Multiple factors modulating the formation of toroidal membrane pores

Denys Biriukov

Robert Vácha Group, CEITEC MUNI

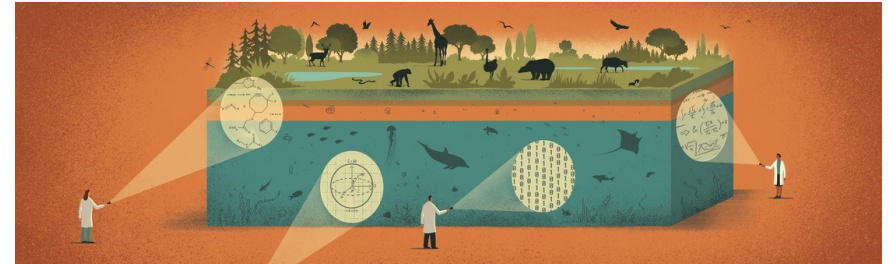NIVB Meeting 2023, 2nd – 5th October 2023, Kutná Hora, Czech Republic

National Institute
of Virology and Bacteriology

# Virology, Bacteriology, and Physics

- Answering biological questions with physics – yes or no?

- Topics of today and later:
  - Bacterial transcription
  - Antimicrobial resistance
  - Cancer immunotherapy
  - Immune response to infections
  - Virus replication
  - …

- No mention of physics, right?

Surface representation of cryo-EM reconstruction of the LRV1 capsid, rainbow colored based on distance from particle center

Procházková, M., ..., **Vácha, R.**, Plevka, P. J. Virol. 95, e01957–20 (2021)

cryo-EM image of E-coli bacteria

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CZECH RECOVERY PLAN

Virology, Bacteriology, and Physics

- Physics is always there
  - Structural organization in biological systems (cellular membranes, cytosol, extracellular matrix, …)
  - Interactions of biomolecules (proteins, drugs, …)

https://elifesciences.org/subjects/physics-living-systems

- Biophysics – the field that had it boom but currently in its middle age crisis
  - Why? Miscommunication of biologists and physicists

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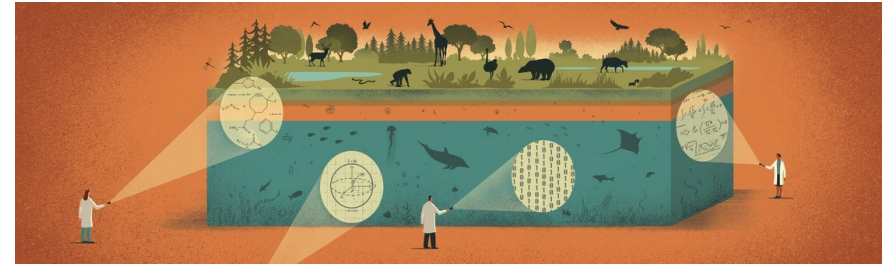
NIVB is an excellent platform to find the common language

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Molecular Simulations: Cartoon or …?

- What are molecular simulations?
  - Visualize experiments?
  - Reproduce experiments?
  - Confirm experiments?

- A molecular simulation is a well-controlled silico experiment
  - "Computational microscope"

- A simulation model = fundamental physics behind it (approximation of fundamental physical laws)
  - Classical Newton's equations of motion
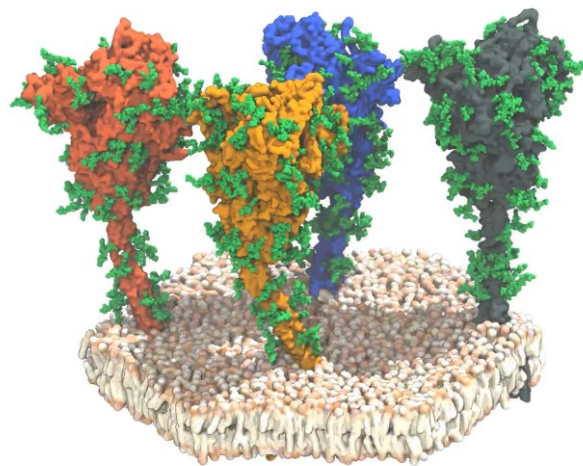
A computational model of spike protein of SARS-CoV-2

Turoňová et al. Science, 370, 203–208 (2020)
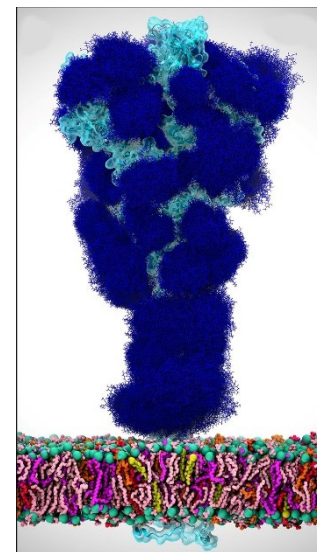
Glycan shield of spike protein of SARS-CoV-2

Casalino et al. ACS Cent. Sci., 6, 1722–1734 (2020)

Resolution and Applications

- Molecular simulation models have different resolution

- Coarse-grained models
  - Larger length-scale, longer time-scale
  - Chemical specificity is mostly lost

- All-atom models
  - Atomistic resolution, atoms are dancing
  - Smaller length-scale, shorter time-scale
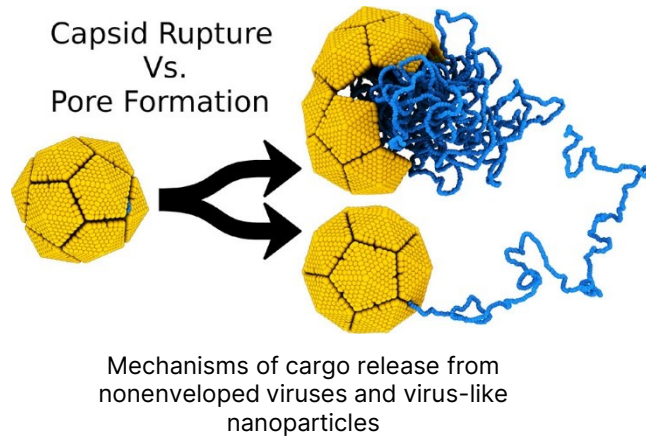
- Do not choose, apply synergy and creativity
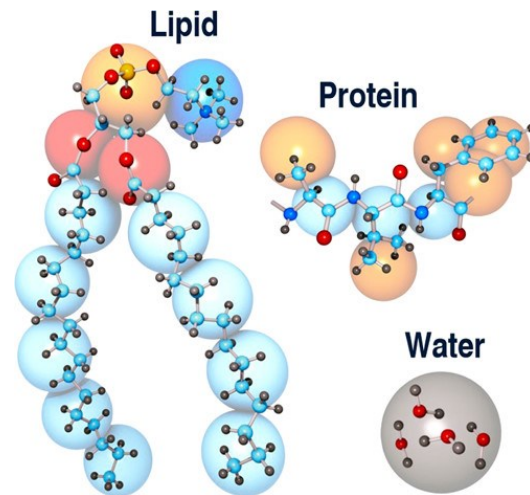
Different representation of atoms/molecules

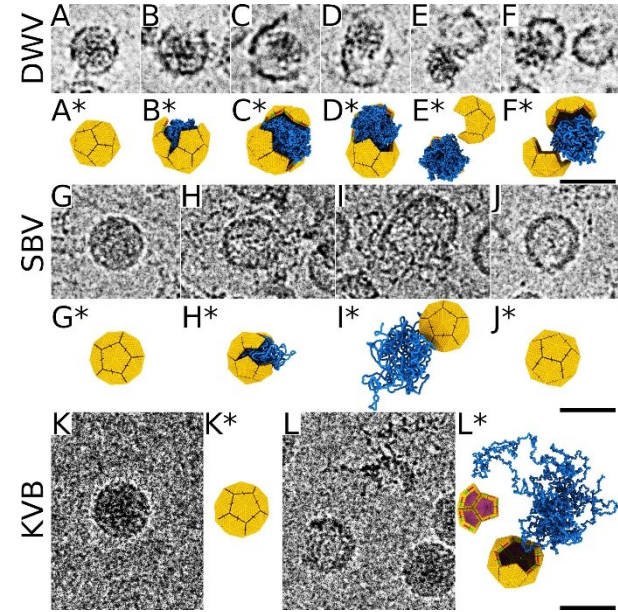Kmiecik et al. Chem. Rev., 116, 7898–7936 (2016)

Coarse-Grained: Virus Genome Release

- Coarse-grained models allow to probe how viruses deliver their content into a cell

Mechanisms of cargo release from nonenveloped viruses and virus-like nanoparticles

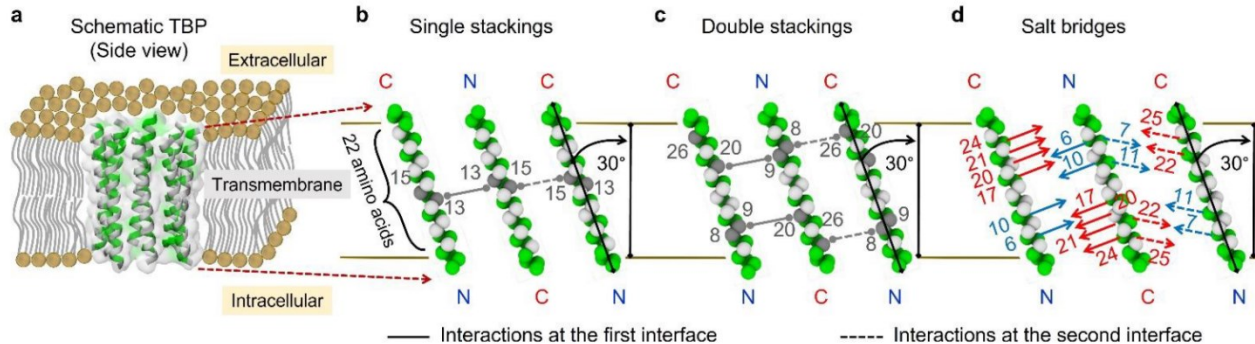Škubník, K., ..., **Vácha, R.**, Plevka, P. Science Advances, 7, eabd7130 (**2021**)

Sukeník, L., ..., **Vácha, R.** ACS Nano, 15, 19233–19243 (**2021**)

Comparison of selected viral capsids from cryo-EM micrographs (A–L) and similar structures obtained in simulations

All-Atom: Designing Peptide-Killers

- All-atom modeling of peptides forming pores
  - Atomistic fingerprints in multiple sequence patterns
  - Atomic force microscopy, fluorescent dye leakage, and cryo-EM experiments confirm the results
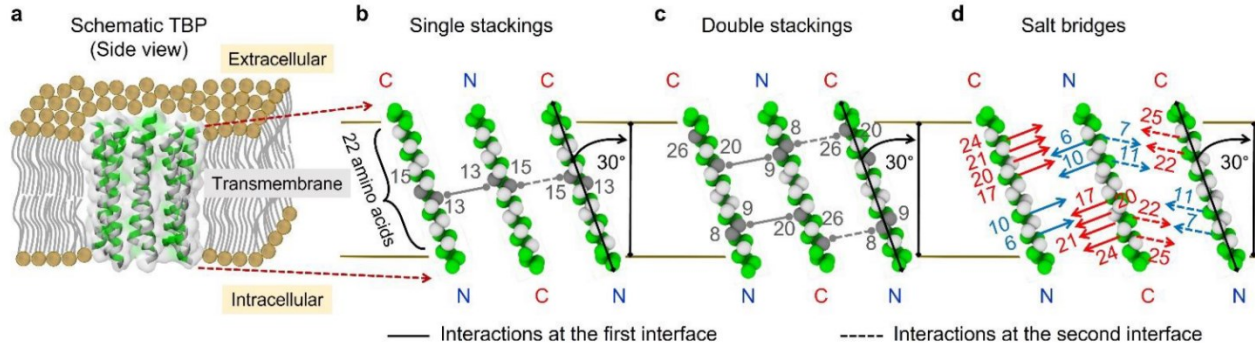

Rational design of peptides that form transmembrane barrel pores

Deb, R., ..., Vácha, R., *biorxiv, in peer-review*

https://doi.org/10.1101/2022.05.09.491086 (**2023**)

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All-Atom: Designing Peptide-Killers

- All-atom modeling of peptides forming pores
  - Atomistic fingerprints in multiple sequence patterns
  - Atomic force microscopy, fluorescent dye leakage, and cryo-EM experiments confirm the results

- **Rational design – what is this?**
  - **Peptide sequence**
  - **Peptide size**
 - **Selectivity/toxicity**
  - **Mechanism of action**
  - … and more

Rational design of peptides that form transmembrane barrel pores

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https://doi.org/10.1101/2022.05.09.491086 (**2023**)

Pores in Cellular Membranes

- **Enveloped viruses and all bacteria have membranes (one or even two)**

- Pores can be categorized into:

  - Barrel pores (peptide–peptide interactions are essential)

  - Toroidal pores (peptide–lipid interactions are essential)

  - Carpet model (foggy concept, the rest what is not barrel/toroidal)

- Different pores – different mechanisms – different peptides – different drugs

  - Chaotic design – flipping a coin

  - Rational – monumental task but worth it; patterns, not single occasions
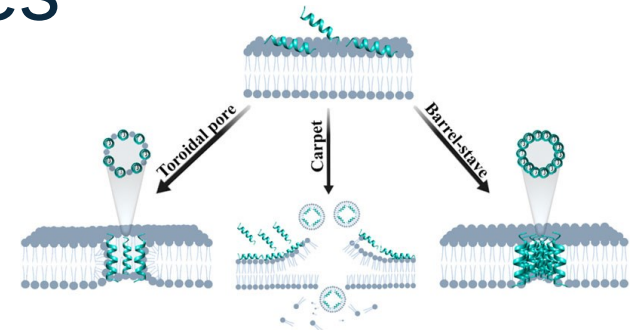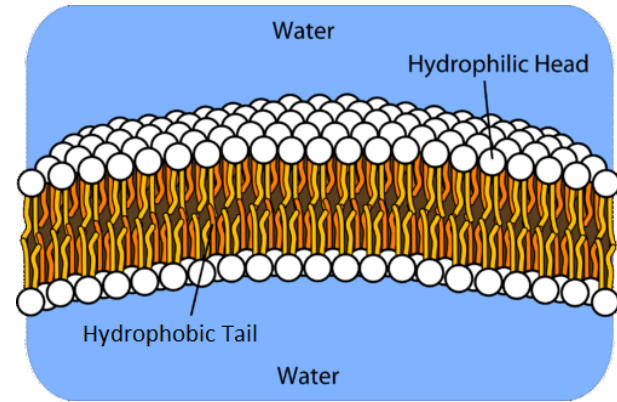

Pores induced by peptides   Amorim-Carmo et al. Front. Mol. Biosci., 9, 887763 (2022)
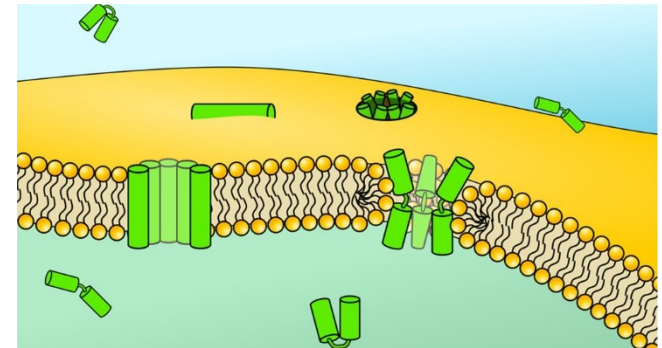
Kabelka, I., Vácha, R., Acc. Chem. Res. 54, 2196–2204 (**2021**)

Energetics of Pore Formation

- We usually do not have holes in our cells
  - High energy penalties
  - Shape of lipids and membranes
 - Interplay of hydrophobic and hydrophilic interactions

Schematical representation of a membrane

https://chem.libretexts.org/

Energetics of Pore Formation

- We usually do not have holes in our cells
  - High energy penalties
  - Shape of lipids and membranes
  - Interplay of hydrophobic and hydrophilic interactions
- Free energy drives reactions and processes
  - Lower – better
- Peptides that counter-act the energy penalties
 - Interactions with exposed to the solution lipids
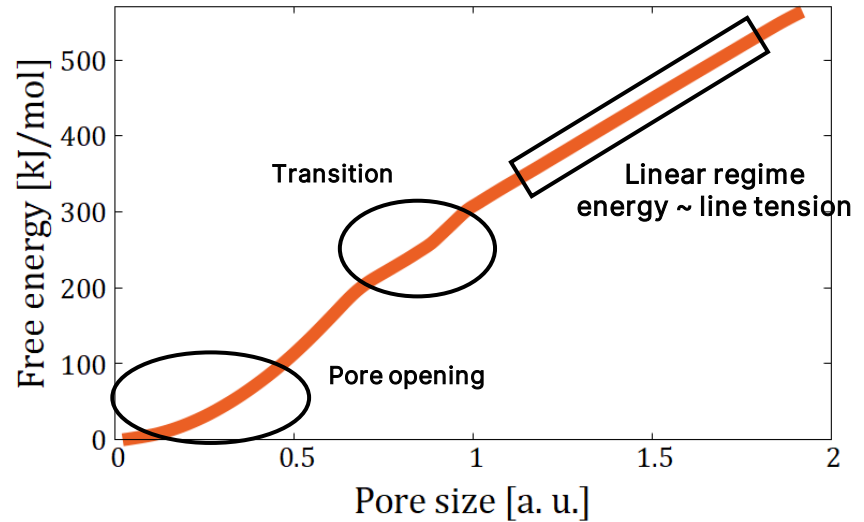
Free energy as a function of pore size in bacterial-mimic membrane from molecular simulations

Rivel, T., …, Vácha, R., *to be submitted* (**2023**)

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Line Tension Defines Pore Stability

- Line tension describes an energy cost to have the membrane edge in the pore to be exposed

- Even we opened a pore, it will close quite rapidly

**Pore closure driven by line (a.k.a. rim, edge) tension of the membrane**
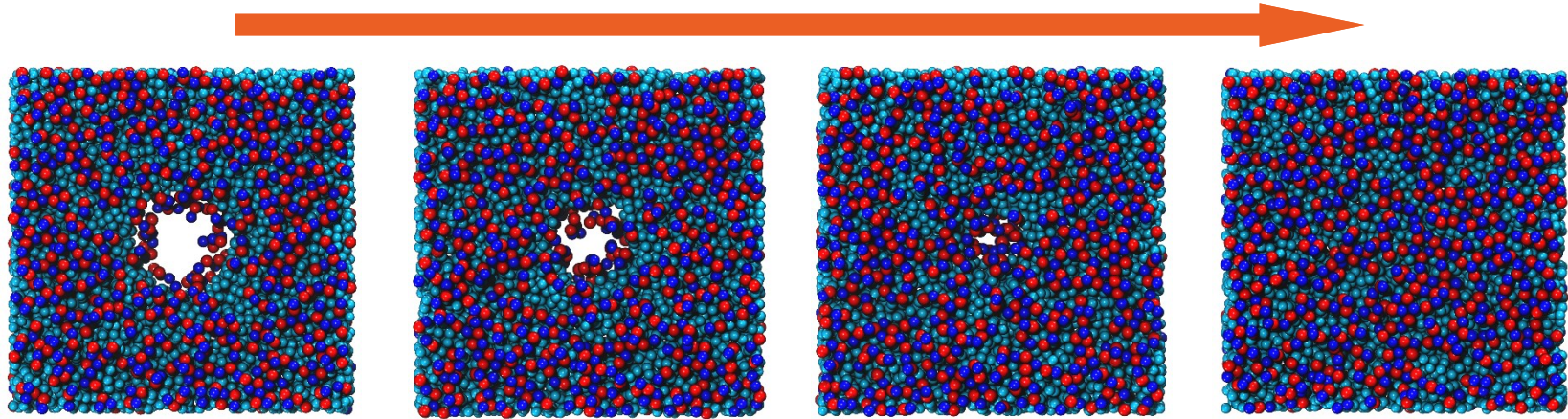
Rivel, T., …, Vácha, R., *to be submitted* (**2023**)

Line Tension Defines Pore Stability

- Line tension describes an energy cost to have the membrane edge in the pore to be exposed

- Even we opened a pore, it will close quite rapidly

- **Typical for all cells**
  - Yet, devil in details

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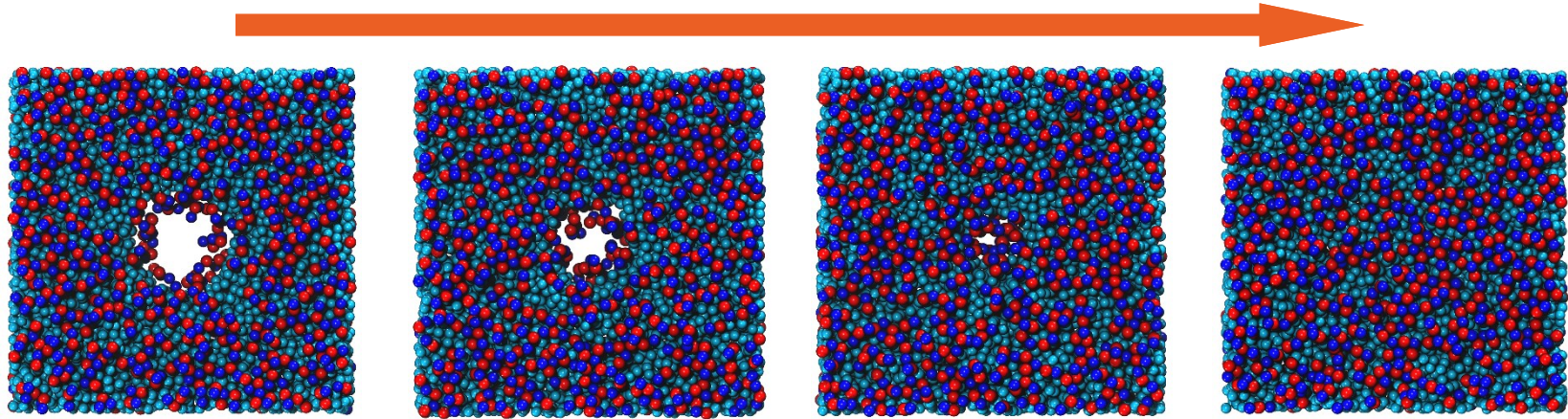
Rivel, T., ..., **Vácha, R.**, *to be submitted* (**2023**)

Line Tension from Molecular Simulations

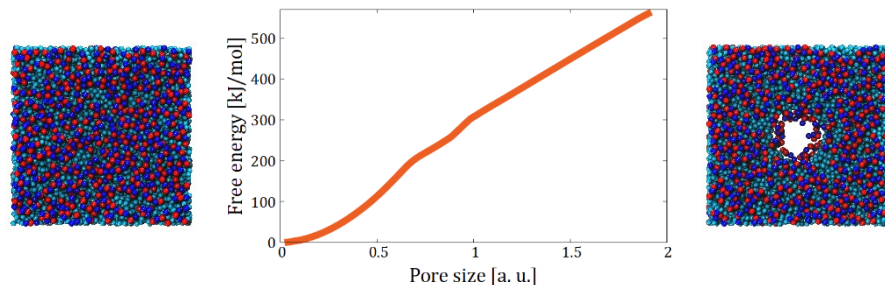- Predictions of line tension from molecular simulations – can we do it?
  - Yes, we established two methods
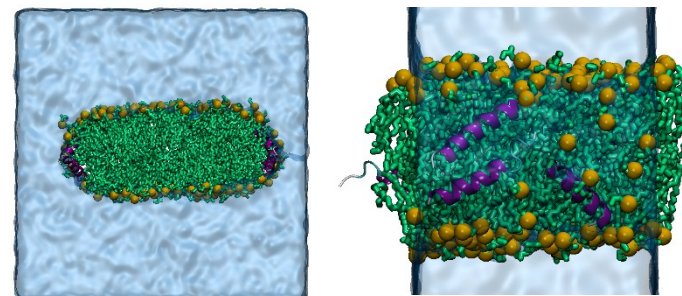
- Free energy predictions
  - Expensive method – full path of pore opening
  - Cheap one – simulations of an infinite pore, line tension screening

- Mutual agreement of methods
 - Synergy, not competition

Rivel, T., ..., Vácha, R., *to be submitted* (2023)

Biriukov, D., ..., Vácha, R., *to be submitted* (2023)

Line Tension & Lipid Composition

- Bacterial cell membranes have a distinct membrane lipid composition
  - More of negatively charged phosphatidylglycerol (PG) lipids
  - Implies lower line tension than in mammalian cells (good for us)

- Molecular simulation of PG-containing lipid membranes
  - Coarse-grained models fail
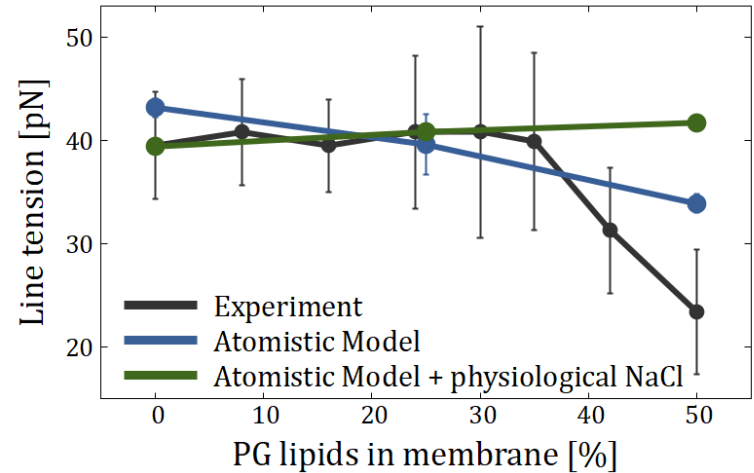 - All-atom models are good
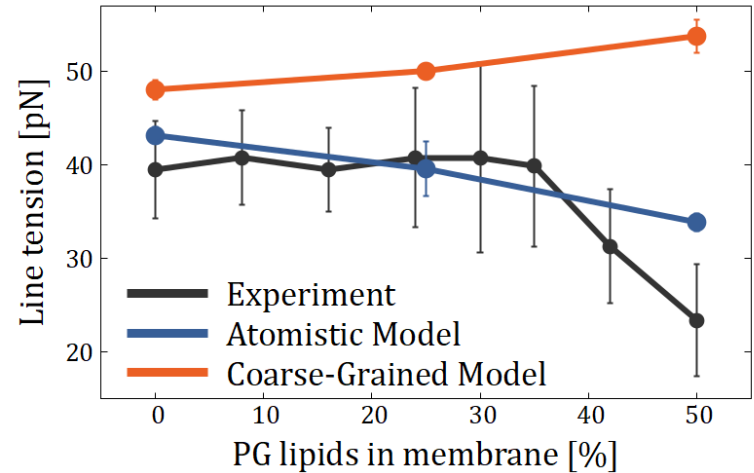
Ability of molecular simulation models to capture the line tension of bacteria-mimic membranes

Biriukov, D., ..., Vácha, R., *to be submitted* (2023)

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Line Tension & External Environment

- Simulations are "the cleanest" experiments
  - We know and control what is inside

- Physiological concentration of NaCl can alter the line tension

- Garbage in / Garbage out
  - Applicable to both simulations and experiments

- All experiments have various buffers, additives, etc.
  - Often necessary for technical reasons
 - Does it matter though? Philosophical question

Ability of molecular simulation models to capture the line tension of bacteria-mimic membranes

Biriukov, D., ..., Vácha, R., *to be submitted* (2023)

Rational, not a Single Shot

- Amphiphilic helical peptides seem not to have an effect (preliminary data)
  - Not suitable sequence or properties?
  - Other amino acids must be involved?

- A random peptide that worked
  - KKFKKLAKKFKKALKKFKAK
    - Little amount decreases line tension by 10% (not much, but good for starters)

- Why? Good question
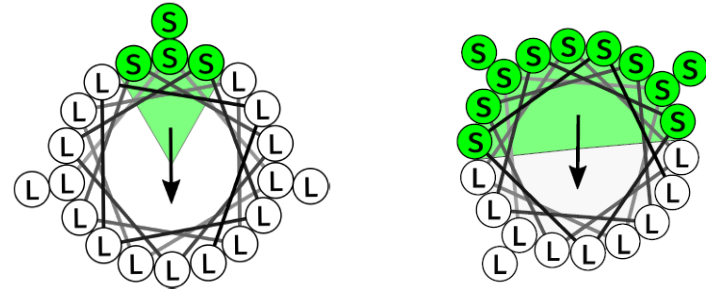 - That is why the design should be rational
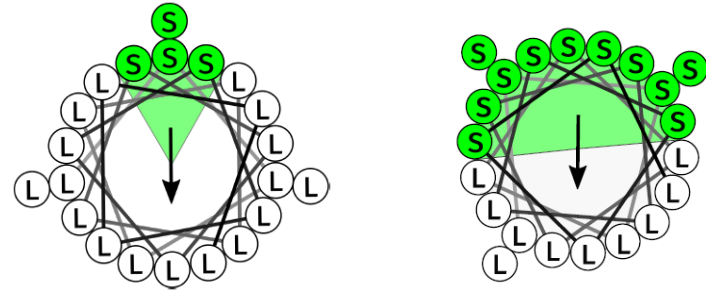
Two-component amphiphilic helical peptides – a more hydrophobic (left) and a more hydrophilic (right) – have little effect on the line tension

Acknowledgments to **Peter Pajtinka**

Rational, not a Single Shot

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Two-component amphiphilic helical peptides – a more hydrophobic (left) and a more hydrophilic (right) – have little effect on the line tension

Acknowledgments to **Peter Pajtinka**

- **What is the chief ingredient?**
  - K (positively charged amino acids)?
  - F (aromatic amino acids)?
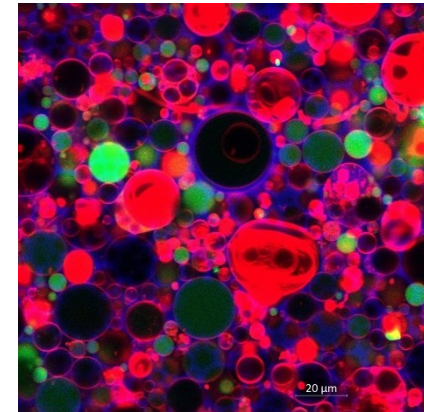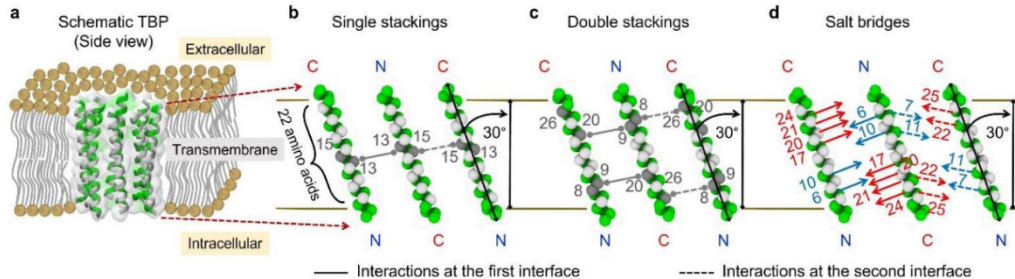 - Their position along the sequence?
  - …

Synergy of Simulations and Experiments

- Are you wondering – does this strategy work?

  - YES!

- Simulations stimulate experiments

- **Several peptides were designed based on patterns from simulations**

- **They were found to translocate through lipid vesicles**

Rationally designed peptides (green) translocated through lipid vesicles (red) as seen in fluorescent microscopy

a — Schematic TBP (Side view), Extracellular, Transmembrane, Intracellular, 22 amino acids

b — Single stackings

c — Double stackings

d — Salt bridges

—— Interactions at the first interface   ----- Interactions at the second interface

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Future Directions

- Rational design of peptides that decrease line tension and increase the stability of toroidal pores

- Protocol and models are tested and established
  - Peptides are entering the workflow

- Do you have a drug? We may already have a taxi driver it
  - Contact us!

?

- Rational design
  - Peptide sequence
  - Peptide size
  - Targeting bacteria
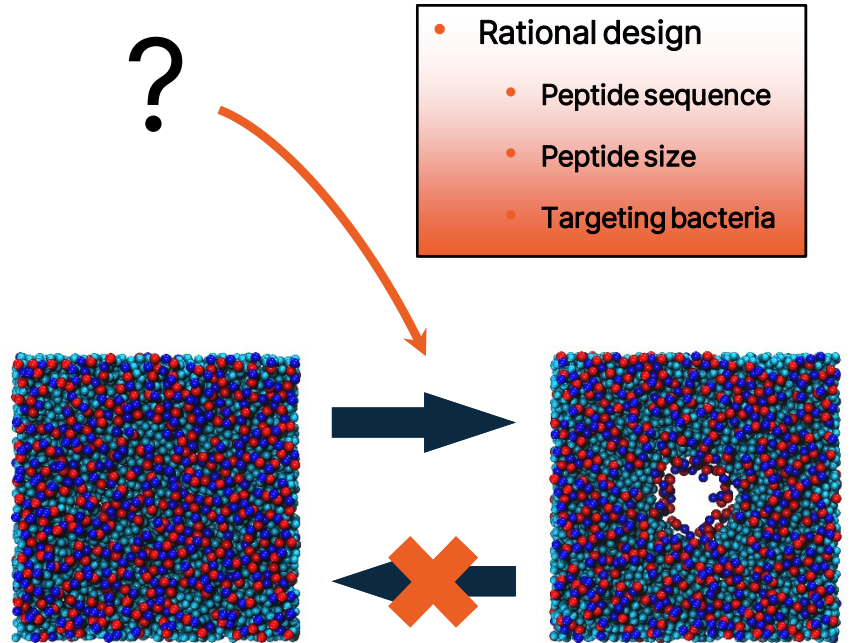
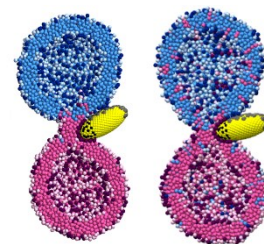**Biriukov, D., ..., Vácha, R.,** *work in progress*

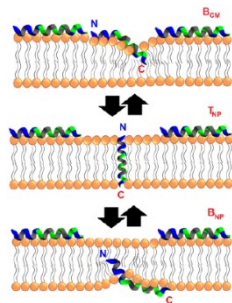... and many other team members

Visit posters from our group!

yesterday :(

P-21: Designing nanoparticles for membrane fusion
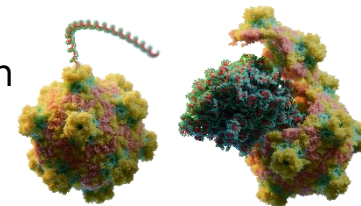

today!

P-22: Membrane Adsorption Enhances Translocation of Antimicrobial Peptide Buforin 2

yesterday :(

P-23: Impact of Interaction Distribution between Capsid Building Blocks on Genome Release Pathways in Picornaviruses

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Thank you for your attention!

National Institute
of Virology and Bacteriology

Do not look for me, I'm not here, I'm a fresh gun

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