

IUCrJ

Volume 8 (2021)

Supporting information for article:

Insights into the structure of mature streptavidin C1 from *Streptomyces cinnamonensis* reveal the self-binding of the extension C-terminal peptide to biotin-binding sites

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Table S1 Primers used in this study. Restriction sites are shown in italic type.

Primer	Sequence (5'- to '3)
C1F-NdeI	<i>CATATGGTACGCGTGTACGCCAA</i>
C1R-XhoI	<i>CTCGAGCTCCCCGTCGGAGGC</i>
C2F-NdeI	<i>CATATGGTTCGACATGCGCAAGATCG</i>
C2R-XhoI	<i>CTCGAGCTGCTGGACGGCGTCGAGCG</i>
ΔC1R-XhoI	<i>CTCGAGGGGCTTGACCCGGGTGAA</i>
ΔC2R-XhoI	<i>CTCGAGCGGCTTCACCTTGGTGAA</i>

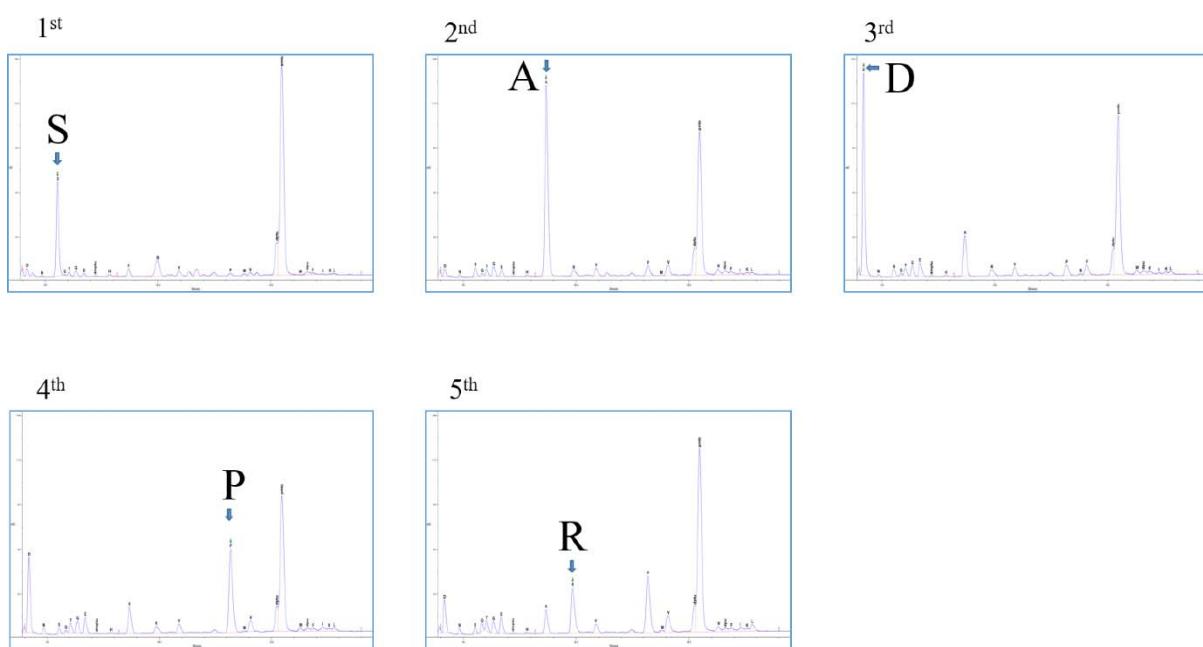


Figure S1 Determination of the N-terminal sequence of recombinant streptavidin C1 using the standard Edman degradation method. The first five amino acids in the N-terminal region of streptavidin C1 were evaluated. Diphenylthiourea is the byproduct of the Edman degradation reaction.

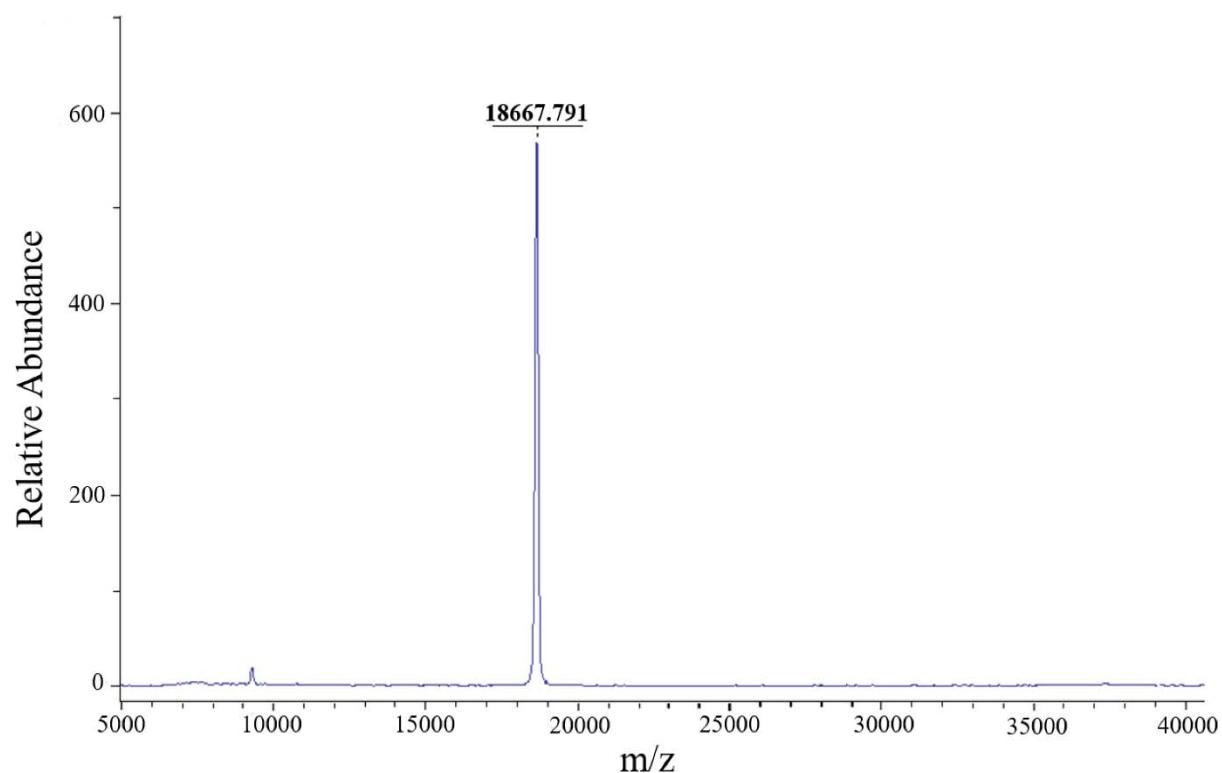


Figure S2 MALDI-TOF mass spectrum of recombinant streptavidin C1. The measurement was performed via MALDI-TOF mass spectrometer using bovine serum albumin as an external standard calibration and sinapinic acid as a matrix.

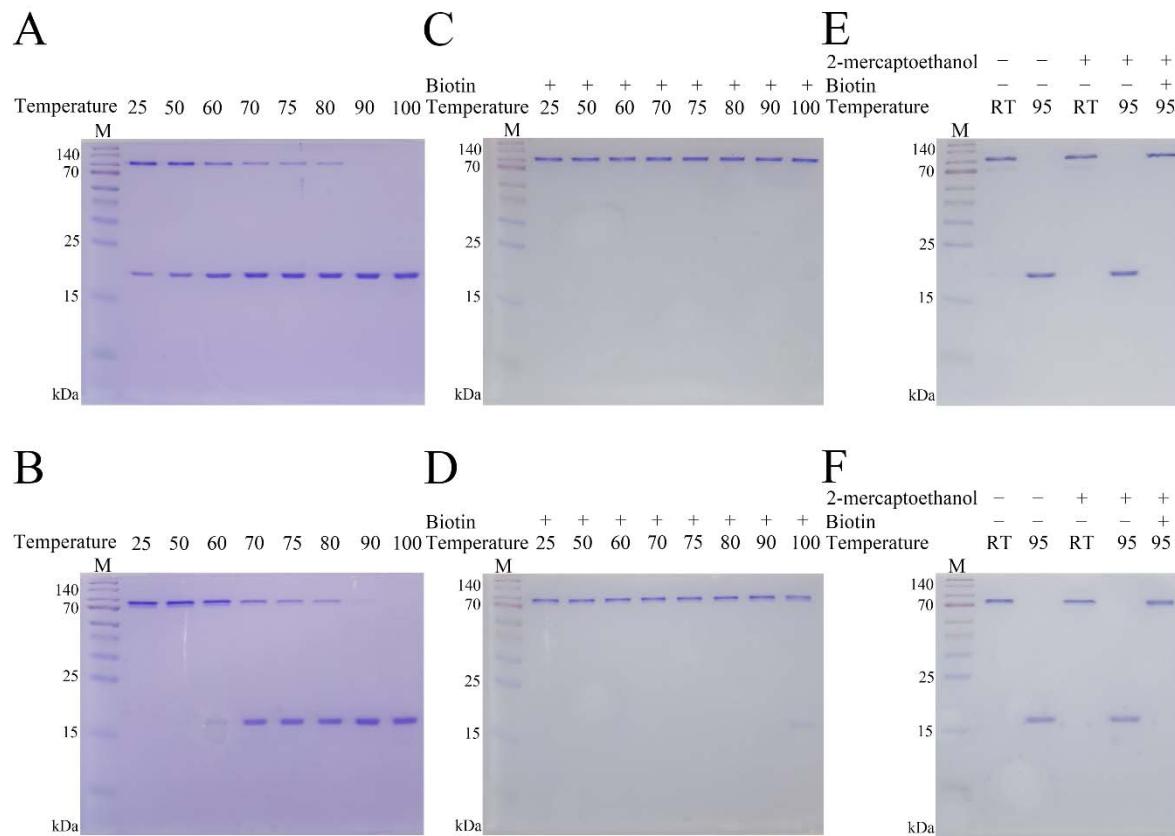
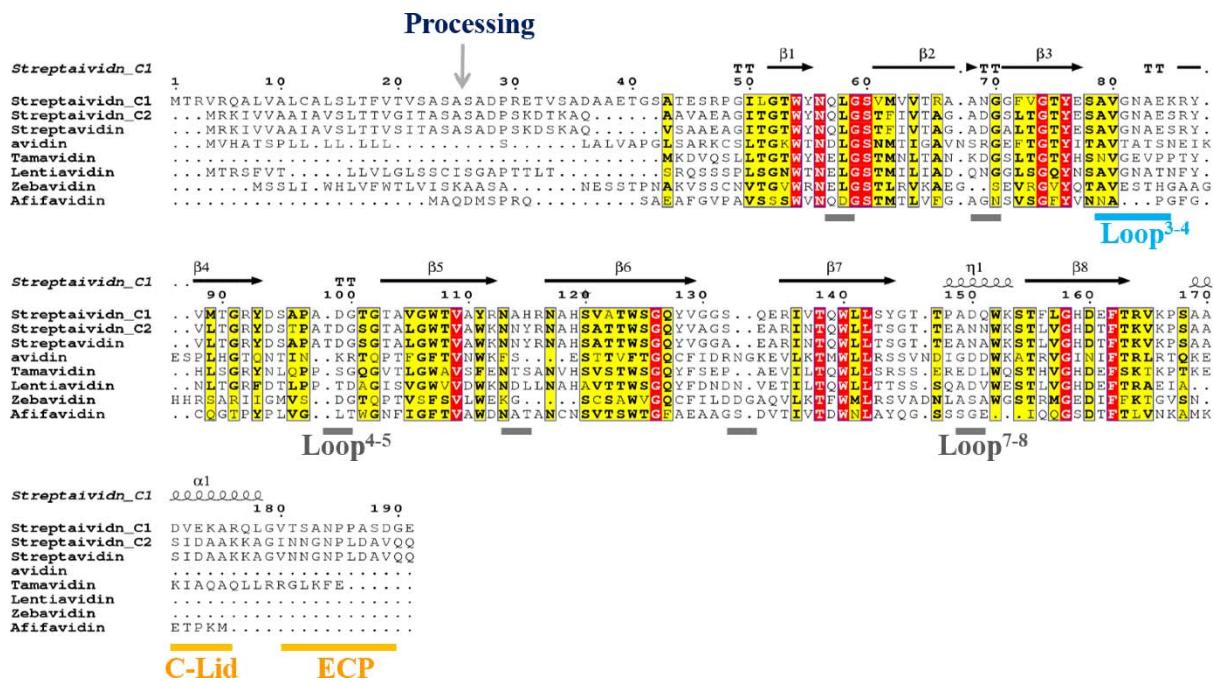


Figure S3 Thermal stability of streptavidin C1 and streptavidin C2 expressed in *Escherichia coli*. Transition temperatures (Tr) of (A) streptavidin C1 and (B) streptavidin C2 at various temperatures without D-biotin. Transition temperatures (Tr) of (C) streptavidin C1 and (D) streptavidin C2 at various temperatures with D-biotin. (E) Streptavidin C1 and (F) streptavidin C2 were incubated with or without β -mercaptoethanol in 1 \times SDS sample buffer at room temperature (RT) or at 95 °C with or without biotin.



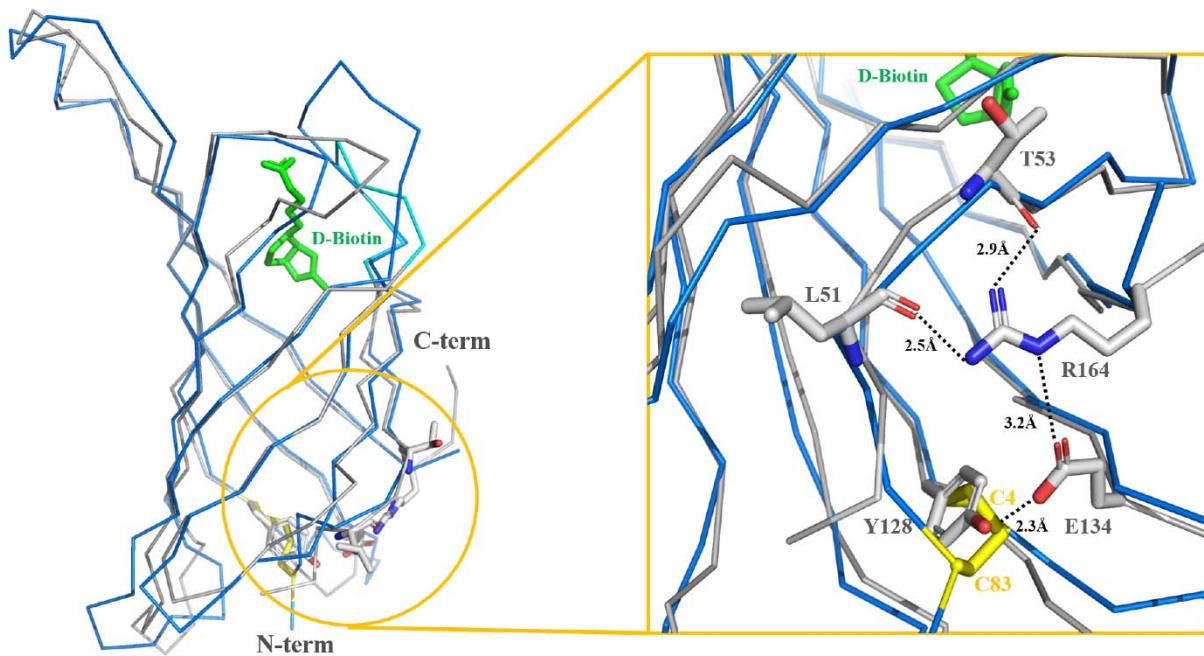


Figure S5 The structural comparison of streptavidin C1 and dimeric avidin structure (PDB entry: 5irw). Streptavidin C1 is different from the dimeric avidin structure as it lacks a cysteine residue. Y128, instead of the cysteine residue, interacts with several residues (E134, R164, and L51) in the crystal structure.

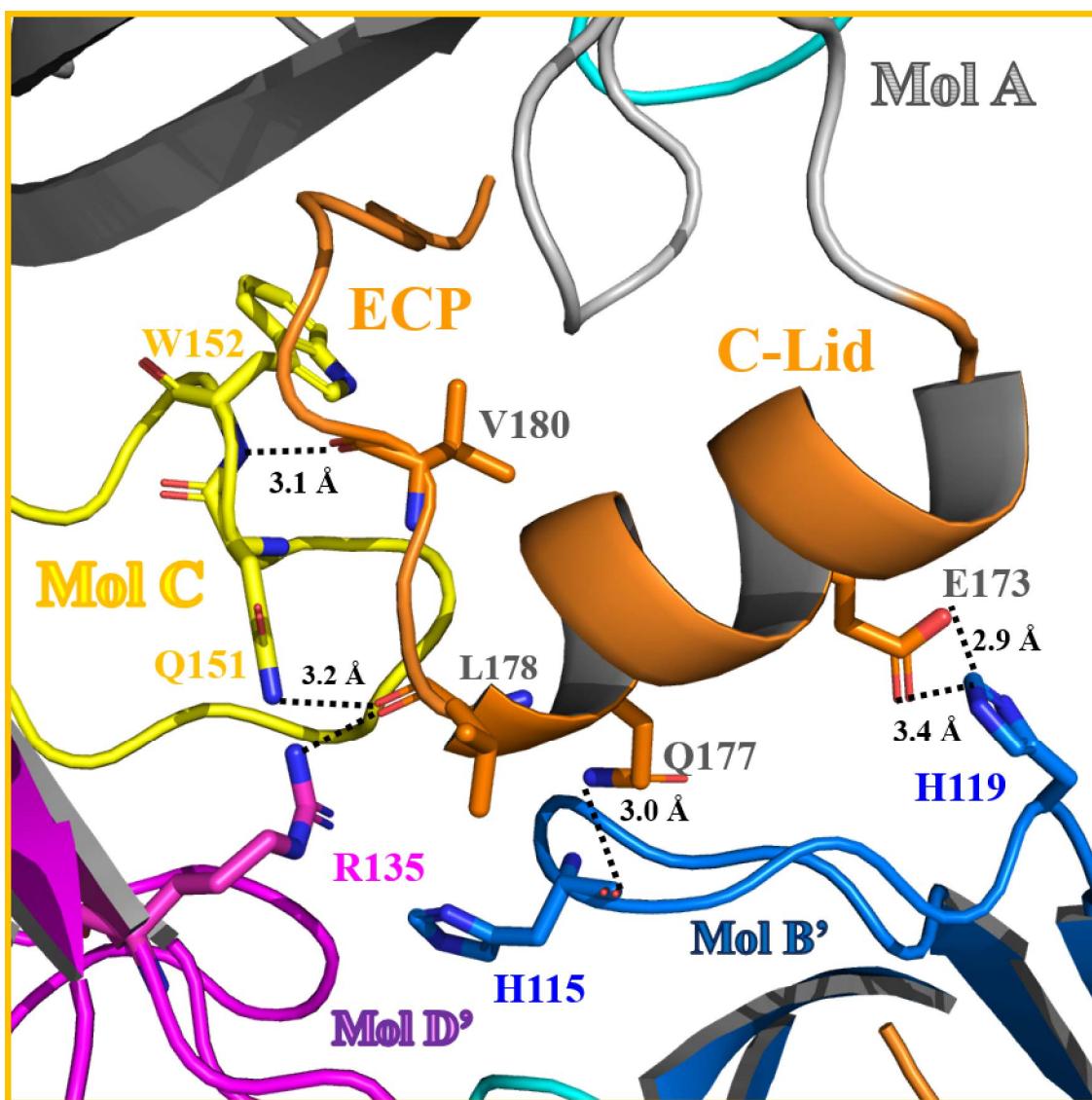


Figure S6 Zoomed view of neighbouring residues of C-Lid and ECP with crystallographic symmetry protomers (Mol B' and Mol D').