STRUCTURAL BIOLOGY
COMMUNICATIONS

Volume 76 (2020)

Supporting information for article:

Crystal structure of monomeric Amuc_1100 from *Akkermansia muciniphila*

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S1. Materials and methods

S1.1 N-terminal sequencing

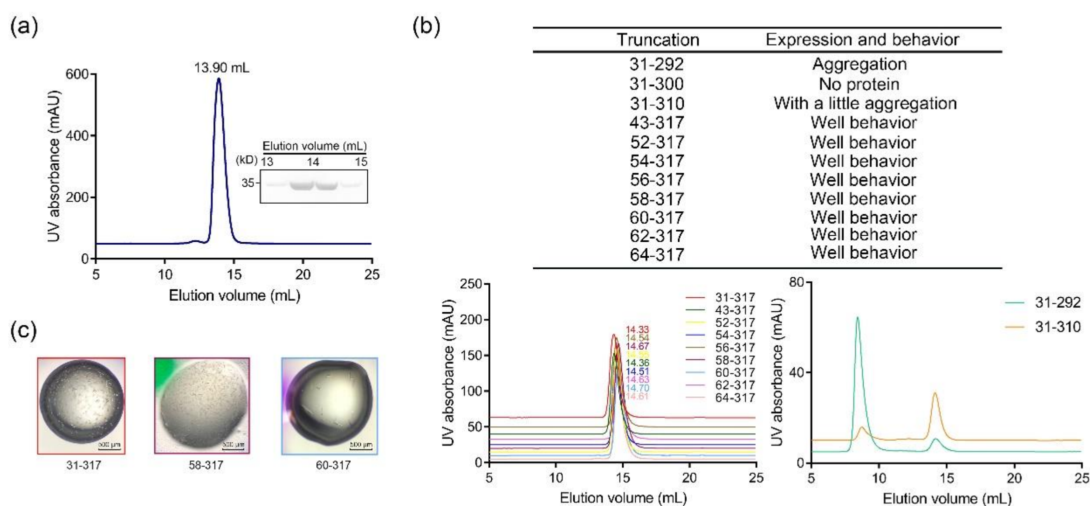
Approximately 3000 crystals were washed with the mother solution containing 0.2 *M* K_2HPO_4, 1.8 *M* NaH_2PO_4 and 0.1 *M* Na_2HPO_4/citric acid at pH 4.2 and dissolved in 2x SDS gel-loading buffer. The wash buffer was refreshed every 20 crystals. The protein sample was separated using tricine-SDS-PAGE and then further transferred to a PVDF membrane (Millipore) at room temperature in buffer containing 10 m*M* CAPS pH 11.0 and 20% methanol. The membrane was further stained with Coomassie Blue R-250. The major band (approximately 28 kDa) was excised and used for analysis (Sangon Biotech, China).

S1.2 Analytical ultracentrifugation

The sedimentation velocity (SV) of Amuc_1100* was measured by analytical ultracentrifugation (AUC). The SV-AUC experiment was performed by using two-channel centerpieces in a Beckman Coulter XL-A/I analytical ultracentrifuge. Amuc_1100* (0.5 mg) was diluted in the solution containing 25 m*M* Tris pH 8.0 and 150 m*M* NaCl. The data were collected by a UV absorbance detector at a rotor speed of 45,000 rpm (147,420 g) at 291 K for 4 h. The SV-AUC data were analyzed using the SEDFIT program. All data were fitted to a continuous $c(s)$ distribution model to determine the molecular weight of Amuc_1100*.

S1.3 Static light scattering

Amuc_1100* at a concentration of 5 mg/mL was loaded onto a Superdex 200 10/300 Increase column (GE Life Sciences) connected to a HELEOS multi-angle light scattering instrument (WYATT Technology). The protein was eluted with buffer containing 25 m*M* Tris pH 8.0 and 150 m*M* NaCl at a flow rate of 0.4 mL/min, and the refractive index was 1.331. Each fraction was automatically analyzed via multi-angle light scattering. Data were then analyzed in Origin 8.0.



Truncation	Expression and behavior
31-292	Aggregation
31-300	No protein
31-310	With a little aggregation
43-317	Well behavior
52-317	Well behavior
54-317	Well behavior
56-317	Well behavior
58-317	Well behavior
60-317	Well behavior
62-317	Well behavior
64-317	Well behavior

Figure S1 Characterization of truncated versions of Amuc_1100

(a) Size-exclusion chromatography (SEC) of Amuc_1100* in Superdex 200 Increase with the elution volume at 13.90 mL. Peak fractions are qualified with SDS-PAGE analysis. (b) Other truncations of Amuc_1100 tested for crystallization. All representative truncations are shown in the table, with gel filtration results in the graphs below. The N-terminal truncations with good behavior are shown in the left panel, colored with different colors. And the C-terminal truncations are shown in the right panel, colored with light cyan (31-292) and orange (31-310). (c) Various shapes of crystals in different conditions. Color of the frame of each drop is consistent with its gel

filtration curve.

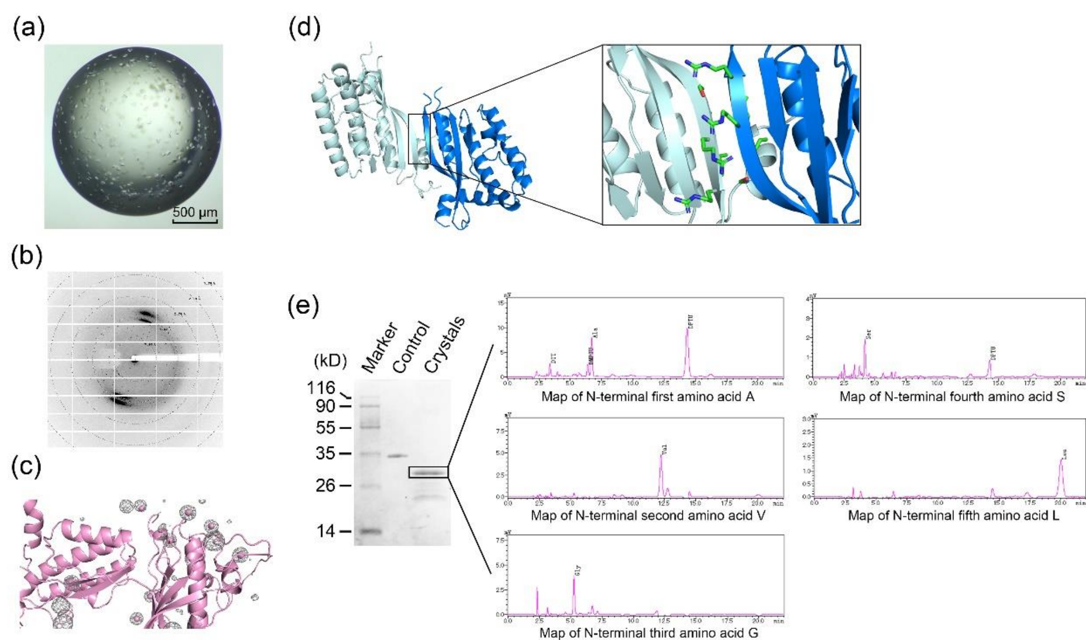


Figure S2 Crystals of Amuc_1100* and N-terminal sequencing of crystallized protein

(a) Crystals of Amuc_1100* in triangle shape. (b) Diffraction image of native crystal at 2.1 Å resolution. (c) Electronic density of heavy atom (Br atom is represented by sphere grid). (d) Predicted dimer interface of Amuc_1100*. (e) SDS-PAGE analysis of Amuc_1100* crystals and N-terminal sequencing result. In the left panel, major band of crystals in the gel is surrounded by rectangle. Five maps of N-terminal amino acids are in the right panel.