From inhibitor to affinity probe: Pepstatin A derivates enable AfBPP of plant aspartic proteases



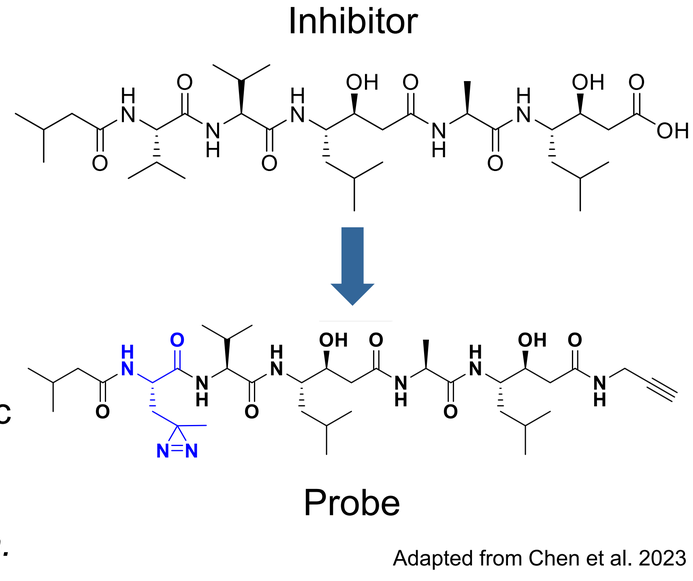
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Introduction

- Plant proteases regulate diverse biological processes, including growth, development, and immunity.
- Five major mechanistic classes of proteases.
- Aspartic proteases are more numerous and diverse in plants than in humans.
- Pepstatin A is a natural inhibitor of aspartic proteases.
- Pepstatin A-based probes were developed for affinity-based profiling (AfBPP) of human aspartic proteases.
- Aspartic protease 1 (APA1) confers drought tolerance in the model plant *Arabidopsis thaliana.*



Adapted from Chen et al. 2023

Aim

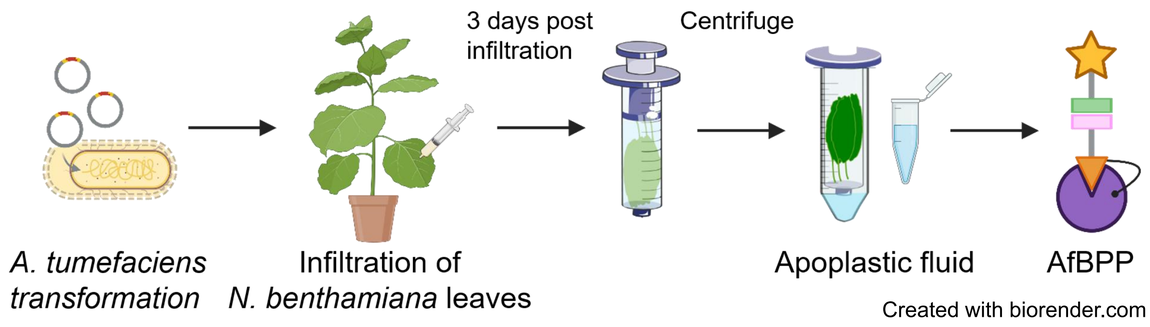
Adapt existing Pepstatin A-based probes for AfBPP of plant aspartic proteases.

→ Elucidate their roles in plant physiology.

Mechanistic class	Year of development for plant ABPP
Cysteine	van der Hoorn et al. 2004
Serine	Kaschani et al. 2009
Threonine	Kolodziejek et al. 2011
Metalloproteases	Lenger et al. 2012
Aspartic	**Bena et al. in preparation**

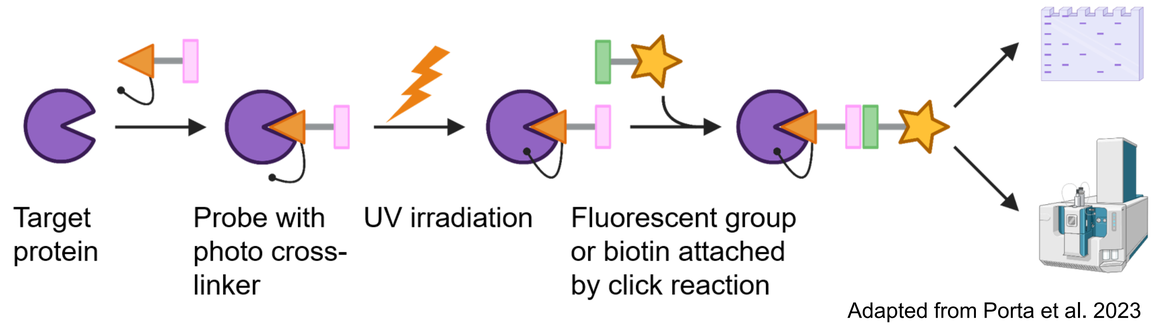
Key methods

Transient expression of plant aspartic protease APA1



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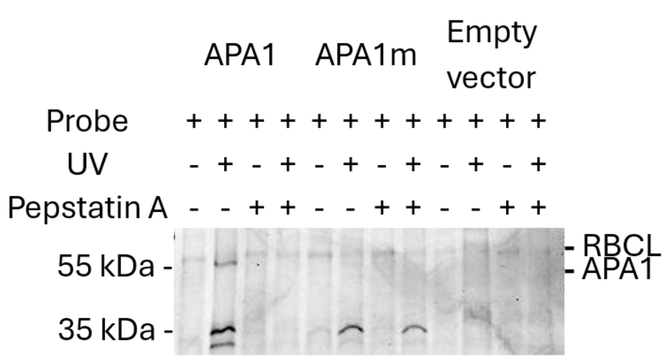
Principle of AfBPP

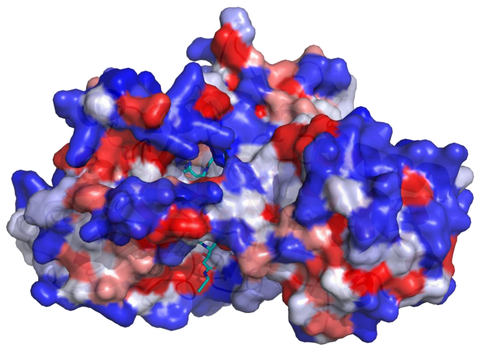


Adapted from Porta et al. 2023

Results

Probe binds specifically active APA1

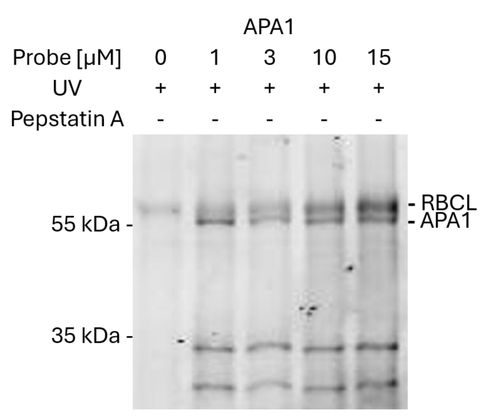


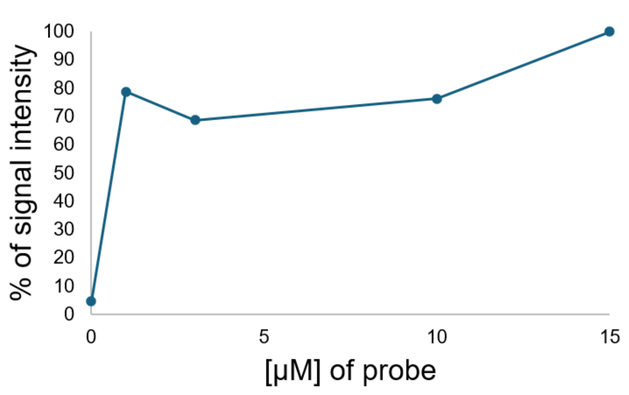


Boltz-2 (AlphaFold3) model of APA1 with probe

- No UV irradiation → no binding
- Pepstatin A → blockage of the binding pocket
- APA1m → catalytically dead version
- RBCL → rubisco, enzyme responsible for photosynthesis

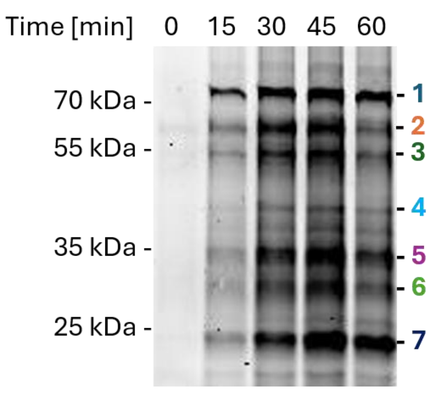
1 µM of probe is sufficient for efficient labelling

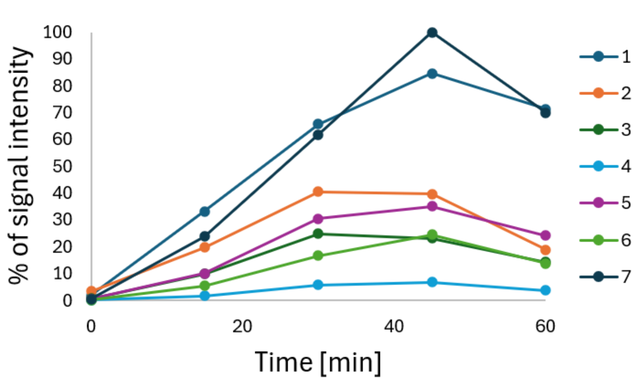




- Highest signal intensity set at 100%

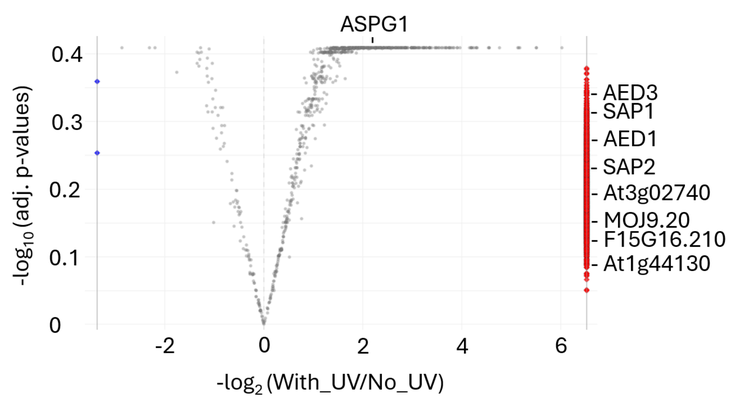
Efficient labeling is achieved after 30 min of UV irradiation





- Labelling of apoplastic fluid of *N. benthamiana* leaves

AfBPP-MS enriches eight plant aspartic proteases



- Labelling of total extract of *A. thaliana* leaves

Conclusion

- Pepstatin-based AfBPP probes selectively label APA1, with efficient labeling achieved using 1 µM probe and 30 min of UV irradiation and enabled the enrichment of eight aspartic proteases from plant leaves.

Outlook

- Apply the probe to investigate the biological roles of plant aspartic proteases.
- Use computational modelling and docking to design subgroup-selective probes for plant aspartic proteases.

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