Figure 1 Example for the optimization of IDE/insulin complex at 2:1 molar ratio. A. The profile of fluorescence signal of IDE-insulin complex in the presence and absence of trimethylamine N-oxide (TMANO), an additive from crystallization additive screen. Note the right shift of melting curve, indicative of increase of melting temperature (Tm) and a slight increase of steepness of rising phase of melting curve, indicative of the increase of DeltaH value. B. CryoEM micrographs for IDE-insulin complex under the condition that the molar ratio of IDE to insulin is 2 to 1 in the presence or absence of TMANO.

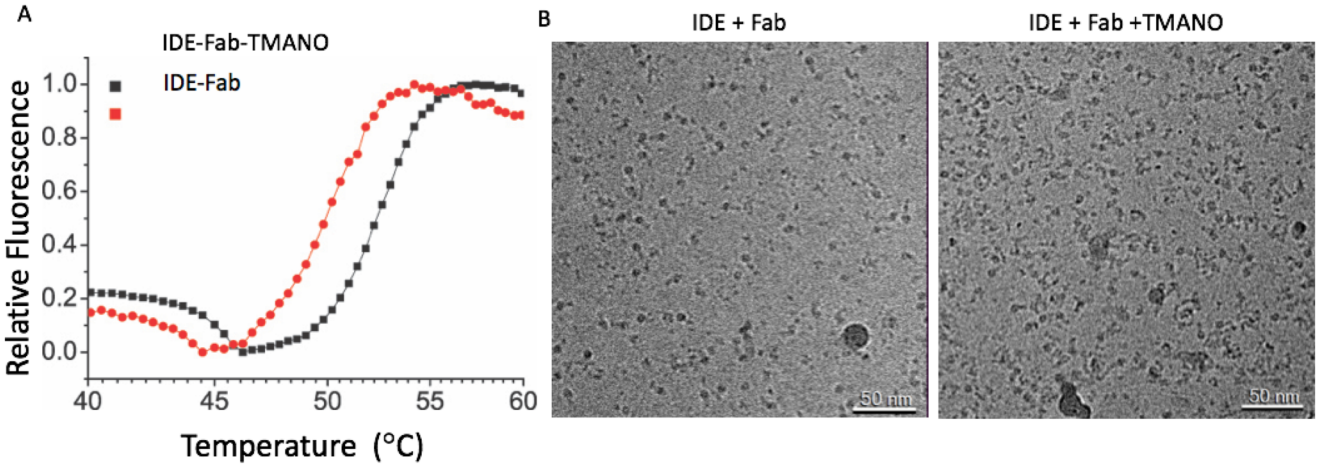

Figure 2 Comparison for the use of two IDE Fabs for the Fab-assisted cryoEM analysis. A. The profile of fluorescence signal of IDE-insulin complex in the presence of Fab1 and Fab-H11. Fab1 was used for the crystal structure study of Fab1-bound IDE (McCord et al PNAS 2013) while Fab-H11 was used for the cryoEM study of apo- and insulin-bound IDE (Zhang/Liang et al eLife 2018). B. CryoEM micrographs for IDE-insulin complex under the condition that the molar ratio of IDE to insulin is 2 to 1 when two different Fab were used.