Supplemental Information

Methods

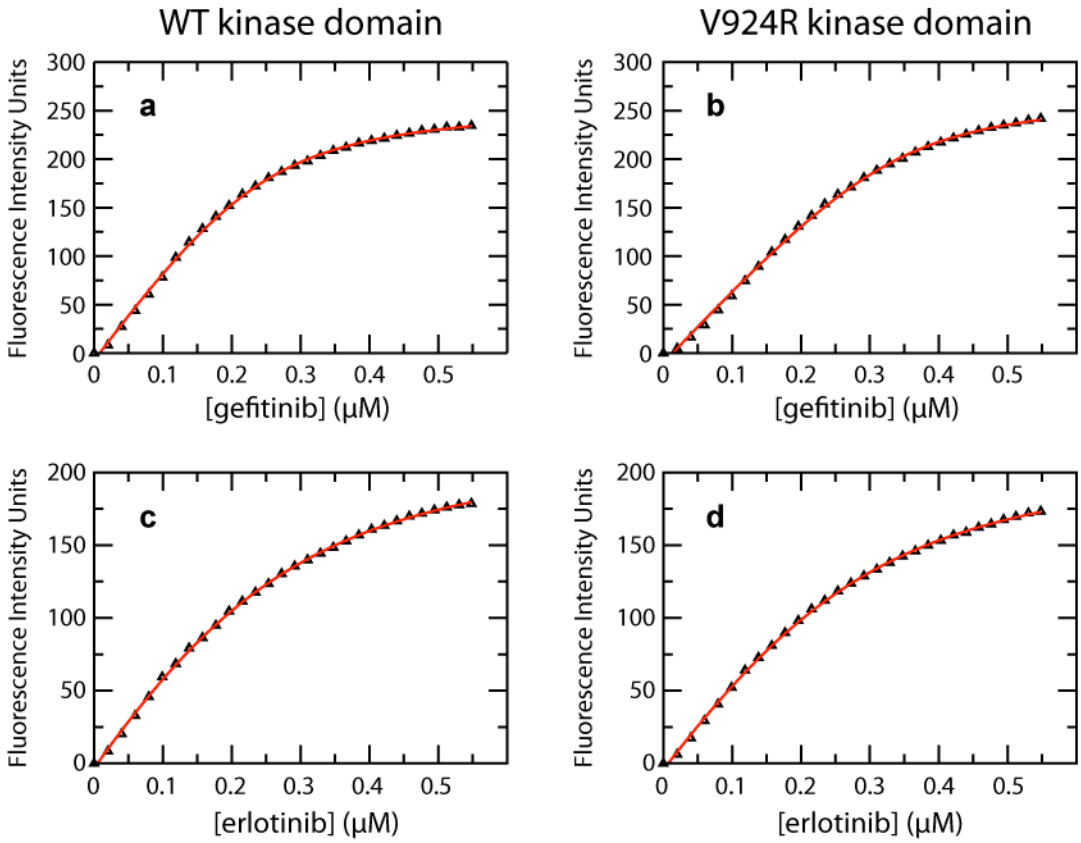
Fluorecence-based Kinase Inhibitor Binding Assay

Kinase inhibitors were dissolved in 100% DMSO to make 10 mM stock solutions. Stock solutions were further diluted to 10 µM with degassed and Argon-aerated assay buffer containing 20 mM Tris pH 8.0, 0.5% glycerol, 250 mM NaCl and 1 mM DTT. The purified EGFR kinase (kindly provided by Drs. Cai-hong Yun and Michael J. Eck, Dana-Farber Cancer Institute, Boston, MA) was diluted to 200 nM with the same assay buffer supplemented with 0.1% DMSO to match the DMSO concentration in inhibitor solutions. To kinase solution (2.5 ml) in a 1.0 cm quartz cuvette with a micro stir bar, 5 µl of inhibitor was added every 30 seconds. Tryptophan fluorescence was monitored with a Perkin Elmer LS-55 fluorescence spectrometer at excitation wavelength of 280 nm and emission wavelength of 341 nm. Experimental data were fit to

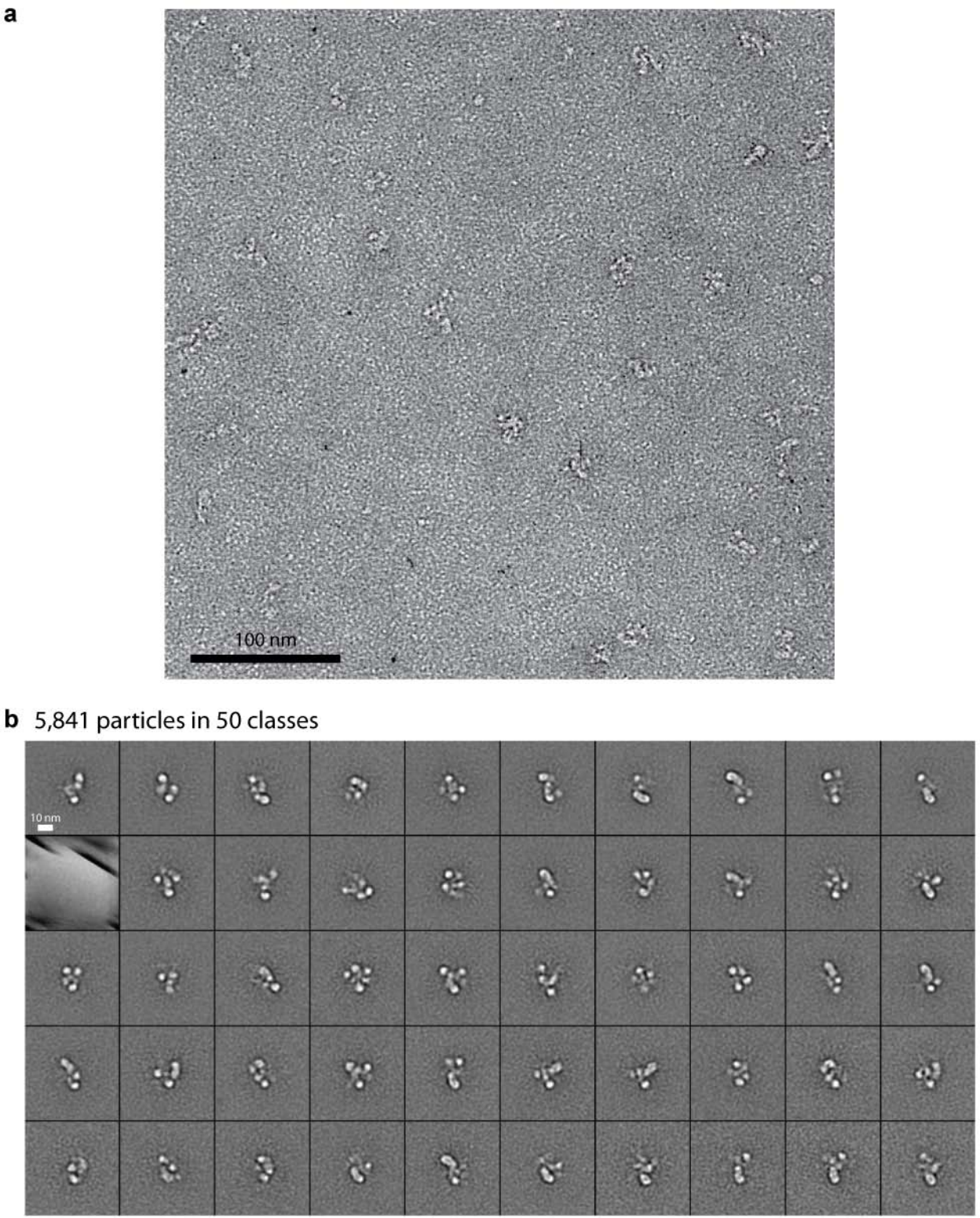
$$\Delta F = \frac{A}{1 + \frac{K_d}{[I]}} - B[I]$$

Where, $$[I] = \frac{-([E_t] - [I_t] + K_d) + \sqrt{([E_t] - [I_t] + K_d)^2 + 4K_d[I_t]}}{2}$$

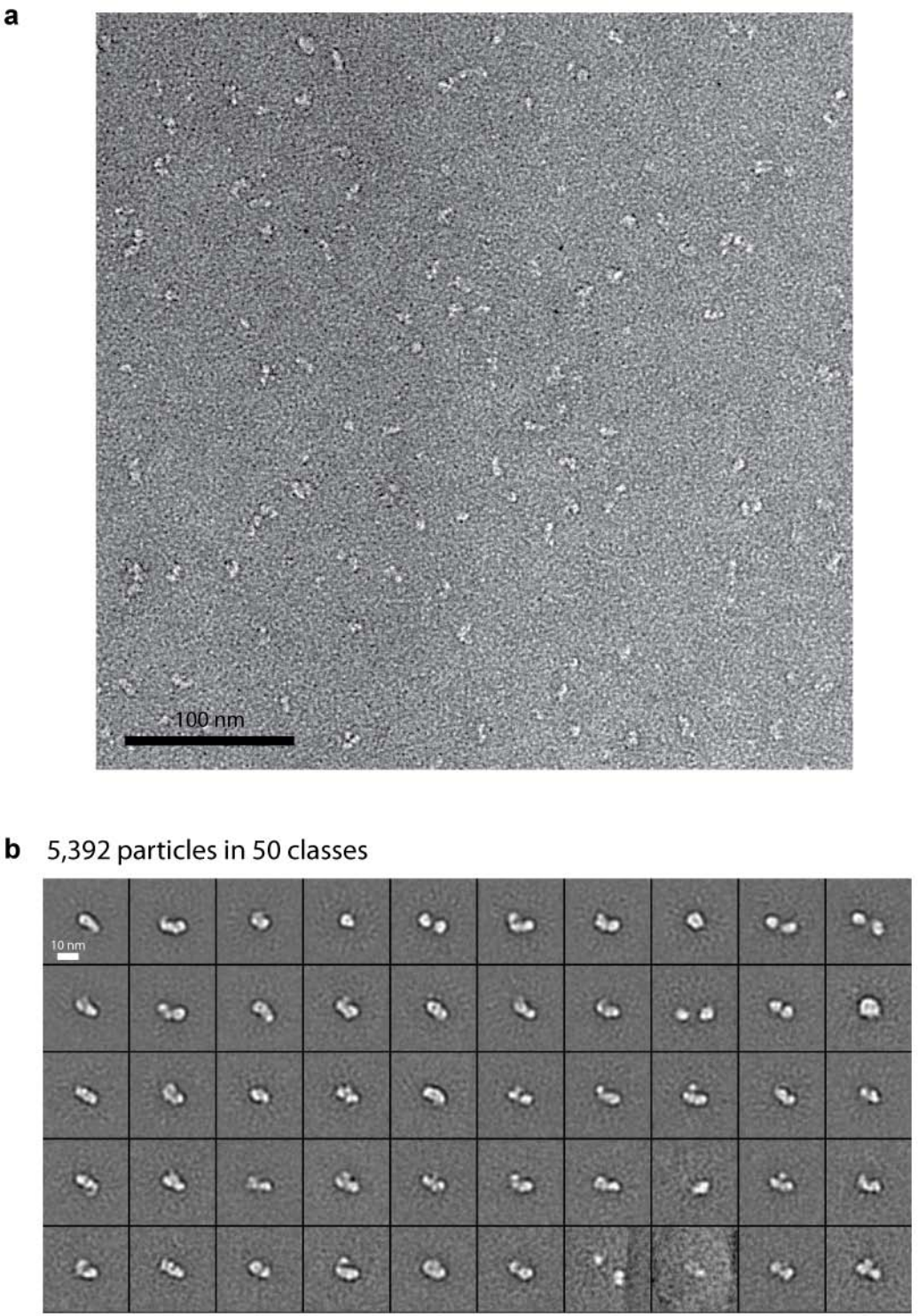
ΔF is the change of fluorescent intensity. A and B are constant parameters. K_d is the dissociation constant, while [I], $[E_t]$, and $[I_t]$ are the free inhibitor concentration, the total kinase concentration, and the total inhibitor concentration, respectively.



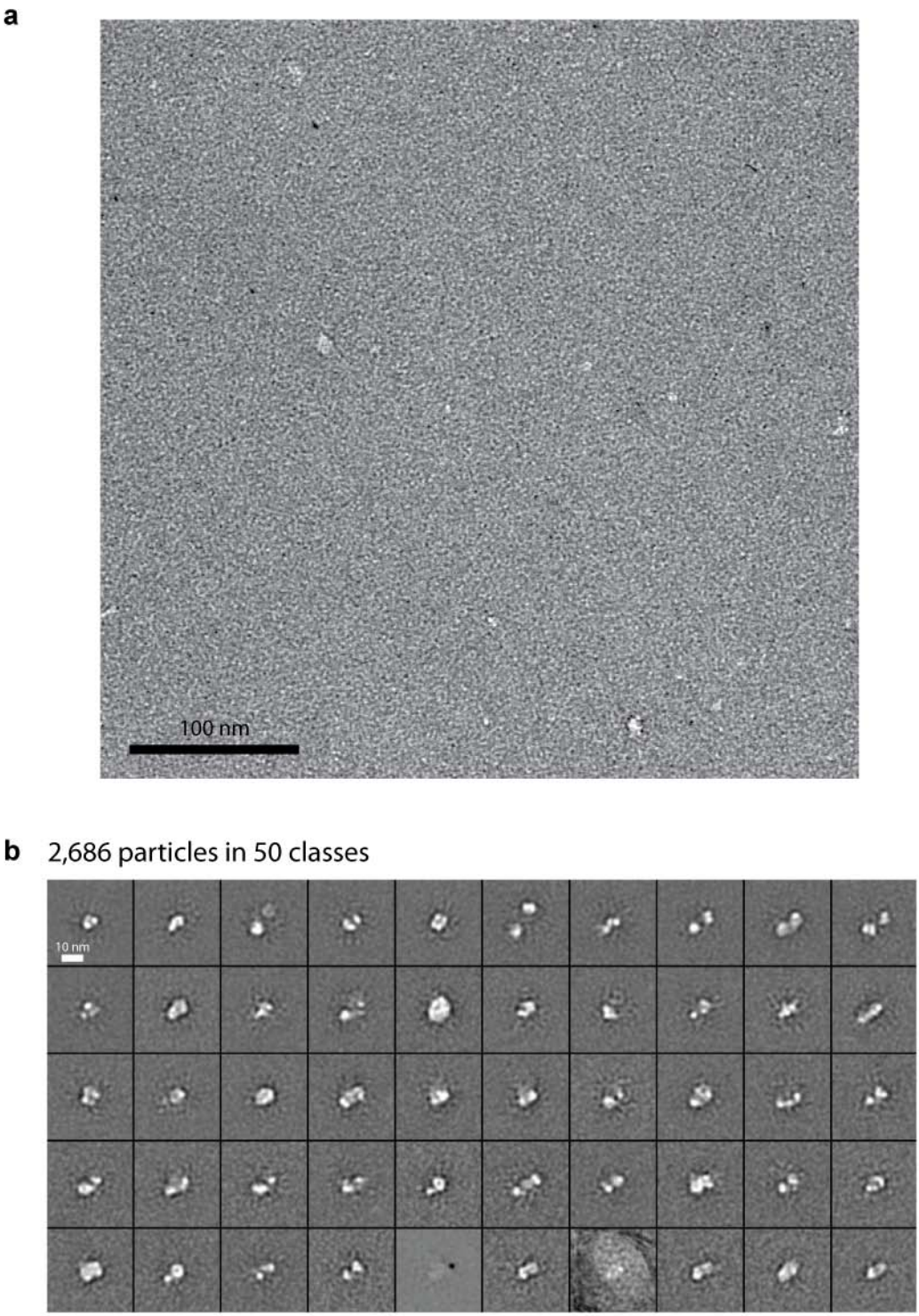
Supplemental Figure S1. Kinase inhibitor binding to EGFR kinase domain. (a,b) The binding of gefitinib to EGFR wt (a) or V924R mutant (b) kinase domain measured with a fluorescence-based assay. (c,d) The binding of erlotinib to EGFR wt (c) or V924R mutant (d) kinase domain measured with the same assay. The binding curves were fit with the equations shown above.



Supplemental Figure S2. EGFR Δ998 in complex with PD168393. Representative micrograph area (a) and class averages (b). Averages are ranked (left to right in each row, from top to bottom row) by numbers of particles in each class.

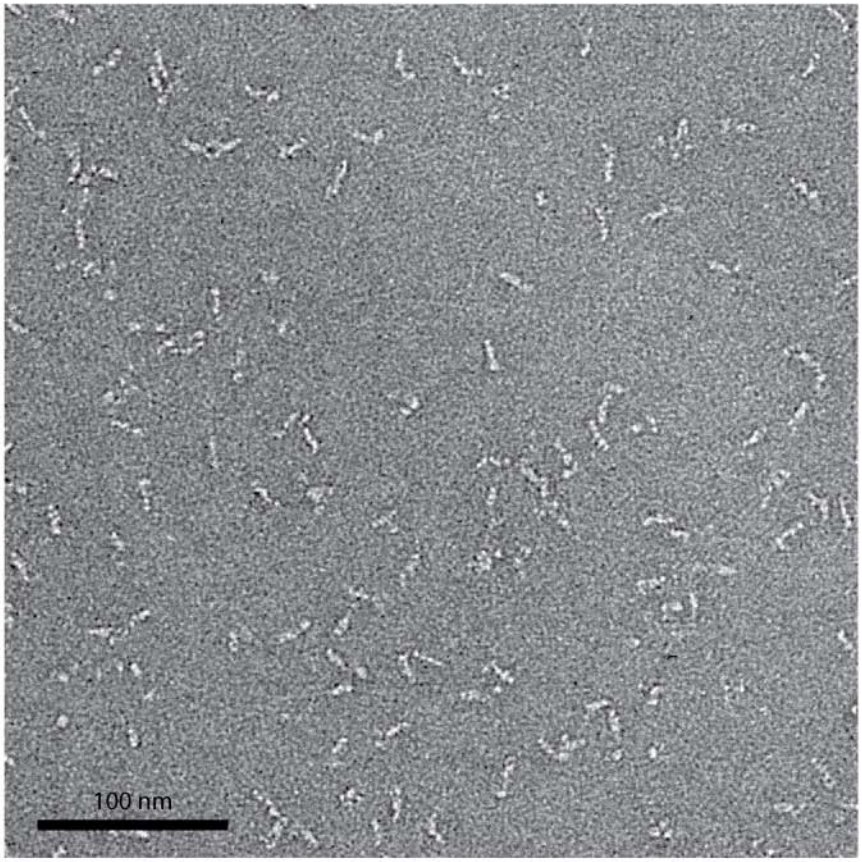


Supplemental Figure S3. EGFR ΔectoΔ998 in complex with PD168393. Representative micrograph area (a) and class averages (b). Averages are ranked (left to right in each row, from top to bottom row) by numbers of particles in each class.

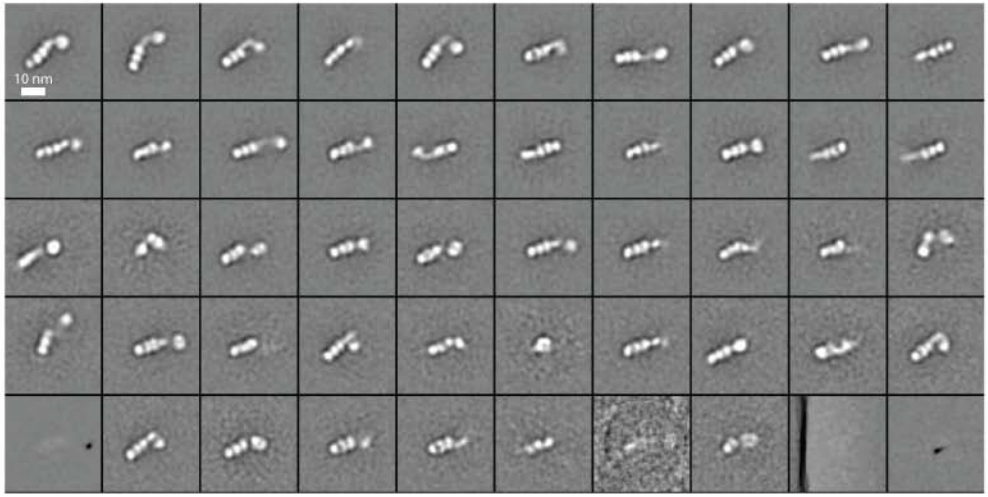


Supplemental Figure S4. EGFR ΔectoΔ998. Representative micrograph area (a) and class averages (b). Averages are ranked (left to right in each row, from top to bottom row) by numbers of particles in each class.

a

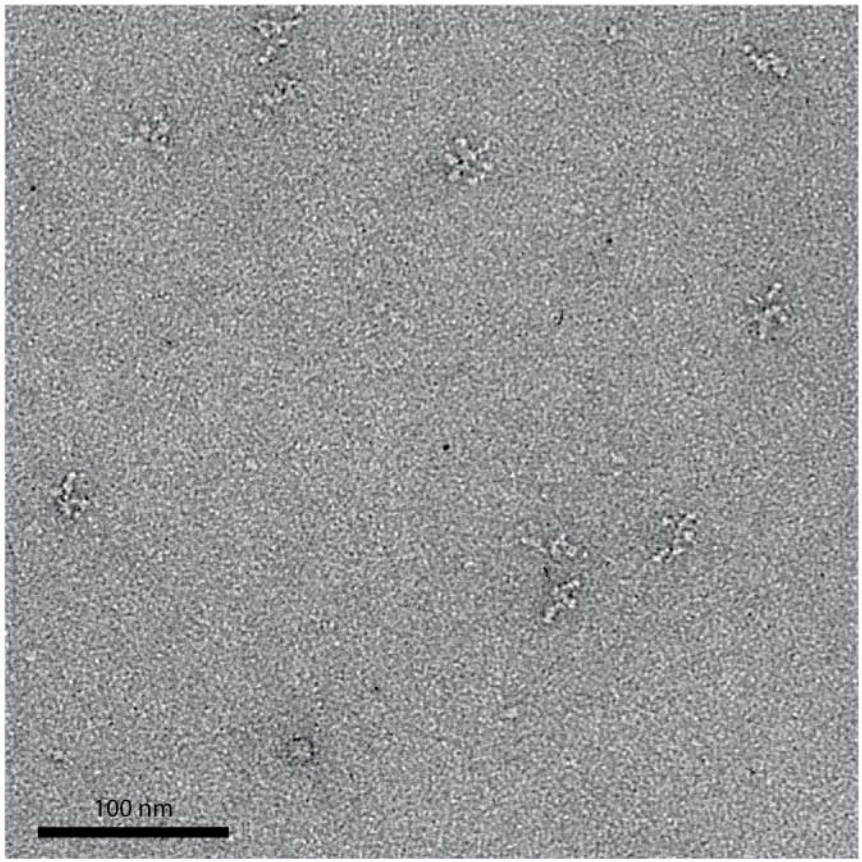


b 8,439 particles in 50 classes

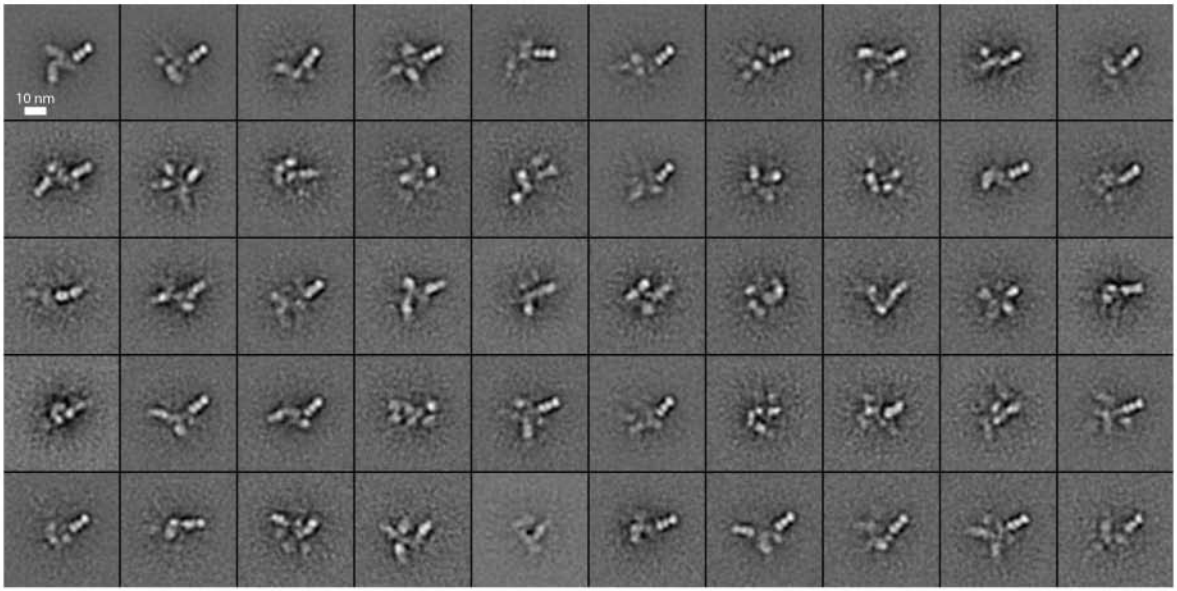


Supplemental Figure S5. EGFR (de 2-7) Δ998 in complex with cetuximab Fab. Representative micrograph area (a) and class averages (b). Averages are ranked (left to right in each row, from top to bottom row) by numbers of particles in each class.

a



b 5,028 particles in 50 classes



Supplemental Figure S6. EGFR Δ998 in complex with PD168393 and cetuximab Fab. Representative micrograph area (a) and class averages (b). Averages are ranked (left to right in each row, from top to bottom row) by numbers of particles in each class.