# On-line electrochemical reduction of inter- and intramolecular disulfide bonds for antibody analysis

Martijn VanDuijn[1], Pablo Sanz de la Torre[2], Jean Pierre Chervet[2], Theo Luider[1]
[1]Erasmus MC, Rotterdam, Netherlands; [2]Antec Scientific BV, Zoeterwoude, The Netherlands

In routine proteomics analysis, the reduction of disulfide bridges is achieved by chemical additives such as dithiothreitol (DTT), β-mercaptoethanol (β-ME) or tris(2-carboxyethyl) phosphine (TCEP). However, reduction by an on-line electrochemical method can offer several advantages. Until now, the cleavage by electrochemical methods of the disulfide bond between heavy and light chain components of antibodies was demonstrated in literature, but the reduction of additional internal disulfide bonds within these chains remained unsuccessful. We now demonstrate the full electrochemical reduction of the monoclonal antibody Avastin as it elutes from a chromatography system. The reduction is achieved in a flowcell (µPrepcell-SS) with a titanium working electrode and a platinum counter electrode. In this system, the intramolecular disulfides could successfully be reduced, as shown by the distribution of charge states, and also by detailed analysis of the top-down MS1 spectra. In addition, it was demonstrated that the electrochemical reduction permitted the acquisition of richer MS-MS spectra, which showed fragment signals that originated from the protein chain between the cysteine residues that are normally bridged. In a sample with closed disulfide bridges, signals from that region were suppressed in the data. This shows that complete protein reduction is important for MSMS-based sequence analysis. The electrochemical reduction conveniently provides this reduction, without a requirement for careful protein denaturation and alkylation as used in chemical reduction protocols for complete and durable reduction of protein chains. We will apply this methodology on the analysis of antibody mixtures, which can now be separated by chromatography as paired heavy and light chains, but analyzed by mass spectrometry as separate chains co-eluting from the flow-cell as cognate pairs.