APMC13 BRISBANE 2025
13th Asia Pacific Microscopy Congress
Brisbane, Australia, February 2-7, 2025

Observing Conformational Distribution of Protein using Nanoparticle-Aided Cryo-Electron Microscopy Sampling

Yeeun Kim[1], Changin Kim[2,3,†], Yongsoo Yang[1,4*] and Hyotcherl Ihee[2,3*]

[1] Korea Advanced Institute of Science and Technology (KAIST), Department of Physics, Daejeon, Republic of Korea
[2] Korea Advanced Institute of Science and Technology (KAIST), Department of Chemistry, Daejeon, Republic of Korea
[3] Institute for Basic Science (IBS), Center for Advanced Reaction Dynamics, Daejeon, Republic of Korea
[4] Korea Advanced Institute of Science and Technology (KAIST), Graduate School of Semiconductor Technology, School of Electrical Engineering, Daejeon, Republic of Korea
[†] Present address: Korea Institute of Science and Technology (KIST), Advanced Analysis Center, Seoul, Republic of Korea
*** Corresponding author: yongsoo.yang@kaist.ac.kr, hyotcherl.ihee@kaist.ac.kr**

Author's ORCID iD's
Yeeun Kim: https://orcid.org/0000-0002-2560-6952
Changin Kim: https://orcid.org/0000-0002-6248-6101
Yongsoo Yang: https://orcid.org/0000-0001-8654-302X
Hyotcherl Ihee: https://orcid.org/0000-0003-0397-5965

Characterizing the structural heterogeneity of small proteins is essential for understanding their biological roles. Conventional methods have been used to investigate the conformational distribution of small proteins. However, this remains challenging due to structural flexibility of small protein [1, 2, 3]. To address this, we developed the Nanoparticle-Aided Cryo-Electron Microscopy Sampling (NACS) technique, which measures the interparticle distance distribution (IPD) between two gold nanoparticles (AuNPs) labeled to proteins by capturing cryo-electron microscopy (cryo-EM) images (Fig. 1) [4, 5]. Three-dimensional IPD (3D-IPD) can be measured by capturing cryo-EM images at multiple tilt angles (MT-NACS), while only the projected IPD (Proj-IPD) can be obtained by capturing the cryo-EM image at a single tilt angle (ST-NACS). To validate capabilities of NACS, we selected calmodulin (CaM) as a model system and observed structural changes in CaM due to Ca^{2+} ions binding under different salt concentrations using both MT-NACS and ST-NACS. Additionally, we measured structural changes in CaM due to amyloid-beta (Aβ) peptide binding, which had never been experimentally measured before (Fig. 2) [4]. We found that IPD changes in CaM measured by ST-NACS and MT-NACS were similar, indicating that NACS can reliably measure IPD by taking only a single-angle image. NACS technique offers a new method for studying the structural heterogeneity and dynamics of flexible biomolecules and small proteins.

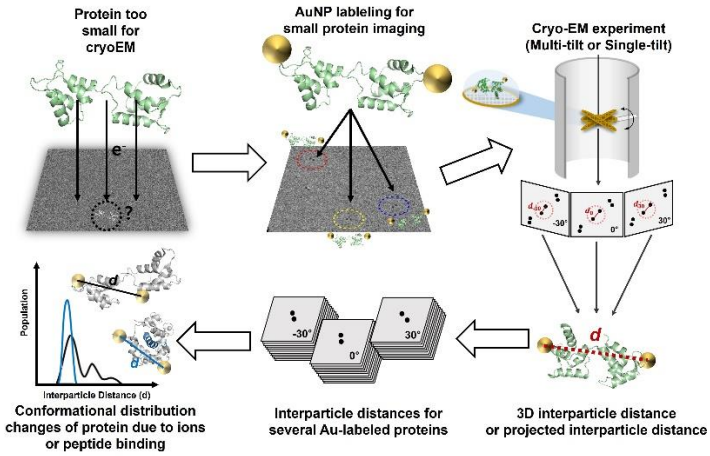

Fig. 1. *Schematic diagram of* Nanoparticle-Aided Cryo-Electron Microscopy Sampling *(NACS) process. Because small proteins are invisible in cryo-electron microscopy (cryo-EM) images due to their low imaging contrast, gold nanoparticles (AuNPs) are labeled to specific residues of protein, and their images are captured using* cryo-EM at multiple tilt angles (MT-NACS) or a single tilt angle (ST-NACS)*. The interparticle distance between two AuNPs attached to the protein can be calculated. By repeating this process for several AuNP-labeled proteins, we can measure interparticle distance distribution (IPD) of AuNP-labeled protein. We measured the IPD changes of the protein before and after binding with ions or peptides [4].*

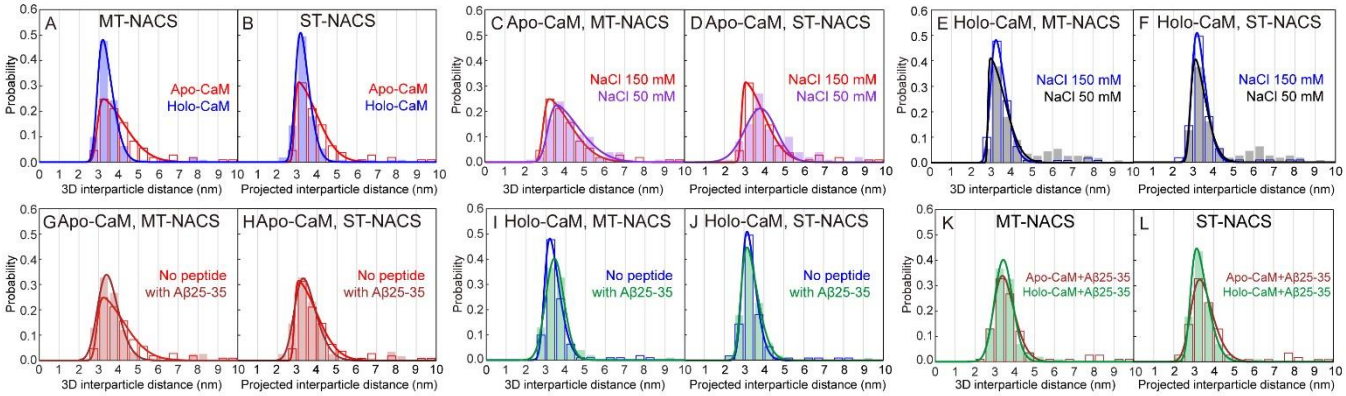

Fig. 2. *Comparison of structural changes measured using MT-NACS and ST-NACS. (A, B) Interparticle distance distribution (IPD) changes in CaM due to Ca^{2+} binding under 150 mM NaCl condition. CaM without Ca^{2+}: apo-CaM; CaM with Ca^{2+}: holo-CaM. (C–F) IPD changes in apo-CaM (C, D) and holo-CaM (E, F) due to NaCl concentration changes. (G–J) IPD changes in apo-CaM (G, H) and holo-CaM (I, J) due to amyloid-beta (Aβ) peptide binding. (K, L) IPDs of apo-CaM with Aβ peptide and holo-CaM with Aβ peptide. Bar histograms and fitted curves using skewed Gaussian functions (solid lines) are displayed in panels (A–L) [4].*

References

1. R. Steinberg et al., *FEBS J*. **288** (2021), p. 7002−7024. https://doi.org/10.1111/febs.15845
2. X. Yang et al., *Genome Res.* **21** (2011), p. 634−641. https://doi.org/10.1101/gr.109280.110
3. D. K. Sasmal et al., *Nanoscale* **8** (2016), p. 19928– 19944. https://doi.org/10.1039/C6NR06794H

4. C. Kim et al., *Nano Letters* **23** (2023), p. 3334-3343. https://doi.org/10.1021/acs.nanolett.3c00313
5. H. Ki et al., J. Phys. Chem. Lett. **12** (2021), p. 6565–6573. https://doi.org/10.1021/acs.jpclett.1c01277