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# In vitro Protein Folding and Stability Using Thermostable Exoshells

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**Abstract :** Folding and stabilization of recombinant proteins remain a consistent challenge for industrial and therapeutic applications. Proteins derived from thermophilic bacteria often have superior expression and stability qualities. To develop a generalizable approach to protein folding and stabilization, we tested the hypothesis that wrapping a thermostable exoshell around a protein substrate would aid folding and impart thermostable qualities to the internalized substrate. To test the effect of internalizing a protein within a thermostable exoshell (tES), we tested in vitro folding and stability using green fluorescent protein (GFPuv), horseradish peroxidase (HRP) and renilla luciferase (rLuc). The 8nm interior volume of a thermostable ferritin assembly was engineered to accommodate foreign proteins and either present a positive, neutral or negative interior charge environment. We further engineered the tES complex to reversibly assemble and disassemble with pH titration. Template proteins were expressed as inclusion bodies and an in vitro folding protocol was developed that forced proteins to fold inside a single tES. Functional yield was improved 100-fold, 100-fold and 150-fold with use of tES for GFPuv, HRP and rLuc respectively and was highly dependent on the internal charge environment of the tES. After folding, functional proteins could be released from the tES folding cavity using size exclusion chromatography at pH 5.8. Internalized proteins were tested for improved stability against thermal, organic, urea and guanidine denaturation. Our results demonstrated that thermostable exoshells can efficiently refold and stabilize inactive aggregates into functional proteins.

**Keywords :** thermostable shell, in vitro folding, stability, functional yield
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