# DYNAMICS IN 'RIGID' DISULFIDE PROTEINS

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Small disulfide proteins (50-60 aa) like PAF are efficient antifungals and some have anti-*Candida* (PAFC) or anti-corona virus activity (PAF, PAFB).[1] However, their mode of action is not yet fully understood.[2] Their β-barrel tertiary structures are stabilised by 3-4 disulfide bridges lending apparent rigidity to the structures. Still, intrinsic dynamics persists as shown[3] by NMR $^{15}$N-relaxation, $^{15}$N-CEST and MD calculations that are now supported by stress induced reversible unfolding and natural abundance $^{13}$C relaxation studies. Besides thermal unfolding we show DMSO induced transitions in PAF and variants as detected by NMR and DSC microcalorimetry.

Partially unfolded reversible states can be biologically relevant, e.g. connected to disulfide shuffling or other thiol related transitions, while dynamic intermediates can be preferred for conformational-selection mode of molecular recognition. Practical consequences may have impact on the validation of MD simulations or protein concentration measurements.

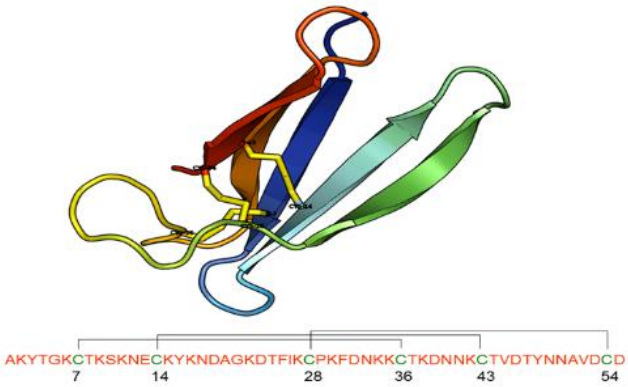


**Figure 1.** Solution structure of the antifungal protein PAF with three disulfide bonds.

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