[1] D.K. Smith, *Powder Diffraction* **1998**, *13*, P. 2–19. [2] A. Ilieva, B.Mihailova, Z.Tsintsov, O.Petrov, *American Mineralogist* **2007**, *92,* 1325–1333. [3] E. Fritsch, *European Journal of Mineralogy* **2004,** *16*, 743-752. [4] J. Pense *Zeitschrift Deutsche Geselschaft Edelsteinkunde*. **1964,** *50,* 25-29.

**Keywords: natural silicate, TEM, AFM**

## MS49.P25

*Acta Cryst.* (2011) A**67,** C542

**In situ investigation of aragonite formation in atomic resolution by FM-AFM**

Yuki Araki,[a] Katsuo Tsukamoto,[a] Ryosuke Takagi,[b] Tomoyuki Miyashita,[b] Noriaki Oyabu,[c] Kei Kobayashi,[c] Hirohumi Yamada,[c] [a]*Graduate School of Science, Tohoku University, Sendai.* [b]*Department of Molecular Genetics, Kinki University, Wakayama,* [c]*Graduate School of Engineering, Kyoto University, Kyoto, (Japan).* E-mail: y-araki@s.tohoku.ac.jp

The crystallization by the interaction between organic materials and in organic minerals is called “biomineralization”. The formation of bivalve shell is a biomineralization of calcium carbonate ($CaCO_3$) crystal. Some kinds of bivalve shell consist of calcite and aragonite which are polymorpfs of $CaCO_3$ crystal. Although aragonite is metastable at normal temperature and pressure, some mollusks make aragonite stable by organic matrices [1], [2]. Amino acid sequences of some special proteins were reveal which are contained in the organic matrices of the shell, and it was confirmed that many aspartic acids are included in the proteins [3], [4].

In this study, we carried out in situ observation of aragonite formation by addition of the synthetic polypeptide which consisted of 15 amino acid residues and had periodic arrangement of six aspartic acids [3].

First of all, supersaturated solution of $CaCO_3$ with the synthetic polypeptide and magnesium($\sigma = 0.86$, $[Mg^{2+}] = 0.05$ M, the concentration of the polypeptide = 50 μg/ml) was loaded on the calcite crystal which was cleaved on (10-14) face, then the surface was observed in atomic level. In order to observe the change of the surface pattern in atomic resolution, *in situ* and in solution, the Frequency Modulation Atomic Force Microscopy (FM-AFM) was employed. In this solution, we succeeded to observe the moment of aragonite formation at 90 minutes after the solution was loaded. The boundary of the atomic patterns of calcite and aragonite was observed in 10 $nm^2$, and aragonite and calcite were in the same layer.

Secondly, the concentration of the synthetic polypeptide was lowered to 30 μg/ml. In this solution, some adsorbents were observed on only calcite surface, and they adsorbed along calcium sequence of calcite [010]. The length of adsorbents is 3 - 6 nm. This is close to the length of monomolecular of the synthetic polypeptide.

These results show that calcite transforms to aragonite in the surface layer of seed crystal, and the adsorbents are likely to be the synthetic polypeptide, because aspartic acids in the synthetic polypeptide can bond to calcium atoms on the surface by carboxyl group.

Our results strongly suggest the new model of aragonite formation under normal condition by the transformation.

[1] G. Falini, S. Albeck, S. Weiner, L. Addadi, *Science* **1996**, *271*, 67-69. [2] A.M. Belcher, X.H. Wu, R.J. Christensen, P.K. Hansma, G.D. Stucky, D.E. Moese, *Nature* **1996,** *381*, 56-58. [3] R. Takagi and T.Miyashita, *Zoological Science* **2010**, *27*, 416-426. [4] M. Suzuki, K. Saruwatari, T. Kogure, Y. Yamamoto, T. Nishimura, T. Kato, H. Nagasawa, *Science* **2009**, *325*, 1388-1390.

**Keywords: biomineralization, atomic force microscopy**

## MS49.P26

*Acta Cryst.* (2011) A**67,** C542

**Quick Pt nanocrystal growth and coalescence on exposition to NO**

Zbigniew Kaszkur, *Departament of Catalysis on Metals, Institute of Physical Chemistry, PAS, Warszawa (Poland).* E-mail: zkaszkur@ichf.edu.pl

The morphology of Pt nanocrystals (10% wt., initial crystallite size approx. 3nm) supported on amorphous silica was studied via in situ XRD during treatment at normal pressure in He, $H_2$, $O_2$ and NO. A specially designed experiment and its analysis enabled detecting of subtle changes in XRD pattern that could be interpreted via atomistic simulations. At the same time a composition of the gas phase could be monitored via Mass Spectrometry (MS). Evolution of Pt fcc peaks position, intensity and width proved to be sensitive to a surface effect and we were able to observe and analyze surface reconstruction of Pt nanocrystals on exposition from $H_2$ to He [1]. On exposition to NO at temperature 100$^{O}$C we observe a relatively quick (in a few hours) Pt crystallite growth process. As a result a distribution of an ordered atom column length (closely related to the crystallite size) becomes evidently bimodal with mode size ~3.4nm and ~16.8nm (from 111 reflection). Qualitative analysis (Williamson-Hall plot) revealed only small contribution of microstrain to the peak broadening. Analysis of three reflections 111, 200 and 220 suggests crystal growth process to be anisotropic with preferential growth in 111 direction. During this process MS reveals evolution of $N_2$, $O_2$ and some amount of $N_2O$ with changing rate (the latter present also in a supplying gas pressure bottle).

A precise analysis shows that the peak position also evolves anisotropically during the growth process. For nanoparticles Bragg low is no longer obeyed precisely and the peak position does not point to the interlayer distance [2,3]. The peak position depends on the crystallites size and on the state of its surface evolving with surface relaxation (and chemisorption phenomena). This also results in a slightly shifted contributions to the peak profile originating from smaller and larger atomic column lengths. Such dependence of a peak position with the crystal size makes classical analysis using Warren-Averbach approach very difficult and sometimes misleading [4].

The observed quick growth of Pt nanocrystals slows down and stops after few hours and the most likely mechanism is coalescence of larger crystallites swallowing the smaller neighbors.

[1] P. Rzeszotarski, Z. Kaszkur, *Physical Chemistry Chemical Physics* **2009**, *11*, 5416-5421. [2] Z. Kaszkur, *journal of Applied Crystallography* **2000,** *33*, 87-94. [3] Z. Kaszkur, *Zeitschrift fur Kristallographie* **2006**, *23*, 147-154. [4] Z. Kaszkur, *Journal of Applied Crystallography* **2005**, *38*, 266-273.

**Keywords: in-situ diffraction, crystal growth, surface relaxation**

## MS49.P27

*Acta Cryst.* (2011) A**67,** C542-C543

**Synthesis of Nd-Fe-B nanocomposite magnets by reactive mechanical milling**

Shoichi Kumon, Ryuji Tamura, *Departament of Material Science and Technology, Tokyo University of Science, Chiba, (Japan).* E-mail: shoichi.kumon@gmail.com

Nd-Fe-B nanocomposite magnets composed of a $Nd_2Fe_{14}B$ hard magnetic phase and soft magnetic phases such as α-Fe and/or Fe-B compounds are known to exhibit excellent magnetic properties due to strong ferromagnetic coupling between the magnetic phases [1]. Hydrogenation-decomposition-desorption-recombination (HDDR)

process is known as a method of synthesizing homogeneous $Nd_2Fe_{14}B$ single phase powders [2]. In the HDDR process, $Nd_2Fe_{14}B$ grains disproportionate into a mixture of α-Fe, $NdH_2$ and $Fe_2B$ by hydrogenation under high temperature hydrogen atmosphere. The subsequent desorption of hydrogen results in the recombination of the $Nd_2Fe_{14}B$ sub-micron grains. On the other hand, nano-structurization and hydrogenation of the Nd-Fe-B alloys by reactive mechanical milling have been successfully performed [3, 4], therefore, the synthesis of the nano-structure is expected to be fulfilled by the reactive mechanical milling [5]. In this work, we aim to synthesize Nd-Fe-B nanocomposite structure by the reactive mechanical milling process in a nanocomposite composition.

As a result of reactive mechanical milling, the $Nd_2Fe_{14}B$ grains result in a mixture of α-Fe, $NdH_2$ and amorphous, and the FWHM of the α-Fe and $NdH_2$ peaks were found to be in inverse proportional to the milling time. The change of the FWHM suggests that grains were refined by reactive mechanical milling. For this reason, it is expected that a nano-structure Nd-Fe-B powders will be obtained after recombination of the α-Fe and $NdH_2$ grains by a subsequent heat treatment. Detailed relationships among the microstructure, mechanical milling conditions, heat treatments and magnetic properties of the nanocomposites will be discussed at the symposium.

[1] R. Coehoorn, D.B. De Mooij, C. De Waard, *J. Mag. Mag. Mater.* **1989**, *80*, 101-104. [2] R. Nakayama, T. Takeshita, M. Itakura, N. Kuwano, K. Oki, *J. Appl. Phys.* **1994**, *76*, 412-417. [3] L. Schultz, J. Wecker, E. Hellstern, *J. Appl. Phys*, **1987**, *61*, 3583. [4] T. Harada, T. Kuji, *J. Jpn. Soc. Powder Powder Metal.* **1993**, *40*, 851-854. [5] S. Gang, H. Lianxi, W. Erde, *J. Mag. Mag. Mater* **2006**, *301*, 319-324.

**Keywords: nanocrystal, composite, magnet**

## MS50.P01

*Acta Cryst.* (2011) A**67,** C543

**Structural Biology of TIM proteins: A family of cell surface phosphatidylserine receptors that regulate immunity**

José M. Casasnovas,[a] Angela Ballesteros,[a] César Santiago,[a] Meriem Echbarthi,[a] Gerardo G. Kaplan,[b] Gordon J. Freeman,[c] *[a]Centro Nacional de Biotecnologia, CSIC, Campus UAM, 28049 Madrid (Spain). [b]Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD 20892 (USA). [c]Department of Medical Oncology, Dana-Farber Cancer Institute, Department of Medicine, Harvard Medical School, Boston, MA 02115 (USA).* E-mail: jcasasnovas@cnb.csic.es

The T-cell immunoglobulin and mucin domain (TIM) proteins are involved in the regulation of immune responses by modulating effector Th1 and Th2 cell functions. The TIM gene family is located in a genomic locus linked to autoimmune disease and asthma both in mouse and humans and it has been associated with immune-related diseases, cancer and viral infections. The TIM are type I membrane proteins with an N-terminal immunoglobulin (Ig) domain followed by a heavily glycosylated mucin domain in the extracellular region, a single transmembrane region and a cytoplasmic tail with tyrosine phosphorylation motifs except in TIM-4. Whereas sequence identity among the Ig domains is high (40-60%), there are large differences in the length of the mucin domains. The N-terminal Ig domain is engaged in ligand binding with some contribution of the mucin domain. The structures of the Ig domains determined by our group provided relevant insights on ligand recognition by the TIM proteins. A loop disulphide linked to the CFG β-sheet of the Ig domain is a distinctive structural feature of the TIM proteins (1). Moreover, the structures identified a unique ligand-binding pocket with a metal ion to which ligands coordinate (Metal Ion-dependent Ligand Binding Site, MILIBS) (2). The TIM proteins use the MILIBS to bind to phosphatidylserine (PtdSer) and to mediate phagocytosis of apoptotic cells (2-4). A model for TIM protein binding to PtdSer in cellular membranes was built based on structural and functional data (3). In this model the tip of the N-terminal Ig domain of the protein penetrated into the membrane, whereas the hydrophilic moiety of PtdSer entered into the MILIBS. Moreover, we have seen that the MILIBS modulate the biology of the TIM proteins, such as the trafficking of TIM-1 to the cell surface, TIM-TIM interactions and the conformation of the proteins on the cell surface. Therefore, the MILIBS is a critical determinant of the ligand binding specificity and functional properties of the TIM family, such as the recognition of PtdSer on the surface of apoptotic cells. Overall, we conclude that the unique structure of TIM IgV domains suggests that the TIM molecules evolved as a family of pattern recognition receptors for PtdSer that determine whether apoptotic cell recognition leads to immune activation or tolerance, depending on the TIM molecule engaged and the cell type it is expressed on (5).

[1] C. Santiago, A. Ballesteros, C. Tami, L. Martinez-Munoz, G. Kaplan, J.M. Casasnovas, *Immunity* **2007,** *26,* 299-310. [2] C. Santiago, A. Ballesteros, L. Martinez-Munoz, M. Mellado, G.G. Kaplan, G.J. Freeman, J.M. Casasnovas, *Immunity* **2007,** *27*, 941-951. [3] R. H. DeKruyff, X. Bu, A. Ballesteros, C. Santiago, Y.-L.E. Chim, H.-H. Lee, P. Karisola, M. Pichavant, G.G. Kaplan, D.T. Umetsu, G.J. Freeman, J.M. Casasnovas, *J Immunol* **2010,** *184*, 1918-1930. [4] N. Kobayashi, P. Karisola, V. Pena-Cruz, D.M. Dorfman, M. Jinushi, S.E. Umetsu, M J. Butte, H. Nagumo, I. Chernova, B. Zhu, A. H. Sharpe, S. Ito, G. Dranoff, G.G. Kaplan, J.M. Casasnovas, D.T. Umetsu, R.H. Dekruyff, G.J. Freeman, *Immunity* **2007,** *27,* 927-940. [5] G.J. Freeman, J.M. Casasnovas, D.T. Umetsu, R.H. DeKruyff, *Immunological Reviews* **2010,** *235,* 172-189.

**Keywords: immunology, immunoglobulin, receptor**

## MS50.P02

*Acta Cryst.* (2011) A**67,** C543

**Substrate recognition by complement convertases revealed in the C5-CVF complex**

Gregers Rom Andersen , Nick Stub Laursen, *Department of Molecular Biology, Aarhus University (Denmark).* E-mail: gra@mb.au.dk

The activation of complement triggers cleavage of the proteins C3 and C5 by proteolytic enzymes, the convertases. These contain a non-catalytic substrate contacting subunit (C3b or C4b) in complex with a protease subunit (Bb or C2a). We determined the crystal structures of the C3b homologue Cobra Venom Factor (CVF, 160 kDa) in complex with the 190 kDa protein C5, and in complex with C5 and the 23 kDa S. aureus inhibitor SSL7 at 4.3 Å resolution [1]. The structures reveal a parallel two-point attachment between C5 and CVF, where the presence of SSL7 only slightly affects the C5-CVF interface explaining the IgA-dependence for SSL7 mediated inhibition of C5 cleavage. CVF functions as a relatively rigid binding scaffold inducing a conformational change in C5 which positions its cleavage site in proximity to the serine protease Bb. A general model for substrate recognition by the convertases is presented based on the C5-CVF and the C3b-Bb-SCIN structures [2]. Prior knowledge concerning interactions between the endogenous convertases and their substrates is rationalized by this model.

[1] N.S. Laursen, K.R. Andersen, I. Braren, E. Spillner, L. Sottrup-Jensen, G.R. Andersen, *EMBO J.* **2011,** *30,* 606-16. [2] S.H. Rooijakkers, Wu J, Ruyken M, van Domselaar R, K.L. Planken, A. Tzekou, D. Ricklin, J.D. Lambris, B.J. Janssen, J.A. van Strijp, P. Gros *Nature Immunology***, 2009,** *10***,** 728-733.

**Keywords: proteolysis, innate immunity, macromolecular crystallography**