# Rapid microscale in-gel processing and digestion of proteins using surface acoustic waves†

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**A new method for in-gel sample processing and tryptic digestion of proteins is described. Sample preparation, rehydration, *in situ* digestion and peptide extraction from gel slices are dramatically accelerated by treating the gel slice with surface acoustic waves (SAWs). Only 30 minutes total workflow time is required for this new method to produce base peak chromatograms (BPCs) of similar coverage and intensity to those observed for traditional processing and overnight digestion. Simple set up, good reproducibility, excellent peptide recoveries, rapid turnover of samples and high confidence protein identifications put this technology at the forefront of the next generation of proteomics sample processing tools.**

The digestion of proteins is of fundamental importance in virtually all proteomic workflows that involve mass spectrometric interrogation of protein identity. In particular, this is becoming increasingly important for relative or absolute quantitation of proteins in comparative proteomics experiments.[1] In recent years, significant advances have been made to accelerate the various stages involved in protein digestion. While many methods have emerged which accelerate tryptic digestion of proteins in-solution including microwave devices,[2] spin columns and immobilized enzymes[3] techniques available for in-gel digestion are still quite limited.[4] In-gel digestion of proteins has become a vital part of SDS-PAGE proteomics primarily because one and two dimensional gel electrophoreses are amongst the most common protein separation techniques. Current in-gel protocols require lengthy sample processing times (up to five hours) followed by overnight tryptic digestion.[5]

We were interested in exploring the possible application of a new microfluidic technology for enhancing chemical reactions,[6] based on the use of SAWs as an energy source, to the acceleration of all the processes involved including desorption and hydration as well as chemical and enzymatic reactions.[5] The acceleration of each stage proved to be dramatic, with the overall time reduced to 30 minutes whilst maintaining excellent, reproducible results.

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SAWs are acoustic waves that propagate along the surface of a piezoelectric material. In this study a 127.86° *y–x* cut lithium niobate (LN) single crystal was used as the piezoelectric substrate. The transduction from electrical energy to mechanical energy (manifested as SAWs) is accomplished using a pair of interdigital transducers (IDTs) in the form of 250 nm thick Ti–Al electrodes patterned at each end of the SAW device (Fig. 1) using standard photolithography. A distinct monochromatic and coherent SAW is then generated using the fundamental resonance frequency, determined by the spacing between IDTs which was set at 19.65 MHz for this study.[7]

A comparison of workflow times for the new, SAW-based method reported here with a standard protocol[5] is shown schematically in (Fig. 2). In our new method, processing of a gel band, including destaining, disulfide reduction and capping, requires a total of only 15

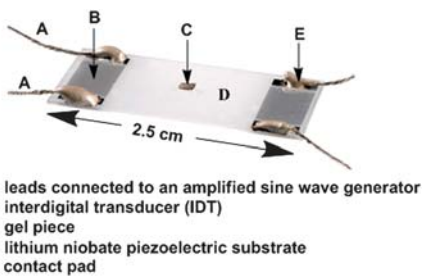


**Fig. 1** Schematic of a gel piece placed on a SAW device.

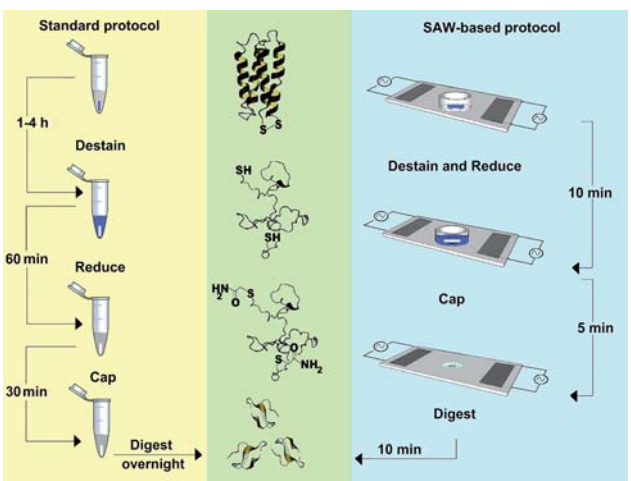


**Fig. 2** Flow diagram comparing the SAW-based and traditional protocols.

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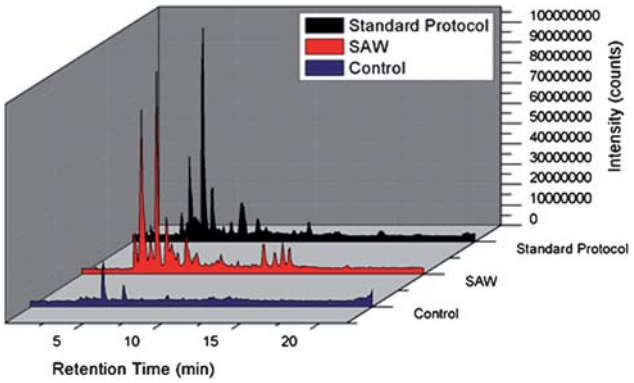


**Fig. 3** Base peak chromatograms from a LC-MS/MS separation of a SAW assisted in-gel digestion, control and traditional overnight digest. Data shown are for 100 ng of BSA (data for 1 μg, 500 and 250 ng BSA are shown in Fig. S1, the peptides and sequence coverage from these digests are shown in Tables S1 and S2 and Fig. S2 in ESI†).

**Table 1** Protein sequence coverages for different gel loadings of BSA[a]

| Sequence coverage | BSA 1 μg | BSA 500 ng | BSA 250 ng | BSA 100 ng |
|---|---|---|---|---|
| SAW | 23% | 18% | 26% | 9% |
| Control | 10% | 1% | 2% | 2% |
| Overnight | 27% | 23% | 18% | 13% |

[a] Bovine serum albumin.

minutes. This compares very favourably with standard protocols which typically require approximately 5 hours to complete. The acceleration of tryptic digestion is even more dramatic. In a total of only 10 minutes, (i) gel rehydration, (ii) protein digestion and (iii) peptide recovery were achieved using our SAW-based protocol, a drastic reduction in time from the overnight protocol employed in many traditional in-gel digestions.[5]

Base peak chromatograms (Fig. 3) for the SAW assisted in-gel tryptic digestion products of 100 ng (1.44 pmol) to 1 μg (14.4 pmol) samples of BSA indicated that the SAW assisted process yielded equivalent tryptic peptide maps to a standard overnight digestion and extraction procedure.

Indeed, the number of peptides detected using the new SAW-based protocol was comparable to that obtained after the standard overnight digest (see Table 1, Fig. S1 and S2 and Tables S1 and S2†). A search performed on the BSA protein digest failed to identify any non-tryptic peptide matches indicating our new method does not generate non-specific cleavage products of the protein substrate.

To highlight the generality of our new technology five proteins selected randomly from 2D-SDS PAGE gels from disparate sources (Table 2) were next processed and digested under the conditions described above. After a database query with MASCOT® high sequence coverages were obtained for each of the proteins. The proteins identified include often difficult to assign membrane proteins of mid to low masses (see Tables S3–S10 and Fig. S3–S7†).

The actual mechanism causing the dramatic reduction in tryptic digest times is under investigation. Tryptic digests in solution are typically very fast and comparable to those observed in our gel-based digests. One plausible mechanism for the acceleration, based on effects induced by the SAW-generated high frequency mechanical energy, is that the consequent increased mobility of the trypsin results in increased diffusion through the gel and the probability of trypsin and the proteins coming into contact.

In summary, this new SAW-based technology, when applied to processing and in-gel tryptic digestion of protein samples, delivers remarkable reductions in reaction times. Protease specificity is retained whilst excellent coverage of the protein is maintained. The dramatically enhanced digest efficiency removes a serious bottleneck in proteomics sample handling and allows for standardization of digestion across samples. Overall, this leads to significant improvements in quantitative workflows where efficient proteolysis prior to chemical labeling or label free peptide and protein quantitation is required.[8]

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**Table 2** MASCOT®[a] search results from SAW assisted tryptic digest of random protein gel bands

| Accession number | Protein name | MASCOT score | Coverage (%) | No. of peptides identified | $M_w$ (Da) |
|---|---|---|---|---|---|
| TO401_ARATH | Mitochondrial import receptor subunit TOM40 homolog 1 | 724 | 51 | 12 | 34 228 |
| 1A01_HUMAN | HLA class I histocompatibility antigen, A-2 alpha chain | 962 | 48 | 21 | 41 181 |
| B2MG_GORGO | Beta-2-microglobulin | 205 | 37 | 4 | 13 820 |
| HA11_MOUSE | H-2 class I histocompatibility antigen, D-B alpha chain | 586 | 27 | 13 | 41 095 |
| OMPF_ECOLI | Outer membrane protein F | 454 | 25 | 6 | 39 309 |

[a] MASCOT is a mass spectral search algorithm that uses mass spectrometry data to identify proteins from primary sequence databases.

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