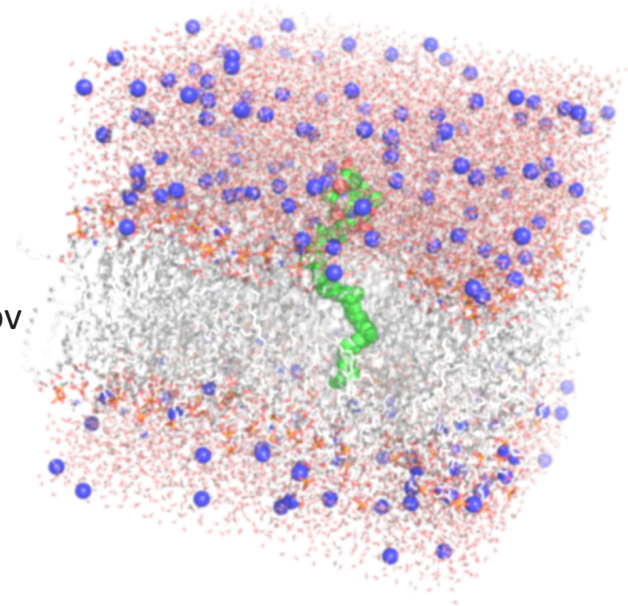
Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow
Scientific Research Institute for Systems Biology and Medicine, Moscow
Frumkin Institute of Physical Chemistry and Electrochemistry RAS, Moscow

Atomistic Insights into a Lantibiotic's Mechanism: an Integrated Molecular Dynamics and Biophysics Study

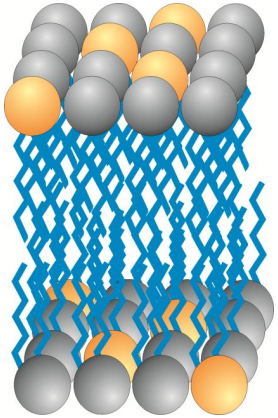


I.S. Panina, V.P. Shendrikov, A.O. Chugunov, I.A. Boldyrev, R.G. Efremov

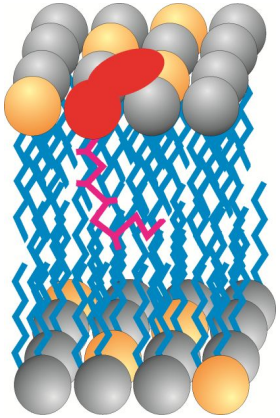
irinaspanina@gmail.com

BCADD-2026

Lantibiotics: the target buys 1000 × potency



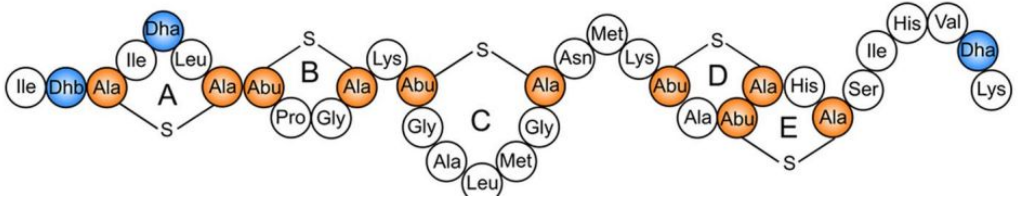
Conventional AMP: unspecific, active at
~10^{-6} M



Lantibiotic: docks onto lipid II, active at
~10^{-9} M

THE PEPTIDE

Nisin: two modules in 34 residues



Blue: dehydro-amino acids (Dha, Dhb). Orange: (methyl)lanthionine. Rings A–E.

Rings A + B (1–12): the recognition module

Capture the pyrophosphate of lipid II. Antibacterial on their own.

Rings C–E + hinge (20–34): the pore module

Insert into the bilayer. Hinge mutations kill pore formation but not antibacterial activity.

- Ribosomally synthesised, post-translationally modified peptide
- Thioether (lanthionine) rings — rigid peptides
- Dual kill: sequestering lipid II blocks cell-wall synthesis, and the same complex nucleates a pore
- A food preservative since 1953, no resistance in 70 years

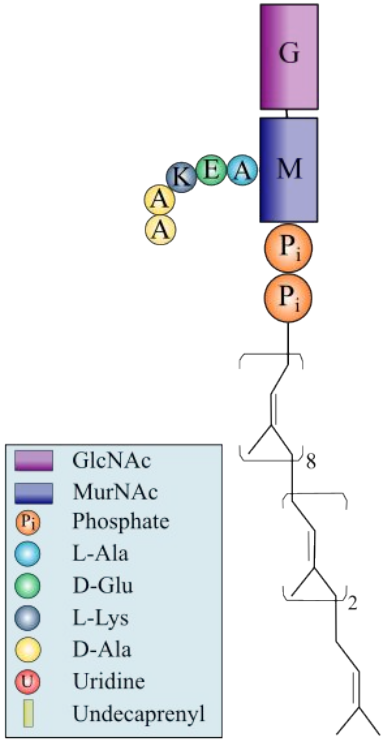
…yet nisin never reached the clinic. To engineer a better one we need the recognition mechanism at atomic resolution.

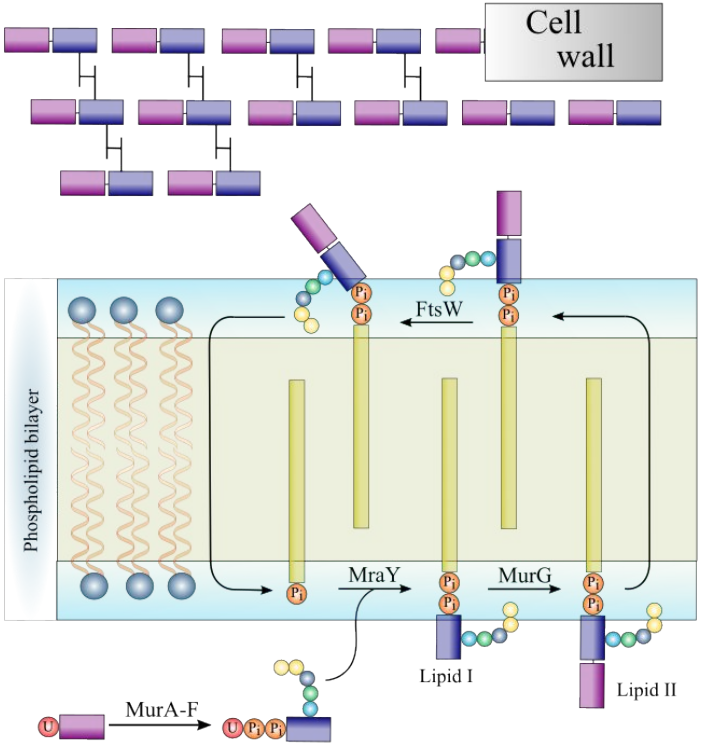
THE TARGET

Lipid II — the Achilles' heel

- Head group: GlcNAc–MurNAc + pentapeptide
- Linker: pyrophosphate (PPi)
- Anchor: C_{55} bactoprenol tail
- Found only in bacteria; chemically invariant across species
- Only tens of thousands of copies per cell — a real bottleneck

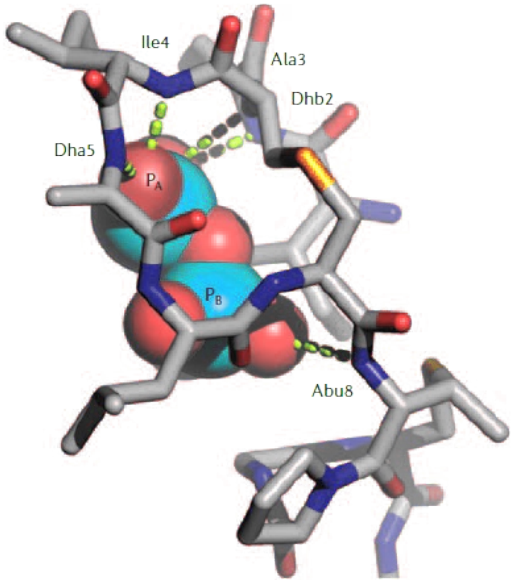
The pyrophosphate is what every nisin-type lantibiotic grips — and what evolution cannot afford to change.





What is known — and what is missing

Pyrophosphate ‘cage’



Hsu et al., Nat. Struct. Mol. Biol. 11, 963 (2004)

- The only high-resolution complex was solved in DMSO, with a truncated lipid II (Hsu et al., Nat. Struct. Mol. Biol.2004).
- Solid-state NMR in liposomes and bacterial membranes contradicts that geometry — but offers no alternative structure (Medeiros-Silva et al., Nat. Commun.2018).
- Lipid II is long, flexible, amphiphilic: its shape is set by the water–lipid milieu, which DMSO does not reproduce.

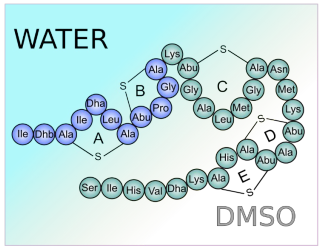
No experimental structure of the full nisin–lipid II complex in a native-like bilayer exists.

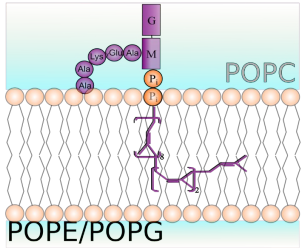
THIS WORK

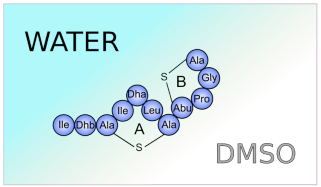
Aim and strategy

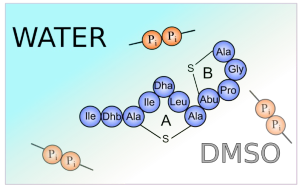
Build and validate an atomistic model of nisin–lipid II recognition in a model Gram-positive membrane using MD simulations.

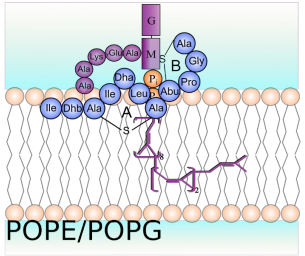
The simulation set-up











I
isolated molecules in their parent and control environments

II
nisin "recognition module" in presence and absence of PPi

III
nisin "recognition module" / lipid II in the model bacterial membrane

I — isolated molecules

lipid II in POPG/POPE and in POPC; nisin in water and in DMSO as published controls

II — recognition module ± PPi

$nisin_{1-11}$ with 0, 1 or 3 dimethyl-pyrophosphate ions (DMPPi) — a minimal mimic of the lipid II linker; free docking, no restraints

III — the complex in the membrane

trap structure superimposed on membrane-bound lipid II, then re-simulated for 1 µs

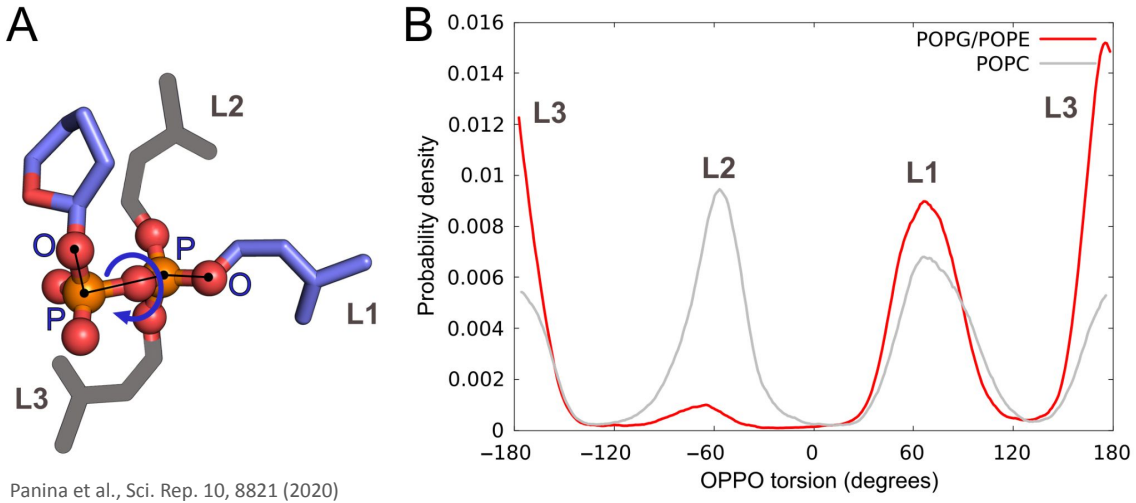
Simulations

- GROMACS
- united-atom GROMOS 43a2
- 500 ns runs in water, 1000 ns in the membrane

FAST. FLEXIBLE. FREE.
GROMACS

Panina et al., Sci. Rep. 10, 8821 (2020)

Result 1 — the membrane selects three PPi states



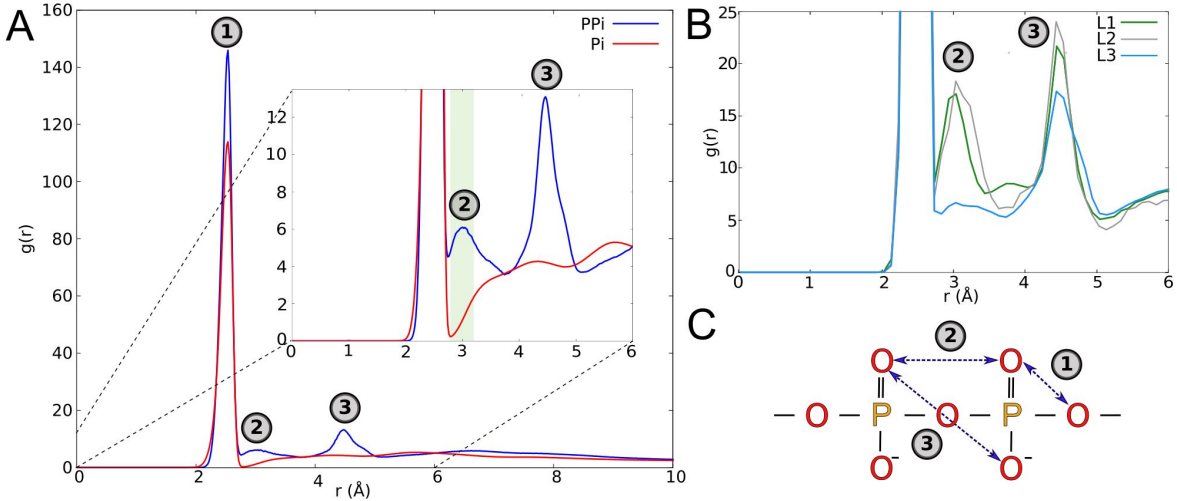
Panina et al., Sci. Rep. 10, 8821 (2020)

Rotation about the O–P–P–O torsion defines states L1, L2, L3. Red: bacterial POPG/POPE bilayer; grey: POPC.

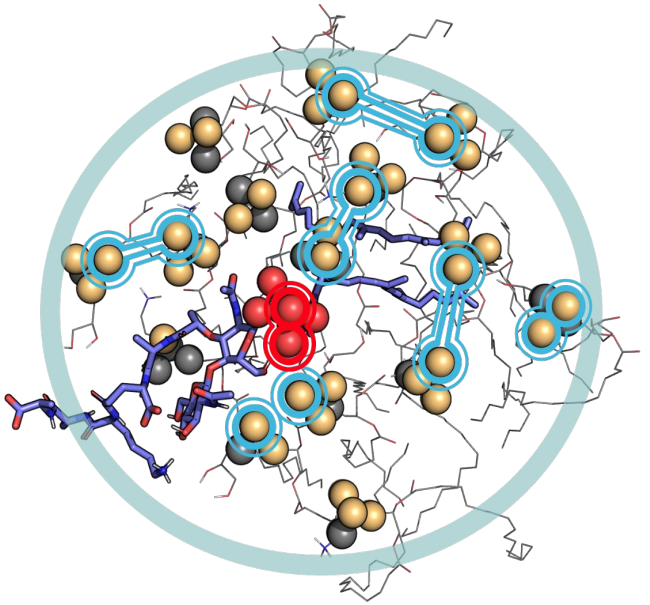
- In bulk water lipid II samples many conformations; in the bilayer only three survive.
- The bacterial lipid composition shifts the populations: L2 is nearly abolished, L1 and L3 dominate.

Only L1 and L2 expose the PPi oxygens in a geometry nisin can grip.

Result 2 — the pyrophosphate pharmacophore



Oxygen–oxygen radial distribution functions for solvent-accessible oxygens of the pyrophosphate (blue) and of a phosphate (red).



A pyrophosphate carries three characteristic O···O distances:

① 2.5 Å within one phosphate

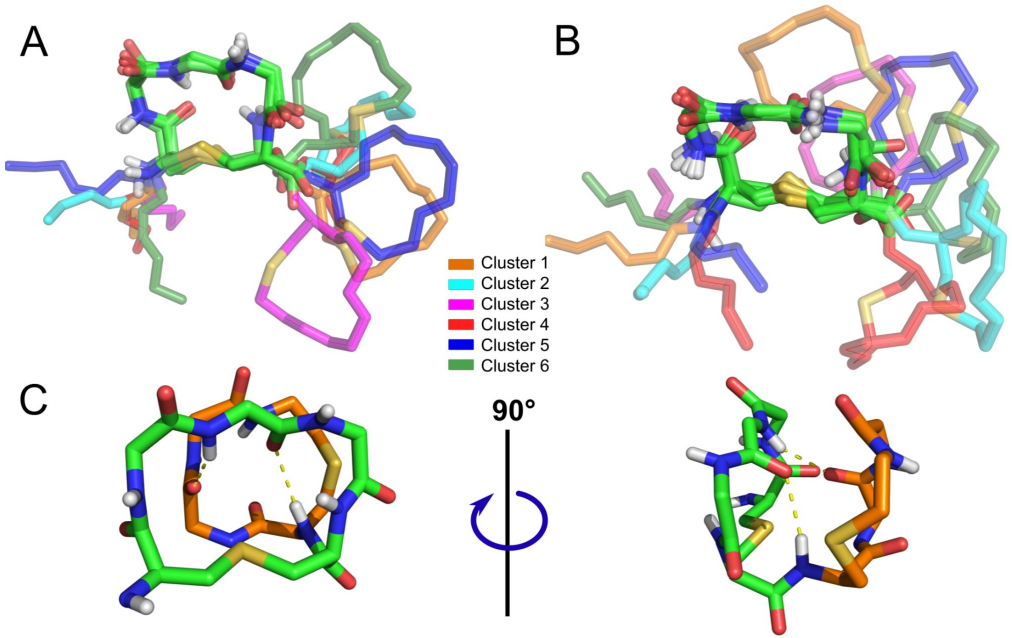
② ~3.0 Å across the O-bridge

③ ~4.5 Å between the two phosphates

Peak ② does not exist for a monophosphate — the lipid head groups cannot imitate it.

A unique, membrane-displayed set of H-bond acceptors: the basis of nisin's selectivity.

Result 3 — a pre-organised recognition module

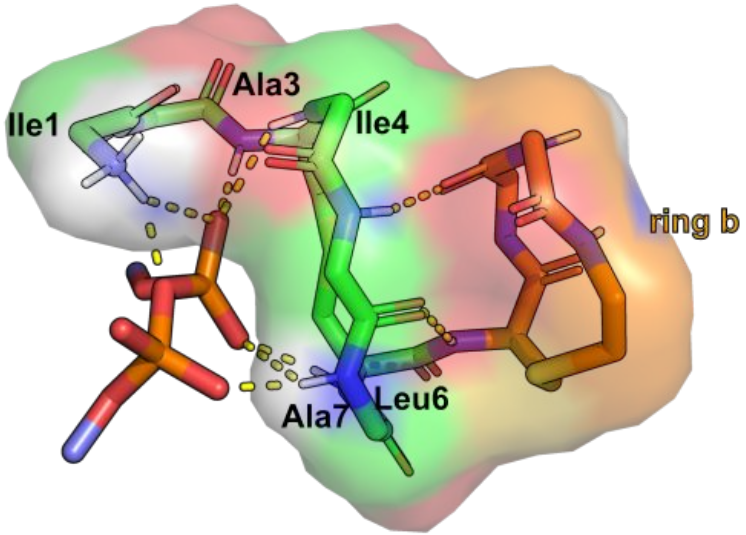


- Clustering the 1–11 backbone over 5 × 500 ns gives six conformations.
- Ring A is conformationally stable (backbone RMSF < 0.7 Å). The direction of H-bond donors, i.e. amide NH groups, is well-defined
- Ring B exhibits higher backbone flexibility.
- In the DMSO (cage) state exists in the free ensemble, but is populated only 7 %.

The peptide does not have to be folded by the target — it already visits the binding-competent shape.

Panina et al., Sci. Rep. 10, 8821 (2020)

Result 4 — Pyrophosphate ‘trap’, not a ‘cage’



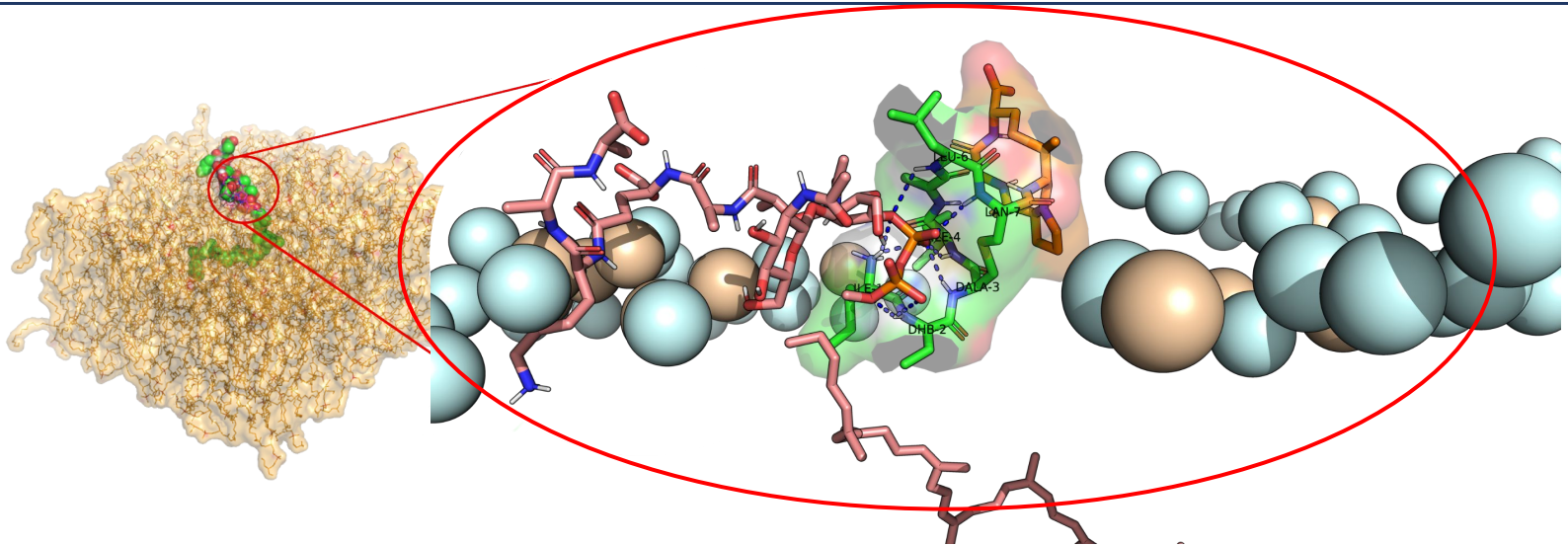
Complex with the DMPPi; hydrogen bonds dashed.

Backbone amides of ring A converge on the PPi.

- DMPPi binds in every run, with no restraints and no bias.
- 2–5 simultaneous backbone hydrogen bonds, plus a salt bridge to the free N-terminus.
- Ring B is only rarely involved — unlike the DMSO structure, where it closes the cage.

Ring A alone forms an open “pyrophosphate trap”, reconciling the DMSO structure with the solid-state NMR data.

Result 5 — the complex holds at the membrane surface

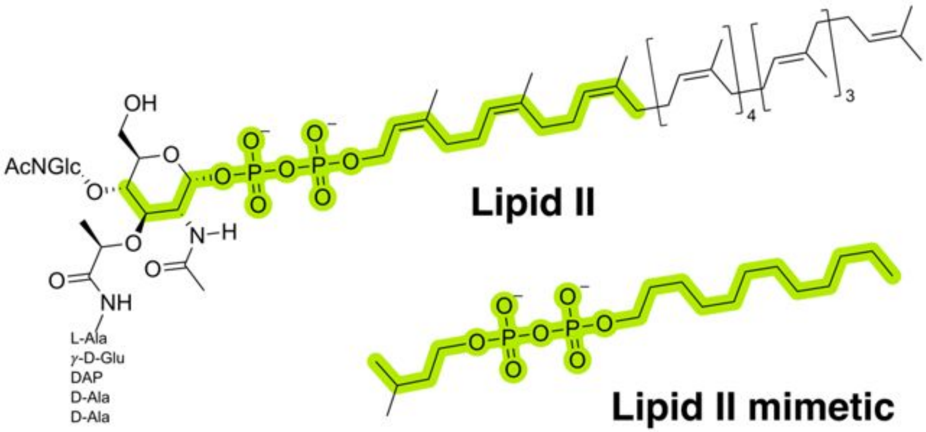


▸ Superimposing the trap on the membrane-bound L1 and L3 states of lipid II — through the pyrophosphate — gives sterically unhindered complexes; 1 µs of MD leaves them intact.

The trap works at the interface, where the real reaction happens: a water-soluble peptide reads a membrane-embedded target without extracting it.

Testing the model: a minimal synthetic target

The simulations result: **the pyrophosphate is the minimal structural element required for recognition.**



iAmPPiC14

mono-isoamyl pyrophosphate-C14: a disubstituted pyrophosphate with one membrane anchor and no sugar–peptide head group.

- keeps the PPi linker and the lipid anchor
- removes MurNAc, GlcNAc and the pentapeptide

In vitro

- Black lipid membranes (BLM) — does binding result in the pore formation, at lantibiotic-like concentrations?

TAKE-HOME

Conclusions

The membrane selects the conformation of target.

In a bacterial POPG/POPE bilayer lipid II keeps only three pyrophosphate states; two are binding-competent.

The pyrophosphate presents a pharmacophore phospholipids cannot copy.

Three characteristic O···O distances — notably ~3 Å between the two phosphates — explain nisin's selectivity on a crowded membrane surface.

Nisin grips lipid II with an open trap, not a closed cage.

Ring A's backbone amides converge on the PPi; ring B is largely a spectator. The complex survives 1 µs at the bilayer surface and reconciles the DMSO and solid-state NMR pictures.

Experiment confirms the minimal recognition element.

A synthetic pyrophosphate with no sugar–peptide head group suffices for nisin pore formation in BLM — while its smaller, shorter-lived pores show the full head group shapes the mature pore.

Next: *full-length nisin + lipid II at the POPG:POPE interface; a concentration series and I–V curves in BLM; synthesis of the Dha5Lys variant.*

Acknowledgements

Laboratory of Biomolecular Modelling, Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry RAS

A.O. Chugunov, R.G. Efremov, N. Krylov, D. Nolde

Frumkin Institute of Physical Chemistry and Electrochemistry RAS

V.P. Shendrikov, I.A. Boldyrev — synthesis of the lipid II mimetic and the BLM experiments

Thank you — questions welcome.