Probing the stability of the C-terminal domain of type IV pilins under external force

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Type IV pilins are "ladle-shaped" proteins that assemble into long bio-filaments called type IV pili, which emanate from the surface of bacterial cells. These filaments are involved in a variety of functions for bacteria, including surface adhesion, twitching motility, and infection. The C-terminal domain of type IV pilin subunits is the adhesive domain that allows the filament to directly interact with its environment. Therefore, the structural stability of the C-terminal domain of type IV pilins is of critical importance for the proper functioning of these bacterial filaments. Type IV pilin subunits utilize a number of strategies for stabilizing their C-terminal domains. The most prevalent stabilization strategy is a disulfide bond that attaches the pilin C-terminus to the rest of the C-terminal domain. Other C-terminal stabilization strategies include metal ion coordination, hydrogen bond networks, and specific water molecules.

In order to probe the role of disulfide bonds or hydrogen bond networks in pilin C-terminal stabilization, we carry out implicit solvent all-atom molecular dynamics simulations of type IV pilin subunits from two organisms (the GC-pilin of *N. gonorrhoeae* and the pilin PilA1 from the NAP08 strain of *C. difficile*). Steered molecular dynamics (SMD) is used to stretch on wild type systems and systems in which the disulfide bond (or hydrogen bonds) have been disrupted through amino acid substitution. For both systems, we observe the breakdown of both secondary and tertiary structure as the pilin proteins are stretched out. Force-extension curves obtained from SMD simulations provide insight into the role of disulfide and hydrogen bonds on the stabilization of each pilin's C-terminal domain. Therefore, these simulations can lead to new insights into the role of specific interactions in pilin subunit stability.

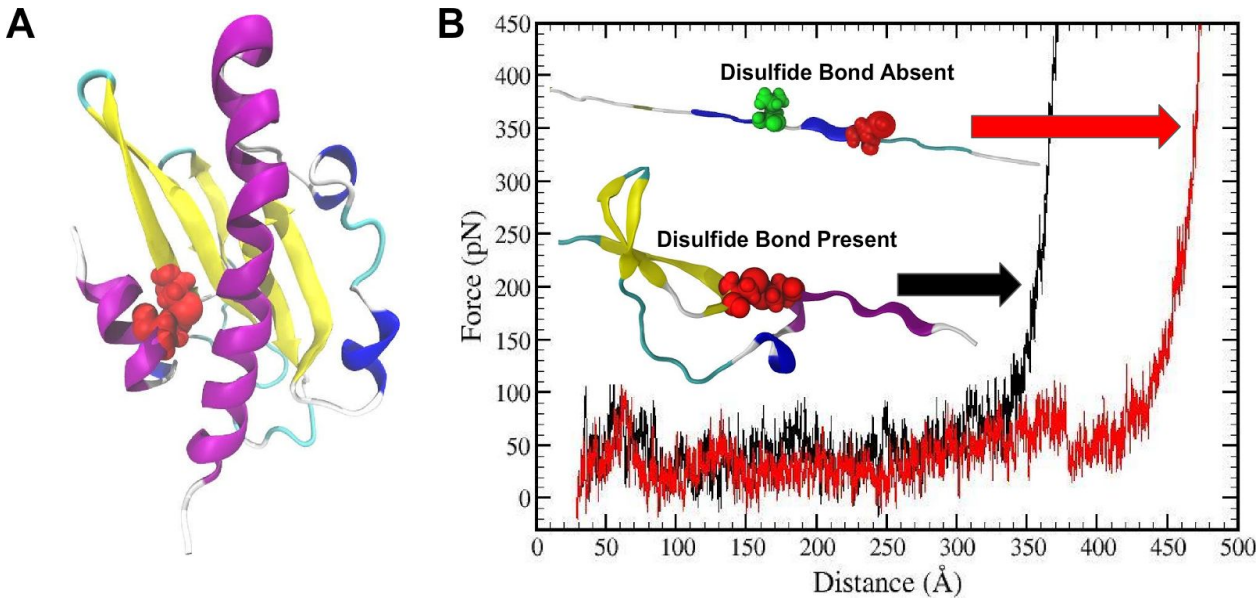

Figure 1. (A) Type IV pilin (wild type) from *N. gonorrhoeae* (GC-pilin), with the disulfide bond forming cysteine residues shown in red. (B) Force vs extension curves obtained from SMD simulations of GC-pilin with disulfide bond present (black) and absent (red). In the model with the disulfide bond absent, green atoms denote an alanine mutation. In the wild type GC-pilin system, the bond between the two cysteine residues (red atoms) limits the extension of the pilin under force compared to when the disulfide bond is absent.