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THE CHANGE OF CONDITIONS DOES NOT AFFECT ROS87 DOWNHILL FOLDING MECHANISM

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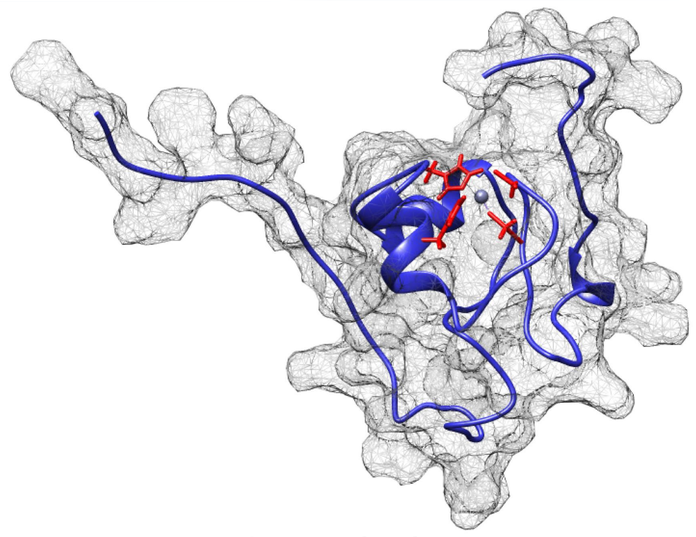
ABSTRACT

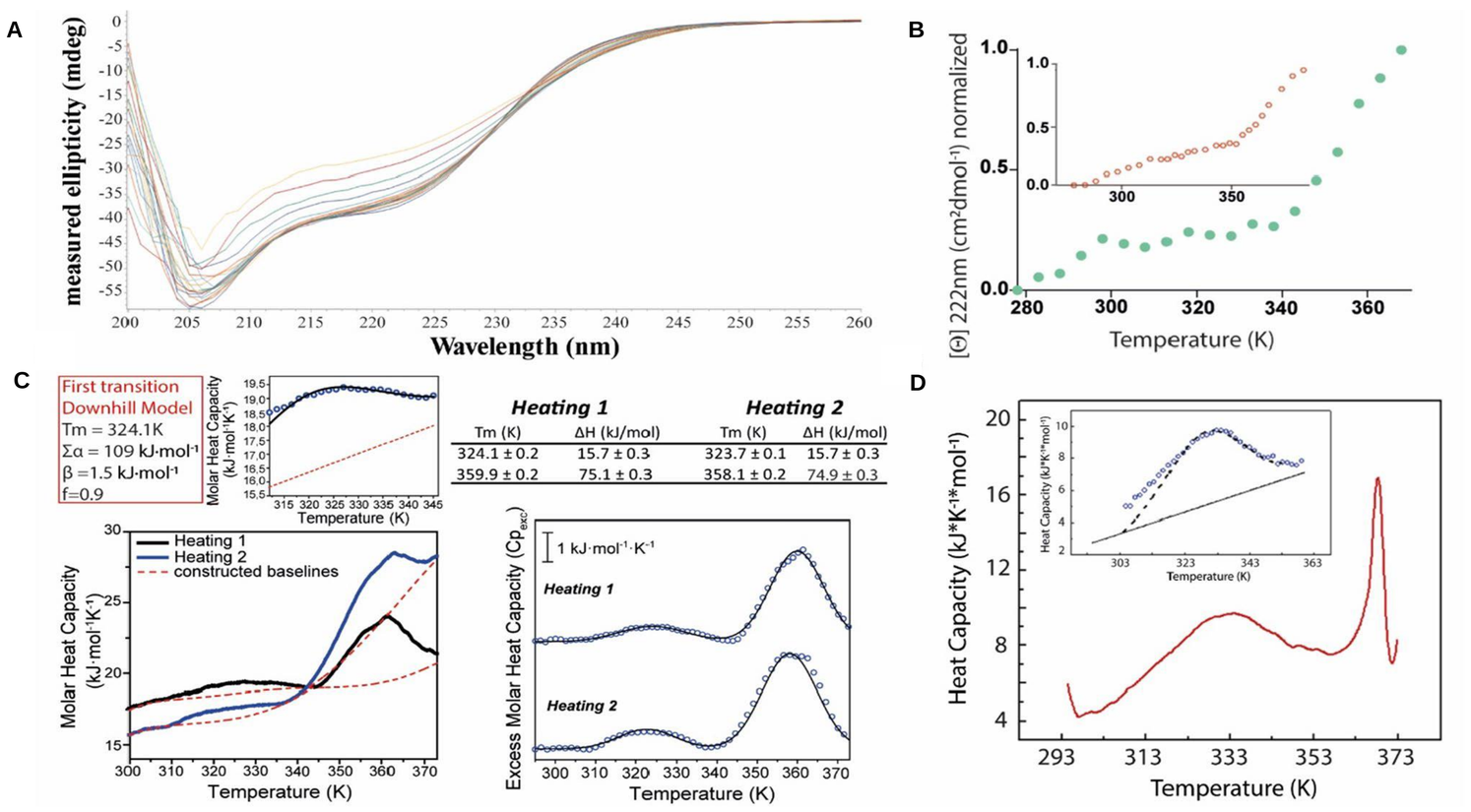
Downhill folding has been defined as a unique thermodynamic process involving a conformations ensemble that progressively loses structure with the decrease of protein stability [1]. Downhill folders are quite rare in nature because there isn't an energetically substantial folding barrier that can protect against aggregation and proteolysis [2-4]. We have previously demonstrated that the prokaryotic zinc finger protein Ros87 shows a folding/unfolding process in which a metal binding intermediate converts to the native structure through a delicate barrier-less downhill transition [5]. Significant variation in folding scenarios can be detected within protein families with high sequence identity and very similar folds and for the same sequence by varying conditions. For this reason, here we show, by means of DSC, CD and NMR, that also in different pH and ionic strength conditions Ros87 is capable to conserve its partly downhill folding mechanism demonstrating that the downhill mechanism can be found under a much wider range of conditions. We also show that mutations of Ros87 zinc coordination sphere produces a different folding scenario demonstrating that the organization of the metal ion core is determinant in the folding process of this family of proteins.

Ros zinc finger domain structure



Ros87 structure by solution NMR

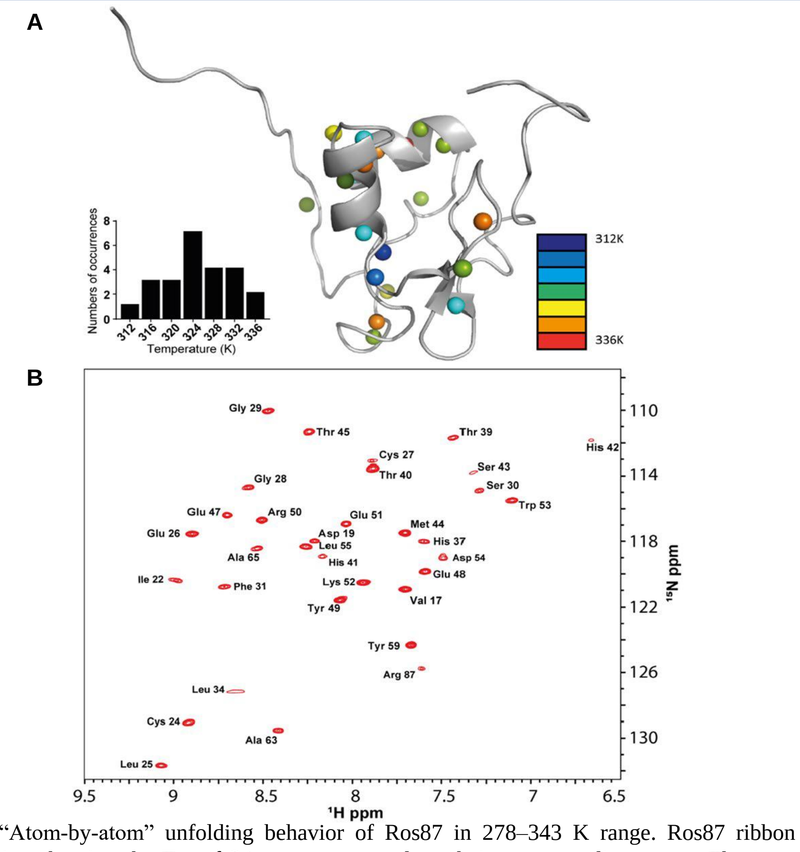
Ros87 thermal unfolding



Heating 1		Heating 2	
Tm (K)	ΔH (kJ/mol)	Tm (K)	ΔH (kJ/mol)
324.1 ± 0.2	15.7 ± 0.3	323.7 ± 0.1	15.7 ± 0.3
359.9 ± 0.2	75.1 ± 0.3	358.1 ± 0.2	74.9 ± 0.3

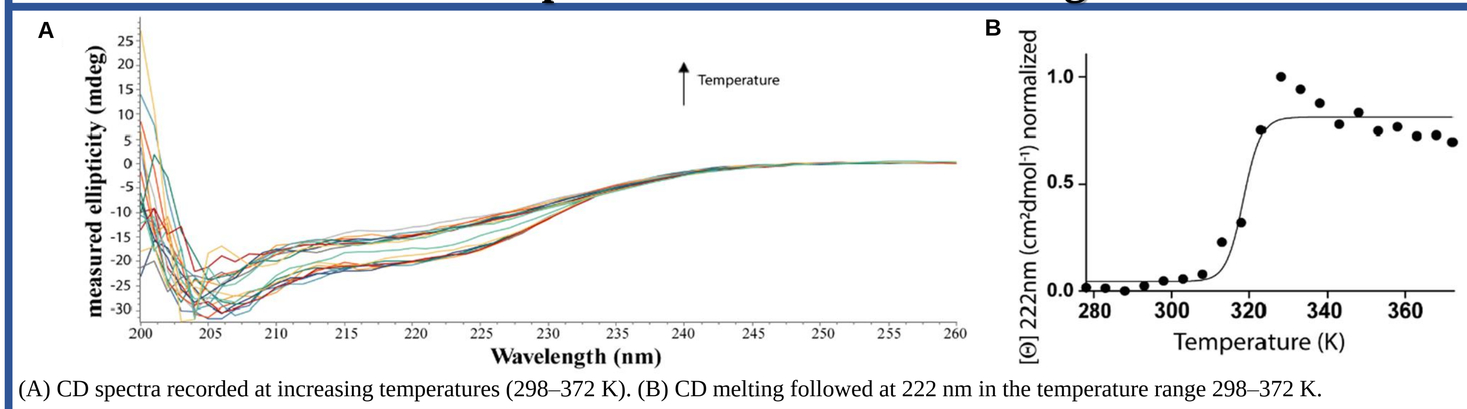
(A) CD spectra recorded in Tris buffer at pH = 6.5 in the temperature range 298 K-372 K. (B) CD melting in Tris buffer followed at 222 nm in the temperature range 298–372 K; the inset shows the melting followed at 222 nm in phosphate buffer at pH = 6.515. (C) DSC thermal unfolding curves and fitting of two different heating cycles in Tris buffer. Baselines are reported with a dashed line. (D) DSC thermal unfolding curves and fitting in phosphate buffer

Ros87 NMR unfolding



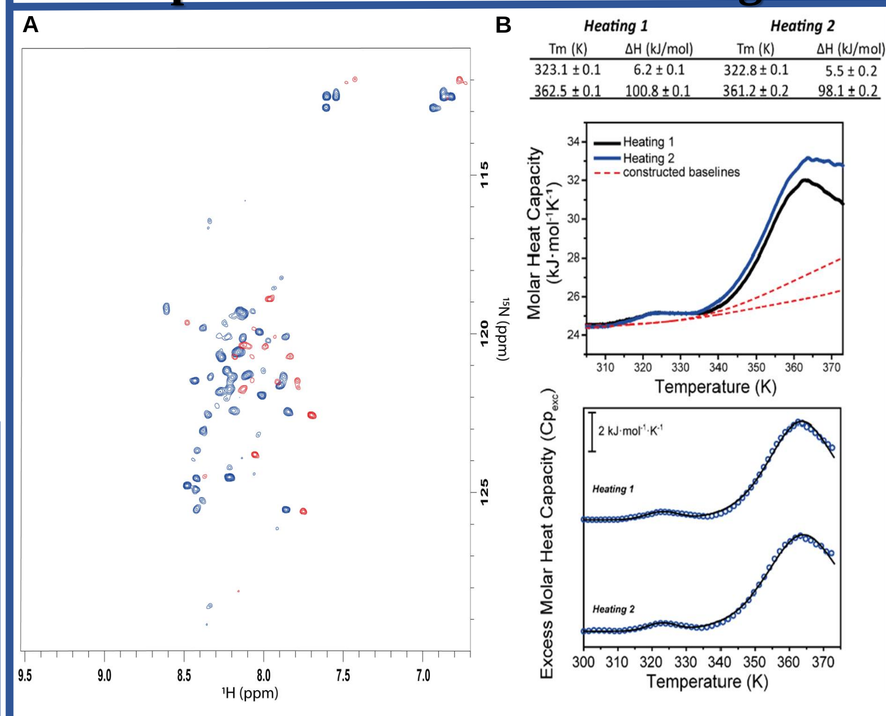
(A) "Atom-by-atom" unfolding behavior of Ros87 in 278–343 K range. Ros87 ribbon drawing showing the Tm of 24 protons mapped on their corresponding atoms. The inset shows the Tm scale. Each atom color corresponds to the Tm of its sigmoidal transition. (B) 1H–15N HSQC spectrum at 343 K.

Apo-Ros87 thermal unfolding



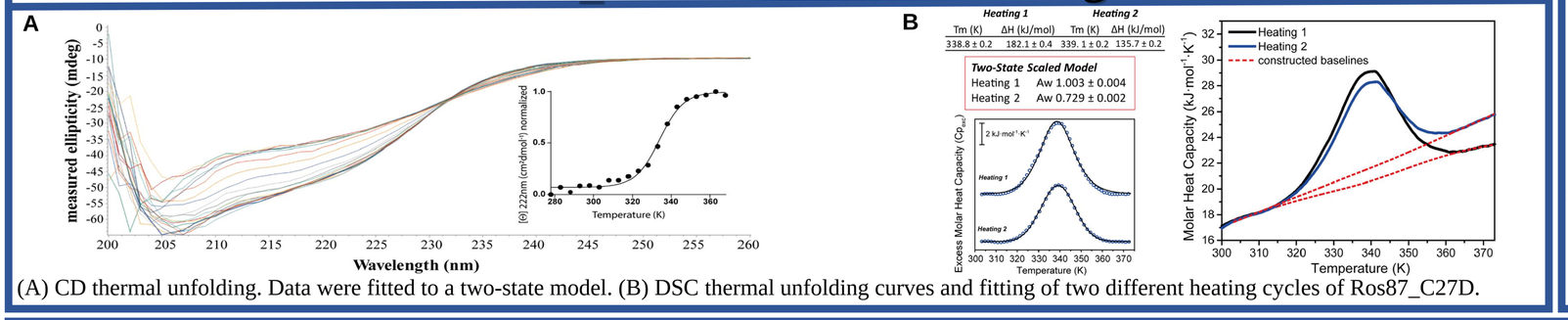
(A) CD spectra recorded at increasing temperatures (298–372 K). (B) CD melting followed at 222 nm in the temperature range 298–372 K.

Apo-Ros87 thermal unfolding



Heating 1		Heating 2	
Tm (K)	ΔH (kJ/mol)	Tm (K)	ΔH (kJ/mol)
323.1 ± 0.1	6.2 ± 0.1	322.8 ± 0.1	5.5 ± 0.2
362.5 ± 0.1	100.8 ± 0.1	361.2 ± 0.2	98.1 ± 0.2

Ros87_C27D thermal unfolding



(A) CD thermal unfolding. Data were fitted to a two-state model. (B) DSC thermal unfolding curves and fitting of two different heating cycles of Ros87_C27D.

(A)Overlay of the 1H–15N HSQC spectra of apo-Ros87 at 298 K (blue) and at 318 K (red). (B) DSC thermal unfolding curves and fitting of two different heating cycles of apo-Ros87.

Conclusions

The body of data collected in the present work clearly shows that change of buffer, pH and ionic strength does not influence Ros87partly downhill unfolding mechanism. Differences with what was previously seen in phosphate buffer are found in the second transition and are likely due to the different environment found by the cysteines during metal ion release. This however does not influence the folding/unfolding behavior of the protein described by the first DSC transition indicating that the rate-limiting step in Ros87 folding is represented by zinc recruitment and, once metal ion is recruited, a partially folded intermediate forms and the protein moves toward its native functional state with a non-cooperative downhill mechanism. We aim at contributing to the general discussion indicating that, at least in metallo-proteins, downhill folding can be commonly found under a much wider range of conditions and coupled to other different transitions. The key event in determining the mechanism of folding is the organization of metal ion center that not only stabilizes the native state but is determinant in the folding process of this proteins family. The finding that mutations of Ros87 zinc coordination sphere trigger different folding mechanisms resembling a more common two-state process suggests that the complex two-step folding mechanism found in Ros87, including a stable downhill scenario, is a precise evolutionary result. The biological advantage of evolving such bipartite folding process needs further investigations but it could have been driven by the necessity to recruit and retain the metal ion in the most diverse chemical conditions.

References

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