Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information

Single-molecule level dynamic observation of disassembly of the apo-ferritin cage in solution

Basudev Maity[†], Zhipeng Li[†,‡], Kento Niwase[†], Christian Ganser,[§] Tadaomi Furuta[†], Takayuki Uchihashi,[§, §§] Diannan Lu,[‡] Takafumi Ueno[†]**

[†]Department of Life Science and Technology, Tokyo Institute of Technology, Nagatsuta-cho, Midori-ku, Yokohama 226-8501, Japan

[‡]Ministry of Education Key Laboratory of Industrial Biocatalysis, Department of Chemical Engineering, Tsinghua University, Beijing 100084, China

[§]Exploratory Research Center on Life and Living Systems (ExCELLS), National Institutes of Natural Sciences, 5-1 Higashiyama, Myodaiji, Okazaki, 444-8787, Japan

[§§]Department of Physics, Nagoya University, Nagoya, Aichi 464-8602, Japan

*E-mail: tueno@bio.titech.ac.jp; ludiannan@tsinghua.edu.cn

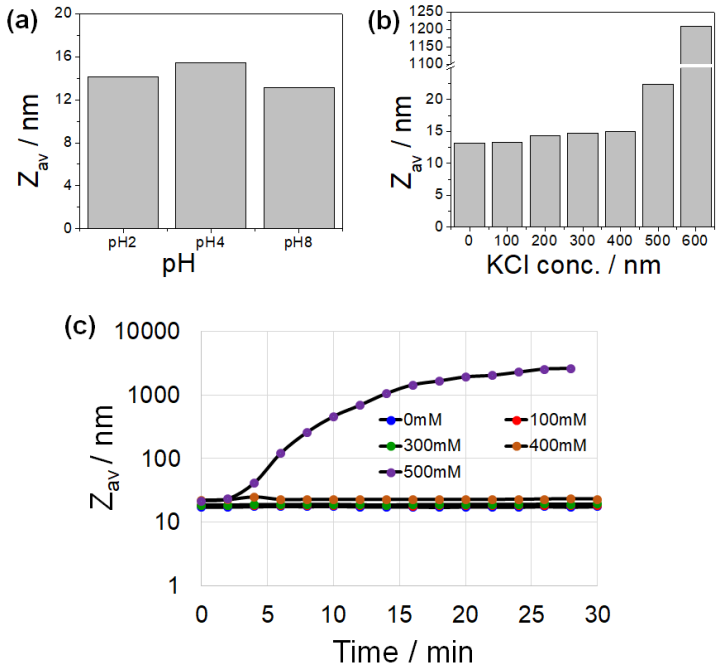

Figure S1: DLS measurement on ferritin particles in solution. (a) Averaged hydrodynamic diameter (Z_{av}) of ferritin particles at various pHs. Equilibration time for the measurement was 60s. (b) Effect of various KCl concentrations on Z_{av} at pH 2.3. Equilibration time for the measurement was 60s. (c) Changes of Z_{av} with time for ferritin particles in a solution of pH 2.3 containing various KCl concentrations.

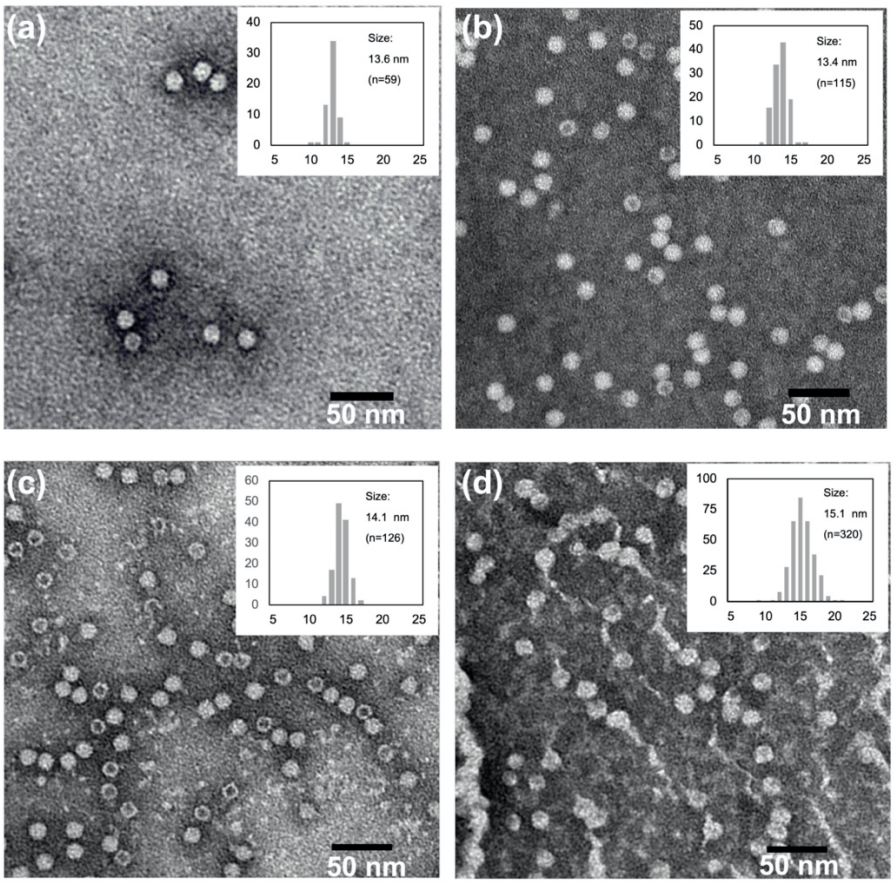

Figure S2: TEM images of the apo-rHLFr cage at various pH and salt concentrations. (a) pH 8, 100 mM KCl (b) pH 4, 100 mM KCl (c) pH 2.3, 100 mM KCl and (d) pH 2.3, 500 mM KCl. The incubation time before 1% Methyl amine tungstate (Nanoprobes) staining was 1 min. Staining time: 1 min. Size distributions (Count vs Size/nm) of the apo-rHLFr cage are shown in the inset.

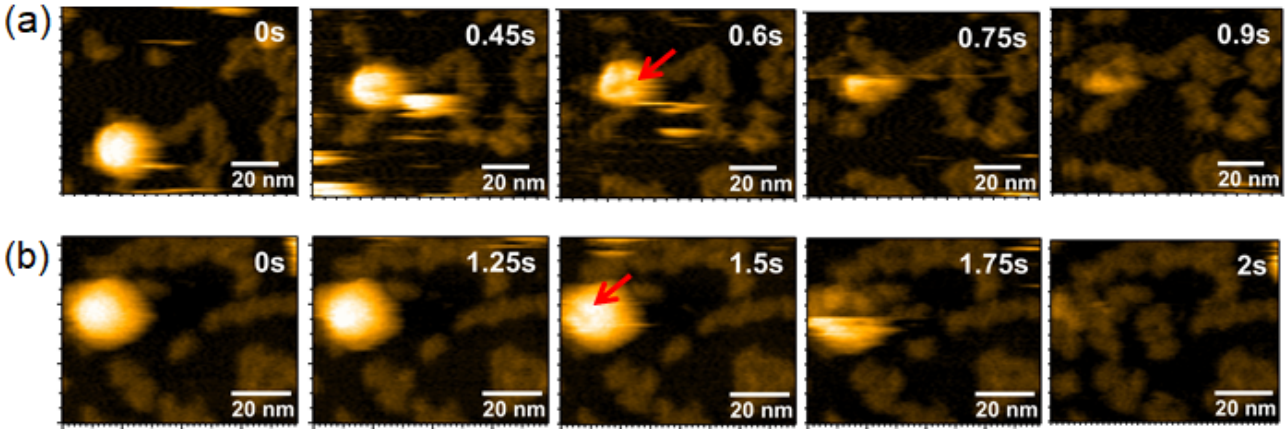

Figure S3: Time course of the disassembly process of single ferritin cage molecules. (a-b) Snapshots of the HS-AFM measurement at various time intervals during the disassembly of two independent ferritin cage molecules. Measurement condition: pH 2.3-100mM KCl at RT.

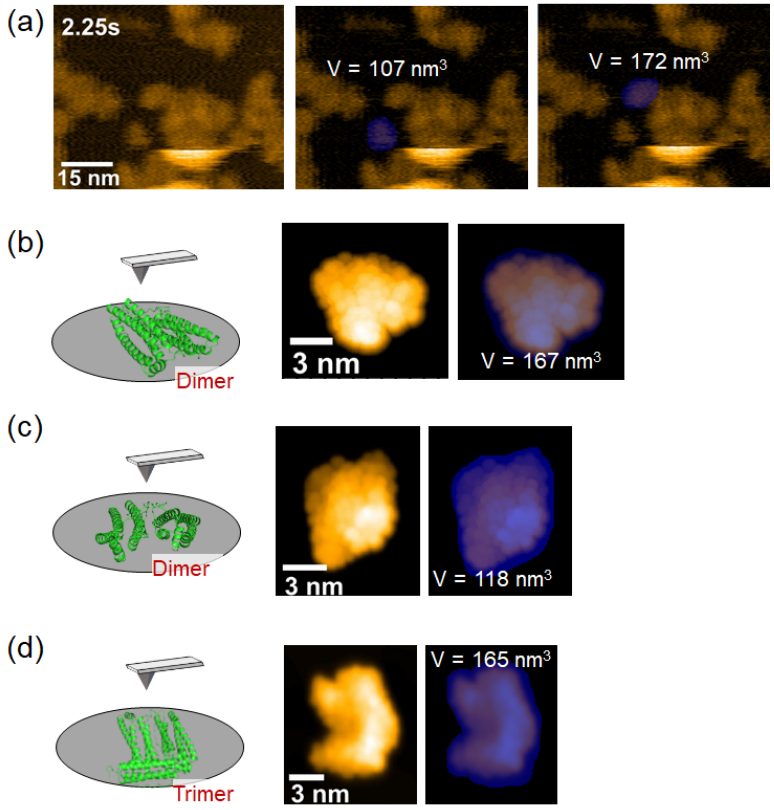

Figure S4: Estimated volumes of the apo-rHLFr fragments after the disassembly. (a) Estimation of volumes in the HS-AFM image of two identifiable subunit fragments (shaded by blue) which were created by the apo-rHLFr cage disassembly at 2.25s. (b-d) Models of the AFM simulation of a dimer with two different angles on the flat surface and a trimer unit with the simulated AFM images and the selected area (marked in blue) for the volume calculation. The probe (Z-axis) and the X-Y plane is represented by a cartoon and a grey circle, respectively. The pdb of the dimers and the trimer were obtained from the crystal structure (1DAT).

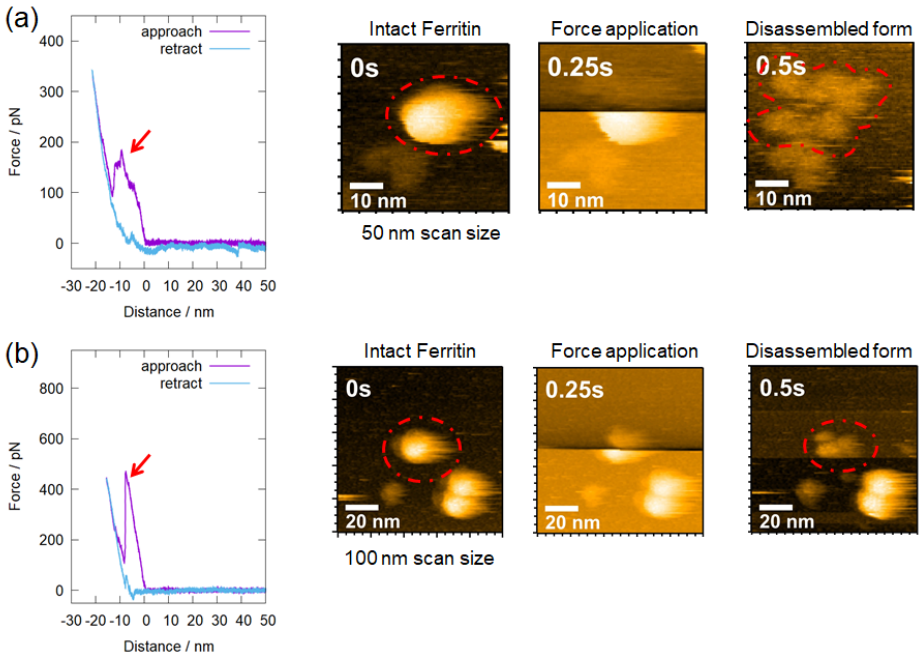

Figure S5: Mechanical loading of the apo-rHLFr cage during HS-AFM imaging, including the corresponding force curves. (a) Mechanical disassembly at pH 2.3 and (b) at pH 4. The red arrows indicate the assumed position to disassemble the cage by the tip-induced mechanical force.

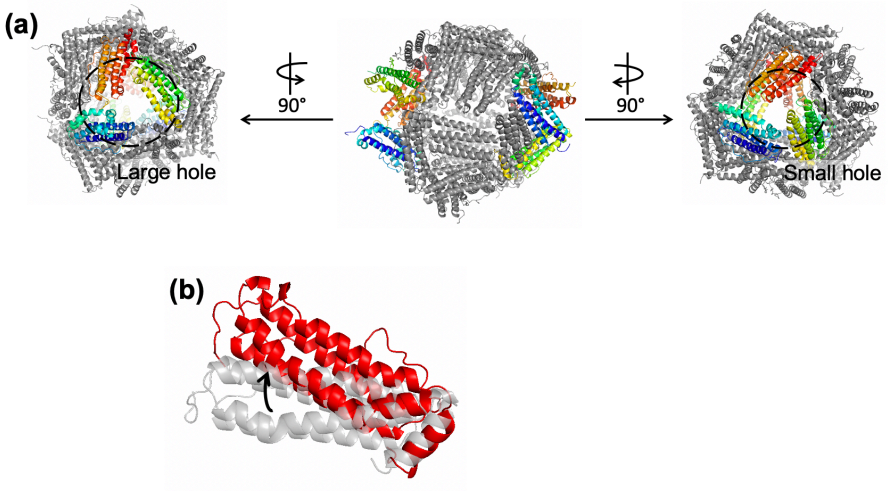

Figure S6: (a) Symmetry disruption at the 3-fold symmetry causing the opening of the pore at the 3-fold pore opposite to each other at pH 2 (50 ns). (b) Displacement of a monomeric subunit at pH 2 (Red) from its original position at pH 7 (Grey) during the opening of 3-fold pore.

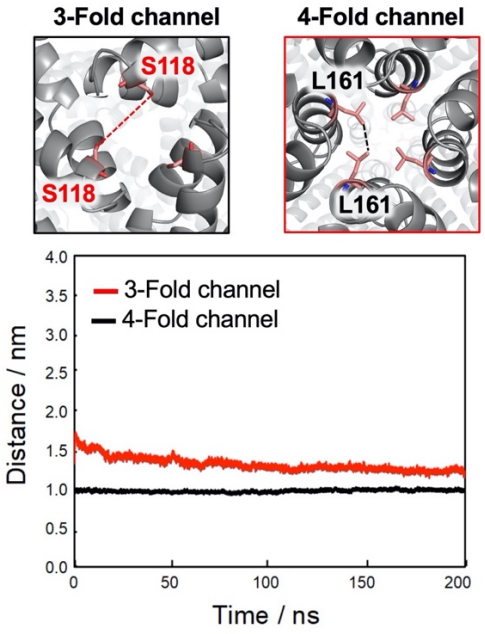

Figure S7: (a) Fluctuations of Ser118-Ser118 distance as a representative of the diameter of 3-fold channel during simulations at pH7 and (b) Fluctuations of Leu161-Leu161 distance as a representative of the diameter of 4-fold channel at pH 7.

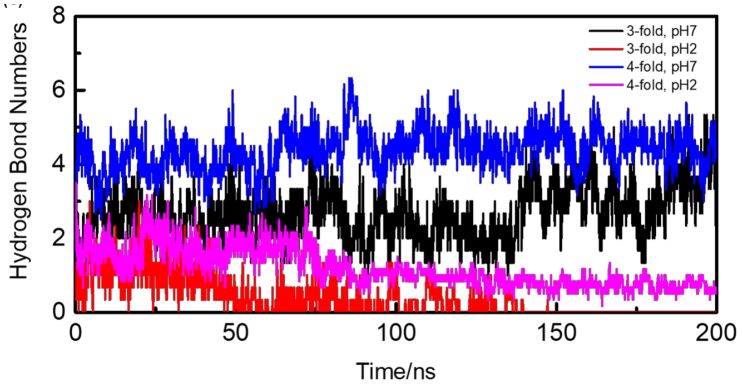

Figure S8: Changes in the number of hydrogen bonds in 3- and 4-fold channels during the simulation period of 200ns.

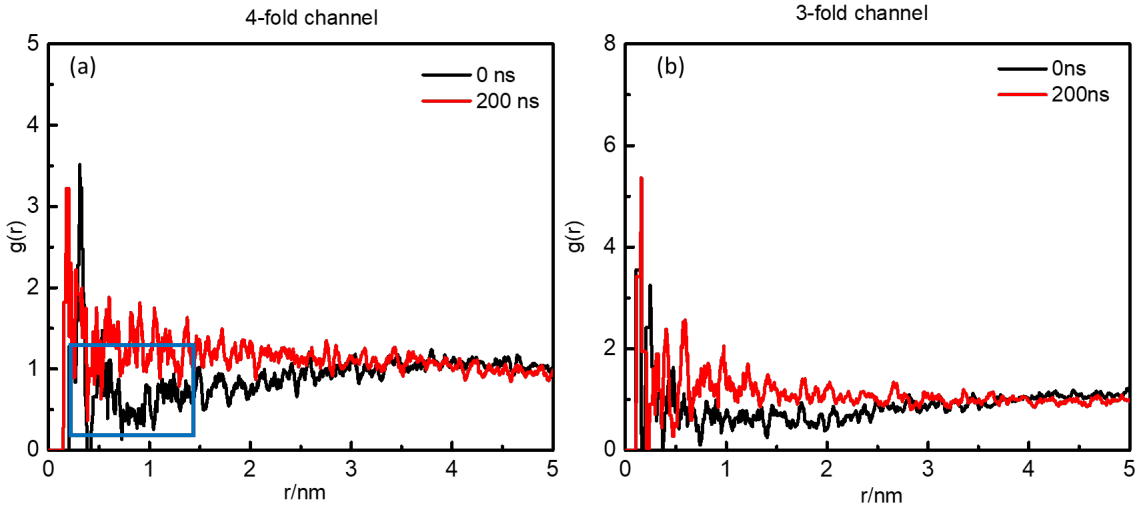

Figure S9: The radial distribution function (RDF) of water molecules around the mass center of (a) 4-fold channel and (b) 3-fold channel within 5 nm for 0 ns and 200 ns at pH 2.

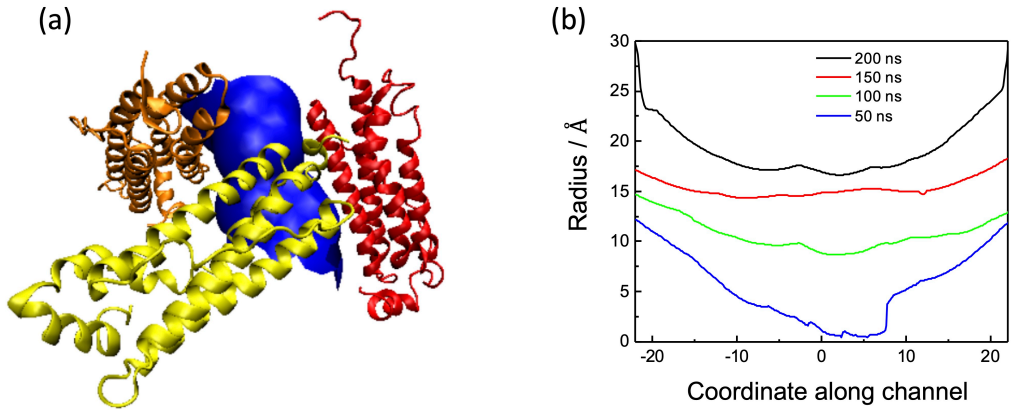

Figure S10: (a) The schematic diagram of channel volume of 3-fold channel. (b) The radius of the channel from 50 ns to 200 ns.

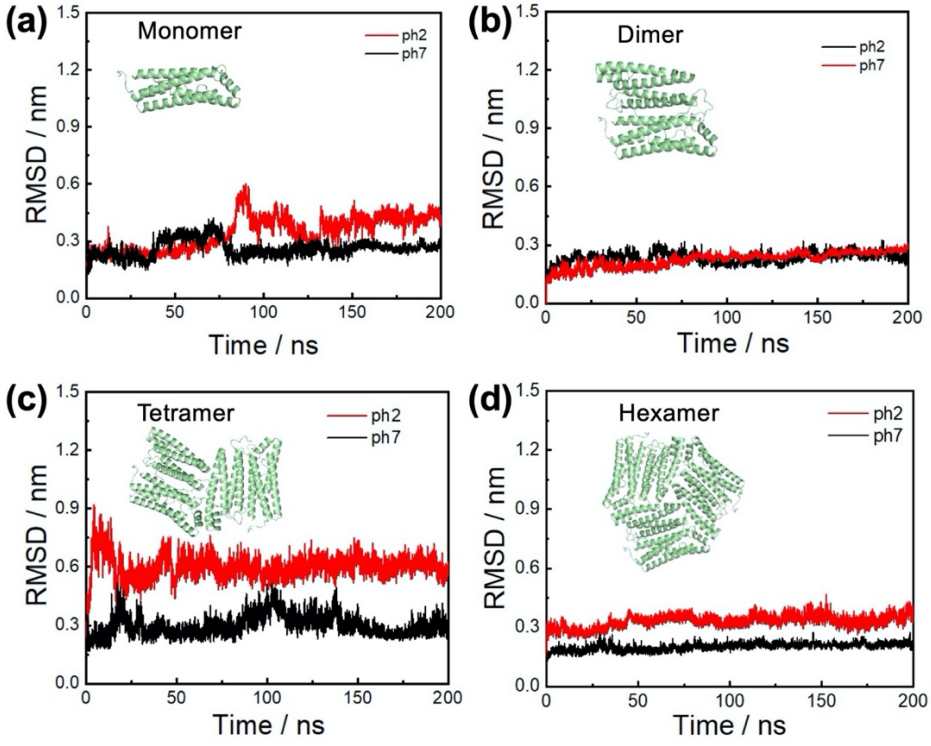

Figure S11: RMSD of various apo-rHLFr cage fragments. (a) monomer, (b) dimer, (c) tetramer and (d) hexamer.

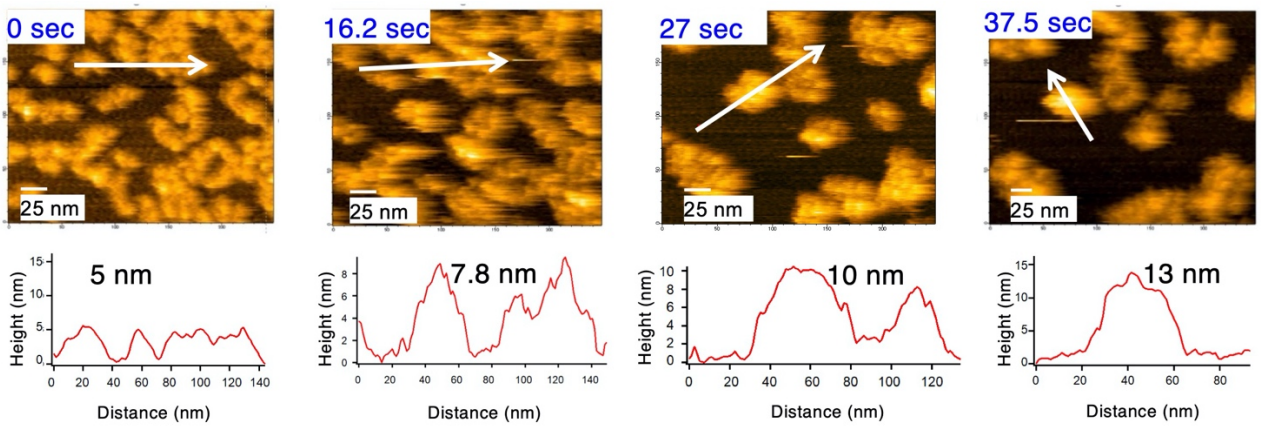

Figure S12: HS-AFM images showing the time course reconstruction of the spherical apo-rHLFr structure from aggregated subunits during the pH jump from 2.3 to 7.7. The corresponding height profiles along the arrow on the image are shown below to the respective images. Initially the apo-rHLFr was at 50mM Gly-HCl, 500mM KCl and the pH jump was achieved by the addition of Tris-HCl (pH 8).