Hierarchical structures of HIV Integrase: Drug-induced Aggregates of HIV Integrase are Weak Gels

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The major effect of allosteric HIV integrase (IN) inhibitors (ALLINIs) is observed during virion maturation, where ALLINI treatment results in IN aggregation and the formation of aberrant particles. We previously determined the structure of full-length HIV IN bound with an ALLINI at 4.4 Å resolution, studies that we have since extended with the ALLINI BI-D and resistance mutations W131C and N222K. Together these structures define the structural features of the ALLINI-mediated interactions that initiate aggregation. To develop a more holistic view of the structural changes in IN that accompany oligomerization and drug-induced aggregation, we have employed an additional battery of biophysical methods and approaches. Using SEC-SAXS with the EFA-SVD analytical method, we computationally decompose, characterize, and model the monomers, dimers, and tetramers formed by recombinant IN in solution. Comparisons with SAXS/SANS contrast variation analyses of reconstituted IN-DNA complexes indicate that the IN tetramers formed in the absence of DNA are distinct to those formed within the intasome. Using SANS, scanning electron microscopy (SEM), and rheology, we have discovered that the higher-order aggregates induced by the prototype ALLINIs BI-D and BI-224436 have the characteristics of weak, reversible 3D-gels; their formation is inhibited by the host factors LEDGF/p75 and TNPO3, as well as resistance mutations. Our results suggest a novel structural model for IN oligomerization and drug-induced aggregation. Characterization of the higher order IN-ALLINI complexes and resulting escape mutants provides important data for optimizing ALLINI drug design and understanding mechanisms of resistance.