



**Ikarugamycin biosynthesis and biochemical and biophysical study of IkaA.** a. Ikarugamycin biosynthesis showing IkaA making tetramic acid intermediate (pro-ikarugamycin) which is processed to pre-ikarugamycin and ikarugamycin by reductases IkaB and IkaC respectively. b. IkaA analysed by size exclusion chromatography on Superose 6 increase column (UV at 280 nm). The purified sample is monodispersed with an elution volume of 12.9 ml, corresponds to ~700kDa(dimeric). The SDS gel on the left shows the sample is highly pure (~341kDa). c. IkaA is active in adenylation of L-ornithine, as shown by a continuous (pyro)phosphate release assay. d. IkaA + IkaB biosynthetic activity coupled with confirmed by LC-MS. Extracted-ion chromatogram at m/z =477 indicates the formation of pre-ikarugamycin. e. IkaA keto-reduction activity confirmed by NADPH consumption assay(relative absorbance at 340nm). f. IkaA analysed by dynamic light scattering (DLS) indicates monodispersed sample with ~10nm diameter. g. Class averages of IkaA particles (cryoSPARC). h. Zoom-in of a segment of the KS domain in high resolution maps. i. Low pass filtered maps show the NRPS and PKS portions of IkaA.