Nep1-like protein – plant membrane interactions

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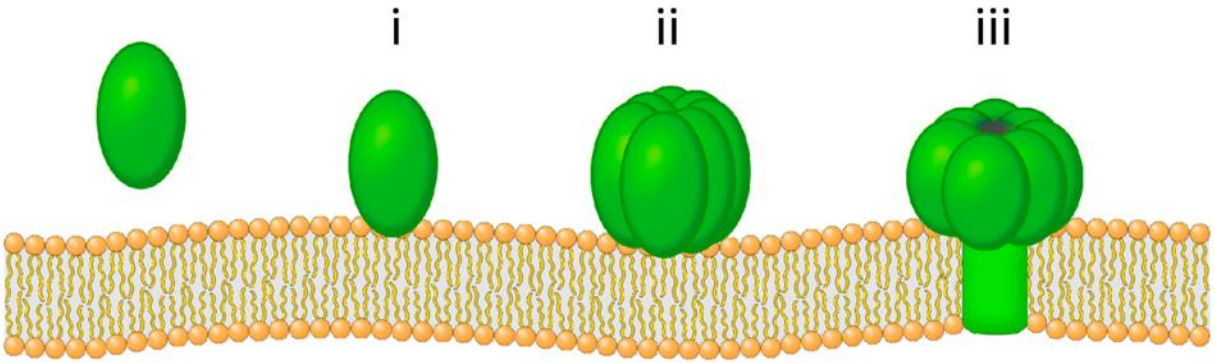
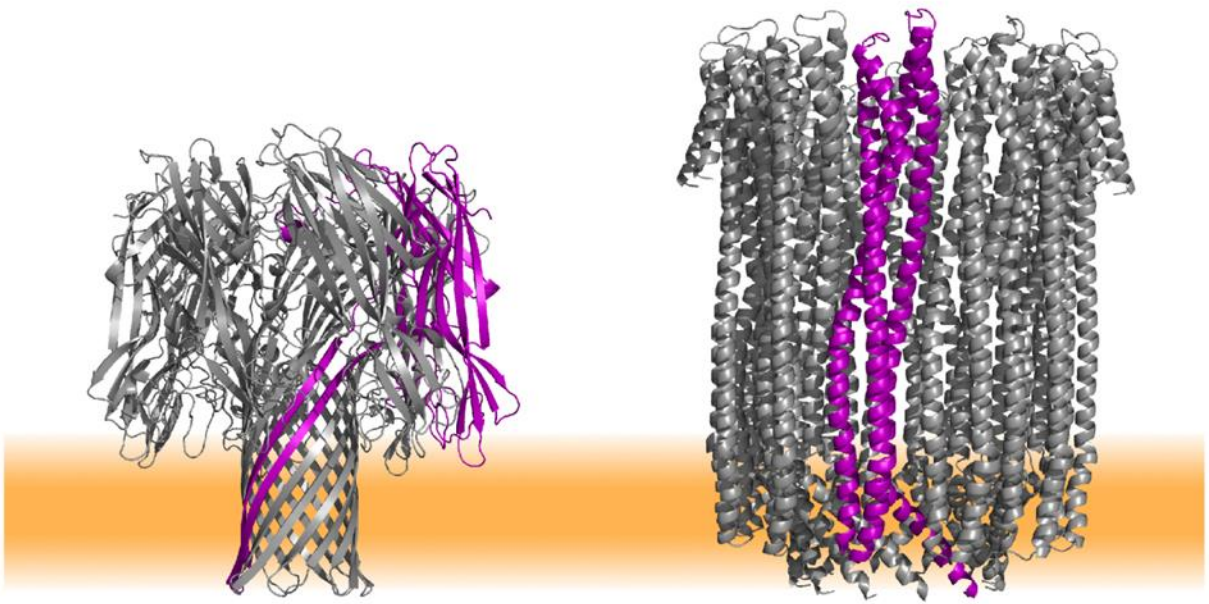
Nep1-like proteins; cytolysins; glycosyl inositol phospho ceramides

KEMIJSKI INŠTITUT

pore forming proteins





α-toxin
Staphylococcus aureus

ClyA
Escherichia coli

Properties

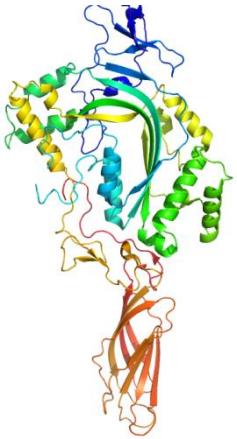
Effects on cells

Molecular tools

Rojko N and Anderluh G (2015) ***Accounts Chem. Res.*** 48: 3073-3079

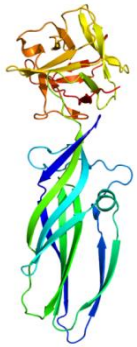
pore forming proteins

Perforin
MACPF



Listeriolysin O
CDC

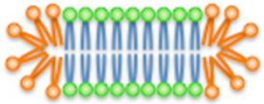
Lysenin
Aerolysin-like



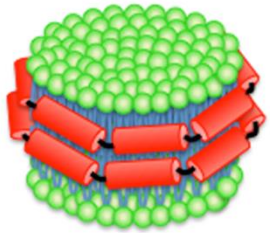
Equinatoxin II
Actinoporins

micelle

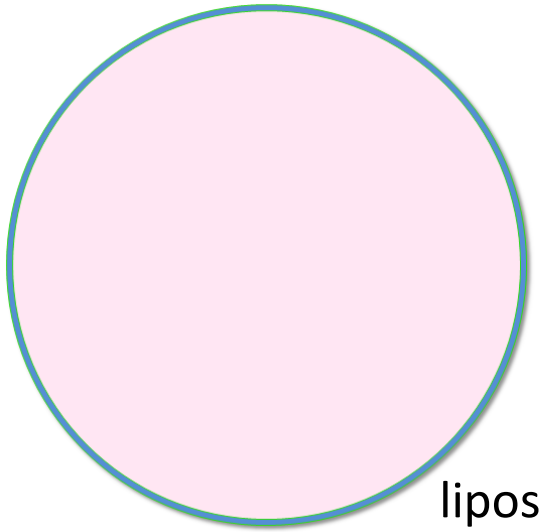
bicelle



nanodisc



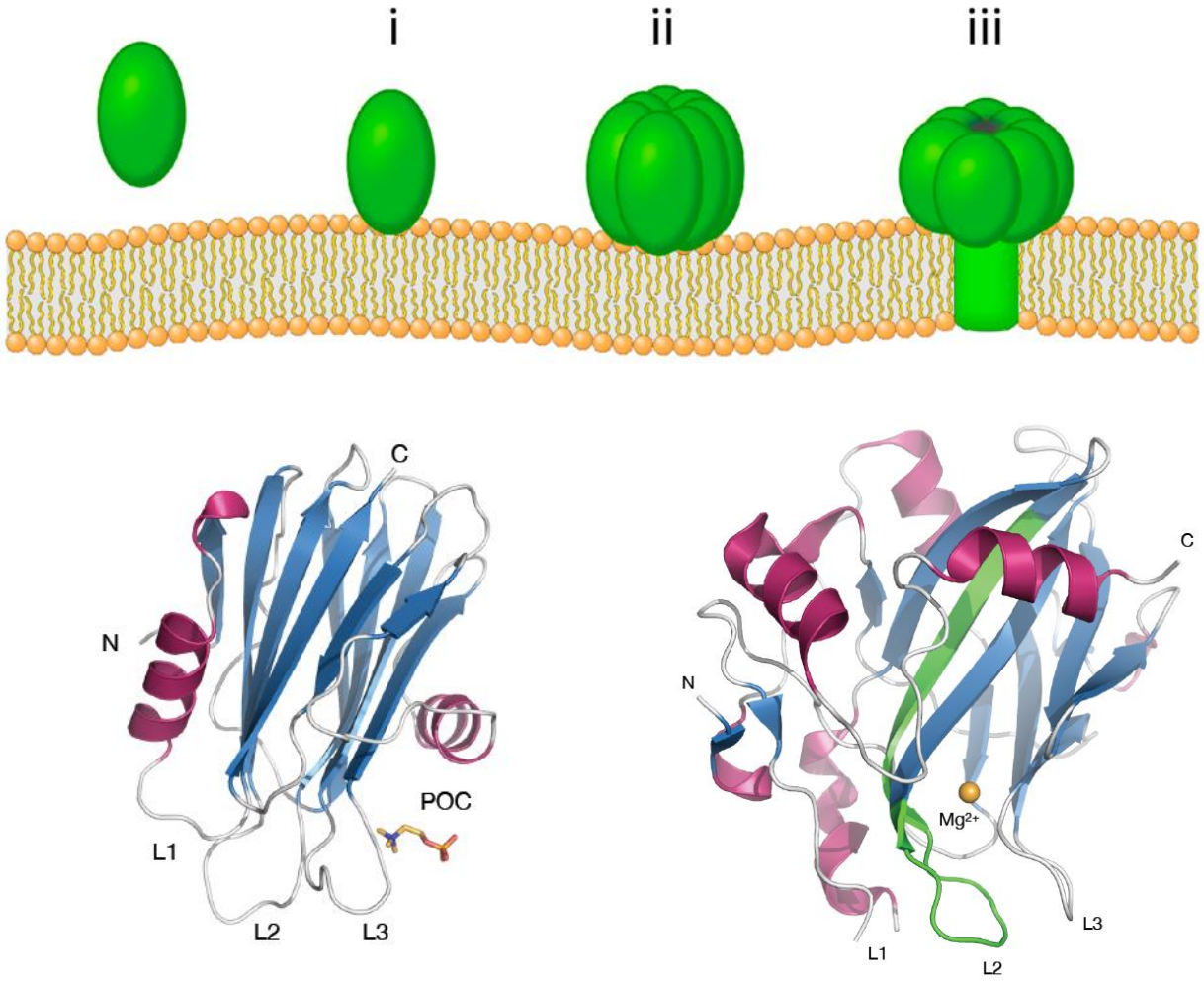
app. 10 nm



liposomes

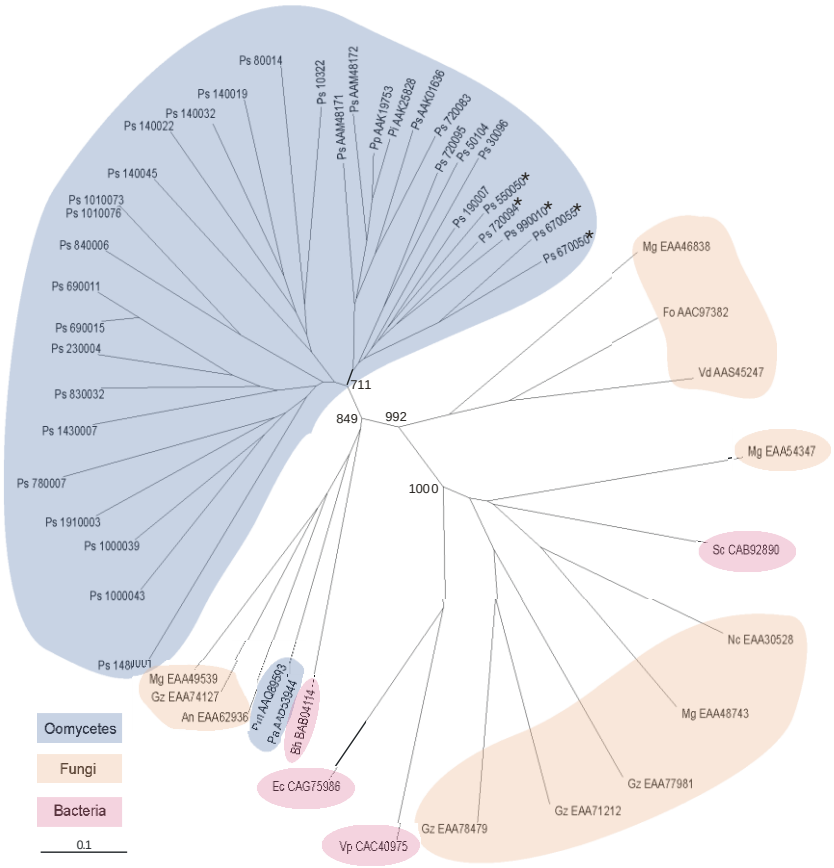
liposome

actinoporins and actinoporin-like proteins



Hong *et al.* (2002) ***J. Biol. Chem.*** 277: 41916-41924
Rojko N *et al.* (2016) ***Biochem. Biophys. Acta*** 1858: 446-456
Ottmann C *et al.* (2011) ***Proc. Natl. Acad. Sci. USA*** 106: 10359-10364

Necrosis and ethylene-inducing protein 1 (NEP1)-like proteins



Constitute one of the largest microbial protein families found to be secreted by bacteria, fungi and oomycetes.

No sequence similarity to known proteins.

Secreted microbial proteins (~ 25 kDa, non-glycosylated).

Act as microbial virulence factors (effectors).

NLPs harbor a partially surface-exposed immunogenic 20-mer peptide fragment (nlp20).

Cytotoxic NLPs are produced by hemibiotrophs and necrotrophs at the onset of host cell death; biotrophic plant pathogens produce non-cytotoxic NLPs at the initial phase of infection.

NLPs trigger plant immunity-associated defenses likely by causing the release of damage-associated molecular patterns from host plants

Kufner I *et al.* (2009) ***Plant Signal. Behav.*** 4: 977-979

NLPs are necrosis and virulence-promoting proteins

tobacco

Arabidopsis

Commelina communis

wheat

NLPs are not cytotoxic on

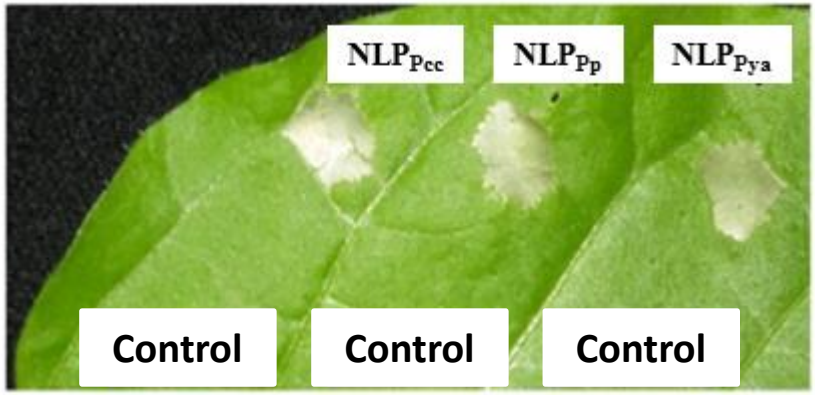
COS-7 cells
human fibroblasts
sheep erythrocytes
Pichia pastoris
Physcomitrella patens cells
monocotyledonous plant cells

Z MBP

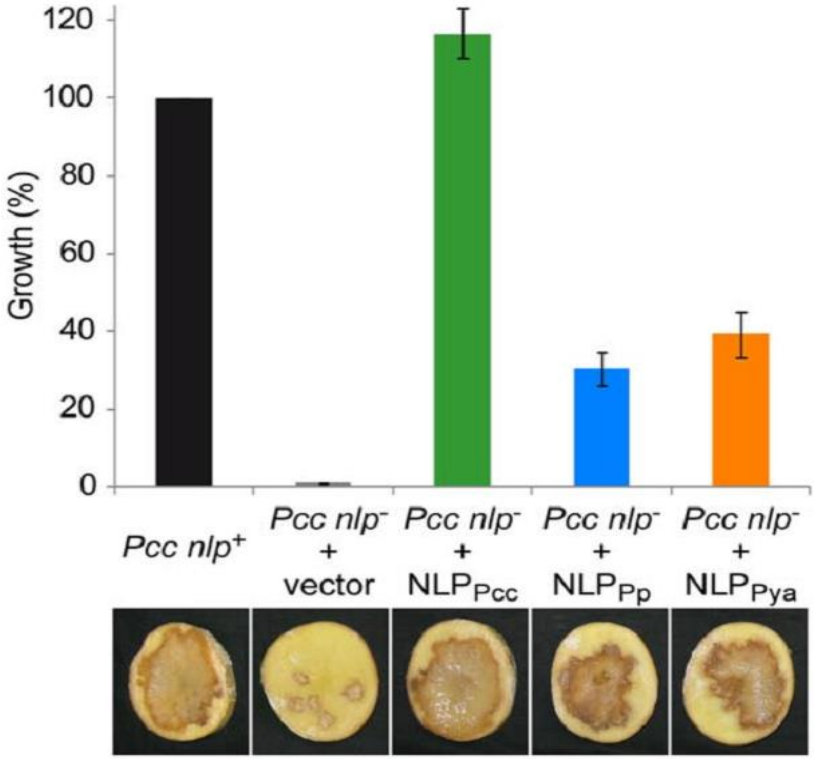
NLPs are necrosis and virulence-promoting proteins

Cytotoxic NLPs are microbial virulence factors

Pectobacterium carotovorum
Fusarium oxysporum
Phytophthora sojae (Dong S *et al.* (2012) **Mol. Plant. Microbe Int.** 25: 896-909)
Verticillium dahliae (Santhanam P *et al.* (2013) **Mol. Plant. Microbe Int.** 26: 278-286)



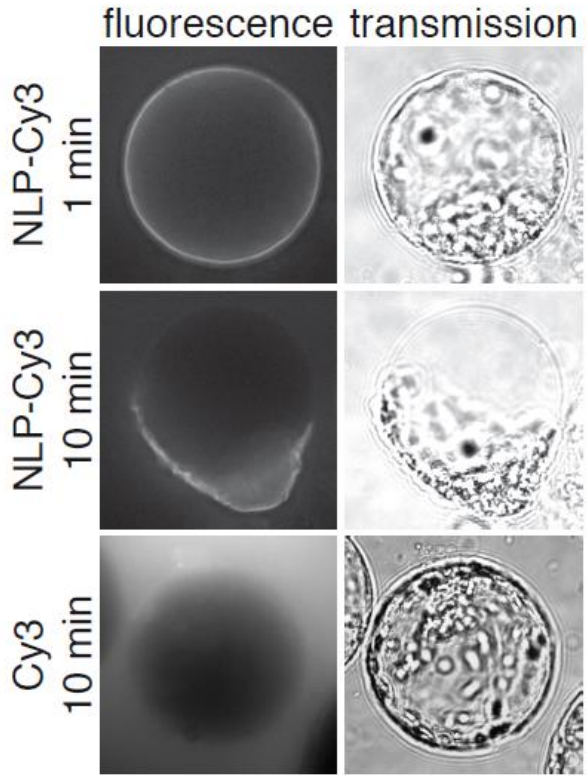
NLP$_{Pcc}$ (*Pectobacterium carotovorum*)
NLP$_{Pp}$ (*Phytophthora parasitica*)
NLP$_{Pya}$ (*Pythium aphanidermatum*)



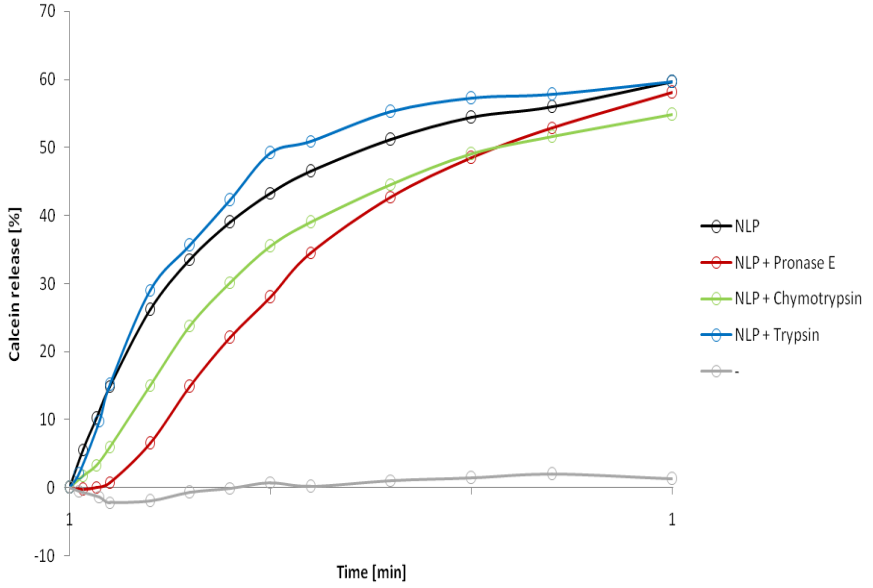
Are NLPs cytolytic toxins (cytotoxins)?

Ottmann C *et al.* (2011) ***Proc. Natl. Acad. Sci. USA*** 106: 10359-10364

NLPs possess cytotoxic activity

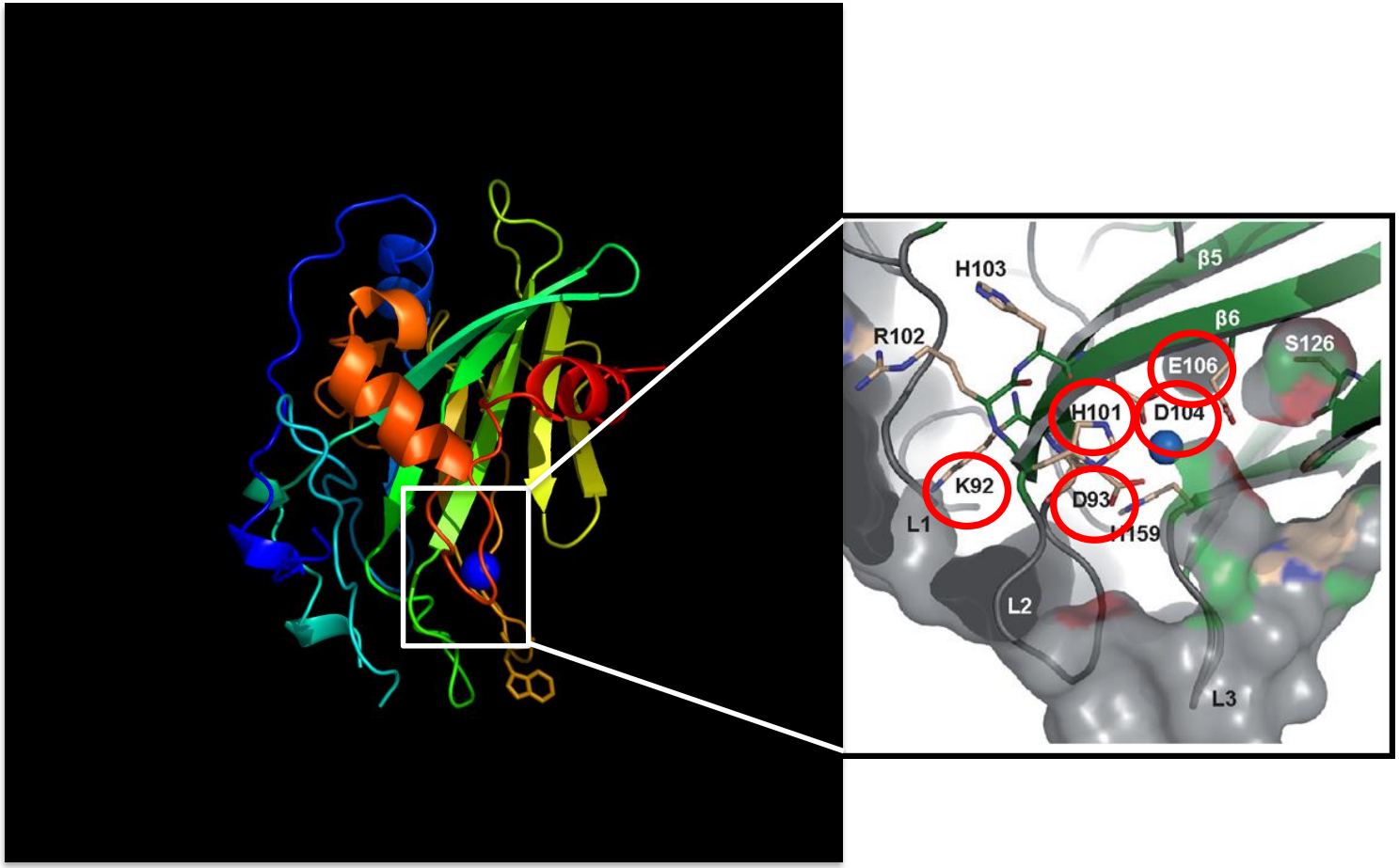


Plasma membrane vesicles filled with the fluorescent dye calcein (measure of membrane integrity)



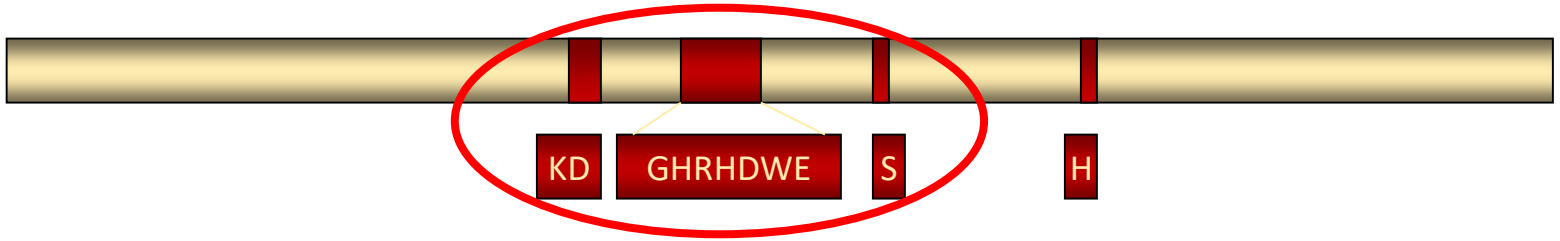
Z MBP

importance of a conserved ion-binding motif

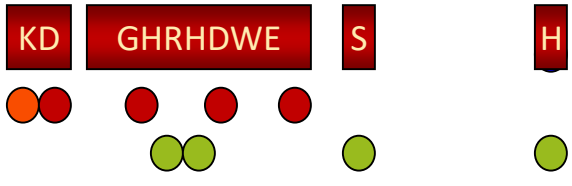


Ottmann C *et al.* (2011) ***Proc. Natl. Acad. Sci. USA*** 106: 10359-10364

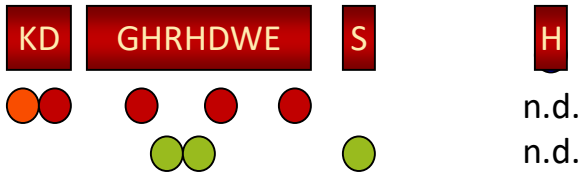
NLP cytotoxicity is required for *Pectobacterium carotovorum* virulence

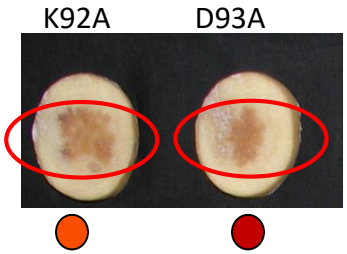


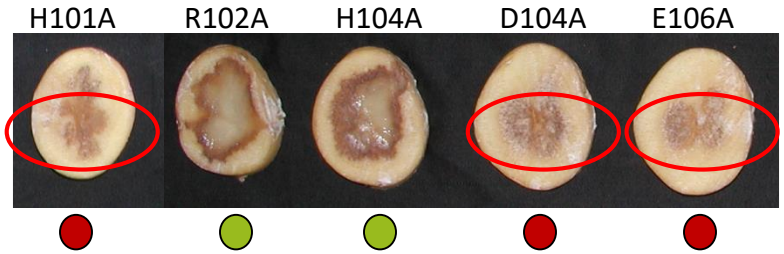
cytotoxicity
- inactive
- active

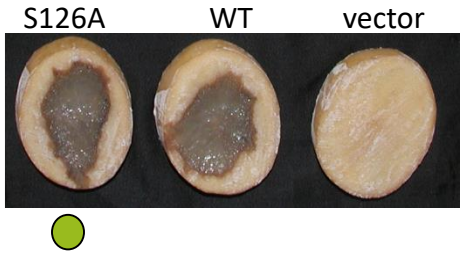


virulence-promoting activity
- inactive
- active



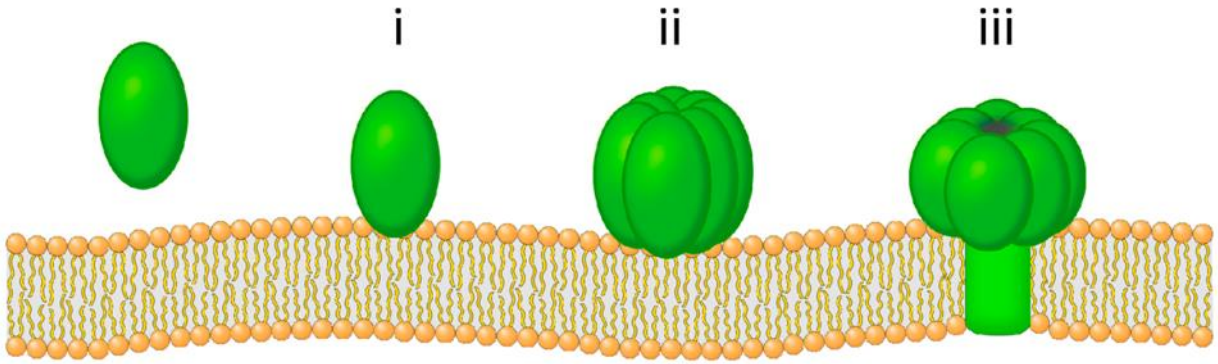


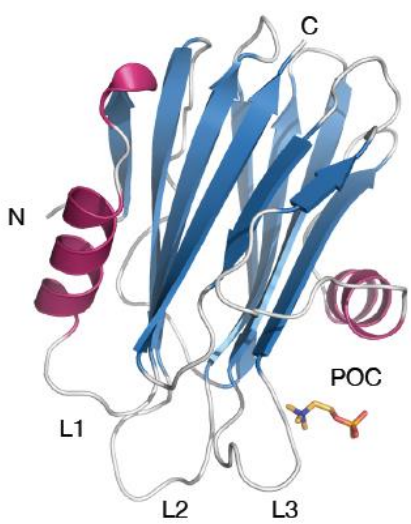


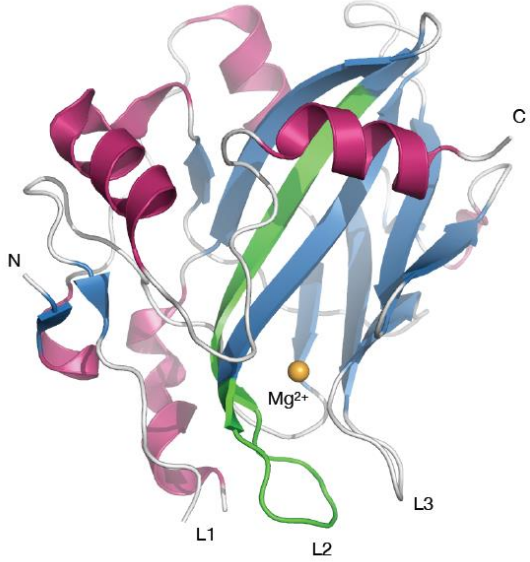


MBP

mechanism of membrane damage by NLPs







Hong *et al.* (2002) ***J. Biol. Chem.*** 277: 41916-41924
Rojko N *et al.* (2016) ***Biochem. Biophys. Acta*** 1858: 446-456
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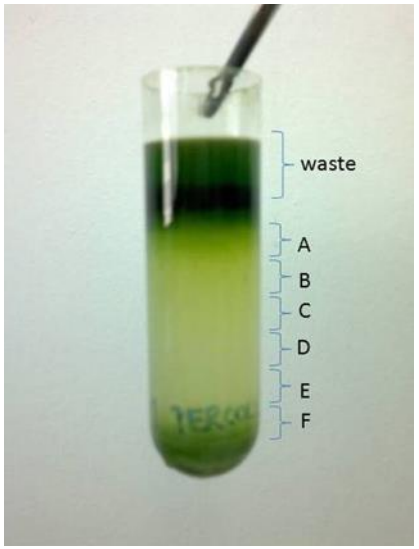
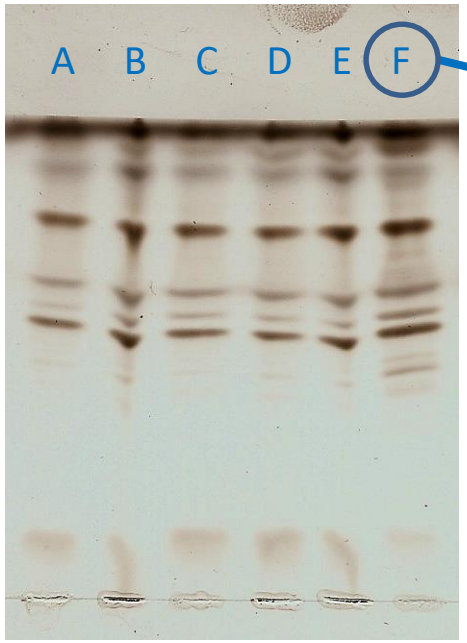
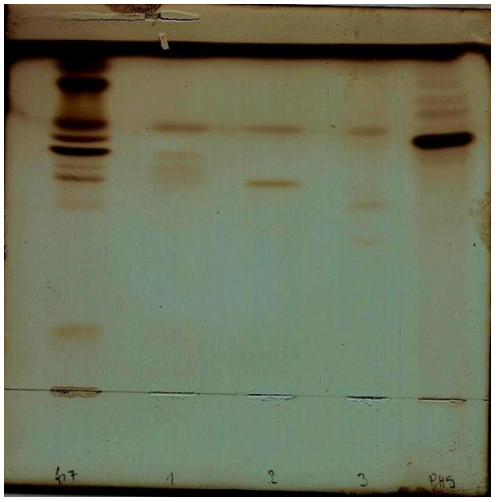
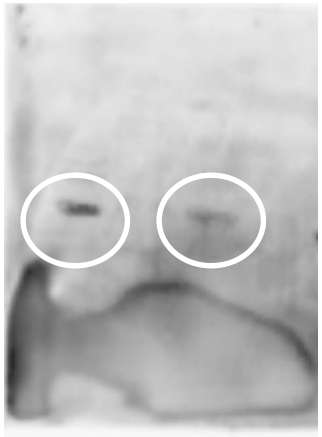
lipids as receptors for NLPs?





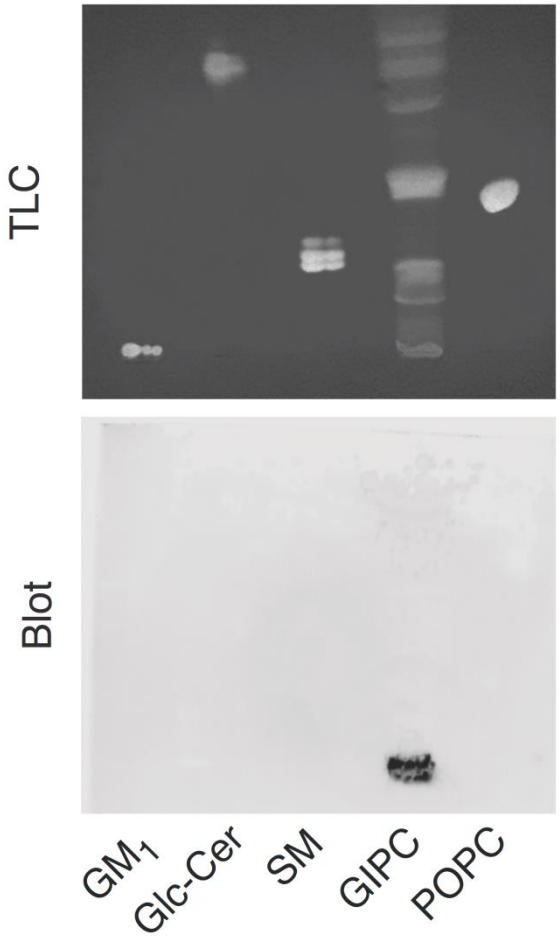


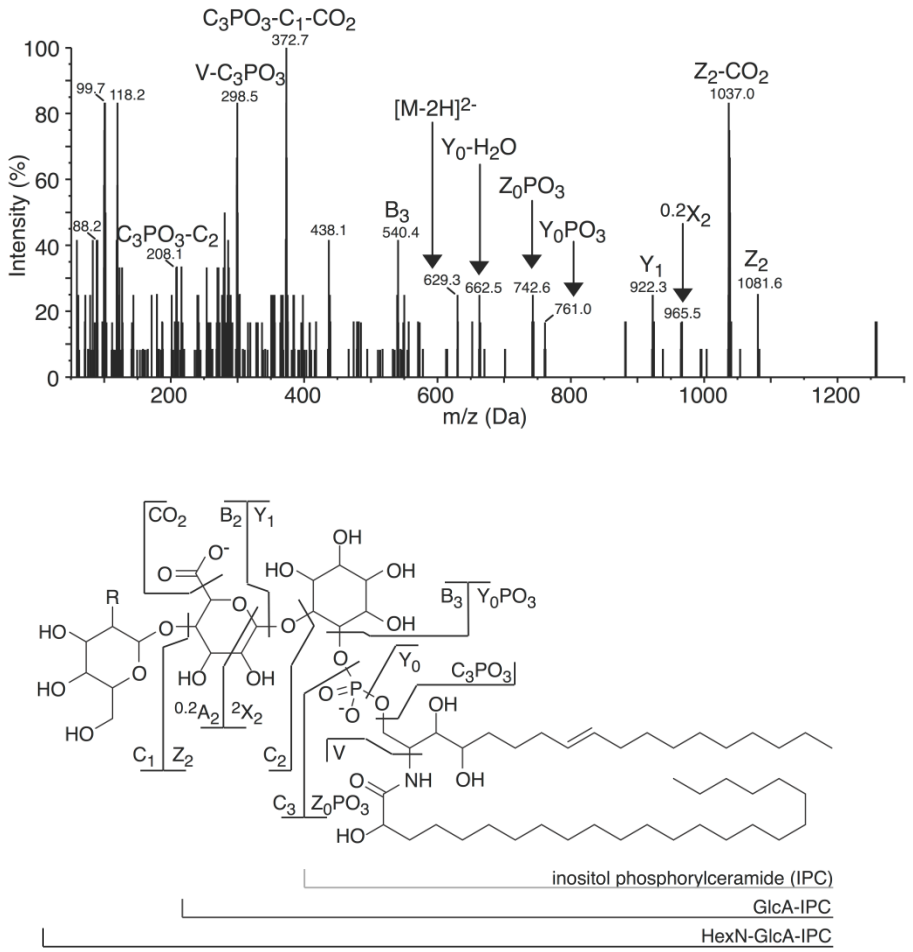
fr F F-1 F-2 F-3



fr F F-1 F-2 F-3

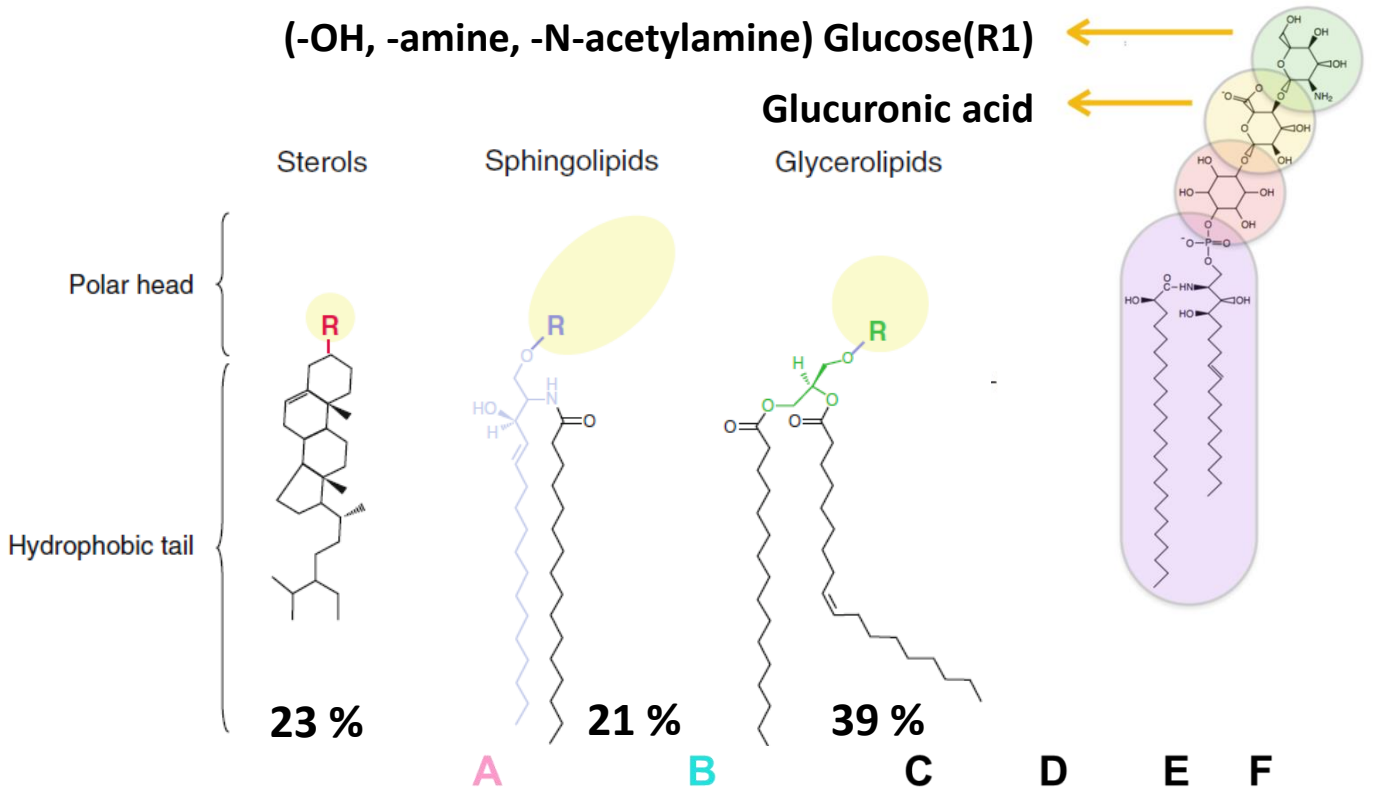
GIPCs ARE receptors for NLPs



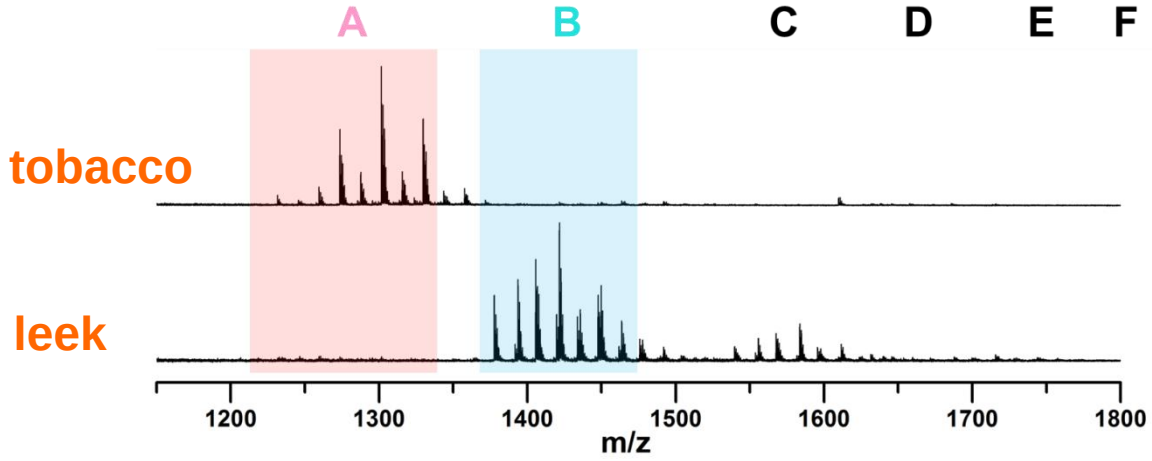


GIPC: glycosyl inositol phospho ceramides

GIPCs ARE receptors for NLPs

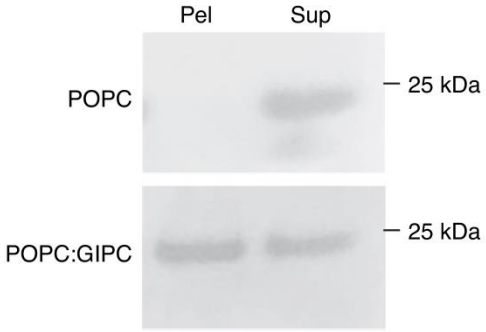


Cer-P-Ins-Hex
Series A
Cer-P-Ins-Hex-Hex(R1)
Series B
Cer-P-Ins-HexA-Hex(R1)-Hex
Series C
Cer-P-Ins-HexA-Hex(R1)-Hex-Pen
Cer-P-Ins-HexA-Hex(R1)-$(Hex)_2$
Series C'
Cer-P-Ins-HexA-HexA-HexA-Hex
Cer-P-Ins-HexA-HexA-HexA-HexA
Series D
Cer-P-Ins-HexA-Hex(R1)-Hex-$(Pen)_2$
Cer-P-Ins-HexA-Hex(R1)-$(Hex)_2$-Pen
Series E
Cer-P-Ins-HexA-Hex(R1)-Hex-$(Pen)_3$
Cer-P-Ins-HexA-Hex(R1)-$(Hex)_3$-Pen
Cer-P-Ins-HexA-Hex(R1)-$(Hex)_2$-$(Pen)_2$
Series F
Cer-P-Ins-HexA-Hex(R1)-Hex-$(Pen)_4$

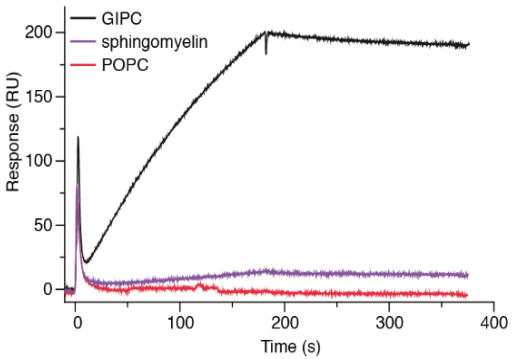


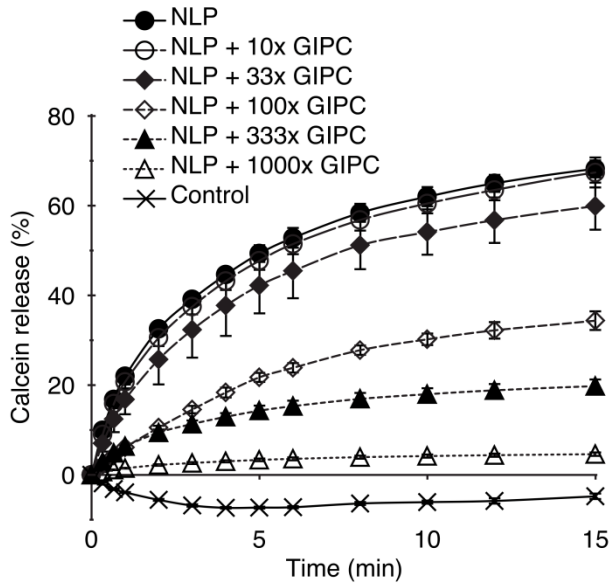
Furt F *et al.* (2011) Lipids of the Plant Plasma Membrane, In The Plant Plasma Membrane, Springer, 3-30

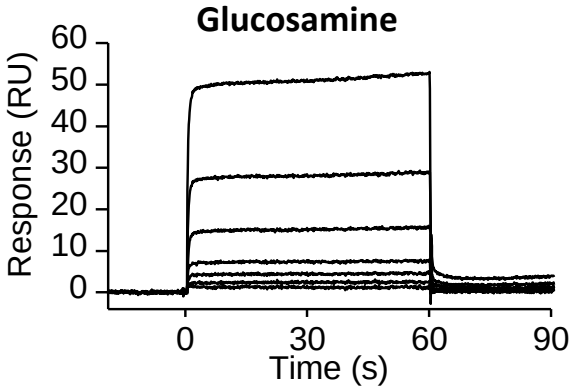
specificity of NLPs



	K_D NLP_{Pya}	K_D NLP_{Pp}
A. thaliana	276 ± 101 nM	323 ± 103 nM
N. tabacum	448 ± 62 nM	250 ± 74 nM
B. oleracea	299 ± 162 nM	253 ± 34 nM

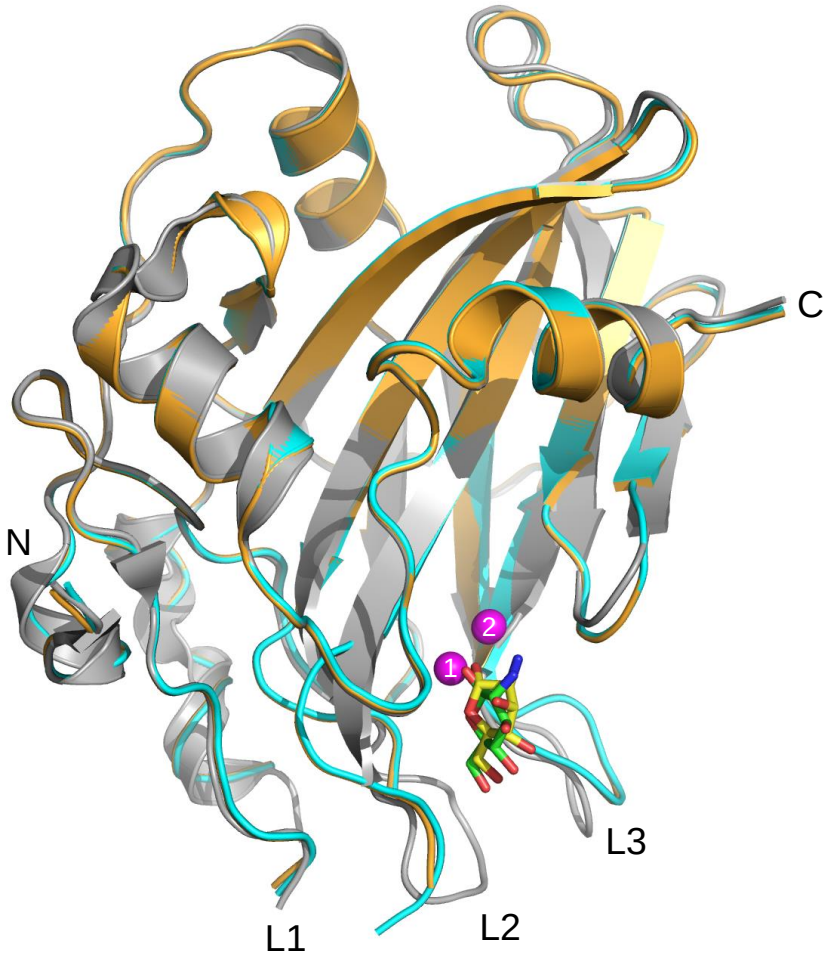


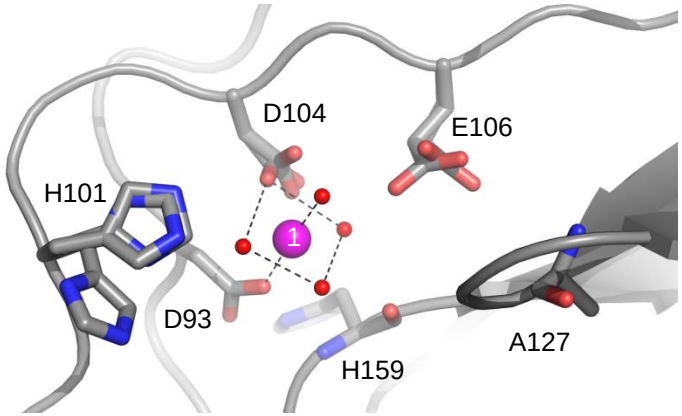


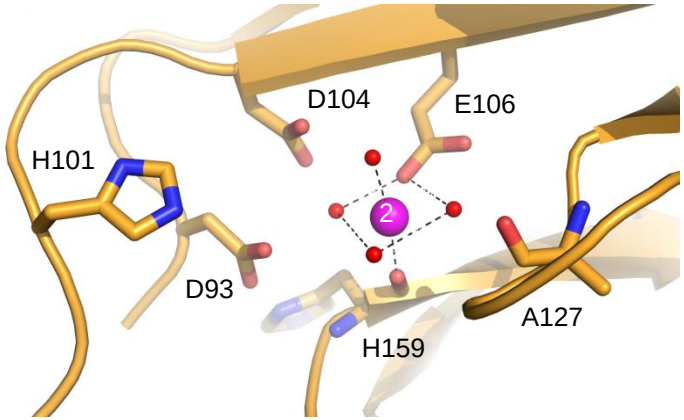


Lenarčič T *et al.* (2017) ***Science*** 358:1431-1434

specificity of NLPs





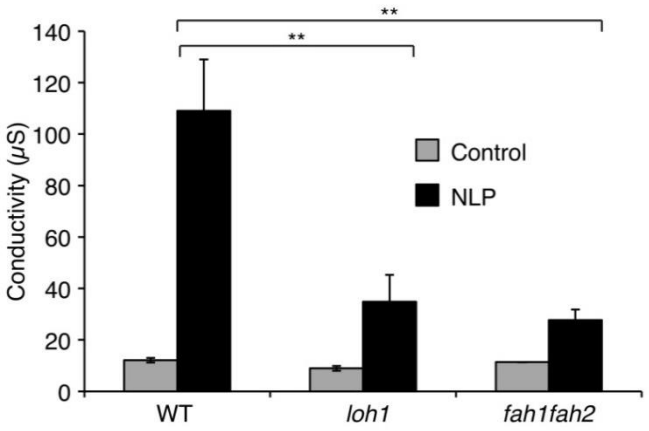


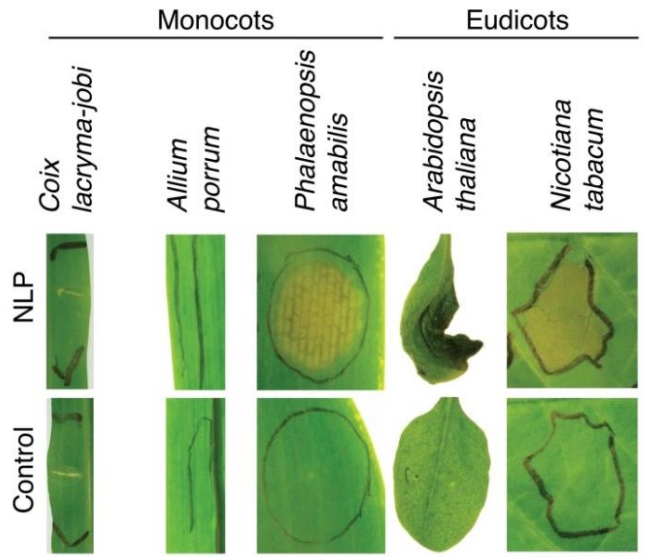
apoNLP$_{Pya}$
NLP$_{Pya}$-glucosamine
NLP$_{Pya}$-mannosamine

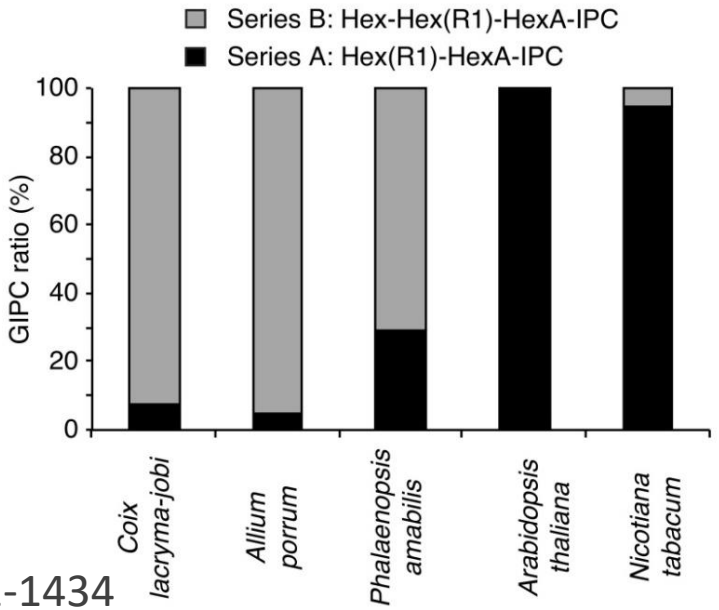
Tea Lenarčič – oral presentation

Lenarčič T *et al.* (2017) ***Science*** 358:1431-1434

GIPC lipids determine specificity







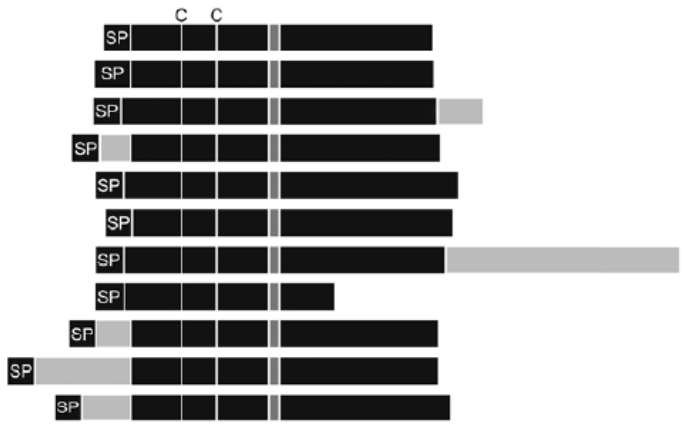
loh1 - ceramide synthase mutant
fah1-2 - fatty acid hydroxylase mutant

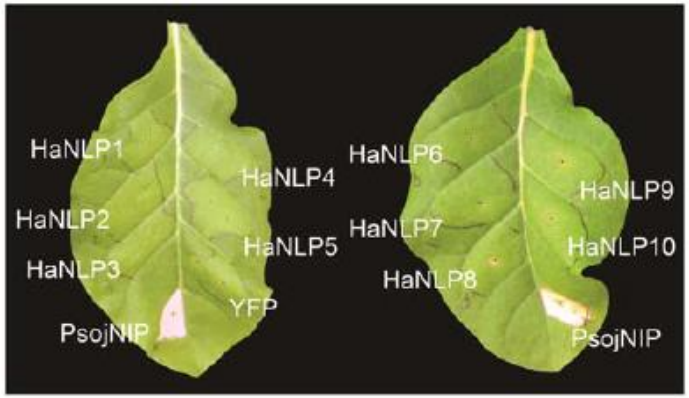
MBP Lenarčič T *et al.* (2017) ***Science*** 358:1431-1434

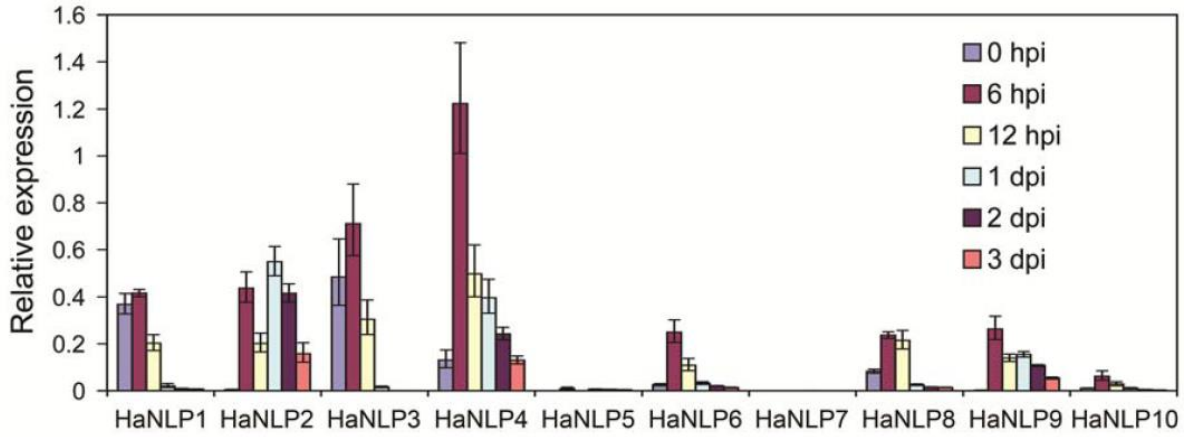
structural studies of non-toxic NLP

Downy mildew pathogen *Hyaloperonospora arabidopsidis*
12 genes coding for NLP proteins and 15 pseudogenes

Name	Size (aa)	SP-score	Heptapeptide
PsojNIP	237	0,998	GHRHDWE
HaNLP1	245	0,999	SVRHSWE
HaNLP2	281	1,000	GHRYDWE
HaNLP3	266	0,393	GHRHDWE
HaNLP4	262	0,999	GHRHDWE
HaNLP5	250	0,998	SRRHDWA
HaNLP6	419	1,000	GHRYDWE
HaNLP7	173	0,998	WIRHVWN
HaNLP8	267	0,999	GYRHAFE
HaNLP9	308	1,000	DDRHDWE
HaNLP10	286	1,000	GQRHDWE

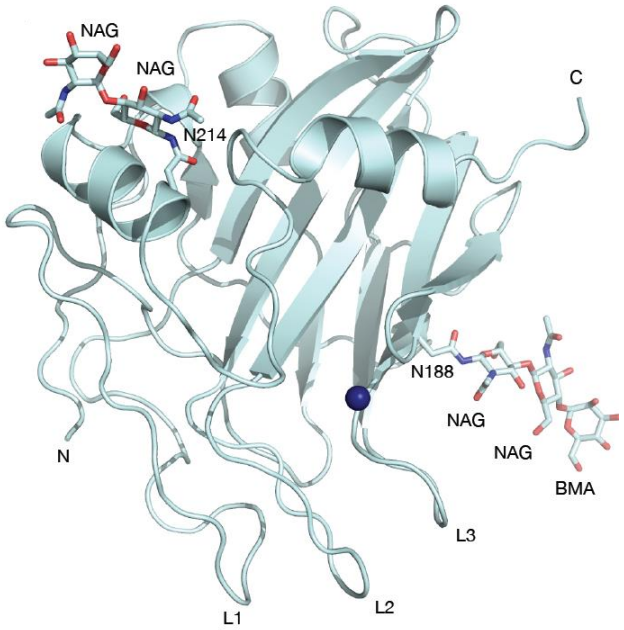


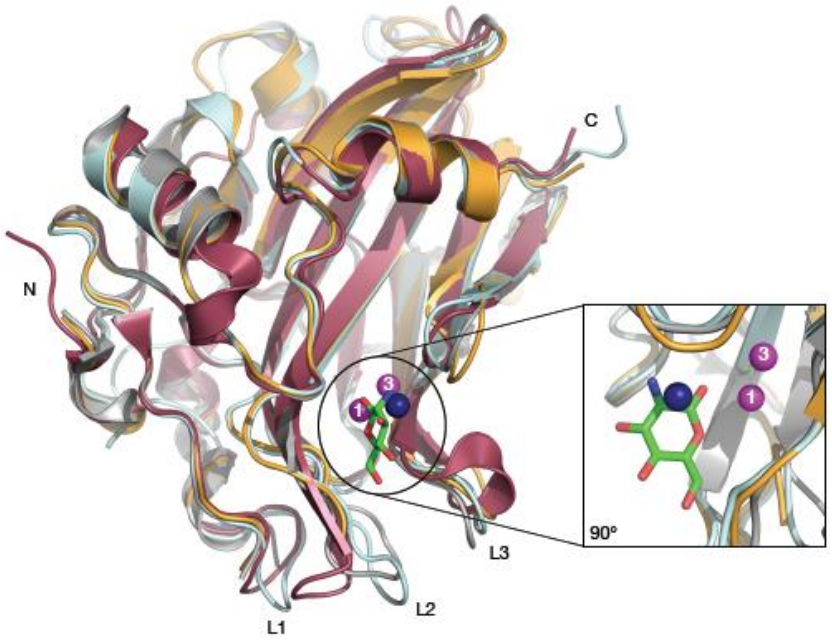


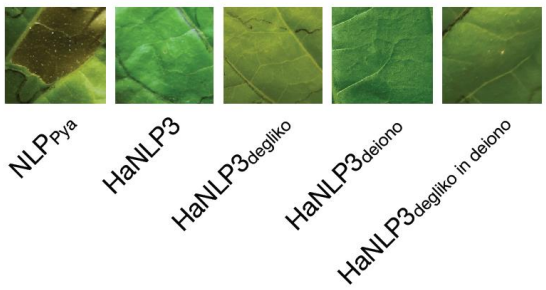


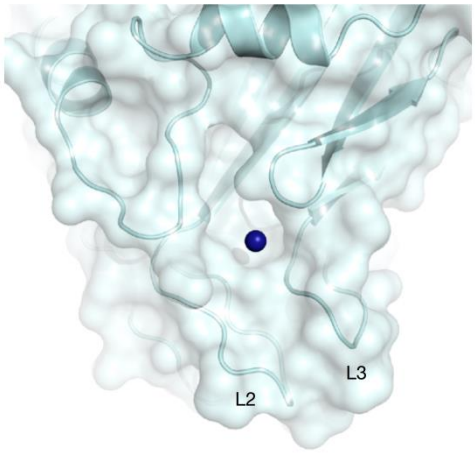
Cabral A *et al.* (2012) ***MPMI*** 25: 697-708

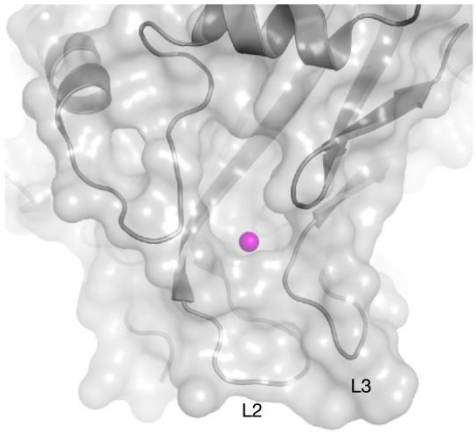
structural studies of non-toxic NLP



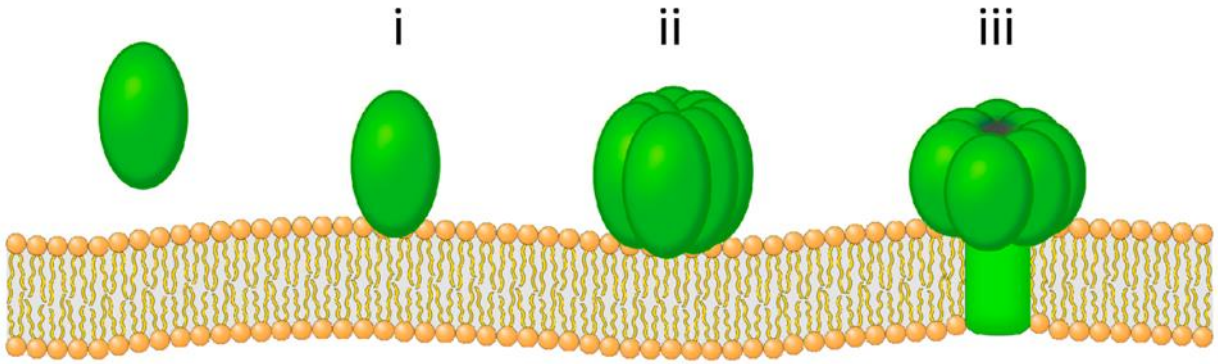


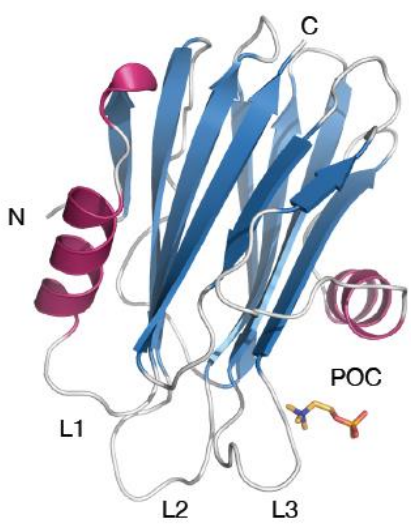


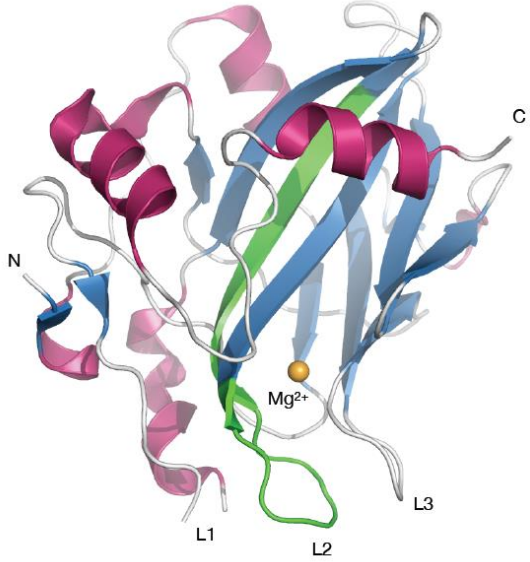




model systems to study toxicity of NLPs







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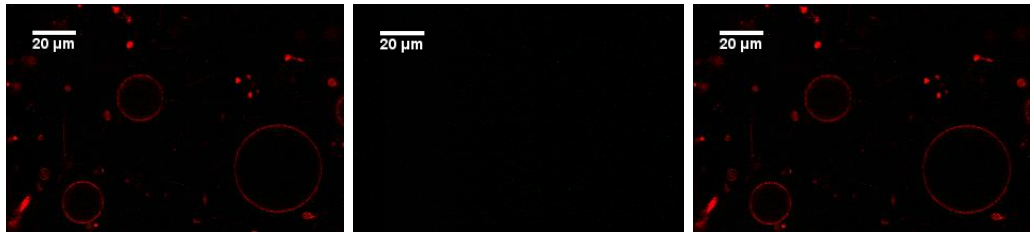
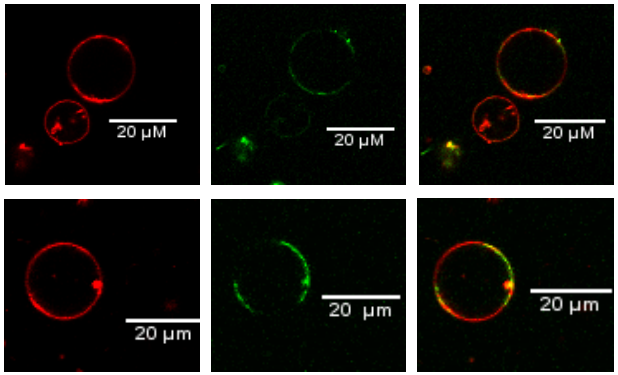
model systems to study toxicity of NLPs

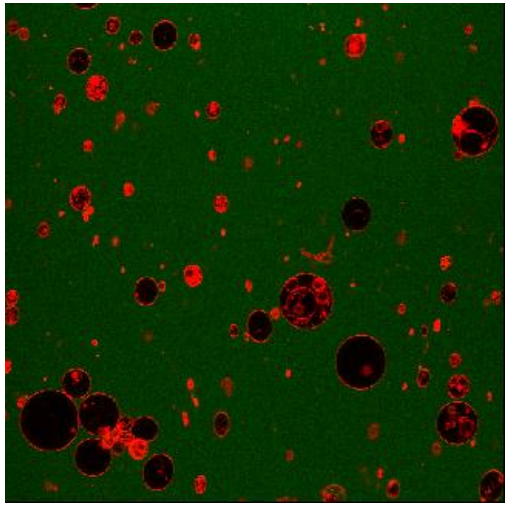
GIPC:POPC 2:1 (w/w)



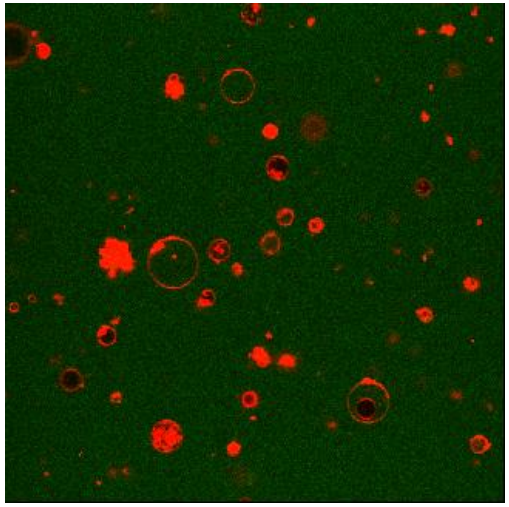
GIPC:POPC 2:1 (w/w) + NLP_{Pya}- Alexa488

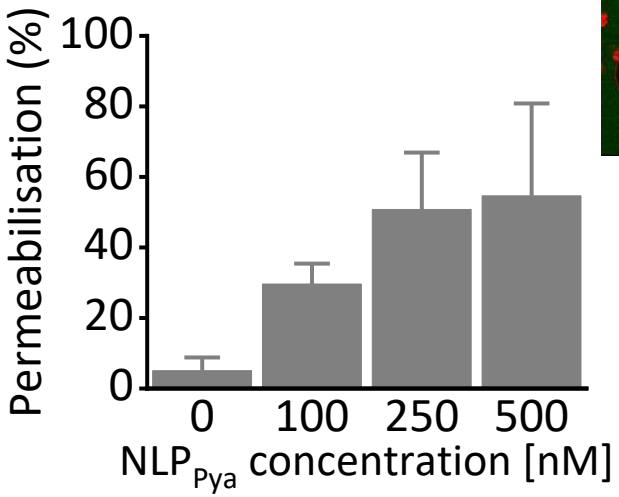


GIPC:POPC 2:1 (w/w), FD4

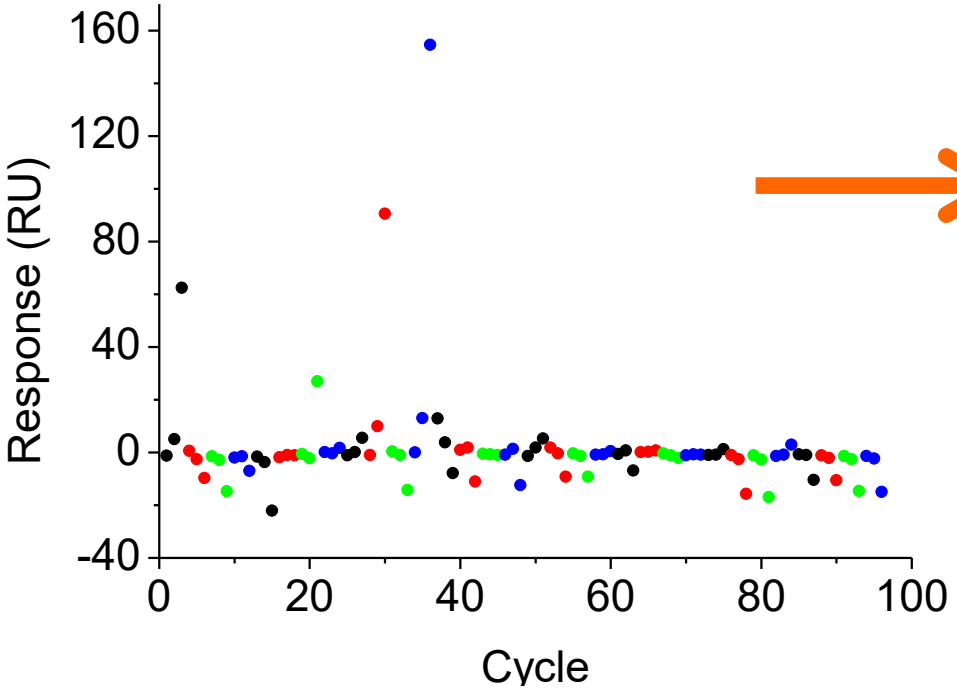


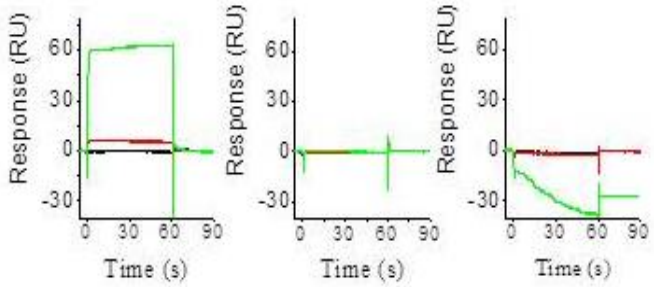
GIPC:POPC 2:1 (w/w), FD4 + NLP_{Pya}

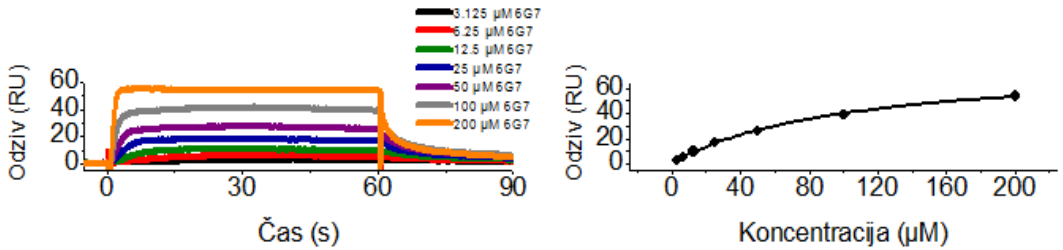


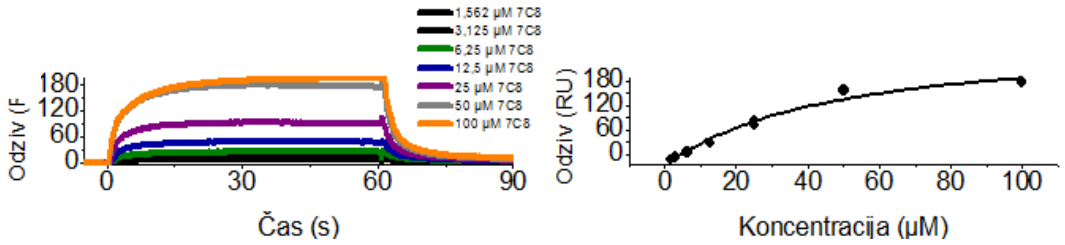


Tina Snoj – poster









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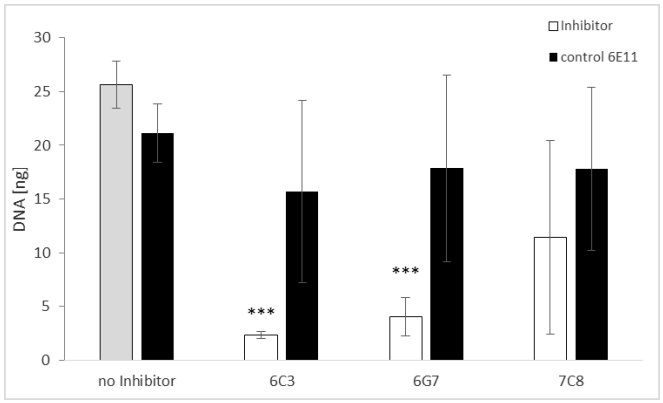
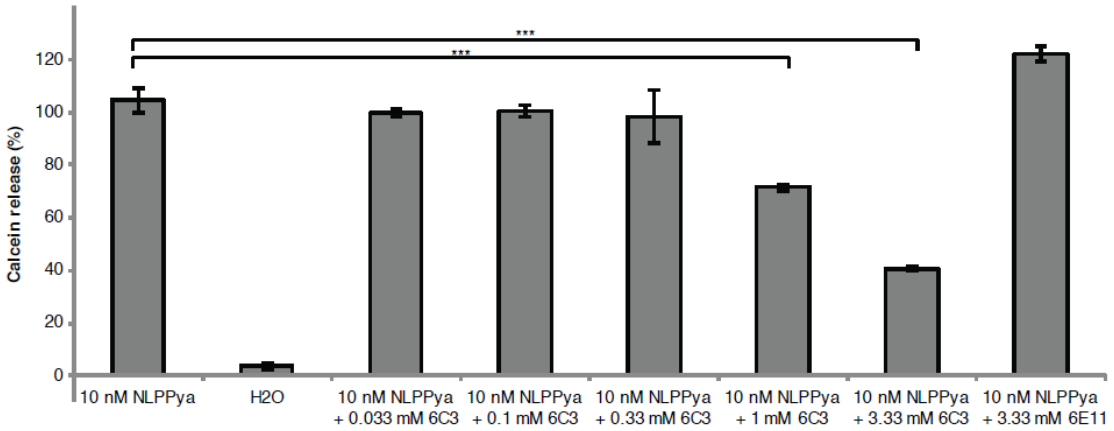
applied value





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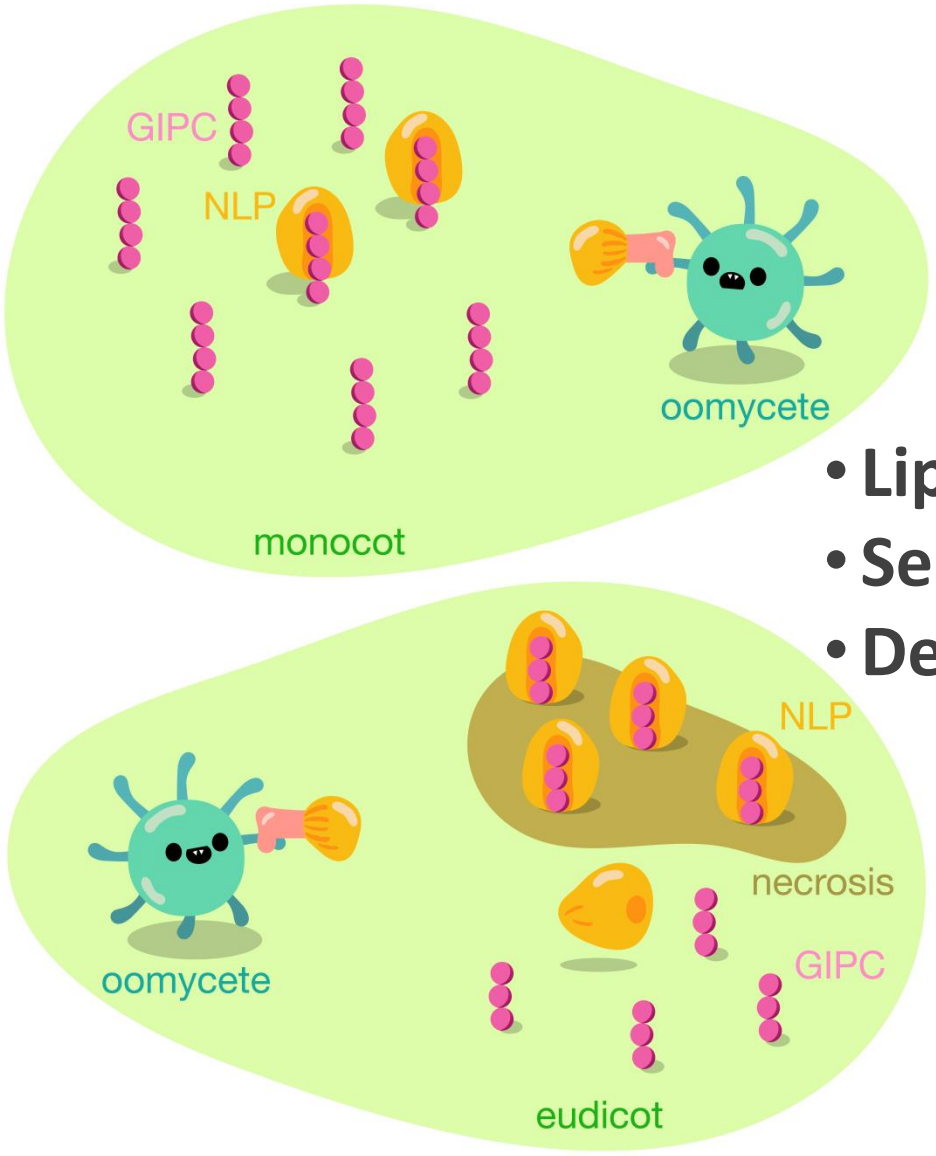

- **Lipid receptor**
- **Sensitivity of plants**
- **Development of inhibitors**

ARBRE MOBIEU
BETWEEN ATOM AND CELL

Porto, 29.10.-3.11.2018

Biomolecular interaction analysis 2018: from molecules to cells

acknowledgements

National Institute of Chemistry, Ljubljana, Slovenia

Tea Lenarčič
Katja Pirc
Vesna Hodnik
Marjetka Podobnik
David Pahovnik
Ema Žagar
Gregor Anderluh

RIKEN Tokyo, Japan

Akiko Yamaji
Peter Greimel
Toshihide Kobayashi

Center for Plant Molecular Biology, Eberhard-Karls-University, Tübingen, Germany

Isabell Albert
Hannah Böhm
Thorsten Nürnberger

Javna agencija za raziskovalno dejavnost Republike Slovenije

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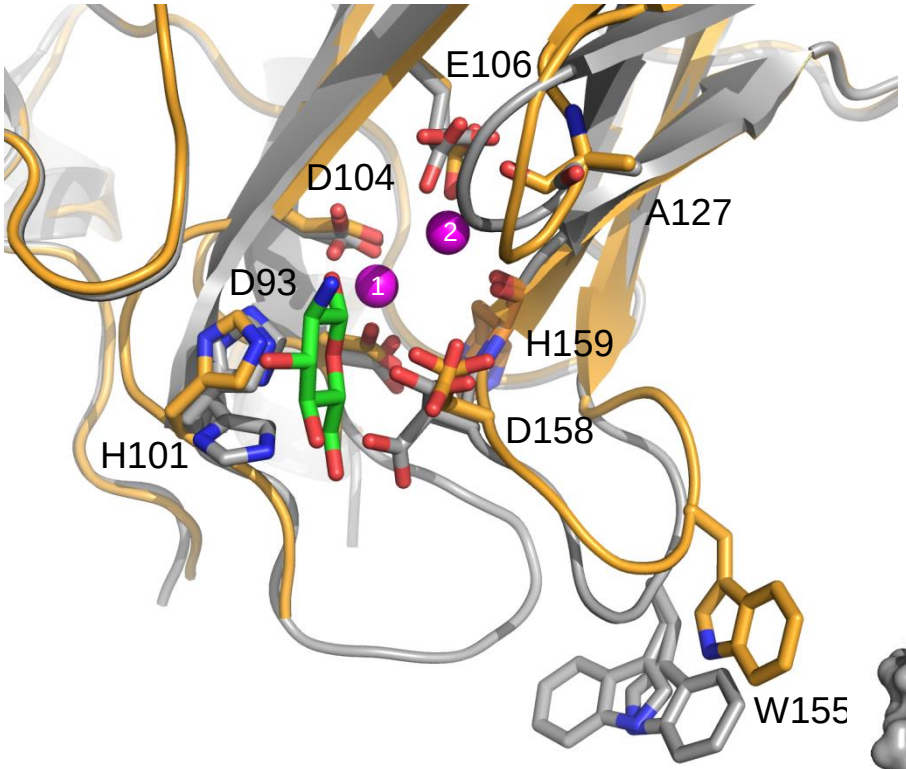
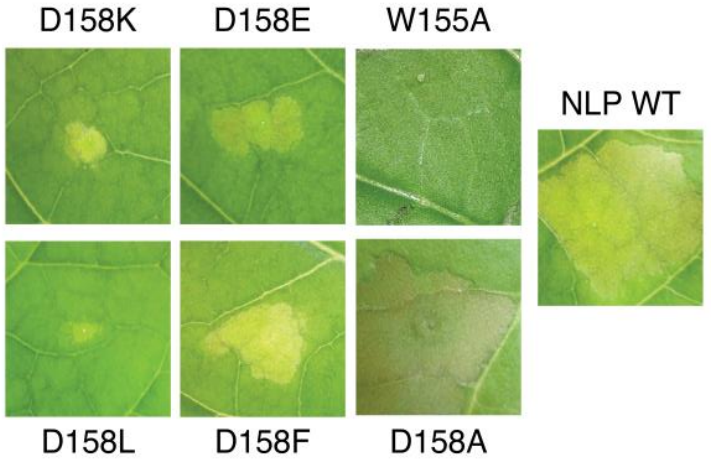
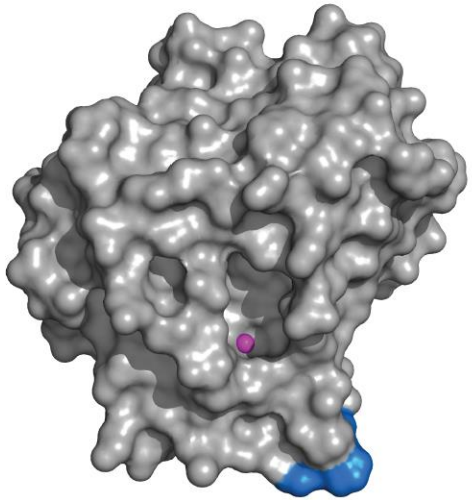
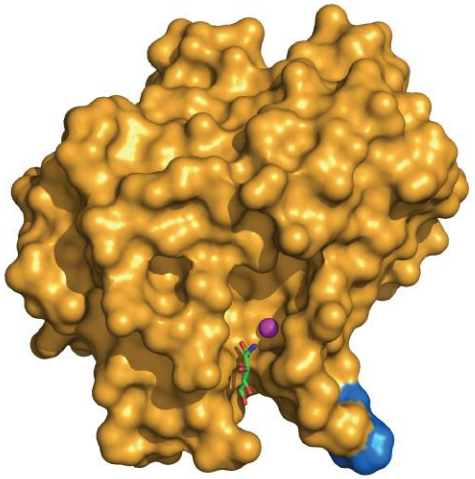
BIA separations
Leaders in monolith chromatography

specificity of NLPs









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