SINGLE MOLECULE 3D STRUCTURES DETERMINED BY INDIVIDUAL-PARTICLE ELECTRON TOMOGRAPHY

♦ Gang Ren (100 %)

gren@lbl.gov

♦The Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

ORCID ID: 0000-0002-8036-2321

DOI: http://doi.org/10.22580/iSciNoteJ9.9.1

Macromolecules, such as proteins and nucleic acids, play essential roles in cellular functions through dynamic structural changes. Understanding these functions requires detailed characterization of structural dynamics. While techniques like X-ray crystallography and cryo-EM resolve high-resolution static structures, they struggle to capture low-resolution, flexible structures and the full distribution of conformations during chemical reactions. These limitations arise from averaging processes that enhance signal-to-noise ratio (SNR) but exclude flexible regions, distort resolution, and miss rare high-energy states. To address this, we developed individual-particle electron tomography (IPET), a method for determining 3D structures of single particles at low-to-intermediate resolution (up to 2 nm) without averaging. IPET reconstructs a detailed 3D density map by capturing images at multiple tilt angles, facilitating flexible model fitting and revealing unique particle structures. This method reveals unbiased structural distributions, enhancing the study of molecular dynamics, phase transitions, and structural alterations during chemical reactions and self-folding.

Figure 1 Tertiary structure of single RNA molecule determined by IPET | The left side of the figure shows the process of IPET where RNA samples in frozen TEM grid are imaged by tilting the sample at various angles. The right side of the figure shows the 3D density maps of 72 individual RNA molecules, which reveal the individual helices within each RNA particle. Figure adapted from Ref. [1].

Dynamic motion is fundamental to life, with cells and their components, including macromolecules and atoms, constantly in motion. Molecular dynamics (MD) simulations provide insight into molecular vibrations, while nuclear magnetic resonance (NMR) captures a limited number of coexisting structures for small molecules. However, primary structural techniques such as X-ray crystallography and cryo-electron microscopy (cryo-EM) single-particle analysis primarily produce static structures. These methods can determine atomic-resolution structures of biomolecules, however capturing low-resolution 3D structures of macromolecules exhibit intrinsic flexibility and continuous conformational changes remains challenging. This challenge arises from the reliance on averaging processes, where a small, homogeneous subset of particles is selected from a heterogeneous population and averaged to generate a static 3D structure. While averaging improves the SNR and enables high-resolution reconstruction of rigid domains, it often results in the loss of density in flexible regions and introduces anisotropic resolution in the resulting 3D map. For instance, two ankyrin repeated regions of TRPV1 were absent in its atomic resolution 3D density map [2]. As a result, the structural variety for the majority of unselected particles remains inaccessible, limiting the ability to fully explore macromolecular flexibility. Therefore, single-molecule 3D structure determination, without the need for averaging, is a highly sought-after approach for understanding macromolecular dynamics and mechanisms during chemical reactions.

A fundamental experimental approach to studying the structure of flexible macromolecules involves determining the structure of each macromolecule individually, without relying on averaging across multiple copies [3].

Electron tomography (ET) enables high-resolution imaging of single objects by capturing a series of tilted views from different angles [4-6]. While ET has been successfully applied to reconstruct the 3D structure of cellular sections and entire bacteria at nanometer-scale resolutions [7], achieving reliable 3D reconstructions of individual macro-molecules remains a significant challenge. The first reported 3D reconstruction of an individual macromolecule, a fatty acid synthetase was achieved using negative staining (NS) by Walter Hoppe's group in 1974 [5], marking the beginning of ET. However, the validity of this reconstruction has been questioned because the molecule was exposed to a radiation dose far exceeding the established damage threshold measured on protein crystals at that time (~1 $e^-/Å^2$ for 7Å resolution), by orders of magnitude [8,9]. Although several subsequent studies attempted reconstructions of individual molecules [5, 10-17], skepticism persisted regarding whether a single protein particle could provide sufficient signal to produce a meaningful 3D structure at adequate resolution. For decades, this skepticism reinforced the belief that individual macromolecules lack the necessary signal-to-noise ratio for reliable and interpretable 3D reconstructions.

The possibility of achieving an intermediate-resolution (1–3 nm) 3D structure from multiple low-contrast ET images of an individual macromolecule under low-dose condition remains uncertain. Ren and colleagues addressed this uncertainty by re-examining the problem using simulated ET data, as well as experimental NS and cryo-electron tomography (cryo-ET) images (3). Achieving a 3D image of a single molecule is highly challenging due to the extremely low SNR, resulting from the limited electron dose required to prevent radiation damage. To address this challenge, IPET employs a robust iterative refinement process that integrates automatically generated dynamic filters and soft masks. This approach eliminates the need for a pre-specified initial model, class averaging of multiple macromolecules, or an ordered lattice arrangement. Instead, IPET tolerates measurement errors such as tilt inaccuracies and image distortions. By systematically reducing the reconstruction image size, it minimizes the impact of these errors and large-scale distortions in ET micrographs on the 3D reconstruction. Their findings demonstrated that intermediate-resolution 3D structures (1–3 nm) can potentially be achieved for individual protein particles using the iterative refinement approach known as Individual-Particle Electron Tomography (IPET) [3,16,18,19].

Recent refinements in the IPET approach have incorporated several TEM-based advancements to enhance single-molecule 3D imaging including: Image contrast enhancement [20]; Automated data acquisition [21]; Missing-wedge correction [22]. IPET has been used to characterize low- to intermediate-resolution (~2 nm) dynamic structures of various flexible macromolecules, including: antibodies [16, 18, 23, 24], DNA / RNA -related nanostructures [25-28], lipoproteins [29-31], neuronal proteins [19, 32-34]. These advancements position IPET as a promising tool for structural studies of single molecular particles, providing deeper insights into the dynamic behaviors and mechanisms of flexible macromolecules.

The IPET approach has been recently further refined to examine large-scale, continuous conformational changes of RNA origami nanoparticles at tertiary structural resolution during their self-folding process [1]. RNA self-folding presents significant challenges for structural studies due to extensive conformational changes involved. By optimizing cryo-ET acquisition parameters and eliminating the need for particle selection, classification, averaging, or chemical fixation, Liu et al., successfully reconstructed 120 individual 3D density maps from 120 folding RNA nanoparticles (Fig. 1). These nanoparticles were designed to form a 6-helix bundle with a clasp helix. The reconstructed maps revealed distinct tertiary structures for each RNA helix arrangement, statistically confirming two previously known conformations while identifying additional intermediate and highly compact states. This structural variation suggests a maturation pathway likely driven by helix-helix compaction interactions, offering new insights into RNA folding dynamics.

The IPET-based approach demonstrates broad applicability for studying flexible biomolecular complexes, including tracking intramolecular conformational changes in tetra-nucleosome arrays during phase transitions. In this study, we applied the IPET technique to investigate the inner and intramolecular conformational changes of tetra-nucleosome arrays during phase transitions [35, 36]. Zhang et al. identified that key determinant of chromatin array structure is the angle between the entry/exit DNA strands and their tangents relative to the nucleosomal disc. Furthermore, Zhang et al., found that the phase transition involves the exposure of hydrophobic nucleosomal surfaces, altering inter-nucleosome interactions. These insights illuminate the initial stages of intra-array compaction and suggest a key precursor to chromatin condensation, providing a physical mechanism by which chromatin transitions from interphase to metaphase structures.

In summary, the IPET approach offers a unique tool for determining the 3D structure of individual biomolecular particles without the need for averaging across multiple particles. By analyzing hundreds of 3D structures from distinct particle, IPET provides an unbiased representation of structural dynamics,

allowing visualization of the full spectrum of macromolecular motions and kinetic processes in solution. This capability is essential for understanding critical biomolecular processes such as macromolecular synthesis, folding, and chemical reactions.

Acknowledgments | The author would like to express gratitude to Drs. Jianfang Liu, Ewan K.S. McRae, Meng Zhang, Cody Geary, Carlos Bustamante, and Ebbe Sloth Andersen for their collaboration on nucleotide structure projects, and to Drs. Hao Wu, Xiaobo Zhai, Lei Zhang, Xing Jin, Xuefeng Cheng, and Dongsheng Lei for their collaboration on methodology development. The work conducted at the Molecular Foundry, LBNL, was supported by the Office of Science, Office of Basic Energy Sciences of the United States Department of Energy (contract no. DE-AC02-05CH11231). I also acknowledge the following research grants: the US National Institutes of Health grants R01HL115153, R01GM104427, R01MH077303, and R01DK042667.

REFERENCES

1. J. Liu et al., **Nat. Comm.** 15, 9084 (2024).
2. M. Liao et al., **Nature** 504, 107-112 (2013).
3. L. Zhang et al., **PLoS One** 7, e30249 (2012).
4. J. L. Milne et al., **Nature Rev. Microbiol.** 7, 666-675 (2009).
5. W. Hoppe et al., **Hoppe-Seyler's Zeitschrift fur physiologische Chemie** 355, 1483-1487 (1974).
6. W. Hoppe et al., **Ann. Rev. Biophys. Bioengg.** 10, 563-592 (1981).
7. A. Komeili et al., **Science** 311, 242-245 (2006).
8. J. Frank, **Methods for Three-Dimensional Visualization of Structures in the Cell.** (Springer, New York, NY, 2006).
9. P. N. Unwin and R. Henderson, **J. Mol. Biol.** 94, 425-440 (1975).
10. U. Skoglund et al., **Nature** 319, 560-564 (1986).
11. H. Mehlin et al., **Cell** 69, 605-613 (1992).
12. S. Sandin et al., **Structure** 12, 409-415 (2004).
13. U. Skoglund et al., **Jl. Struc. Biol.** 117, 173-188 (1996).
14. Q. Zhao et al., **Expt. Cell Res.** 300, 190-201 (2004).
15. A. Fera et al., **Jl. Compara. Neuro.** 520, 4218-4225 (2012).
16. H. Tong et al., **Sci. Rep.** 3, 1089 (2013).
17. K. Iwasaki et al., **Jl. Struc. Biol.** 150, 259-267 (2005).
18. X. Zhang et al., **Sci. Rep.** 5, 9803 (2015).
19. Z. Lu et al., **Jl. Biol. Chem.** 289, 34530-34542 (2014).
20. H. Wu et al., **Sci. Rep.** 8, 16711 (2018).
21. J. Liu et al., **Sci. Rep.** 6, 29231 (2016).
22. X. Zhai et al., **Sci. Rep.** 10, 10489 (2020).
23. D. Lei et al., **Sci. Rep.** 9, 8864 (2019).
24. H. M. Zhang et al., **Anal. Chem.** 89, 13494-13501 (2017).
25. E. K. S. McRae et al., **Nat. Nanotech.** 18, 808-817 (2023).
26. S.-T. Wang et al., **Nat. Commun.** 12, 3702 (2021).
27. D. Lei et al., **Nat. Commun.** 9, 592 (2018).
28. L. Zhang et al., **Nat. Commun.** 7, 11083 (2016).
29. D. Lei et al., **Biochim. Biophys. Acta Mol. Cell Biol. Lipids** 1864, 260-270 (2019).
30. Y. Yu et al., **Jl. Lipid Res.** 57, 1879-1888 (2016).
31. M. Zhang et al., **Sci. Rep.** 5, 8741 (2015).
32. H. Lee et al., **Jl. Biol. Chem.** 299, 104586 (2023).
33. J. Liu et al., **Jl. Mol. Biol.** 430, 4325-4343 (2018).
34. Z. Lu et al., **Jl. Biol. Chem.** 291, 24133-24147 (2016).
35. M. Zhang et al., **Nat Commun**. 15, 4395 (2024).
36. M. Zhang et al., **Mol. Cell** 82, 3000-3014 e3009 (2022).

Editor's Views | | This short article by Gang Ren concisely summarizes the IPET-related works and clearly states the issue that IPET can address (i.e., 3D structure determination of biomolecules without averaging across multiple particles). The applications of IPET span the structural determination of a diverse range of biological macromolecules and molecular complexes, offering unique insights into their dynamic behaviors.