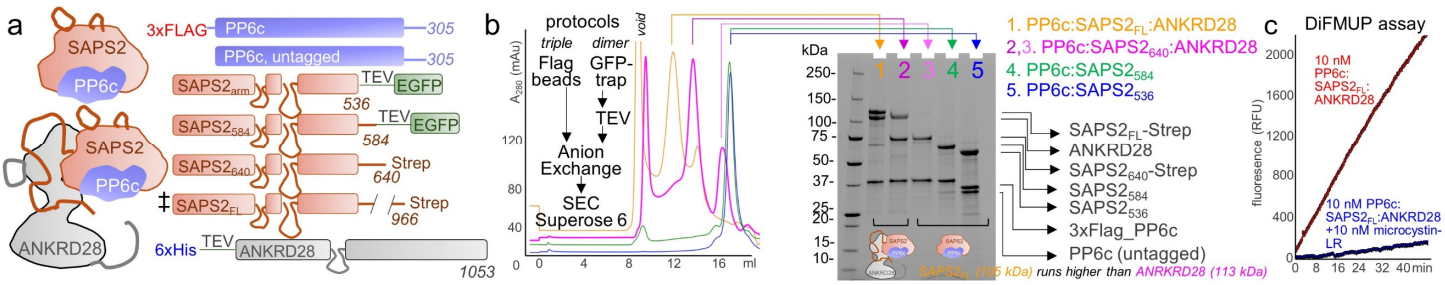

Fig. 1. Purification of PP6 core dimers and holoenzyme trimers. a. PP6 expression constructs (protocols used for grid preparations described in Aim 1 & 2: PP6c, 3xFLAG or untagged; SAPS, C-term Strep or TEV-EGFP; ANKRD, 6xHis_TEV). **b**. Overlay of SEC chromatograms (Superose 6 Increase 10/30 GL) and corresponding SDS-PAGE gel of PP6 dimer/trimer complexes. PP6c:SAPS2$_{640}$:ANKRD28 forms both a holoenzyme trimer (2) and core dimer (3) peak, demonstrating that *even when co-expressed, ANKRD subunits are limiting*. **c**. DiFMUP assay of PP6c:SAPS2$_{FL}$:ANKRD28 without (red) or with (blue) the non-specific PPP inhibitor microcystin-LR.

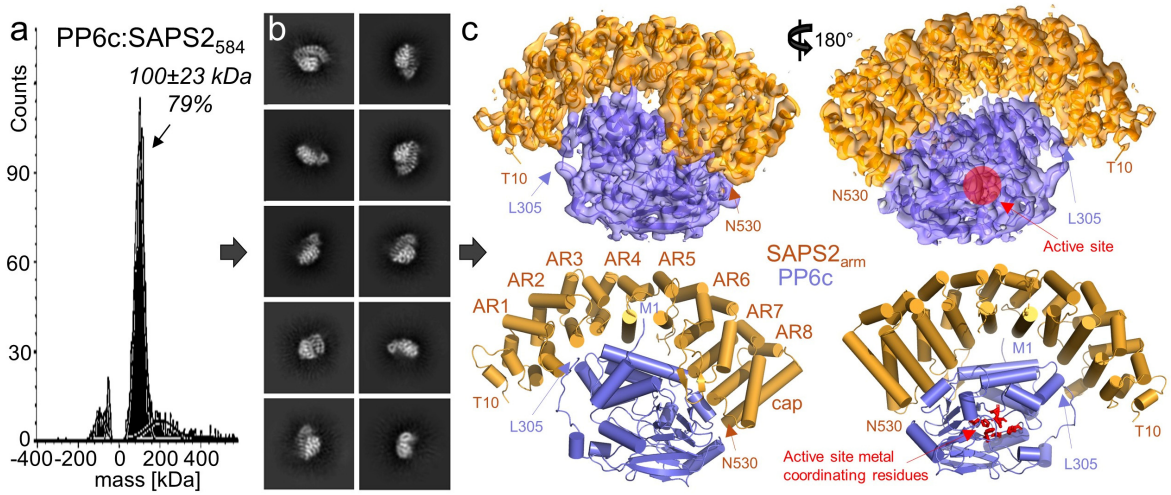

Fig. 2. Model of the PP6c core dimer. **a**. Refeyn data for PP6c:SAPS2$_{584}$ (Fig. 1b, lane 4), expected complex MW 101 kDa. **b**. Representative 2D class averages of the PP6c:SAPS2$_{584}$ core dimer. **c**. Preliminary map fit with PP6c:SAPS2$_{584}$ (upper) and the initial model (lower) show the extensive interaction of the complex and that, as expected, the PP6c active site (red) is accessible. AR1-8: arm repeats 1-8 and cap.

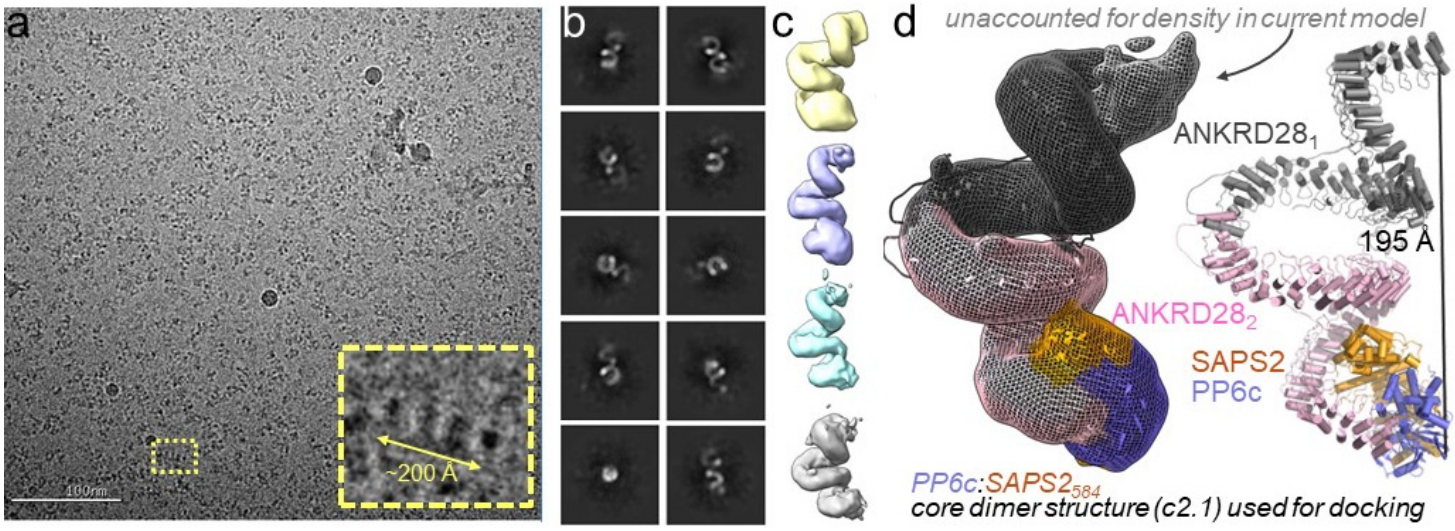

Fig 3. PP6c:SAPS2:ANKRD28 dimerizes via ANKRD28. **a**. Representative micrograph of PP6c:SAPS2$_{640}$:ANKRD28 holoenzyme triple complex (PP6c:SAPS2$_{FL}$:ANKRD28 is similar, with increased tendency for aggregation at hole edges; see lanes 1,2 (Fig. 5) for sample purity. **b**. Representative 2D class averages of micrographs in a. **c**. Representative reconstructions from homogeneous refinement using CryoSPARC using various strategies for particle picking. Some maps suggest that partial occupancy of the second core dimer (lavender/cyan), with others (yellow, grey) suggesting both ANKRD28 domains may be fully occupied by core dimers. **d**. Lavender reconstruction docked with two ANKRD$_{AF}$ (AlphaFold) subunits and 1 core dimer, illustrating the additional density at the second ANKRD28 domain. Our current model suggests that dimerization is mediated by the ANKRD28 C-terminal cap.