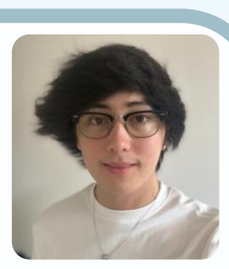
smFRET and PELDOR reveal an alternate conformation of the BAM Complex in lipid membranes



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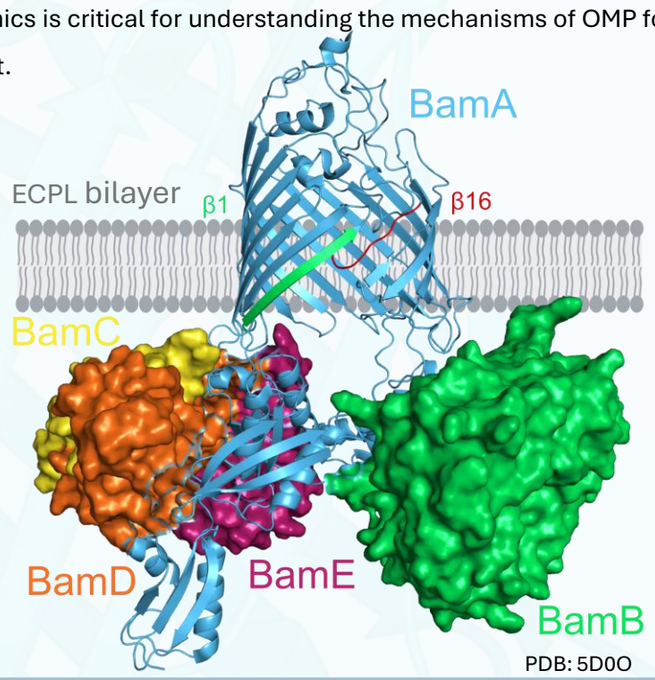
1 Background: The BAM complex

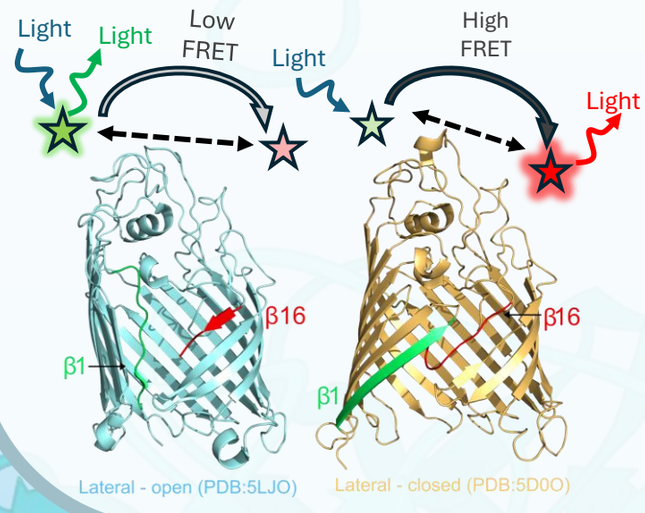
- Four diderm species accounted for 51.3% (2.16–2.66 million) of all antimicrobial resistance (AMR)–associated deaths in 2019(1).
- The β-barrel assembly machinery (**BAM**) complex is essential for outer-membrane protein (**OMP**) biogenesis and is conserved across diderm bacteria.
- Opening and closing of the **lateral gate**, formed between β1 and β16 of BamA, is a key mechanistic step in BAM function.
- While cryo-EM has provided important structural insights into the OMP insertion mechanism, the **timescale** of this process and the **rate of conformation dynamics** remain poorly understood. Resolving these dynamics is critical for understanding the mechanisms of OMP folding and for new antibiotic development.



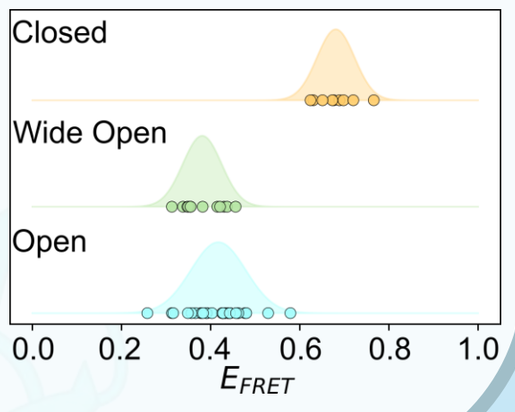
2 Method: Single-molecule FRET & EPR

- Single-molecule Förster resonance energy transfer (**smFRET**) enables real-time interrogation of conformational dynamics, with energy transfer efficiency scaling as $1/R^6$ with inter-dye distance (R).
- Using **PIE**, FRET efficiency (E_{FRET}) and stoichiometry (**S**) can be measured enabling us to track the populations of multiple conformations, and the **rates of interchange** between them on a **microsecond** to **millisecond** timescale.
- Using **PELDOR**, conformational states can be confidently assigned linking dynamics to conformation.
- Cysteine sites were determined by **accessible volume** calculations on the canonical lateral-open (PDB: 5LJO) and lateral-closed (PDB: 5D0O) structures of BamA, and were introduced into wild-type (**WT**) and a closed-biased mutant (**LGc**) to monitor lateral gate dynamics.
- Predicted FRET efficiency **(pE_{FRET}) ranges** for each **conformational state** were then determined using the **rotamer library** implemented in **FRETpredict** across all published cryo-EM structures of BAM[2].

smFRET



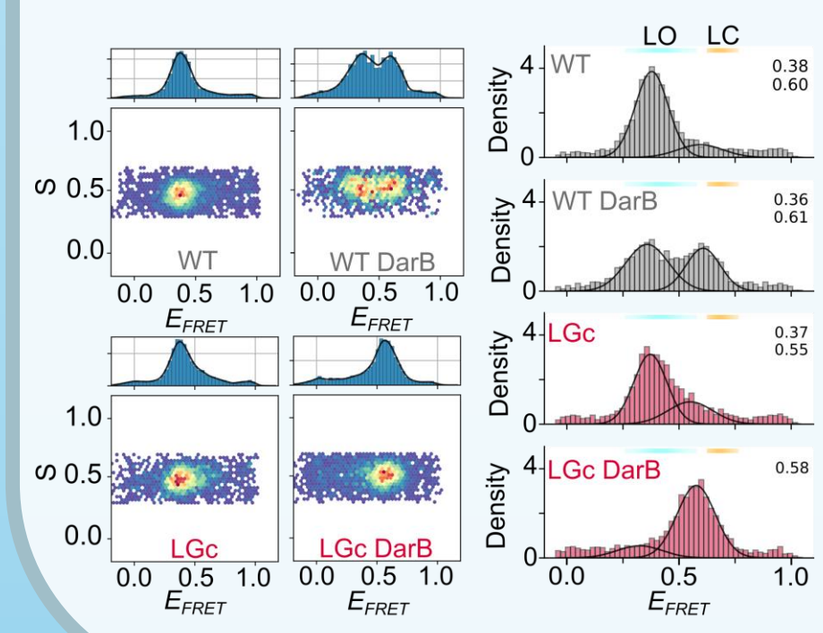
FRETpredict



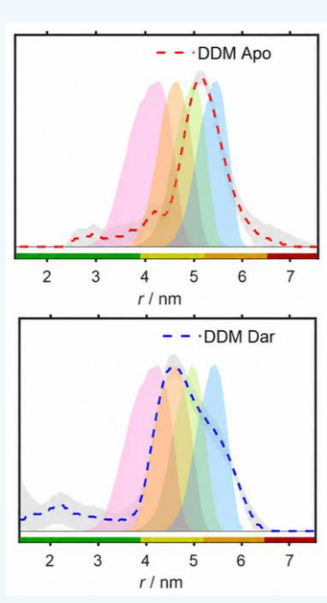
3 Results: Characterising E_{FRET} states in DDM

- The **predicted E_{FRET}** from FRETpredict were ***E_{FRET}* ≈ 0.36-48** (open) and ***E_{FRET}* ≈ 0.64-0.72** (closed).
- To validate these predictions, we measured smFRET of BAM in the absence (WT) or presence (WT + DarB) of Darobactin-B (DarB), a peptide that induces the lateral-closed state(3).
- The open population (**O**) at ***E_{FRET}* ≈ 0.38** that closely matched predictions, and addition of DarB induced a partially-occupied closed state (**C**) at ***E_{FRET}* ≈ 0.60** slightly lower than predicted.
- As the populations were approximately evenly split, we next examined a closed-biased mutant (LGc), which partially occupied the closed state in the apo condition and fully closed upon DarB addition.
- **PELDOR** analysis of the wild-type reflect our smFRET observations, shifting from an open-population evenly distributed between lateral-open states (5LJO and 9CNW), to the canonical lateral-closed state (5D0O) upon addition of DarB.

smFRET

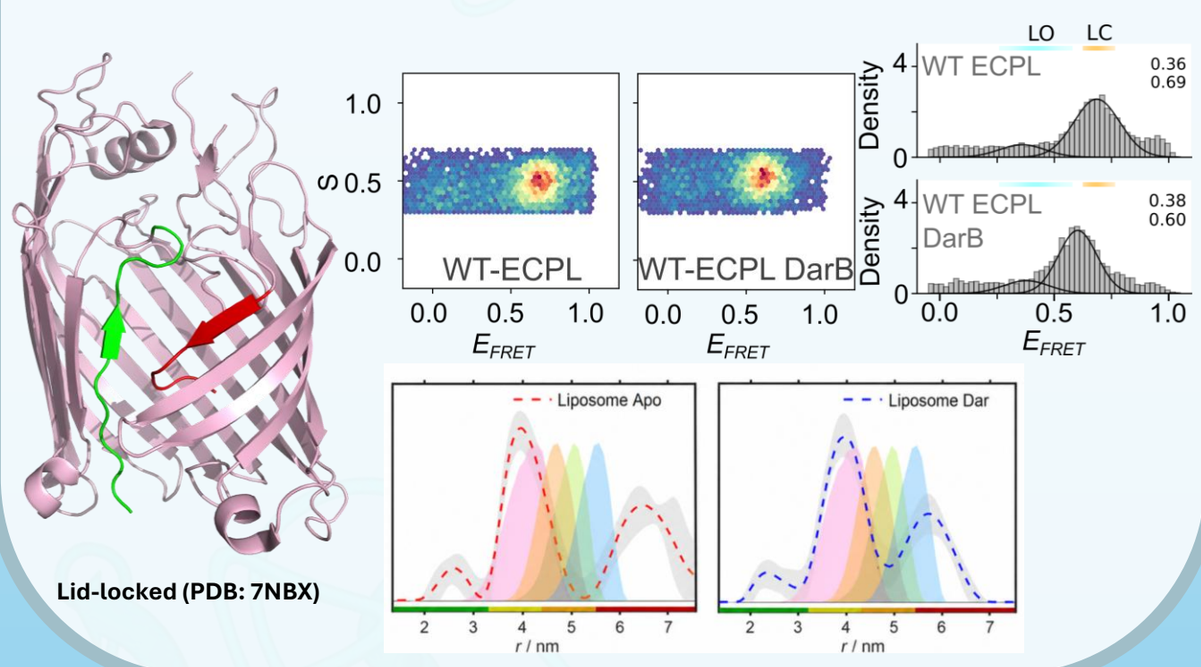


PELDOR



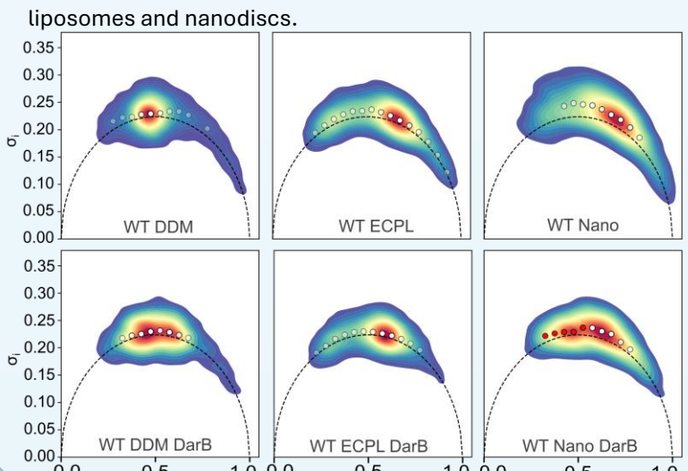
4 Results: BAM adopts a new conformation in ECPL liposomes

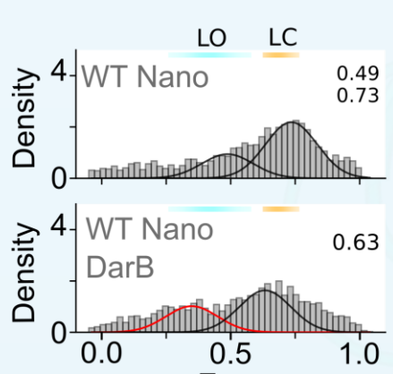
- To examine BAM under more physiological conditions, we reconstituted the smFRET system into ***E. coli* polar lipid extract liposomes** (**WT ECPL**).
- A peak of ***E_{FRET}* ≈ 0.68** was observed, closely matching predictions for the lateral-closed conformation.
- Interestingly, addition of DarB shifted ***E_{FRET}* to ≈ 0.60**, consistent with the peak observed in DDM micelles and likely corresponding to the **canonical lateral-closed state (5D0O)**.
- Reanalysis of the predicted E_{FRET} distributions revealed a **lid-locked construct** (PDB:7NBX) that best described the observed apo population, adopting a **lateral-open state with inward β1-2 bending.**
- **PELDOR** analysis of the wild-type in ECPL liposomes reflected our observation, with the fit distance distributions matching the predicted distributions of the lid-locked state rather than the lateral-closed state.
- Addition of Dar-B induced a 3-4 Å shift of the main population towards the lateral-closed distribution, suggesting that BAM adopts an alternative lateral-open conformation with significant **inward β1-2 bending** in ECPL bilayers.



5 Results: BAM dynamics change across membrane environments

- smFRET reveals that BAM adopts a similar altered conformation in nanodiscs to liposomes, and addition of DarB shifts the ensemble towards the lateral-closed state, matching the other environments.
- **Burst variance analysis (BVA**) shows that BAM rapidly samples its conformational landscape across all membrane environments, with conformational dynamics increasing progressively from DDM micelles to liposomes and nanodiscs.





6 Conclusions

- **Membrane environment is a major determinant** of BAM conformational equilibria, shifting the population away from the detergent-state ensemble.
- BAM exists as a **dynamic ensemble** rather than discrete static conformations, with rapid interconversion occurring on timescales accessible to smFRET.
- Developed a **smFRET platform** capable of probing **BAM dynamics** across **membrane environments** and probing the impact of **BAM mutations** on dynamics.
- By combing **PELDOR** and **smFRET** a newly identified **altered lateral-open** state expands the current model of the BAM conformational cycle and may represent a previously uncharacterised intermediate relevant to OMP biogenesis.
- Provides a **platform for studying the OMP insertion cycle** and for **screening mutants** to uncover mechanisms underlying altered function, both in OMP insertion and drug resistance.

British Biophysical Society

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[1] Naghavi, M. *et al.* Global burden of bacterial antimicrobial resistance 1990-2021: a systematic analysis with forecasts to 2050. *Lancet* **404**, 1199–1226 (2024).

[2] Birtles, D. *et al.* Integration of Membrane Proteins into the Outer Membrane of Diderm Bacteria by the BAM Complex. Chem. Rev. 126, 4036–4061 (2026).

[3] Imai, Y. *et al.* A new antibiotic selectively kills Gram-negative pathogens. *Nature* **576**, 459 (2019).