Steric Hindrance-Based, Digital Imprinting of RNA Recognition and Processing on a Self-Assembled, Nucleic Acid Matrix

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In this seminar I will present a novel nanoscale effect that enables an unprecedented approach for detecting and analysing RNA recognition and processing on a surface matrix. Specifically, a self-assembled monolayer of a double-stranded(ds) RNA-DNA chimera, laterally confined by means of atomic force microscopy (AFM)-based nanomanipulation, can serve as a molecular matrix for the permanent 'imprinting' of the action of specific 'inputs', including an RNA processing nuclease and an RNA-binding protein, each of which interact with the surface-exposed dsRNA in qualitatively distinct modes. The dsRNA-specific inputs can be captured by the action of a restriction endonuclease (Bam HI) that cleaves an ancillary 'reporter' site within the dsDNA segment, causing a matrix height decrease with respect to the reference monolayer surface, as measured by AFM. We found that, in general, the action of a restriction endonuclease is surface-density-dependent, and in this case, the inputs can effectively act as steric regulators of Bam HI action, providing an output as a step (i.e. digital) function of the dsRNA-specific input. Specifically, we find that the height change (output) of the ds[RNA-DNA] matrix is a permanent imprint that can be detected by AFM topographic imaging, even several hours following removal of the input. We chose a surface-bound, dsRNA-containing probe because the ability to detect and characterize dsRNA and/or dsRNA-related biomolecules in small (e.g. single-cell) volumes is of significant value for diagnostic and functional genomic studies, as RNA-related biomarkers are relevant to viral infection, neoplastic disease, and inflammation. In this work, we also are inspired by the fact that very little is known of the effect of confinement on the functional behaviour of biomolecules. We believe that in-depth investigations of crowding and confinement will be extremely useful in the design and application of functional nanoscale systems to solve biomedical problems. In conclusion, we believe that our studies have important implications for the development of selfassembled, molecular nano-devices for future applications in nanomedicine and nanobiotechnology.

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