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# Structural and biochemical characterizations of an intramolecular tandem coiled coil protein

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ABSTRACT

Coiled coil has served as an excellent model system for studying protein folding and developing protein-based biomaterials. Most designed coiled coils function as oligomers, namely intermolecular coiled coils. However, less is known about structural and biochemical behavior of intramolecular coiled coils where coiled coil domains are covalently linked in one polypeptide. Here we prepare a protein which harbors three coiled coil domains with two short linkers, termed intramolecular tandem coiled coil (ITCC) and characterize its structural and biochemical behavior in solution. ITCC consists of three coiled coil domains whose sequences are derived from Coil-Ser and its domain swapped dimer. Modifications include positioning E (Glu) residue at "*e*" and K (Lys) at "*g*" positions throughout heptad repeats to enhance ionic interaction among its constituent coiled coil domains. Molecular modeling of ITCC suggests a compact triple helical bundle structure with the second and the third coiled coil domains forming a canonical coiled coil. ITCC exists as a mixture of monomeric and dimeric species in solution. Small-angle X-ray scattering reveals ellipsoidal molecular envelopes for both dimeric and monomeric ITCC in solution. The theoretically modeled structures of ITCC dock well into the envelopes of both species. Higher ionic strength shifts the equilibrium into monomer with apparently more compact structure while secondary structure remains unchanged. Taken together, our results suggest that our designed ITCC is predominantly monomeric structure through the enhanced ionic interactions, and its conformation is affected by the concentration of ionic species in the buffer.



## 1. Introduction

Coiled coil is a common structural domain found in a wide range of proteins, and characterized by the hydrophobic core and the surrounding electrostatic periphery. Coiled coil has also been implicated in a milieu of biological functions, ranging from transcription to amyloid [1]. The fundamental sequence domain of the coiled coil is a heptad repeat which consists of seven residues with the first ("*a*") and the fourth ("*d*") positions being hydrophobic one, specifically leucine. Coiled coils form amphipathic α-helices and the driving forces to hold those helices together are the hydrophobic interactions among residues in "*a*" and "*d*" positions and surrounding electrostatic ones. Such two major interactions form basis for forming oligomers with monomers containing the coiled coil domain arranged in either parallel or anti-parallel fashion. In addition to biological involvement, coiled coil has served as a model system to understand protein folding and design, the latter being extended to protein-based material development including nanotubes, cages and much more [2].

Designed coiled coils have served as an excellent model system to understand the basic principles of protein folding and stability. Most designed coiled coils feature intermolecular coiled coils, often leading to multiple oligomeric states. One of early examples involves coiled coils derived from yeast transcription factor GCN4. Coiled coil peptide from GCN4 shows parallel dimeric arrangement [3]. However, when buried hydrophobic residues were mutated, those mutant GCN4 peptides form trimers and parallel tetramers [4]. Coil-Ser, which has 29 residues with four heptad repeats, forms anti-parallel trimers or helical bundles [5]. By contrast, fewer cases have been reported to have intramolecular coiled coils. A "coiled coil stem loop" design with 56 residues constituting two helices connected by a stem loop led to a monomeric intramolecular anti-parallel coiled coil [6]. Domain swapped dimer (DSD), and domain swapped aggregates (DSAg) have three helix bundles being held by both intermolecular and intramolecular

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interactions: Two helices (domains I and II) are from one polypeptide and the third one (domain III) is from another polypeptide. Redistribution of the "*e*" and "*g*" positions in the heptad repeats presumably drives the topological difference of the starting monomer, which in turn leads to either DSD or DSAg [5,7].

Electrostatic interactions in coiled coil design have served as key regulatory elements to control conformation and oligomeric state of the resulting coiled coils. For instance, a negatively charged 30-residue peptide where E (Glu) occupies the positions "*e*" and "*g*" formed a coiled coil at acidic pH and high salt [8]. A set of 35-residue peptides with K (Lys)–E (Glu) pairs revealed that ionic interactions are critical in forming intra- and inter-helical coiled coil pairs [9]. In this case, salt concentrations modulate protein stability.

We aim to prepare an intramolecular tandem coiled coil (ITCC) which is longer than the coiled coil stem loop so that ITCC can be used as a larger building block in biomaterial construction. Also desired is the property that ITCC could form an oligomer comparable to fibrils in size when necessary, similar to the relationship between DSD and DSAg. To achieve these goals, we construct ITCC containing three coiled coil domains whose sequences are derived from Coil-Ser and DSD. Charged residues in the "*e*" and "*g*" positions are introduced for the purpose of conformational and oligomeric state regulation. We characterize ITCC both structurally and biochemically in solution using combination of molecular modeling, small-angle X-ray scattering, size exclusion chromatography and circular dichroism. Our ITCC construction and characterization will add one more component in the repertoire of coiled coil-based biomaterials.

## 2. Materials and methods

### 2.1. Cloning, protein expression and purification

Primers for PCR were chemically synthesized (Table S1). DNA encoding NZIP was first amplified from the NZIP template by the primers NZ-*Nde*I and NZ-*Bam*HI. The amplified DNA was digested with *Nde*I and *Bam*HI, and cloned into the polylinker region of pET28a (Novagen). The resulting plasmid, pNZIP, was used to clone the second NZIP at *Eco*RI and *Hind*III sites. The second NZIP DNA was amplified with NZ-EK and NZ-Hter: the former contains *Eco*RI and *Kpn*I sites at its 5′-end and the latter contains *Hind*III sites at the 3′-end and a termination codon just after the last codon of the NZIP. This second NZIP DNA was cleaved by *Eco*RI and *Hind*III and ligated to the *Eco*RI/*Hind*III-cut pNZIP. The resulting pNZ-NZ contains an extra *Kpn*I site that would be used for next cloning step for CZIP-coding DNA. Finally, DNA coding for CZIP was amplified with CZ-*Bam*HI and CZ-*Kpn*I and subsequently treated with *Bam*HI and *Kpn*I. The enzyme-cleaved CZIP DNA was ligated to the *Bam*HI/*Kpn*I-cut pNZ-NZ plasmid DNA. The final plasmid, pHis-ITCC, contains the N-terminal His-tag followed by NZIP, CZIP and NZIP in order. GSGG and GTGG linkers were placed between the first NZIP and CZIP, and CZIP and the second NZIP for flexibility, respectively.

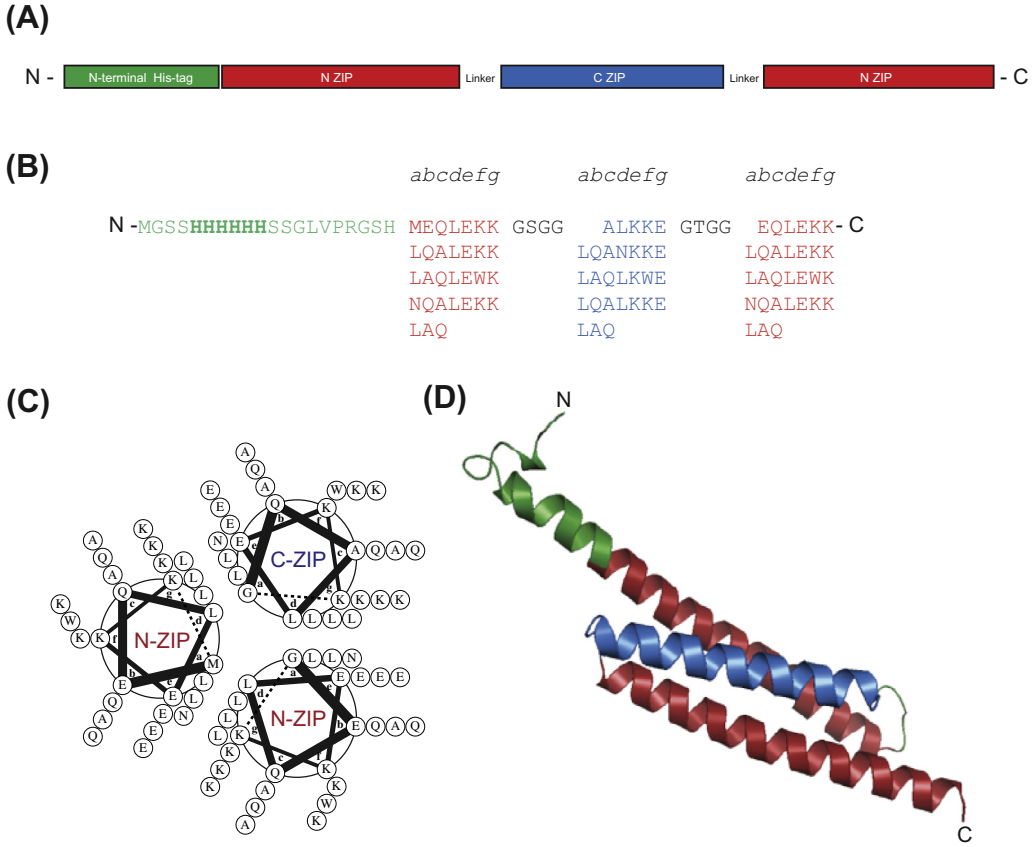


**Fig. 1.** Sequence and structural model of ITCC. (A) Sequence and domain architecture of the ITCC. N-terminal His-tag is colored green, N-ZIP red, and C-ZIP blue. (B) Primary sequence of the ITCC with the same coloring scheme as in (A). Heptad repeats, $(abcdefg)_n$, shown in columns with positions above. Linker sequence is shown in black. (C) Helical wheel diagram of the ITCC generated by *DrawCoil* [21], Note the perfect pairing of acidic residues (Glu) in the "*e*" position and basic ones (Lys) in the "*g*" position. (D) An *rigid body* model of the monomeric ITCC generated by the Robetta server. Ribbon representation of the model was prepared using PyMOL (Schrödinger). Coloring scheme is the same as in (A). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)