

## **Description of Additional Supplementary Files**

**File name: Supplementary Movie 1**

**Description: Cryo-EM structure of the Pex1/Pex6 (class 3; “single-seam” state).** Color code as in Figure 1a.

**File name: Supplementary Movie 2**

**Description: Structural comparison between “twin-seam” and “single-seam” state.** Color code as in Figure 1a. Note that the transition from twin-seam to single-seam state in the D2 ring induces a swing-in of the Pex1(D1/N2)/Pex6(D1/N2/N1) domains in the D1 ring (see Fig. 4d-e).

**File name: Supplementary Movie 3**

**Description: Structural comparison of the Pex1(D2)/Pex1(D1) interface between “twin-seam” and “single-seam” state.** (see Fig. 4g).