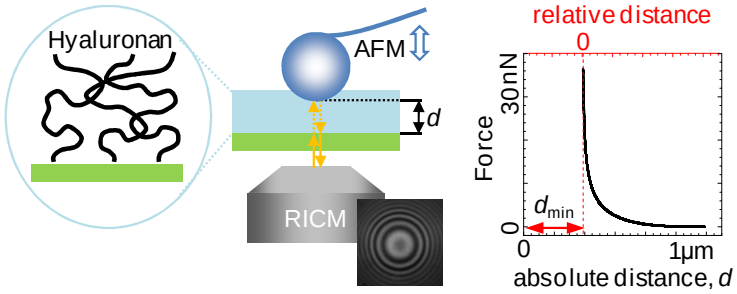
Mechanical properties of hyaluronan-rich pericellular matrices – A study on a biomimetic model system combining colloidal probe atomic force and reflection interference contrast microscopy

Seetharamaiah Attili[1,3] and Ralf P. Richter[1,2,3]

[1]CIC biomaGUNE, Biosurfaces Unit, San Sebastian, Spain; [2]12BM, Department of Molecular Chemistry, J. Fourier University, Grenoble, France; [3]Max-Planck-Institute for Intelligent Systems, Stuttgart, Germany. Email: sattili@cicbiomagune.es



Hyaluronan (HA) is a naturally occurring linear, negatively charged polysaccharide that plays a vital role in the mechanical integrity and function of pericellular matrices (PCM) surrounding many cell types and that is becoming increasingly popular in biomedical applications. Elucidating the mechanical properties of the highly hydrated HA-rich matrices would be valuable to understand how PCMs are organized and how they function. For a thorough investigation of the physical principles underlying the biological function of HA-rich pericellular matrices and provided the instrumental limitations in studying these highly hydrated systems *in vivo*, we have studied a model system that is based on films of HA that is end-grafted to a supported lipid bilayer.

In this study we combine colloidal probe atomic force microscopy (AFM), a widely used analytical approach to determine the behavior of molecules or thin films under mechanical force and reflection interference contrast microscopy (RICM), an established microinterferometric technique to determine the thickness of soft hydrated films into one instrument. The combination provides interaction forces as a function of the *absolute distance* between the two approaching surfaces, information that may not easily be obtained with either technique alone (Attili and Richter 2012, Langmuir, 28:3206-16). On a well defined model system based on supported lipid bilayers (Richter et.al. 2007, JACS, 129:5306-7), we employ the combined setup to quantify the thickness of end-grafted HA films, and their resistance to compression forces as a function of salt concentration. We find a swelling behavior that is consistent with expectations for a polyelectrolyte brush (Attili et.al. 2012, Biomacromolecules, 13:1466-77). Intercalation of aggrecan, a HA-binding proteoglycan prominent in the PCM of chondrocytes, drastically increased the thickness and resistance of HA films to compression. The combined AFM/RICM setup can serve as a powerful tool to study the mechanical properties of surface-confined hydrated films in general.