New Electrochemical Cell for Superior Reduction of Protein Disulfide Bonds in HDX-MS

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The use of on-line electrochemical (EC) reduction of disulfide bonds in HDX-MS has shown great potential for superior characterisation of proteins [1-2], making the use of interfering reducing agents such as TCEP or DTT obsolete.

So far, the electrochemical reduction was based on flow through cells using a 3-electrode configuration with Titanium based working and counter electrodes and Pd/H2 reference electrode. Though the initial reduction efficiency was good, in practice, this 3-electrode cell configuration resulted in short longevity due to electrode contamination (fouling) even when a cleaning pulse was applied. Another shortcoming was the observed undesired oxidation of e.g., methionine, under reductive conditions.

Therefore, a new electrochemical cell was developed. The cell consists of a dual electrode set-up, i.e., working electrode and counter electrode but without a reference electrode. The inlet block of the cell is made out of titanium and serves as working electrode, meanwhile the lower part of the cell contains the counter electrode made of platinum.
In practice we have noticed that this new configuration results in distinguished advantages such as: superior stability and longevity, no undesired peptide/protein oxidation during the reduction and overall much easier in use. Another advantage of the new design is the much higher pressure stability of up to 350 bar, allowing for its routine on-line use in HDX-MS.

Results will be shown on the robustness of the new cell for the reduction of S-S bonds in proteins and its application in HDX-MS.

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