

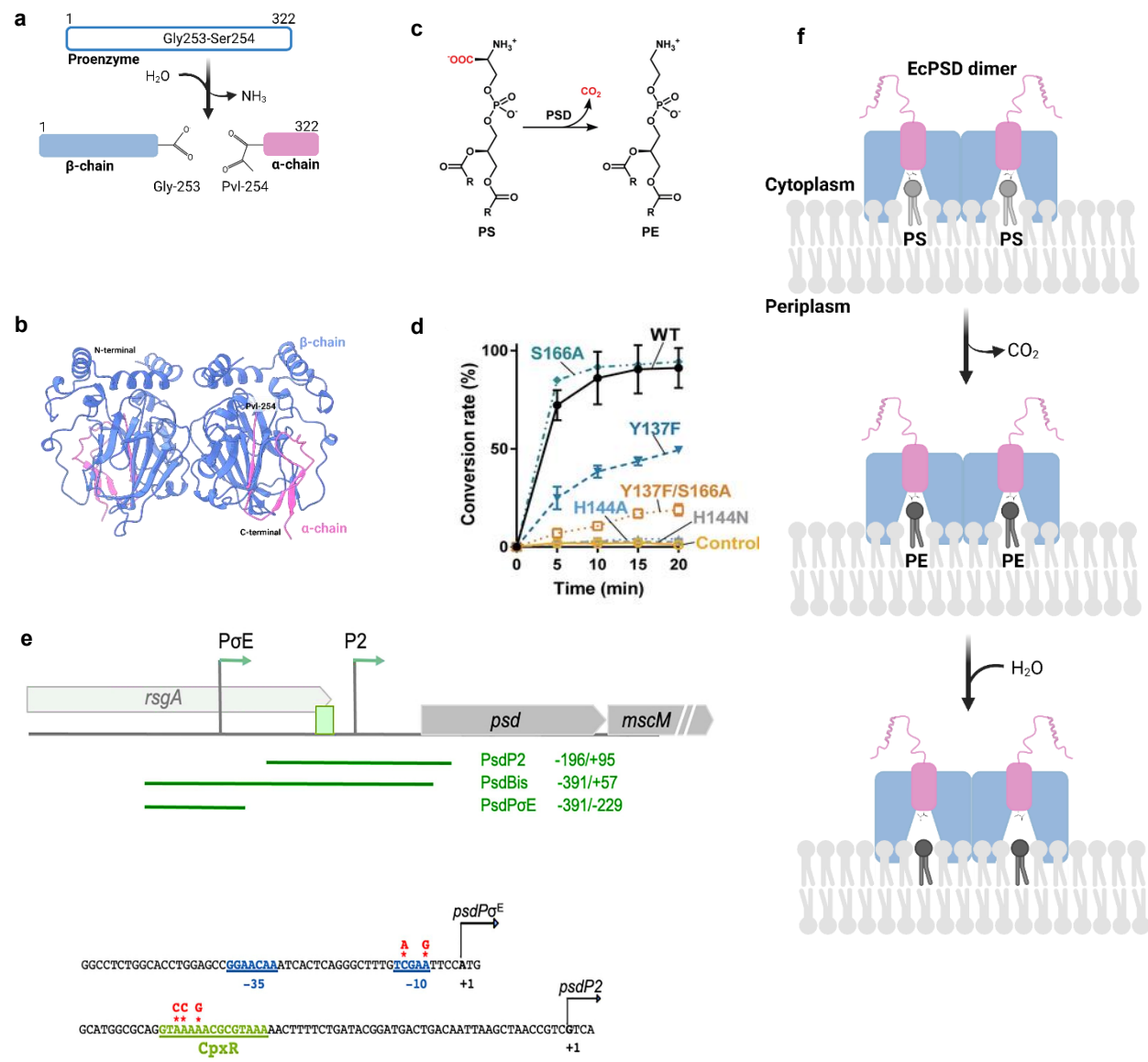
Investigating the catalytic mechanism of a membrane-associated enzyme by EPR spectroscopy and MD simulations

Gaurav Nanakwani, Joshua L. Wort, Qaiser Waheed, Christos Pilotas

Division of Molecular and cellular function, FBMH, University of Manchester

Introduction and Objectives

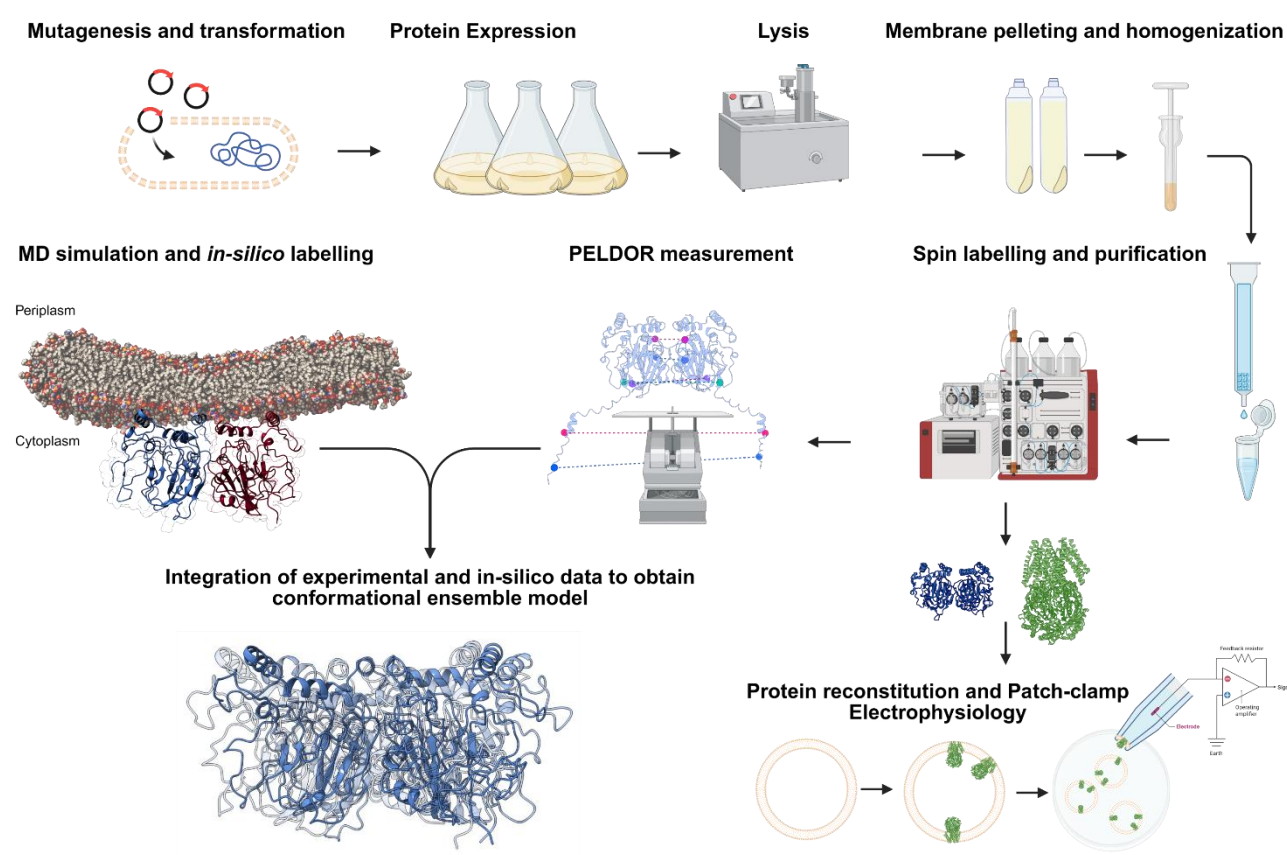
- Phosphatidylserine decarboxylase (PSD) is a membrane-associated enzyme^{1,2}
- PSD catalyses the biosynthesis of phosphatidylethanolamine (PE) from phosphatidylserine (PS)^{1,2}
- Exists as a homodimer, with each protomer comprising one α -chain and one β -chain^{1,2}
- Shares an operon with mechanosensitive ion channel MscM³



a. Autoproteolysis, b. A structure of active PSD $\alpha_2\beta_2$ heterotetramer, c. Scheme of PS decarboxylation, d. Promoter region of *psd-mscM* operon, e. Enzyme kinetics, f. A model of PSD-membrane association and kinetics.

- PELDOR/DEER spectroscopy⁴ and atomistic MD simulations were used to characterise the conformational ensemble of the lipid-bound enzyme and identify EPR-consistent minimal conformational states under different conditions
- Single-channel electrophysiology was employed to assess how PSD modulates mechanosensitive ion channel activity, linking lipid biosynthesis enzymes to ion channel regulation

Methodology

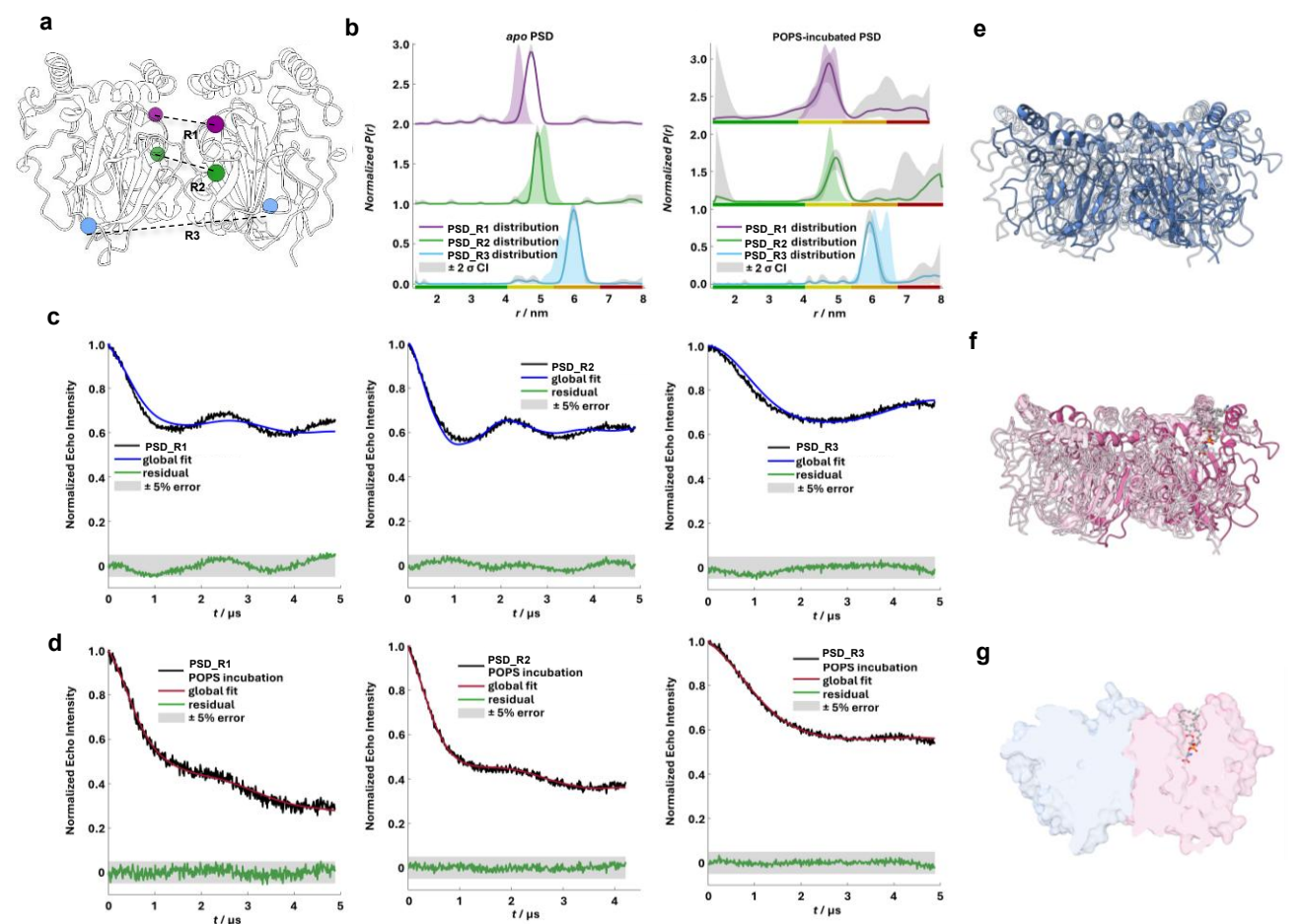


Conclusions and Future-Outlooks

- Pulse dipolar spectroscopy distinguishes apo- and substrate-bound states of PSD**
By combining PELDOR spectroscopy, MD simulations, and structural modeling, we propose conformational ensembles for both apo and substrate-bound PSD states. These models provide insights into the dynamic structural transitions that are not captured in available crystal structures.
- The C-terminal region of the PSD α -chain is highly dynamic and may play a functional role**
The C-terminal loop, unresolved in previous X-ray structures, was modeled using AlphaFold3 under different conditions. PELDOR and MD analyses indicate substantial flexibility in this region, with preliminary PELDOR data supporting a highly dynamic conformational behavior.
- PSD function is linked to mechanosensitive (MS) channel activity**
Given that PSD shares an operon with an *E. coli* MS channel, we investigated a potential functional relationship between the two proteins. Electrophysiological measurements suggest that PSD modulates MS channel activity, and ongoing experiments aim to further validate this connection.

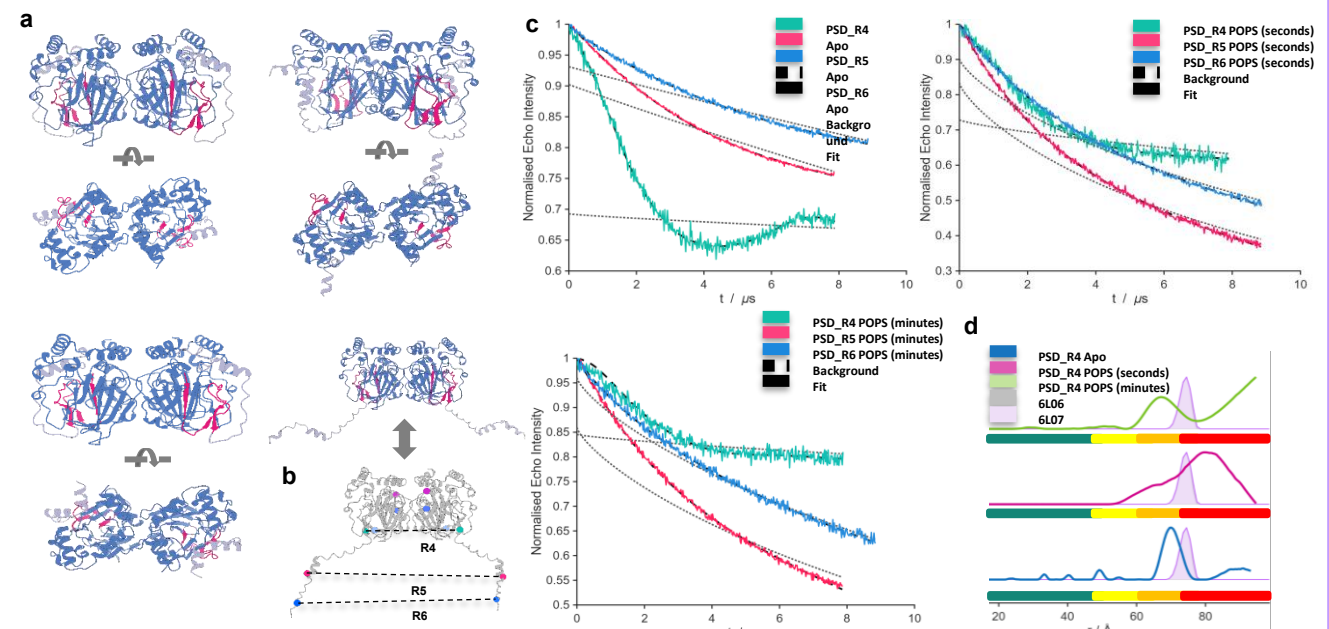
Results

Pulse dipolar EPR spectroscopy distinguishes apo- and substrate-bound PSD



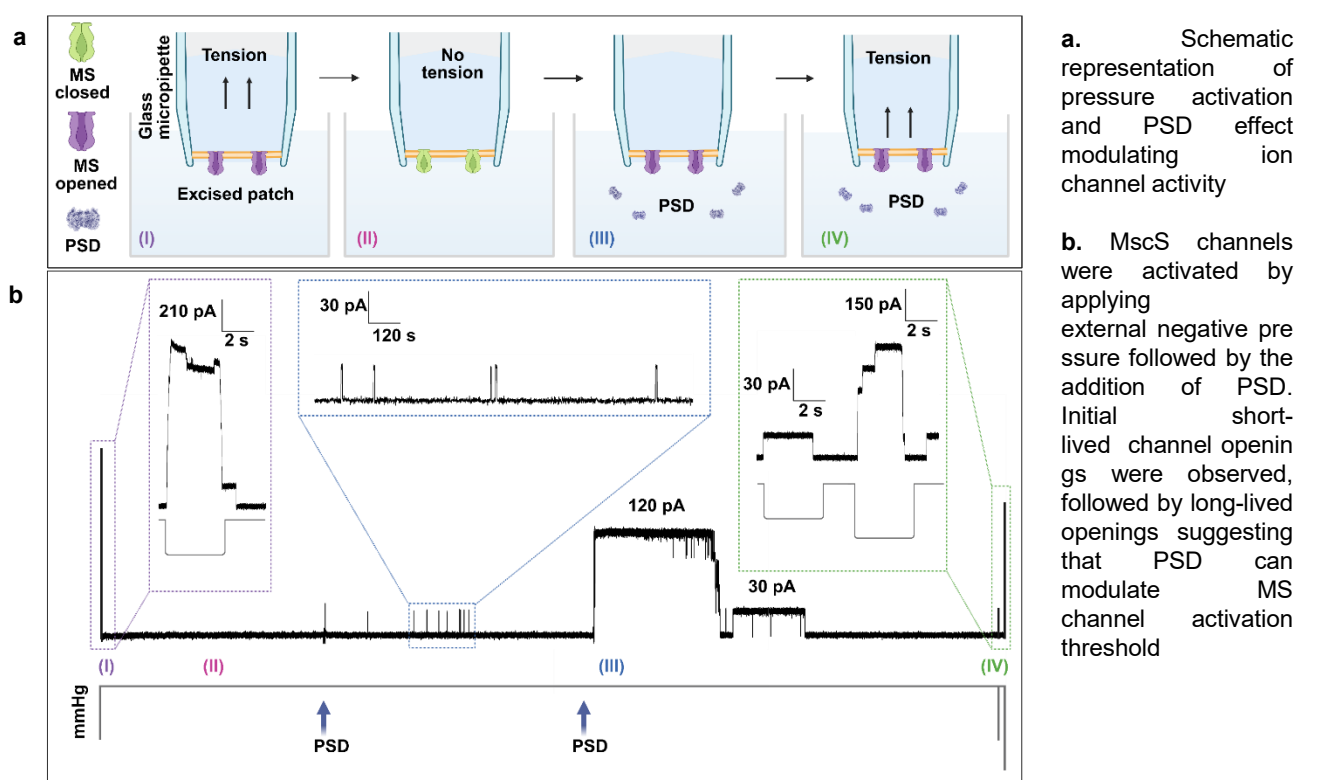
a, Cartoon representation of the PSD dimer, with spheres representing α -carbons of residues spin labelled. b, PELDOR distance distributions of the apo-, and substrate-bound states. c, Global linear fitting of experimental PELDOR traces corresponding to apo with simulated traces using ChiLife. d, Experimental PELDOR traces corresponding to substrate-bound condition, with simulated traces using ChiLife. e, Cartoon representation of the complete apo PSD conformational ensemble informed by PELDOR. f, Cartoon representation of the substrate-bound PSD conformational ensemble informed by PELDOR. g, Surface representation of the PSD dimer, in blue and magenta, with a substrate molecule in ball-and-stick representation and inset indicating substrate binding pocket.

C-terminus loop dynamics



a, Cartoon representation of alphaFold3 generated initial models of full-length PSD dimer with unresolved disordered c-terminus loop (grey). b, PSD dimer, with spheres representing α -carbons of residues spin labelled. c, PELDOR time traces of the apo-, and substrate-bound states. d, PELDOR distance distributions of the apo-, and substrate-bound states.

PSD modulates the activity of mechanosensitive ion channels



a. Schematic representation of pressure activation and PSD effect modulating ion channel activity

b. MscS channels were activated by applying external negative pressure followed by the addition of PSD. Initial short-lived channel openings were observed, followed by long-lived openings suggesting that PSD can modulate MS channel activation threshold

References and Acknowledgements

- Cho, G., Lee, E. & Kim, J. Structural insights into phosphatidylethanolamine formation in bacterial membrane biogenesis. *Sci Rep* 11, 5785 (2021).
- Kefauver, J. M., Ward, A. B. & Patapoutian, A. Discoveries in structure and physiology of mechanically activated ion channels. *Nature* 587, 567–576 (2020).
- Berrier, C., Besnard, M., Ajouz, B., Coulombe, A. & Ghazi, A. Multiple Mechanosensitive Ion Channels from Escherichia coli, Activated at Different Thresholds of Applied Pressure. *Journal of Membrane Biology* 151, 175–187 (1996).
- Pilotas, C. et al. Conformational state of the MscS mechanosensitive channel in solution revealed by pulsed electron-electron double resonance (PELDOR) spectroscopy. *Proceedings of the National Academy of Sciences* 109, (2012).