# ISOELECTRIC POINT CHARACTERIZATION OF PROTEINS BY ATOMIC FORCE MICROSCOPY

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## Abstract

Biofilm forming, bacterial adhesion, and settlement of fouling species e.g. in marine environments depend on several physico-chemical parameters related to the surface of the substrates and the foulants. The first steps of fouling is often related to protein adsorption. Thus knowledge of the Coulombic charge and protein isoelectric point values are of pivotal importance to understand and control fouling (and protein attachment). However, often only trace amounts of unknown (adhesion) proteins are available for characterization, which poses a challenge to conventional analytical techniques. Here we introduce a new method that requires the use of only minute quantities (on the order of hundreds to a few thousands) of macromolecules covalently immobilized to the surface of AFM colloidal probes to determine isoelectric points of proteins [1]. We first validate our approach using well-known structures, and then study the surface charge of “footprint” adhesion proteins secreted by barnacle cyprid larvae of Amphibalanus Amphitrite during their search of settlement spots in sea water. We measure adhesion forces of the tip- immobilized proteins against positively and negatively charged robust and smooth surfaces [2], made by sequential deposition of polyelectrolytes, as a function of pH. The use of such reference substrates allowed us to study the influence of only one parameter (surface charge) while keeping the others (roughness, chemical composition) essentially unchanged. Examples for combating marine and bacterial fouling will be shown to illustrate possible applications.

[1] Guo, S., Zhu, X., Janczewski, D., Lee, S.S.C., He, T., Teo, S.L.M., Vancso, G.J. Measuring protein isoelectric points by AFM¬based force spectroscopy using trace amounts of sample (2016) Nature Nanotechnology, 11 (9), pp. 817¬823.

[2] Zhu, X., Janczewski, D., Guo, S., Lee, S.S.C., Parra Velandia, F.J., Teo, S.L.¬M., He, T., Puniredd, S.R., Julius Vancso, G.J. Polyion multilayers with precise surface charge control for antifouling (2015) ACS Applied Materials and Interfaces, 7 (1), pp. 852¬861.
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