## Assessment of susceptible chemical modification sites of trastuzumab and endogenous human immunoglobulins at physiological conditions

The quality control testing of chemical degradations in the bio-pharmaceutical industry is currently under controversial debate. Here we have systematically applied in vitro and in vivo stress conditions to investigate the influence of protein degradation on structure-function. Extensive purification and characterization enabled identification and functional assessment of the physiological degradation of chemical modification sites in the variable complementarity-determining regions (CDRs) and conserved region of trastuzumab. We demonstrate that the degradation of the solvent-accessible residues located in the CDR and the conserved fragment crystallizable region (Fc) occurs faster in vivo (within days) compared to the levels observed for bio-process and real-time storage conditions. These results hence question the rationality of extreme monitoring of low level alterations in such chemical modifications as critical patient safety parameters in product quality control testing, given that these modifications merely mirror the natural/physiological aging process of endogenous antibodies.