# Biosilica Synthesis in Sponges: New insights in the biology/chemistry and application in nano-biotechnology

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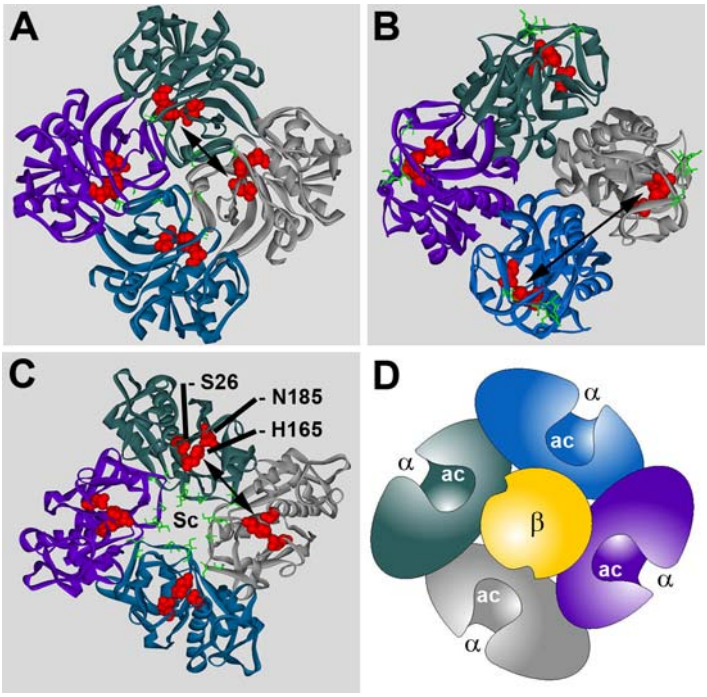
Biomineralization, biosilicification in particular (i.e. the formation of biogenic silica, $SiO_2$), has become an exciting source of inspiration for the development of novel bionic approaches following “nature as model”. Siliceous sponges are unique among silica forming organisms in their ability to catalyze bio-silica, a material with increasing applied potential in bio-medicine and bio-optics. The sponges (phylum Porifera; diploblasts), as the phylogenetically oldest metazoan phylum, are characterized by a distinct body plan, which is organized – in contrast to that of most other, higher metazoan phyla – along only one body axis. It runs in apical-basal direction, while most triploblastic metazoans are organized along an antero-posterior and a dorso-ventral axis. Surely the form and hence the body plan of sponges is genetically controlled, since the size and shape of a given species are defined. In the last years a bulk of information became available about the genetic basis of cell-cell and cell-tissue interactions in sponges, e.g. about the integrin system, the differentiation of cells, e.g. stem cells, the axis formation of the body, e.g. the Wnt pathway, or the immune system, e.g. cytokines or immunoglobulin. Most of the studies were performed with the demosponge *Suberites domuncula*.

The siliceous spicules of sponges (Porifera) show great variations of sizes, shapes and forms; they constitute the chief supporting framework of these animals; these skeletal elements are synthesized enzymatically by silicatein. Each sponge species synthesizes at least two silicateins, which are termed –α and –β. In the present study, using the demosponge *Suberites domuncula*, we studied if the silicateins of the axial filament contribute to the shape formation of the spicules. For these experiments native silicateins have been isolated by a new Tris/glycerol extraction procedure. It is shown that the Tris/glycerol extracted silicateins are monomeric (24 kDa), but readily form dimers through non-covalent linkages. The Tris/glycerol extracted silicateins show a considerable proteolytic activity that increases during the polymerization phase of the protein. The assembled silicateins (dimers, tetramers as well as hexamers) can be demonstrated in zymograms. The filament/aggregate formation from disassembled silicatein can be visualized by both light and transmission electron microscopic (TEM) analyses. Since in *S. domuncula* silicatein-α is 4-times more abundant in the axial filament than silicatein-β we propose that four silicateins form a platform with serine clusters directed to the center. These serines of the con-axially arranged silicateins interact with silicatein-β. We conclude that the silicateins re-assemble initially chaotically, and, in the second phase assemble to fractal-like structures, which subsequently form the filaments.

The data presented will contribute to a controlled enzymatic synthesis of biosilica, applicable for biomedical use.




Schematic representation of one planar silicatein-α tetramer (α; green-grey-blue-pink); with one silicatein-β (β in the center [yellow]). It is proposed that the orientations of the silicatein units are identical in each of the three configurations. The amino acids involved in the active centers of the silicateins (the amino acids are counted within the mature molecule) are marked; serine (S26), histidine (H165) and asparagine (N185). (**A**) The silicatein molecules are oriented with their active centers towards the center of the axis (syn-axial), or (**B**) in the opposite direction of the axis (anti-axial), or, (**C**) the entrances of the con-active centers are located in the plane of the tetramer (con-axial). Only in the axial-orientation the Ser-rich clusters (Sc) are directed towards the center of tetramer. The double arrow is showing the distance between one pair of histidines between two silicatein-α molecules (H165). (**D**) Graphical model of the silicatein-α tetramer with the silicatein-β in the center. The active centers in the silicatein-β molecules are marked (ac). (**D** and **F**) The distortion of the silicatein-β molecules within the tetrameric plane is illustrated.

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