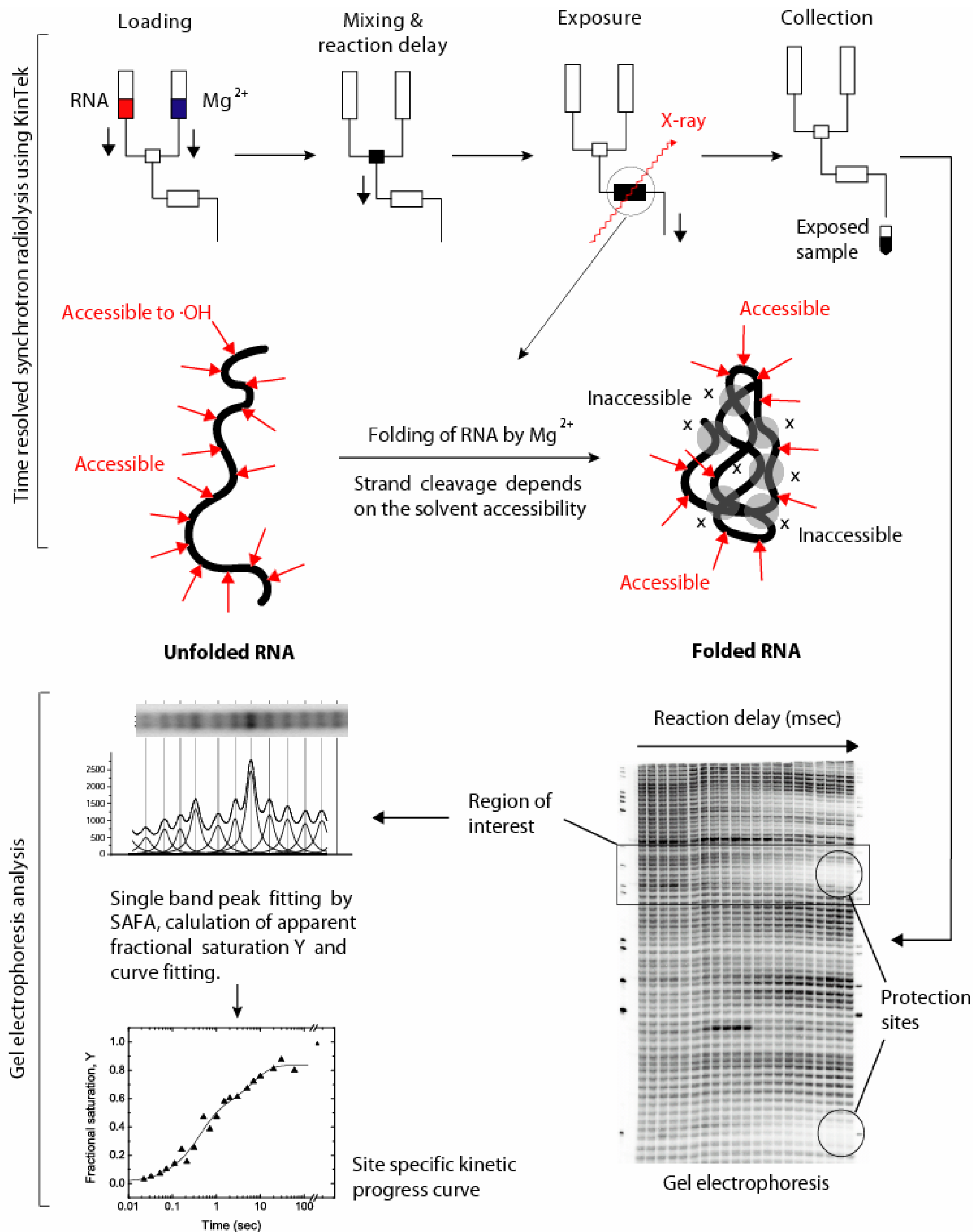

Schematic representation of nucleic acid footprinting using synchrotron radiolysis and quantitative PAGE. The upper panel shows the method for conducting a kinetic experiment using the KinTek apparatus. Unfolded RNA and Mg^{2+} are mixed through a T-mixer and allowed to react for various time intervals or delay times. After each reaction delay the samples are exposed to X-rays for 10 or 15 ms in the flow cell. When RNA folds, it can form discrete tertiary regions of contact which become inaccessible to the solvent; hence those regions show less strand cleavages. When PAGE is run with the exposed sample in the presence of appropriate controls, as indicated in the lower panel, the reduced strand cleavages appear as reduced band intensities. These regions of reduced intensity are termed `protections'. The `single-band analysis' technique is used for the quantification of the bands. By this procedure, total intensities of the bands are converted to `Y', fractional saturation. Y is proportional to the amount of cleavage in the nucleotide backbone. Any time-resolved experiment generates a set of Y values with respect to various reaction times. A non-linear regression curve fitting of Y against time reveals the kinetic progress curve of the folding process with a single nucleotide. (Reproduced from Gupta et al. *J Synchrotron Radiation* 2007 May;14(Pt 3):233-43).