experimental data. At the same time, pulling of antiparallel in-register β sheet provides much higher rupture force, suggesting that CGNNQNY monomers adopt the out-of-register arrangement in the dimer. Similar analysis applied to Aβ14-23 peptide demonstrated that the out-of-register antiparallel β-sheet arrangement of monomers is realized for this peptide as well. The elevated rupture forces for Aβ14-23 compared to CGNNQNY dimers is explained by elevated contribution of hydrophobic, salt bridge and aromatic interactions in Aβ peptide.

1978-Pos Board B708
Exploring the Formation, Lifetime and Dissociation Statistics of Acid-Amine Bonds
Sangeetha Raman, Markus Valtiner.
Max Planck Institut für Eisenforschung GmbH, Düsseldorf, Germany.

Acid–amine interactions are non-covalent, long-range interactions, contributing to the structural integrity in manmade adhesives and to serve complex life functions in several biological systems. Understanding how these interactions develop and alter over time in an aqueous environment, especially when presented across an interface, is vital when it comes to designing functional surfaces for biomedical applications. We use single molecule force spectroscopy to investigate the contact dynamics of molecular bonds under near-physiological conditions. We explore the interactions of NH₂/COOH bonds that are presented across the atomic force microscopy (AFM) tip-lipid bilayer surface interface, with much focus on the dissociation of these bonds by studying specific signatures obtained during the force measurements. Since the approach permits us to have an exquisite of control over the interface, a number of experimental parameters are varied such as the number density of the molecules, ionic strength of the surrounding medium and extension/retraction speed of the tip to vary the loading rate. A statistical evaluation of the interactions and contact dynamics is presented to assess the influence of the experimental parameters on the bond dissociation. The transition rate under zero-load conditions is calculated combining the detachment statistics and Kramer Evans theory. Our results provide new insights into the binding regime and dissociation behavior of acid-amine bonds from non-equilibrium to near-equilibrium conditions as a function of the loading rate on a logarithmic scale in aqueous environments of varying concentration.

1979-Pos Board B709
Single Molecule Force Spectroscopy of CNGA1 Channels “In Situ” Reveals Major Conformational Changes upon Gating
Sourav Maiti1, Monica Mazzolini1,2, Paolo Fabris1, Marco Lazzarino1,2, Alejandro Valbuena1, Vincent Torre1.
1Scuola Internazionale Superiore Di Studi Avanzati, Trieste, Italy, 2CBM S.r.l., Area Science Park, Basovizza, Trieste, Italy, 3CNR-IOM Basovizza, Trieste, Italy.

Single Molecule Force Spectroscopy (SMFS) is a powerful tool to investigate the structural properties of proteins avoiding all crystallisation problem. SMFS can be used to study membrane proteins directly in their lipid environment, but so far only few proteins have been investigated in their natural environment, i.e. embedded in natural membranes. Here we investigate the structure and function of cyclic nucleotide gated (CNG) channels, cationic channels, mediating sensory transduction in photoreceptor and olfactory sensory neurons, which open upon binding to cyclic nucleotides. Although the cyclic nucleotide binding (CNB) domain has been localized in the C-terminal, the conformational details still remain unveiled. Here we correlate the functional opening of the channel with a conformational change that occurs in the CNB domain in the FMRFamide. CNGA1 channels were over-expressed in Xenopus laevis oocytes and their functionality was verified by electrophysiology. Membrane patches were subsequently deposited on mica, exposing the cytoplasmic side. Patches were identified by AFM imaging and more than 300,000 force-distance (F-d) traces were acquired for each conditions. Several engineered constructs including I27 and N2B domains were used as SMFS finger-prints. Using the WLC model and a custom developed cluster analysis approach, F-d traces in closed state, and in the open state were compared, the latter showing larger unfolding forces (about 55 pN in the closed state and about 85 pN in the open state) in the trans-membrane domains and two additional force peaks located in the cytoplasmic domain (Lc ≈ 80 nm) and in the pore region (Lc ≈ 148 nm), indicating the formation of differently folded structures. Therefore SMFS can be used to identify the conformational changes of proteins underlying gating of ionic channels in situ.

1980-Pos Board B710
HDL-Lipid Uptake is Regulated by Elastic Properties of the Plasma Membrane

1981-Pos Board B711
Richard Walder1, D. Hern Paik1, Matthew S. Bull1, Thomas T. Perkins1,3.
1JILA, National Institute of Standards and Technology, Boulder, CO, USA, 2Department of Physics, University of Colorado, Boulder, CO, USA, 3Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO, USA.

Single-molecule techniques, such as atomic force microscopy (AFM) and optical traps, are capable of measuring atomic-scale (1 Å) displacement. However, drift limits the utility of these instruments in a number of exciting assays. Prior work from our lab demonstrated atomic-scale stabilization in 3D over 100 s using back-scattered detection (BSD). BSD works by scattering a focused laser off a small silicon post, bead, or AFM tip. The backscattered light is collected by the objective lens and projected onto a quadrant photodiode (QPD). The optical and mechanical conciseness of BSD makes this technique amenable to a wide variety of applications. For instance, to make an ultrastable AFM, we use two lasers; one to stabilize the sample and one the AFM tip. The fundamental limit to positional stability in such assays is the differential point-spread function of the two lasers. In this work, we present a number of technical improvements in BSD to enhance long-term stability. The central idea is to use a single QPD to detect both laser beams, suppressing residual motion of the QPD. To detect both lasers with a single QPD, we modulated each laser at separate frequencies (1 and 2.5 MHz) using acousto-optic modulators and de-convolved the signals using lock-in amplifiers. High-resolution z detection was preserved by implementing active intensity control of modulated laser beams. We demonstrated this system by stabilizing an optical microscope with one laser while measuring the resulting stability with the other, as an out-of-loop monitor. This improved BSD system achieved atomic-scale stability in 3D on 100 s but also sub-nm lateral stability for over an hour (2 and 6 Å RMS in x, and y respectively; Δx ≈ 0.0002-0.1 Hz).