Super-resolution proximity labeling reveals antiviral protein network and its structural changes against SARS-CoV-2 viral proteins

Yun-Bin Lee,[a] Minkyo Jung,[b] Jeesoo Kim,[c] Afandi Charles,[d] Wanda Christ,[e] Jiwoong Kang,[a] Myeong-Gyun Kang,[a] Chulhwan Kwak,[a] Jonas Klingström,[e] Anna Smed-Sörensen,[d] Jong-Seo Kim,[*c] Ji Young Mun,[*b] Hyun-Woo Rhee[*a]

[a] *Department of Chemistry, Seoul National University, Seoul, Republic of Korea.* [b] *Neural Circuit Research Group, Korea Brain Research Institute, Daegu, Republic of Korea*. [c] *School of Biological Sciences, Seoul National Univeristy, Seoul, Republic of Korea.* [d] *Department of Medicine Solna, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden.* [e] *Department of Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden.*

E-mail: yunbin18@snu.ac.kr

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) replicates in human cells by interacting with host factors after infection. To understand the virus and host interactome, proximity labeling methods (biotin ligase or APEX) have been utilized. However, conventional proximity labeling workflow often provides rather ambiguous results likely due to the indirect identification of biotinylated proteins. Herein, we developed a *super-resolution proximity labeling* (*SR-PL*) method with “plug and playable” PL enzyme, TurboID-GFP binding protein (GBP) and we applied it for interactome mapping of GFP-tagged SARS-CoV-2 ORF3a and M proteins, which generated highly perturbed ER structures. Through *SR-PL* analysis of the biotinylated interactome of ORF3a and M, 224 and 272 peptides were robustly determined as ORF3a and M interactomes, respectively. Within the ORF3a interactome, RNF5 co-localized with ORF3a and generated ubiquitin modifications of ORF3a related to protein degradation. We also observed that SARS-CoV-2 infection rate was efficiently reduced by the overexpression of wild-type RNF5 in the host cells compared to cells overexpressing the nonfunctional mutant RNF5^{C42S}. Overall, we introduced a new virus–host interactome mapping workflow using *SR-PL* and we could identify novel anti-viral ubiquitin ligase (RNF5) interfering with SARS-CoV-2 infection in human cells. This interactome data obtained using this *SR-PL* method was presented as a web-based platform (https://sarscov2.spatiomics.org) for readers to make it more accessible. This method contributes to revealing virus–host interactomes of other viruses in an efficient way in the future.

References

[1] Lee Y. B.; Jung M., Kim J.; Charles A.; Christ W.; Kang J.; Kang M. G.; Kwak C.; Klingström J.; Smed-Sörensen A.; Kim J. S.;* Mun J.;* Rhee H. W.* *In Revision*. **2023**.