Complete online electrochemical disulfide bond reduction of antibodies

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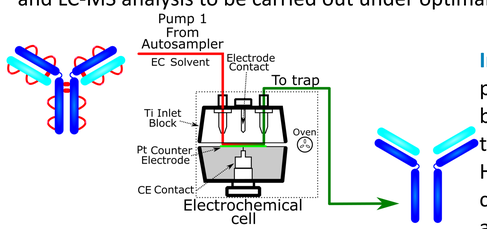
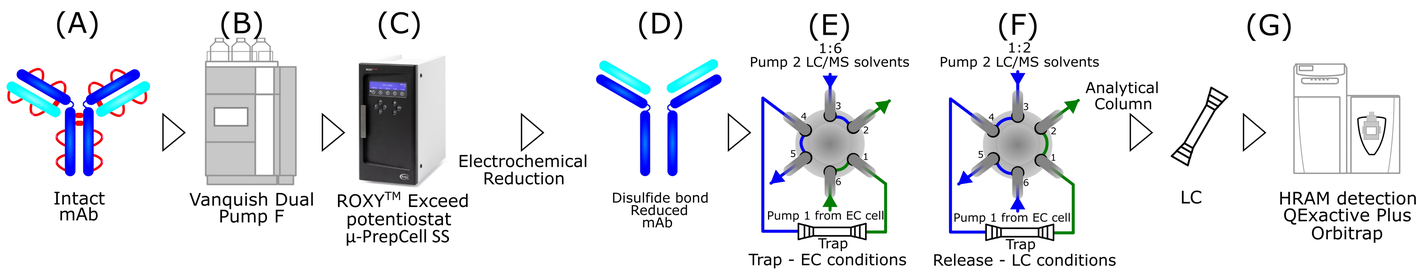
Overview—Electrochemical reduction (EC) of antibodies results in cleavage of all *inter*– and *intra*-chain disulfide bonds. The online EC reduction included a solvent switching step allowing both EC reduction and LC-MS analysis to be carried out under optimal conditions.



ROXY Exceed™ µ-PrepCell SS
Electrochemical reduction
Chromeleon™ software integration
Temperature control
Electrode potential control

Introduction—Middle-down MS methods require complete reduction of the *inter*– and *intra*-chain disulfide bonds present in antibodies. Reducing the antibody to the subunit level allows easier analytical separation, HRMS, and tandem MS methods to be used. Electrochemical reduction of disulfide bonds in proteins using a flow-through reactor cell prior to mass spectrometry has been successfully applied as an alternative method to chemical reduction previously.

Methodology— Integrated online LC-MS EC reduction of Abs. Intact Ab (A) is introduced *via* a Vanquish Dual Pump F system (B). A discrete EC solvent flows into the ROXY™ Exceed potentiostat (C), through the µ-PrepCell SS where EC reduction occurs producing subunits (D). Switching valve setup in 1:6 position (E) traps the subunits onto a short trapping column, before switching positions (F) to backflush the trap with LC-MS compatible solvents onto the LC/MS HRAM Orbitrap™ setup (G).

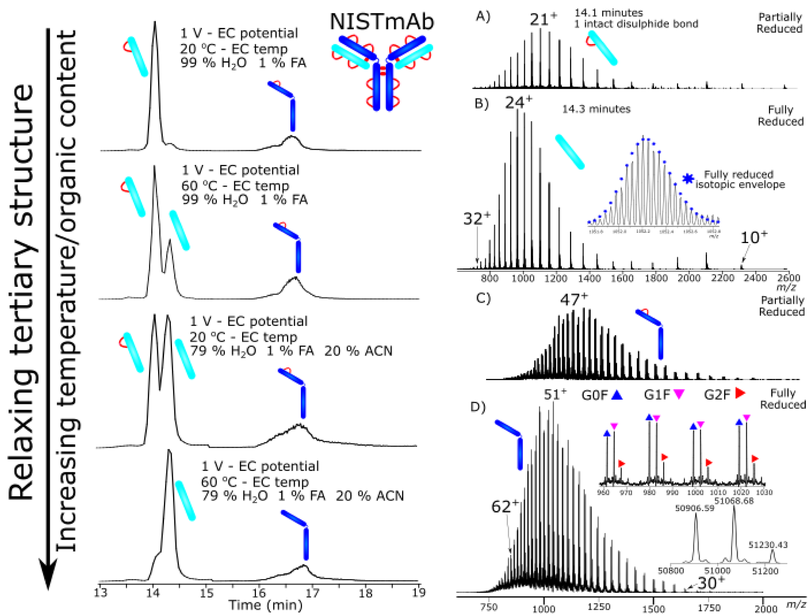


Results—Online EC disulfide bond reduction consistently reduced intact NISTmAb into Lc and Hc subunits. The LC-MS analysis of the antibody could be kept the same as using more traditional chemical reduction techniques as the solvent switching step allowed separate EC and LC solvents to be optimised.

*Intra*chain disulfide bond reduction could be easily monitored using exact masses, isotopic, and charge state distributions. Higher charge states, indicative of increase accessible surface area and relaxed tertiary structure.

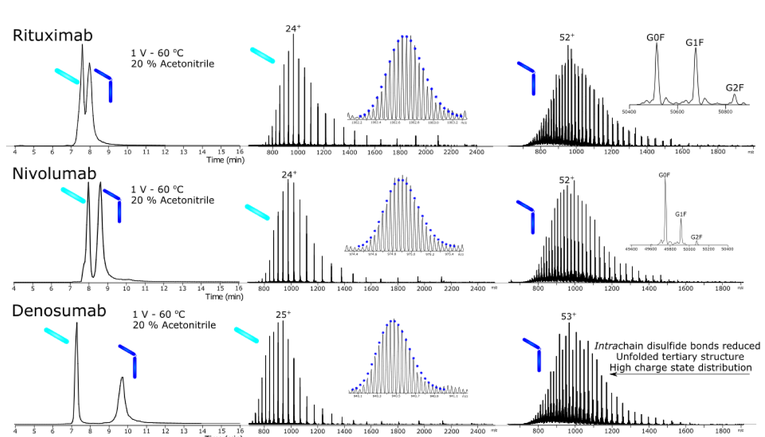
An EC potential of 1 V was effective in reducing disulfide bonds, increasing the potential further showed no further increase in disulfide bond reduction but increased the possibility of solvent electrolysis.

*Inter*chain disulfide bonds were completely reduced consistently for NISTmAb . However, *intra*chain disulfide bonds were still intact at room temperature and in aqueous solvents (A, C).



Increasing the temperature in the electrochemical cell as well as increasing the organic content of the solvent resulted in more complete *intra*chain disulfide bond reduction (B, D). Overall, complete disulfide bond reduction was achieved without the need for addition of chaotropes.

The same analysis carried out on a selection of other antibodies showed consistent electrochemical reduction, of the *inter*– and *intra*– chain disulfide bonds. Nivolumab, Rituximab, and Denosumab were all reduced at 60 °C and 20% acetonitrile.



Conclusion—Online electrochemical disulfide bond reduction consistently reduced both the *inter*– and *intra*– chain disulfide bonds in a selection of antibodies. The use of a solvent switching valve setup allowed electrochemical reduction to be carried out in the optimal electrochemical conditions followed by LC-MS analysis in the optimal analytical conditions.

References—Nicolardi, S.; *et. al.*, J. Am. Soc. Mass. Spectrom. 2013, 24 (12), 1980-7; Cramer, C. N. et. al., Anal. Chem. 2016, 88 (3), 1585-92. Buter, L. et. al.; J. Chromatogr. A 2017, 1479, 153-160. Switzar, L. et. al; J. Am. Soc. Mass. Spectrom. 2016, 27 (1), 50-8. Zheng, Q. et. al; Int. J. Mass spectrom. 2013, 353, 84-92.
Morgan, T., Jakes, C., Brouwer, H-J., Millán-Martín, S., Chervet, J-P., Cook, K., Carillo, S., Bones, J., *Inline electrochemical reduction of NISTmAb for middle-up subunit liquid chromatography-mass spectrometry analysis.* Analyst, 2021, doi.org/10.1039/D1AN01184G.