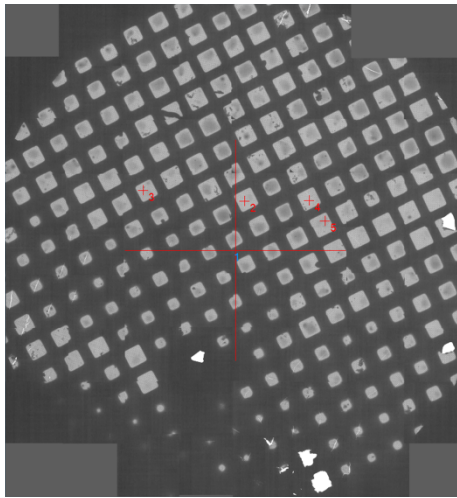


Supplementary Information

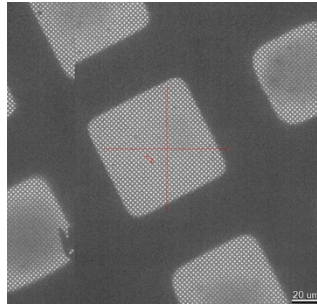
Size of Particles (kDa): 116 kDa (METTL3: 64 kDa; METTL14: 52 kDa)

Symmetry: C1 symmetry

Shape: butterfly shape molecule



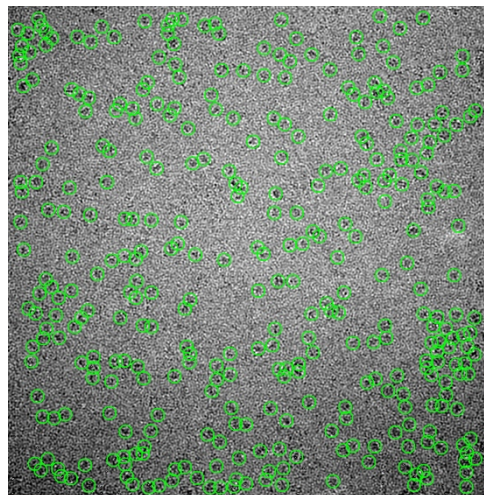
Low Mag 280X Atlas



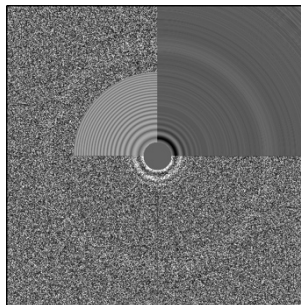
Low Mag 2600x



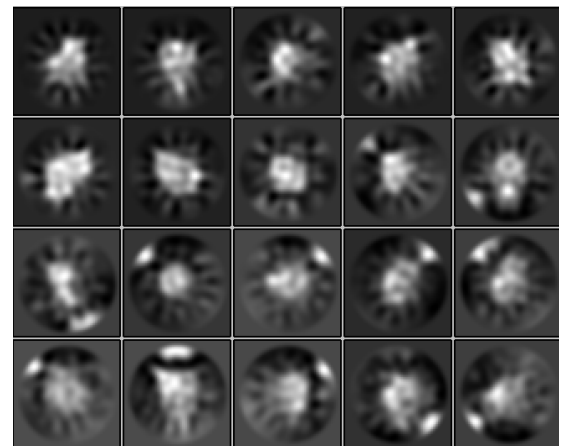
High Mag 120kx Particles



Autopicking



CTF estimation



2D classification

A dataset shown above was collected on Titan Krios microscope using K2 camera at super-resolution counting mode. The collected movies and resulting particles were processed using Relion. During this 2-day data collection session, ~1300 micrographs were collected and ~870,000 particles were picked for 2D classification.

Although the contrast of the micrographs is reasonably good, the 2D classification always show signs of overfitting no matter how we tweak the parameters. There may be several possible reasons and one major concern is that many particles were denatured at the air-water interface. Therefore, we propose to take advantage of chameleon to reduce the exposure time of METTL3-METTL14 to the air-water interface and produce even ice, which can solve the overfitting problem possibly caused by protein denaturation. We believe that once chameleon solves the problem of protein denaturation, we can obtain METTL3-METTL14 structure of high resolution. Therefore, the access to NCCAT's chameleon facilities is essential for the success of this project.