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# Nano-mechanical characterization of tension-sensitive helix bundles in talin rod

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ABSTRACT

Tension-induced exposure of a cryptic signaling binding site is one of the most fundamental mechanisms in molecular mechanotransduction. Helix bundles in rod domains of talin, a tension-sensing protein at focal adhesions, unfurl under tension to expose cryptic vinculin binding sites. Although the difference in their mechanical stabilities would determine which helix bundle is tension-sensitive, their respective mechanical behaviors under tension have not been characterized. In this study, we evaluated the mechanical behaviors of residues 486–654 and 754–889 of talin, which form helix bundles with low and high tension-sensitivity, by employing AFM nano-tensile testing. As a result, residues 754–889 exhibited lower unfolding energy for complete unfolding than residues 486–654. In addition, we found that residues 754–889 transition into intermediate conformations under lower tension than residues 486–654. Furthermore, residues 754–889 showed shorter persistence length in the intermediate conformation than residues 486–654, suggesting that residues 754–889 under tension exhibit separated α-helices, while residues 486–654 assume a compact conformation with inter-helix interactions. Therefore, we suggest that residues 754–889 of talin work as a tension-sensitive domain to recruit vinculin at the early stage of focal adhesion development, while residues 486–654 contribute to rather robust tension-sensitivity by recruiting vinculin under high tension.



## 1. Introduction

Focal adhesion-mediated physical connection between the intercellular actin cytoskeleton and the extracellular matrix (ECM) is crucial in a variety of cellular processes such as cell growth, motility, and differentiation [1–4]. The architecture of focal adhesions exhibits sophisticated molecular layers of integrin, paxillin, talin, vinculin, zyxin, VASP, α-actinin, and so on [5]. As the size of focal adhesions corresponds to intercellular tension [6], focal adhesions are endowed with a feedback system to remodel their molecular architecture by reacting to intercellular tension. The tension-induced architectural remodeling is modulated by the tension-sensitivity of talin to recruit vinculin [7–10], which associates with actin filament [11]. The tension-sensing domain, called as a rod domain, forms helix bundles in series, some of which contain cryptic vinculin binding sites (VBSs) [12–14]. VBS-containing helix bundles unfurl under tension to expose the VBSs [15–18]. Thereby, their mechanical stabilities would be critical for determining which helix bundle is sensitive or insensitive to tension. According to the previous study [19], N-terminal helix bundles in a talin rod (residues 482–911) form a particularly tension-sensitive region. Furthermore, the tension sensitivities of the N-terminal helix bundles are known to vary according to their thermal instabilities [20]; thermally instable residues 754–889 exhibit higher tension-sensitivity than residues 486–654. However, little is known about the nano-mechanical behaviors of tension-sensitive helix bundles under tension that direct the tension-sensitivity.

Nano-tensile testing, or single-molecule force spectroscopy, is a powerful tool for investigating the forces and motions associated with biological molecules and enzymatic activities [21,22]. In nano-tensile testing using atomic force microscopy (AFM), biomolecules modified on the substrate are directly tested by the AFM tip. The force $F$ versus extension $\Delta L$ curves obtained in the testing contain a lot of valuable information about the mechanical behaviors of

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biomolecules under tension, such as their mechanical stabilities, unfolding pathways with multiple intermediate states, and mechanical properties as a polymer chain [23–26]. This technique is thus occasionally described as “mechanical fingerprinting” [27]. AFM nano-tensile testing has been utilized to explore the mechanical behaviors of α-catenin [28], a tension-sensor at adherens junctions recruiting vinculin under tension, similarly to talin.

In this study, we employed AFM-based nano-tensile testing to characterize the nano-mechanical behaviors of residues 486–654 and 754–889 of talin, which form helix bundles with low and high tension-sensitivities. Based on the force curves obtained in nano-tensile testing, we evaluated 1) the unfolding energy for the complete unfolding of talin molecules, 2) the unfolding force required to transition to intermediate conformations, and 3) the persistence length, which describes the flexibility of intermediate conformations as a worm-like chain (WLC) polymer.

## 2. Materials and methods

### 2.1. Protein purification

In this experiment, we examined residues 486–654 and 754–889 of talin. The tension-sensitive rod domain of talin, as shown in Fig. 1a, consists of serially connected helix bundles (purple, cyan, and green boxes). Some of the helix bundles contain cryptic VBSs (magenta ellipses) inside their tertiary structures. The three dimensional structure of helix bundles formed by residues 486–654 and 754–889 are shown in Fig. 1b [13,14]. DNA fragments of human talin (residues 486–654 and 754–889) were amplified by PCR and cloned into the pGEX6P-3 vector (GE Healthcare). The plasmids were verified by DNA sequencing and transformed into *Escherichia coli* strain BL21Star (DE3) cells (Invitrogen). BL21Star cells, expressing talin molecules at 20 °C in Luria–Bertani medium with 0.1 mM isopropyl-β-D-thiogalactopyranoside, were suspended in 20 mM Tris-HCl buffer (pH 8.0) containing 150 mM NaCl and disrupted by sonication. After ultracentrifugation, the supernatant was applied to a Glutathione Sepharose 4B column (GE Healthcare) to allow the binding between glutathione and GST-tag at the N-terminus of talin molecules. Eluted proteins were further purified by anion exchange (HiTrap Q HP, GE Healthcare) and then gel filtration chromatography (Superdex 200 pg, GE Healthcare).

### 2.2. Chemical modification for glass substrates and AFM tips

For nano-tensile testing using AFM, glass substrates and AFM tips were treated in a chemical modification process, as illustrated in Fig. 1c. The glass substrates were modified with talin at its C-terminus and the AFM tips were modified with glutathione, which associates with the GST-tag of talin at its N-terminus. The glass substrates were cleaned in a plasma cleaner and treated for 15 min with 2% MPTMS/ethanol, a silanization agent, to introduce thiol groups into the glass surface. The substrates were then treated with 2 mM maleimide-C3-NTA (Mal-C3-NTA; DOJINDO Lab.)/PBS for 30 min, with 10 mM $NiCl_2$ (Wako Pure Chemical Industries)/Milli-Q for 30 min, and washed with PBS. Talin fragments (100 μM for each fragment) were modified by NTA-$Ni^{2+}$-$His_6$ binding for 1 h and finally washed with working buffer (10 mM HEPES, 150 mM NaCl, pH 7.2). Silicon nitride AFM tips (OMCL-TR400PSA-1; spring constant, 0.02 N/m, Olympus Co.) were first cleaned in a plasma cleaner and treated for 15 min with 2% APTES/ethanol, a silanization agent, to introduce amine groups into the glass surface. The AFM tips were then treated with 1.5 mM Mal-PEG-NHS ester/PBS for 30 min and with 10 mM glutathione/PBS for 1 h. The remaining maleimide was quenched with 50 mM 2-mercaptoethanol/HEPES and finally washed with working buffer.

### 2.3. AFM nano-tensile testing

For nano-tensile testing using AFM, we approached the talin-modified glass substrate with the glutathione-modified AFM cantilever and retracted the cantilever to load talin molecules directly. For this approach, we set a sampling grid on the substrate with an interval of >100 nm, so that every molecule was tested at only one time. The piezo moving speed was kept constant at

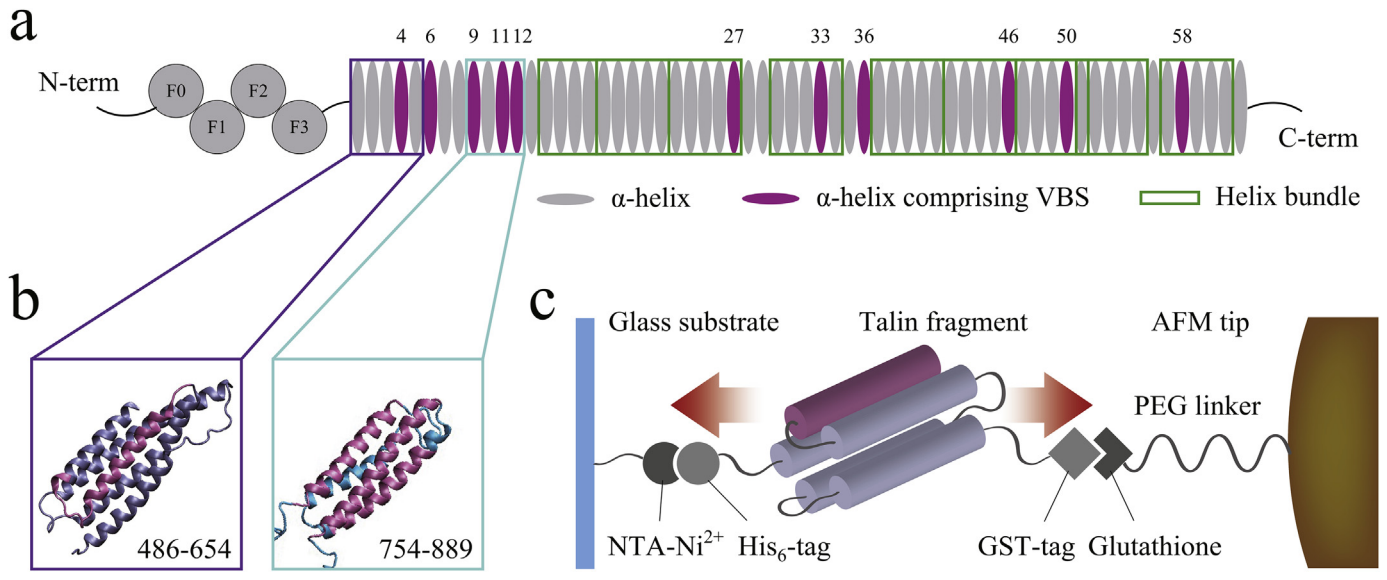


**Fig. 1. Nano-tensile testing of helix bundles in talin rod employing AFM. (a)** The structural domains of talin. The tension sensitivity of talin is modulated by a central rod domain, which consists of 62 α-helices (gray and magenta ellipses). Most vinculin binding sites (VBSs; magenta ellipses) are embedded in helix bundles (purple, cyan and green boxes), which are formed by four or five amphipathic α-helices based on hydrophobic interaction. **(b)** Crystal structures of residues 486–654 (left panel, PDB code: 1SJ7) and 754–889 (right panel, PDB code: 1U89) of talin. In both panels, VBSs are shown in magenta. **(c)** Schematics of nano-tensile testing employing AFM. Single talin molecules, which were chemically modified on the glass substrate by $His_6$-$Ni^{2+}$-NTA affinity, were captured by the AFM tip using GST-glutathione affinity and directly loaded. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)