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Title: Integrative Modeling of Protein/DNA Complexes at the Replication Fork

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Processivity clamps such as proliferating cell nuclear antigen (PCNA) and the checkpoint sliding clamp Rad9/Rad1/Hus1(9-1-1) act as versatile scaffolds in the recruitment of proteins involved in replication, cell-cycle control and DNA repair. We have employed integrative modeling methods to address two outstanding aspects of PCNA biology: (i) PCNA ubiquitination and (ii) the structures of the ternary FEN1/sliding clamp/DNA complexes. 1) PCNA ubiquitination in response to DNA damage leads to the recruitment of specialized TLS polymerases to the damage locus. This constitutes the initial step in translesion synthesis ? a critical pathway for cell survival and for maintenance of genome integrity. To determine the conformations of Ub-PCNA in solution, we combined multiscale computational modeling and small angle X-ray scattering (SAXS). This strategy revealed alternative positions for ubiquitin to reside on the PCNA surface, distinct from the positions identified in a recent x-ray structure. These computationally-derived positions, in an ensemble with the crystallographic and flexible positions, provided the best fit to the solution-phase scattering, indicating that ubiquitin is dynamically associated with PCNA and capable of transitioning between a few preferred sites on the PCNA surface. 2) Association and handoff of DNA-editing enzymes such as flap endonuclease 1 (FEN1) with sliding clamps are key processes in biology, which are incompletely understood from a structural perspective. Herein, we have chosen an integrative computational and experimental approach to define the assemblies of FEN1 with double-flap DNA and PCNA or the checkpoint clamp 9-1-1. Fully atomistic models of these ternary complexes were developed and refined through extensive MD simulations. The models were subsequently used in conjunction with single particle electron microscopy to generate an EM map of the 9-1-1/FEN1/DNA assembly determined at 18 ≈ resolution.