SUPPLEMENTARY DATA FOR

Structural complementation of the catalytic domain of *Pseudomonas* exotoxin A

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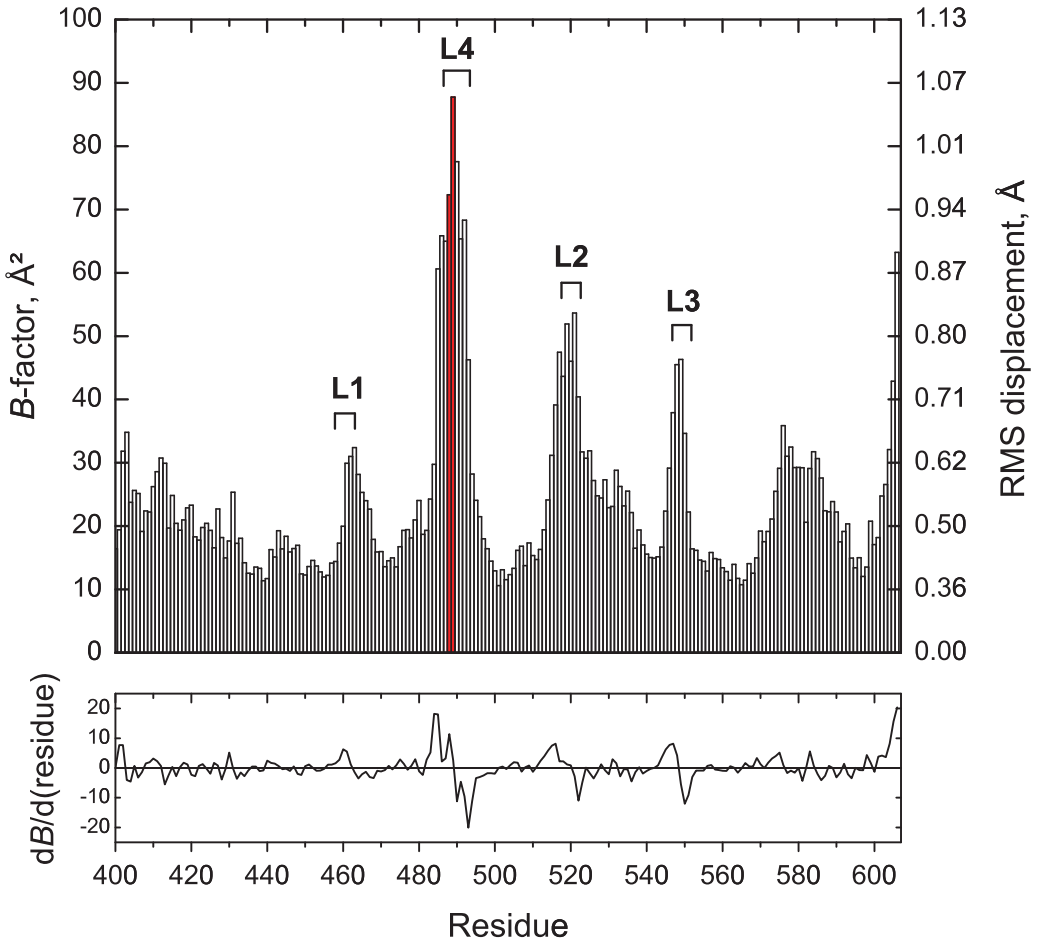

Figure S1. **Main-chain *B*-factors along the intact PE3 (residues 400 to 613) sequence.** *B*-factors are extracted from the PE3 apoenzyme (PDB 1IKQ). The root-mean-square (RMS) displacement (assuming isotropic vibration) and a first-derivative analysis are shown for reference. The four mobile loops identified with locally elevated *B*-factors are labeled L1 to L4. Bars bracketing to the site of dissection, Asp^{488}/Ala^{489}, are colored red.

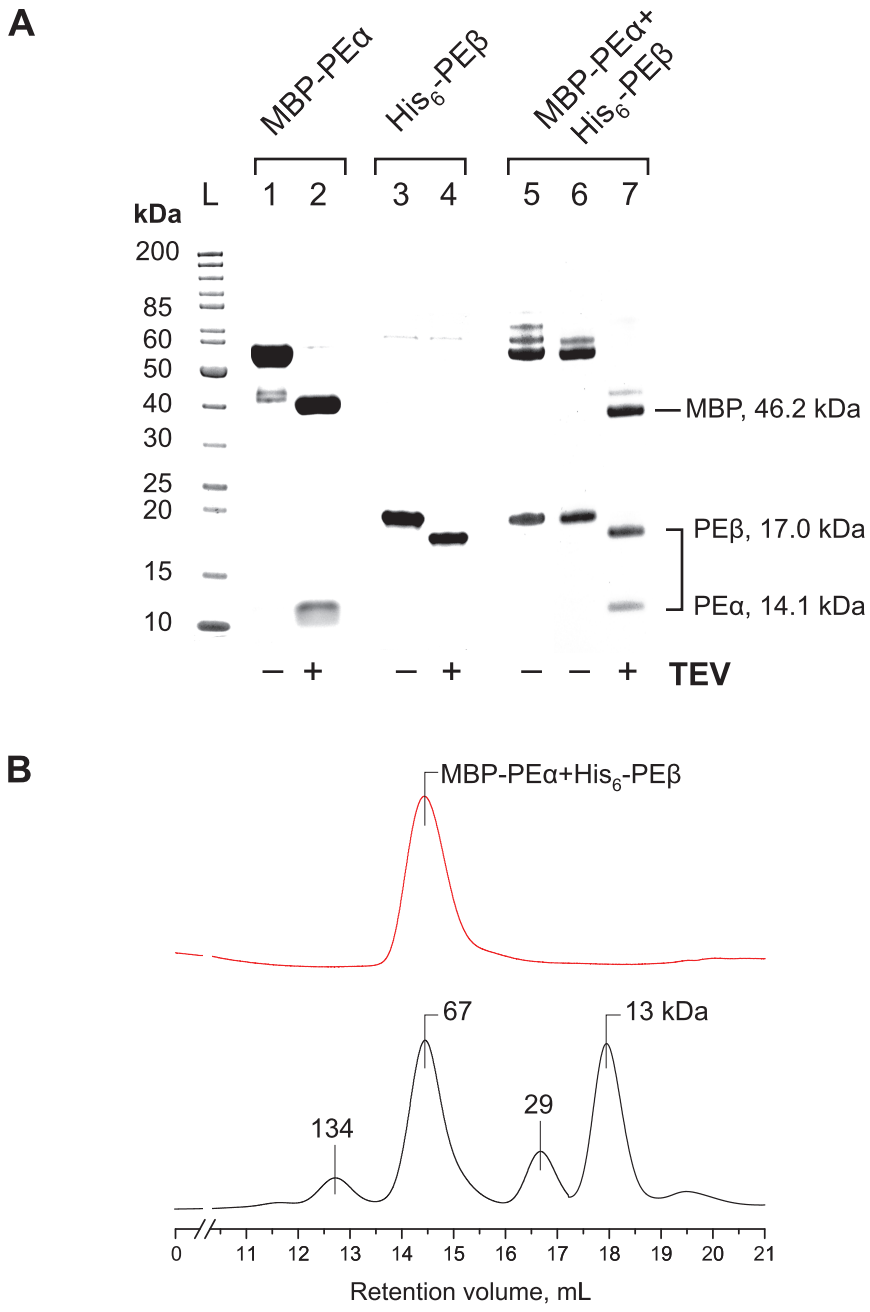

Figure S2. **Split PE3 self-assembles spontaneously in solution.** *A*, SDS-PAGE analysis of split PE3 fragments expressed individually (Lanes 1, and 3) and together (Lane 5) in *E. coli*. MBP-tagged PEα fragment was purified on amylose-agarose (Lane 1) and His_6-tagged PEβ on Co-NTA agarose (Lane 3), respectively. Cleavage by TEV protease (not visible) yields the untagged constructs (Lanes 2, 4). To confirm noncovalent association of the two fragments, co-expressed MBP-PEα and His_6-PEβ fragments were purified on Co-NTA agarose (Lane 5) and subsequently pulled down on amylose-agarose (Lane 6) before cleavage with TEV (Lane 7). *B*, Size-exclusion chromatography of co-purified PEα and PEβ fragments (from Lane 6 in Panel A; red trace). The column was calibrated (black trace) with BSA (134 and 67 kDa, corresponding to dimeric and monomeric forms, respectively), carbonic anhydrase (29 kDa), and cytochrome C (13 kDa).

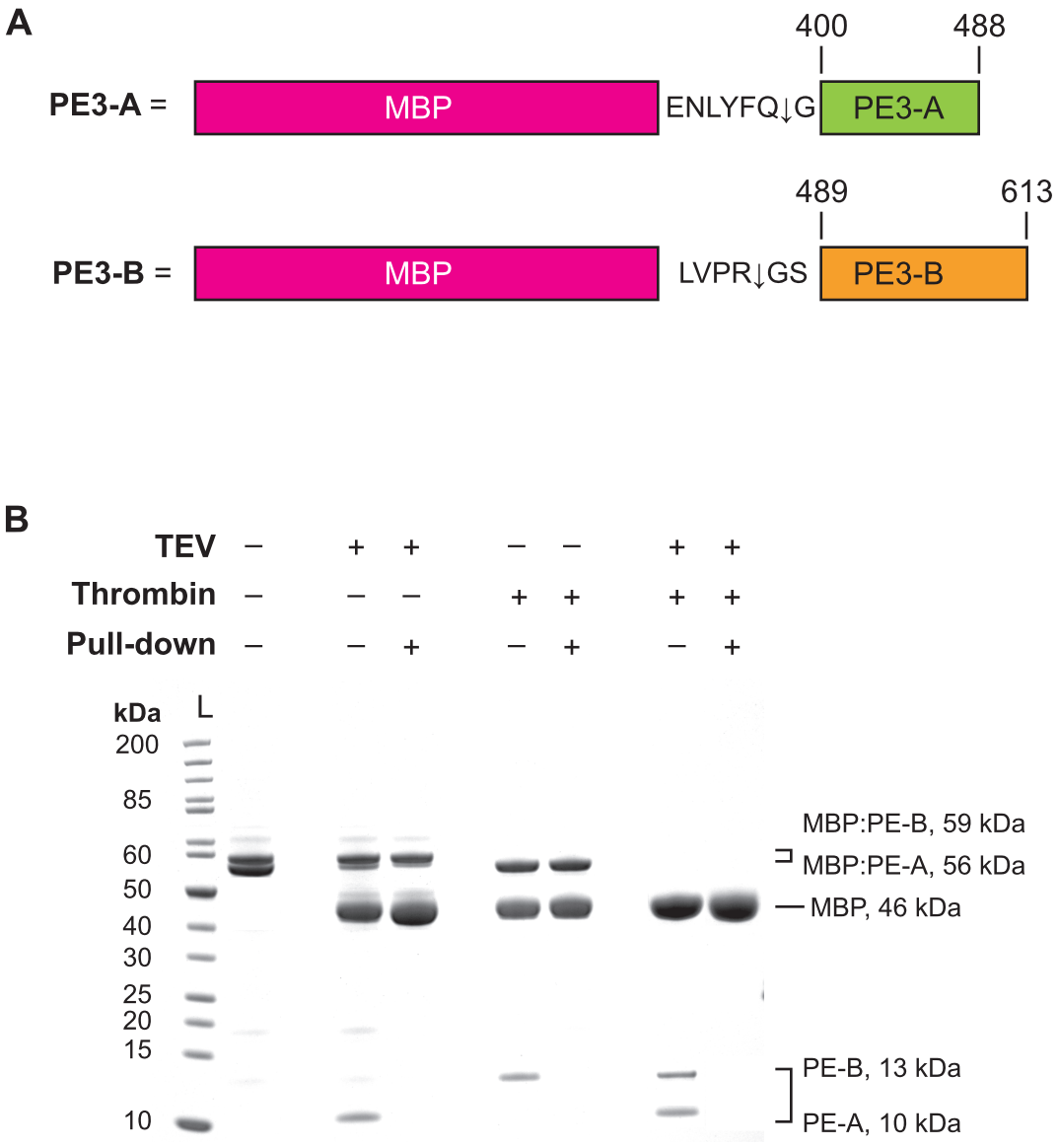

Figure S3. **Structural complementation of split PE3 requires a dimerization domain.** *A*, Split PE3 fragments without a heterospecific coiled coil (PE3-A and PE3-B) were cloned and expressed as C-terminal fusions to maltose-binding protein (MBP). The coiled-free fragments were spaced from their MBP partners by a specific cleavage site for TEV protease (ENLYFQ↓G for PE-A) or thrombin (LVPR↓GS for PE-B). PE-A and PE-B were co-expressed and purified from *E. coli*. *B*, After treatment with TEV protease or thrombin, the mixture was pulled down on amylose-agarose (which binds MBP) and analyzed by SDS-PAGE. Whereas coil-bearing split PE3 fragments were efficiently co-precipitated (c.f. Figure 2A in the main text), neither coil-free construct could be pulled down without its MBP partner, indicating that PE3-A and PE3-B do not associate without a dimerization domain. This observation is corroborated by the lack of ADPRT activity in coil-free PE3-A and PE3-B (Figure S3).

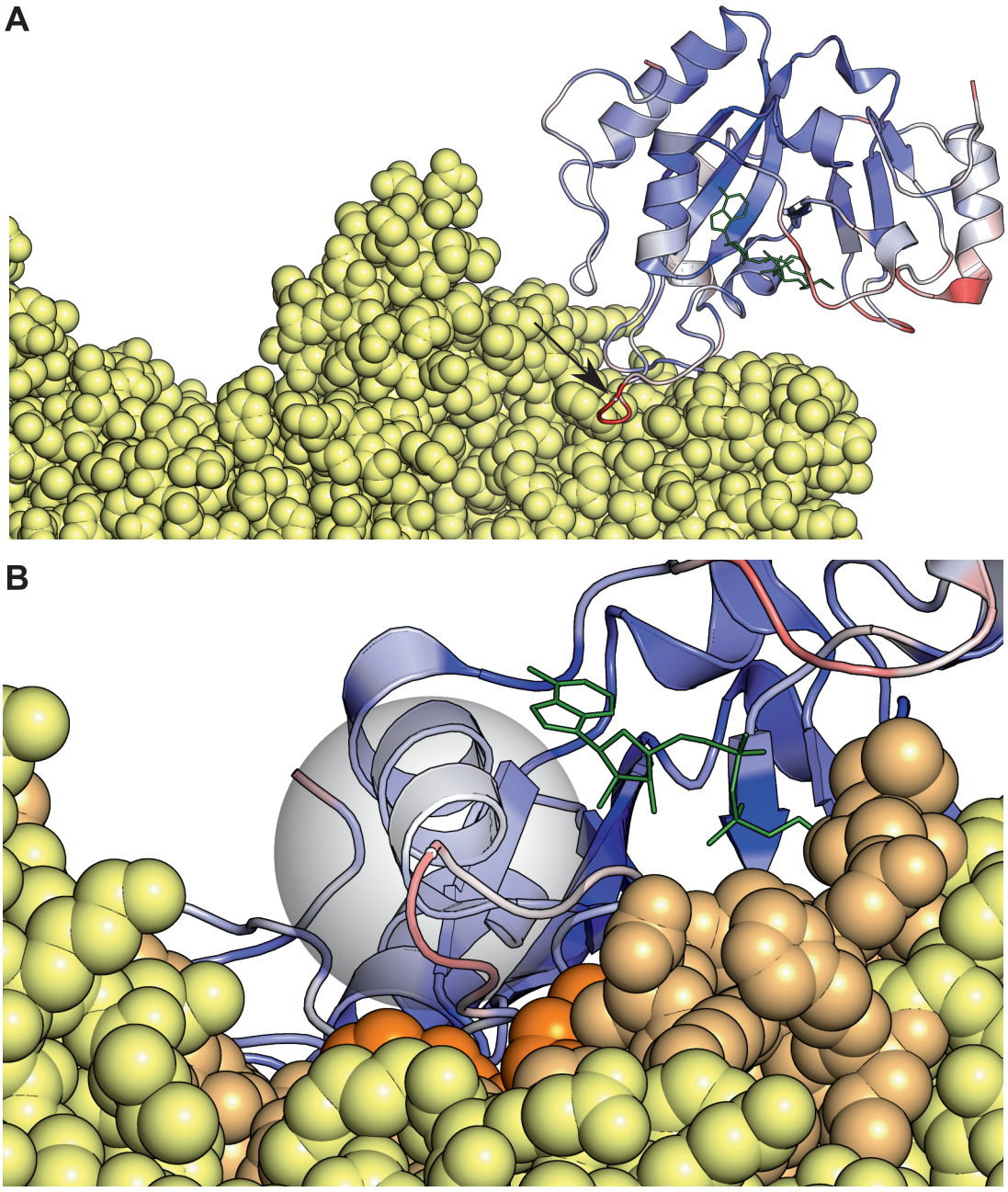

Figure S4. **Structural analysis of the dissection site in split PE3.** *A*, The co-crystal structure of eEF2-PE3-NAD^+ (PDB ID: 2ZIT) is rendered to show the topology of the dissection site (Asp^{488}/Ala^{499}, arrow) in split PE3. PE3 is shown in cartoon and colored by *B*-factor of the backbone C^{α} as in Figure 1A (red = most dynamic). NAD^+ is shown in green and eEF2 is shown as yellow spheres by their van der Waals radii. The dissection site is located in a highly dynamic segment and points away from eEF2 (towards the reader). *B*, The dissection site does not contact any eEF2 atom within 6.5 Å (gray sphere). eEF2 residues within 10 Å of the dissection site are colored orange, those within 20 Å are beige, and all other distal residues are yellow.