PROBING EFFECTS OF AMINO ACID SUBSTITUTIONS ON HIV-1 PROTEASE CONFORMATIONAL FLEXIBILITY WITH PULSED EPR SPECTROSCOPY Gail E. Fanucci, University of Florida, Department of Chemistry, PO Box 117200, Gainesville, FL 32611-7200

We have recently shown that conformational sampling in HIV-1 protease (HIV-1PR) can be captured with site-directed spin labeling (SDSL) and pulsed electron paramagnetic resonance (EPR) methods. Specifically a nitroxide spin-label is incorporated into the flap region of the protease. By combing results from experimental distance measurements with molecular dynamic simulations models regarding four predominant conformational states are seen, and the relative population of these states is modulated by amino acid substitutions. Results for select natural polymorphism subtypes, drug pressure selected mutations and co-evolved non-active site mutations will be presented and discussed. Results demonstrate that mutations distal from the active site that modulate enzymatic activity have a dramatic effect on the relative populations of the conformational states. We are in the process of correlating changes in conformational sampling with enzymatic activity.