Structural basis of Zn^{2+}-mediated tethering that stabilizes ERp44-client complexes in protein quality control

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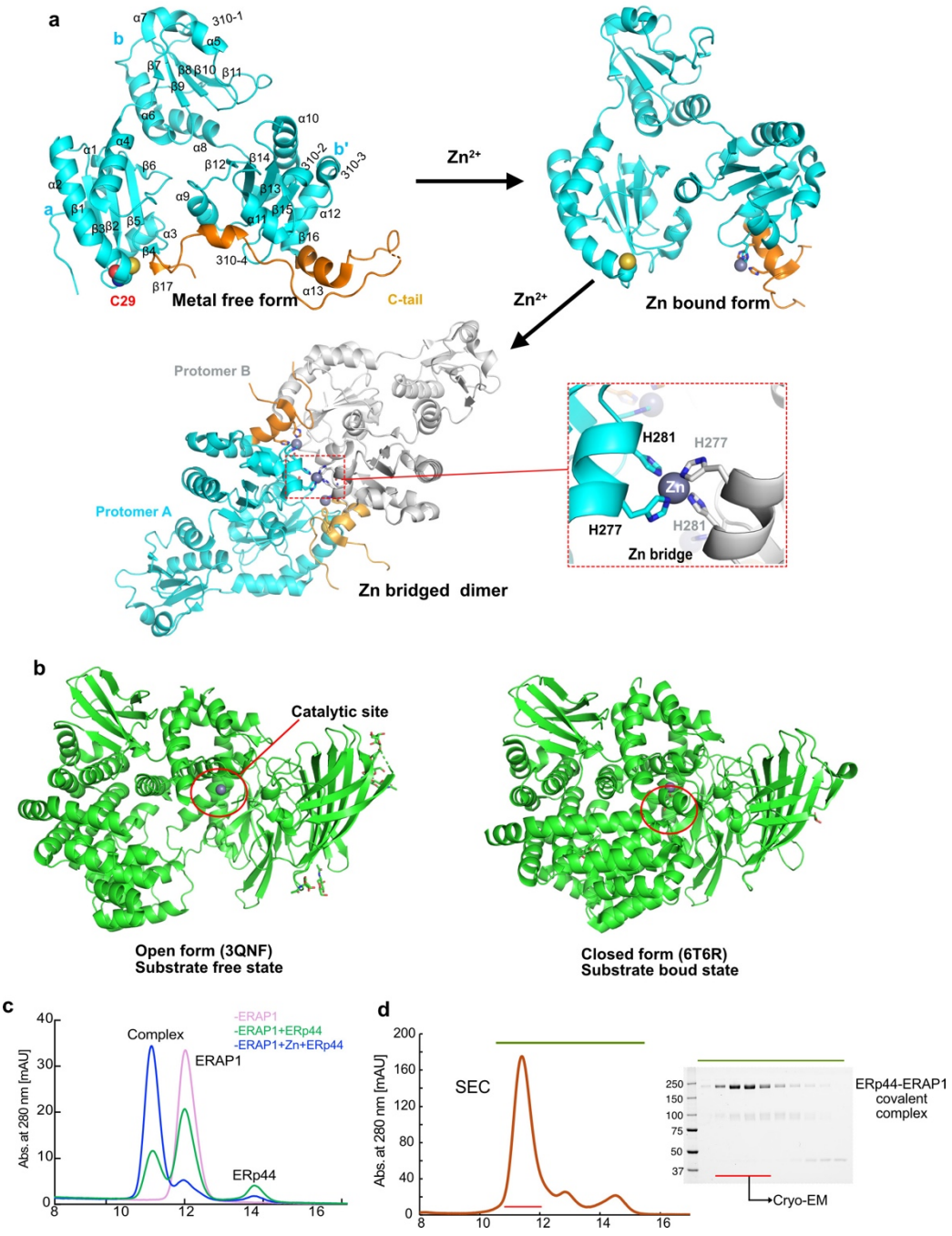
Supplementary Figures 1-10



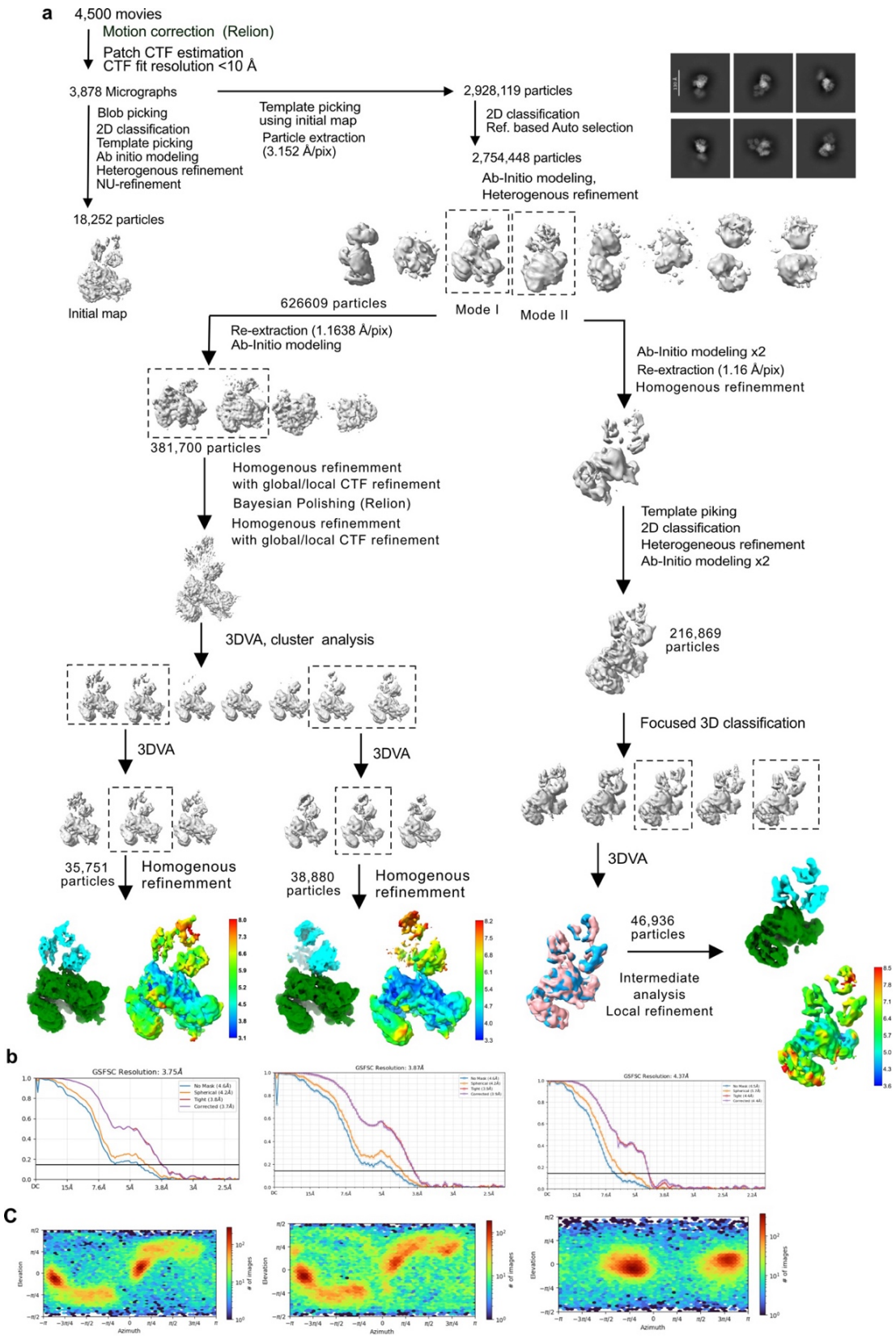
Supplementary Fig. 1 Preparation of the ERp44-ERAP1 complex

a, Zn^{2+}-dependent conformational changes of ERp44 revealed by our previous crystal structure analysis of ERp44[28].

b, Crystal structures of ERAP1 adopting an open conformation in the substrate-free state, and a closed conformation in the substrate-bound state.

c, SEC analysis of Zn^{2+}-dependent complex formation between ERp44 and ERAP1.

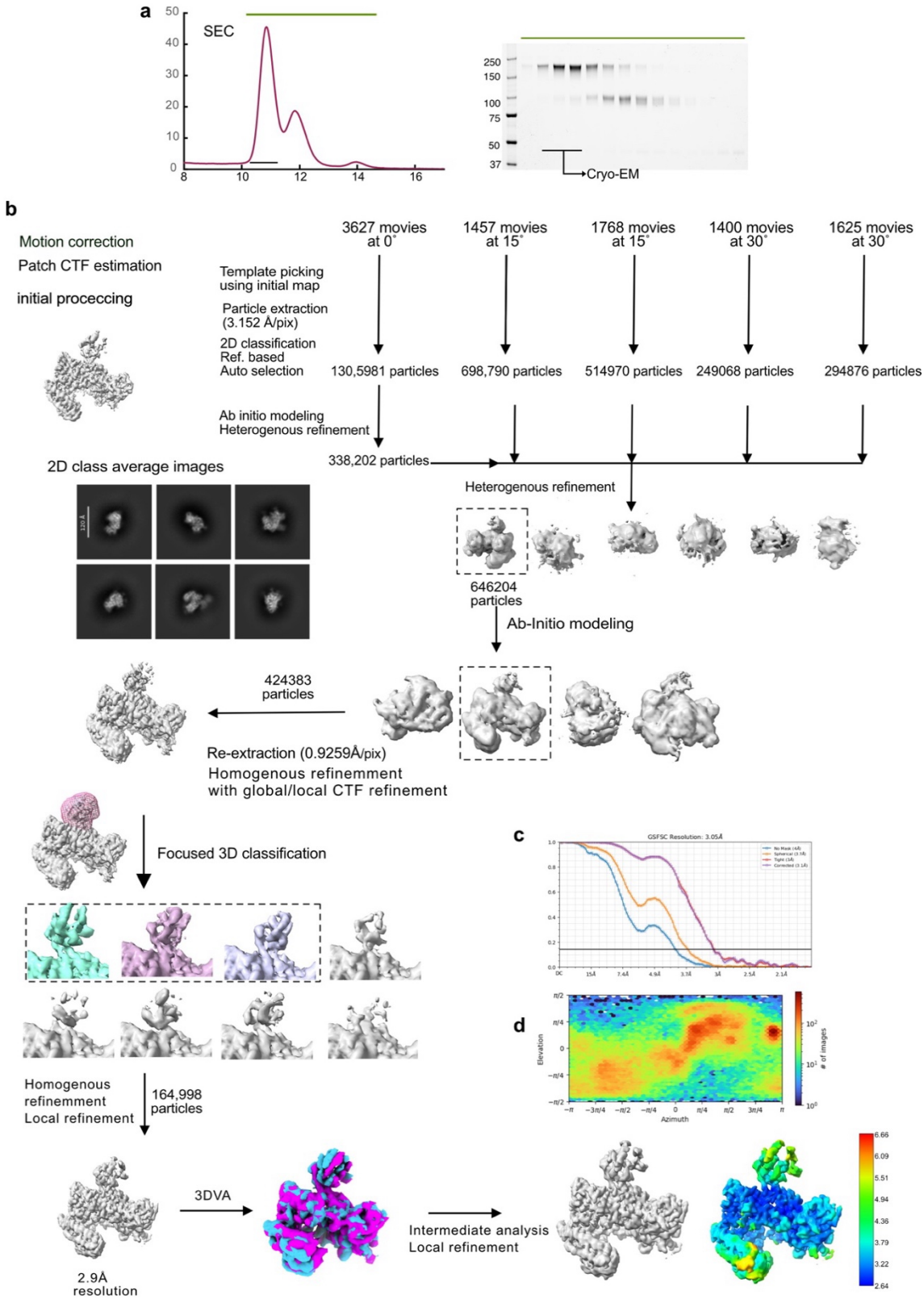
d, SEC profile (left) and non-reducing SDS-PAGE analysis (right) of the ERp44-ERAP1 complex for cryo-EM analysis. The protein bands were visualized with stain-free technology (Bio-Rad).



Supplementary Fig. 2 Overall workflow of cryo-EM data processing of the ERp44-ERAP1 complex

a. Workflow of cryo-EM data processing

b, c. Gold standard Fourier shell correlation (GSFSC) curve (b) and Euler angle distribution (c) for the final reconstruction of each map

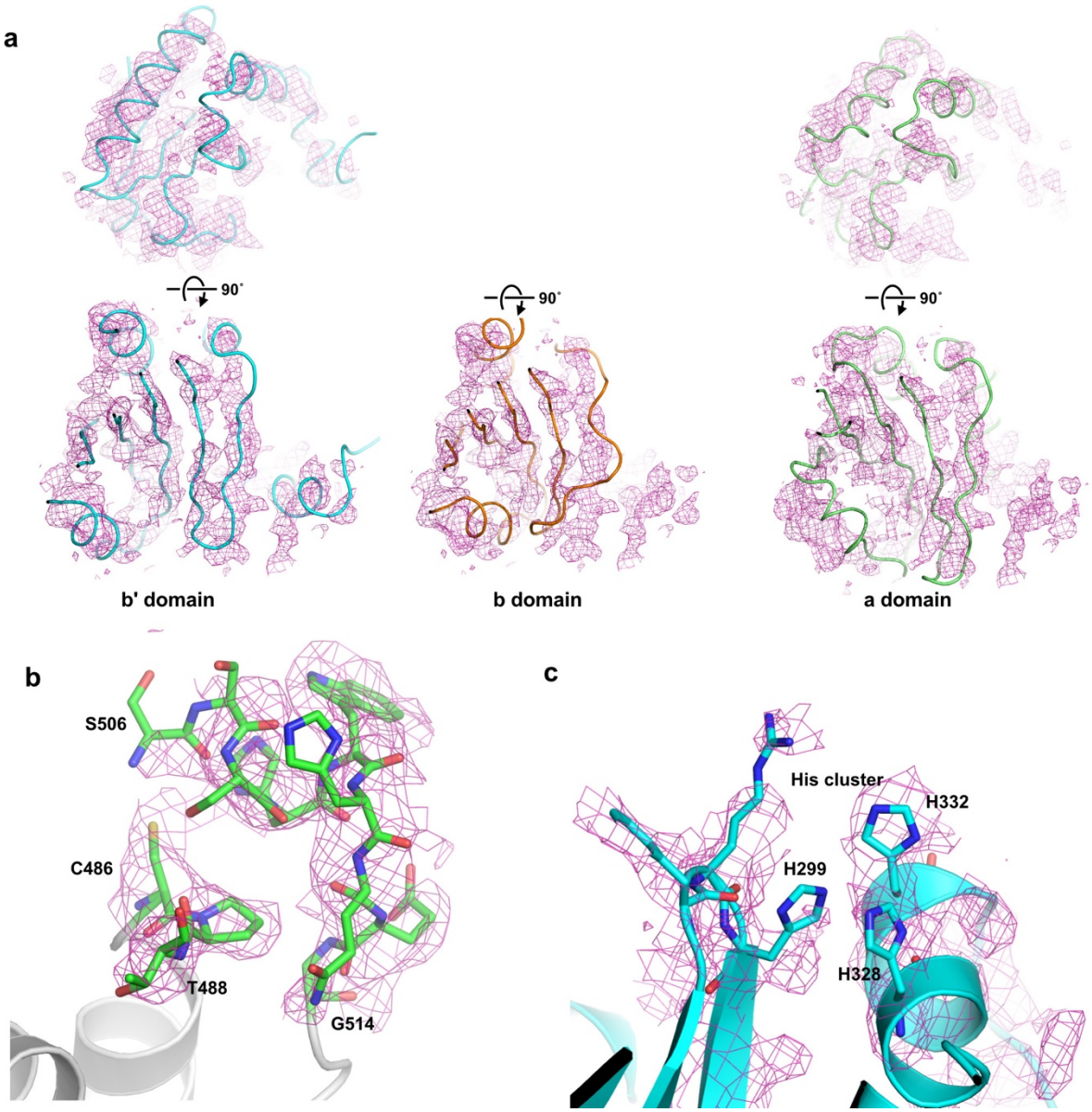


Supplementary Fig. 3 Overall workflow of cryo-EM data processing of the PEG4-treated ERp44-ERAP1 complex

a. SEC profile and non-reducing SDS-PAGE analysis of the PEG4-treated ERp44-ERAP1 complex for cryo-EM analysis. The protein bands were visualized with stain-free technology.

b. Workflow of cryo-EM data processing

c, d. GSFSC curve (c) and Euler angle distribution (d) for the final reconstruction

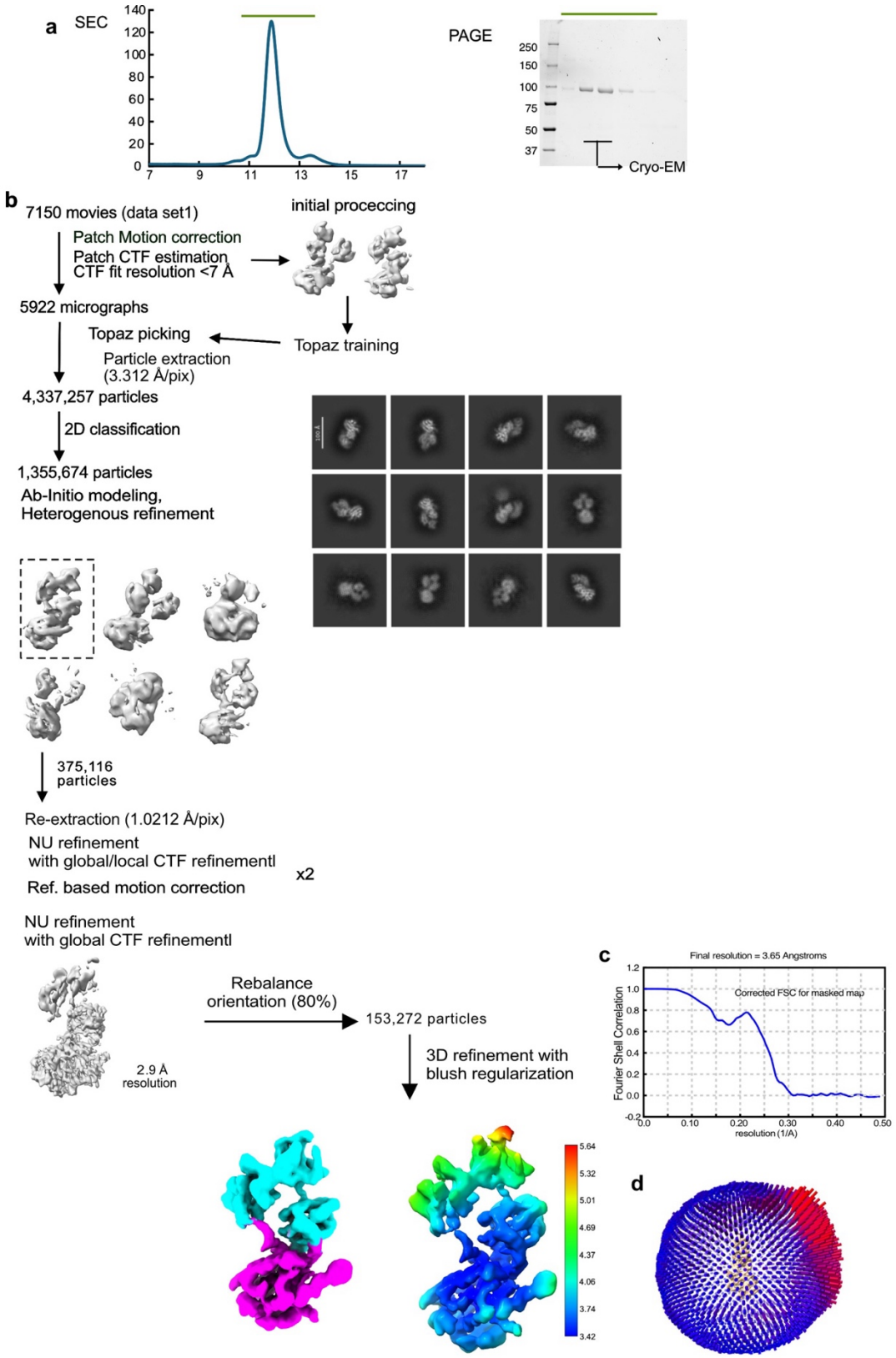


Supplementary Fig. 4 Details of the high-resolution cryo-EM structure of the ERp44-ERAP1 complex

a. Structures of individual ERp44 Trx-like domains overlaid onto the updated cryo-EM density map of the ERp44-ERAP1 complex.

b. Conformation of a part of the ERAP1 loop overlaid on the updated map.

c. Close-up view of the His cluster (His299, His328, His332) of ERp44 overlaid on the updated map.

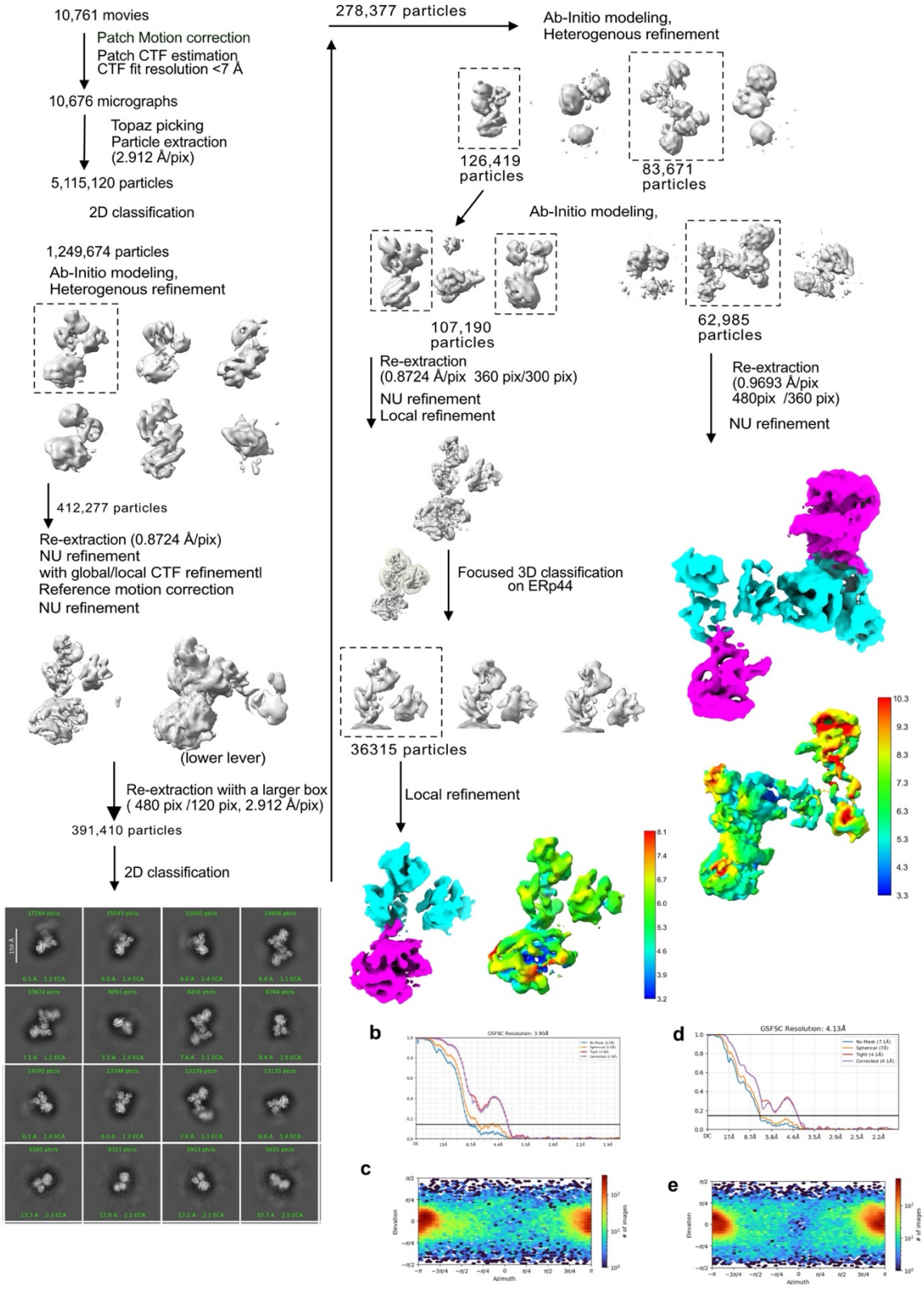


Supplementary Fig. 5 Overall workflow of cryo-EM data processing of the ERp44-Ero1 complex in binding mode I

a. SEC profile and non-reducing SDS-PAGE analysis of the PEG4-treated ERp44-Ero1 complex for cryo-EM analysis. The protein bands were visualized with stain-free technology.

b. Workflow of cryo-EM data processing and overall and locally refined maps

c, d. Fourier Shell Correlation (FSC) curve (c) and Euler angle distribution (d) for final reconstruction.

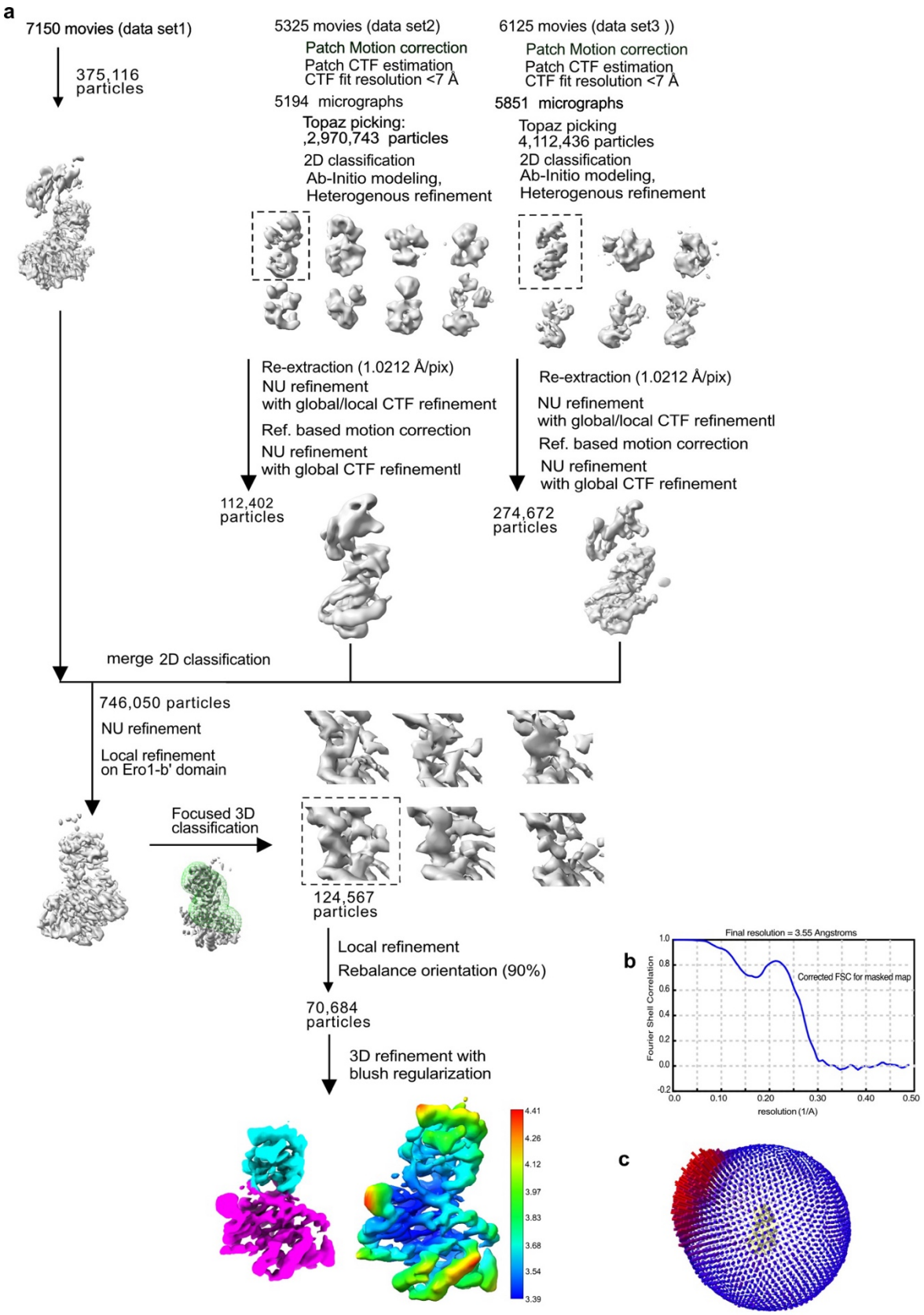


Supplementary Fig. 6 Overall workflow of cryo-EM data processing of the ERp44-Ero1α complex in binding mode I

a. Workflow of image processing of the locally refined structure around the interface between ERp44 **b'** domain and Ero1α

b, c. GSFSC curve (b) and direction distribution (c) for final reconstruction of mode II complex in monomer.

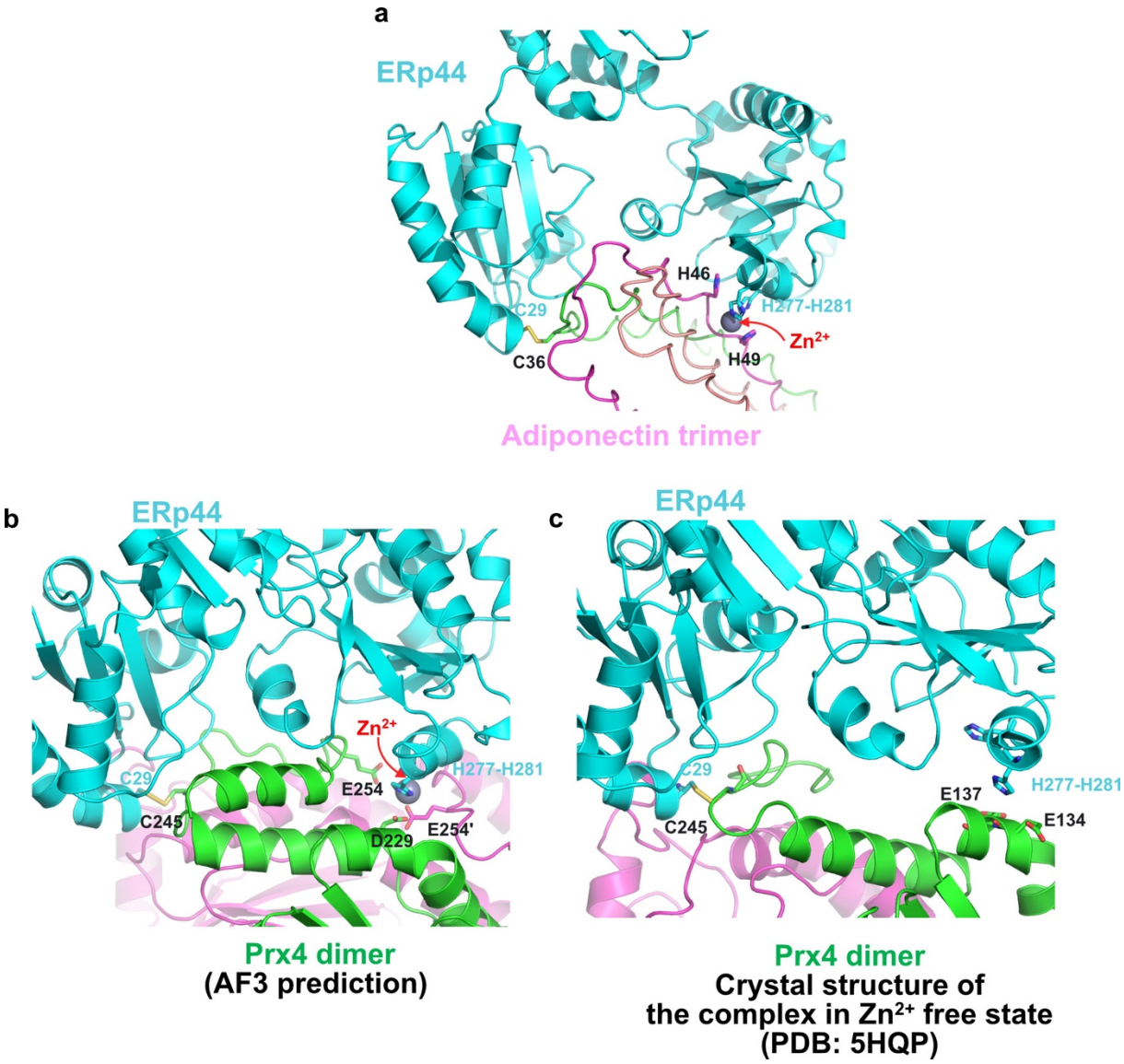
d, e. GSFSC curve (d) and direction distribution (e) for final reconstruction of mode II complex in dimer.



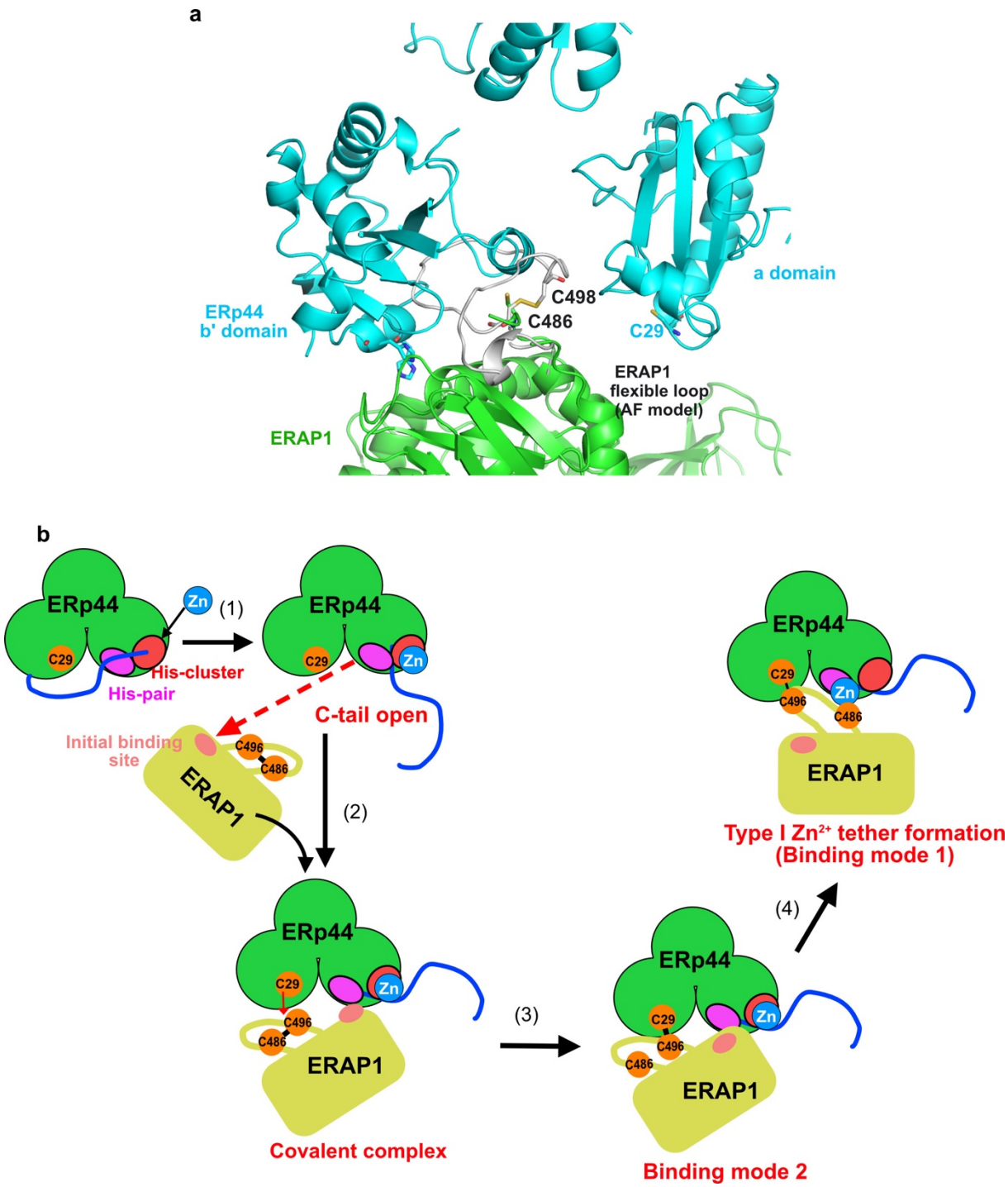
Supplementary Fig. 7 Overall workflow of cryo-EM data processing of the ERp44-Ero1α complex in binding mode II

a. Workflow of cryo-EM data processing and overall and locally refined maps.

b, **c.** FSC curve (b) and Euler angle distribution (c) for the final reconstruction.



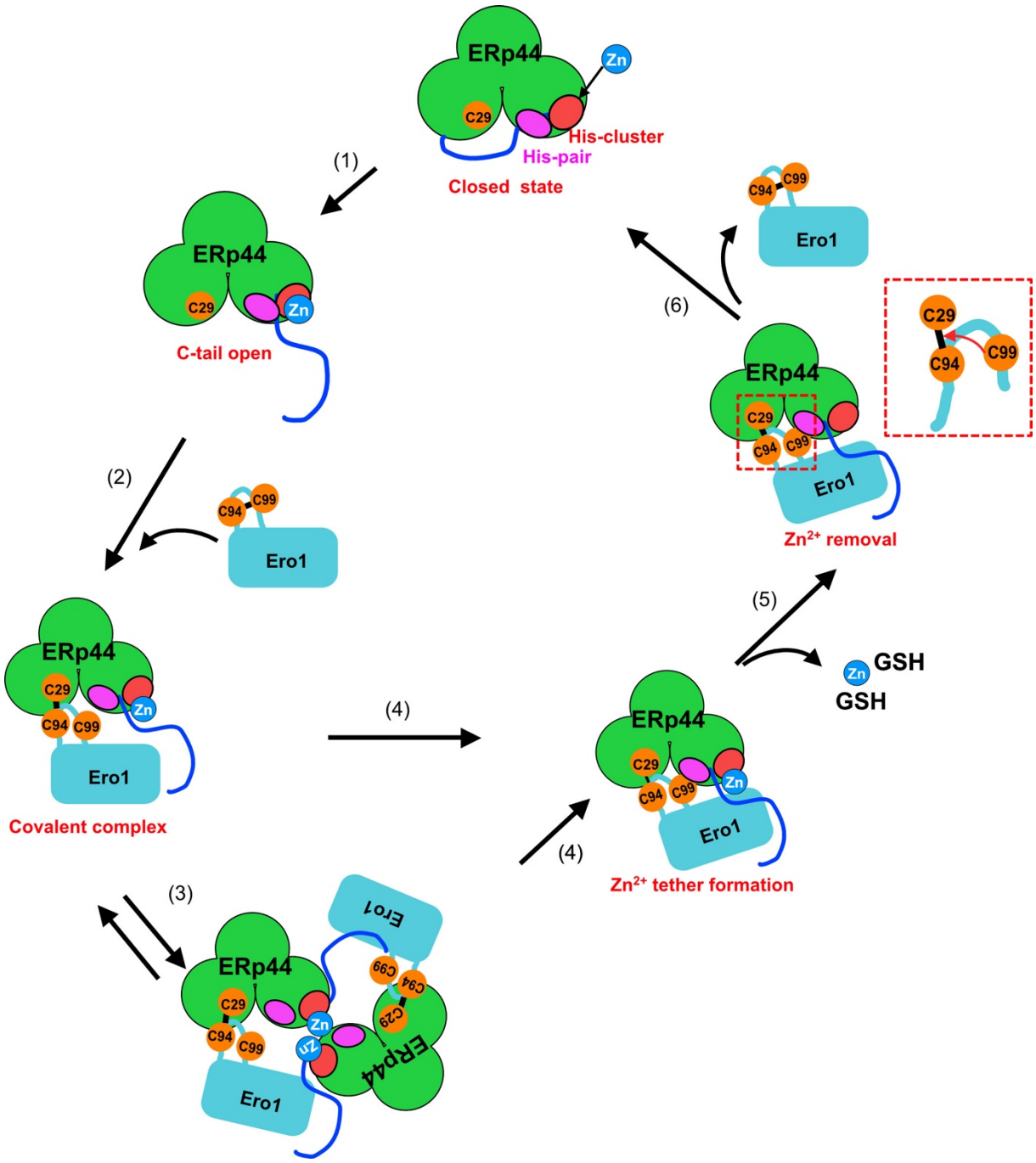
Supplementary Fig. 8 Prediction of Zn^{2+}-mediated interactions between ERp44 and other clients
a. AlphaFold3 (AF3)-predicted model of the complex between ERp44 (cyan) and the adiponectin trimer (green, magenta and pink) in the presence of Zn^{2+} (gray sphere).
b. AF3 predicted model of the complex between ERp44 (cyan) and the Prx4 dimer (green, magenta) in the presence of Zn^{2+} (gray sphere).
c. Crystal structure of the ERp44-Prx4 complex in the absence of Zn^{2+} (PDB ID 5HQP).



Supplementary Fig. 9 Mechanisms of ERAP1 recognition by ERp44

a. Putative placement of the modeled flexible loop of ERAP1 based on the cryo-EM structure of the ERp44-ERAP1 complex

b. Proposed model for Zn^{2+}-dependent, stepwise recognition of ERAP1 by ERp44.



Supplementary Fig. 10 Mechanisms of Ero1α recognition and release by ERp44
Proposed model for Zn^{2+}-dependent, stepwise recognition and release of Ero1α by ERp44.