Development of a robust and reproducible method for detection of citrullination in complex samples

Citrullination is a deamination of arginine, resulting in an increase of hydrophobicity and a charge difference of 1.2 charges per peptide. This method is used to detect citrullinated proteins containing citrulline. Citrullination is a post-translational modification that is catalyzed by peptidylarginine deiminases (PADs).PADs are enzymes that catalyze the deamination of arginine to citrulline, resulting in citrullinated proteins. Citrullinated proteins are found in a variety of diseases, including rheumatoid arthritis (RA) and periodontal disease.

HPLC – Fractionation using HFBA

48 fractions were collected in the initial experiment on one minute fractions from 5 mm additions and mix of files with the sample prior to injection gave better result, until 4 min. Further analysis was performed on an EVO-TIPS system with 4 mm injections of sample, eluting at 45 min with a 50% linear gradient. EVOSEP provided a clean separation of the citrullinated sample graph, whereas the semi-complex sample graph show only high background.

Porphyrmonas gingivalis PepTidyl Arginine Deiminase (PAD)

P. gingivalis is a common oral pathogen that induces citrullination of host proteins. PADs are highly expressed in P. gingivalis and have been implicated in the pathogenesis of periodontal disease.

Citrullination of internal arginines have also been observed, where Anti-Citrullinated Peptide Antibodies (ACPA) are validated on their own and unpaired citrullinations are validated than the 90 min, single run method. Still unique citrullinations were identified, where Anti-Citrullinated Peptide Antibodies (ACPA) are validated on their own and unpaired citrullinations are validated than the 90 min, single run method. Still unique citrullinations were identified.