Canadian Association of Physicists
Association canadienne des physiciens et physiciennes

Contribution ID: **2536** Type: **Oral Competition (Graduate Student) / Compétition orale (Étudiant(e) du 2e ou 3e cycle)**

Atomic force microscopy reveals how structural variations impact the flexibility of collagen

Tuesday, 4 June 2019 08:45 (15 minutes)

Collagen, the most abundant protein in mammalian organisms, is responsible for the cohesion of tissues and organs. It is a major structural component of our extracellular matrix, contributing to the mechanical stability, organization, and shape of a wide variety of tissues. Many collagen types have been reported in humans, all of which are triple-helical proteins that assemble into distinct higher-order organizational structures. To date, there has been greater focus on characterizing the more abundant fibrillar collagen types, leaving the mechanical properties of network-forming collagen type IV comparatively understudied. A key feature that differentiates fibrillar collagens from collagen IV lies in the characteristic triple-helical defining (Gly-X-Y)n sequence of the collagenous domain, where collagen IV has intrinsic discontinuities in its sequence. The role and structure of these interrupted Gly-X-Y regions remains unknown; however, it has been suggested that they play a role in the flexibility of the collagen molecule. To address this question, we used atomic force microscopy (AFM) to sample the two-dimensional conformations adopted by collagen on mica and performed statistical analysis to calculate its persistence length –a mechanical property that is used to quantify the flexibility of a polymer. By assuming homogeneous flexibility across the length of the molecule, we found the persistence length of collagen IV to be less than half of that of the continuously triple-helical fibrillar collagens. To investigate local sequence variations, we developed an algorithm that determines position-dependent persistence length profiles. We found significant variations in persistence length along the contour of the collagen IV molecule, where regions of higher flexibility correlated strongly with interrupted Gly-X-Y regions in its amino acid sequence.

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Session Classification: T1-8 Topics in medical physics and biophysics (DPMB) | Sujets en physique médicale et biophysique (DPMB)

Track Classification: Physics in Medicine and Biology / Physique en médecine et en biologie (DPMB-DPMB)