s1.m5.p17 **The X-Ray Diffraction Structure of a Glycosyl Hydrolase Family 32 protein: 1-Fructan Exohydrolase IIa of Cichorium Intybus.** Maureen Verhaest,[a] Wim Van den Ende,[b] Camiel De Ranter,[a] André Van Laere[b] and Anja Rabijns[a], [a]*Laboratorium voor Analytische Chemie en Medicinale Fysicochemie, K.U. Leuven, E. Van Evenstraat 4, 3000 Leuven, Belgium, and* [b]*Laboratorium voor Moleculaire Plantenfysiologie, K.U.Leuven, Kasteelpark Arenberg 31, 3001 Heverlee, Belgium. E-mail: Anja.Rabijns@pharm.kuleuven.ac.be*

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About 15% of flowering plants use fructans, fructose based oligo- or polysaccharides, as a storage carbohydrate instead of starch or sucrose [1]. In addition, fructans are recognized to enhance cold and drought tolerance of plants. Food industries are interested in fructans for their different health-promoting effects. However, fructan degradation causes an important drawback for industrial harvesting. Fructan exohydrolase (FEH), a glycosyl hydrolase, catalyzes this breakdown. FEH belongs to family 32 of the glycosyl hydrolases, a classification based on general amino acid sequence similarities [2]. At present, no structural information is available for any member of this family. Therefore, elucidating the structure of FEH can not only contribute to a better understanding of its catalytic mechanism, but can also provide a model for the other enzymes of family 32. Consequently, 1-FEHIIa from Chicory (*Cichorium intybus*) [3] has been crystallized using the hanging drop vapor diffusion method [4]. The structure was determined by the Single Anomalous Dispersion method to a resolution of 2.35 Å. The crystals are tetragonal, belonging to space group $P4_12_12$. The structure comprises an N-terminal five fold β-propeller domain followed by a second domain existing of 2 β-sheets. The active site is located completely in the β-propeller structure and is formed by highly conserved amino acids within the glycosyl hydrolase family 32. The 2 β-sheets domain is thought to be important for fructan binding.

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s1.m5.p18 **Structural organization in siliceous spicules from marine sponges.** Croce G.[1], Viterbo D.[1], Milanesio M.[1], Amenitsch H.[2], [1]*DISTA, Univ. del Piemonte Orientale P.za G. Ambrosoli 5, I-15100 Alessandria, Italy;* [2]*Inst. of Biophysics, Austrian Acad. of Science, Schmiedlstr. 6, A-8043 Graz, Austria. E-mail: davide.viterbo@mfn.unipmn.it*

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The biological formation of skeletal structures formed by amorphous hydrated silica is called biosilicification and occurs in a wide variety of organisms. In particular siliceous sponges deposit hydrated silica in needle-like spicules and other structural patterns. Siliceous spicules are produced within specialized cells (sclerocytes) and contain an organic axial filament, which functions as template for silica deposition.This presentation deals with the fiber diffraction structural study of the organization of the axial filaments of monoaxonic spicules from different sponges, carried out by diffraction experiments using a small angle X-ray scattering (SAXS) setup with synchrotron radiation. We carried out our measurements on a number of species belonging to two different sponge families: the Demosponges *P. ficiformis*, *G. cydonium*, and *T. aurantium* and the Hexactinellid *S. joubini.* All these samples present needle-like spicules with a length of some mm. This allowed the collection of fiber diffraction patterns from a bundle of almost parallel spicules. The diffraction patterns show diffraction spots sharper than what can be expected from a regular polymeric fiber, indicating that the protein (silicatein) units in the spicule axial filaments must form highly ordered patterns. As also indicated by TEM investigations, the protein units are packed in a compact hexagonal way. The diffraction patterns of different samples are quite different and the most relevant result, from a phylogenetic point of view, is that all Demosponges have a common hexagonal lattice period a = 58Å, while the Hexactinellid has a longer period a = 84Å. The analysis of the position and distribution of the spots reveals different possible bi- and tri-dimensional arrangements of protein units along the main axis of the spicules.A part from fiber diffraction, the study was carried out also by a unique combination of other techniques such as SEM, thermogravimetry, FTIR. All our results suggest the following possible mechanism for the biosilification process in spicules. The initial step consists in the formation of a very ordered disposition of the silicatein units, forming a regular mesoporous arrangement in a silica matrix, similar to that found in some synthetic materials. In a second step the biosilification process should continue with a deposition of amorphous silica on the outer walls of the mesoporous filament.