Enzymatic Degradation of DNA Probed by In Situ X-ray Scattering

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Label-free in situ X-ray scattering from protein spherical nucleic acids (Pro-SNAs, consisting of protein cores densely functionalized with covalently bound DNA) was used to elucidate the enzymatic reaction pathway for the DNase I-induced degradation of DNA. Time-course small-angle X-ray scattering (SAXS) and gel electrophoresis reveal a two-state system with time-dependent populations of intact and fully degraded DNA in the Pro-SNAs. SAXS shows that in the fully degraded state, the DNA strands forming the outer shell of the Pro-SNA were completely digested. SAXS analysis of reactions with different Pro-SNA concentrations reveals a reaction pathway characterized by a slow, rate determining DNase I-Pro-SNA association, followed by rapid DNA hydrolysis. Molecular dynamics (MD) simulations provide the distributions of monovalent and divalent ions around the Pro-SNA, relevant to the activity of DNase I. Taken together, in situ SAXS in conjunction with MD simulations yield key mechanistic and structural insights into the interaction of DNA with DNase I.

Significance and Impact

Overall, our measurements and analysis yield insights into the nanoscale structure of Pro-SNA and the reaction pathway for enzyme-induced DNA degradation that are inaccessible by conventional fluorescence-based nuclease assays. The findings demonstrate that it is possible to determine the physiological stability of different DNA sequences to a variety of enzymes in different conditions by using the Pro-SNA platform, which can be designed in different configurations to access the desirable probes.

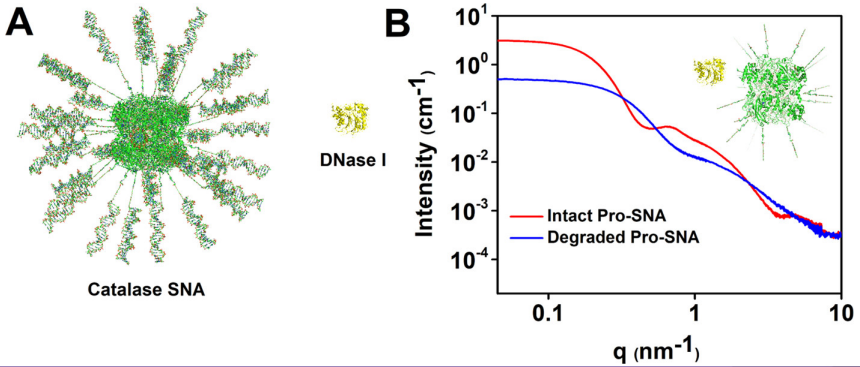

Figure: (A) Schematic for Pro-SNA depicting a Cg Catalase core (~9 nm × 9 nm × 7.5 nm) covalently linked with 40 ds-DNA. Also shown is the nuclease, DNase I (~4.6 nm × 4.2 nm × 3.4 nm), drawn to scale. (B) Background subtracted SAXS intensity profiles from Pro-SNA before (red) and after (blue) 6 h of incubation with DNase I. The intensity profile at 6 h time point (blue) was invariant over an additional 2 h of incubation with the enzyme, indicating that the degradation of Pro-SNA had completed within the first 6 h.

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