# **Supporting Information**

# **Biochip Spray: Simplified Coupling of Surface Plasmon**

# **Resonance Biosensing and Mass Spectrometry**

Sweccha Joshi,<sup>†,‡</sup> Han Zuilhof,<sup>†</sup> Teris A. van Beek,<sup>†</sup> Michel W.F. Nielen<sup>†,</sup>||\*

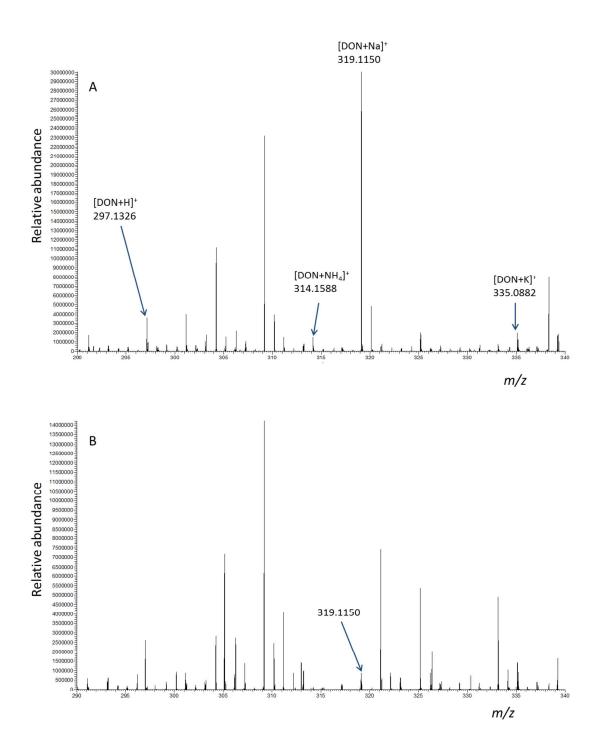
<sup>†</sup>Laboratory of Organic Chemistry, Wageningen University & Research, Stippeneng 4, 6708 WE Wageningen, The Netherlands

<sup>‡</sup>TI-COAST, Science Park 904, 1098 XH Amsterdam, The Netherlands

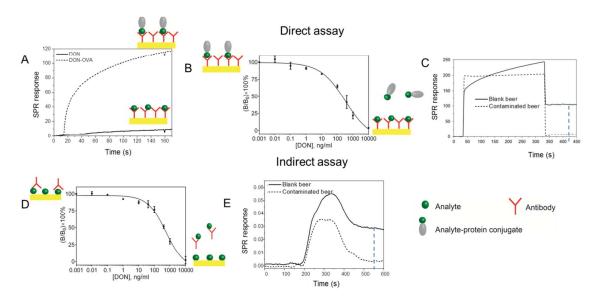
RIKILT Wageningen University & Research, P.O. Box 230, 6700 AE Wageningen, The Netherlands

#### Table of Contents

	Page
Figure S1. Chip Spray mass spectra of (A) DON in methanol and (B) blank	S-2
methanol	
Figure S2. Different SPR assay modes, calibration curves and sensorgrams for	S-3
blank and contaminated beer	
Figure S3. TIC and EIC for DON using a chip containing anti-FB <sub>1</sub>	S-4
Figure S4. Schematic representation of the workflow options for large and small	S-5
molecules by SPR-MS	



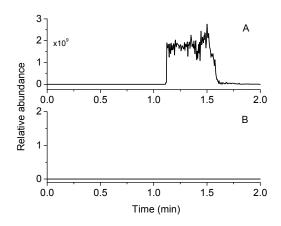
**Figure S1.** Chip Spray mass spectra recorded in positive ion mode from a CMD-modified gold chip spiked with A) 5  $\mu$ L of 1  $\mu$ g/mL DON in methanol and B) 5  $\mu$ L of methanol, drying, and application of 5  $\mu$ L of methanol and 5 kV. In blank methanol an unknown species with m/z 319.1150, i.e., the same m/z as for [DON+Na]<sup>+</sup> was observed.



**Figure S2.** Different SPR assay modes. In a direct SPR assay, anti-DON is immobilized on the surface. The response obtained only for DON was weak (A, solid line, Biacore) or absent (iSPR) thus requiring use of an ovalbumin conjugate of DON (DON-OVA) as a signal enhancer (A, dashed line). B) The response of a fixed concentration of DON-OVA in competition with increasing concentration of DON (in sample) for the immobilized anti-DON is measured to construct a calibration curve. C) SPR response measured for blank beer and contaminated beer using the direct assay of B with DON-OVA as signal enhancer.

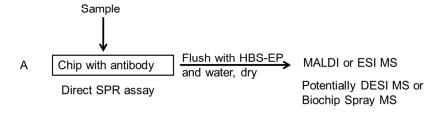
In an indirect SPR assay, DON is immobilized on the surface and the response of a fixed concentration of anti-DON with increasing concentrations of DON (in sample) in competition with the immobilized DON is measured (D). E) SPR response measured for blank beer and contaminated beer using the indirect assay of D.

A near-complete inhibition of the SPR response (taken at time points indicated by blue dashed lines in Figure S2C and E) is seen for the contaminated beer, indicating the presence of DON and/or cross-reacting conjugates. Note: the indirect assay is recommended for routine screening of large numbers of samples as the chips with immobilized DON are much more durable than the ones with immobilized antibodies (see also ref. 29 cited in the main text).

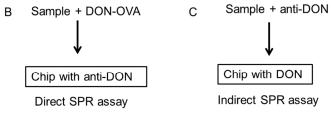


**Figure S3.** A) Total ion chronogram and B) extracted ion chronogram for m/z 297.1333 ([DON+H]<sup>+</sup>). Conditions: results obtained from a CMD-modified gold chip with immobilized anti-FB<sub>1</sub> that was flushed in the flow cell of the iSPR with spiked beer (containing 10  $\mu$ g/mL of DON), followed by washing of the anti-FB<sub>1</sub> chip with buffer and water and transfer of the chip to the Biochip Spray MS set-up.

## For large molecules (e.g. proteins)



## For small molecules (e.g. DON)



Positive or negative for DON

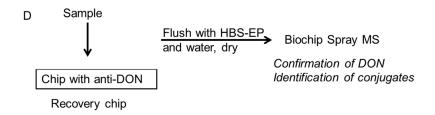


Figure S4. Schematic representation of the workflow for SPR-MS. A) For large molecules such as proteins where the sample could be analysed using direct SPR assay (Figure S2A) without signal enhancer followed by various forms of MS analysis. For small molecules such as DON where the SPR biosensing can be performed in two modes, B) a competitive direct mode, using an anti-DON biochip and the addition of DON-OVA to the beer sample as a signal enhancer and C) a competitive indirect mode using an immobilized DON biochip and the beer sample mixed with anti-DON. D) A sample considered positive from the indirect competitive assay (Figure S2E) or the direct SPR assay (Figure S2C) is re-injected onto a 'recovery chip' containing anti-DON (like in the direct SPR assay format but without the competing DON-OVA conjugate being present, i.e., as in Figure S2A solid line), followed by Biochip Spray MS analysis.