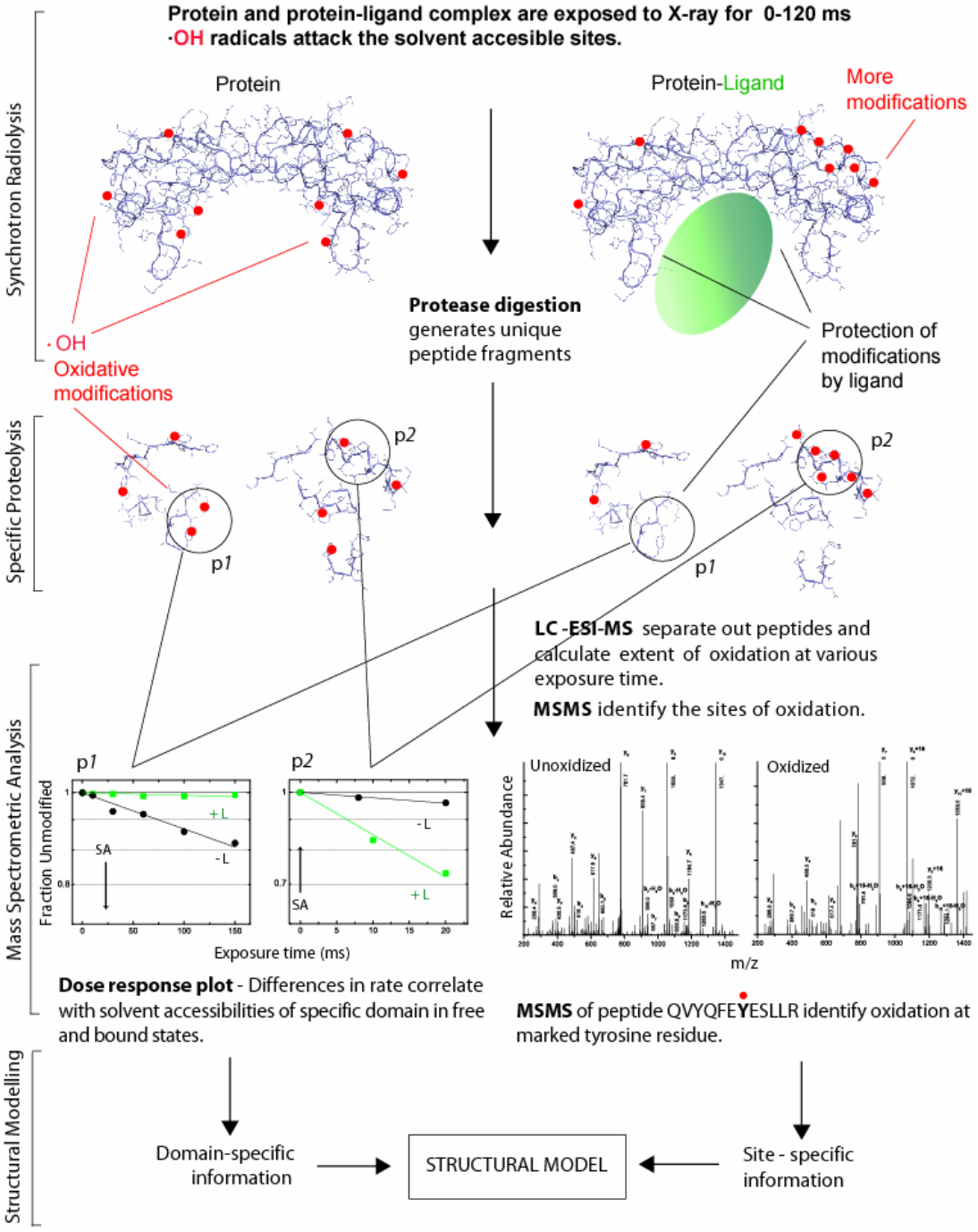

Schematic representation of protein footprinting using synchrotron radiolysis and mass spectrometry. The examples emphasize the protection formed in the interface of a protein-ligand complex as well as allosteric conformation changes that can result in increases in reactivity upon ligand binding; however, the comparison could be for any two (or multiple) functional states of the protein of interest. Two sets of samples, one free protein and the other a protein-ligand complex, are exposed to X-rays for different time intervals. The exposed samples are digested with specific digestion enzymes. The digested fragments are analyzed by ESI-MS to quantitate the extent of modification products and determine the fraction `unmodified' for a specific exposure time. A plot of fraction unmodified *versus* exposure time, known as the dose response plot, fit to a first-order function providing the rate of modification for the specific peptide. Comparisons of the dose response of the same peptide under different conditions provide structural information about ligand binding. MS/MS is used to determine the specific modification site within the peptide and provide side-chain-specific structural resolution. Synchrotron protein footprinting data are often used as one of the constraints in model building for complexes. (Reproduced from Gupta et al. J Synchrotron Radiation 2007 May;14(Pt 3):233-43).