Simultaneous analysis of endogenous protein community members by high-resolution cryo-EM

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Abstract

Cellular function is often underlined by megadalton protein assemblies that organize in local proximity forming communities. Metabolons are protein communities involving metabolic pathways such as protein, fatty acid and thioesters of coenzyme-A synthesis. To perform such composite processes, metabolons are highly heterogeneous, making their structural analysis particularly challenging. Here, we devise a rapid approach to enrich metabolons in native cell extracts. We simultaneously characterize metabolon-embedded architectures of a 60S pre-ribosome, fatty acid synthase, and pyruvate/oxoglutarate dehydrogenase complex E2 cores de novo. Cryo-EM 3D reconstructions are resolved at 3.84-4.52 Å resolution by collecting <3,000 micrographs of a single cellular fraction. At this resolution range, polypeptide hydrogen bonding patterns are discernible. Residing molecular components resemble their purified counterparts from other eukaryotes, but also exhibit substantial conformational variation with potential functional implications. Our results propose an integrated tool that opens doors for structural systems biology spearheaded by cryo-EM characterization of native cell extracts.

Characterization

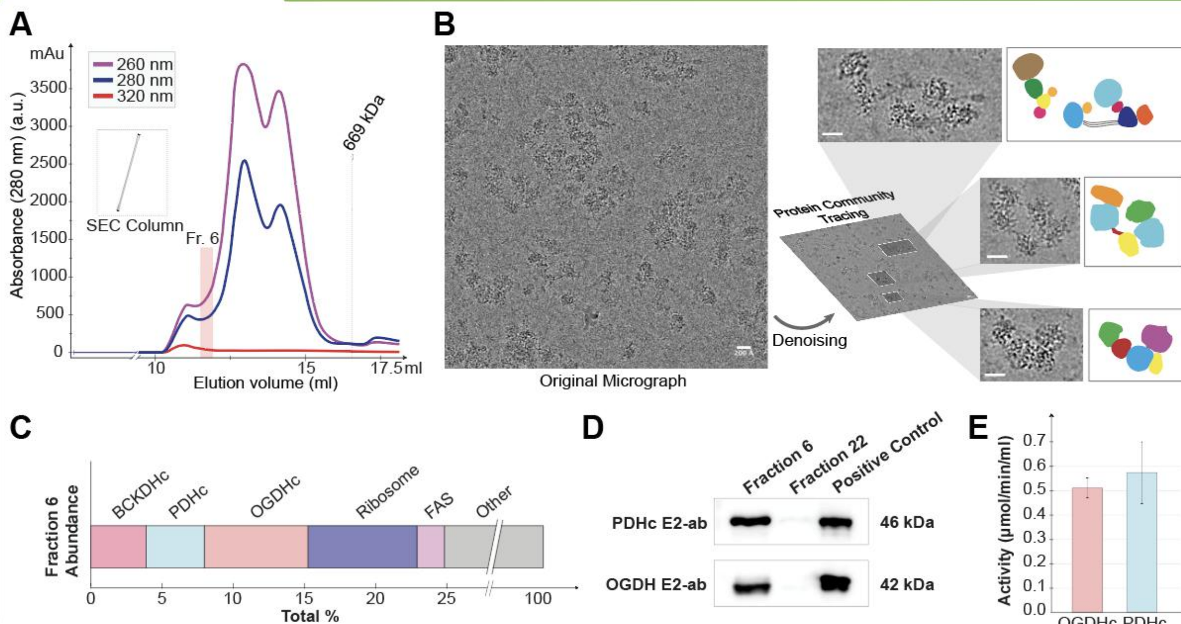

Fig. 1. Biochemical characterization of native protein communities.

(A) Size-exclusion chromatography profile of a native C. thermophilum cell extract. Fraction 6 is located in the MDa weight range.
(B) Cryo-EM of fraction 6 allows, after denoising, to detect and trace various protein community assemblies that remain intact during fractionation and vitrification. Scale bars: 20 nm.
(C) MS data allows for the calculation of the abundance of high molecular weight metabolons in the fraction.
(D) Immunodetection of PDHc E2 and OGDHc E2 proteins in fraction 6. MW (on the right side) corresponds to recombinant proteins used for Ab production.
(E) In-fraction OGDHc and PDHc activity assays. Standard deviation is calculated for 3 technical triplicates.

Signature Detection, Reconstruction and Validation

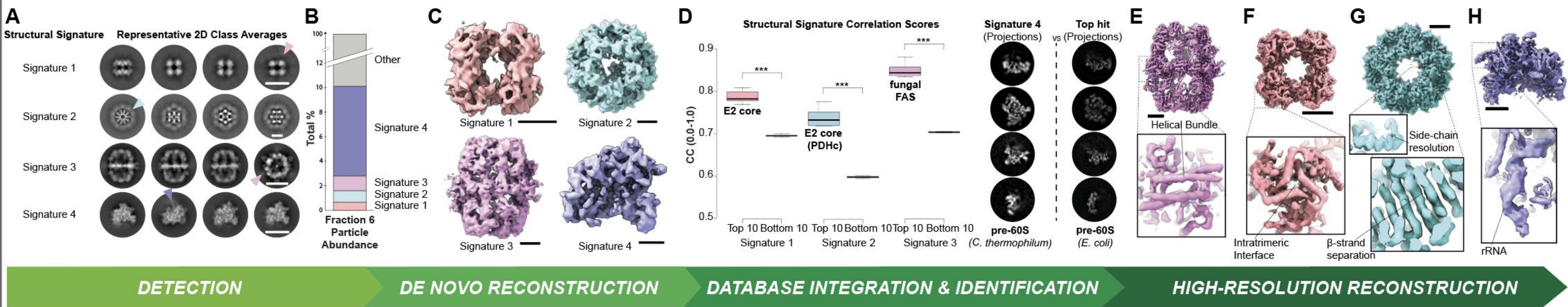

Fig. 2. Signature detection, identification and high-resolution reconstruction

(A) Representative 2D class averages of the most prominent in-fraction structural signatures. Arrows denote diffused densities of lower signal, highlighting structural flexibility or potential binders. Scale bars: 20 nm. **(B)** Particle abundance for each signature, compared to total particles initially picked. **(C)** Ab-initio reconstruction of each signature. Scale bars: 10 nm. **(D)** Cross-correlation comparison among top-10 and bottom-10 of the top 100 hits returned from the Omokage search (Suzuki, Kawabata et al. 2015) for each signature. 2D projections of signature 4 compared to the matching top-10 hits returned from the Omokage search. P-values for the top-10 and bottom-10 comparisons: Signature 1: 3.09E-14, Signature 2: 2.20502E-12, Signature 3: 4.23128E-17 ($P < 0.05$). **(E)** Reconstruction of fatty acid synthase complex. α-Helical bundles and pitch are clearly visible. **(F)** Reconstruction of the oxoglutarate dehydrogenase complex E2 core, where the intratrimeric interfaces at the edge of the core are recapitulated. **(G)** Reconstruction of the pyruvate dehydrogenase complex E2 core. High-resolution structural features, such as side-chain densities and β-strand separation are identifiable. **(H)** Among other features, in the reconstruction of the pre-60S ribosomal subunit densities belonging to the rRNA structural elements are visible. Scale bars: 5 nm.

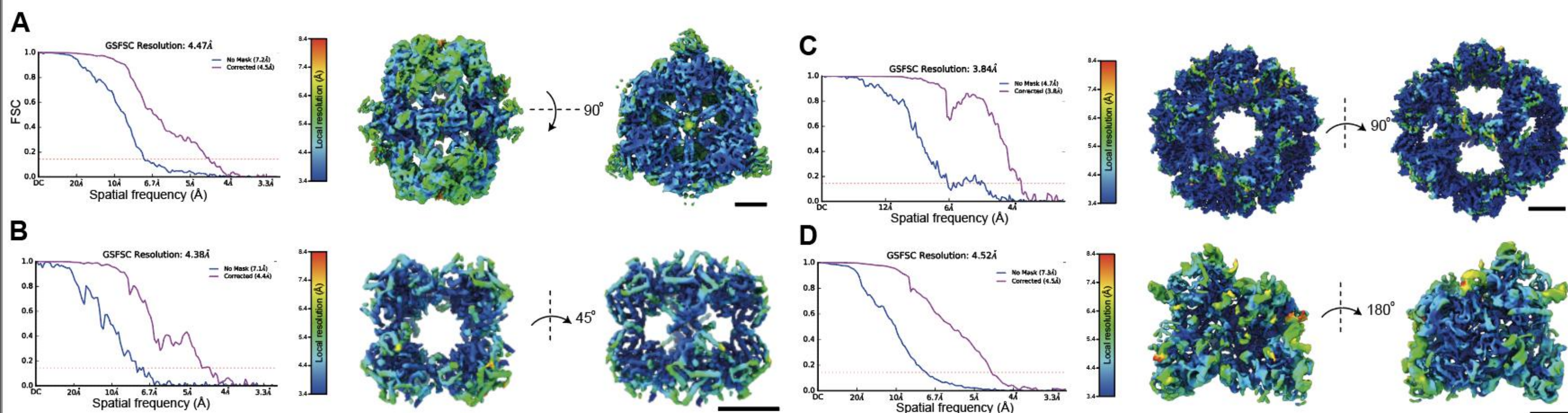

Fig. 3. FSC plots and local resolution distributions for all reconstructed maps.

(A) FSC resolution plot and local resolution distribution for FAS. Central core of the complex demonstrates an overall higher resolution when compared to the external, more flexible densities. **(B)** FSC resolution plot and local resolution distribution for OGDHc E2 core. External densities demonstrate more flexibility compared to the inner part of the complex' core. **(C)** FSC resolution plot and local resolution distribution for PDHc. The reconstruction demonstrates high and uniform resolution distribution. **(D)** FSC resolution plot and local resolution distribution for the pre-60S ribosomal subunit. More flexible, external densities corresponding to the rRNA components are recognizable. Scale bars: 5 nm.

High-Resolution Insights

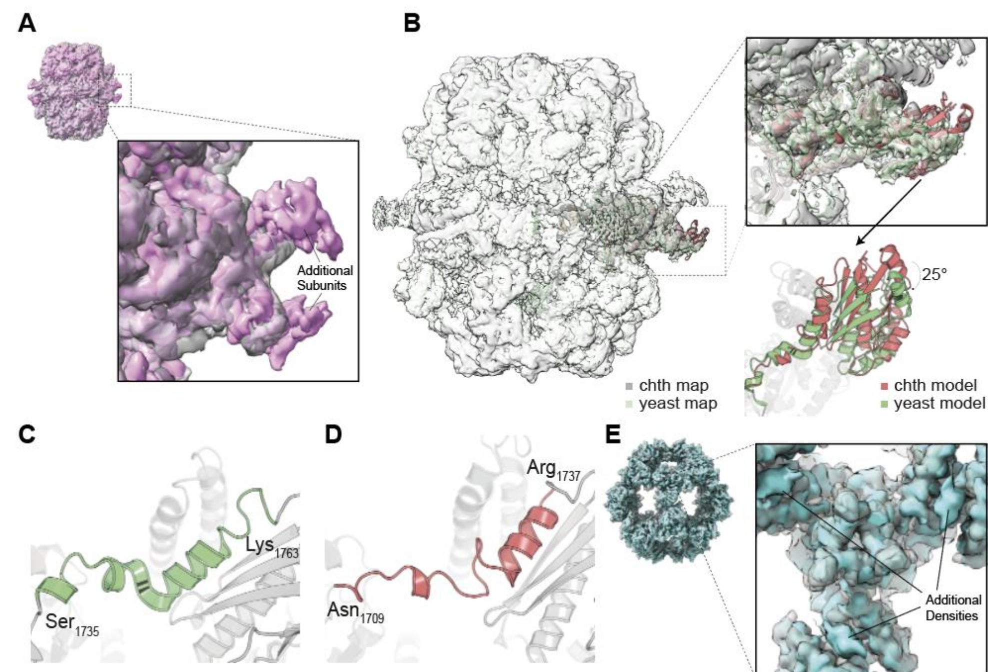

Fig. 4. Structural insights into the FAS and PDHc reconstructed maps.
(A) When compared to previously resolved C. thermophilum FAS, new densities can be identified. **(B)** Comparison of the Acetyl-CoA binding PPT domains of the C. thermophilum (chth) and S. cerevisiae (yeast) reveals the lateral movement of the domain. **(C)** Flexible linker region of the yeast Acetyl-CoA binding PPT domain of yeast. **(D)** Flexible linker region of the yeast Acetyl-CoA binding PPT domain of C. thermophilum. Longer length of unstructured region may provide explanation for the domain's higher flexibility. **(E)** Comparison to the previously resolved C. thermophilum PDHc E2 core reveals the existence of additional densities on the inside of the core, possibly indicating the anchor points of the E3BP.

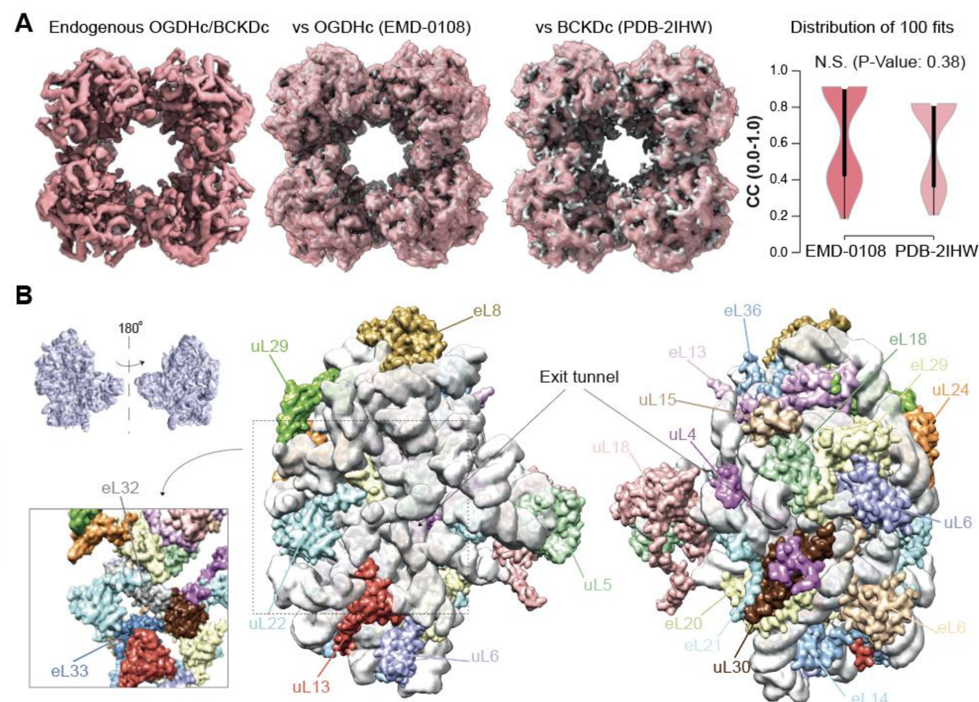

Fig. 5. Structural insights into the hybrid OGDHc/BCKDHc E2 core and pre-60S ribosomal subunit reconstructed maps.
(A) Distribution of fits with overexpressed OGDHc and BCKDHc E2 core maps (gray) does not allow the unambiguous identification of the endogenous reconstructed hybrid OGDHc/BCKDHc map (salmon). **(B)** Identifiable ribosomal subunits taking part in the assembly of the endogenous pre-60S ribosomal subunit. The exit tunnel for newly synthesized polypeptide chain is one of the first features visible during the 60S assembly sequence.

Conclusions and Outlook

- **Employ study of cell extracts as a means of investigating protein communities.**
- **Reconstruct/ identify multiple structural signatures belonging to members of these communities.**
- **Complement with classic biochemical assays, mass spectrometry, independent statistical analysis and bioinformatic methods for further verification.**
- **Newest advances in *de novo* model prediction can be integrated to further metabolon structural knowledge.**

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