemblance to negative staining averages is highlighted in red.