Intricate Folding Pathways of the Bacterial Conjugation Enzyme TrwC Uncovered by Force Spectroscopy

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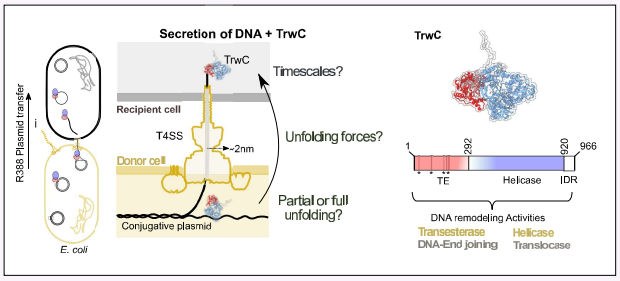
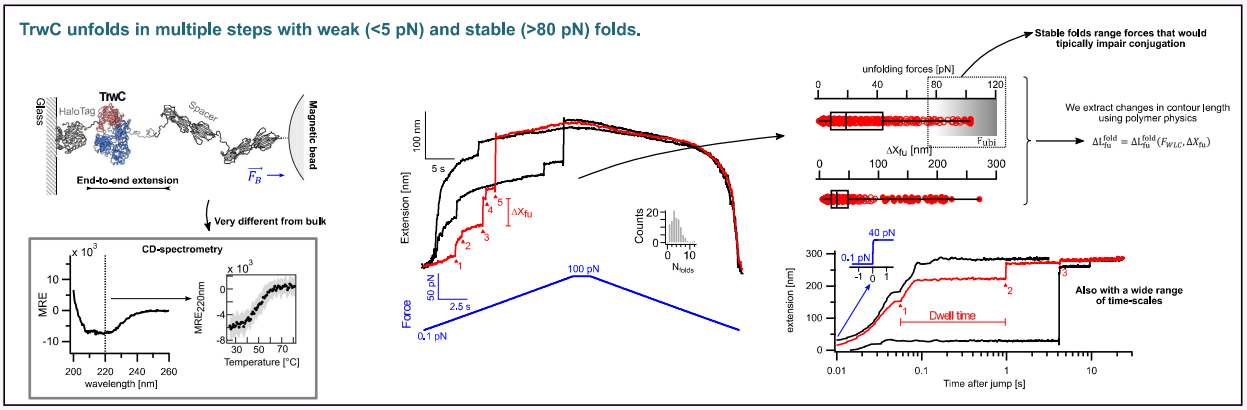
Bacterial conjugation is a gene transfer mechanism essential for spreading of virulence factors and antimicrobial resistances along bacterial populations. Here a donor cell transfers DNA into a recipient cell thanks to the coordinate action of DNA-processing enzymes and specialized secretion machineries. We study a key model enzyme for bacterial conjugation, TrwC, to understand molecular details that might be essential for development of targeted solutions to prevent further spread of antibiotics resistence genes.

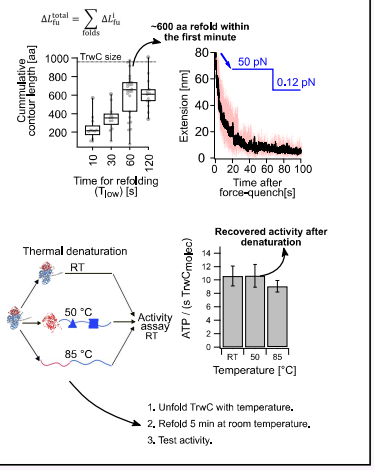
TrwC is a 966 aminoacids multi-domain enzyme with several DNA-remodelling activities. It process and guides DNA in the donor cell thanks to transesterase and helicase activities, and then helps for recircularization of the plasmid in the recipient cell thanks to DNA-End joining and translocase activites. But to reach the recipient cell, TrwC is secreted in complex with a single-strand of the plasmid through constricted channels of the type IV secretion system. This is a highly specialized mechano-chemical process where molecular motors unfold TrwC and translocate along its backbone [1,2]. Our goal is to understand the forces and timescales required to unfold individual TrwC molecules, for which we use as main technique high resolution magnetic tweezers [3].



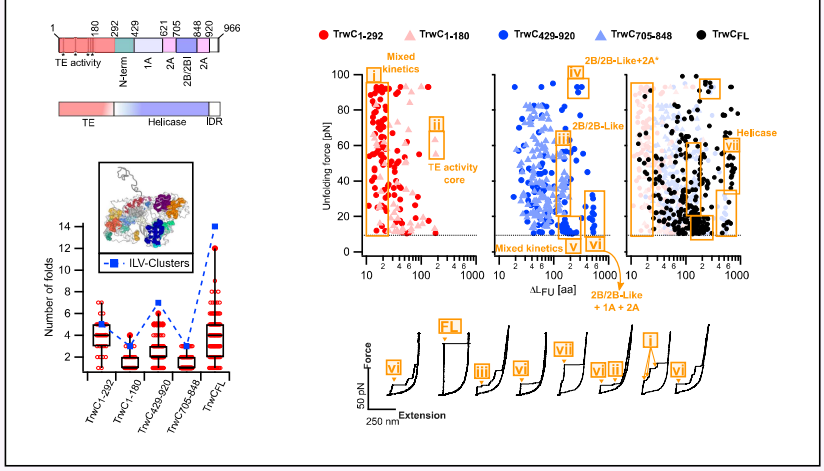
TrwC unfolds in multiple steps with weak (<5 pN) and stable (>80 pN) folds.



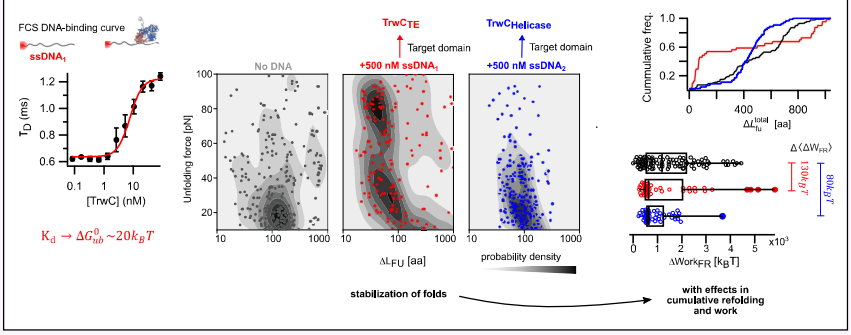
A large extent of TrwC refolds in < 1 min



To give structural context, we annotate common folds with more simple truncated variants



Binding to DNA modulates TrwC mechanics



Our next steps:

1. How individual TrwC unfolding pathways modulate work?
2. Can we identify preferential pathways *in vivo*?
3. Instrument and data analysis upgrades for complex multi-domain proteins.

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