

**Stony Brook University
The Graduate School**

Doctoral Defense Announcement

Abstract

Folding of α -Helical Proteins and Protein Design Using Non-coded Amino Acids

By

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A major goal of protein design is to enhance protein stability. Replacement of helix C-capping glycines which have positive ϕ angles by D-alanine is shown to be a new strategy to increase protein stability. A series of small helical proteins have been targeted and the substitutions stabilize the proteins by 0.6 to 1.2 kcal/mol. Such substitutions can decrease the conformational entropy of the unfolded state without introducing any strain into the native structure. D-valine and D-alanine variants were compared to test for desolvation effect and the results showed that the backbone desolvation has an unfavorable effect on protein stability but is not as large as the favorable entropy effect.

The villin headpiece, HP67, is a small globular protein that consists of two subdomains; the N-terminal subdomain and the C-terminal subdomain, which form an extended hydrophobic core. A salt bridge between E39 and K70 links the two subdomains and is buried in the hydrophobic core. The contribution of this salt bridge to the structure and stability of HP67 was studied by characterizing a series of variants (HP67E39Q, HP67E39L, HP67K70M and HP67E39QK70M). One-dimensional and ^1H - ^{15}N HSQC NMR were used to characterize the structure of the variants and the N-terminal subdomain was found to be partially unfolded in all cases. Thus the buried salt bridge is indispensable to ensure a correct fold for the N-terminal subdomain.

The contribution of electrostatic interactions to protein folding and stability has been studied in the N-terminal domain of ribosomal protein L9 (NTL9) by changing the solvent ionic strength. The Leffler plot of ΔG^\ddagger , the change of the activation free energy upon perturbation, vs. ΔG^0 , the change of the free energy of folding, is linear ($r^2 = 0.918$) with slope equal to 0.45. The relatively low value of the slope indicates that the ionic strength dependent interactions are modestly developed in the transition state.

Date: December 11th, 2007

Time: 12pm

Place: Chemistry, Rm 412

Program: Chemistry

Dissertation Advisor: Daniel P. Raleigh