In situ X-ray fluorescence analysis of barnacle larvae and juvenile barnacles

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Biofouling is the unwanted growth of marine organisms on man-made surfaces with a high ecological and economic impact. Barnacles – as a major macro fouler – contribute substantially to this phenomenon. In order to develop anti-fouling coatings avoiding their settlement on submerged surfaces, it is important to characterize their under-water cement and the mechanism of initial attachment of the motile barnacle larvae. Besides understanding the chemistry side, it is of pivotal importance to decipher the mechanism how the adhesive is able to cure and to adhere to the surface [1]. Here, we are especially interested in the metal distribution in adhesives used by barnacles. In general, studies on underwater cement are hampered by such obstacles as insoluble and/or sticky nature of the sample and poor development of micro-analytical methods for identifying the functions of underwater attachment. While the organic components, namely proteins and polysaccharides are intensively studied [2], inorganic components are barely addressed. Minerals used for calcification are rather obvious (e.g. Ca) and analysis on further metal ions is usually focused on those present in relatively high concentrations (Zn, P) and the accumulation of toxic components in barnacles (Cu, Pb, Cd) [3,4]. While in the adhesive of mussels or algae, iron and vanadium, respectively, play important roles, the function of trace elements in the adhesive of barnacles is not yet clear. Few reports indicate that trace elements, such as iron are present in barnacles [5].

We used X-ray fluorescence at the dedicated X-ray fluorescence end-station FLUO of the synchrotron storage ring ANKA (Karlsruhe Institute of Technology, Germany) to investigate the elemental composition of marine adhesives present in barnacles. The young invertebrates, provided by our biological collaborators (Prof. Clare, Newcastle University) had been settled on an 8 µm Kapton film sealing a small plastic cup filled with artificial seawater to provide a suitable environment for the living marine organisms. Using a focused 17 keV photon beam we were able to obtain elemental distribution maps of the organisms at a spatial resolution of approximately 15 µm. As the X-ray photons are able to penetrate the whole organism, the images correspond rather to a 2D projection of the elemental distribution, with the depth-visibility of every element depending on the corresponding X-ray fluorescence photon energy. In order to distinguish signals originating from the contact area from those coming from the organism behind, we additionally used a confocal measurement setup providing a depth resolution below 20 µm allowing us to focus on the substances in direct contact with the Kapton substrate.

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