Intramolecular Donor Strand Complementation in the *E. coli* Type 1 Pilus Subunit FimA Explains the Existence of FimA Monomers As Off-Pathway Products of Pilus Assembly That Inhibit Host Cell Apoptosis

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Why I think that you should know about this work…

- FimA protein has been exclusively associated with pilus biogenesis.
  - This paper introduces FimA monomers and involvement in host cell apoptosis inhibition.
- This complex structure has been solved by NMR as opposed to X-ray crystallography and electron microscopy (which are techniques used by my lab).
- This paper also provides a detailed structure of full-length FimA.
  - Which was not done in the truncated x-ray crystallography structure due to the spontaneous homo-polymerization of FimA.

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Why am I interested in this work…

- I have been working on calculating the rate of FimA polymerization by the FimD usher.
  - This paper provides a detailed mechanism of the relationship between FimA and DsbA (oxidizing protein).
  - The relationship of DsbA and FimA can play a fundamental role determining the polymerization of FimA in pilus biogenesis.

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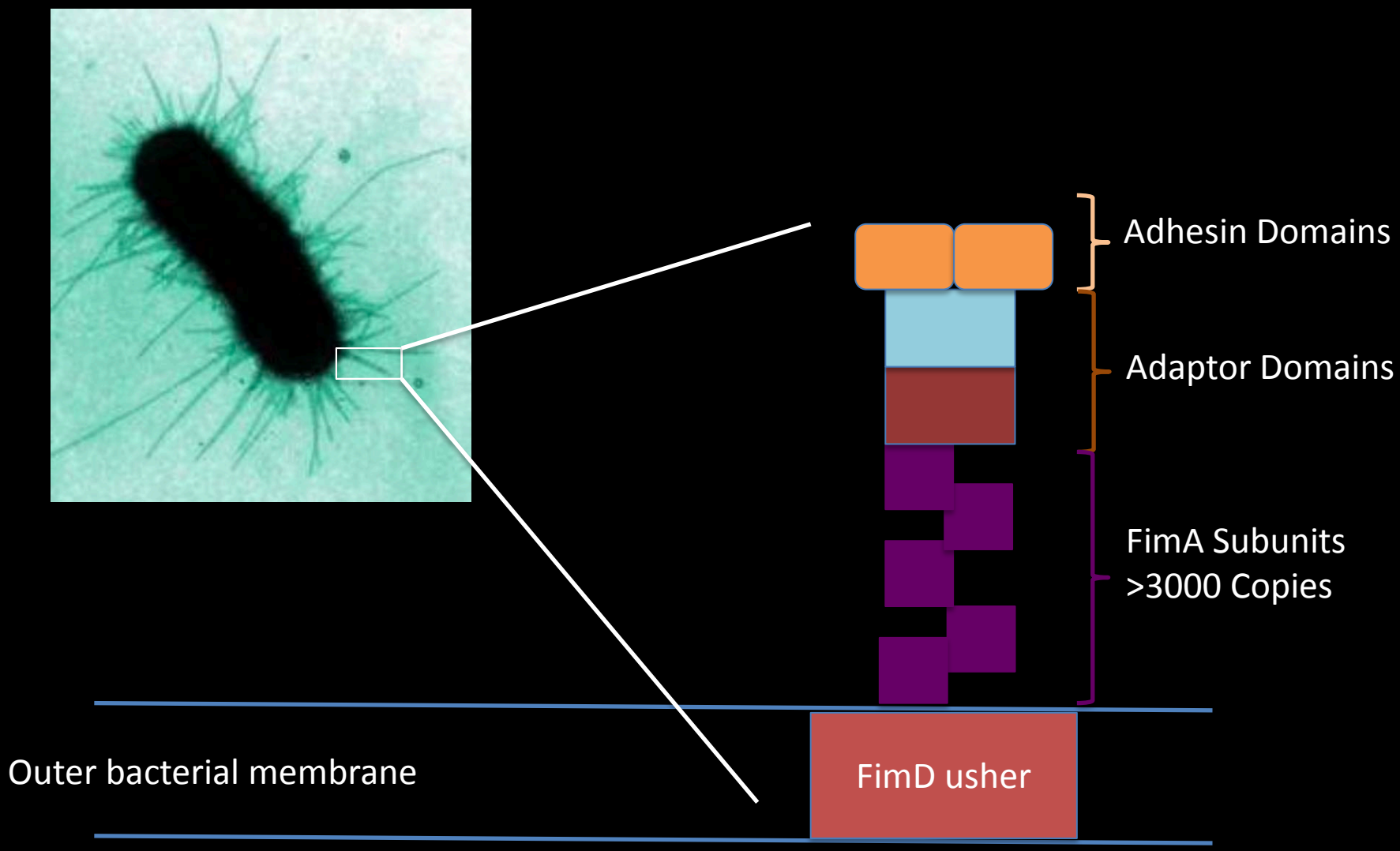
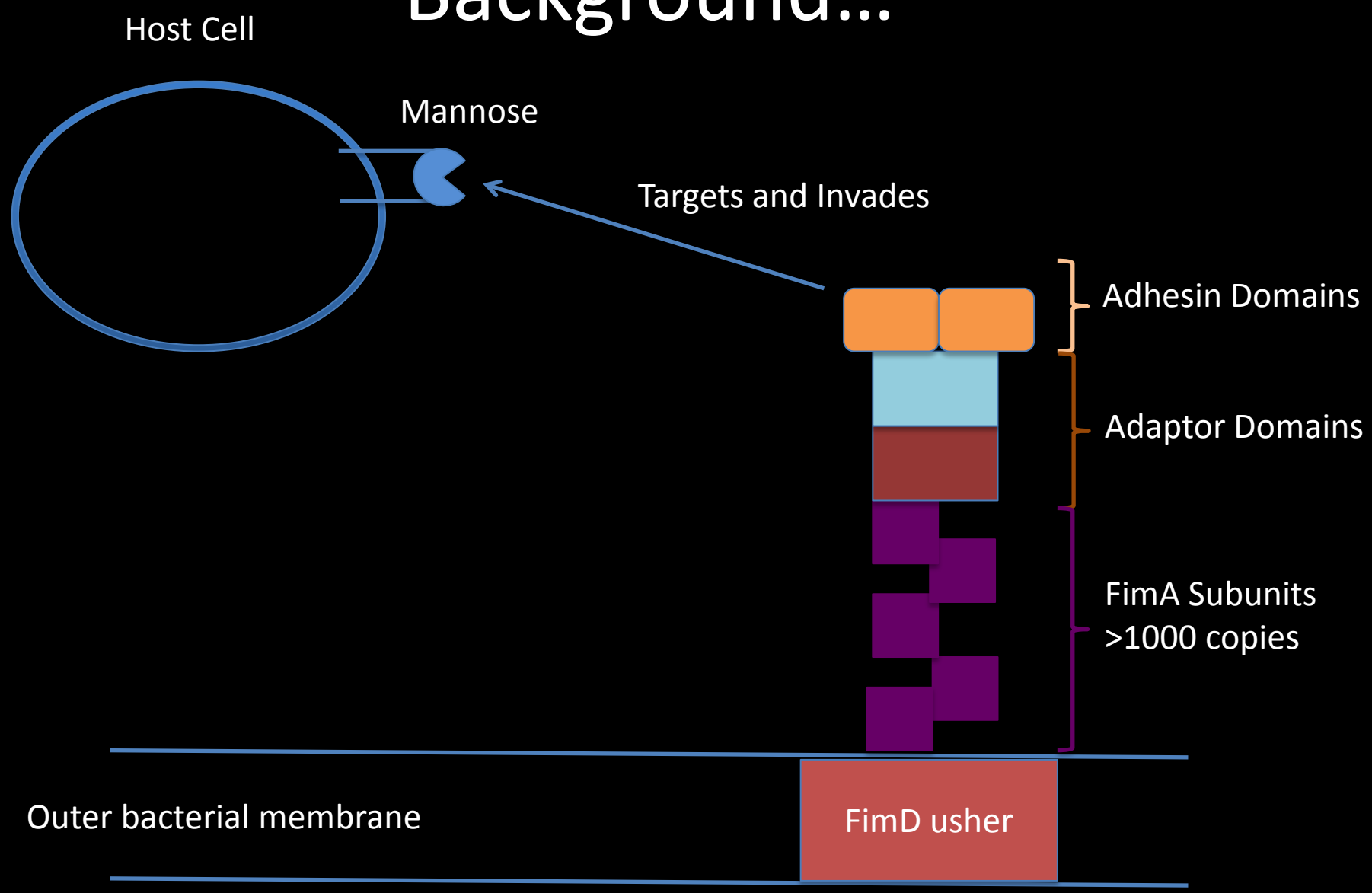
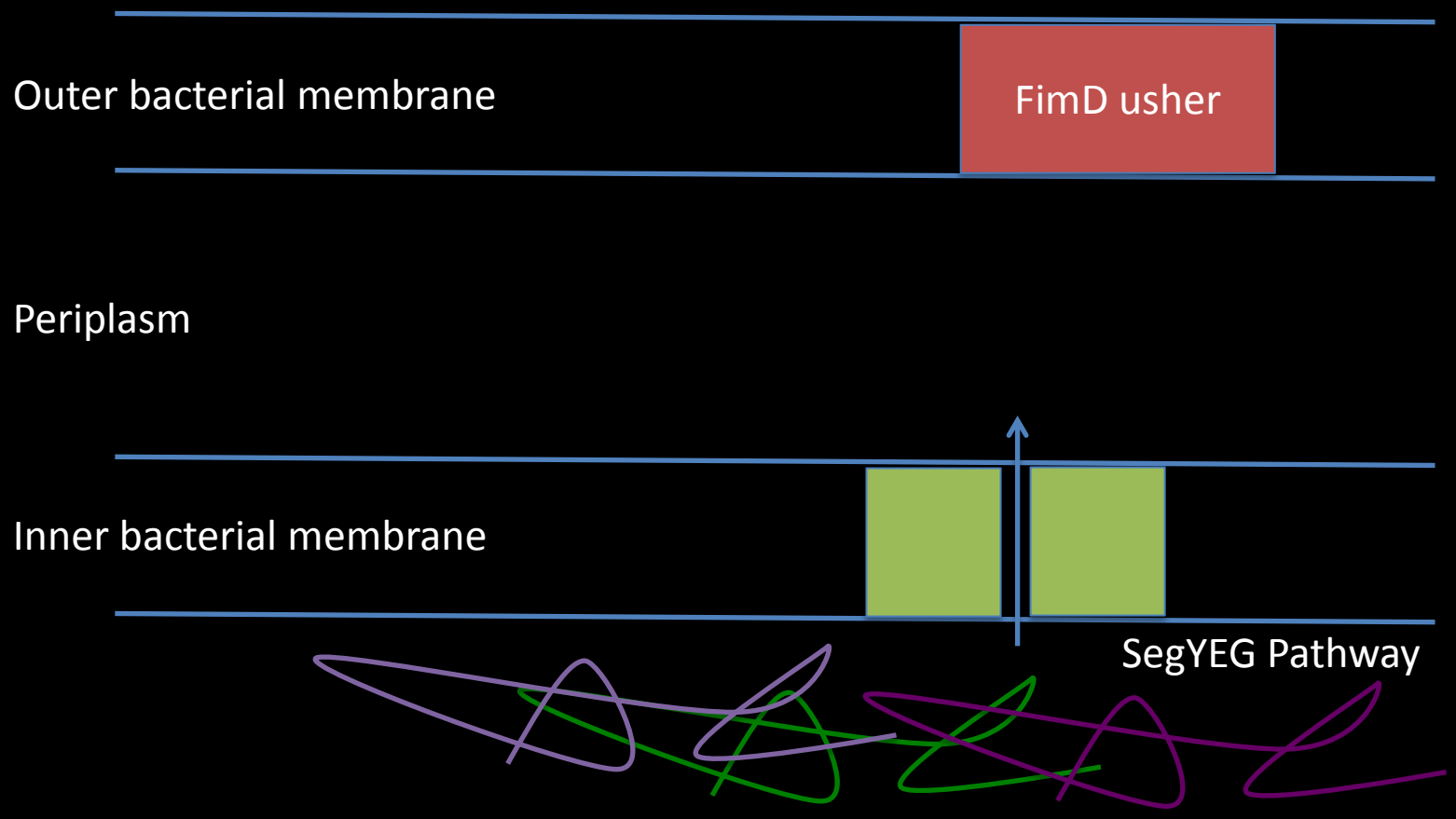
Background…


Adhesin Domains
Adaptor Domains
FimA Subunits
>3000 Copies
Outer bacterial membrane
FimD usher

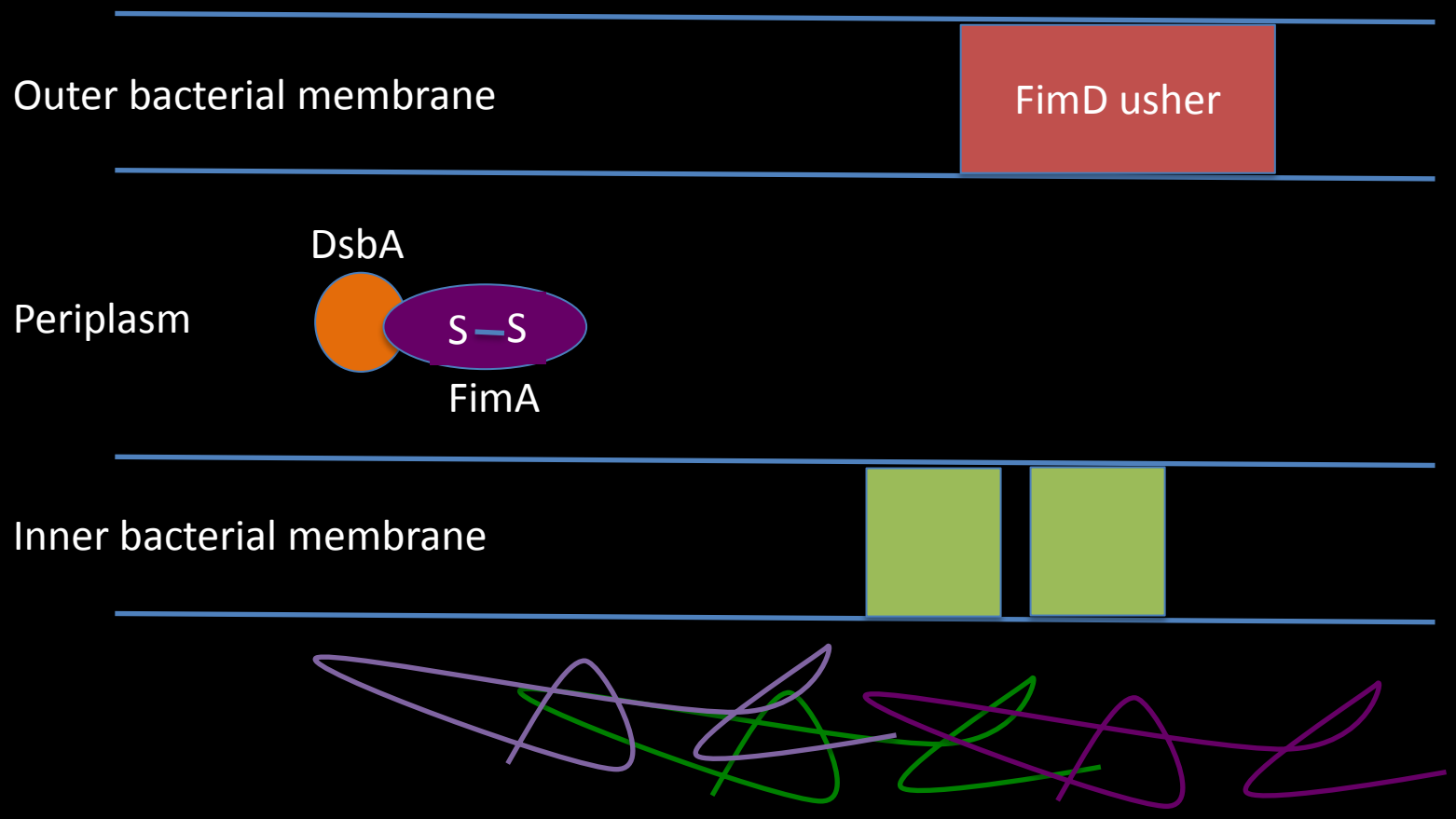
Background…


Host Cell
Mannose
Targets and Invades
Adhesin Domains
Adaptor Domains
FimA Subunits
>1000 copies
Outer bacterial membrane
FimD usher

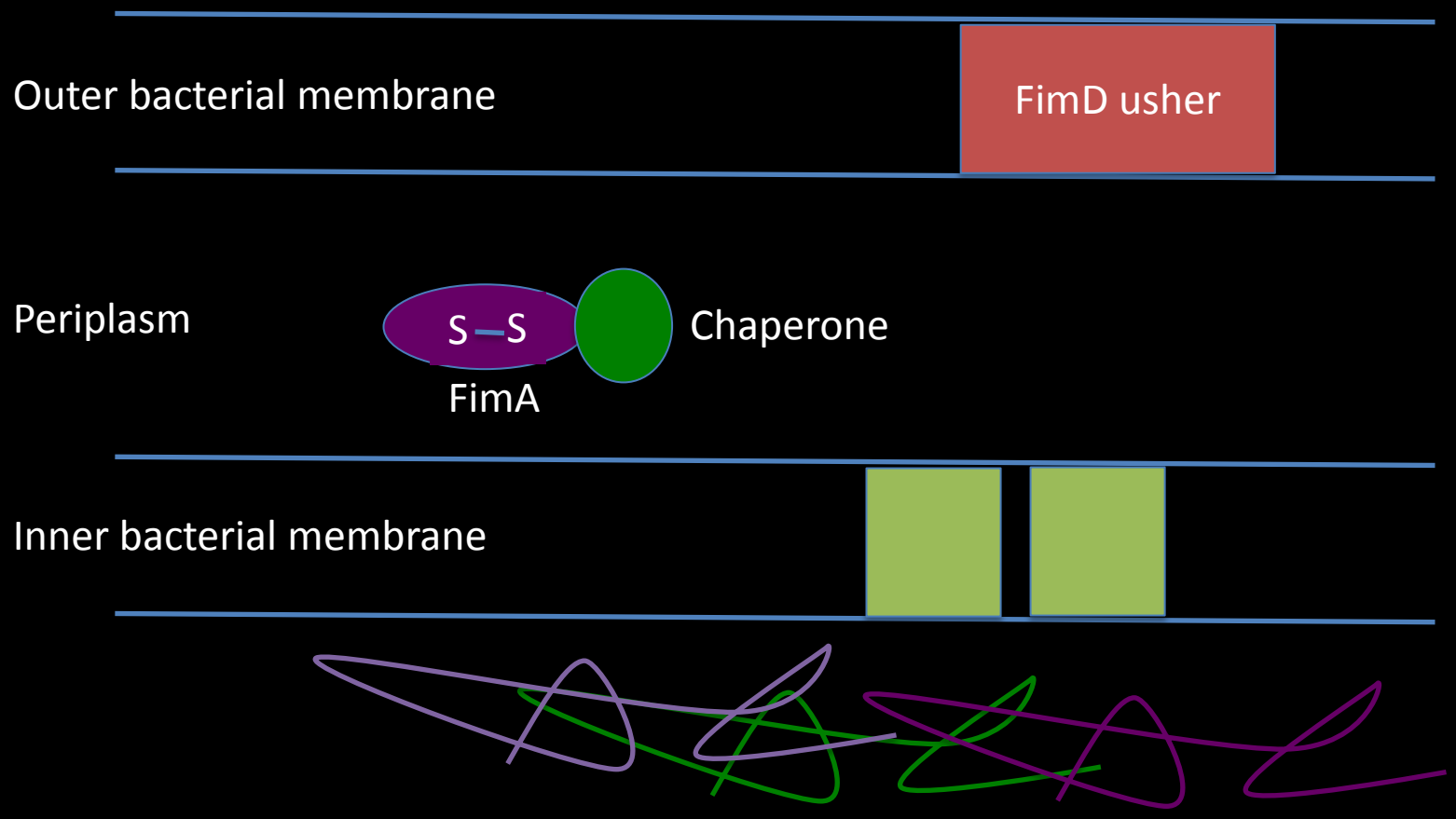
Background…


Outer bacterial membrane
FimD usher
Periplasm
Inner bacterial membrane
SegYEG Pathway

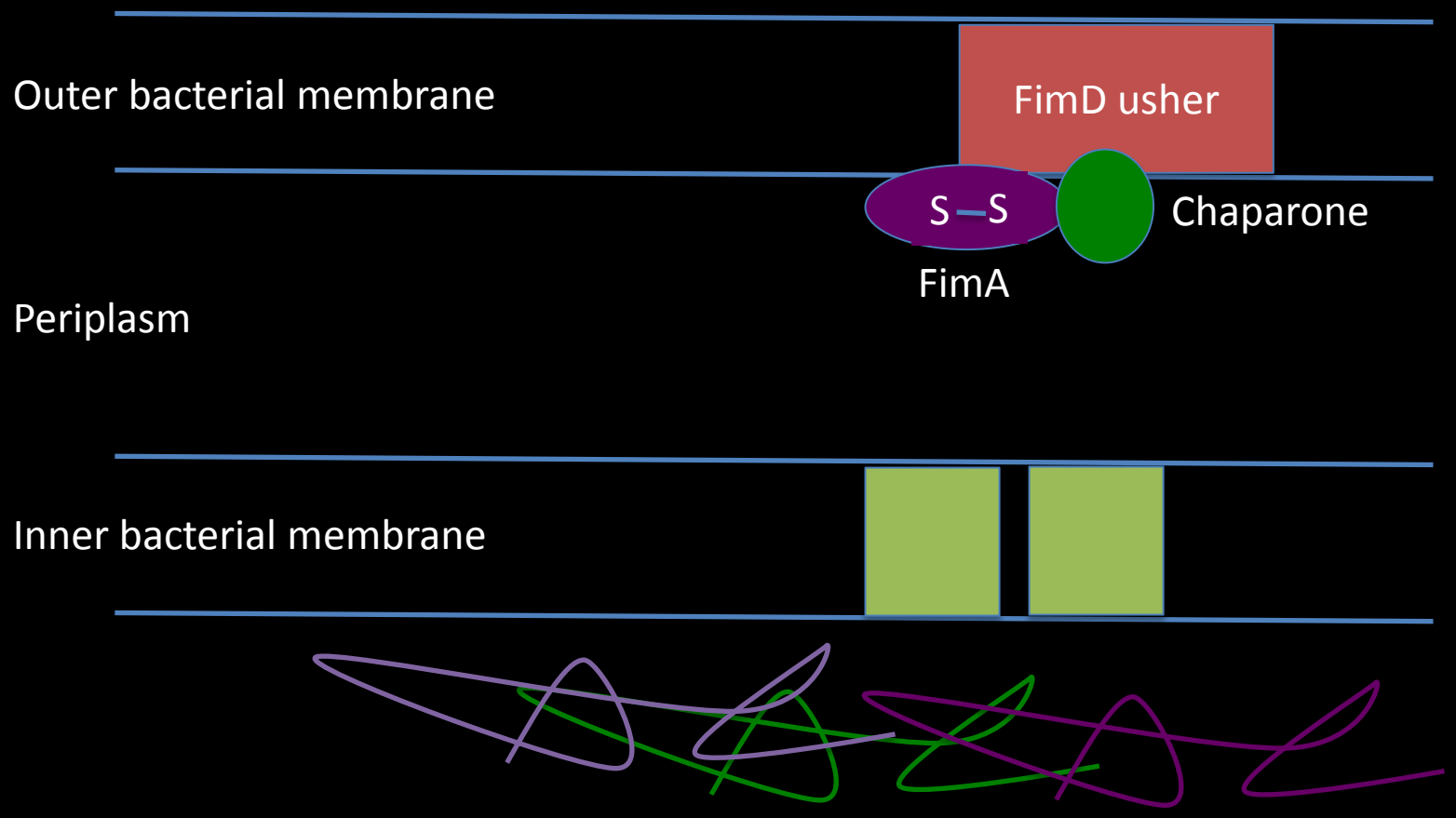
Background


Outer bacterial membrane
FimD usher
DsbA
Periplasm
S—S
FimA
Inner bacterial membrane

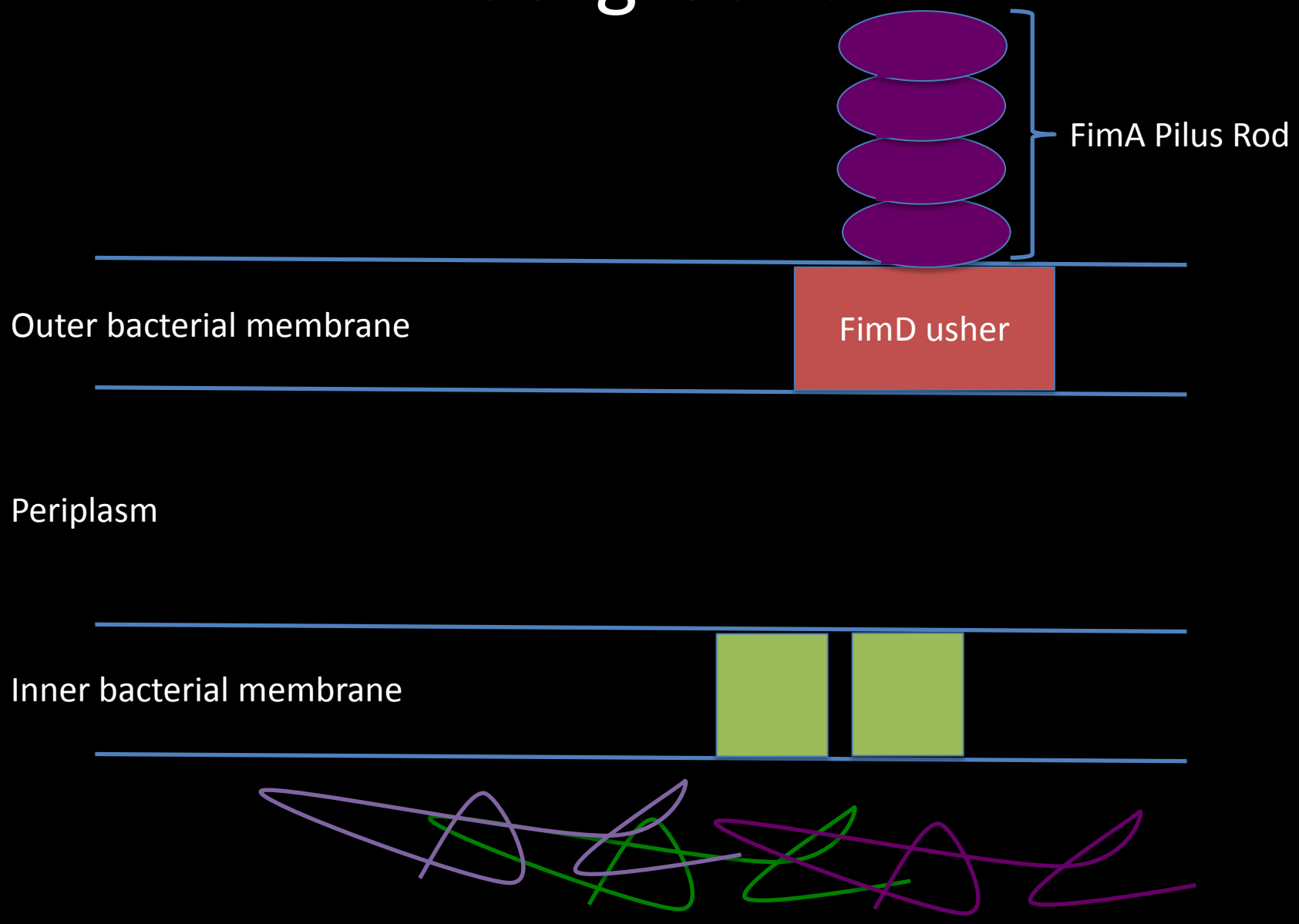
Background


Outer bacterial membrane
FimD usher
Periplasm
S—S
Chaperone
FimA
Inner bacterial membrane

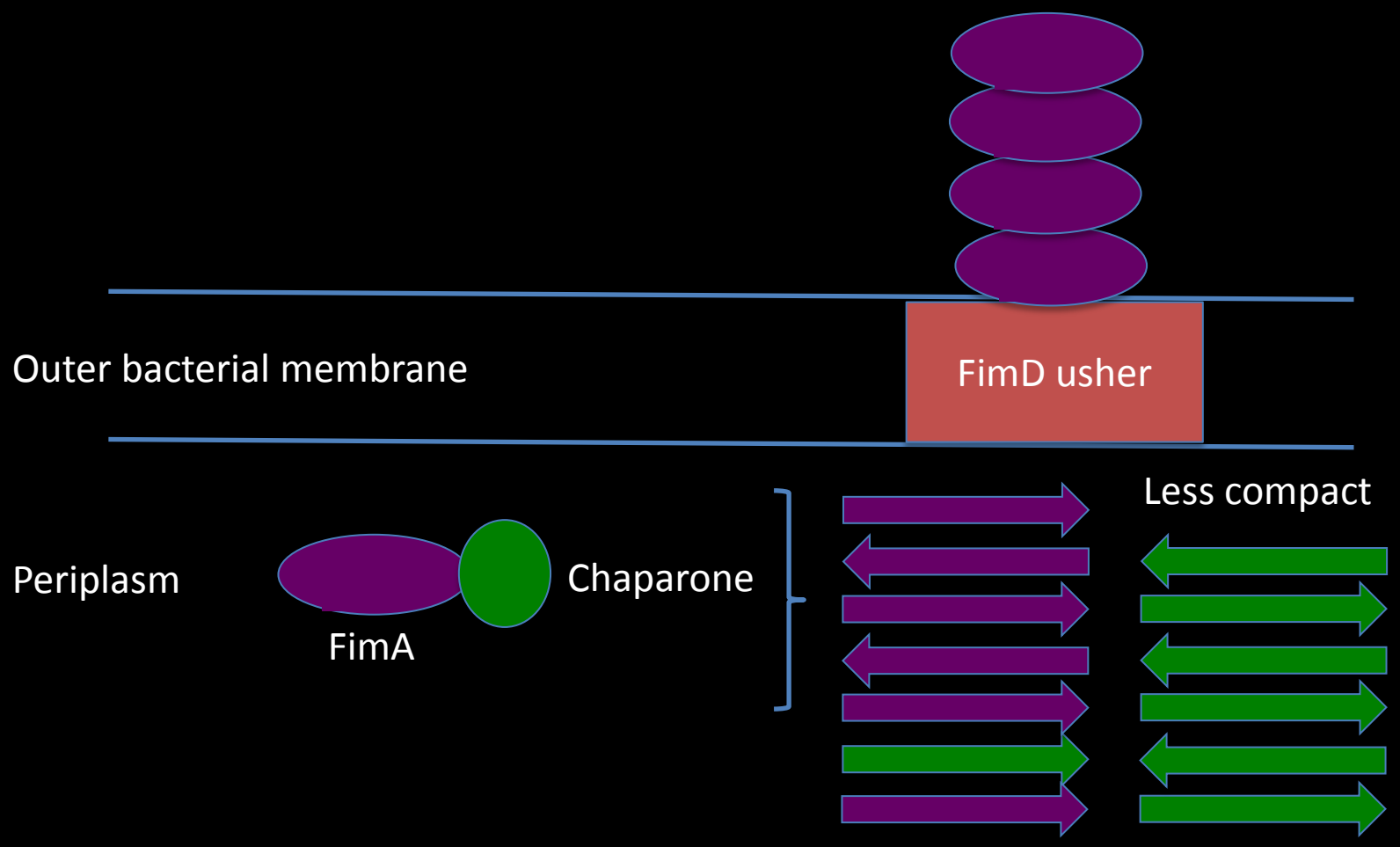
Background


Outer bacterial membrane
FimD usher
S—S
Chaparone
FimA
Periplasm
Inner bacterial membrane

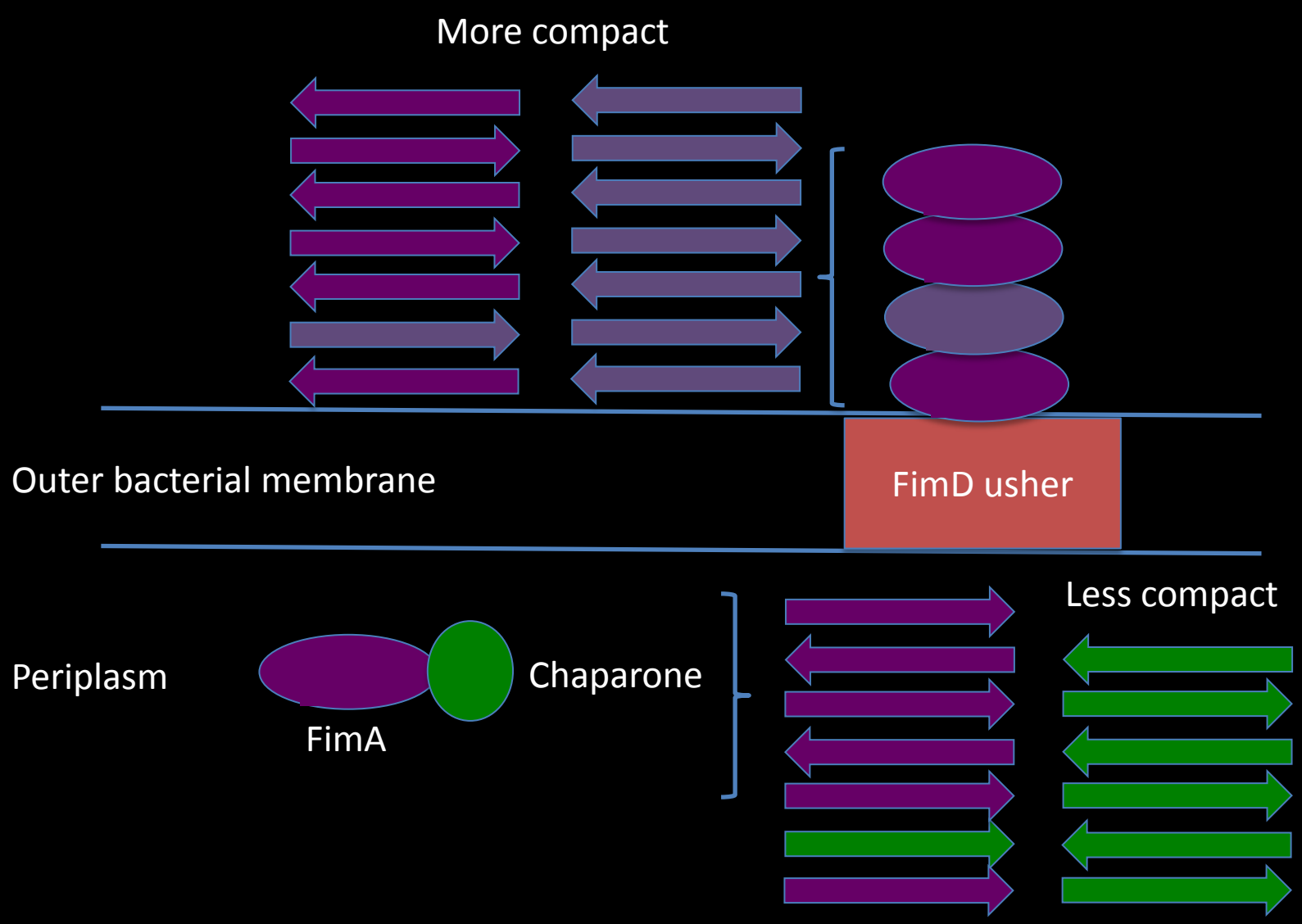
Background


FimA Pilus Rod
Outer bacterial membrane
FimD usher
Periplasm
Inner bacterial membrane

Background…


Outer bacterial membrane
FimD usher
Periplasm
FimA
Chaparone
Less compact

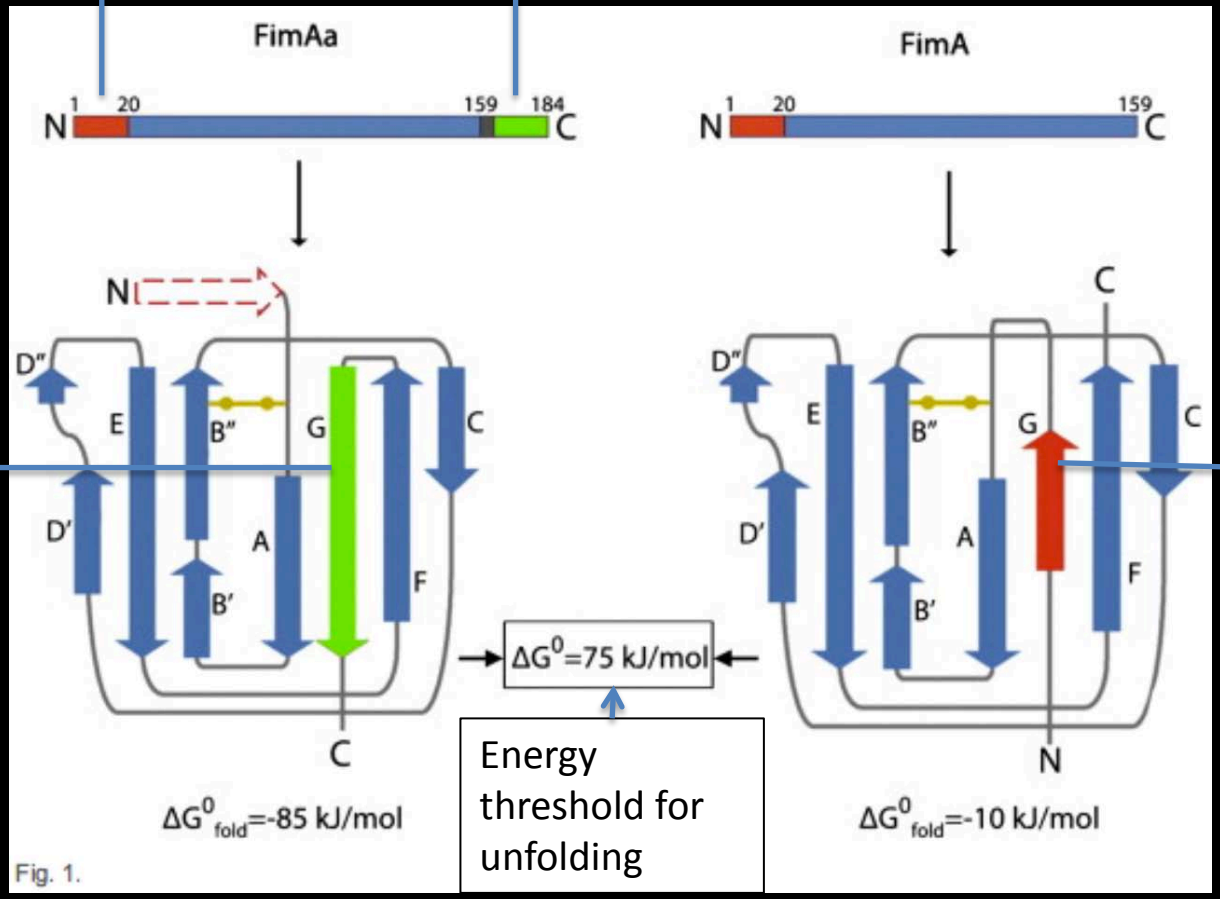
Background…


More compact
Outer bacterial membrane
FimD usher
Less compact
Periplasm
Chaparone
FimA

Results…

Native donor strand

Second copy of donor strand inserted



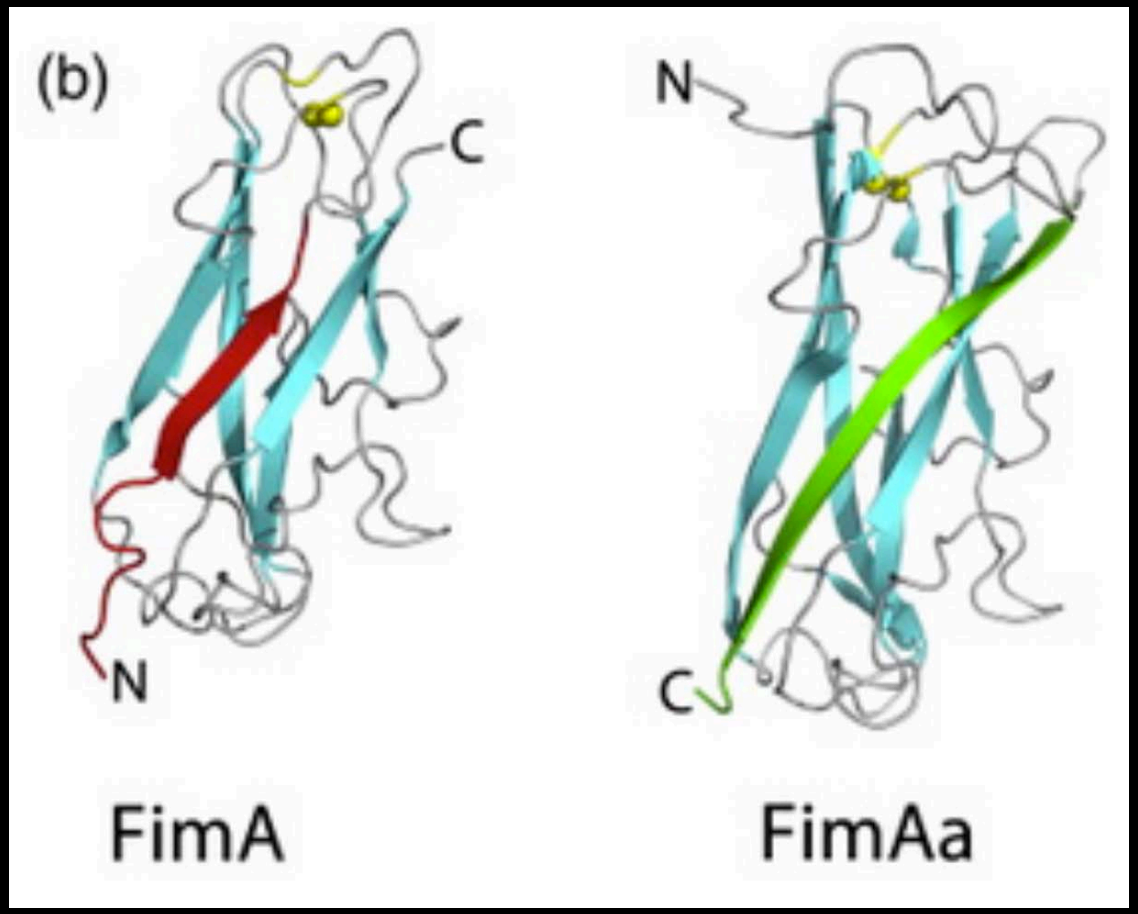
Wild Type

Fills in gap to complete fold to produce stable monomers

Fills in gap to complete fold to produce unstable monomers

Energy threshold for unfolding

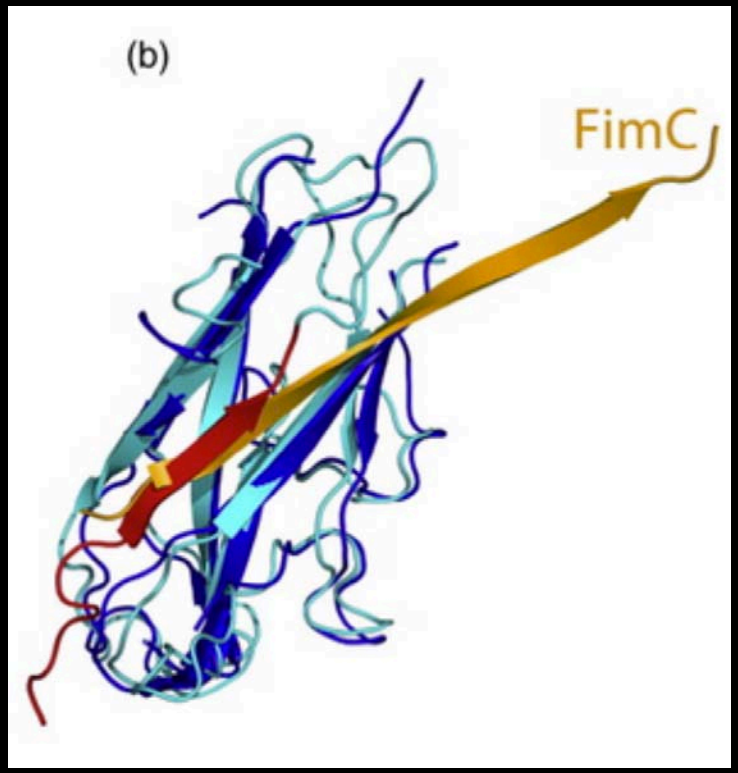
Results…



Parallel Orientation
Solved by NMR

Anti-parallel Orientation
Solved by X-ray crystallography

Results…



- **Why doesn't FimA WT just remain a monomer is solution?**
  - FimA complexes with FimC (chaperone) in the periplasm.
- **Why would FimA allow the chaperone to replaces its own incomplete fold?**
  - The chaperone's donating stand actually is a shared beta strand between FimA and the chaperone.

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Conclusions…

- Eventually all the FimAa and WT FimA will fold into the thermodynamically stable anti-parallel fold.
- WT FimA takes longer than FimAa to reach the stable state because FimAa has the advantage of two donor strands (due to the addition of the second set of residues to the C-terminal domain).
- This WT FimA mechanism explains the mode of storage of FimA monomers in the periplasm because attachment to the chaperone and pilus biogenesis.

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Take home message…

- There is a structure and mechanism that accounts for soluble FimA monomers in solution.
- The FimA monomers are important in interacting with apoptotic proteins to delay and inhibit apoptosis of invaded cells.
  - Delays the integration of Bax into the mitochondrial outer membrane.
  - Strengthens the VDAC-Hexokinase complex.
- This mechanism allows for longer survival of the pathogenic bacteria in the infected cell.

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Thank you!

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