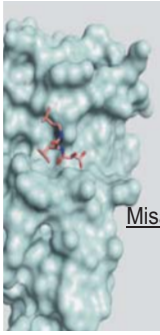

# The small effector Syringolin A inhibits the plant proteasome *in vivo* and during infection

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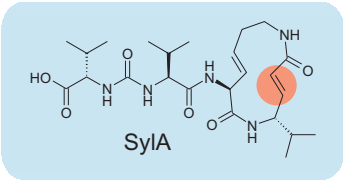
SylA is a small molecule effector of *Pseudomonas syringae* pv. *syringae* that was shown to block the activity of yeast and mammalian proteasomes. Direct evidence of inhibition of the plant proteasome by SylA *in vivo* was still to be demonstrated. Activity-based proteome profiling (ABPP) is a powerful technology to study the activity of the plant proteasome in living plant cells. ABPP is based on the use of fluorescent inhibitors that react with catalytic residues of enzymes in an activity-dependent manner. By using ABPP we demonstrate that SylA selectively inhibits the Arabidopsis proteasome *in vivo* and that bacteria producing SylA are able to inhibit the proteasome of *Nicotiana benthamiana* during infection.

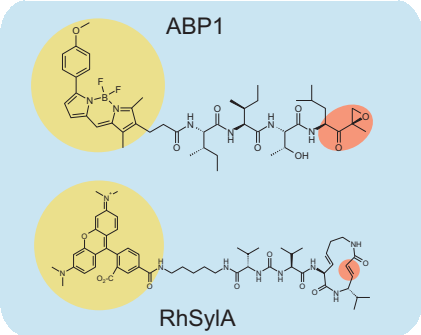
## The small effector molecule Syringolin A




*Pseudomonas syringae* pv. *syringae (Pss)* is a pathogen of snap bean and *Nicotiana benthamiana*. *Pss* strain B728a secretes the small proteasome inhibitor and virulence factor Syringolin A (SylA) (Groll et al., Nature 2008).

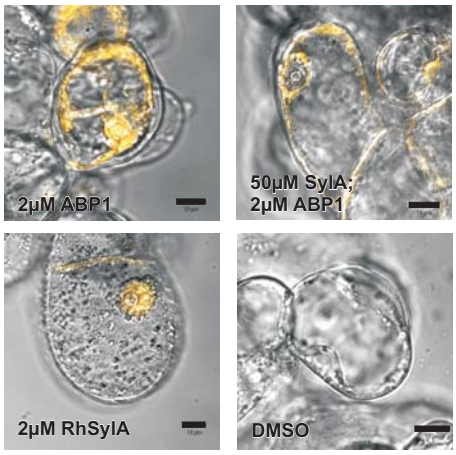
The plant proteasome is a cytoplasmic/nuclear complex containing three catalytic subunits each with differential activities: β1(caspase-like), β2 (trypsin-like) and β5 (chymotrypsin-like).

## Activity-based protein profiling (ABPP)



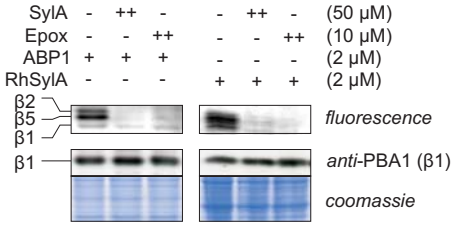

Activity-based probe-1 (ABP1) is based on epoxomicin, a covalent proteasome inhibitor produced by Actinomycetes. Epoxomicin and Syringolin A are irreversible inhibitors of all three catalytic proteasome subunits.

## SylA targets the plant proteasome *in vivo*

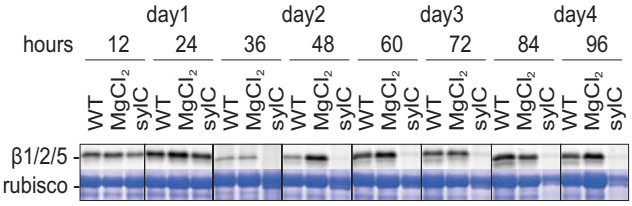



Arabidopsis cell cultures were preincubated for 30 min with the respective inhibitor and labeled for 120 min with the different probes.

Protein extracts of labeled cell cultures were separated by SDS-PAGE and fluorescently labeled proteins were detected by in-gel fluorescent scanning.




## Proteasome suppresion during infection




*Nicotiana benthamiana* plants were infiltrated with 10 mM $MgCl_2$ buffer or wild-type (WT) or SylA-deficient (sylC) strains at $10^5$ bacteria/mL. The proteasome activity at various hours-post-inoculation (hpi) was visualized by labeling extracts with ABP1.

## Conclusions

» SylA inhibits the plant proteasome *in vivo* and during infection.

» ABP1 is a powerful tool to follow the activity of the plant proteasome *in vivo* and *in vitro*.

## Open questions

» Why does Pseudomonas inhibit the proteasome?

» Which (defence) pathway is targeted by proteasome inhibition?

**References:**
Groll M., Schellenberg B. *et al*., (2008) Nature 452, 755-758.
Kurepa J, Smalle J.A. (2008), Biochimie 90, 324-335.
Vinatzer B. *et al*., (2006) Mol. Microbiol. 62, 26-44.

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