Differential Scanning Fluorimetry Illuminates Silk Feedstock Stability and Processability

N. Kasoju,[a] I. C. Dicko,[b] N. Hawkins and F. Vollrath[c]

Abstract

The ability to design and implement silk feedstock formulations for tailored spinning has so far eluded the bioengineers. Recently, the high throughput screening technique of differential scanning fluorimetry (DSF) demonstrated the link between the instability transition temperature (Ti) and the processability of the silk feedstock.[1] Using DSF we screened a large set of chemicals known to affect solvent quality. A multivariate analysis of the results shows that, regardless the diversity of chemicals, three groupings are significantly distinguishable: G1= similar to native silk; G2= largely dominated by electrostatic interactions; and G3= dominated by chelating interactions. We propose a thermodynamic analysis based on a pre and post transition fit to estimate the van't Hoff enthalpies (ΔH_v) and the instability temperature (Ti). Our analysis shows that the ΔTi and ΔHv were distinct: G1 (ΔTi = 0.23 ± 0.2; ΔH_v = -159.1 ± 5.6 kcal/mol), G2 (ΔTi = -7.3 ± 0.7; ΔH_v = -191.4 ± 5.5 kcal/mol); and, G3 (ΔTi = -19.9 ± 3.3; ΔH_v = -68.8 ± 6.0 kcal/mol). Our analysis further combined the ΔTi and the ΔH_v using stability $\Delta\Delta$G to find that G1 only marginally stabilizes native silks ($\Delta\Delta$G = -0.15 ± 0.04 kcal/mol), whereas G2 and G3 destabilize native silk ($\Delta\Delta$G = 3.8 ± 0.11 and $\Delta\Delta$G = 3.8 ± 0.3 kcal/mol respectively). Here our analysis shows that native silk have a complex multistep transition that is possibly non-cooperative. However, all three groupings also show a direct and cooperative transition with varied stabilization effects. This analysis suggests that native silks are able to sample multiple substates prior to undergo (or to delay) the final transition. We conclude by hypothesizing that the observed energetic plasticity may be mediated by a fragile packaging of the silk tertiary structure that is readily lost when the solvent quality changes.

Supplementary Figures

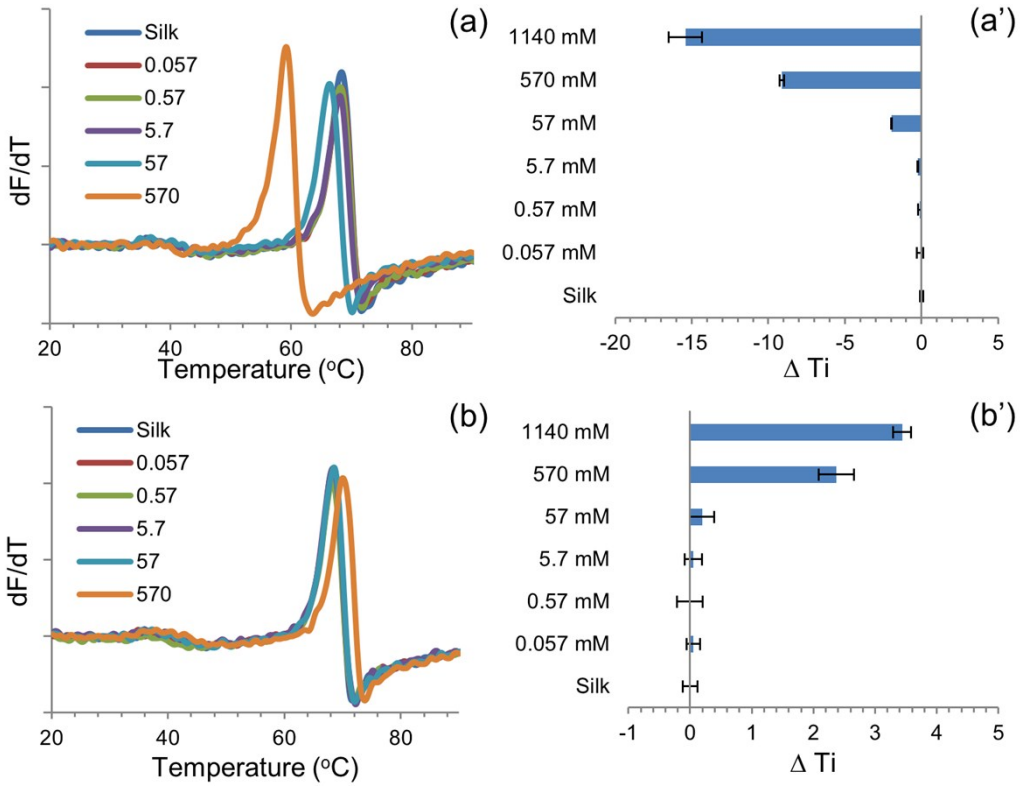

Figure S1. The first derivative fluorescence spectra and change in *Ti* of NSF in presence of guanidinium (a and a' respectively) and trehalose (b and b' respectively) with concentrations ranging from 0.057 to 1140 mM.

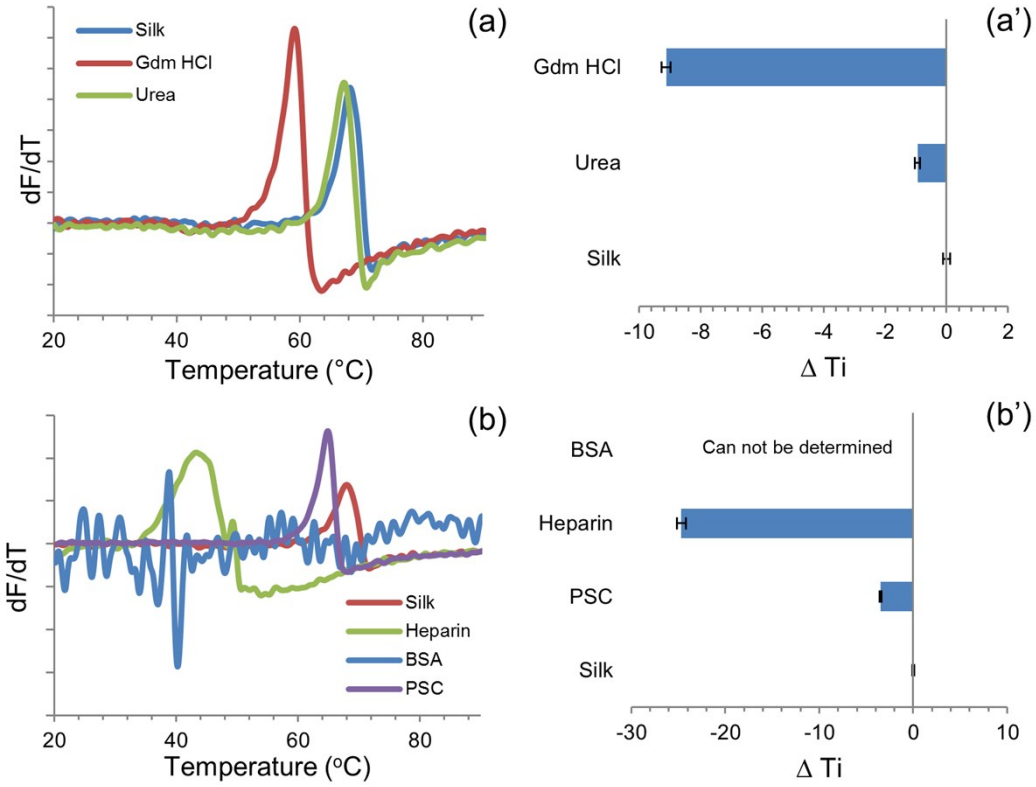

Figure S2. The first derivative fluorescence spectra and change in *Ti* of NSF in presence of popular denaturing (a and a' respectively) and stabilizing (b and b' respectively) agents.

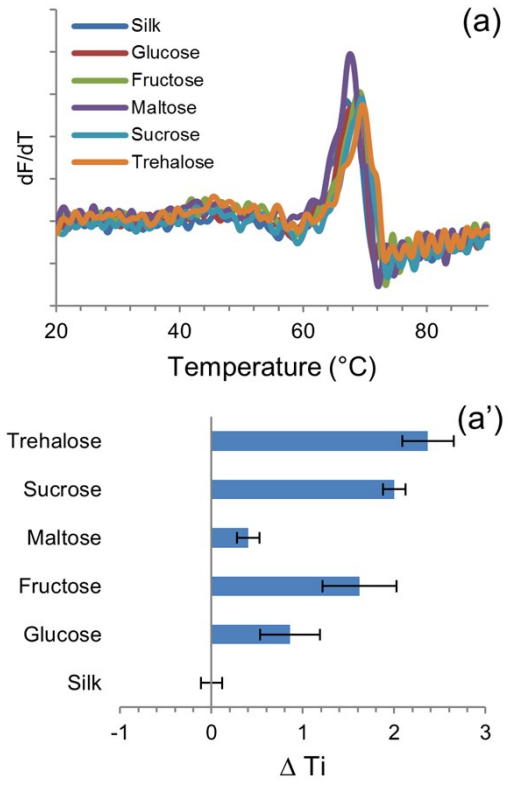

Figure S3. The first derivative fluorescence spectra (a) and change in *Ti* (a') of NSF in presence of sugars.

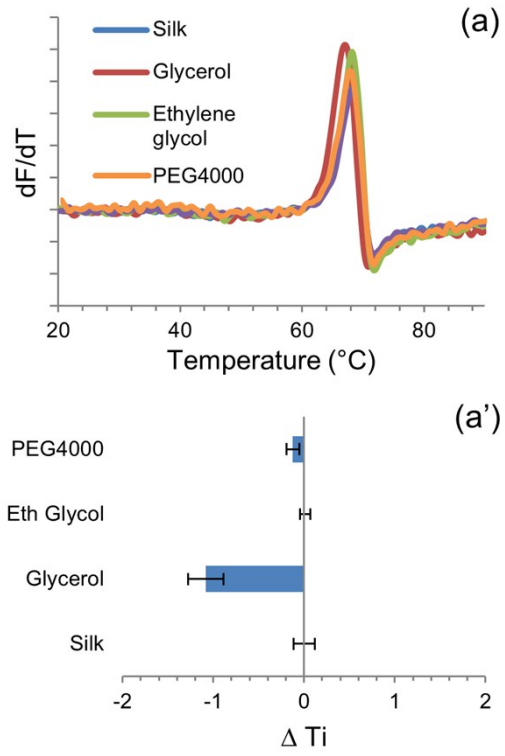

Figure S4. The first derivative fluorescence spectra (a) and change in *Ti* (a') of NSF in presence of cryoprotectants.

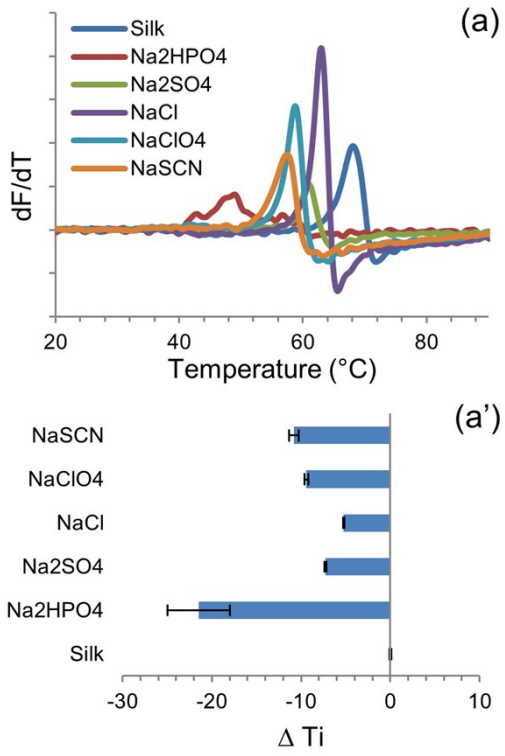

Figure S5. The first derivative fluorescence spectra (a) and change in *Ti* (a') of NSF in presence of hofmeister series of salts.

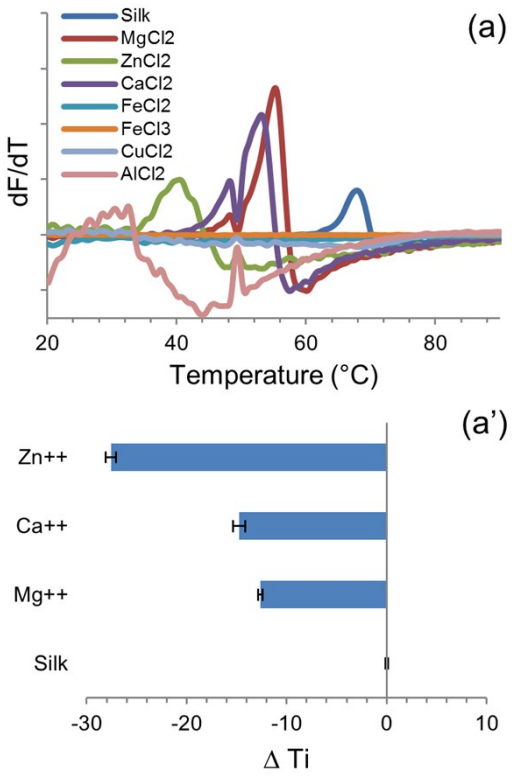

Figure S6. The first derivative fluorescence spectra (a) and change in *Ti* (a') of NSF in presence of coordinating metal ions. The calculation of change in *Ti* of NSF in presence of Fe^{2+}, Fe^{3+}, Cu^{2+} and Al^{2+} was not possible.

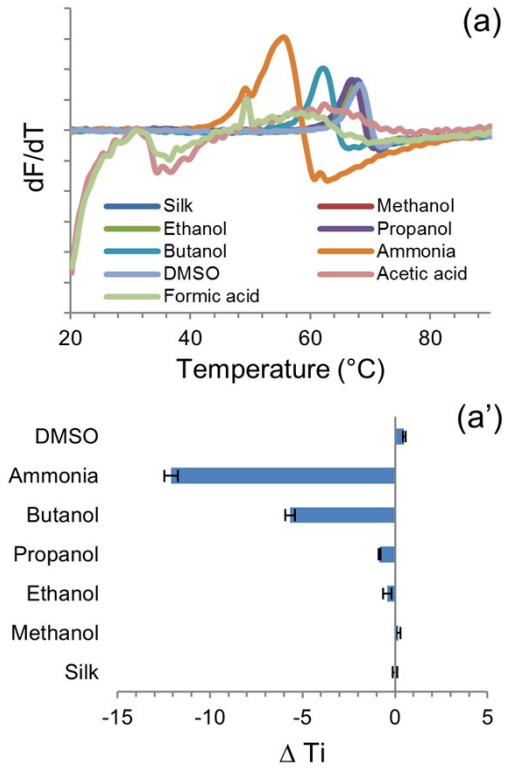

Figure S7. The first derivative fluorescence spectra (a) and change in *Ti* (a') of NSF in presence of protic solvents. The calculation of change in *Ti* of NSF in presence of acetic acid and formic acid was not possible.

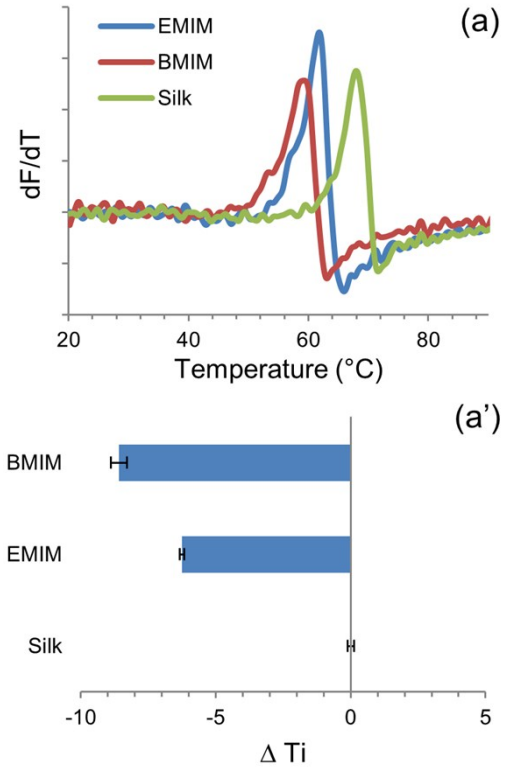

Figure S8. The first derivative fluorescence spectra (a) and change in *Ti* (a') of NSF in presence of ionic liquids.

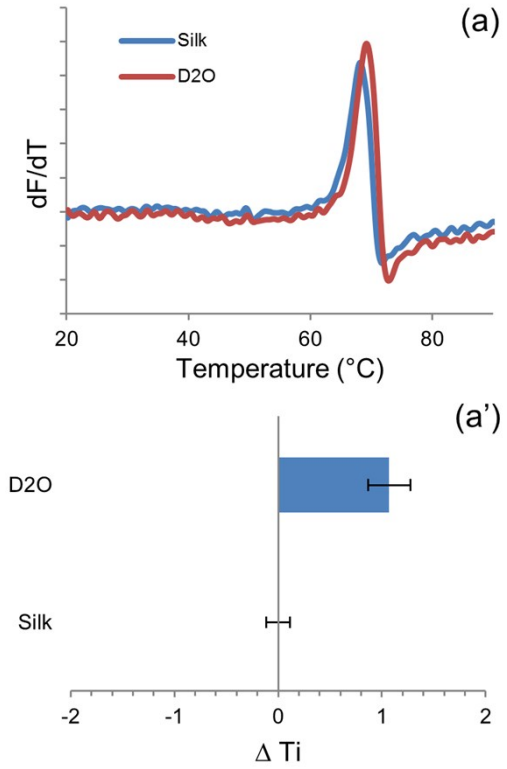

Figure S9. The first derivative fluorescence spectra (a) and change in *Ti* (a') of NSF in presence of deuteriated water.

1. Vollrath, F., et al., *Differential Scanning Fluorimetry provides high throughput data on silk protein transitions.* Sci. Rep., 2014. **4**.