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ICMRBS 2018 participation report

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I participated in the XXVIII International Conference on Magnetic Resonance in Biological System (ICMRBS) in Dublin, which was held from August 19-24, 2018. This travel was partially supported by young researchers travel expenses subsidy from Nuclear Magnetic Resonance Society of Japan. In this opportunity, I presented a poster, which exhibited my research about a spider silk protein in RIKEN. My poster (number :P255) had a title: Conformation and dynamics of soluble prefibrillar repetitive domain of spider dragline silk proteins: insights into β-sheet formation.

Spider dragline silks are fibrous proteins, which are well known for their superior mechanical properties, biodegradability and biocompatibility. These proteins consist of conserved N-terminal (NTD) and C-terminal (CTD) domains separated by a long repetitive domain. The repetitive domain mainly consists of polyalanine region (4-12 alanine residues) and glycine-rich region (-GGX-, where X can be Y, L, Q, P). In silk fiber, the polyalanine region forms β-sheet structure, which is a key structure underlying strong mechanical properties of spider silk **(Keten, S, et al. Nat. Mater, 2010).**

Before spinning, spider silk proteins (spidroins) are stored at high concentration in the spinning dope and they are transformed into insoluble fiber after passing pH- and ions-gradient across the spider gland. Spinning dope contains high concentration of chaotropic ions (Mg^{2+}, Na^{+}, Cl^{-}) and pH in this region is close to neutral (pH ~7), while closer to spinning duct, chaotropic ion concentrations and pH decrease (pH ~5) and kosmotropic ion (PO_4^{3-}) concentration increases **(Andersson, M, et al, PLOS Biol, 2014; Knight, D.P and Vollrath, Naturewissenschaften, 2001).** NTD and CTD of spidroin display strong pH-dependence and are essential for controlling the pH-dependent of fiber assembly **(Askarieh G, et al, Nature, 2010; Hagn F et al, Nature, 2010**). Despite the essential role of NTD and CTD in spider silk self-assembly, conformation and dynamics of the soluble repetitive domain, which dominate the length of protein chain, at different pH and ions are still poorly understood.

In this study, I reported the conformation and dynamics of soluble repetitive domain of *Nephila clavipes* spider dragline silk in different repeat units, pH, temperature, and ions using solution-state NMR, far-UV circular dichroism (far-UV CD) and vibrational circular dichroism (VCD). At pH 7 and temperature 10°C, the soluble repetitive domain of spider dragline silk consist of two major populations, which are ~65% random coil and ~24% PPII helix. PPII helix conformation is distributed over the glycine-rich region, which showed more limited flexibility compared to polyalanine region. The PPII helix in the glycine-rich region is proposed as a soluble prefibrillar, which can subsequently undergo intramolecular interaction (**Oktaviani, N.A et al, Nat. Commun, 2018**). Contrary to CTD and NTD, conformation of soluble repetitive domain is pH independent, indicating that the prefibrillar form of the repetitive domain is not influenced by pH (**Oktaviani, N.A et al, Nat. Commun, 2018)**. Furthermore, our NMR data also demonstrated that chaotropic ions prevent intra- and inter-molecular interactions, which explains the role of chaotropic ions in improving the solubility of spidroin. In contrast, in the presence of high concentration of kosmotropic ion (PO_4^{3-}), β-sheet propensity in the polyalanine region increases. This finding is supported by slower motion of the repetitive domain in the presence of high concentration of phosphate buffer. Together, our study provides the insight into β-sheet formation of spider silk protein.

It was a great opportunity for me to attend this conference because I could share my research progress and at the same time, I could learn about many recent progress from NMR society, which could give me some ideas for my future research.