Supporting Information

Multiplex surface plasmon resonance biosensing and its transferability towards imaging nanoplasmonics for detection of mycotoxins in barley

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Scheme S1 Extraction scheme for three different barley samples (B1, B2, B3) on three different days to obtain 21 blank and 21 spiked samples for validation experiment.

Table S1 Limit of detection (LOD), IC50 and working range of the double-3plex assay (using Biacore) and 6plex (using prototype nanostructured iSPR) for singleplex in buffer, multiplex in buffer and multiplex in barley extract (n=3). All values are expressed in ng of toxin per ml of buffer or barley extract

		Double 3-plex SPR (Biacore)			6-plex nanostructured iSPR		
Toxin	Format	LOD[a]	IC50	Working range[b]	LOD[a]	IC50	Working range[b]
DON	Singleplex	0.6*	5.6	0.6-25	-	-	-
	Multiplex (buffer)	0.3*	3.9	0.3-25	0.6	12	2-60
	Multiplex (barley)	0.8*	15	0.8-100	2	38	6-150
ZEA	Singleplex	0.2	1.5	0.5-5	-	-	-
	Multiplex (buffer)	0.2	1.3	0.5-3	0.8	25	3-150
	Multiplex (barley)	0.2	1.6	0.5-5	3	30	7-250
T-2	Singleplex	0.4*	1.9	0.4-6	-	-	-
	Multiplex (buffer)	0.1*	1.2	0.1-7	0.5	10	1.5-80
	Multiplex (barley)	0.02*	0.5	0.02-9	0.8	18	2.5-120
OTA	Singleplex	0.4	2.0	0.8-5	-	-	-
	Multiplex (buffer)	0.1	1.8	0.4-6	3.8	20	6-70
	Multiplex (barley)	0.1	2.1	0.4-10	5	40	10-180
FB_1	Singleplex	0.3	2.7	0.8-9	-	-	-
	Multiplex (buffer)	0.2	3.5	0.6-20	0.2	8	0.8-70
	Multiplex (barley)	0.07	3.5	0.3-40	0.4	15	1.5-120
AFB_1	Singleplex	0.7	2.5	1-5	-	-	-
	Multiplex (buffer)	0.2	1.7	0.4-8	0.8	10	2-60
	Multiplex (barley)	0.02	0.8	0.1-8	0.3	20	1.5-250

[a]LOD is defined as IC10 (concentration at which 10% inhibition of binding occurs) unless marked with an asterisk

[b]Working range is defined as IC20-IC80 (concentration at which 20%-80% inhibition of binding occurs)

*IC20 is used as LOD, as the fitting does not allow determination of IC10 for this toxin

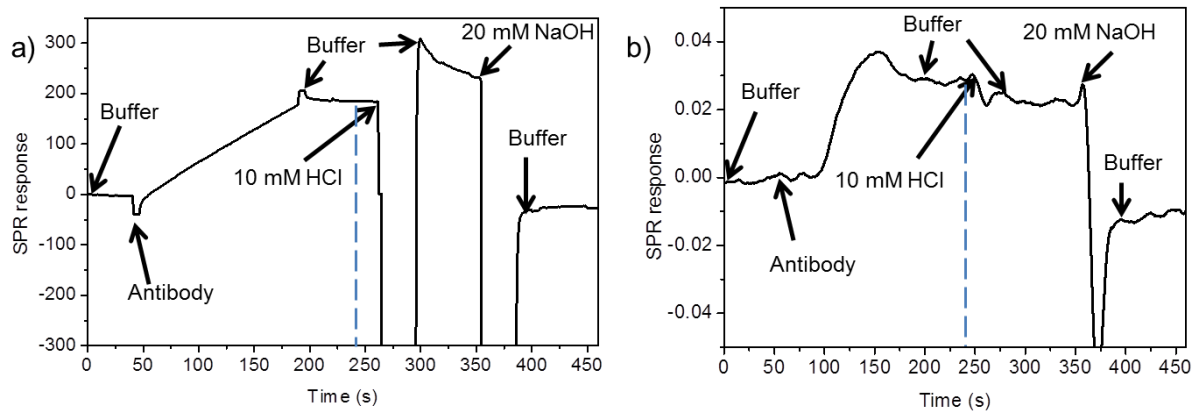

Fig. S1 SPR sensorgrams generated in a channel (for SPR) or ROI (for iSPR) with FB_1-OVA upon injection of a) 0.1 µg/ml aFB_1 measured using a Biacore 3000 with flat gold and b) 5 µg/ml aFB_1 measured using a prototype iSPR instrument with nanostructured gold. Each cycle consists of flushing with buffer, injection of antibody and regeneration with 10 mM HCl (30 s) followed by 20 mM NaOH (30 s). After injection of antibody and regeneration, the chip was flushed with buffer. The blue dotted line shows the time point where the SPR response was recorded.

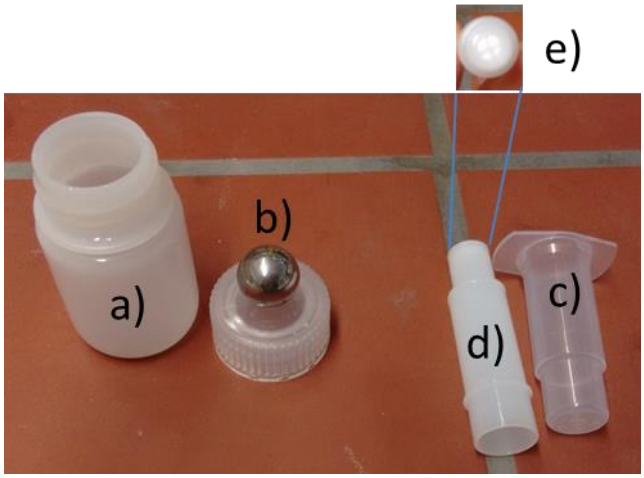

Fig. S2 Kit used for simplified sample preparation suitable for field applications. The extraction was done using a plastic container (a) and a stainless steel ball (b). The extract was collected in the upper tube of the filter device (c) and filtered into the plunger (d) through the filter device (e).

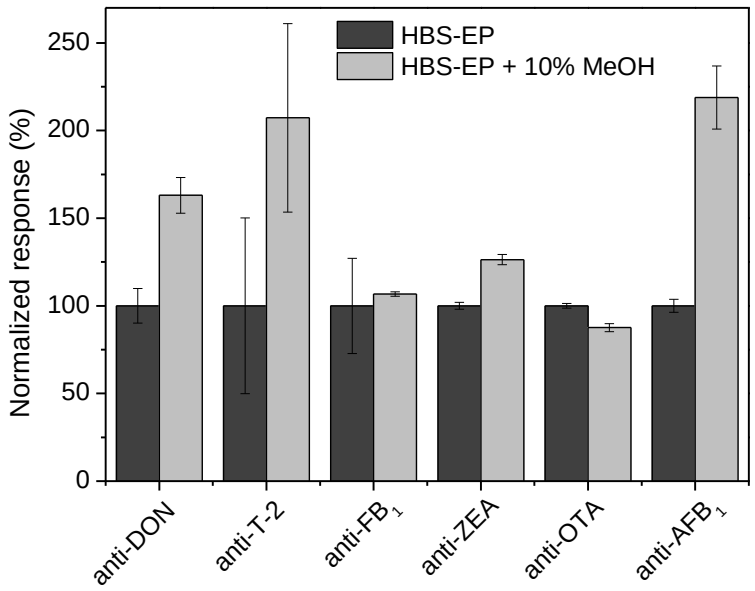

Fig. S3 Comparison of binding of the six antibodies with their corresponding OVA conjugates in HBS-EP without and with 10% MeOH measured with SPR (Biacore) (n=3). The responses are normalized to the binding of the corresponding antibodies in HBS-EP.

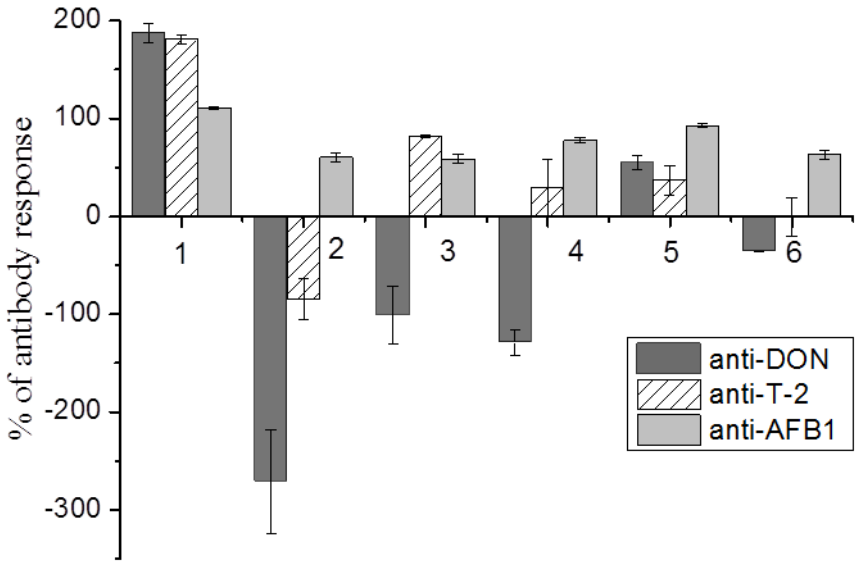

Fig. S4 Regeneration scouting for anti-DON, anti-T-2 and anti-AFB_1 performed using SPR (Biacore). The percentage of antibody response remaining after regeneration is plotted for three different antibodies and six different regeneration conditions (1 = 6 M guanidine HCl, 2 = 20 mM NaOH, 3 = 5 mM NaOH + 0.5% SDS, 4 = 5 mM NaOH followed by 0.1% Tween20, 5 = 10 mM HCl followed by 10 mM NaOH and 6 = 10 mM HCl followed by 20 mM NaOH). All regeneration solutions were injected for 30 s at a flow rate of 100 µL/min (n=3). Please note that the other three antibodies (anti-ZEA, anti-FB_1 and anti-OTA) are not shown here but were successfully regenerated under regeneration condition 6.

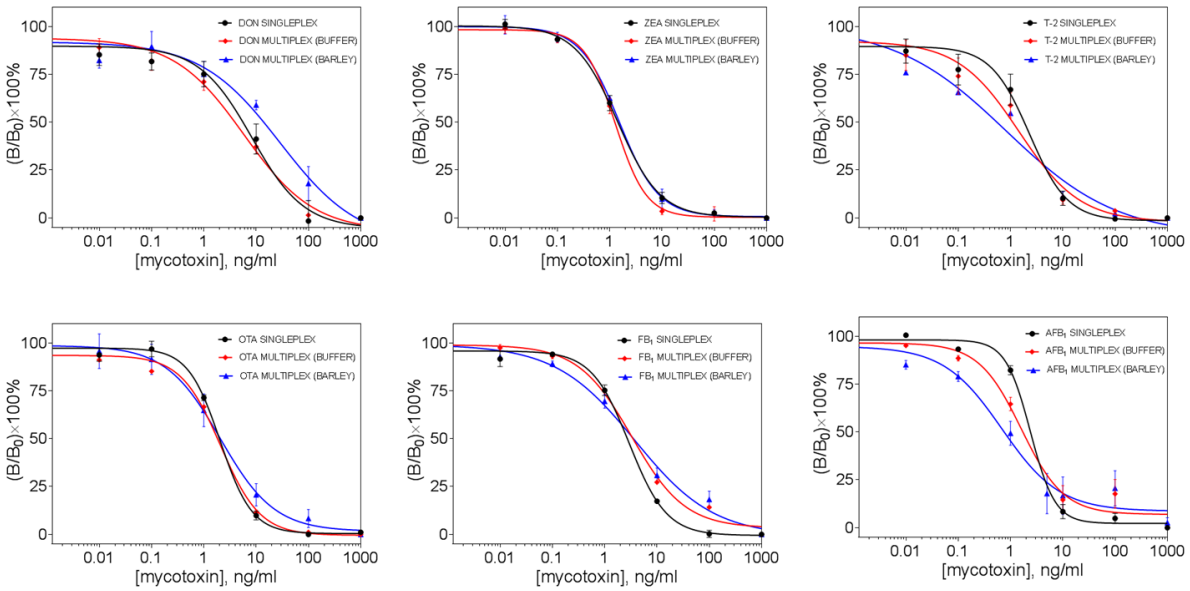

Fig. S5 Calibration curves of the double 3-plex assay using SPR (Biacore) for singleplex in buffer, multiplex in buffer and multiplex in barley extract (n=3). Two carboxymethylated chips were used: one for DON, ZEA and T-2 and another for OTA, FB_1 and AFB_1.

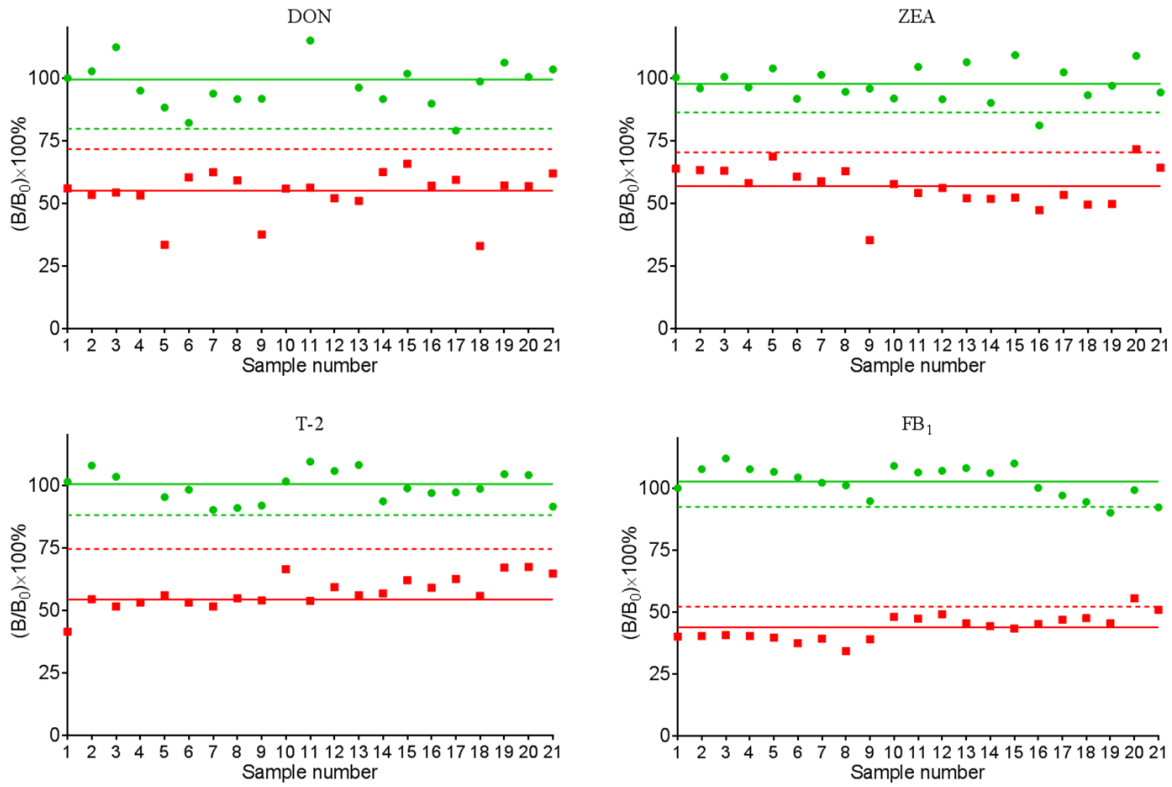

Fig. S6 Validation graphs for four mycotoxins (DON, ZEA, T-2 and FB_1) in spiked barley extract in the double 3-plex assay (using Biacore). The responses of the blank samples (dots) and spiked samples (squares) are relative to the response of a mixed blank barley sample. The averages of the responses of the blank samples (green solid line) and spiked samples (red solid line), the threshold values (green dotted line) and cut-off factors (red dotted line) are also shown in the graph.

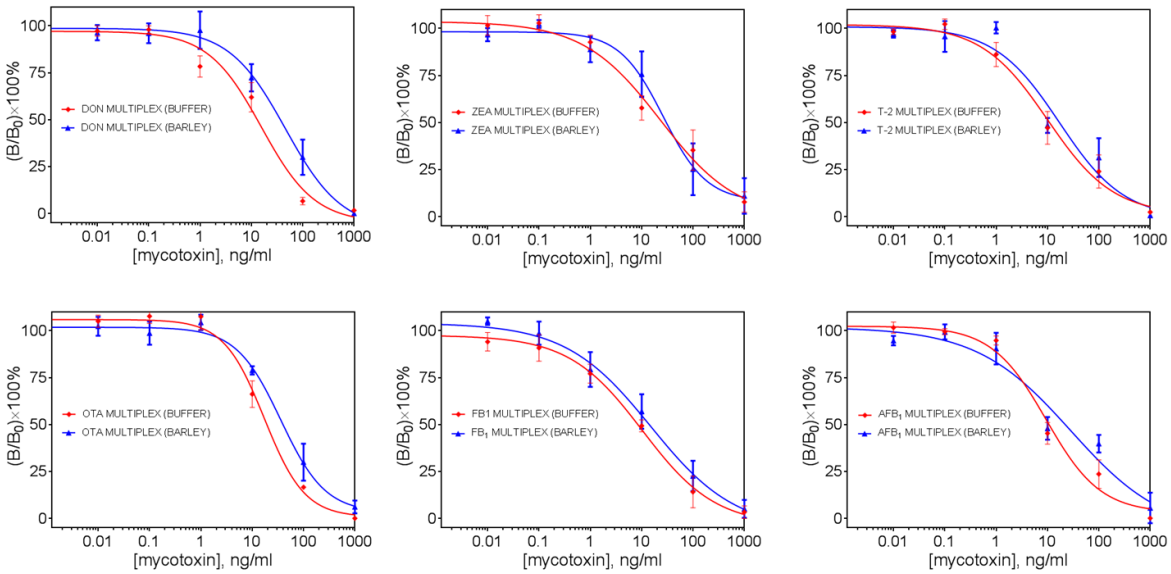

Fig. S7 Calibration curves of the 6-plex assay using the prototype nanostructured iSPR for multiplex in buffer and multiplex in barley extract (n=3). A single PEG3500 chip was used for detection of DON, ZEA, T-2, OTA, FB_1 and AFB_1.

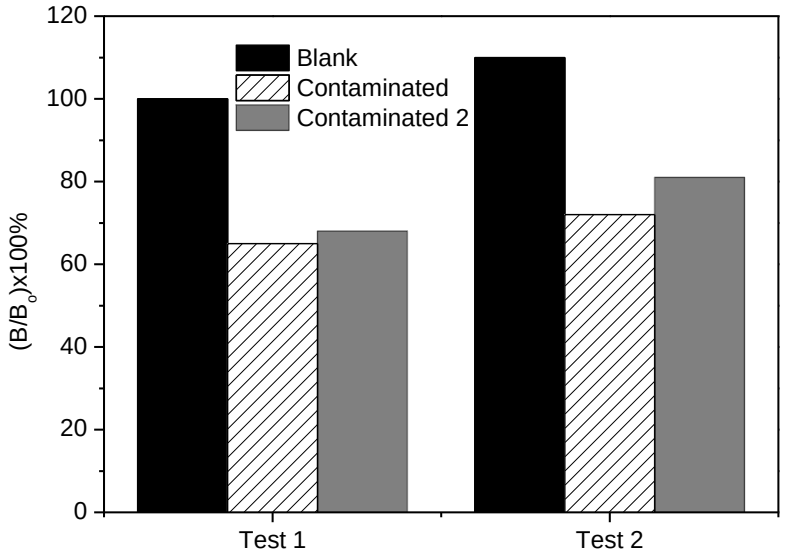

Fig. S8 Relative responses measured in duplicate for naturally contaminated barley sample (CS2) for presence of ZEA in a 6-plex format using the prototype nanostructured iSPR instrument. The responses are relative to the response of a mixed blank barley sample. Contaminated sample 1 was extracted using the laboratory based extraction protocol whereas contaminated sample 2 was extracted using a portable plastic extraction device (see experimental section).