Structural and functional characterisation of plant innate immune receptors

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Plant nucleotide-binding leucine-rich repeat receptors (NLRs) recognize specific effectors secreted by pathogens and elicit defence responses, including localized cell death. NLRs consist of a variable N-terminal signalling domain, a central nucleotide-binding (NB-ARC) domain and a C-terminal leucine-rich repeat (LRR) domain. Several NLRs encompassing the N-terminal Toll/interleukin-1 receptor (TIR) domain form tetrameric oligomers upon effector binding [1, 2]. Oligomerised TIR-NLRs function as nicotinamide adenine dinucleotide (NAD^+)-cleaving enzymes [1-3], producing different signalling molecules [4-5] that are required to trigger cell death in plants. It remains unclear whether NADase activity is a common feature among diverse TIR-NLRs and how the different metabolites derived from NADase activity activate downstream defence pathways. We expressed and purified two closely related flax TIR-NLR receptors, L6 and M, that are known to activate defence responses to rust fungi. Based on size exclusion chromatography, negative stain electron microscopy (EM) and mass photometry analyses, we found that the purified proteins formed oligomeric complexes in a concentration-dependent manner, even in the absence of their cognate effectors (Fig. 1). We are currently focusing on cryo-EM structural analyses with and without their effectors. To test for NADase activity, their TIR domains were expressed and purified. While high NADase activity was detected for $L6^{TIR}$, M^{TIR} showed low levels of activity. Liquid chromatography-mass spectrometry analysis revealed that both $L6^{TIR}$ and M^{TIR} produced 2'cADPR, the proposed biomarker of immune signalling in plants upon NADase activity. At the same time, each of them produced additional distinct metabolites. Transient overexpression of $L6^{TIR}$ elicited cell death in leaves, while M^{TIR} overexpression failed to do so. Further work is required to identify and investigate roles of signalling molecules produced by L6 and establish mechanisms of action by M.

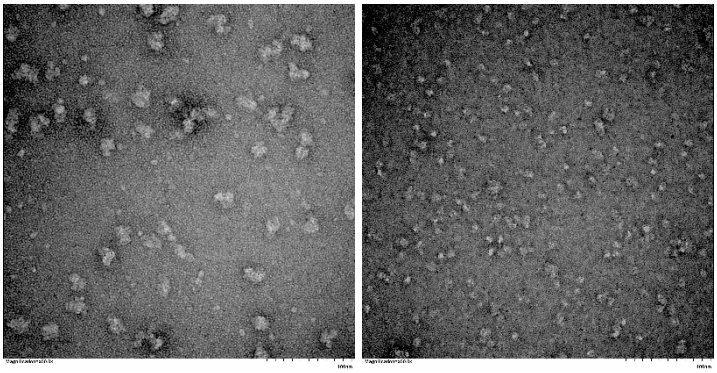

Figure 1. Negative stain EM analyses on oligomeric (left) and monomeric (right) fractions of M in the absence of its effector.

[1] Ma, S., Lapin, D., Liu, L., Sun, Y., Song, W., Zhang, X., Logemann, E., Yu, D., Wang, J., Jirschitzka, J., Han, Z., Schulze-Lefert, P., Parker, J. E. & Chai, J. (2020). *Science.* **370**, 6521, eabe3069.

[2] Martin, R., Tiancong, Qi., Zhang, Haibo., Furong, L., King, M., Toth, C., Nogales, E. & Staskawicz, B. J. (2020). *Science*, **370**, 6521, eabd9993.

[3] Horsefield, S., Burdett, H., Zhang, X., Manik, M. K., Shi, Y., Chen, J., Tiancong, Qi., Gilley, J., Lai, J. S., Rank, M. X., Casey, L. W., Gu, W., Ericsson, D. J., Foley, G., Hughes, R. O., Bosanac, T., Itzstein, M. V., Rathjen, J., Nanson, J. D., Boden, M., Dry, I. B., Williams, S. J., Staskawicz, B. J., Coleman, M. P., Ve, T., Dodds, P. N. & Kobe, B (2019). *Science*, **365**, 6455, 793-799.

[4] Wan, L., Essuman, K., Anderson, R. G., Monteiro, F., Chung, E., Nishimura, E. O., Diantonio, A., Milbrandt, J., Dangl, J. L. & Nishimura, M. T. (2019). *Science*, **365**, 6455, 799-803.

[5] Jia, A., Huang, S., Song, W., Wang, J., Meng, Y., Sun, Y., Xu, L., Laessle, H., Jirschitzka, J., Hou, J., Zhang, T., Yu, W., Hessler, G., Li, E., Ma, S., Yu, D., Gebauer, J., Baumann, U., Liu, X., Han, Z., Chang, J., Parker, J. E., Chai, J (2022). *Science*, **377**, 6605, abq8180.