THZ SPECTROSCOPY AND MOLECULAR MODELING TO INVESTIGATE MEMBRANE PROTEINS

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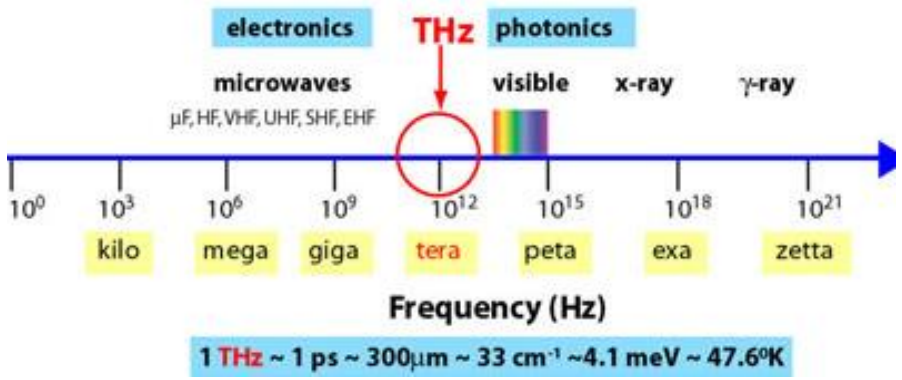
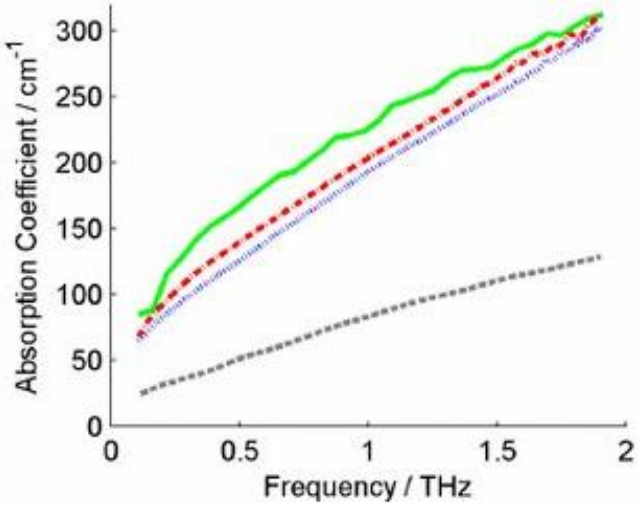
THZ SPECTROSCOPY



https://www.jlab.org/news/releases/experiment-generates-thz-radiation-20000-times-brighter-anyone-else



Fitzgerald AJ, Pickwell-MacPherson E, Wallace VP (2014) PLOS ONE 9(7): e99291

THz spectra of complex systems lack distinct spectral features

Difficult interpretation

Interpretation – molecular modeling

Directly addresses the low frequency and high amplitude modes of biomolecules → High sensitivity to protein structure and flexibility

Molecular modeling workflow

Protein of interest

- Simulate the spectrum - NMA
  - Protein powder
- Build a simulation system with water, ions and membrane → Simulate the spectrum - MD
  - Protein solution
  - Membrane proteins in cells

Comparison of simulated spectra with the experiment

↓

The agreement – **validation** of simulation work

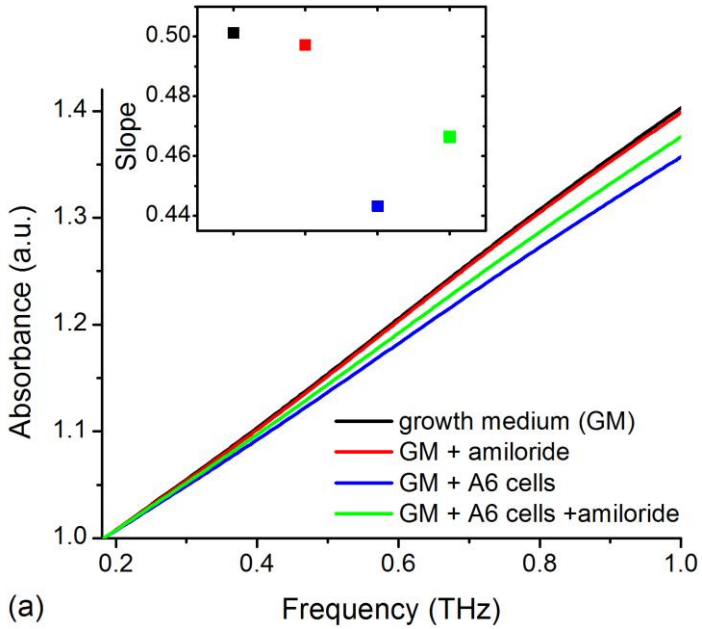
ENAC INTERACTION WITH AMILORIDE

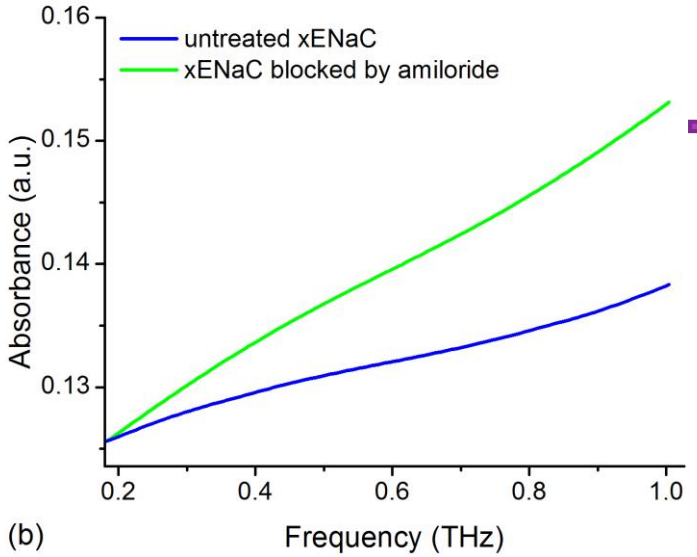
-**ENaC** - highly selective Na^+ channel expressed in water reabsorbing epithelia

-specifically blocked by amiloride

Aim: investigate ENaC blockade by amiloride using THz spectroscopy

Experiments: measurements on *Xenopus laevis* cells expressing ENaC inhibited with amiloride versus cells expressing native ENaC





The THz absorption of cells treated with amiloride is lower than the absorption of cells with amiloride

ENaC is the sole target of amiloride in A6 cells

the increased absorption reflects a flexibility change in ENaC due to amiloride binding

Mernea M, Ulăreanu RȘ, Cucu D, Al-Saedi JH, Pop CE, Fendrihan S, Anghelescu GDC, Mihăilescu DF. Epithelial Sodium Channel Inhibition by Amiloride Addressed with THz Spectroscopy and Molecular Modeling. Molecules. 2022 19;27(10):3271.

ENAC MODELLING AND SPECTRA SIMULATION

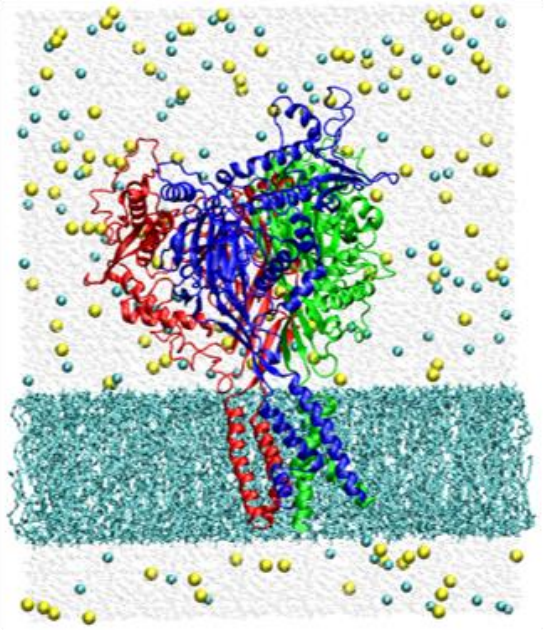
-*Xenopus laevis* ENaC was modeled using as templates:

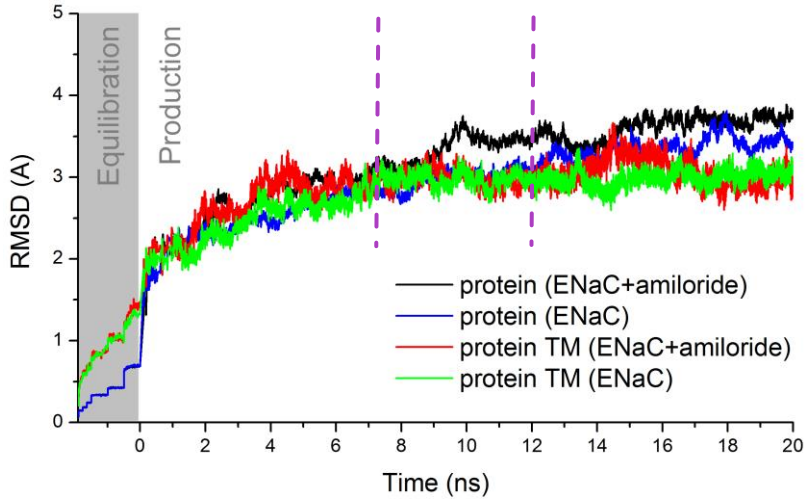
-full-length extracellular domain (ECD) of human ENaC (6WTH)

-the transmembrane (TM) region of cASIC1 in complex with amiloride (4NTX)

-amiloride was docked in the TM region of ENaC

-we simulated 20 ns of production dynamics for ENaC and ENaC + amiloride systems

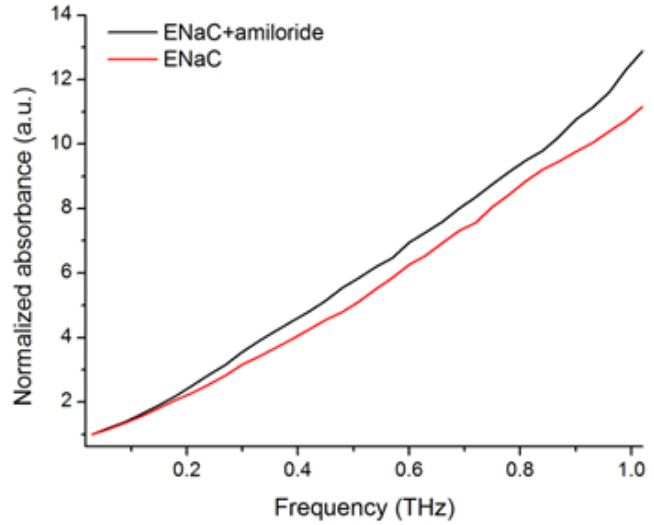




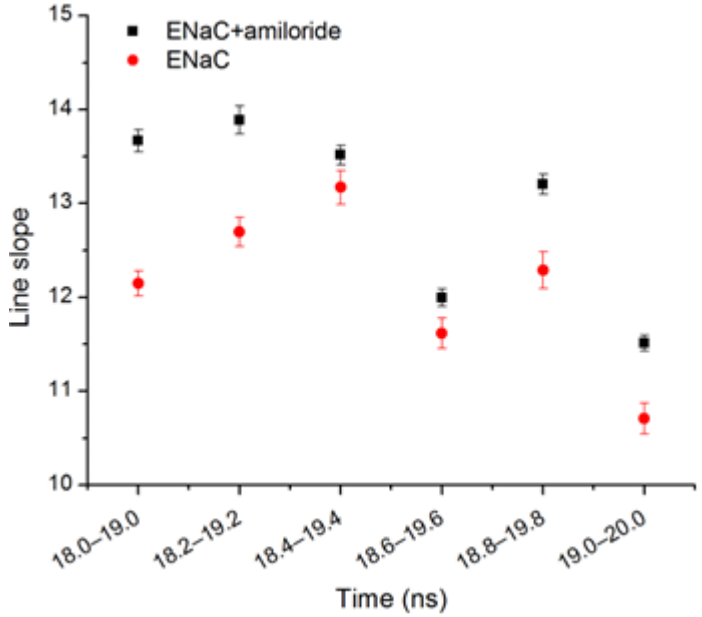
-the convergence addressed in the 12-20 ns time range, considering running windows of 1 ns, from 0.2 to 0.2 ns

-comparison with FastDTW and CDTW methods returned confidence interval of 95%, indicating that the spectra are convergent

Simulated spectra – the last 1 ns of MD



Spectra convergence



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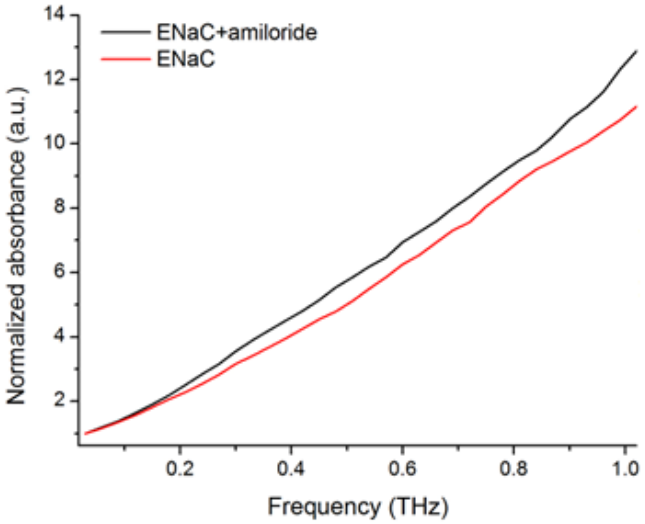
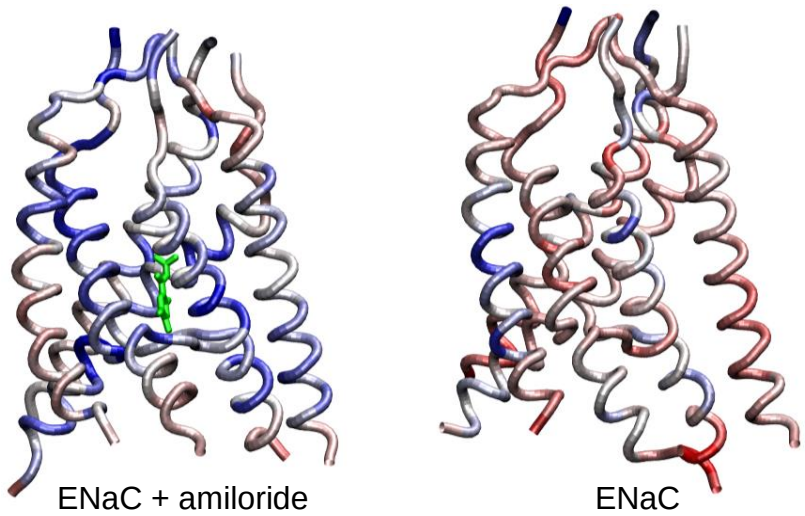
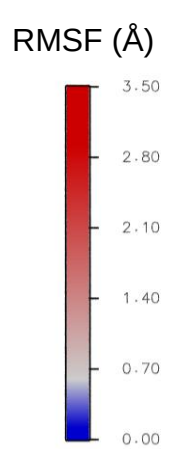
EXPLANATION AND AMILORIDE DYNAMICS

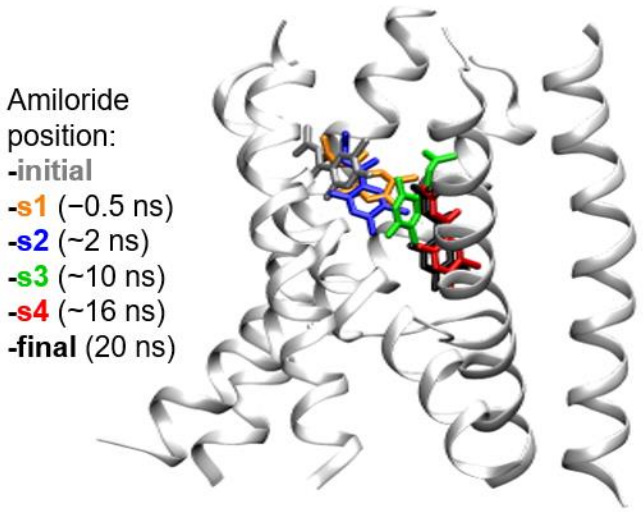


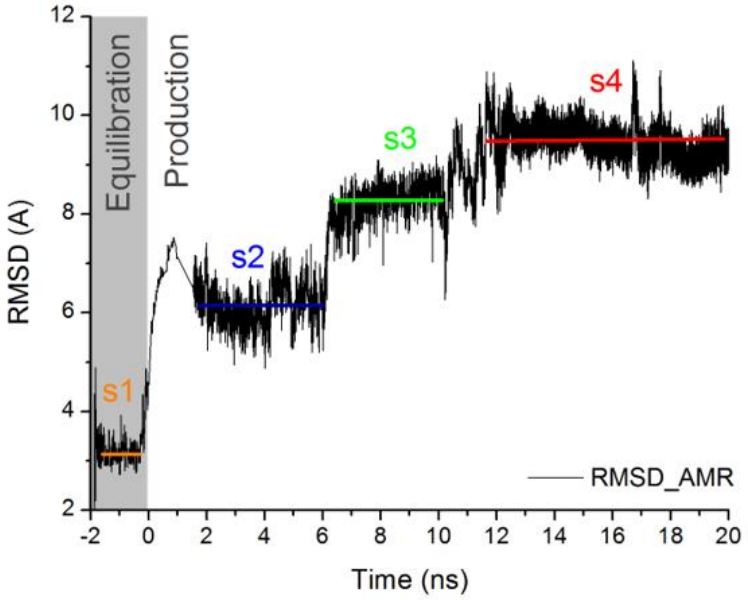




Amiloride binding renders the channel **more rigid** in both TM and ECD, but the interaction with the ligand contributes to an increased THz absorption of the protein–ligand complex.

Amiloride dynamics





-amiloride initial binding – agreement with the template structure;

-binding at the end of the simulation - agreement with the results of previous mutagenesis studies

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CONCLUSIONS

-We successfully used THz spectroscopy and molecular modeling to:

-discriminate between cells comprising glycosylated and unglycosylated TRPM8 channels and to propose a model of TRPM8 N-glycans

-discriminate between native and blocked ENaC channel and to validate a model of ENaC-amiloride complex that is also in agreement with previous mutagenesis studies

ACKNOWLEDGEMENT

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Octavian Calborean
Dan Mihailescu

Dana Cucu

Roxana Ulăreanu (Dragan)

Dan Mihailescu

REFERENCES:

1. Mernea M, Ulăreanu R, Călboreanu O, Chiriţoiu G, Cucu D, Mihăilescu DF. N-glycosylation state of TRPM8 protein revealed by terahertz spectroscopy and molecular modelling. Biochim Biophys Acta Gen Subj. 2020;1864(7):129580.

2. Mernea M, Ulăreanu RȘ, Cucu D, Al-Saedi JH, Pop CE, Fendrihan S, Anghelescu GDC, Mihăilescu DF. Epithelial Sodium Channel Inhibition by Amiloride Addressed with THz Spectroscopy and Molecular Modeling. Molecules. 2022 19;27(10):3271.