the biological crystallization pathway given by a liquid amorphous precursor (PILP), a phase intermediate to the solid ACC precursor phase proposed by Addadi.

Liquid PILP droplets coalesce to patches. Nucleation starts after about 24 hours of PILP formation with tablet crystals with hexagonal shapes and segment assemblages forming within the PILP patches. EBSD analysis shows that c*-axis orientation of the crystals is only slightly out of the plane of growth (by about 5-10 degrees), *a**-axes orientation is almost perpendicular to the plane of growth. For the calcite phase in the given orientation the hexagonal shape is surprising and is produced despite symmetrically not equivalent facets. Thus, crystal shape is also determined by the given space allowance during growth: here a two-dimensional thin film. Assuming that [0001] is the fastest direction of growth, then the largest crystals that form have the [0001]-direction within the plane of the PILP. AFM studies show that these crystals consist of an internal mesostructure consisting of nanoparticles and the hexagonal shape is due to the 2D aggregation of nanoparticles and not to atomic-scale crystal growth of calcite. In addition, we observed calcite crystallite formation from natural ACC in the shell of the modern brachiopod *Megerlia truncata*. We found an unhydrated amorphous domain in direct contact to a solid inclusion that has been incorporated into the shell. The ACC phase has been used as a precursor prior to shell calcite crystallization during shell repair [5]. Under TEM conditions this ACC domain crystallizes to vaterite and calcite [5]. Crystallization starts at the border of a fiber at the organic membrane lining of the fiber [5]. By keeping the electron beam on the amorphous shell region crystals develop in situ on both sides of the fiber, grow towards the center and meet along a growth front in the center of the fiber. The newly formed crystallites highly resemble in morphology, habit and texture the crystallites that form the primary shell layer of *M. truncata* [5].

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Keywords: ACC, PILP, biomimetic calcite growth

MS.35.5

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Crystallization in gels and microgravity: a comparative study

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For crystals grown from solution it is generally accepted that a diffusive mass transport is beneficial for crystal quality as it promotes a slow and regular supply of growth units from the solution to the crystal interface. Microgravity and gels are two methods that have been used to reduce convection in protein crystallization experiments. While microgravity provides a chemically cleaner environment than gels, the associated noise in the microgravity of orbital facilities prevents a convection-free environment in space experiments. In addition, recent studies have shown [1] that gels can even be helpful in reducing impurity effects during protein crystal growth.

In this work we present the results of a comparison of the quality of several model and non-model protein crystals grown in microgravity on the un-manned spacecraft Foton, and in gels on ground. The experiments were performed with the counterdiffusion technique using the Granada Crystallization Facility-2 [2]. To ensure the validity of the comparison critical parameters like protein common batches, thermal history, duration of the experiments and diffraction data collection were carefully controlled. Our results show: 1) that crystals obtained in these experiments are of the highest quality as compared with those obtained in classical techniques, and 2) no statistically clear difference in crystal quality between growth in gels on Earth, and in the excellent microgravity environment on board of Foton, was observed for the studied proteins.

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Keywords: crystallization, gel, microgravity

MS

MS.36.1

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Structure basis of ligand−receptor interaction in the IL-1 family of cytokines

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Interleukin 1 (IL-1) is a family of cytokines consisting of IL-1 α, IL-1 β, IL-1 receptor antagonist (IL-1Ra), IL-18, IL-33 and IL-1F5−IL-1F10, which play significant roles in inflammation and immune regulation [1]. The IL-1 family members interact with ligand-binding receptor (α chain) and co-receptor (β chain) on the surface of target cells, resulting in the formation of a cytokine/receptor ternary complex necessary and sufficient for triggering intracellular signaling [2]. IL-1 β is a prototype member in the IL-1 family. It first binds the ligand-binding α chain−IL-1 receptor type I (IL-1RI) and then recruits co-receptor β chain−IL-1 receptor accessory protein (IL-1RAcP) to form a signaling IL-1 β/IL-1RI/IL-1RAcP ternary complex. The co-receptor IL-1RAcP is also utilized by other agonists in the IL-1 family including IL-1 α, IL-33, IL-1F6, IL-1F8, and IL-1F9 as a necessary receptor chain in their signaling complexes. The IL-1 β signaling is negatively regulated by native antagonist IL-1Ra that competitively binds IL-1RI and further inhibits the association of IL-1RAcP. The decoy receptor IL-1RII also inhibits IL-1 β activities by binding IL-1 β and IL-1RAcP to form a non-signaling IL-1 β/IL-1RII/IL-1RAcP ternary complex.

We recently determined the structure of non-signaling IL-1 β/IL-1RII/IL-1RAcP complex at a resolution of 3.3 Å. The extracellular regions of IL-1RII and IL-1RAcP have a similar domain organization as that of IL-1RI, consisting of three Ig-like domains (D1−D3). However, structural analysis showed that the linker between D2 and D3 domains in IL-1RAcP is not as flexible as that in IL-1RI and IL-1RII, which may prevent IL-1RAcP from binding ligand in the absence of ligand-binding α chain. IL-1RII inhibits the binding between IL-1 β and IL-1RI by directing blocking sites I and II on IL-1 β for interaction with IL-1RI. The IL-1 β −IL-1RII interaction generates a composite surface contributed by both IL-1 β and IL-1RII to associate with IL-1RAcP. Biochemical analysis demonstrated that preformed IL-1 β/IL-1RI and IL-1 β/IL-1RII complexes bind IL-1RAcP in a similar manner, supporting that the signaling IL-1 β/IL-1RI/IL-1RAcP complex has a similar architecture. It also showed the importance of two loops of IL-1Ra in determining its antagonism. These results together provide a structural basis for assembly and activation of IL-1 β with its receptors and offer a general cytokine/receptor architecture that governs the IL-1 family of cytokines.

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Keywords: cytokine, receptor, structure

MS.36.2

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Bacterial cell wall degradation by a staphylococcal autolysin

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Treatment of hospital-acquired Staphylococcus infections remains a challenge as the number of strains with multiple antibiotic resistances has increased steadily over the last decades. This emphasizes the need for a new class of antibiotics that act on new targets. The major autolysins AtlE and AtlA of *S. epidermidis* and *S. aureus*, respectively, represent such targets. Both enzymes take over pivotal roles in cell division where they are responsible for the separation of daughter cells.

The high-resolution structure of AmiE reveals for the first time detailed insights into the enzymatic function of a staphylococcal peptidoglycan (PGN) hydrolase and thereby provides the basis for the formulation of a likely mechanism of catalysis [1]. With the synthesis of PGN fluorescent substrates, a method was established that offers the option to produce substrates of defined length and high purity [1,2]. Easy modifiability of the synthetic substrates also allows probing the influence of amino acid substitutions on substrate recognition. It could be demonstrated that the presence of a third amino acid in the peptide stem as well as the isoform of glutamine in the second position are key motifs and essential for recognition by AmiE. These findings could also be confirmed in a docking model. Structural comparisons with other proteins sharing the amidase-fold reveal common features in the substrate grooves, which points to an evolutionary conserved mechanism of PGN recognition.

Contrary to previous assumptions according to which the cell wall binding region (CBR) of the AtlE amidase contains only two repeat domains, the crystal structure of $R_{3,4}$ reveals the presence of four highly similar domains that belong to the family of SH3b domains. The sequence and structural similarity between every second repeat in the CBR is higher than between adjacent ones. A distinct patch of conserved residues could be located on opposite sides of each repeat in the $R_{3,4}$ tandem repeat. These areas might serve as binding pockets for negatively charged ligands such as teichoic acids. We are currently examining this possibility.

Small angle x-ray scattering (SAXS) experiments of AmiE-R_{1-4} and R_{1-4} reveal that the hinge region between the catalytic domain and the first repeat module is more flexible than the second one separating $R_{1,2}$ from $R_{3,4}$. In conjunction with the available structural data, it is now possible to draw a plausible mechanism of cell wall interaction in which the flexibility of the first linker ensures access of the catalytic domain to several PGN cleavage sites. The second linker serves to separate the two tandem repeats of the CBR. This creates a binding groove in R_3 that can easily be modulated by movement of the RT-loop. In comparison to its counterpart in R_4, the RT-loop of R_3 is not engaged in any contacts with the preceding domain.

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MS.36.3

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Gram-positive bacterial pili: structures, assembly and function

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Bacterial pili are filamentous appendages that are critically involved in adhesion to host cells, leading to colonization of host tissues and establishment of infections. They are built from protein subunits called pilins; the backbone of the pilus is formed by hundreds of copies of a major pilin, while several minor pilins with specialized functions are associated with the backbone. While pili of Gram-negative bacteria such as *E. coli* have been extensively studied, the pili on Gram-positive organisms have been only recently discovered and are fundamentally different in that the pilins are held together by covalent isopeptide (amide) bonds formed by sortase enzymes.

We determined the first atomic structure of a Gram-positive major pilin, Spy0128 from *Streptococcus pyogenes* (Group A Streptococcus; GAS) [1]. The crystal structure showed an elongated molecule comprising two immunoglobulin (Ig)-like domains. The fold of each domain resembles the repeating CnaB domains of the collagen-binding adhesin Cna from *Staphylococcus aureus* and demonstrates a possible evolutionary relationship that links pili to a large family of cell surface proteins involved in binding to the extracellular matrix. In addition, the crystal structure brought two unexpected surprises that brought significantly new understanding of the assembly of Gram-positive bacterial pili. Firstly, the head-to-tail packing of successive molecules in the crystal provides a very persuasive model for pilus assembly, and mass spectrometry of native GAS pili validated this model. Secondly, the structure revealed a previously unknown mechanism for stabilizing proteins, in the form of self-generated intramolecular isopeptide (amide) bonds, which explains the long-known stability of Lancefield T-antigens. We also determined the high resolution crystal structure of a major pilin SpaA from *Corynebacterium diphtheriae* [2]. This reveals a similar modular structure of Ig-like domains, yet with several variations from Spy0128.

Gram-positive bacterial pilin proteins show wide variations in size and sequence, making it difficult to predict structural features based on sequence alone, and X-ray crystallography has played a critical role in elucidating structure and function of Gram-positive pili. There are now several other crystal structures are available, for both major and minor pilins, and these structures clearly point to common principles for many Gram-positive pilus assembly, in which self-generated intramolecular isopeptide bonds complement the sortase-mediated intersubunit bonds. These bonds are strategically located to give strength and stability to the pilin subunits and also facilitate proper assembly of pilus. Within this common theme, however, there are several variations that can account for their functional differences.

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MS.36.4

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Structural investigations of a novel bacterial AB_5 toxin

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