Abstract

The nuclear envelope (NE) is an essential cellular structure that contributes to nuclear stability, organization, and function. Mutations in NE-associated proteins result in a myriad of pathologies with widely diverse clinical manifestations, ages of onsets, and affected tissues. Notably, several hundred disease-causing mutations have been mapped to the LMNA gene, which encodes the intermediate filament proteins lamin A and C, two of the major architectural components of the nuclear envelope. However, how NE dysfunction leads to the highly variable pathologies observed in patient cells and tissues remains poorly understood. One model suggests alterations in the dynamic properties of the nuclear lamina and its associated proteins contribute to disease phenotype. Here, we describe the application of single molecule tracking (SMT) methodology to characterize the behavior of nuclear envelope transmembrane proteins (NETs) and nuclear lamins in their native cellular environment at the single molecule level. As proof-of-concept, we demonstrate by SMT that Halo-tagged lamin B1, Samp1, lamin A, and lamin AΔ50 have distinct binding and kinetic properties, and we identify several disease-relevant mutants which exhibit altered binding dynamics. SMT is also able to separately probe the dynamics of the peripheral and the nucleoplasmic populations of lamin A mutants. We suggest that SMT is a robust and sensitive method to investigate how pathogenic mutations or cellular processes affect protein dynamics at the NE.

KEYWORDS: Lamin A; Laminopathies; NET dynamics; Single particle tracking
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