## Structure determination of the collagen and silk-type environments in the blue mussel *Mytilus edulis* byssal threads

Alexandre A. Arnold and Isabelle Marcotte Department of Chemistry, Université du Québec à Montréal, Montréal, Québec marcotte.isabelle@ugam.ca

Mussels attach themselves to substrates on the seashore via extra-corporeal threads called byssus. These are complex biopolymers essentially made of proteins with unique mechanical properties. Byssal threads are composed of three distinct regions: an initial corrugated and elastic proximal part followed by a smooth and stiff distal part and finally the plaque that attaches the animal to the substrate. The core of the fibre is composed of three pepsin resistant proteins, PreCol-P, PreCol-D and PreCol-NG. Each of these proteins is composed of a collagen centre flanked by elastin-like (PreCol-P), silk-like (PreCol-D) or plant cell wall-like (PCW) (PreCol-NG) regions [1].

In order to correlate the molecular structure of the constituents of this fibre to its macroscopic mechanical properties, the molecular structure of byssal threads of the blue mussel Mytilus edulis was studied by high-resolution solid-state NMR. Measurements were carried out at high field (21.1 T) in order to resolve resonances in the different proteins constituting the fibre. <sup>13</sup>C-<sup>13</sup>C dipolar correlation spectra were recorded and the two-dimensional spectra provided sufficient resolution to assign the various spin systems to the corresponding amino acids (Figure 1). This unique material proves to be well ordered at a molecular level despite its composition heterogeneity as evidenced by the very narrow measured <sup>13</sup>C linewidths below 1.5 ppm. The spectra are dominated by residues in collagen environments as determined from the chemical shift analysis. Chemical shift predictions were carried out using the software *Shift* X and the torsion



**Figure 1**: Ca-C $\beta$  region of a <sup>13</sup>C-<sup>13</sup>C dipolar correlation spectrum recorded at 21.1 T with <sup>13</sup>C-labelled byssal threads. Ca-C $\beta$  correlation peaks and their corresponding amino-acid type assignment are indicated. In the case of alanine, amino-acids present in collagen or silk-type regions can be distinguished.

angles of the collagen regions thus determined [2]. The most abundant glycine and alanine residues can be resolved in up to three different environments. Intense alanine peaks were detected with chemical shifts consistent with an anti-parallel  $\beta$ -sheet structure which are assigned to silk-like regions. This result was further confirmed by the short T<sub>1</sub> <sup>13</sup>C relaxation time measured for the corresponding alanine C<sub> $\beta$ </sub> resonance. Our results, thus, determine the structure of collagen and silk-type domains in byssal threads and provide a detailed assignment of minor residues in collagen helices.

*References:* [1] H. Waite et al., *Phil. Trans. R. Soc. Lond. B* **357** (2002) 143-153. [2] S. Neal et al., *J. Biomol. NMR* **26** (2003)215-240.