Evaluating Antimicrobial Resistance *Acinetobacter baumannii* Porins for Antibiotic Permeability

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Abstract

Background: The sluggish discovery of new antibiotics contributes to the growing crisis of antimicrobial resistance (AMR) in Gram-negative bacteria such as Acinetobacter baumannii. The decreased uptake of antibiotics across the outer membrane, which is mostly mediated by porin channels, is a crucial resistance mechanism. Designing effective therapeutic solutions requires a mechanistic understanding of how various porins either facilitate or limit antibiotic passage.

Methods: To describe the functional characteristics of important porins (OccAB1, OmpA, OmpW, and HMP-BA) isolated from A. baumannii, we used single-channel electrophysiology. The ion and antibiotic conductance characteristics of individual porins were directly evaluated after they were reconstituted into a synthetic lipid bilayer. This method makes it possible to identify the channel properties that affect antibiotic influx and to precisely quantify permeability rates [1-3].

Results: For every kind of porin, our research showed unique single-channel conductance and gating characteristics. Importantly, we found notable variations in the speeds at which different antibiotic compounds pass through these channels. These results offer a quantitative map of how particular porins in *A. baumannii* function as molecular sieves, specifically regulating the intracellular concentration of antimicrobial drugs.

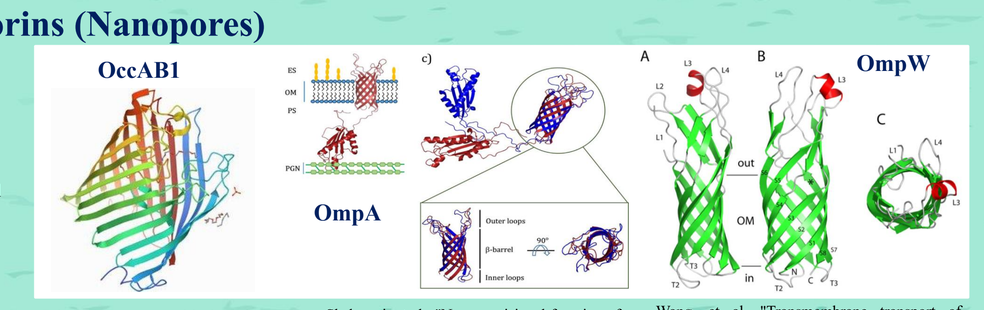
Conclusion: This study establishes a direct connection between the intrinsic antibiotic resistance profile of *A. baumannii* and the function of particular porins. Our approach offers a fundamental paradigm for directing the identification and logical design of novel chemotherapeutic drugs capable of circumventing porin-mediated resistance in this crucial priority pathogen by determining how porins are more permissive or restrictive to important antibiotics.

Materials and Methods

Overview of Target Porins (Nanopores)

Figure 1.

- **OccAB1**
  - General diffusion porin
  - Broad substrate permeability
  - Important for antibiotic uptake
- **OmpA**
  - Dual role: structural stability + small molecule transport
  - Associated with virulence in *A. Baumannii*
- **OmpW**
  - Narrow, selective channel
  - Typically allows uptake of small hydrophobic molecules
  - Limited antibiotic permeability



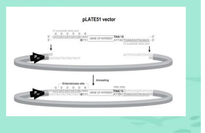
Zahn, et al. "Structural insights into outer membrane permeability of Acinetobacter baumannii." Structure 24, no. 2 (2016): 221-231. https://doi.org/10.1016/j.str.2015.12.009

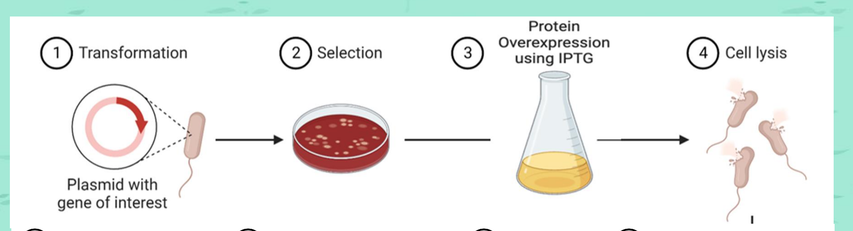
Shahryari, et al.. "New provisional function of OmpA from Acinetobacter sp. strain SA01 based on environmental challenges." Msystems 6, no. 1 (2021): 10-1128. https://doi.org/10.1128/msystems.01175-20

Wang, et al. "Transmembrane transport of polycyclic aromatic hydrocarbons by bacteria and functional regulation of membrane proteins." Frontiers of Environmental Science & Engineering 14, no. 1 (2020): 9. https://doi.org/10.1183/16000617.0068-2022

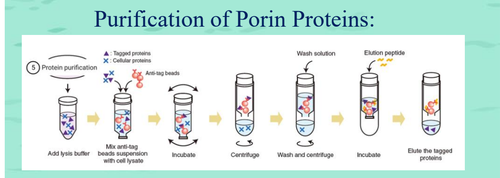
Protein Expression

Figure 2. Protein expression process





Purification of Porin Proteins:



Experimental Setup

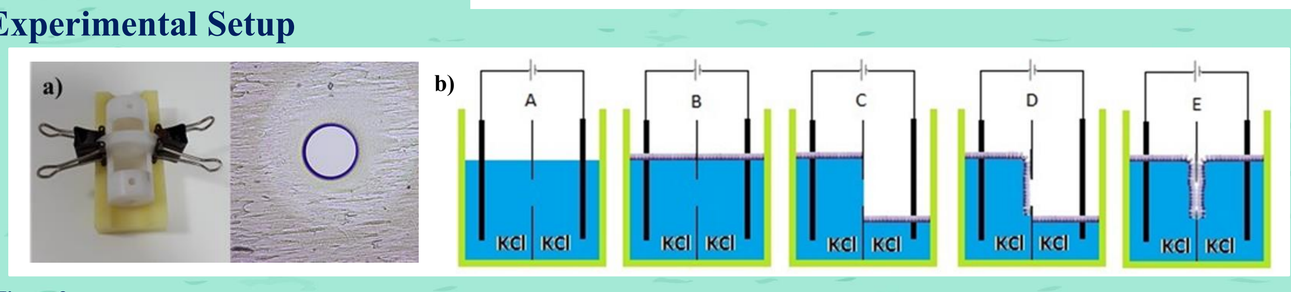


Figure 3.

- **Black Lipid Membrane (BLM) System**
  - Two compartments separated by an artificial lipid bilayer
  - Porin protein reconstituted into bilayer
- **Electrodes & Amplifier**
  - Ag/AgCl electrodes in cis and trans chambers
  - Connected to Axon 200B amplifier for current detection
- **Solution Conditions**
  - 0.5 M KCl, 10 mM HEPES buffer, pH 8.0
  - Provides ionic environment for conductance measurement
- **Data Acquisition**
  - Signals digitized by Axon Digidata 1550B
  - Analyzed using ClampFit software
- **Lipid Bilayer Formation**
  - Lipids dissolved in organic solvent (e.g., decane, hexane).
  - Bilayer formed over a small aperture between cis & trans chambers.
  - Verified by capacitance and baseline current stability.
- **Porin Insertion**
  - Purified porin protein added to cis chamber.
  - Protein spontaneously integrates into lipid bilayer.
  - Channel insertion detected by sudden stepwise current change.
- **Confirmation**
  - Single-channel openings/closings observed.
  - Stable baseline conductance indicates successful reconstitution.

Electrical Recording & Equipment

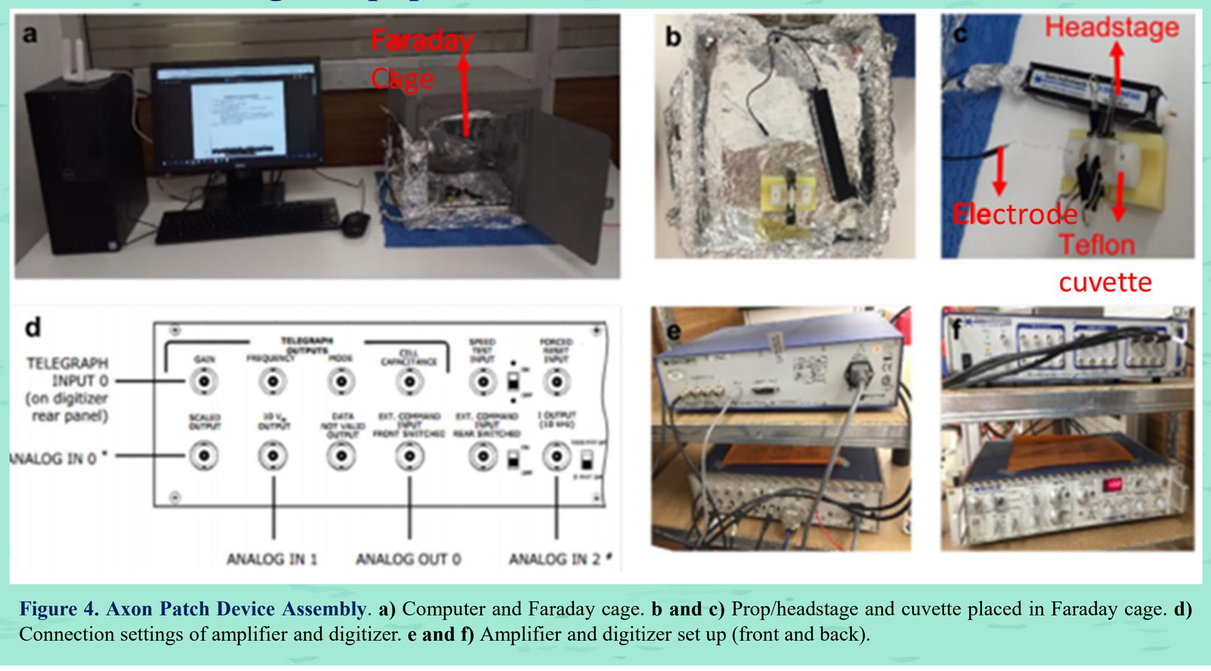


Figure 4. Axon Patch Device Assembly. **a)** Computer and Faraday cage. **b and c)** Prop/headstage and cuvette placed in Faraday cage. **d)** Connection settings of amplifier and digitizer. **e and f)** Amplifier and digitizer set up (front and back).

Results

OccAB1 Single-channel Study

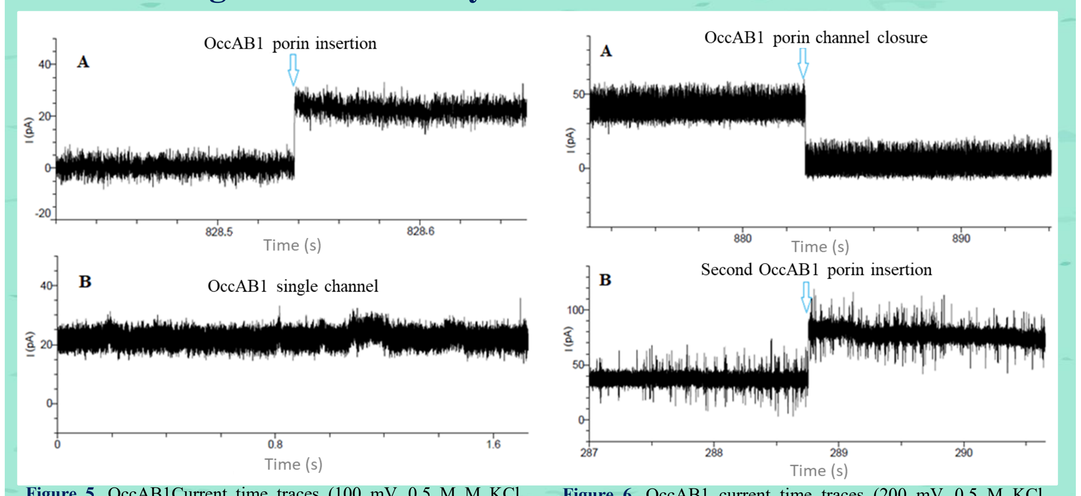


Figure 5. OccAB1Current time traces (100 mV, 0.5 M M KCl, HEPES buffer 10 mM, pH=7.5, sampling frequency 100 KHz, filtering at 5 KHz). A) Porin insertion and single channel formation, B) Single channel current time trace.

Figure 6. OccAB1 current time traces (200 mV, 0.5 M KCl, HEPES buffer 10 mM, pH=7.5, sampling frequency 100 KHz, filtering at 5 KHz). A) OccAB1 channel closure, B) insertion of the second OccAB1porin.

OccAB1 Glysin, Glutamic acid, and Ampicillin Permeability

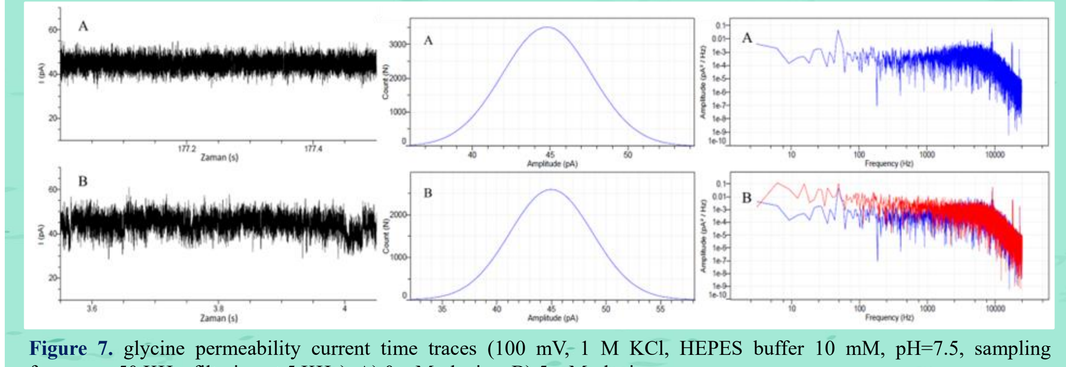


Figure 7. glycine permeability current time traces (100 mV, 1 M KCl, HEPES buffer 10 mM, pH=7.5, sampling frequency 50 KHz, filtering at 5 KHz): A) 0 mM glycine, B) 5 mM glycine.

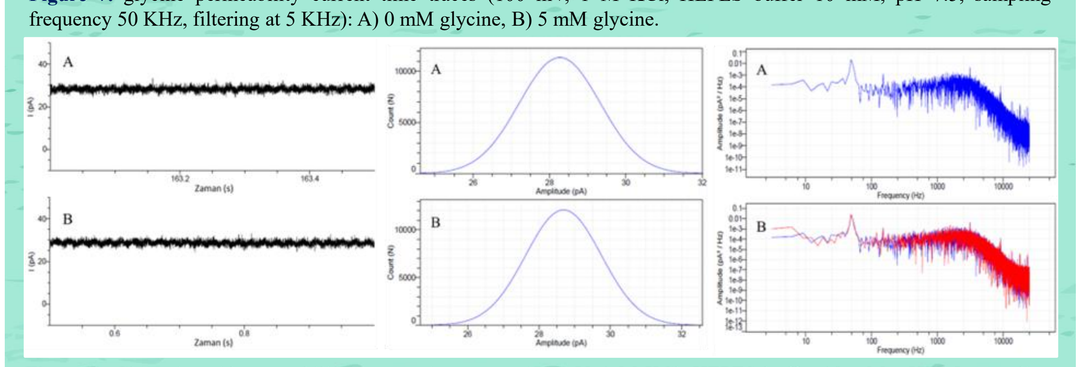


Figure 8. Glutamic acid permeability current time traces (50 mV, 1 M KCl, HEPES buffer 10 mM, pH=7.5, sampling frequency 50 KHz, filtering at 2 KHz): A) 0 mM glutamic acid, B) 5 mM glutamic acid.

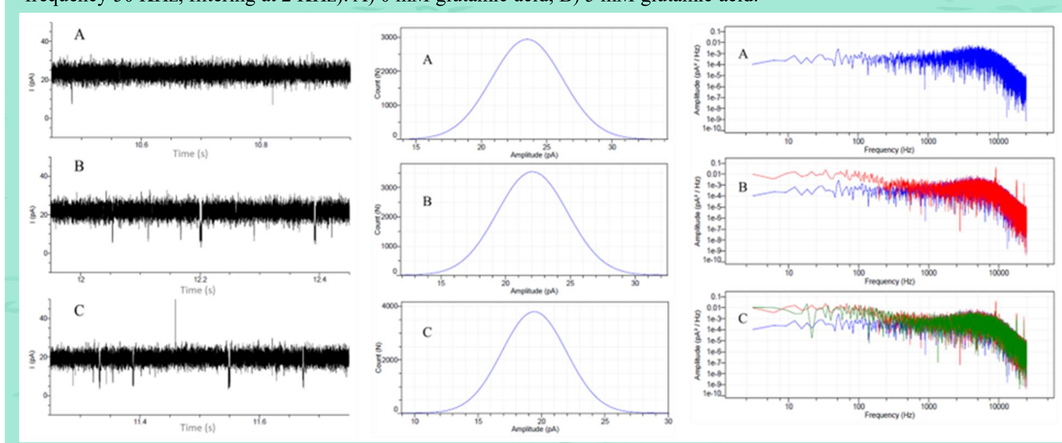


Figure 9. Ampicillin permeability current time traces and power spectromes for OccAB1 Standard porin (100 mV, 0.5 M KCl, HEPES buffer 10 mM, pH=7.5, sampling frequency 100 KHz, filtering at 5 KHz): A) 0 mM ampicillin, B) 20 mM ampicillin, C) 40 mM ampicillin.

Conclusion

This study establishes a direct connection between the intrinsic antibiotic resistance profile of *A. baumannii* and the function of particular porins. Our approach offers a fundamental paradigm for directing the identification and logical design of novel chemotherapeutic drugs capable of circumventing porin-mediated resistance in this crucial priority pathogen by determining how porins are more permissive or restrictive to important antibiotics.

Acknowledgement

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References:

1. Ozturk, Y., et al., Rapid fabrication of teflon apertures by controlled high voltage pulses for formation of free standing planar lipid bilayer membrane. Biomedical Microdevices, 2021. 23: p. 1-9.
2. Ebrahimi, A., et al., Revealing the single-channel characteristics of OprD (OccAB1) porins from hospital strains of Acinetobacter baumannii. European Biophysics Journal, 2023. 52(3): p. 131-143.
3. İzgördü, Ö.K., C. Darcan, and F.D. Guzel, Cloning, expression, and purification of acinetobacter baumannii OccAB1 porin protein in Escherichia coli. Osmaniye Korkut Ata Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 2023. 6(2): p. 1529-1542.

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