

4th Joint Symposium on Nanotechnology

Nanotechnological detectors for mycotoxins in cereals

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Motivation

Mycotoxins: Relevance and global view

Approx. 25% of food worldwide is contaminated with mycotoxins^{1,2}

In some regions 99% of corn samples are positive for at least one mycotoxin³

Contamination levels might increase in following years

Risk factors

- Late harvest
- Inappropriate storage
- Climate change

³ BIOMIN World Mycotoxin Survey 2019



² Eskola *et al.* (2019)

Motivation

Mycotoxins: Relevance and global view

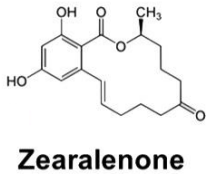
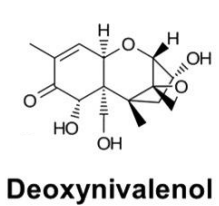
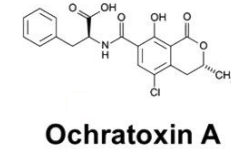
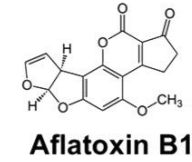
Most relevant mycotoxins

- Aflatoxin B1
- Ochratoxin A
- Zearalenone
- Deoxynivalenol

Transmission into food chain

- Primary transmission
- Secondary transmission
- Carry-over

Various toxic effects and health risks



Adapted from 10.1016/j.jhazmat.2020.122087

Motivation

Current analytics

Lab-based chromatographic analytics

- Quantitative
- Sensitive
- Experienced staff
- Expensive
- Time-consuming

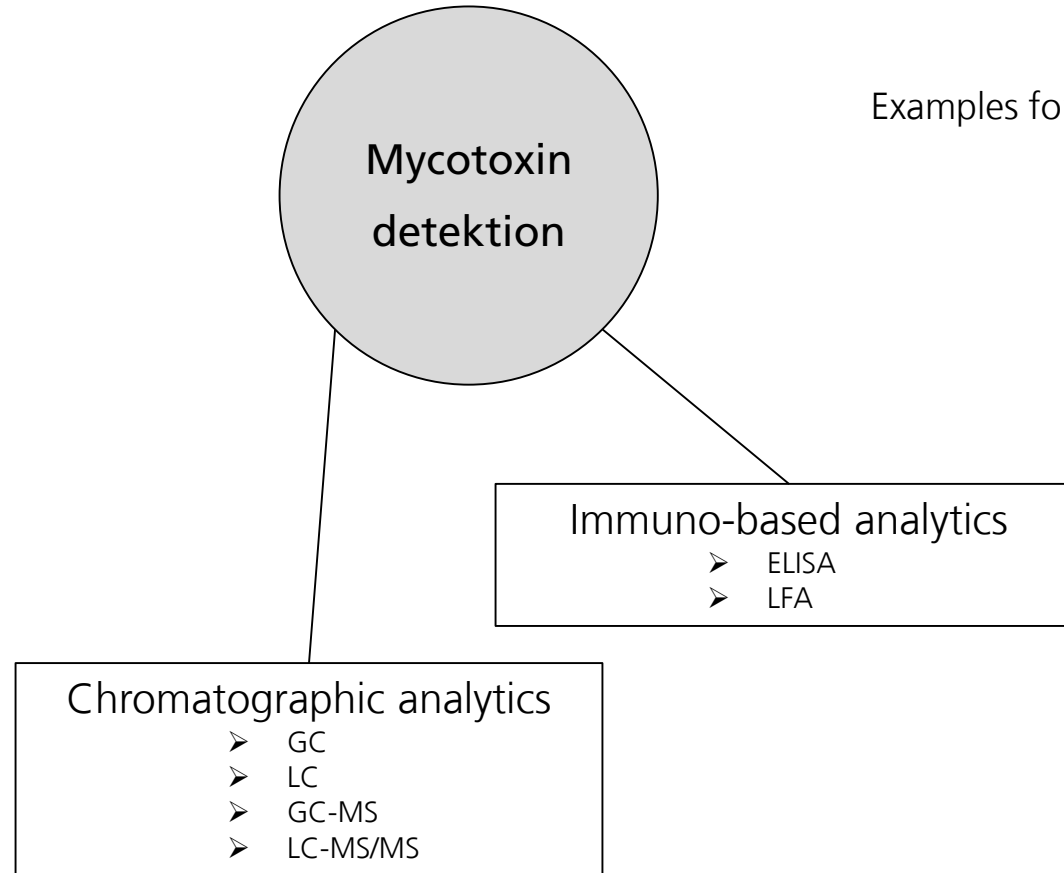
Lab-based immunological analytics

- Quantitative
- Sensitive
- Time-consuming

Mobile immunological analytics

- Lateral-flow-devices (LFD)
- No lab required
- Fast
- Simple assay procedure
- But: Prone to user errors
- Non/semi-quantitative
- Less sensitive

Examples for immunological mycotoxin tests

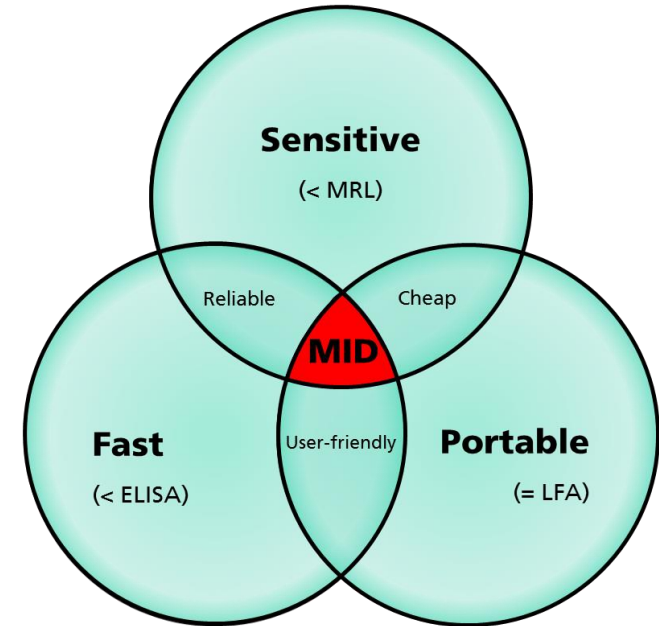


MykoNANO Project: Challenge for fast on-site analytics

Magnetic Immunodetection



- Qualitative and quantitative analytics**
- Extraction-/separation for enrichment**
 - Extraction and concentration of analyte out of sample matrix
 - Reduction of matrix effects
- Multiplex Detection**
 - Differentiation of nanoprobes or multi matrix approach
- Portability**
 - Suitable for on-site analytics
 - No Lab-equipment needed
- User-friendly**
 - App-support: Durch SOP und visuelle Testinstruktion kein geschultes Personal nötig



Competitive Magnetic Immunodetection

How it works

For mycotoxins:

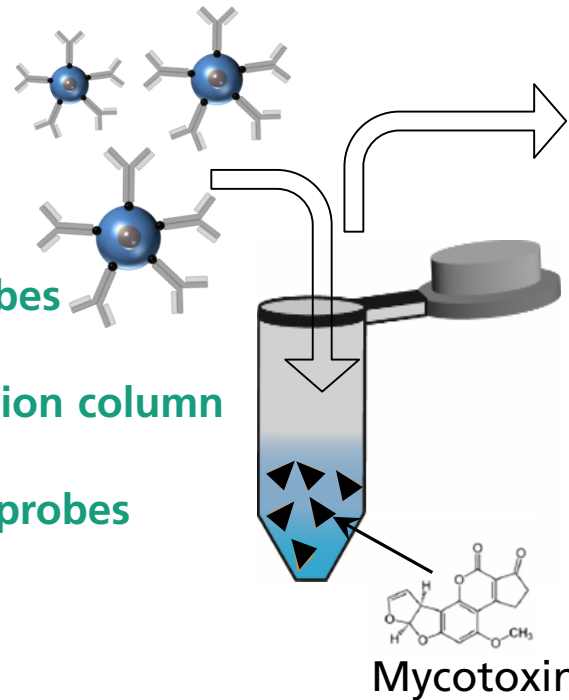
Competitive magnetic assay

Pre-incubation with nanoprob

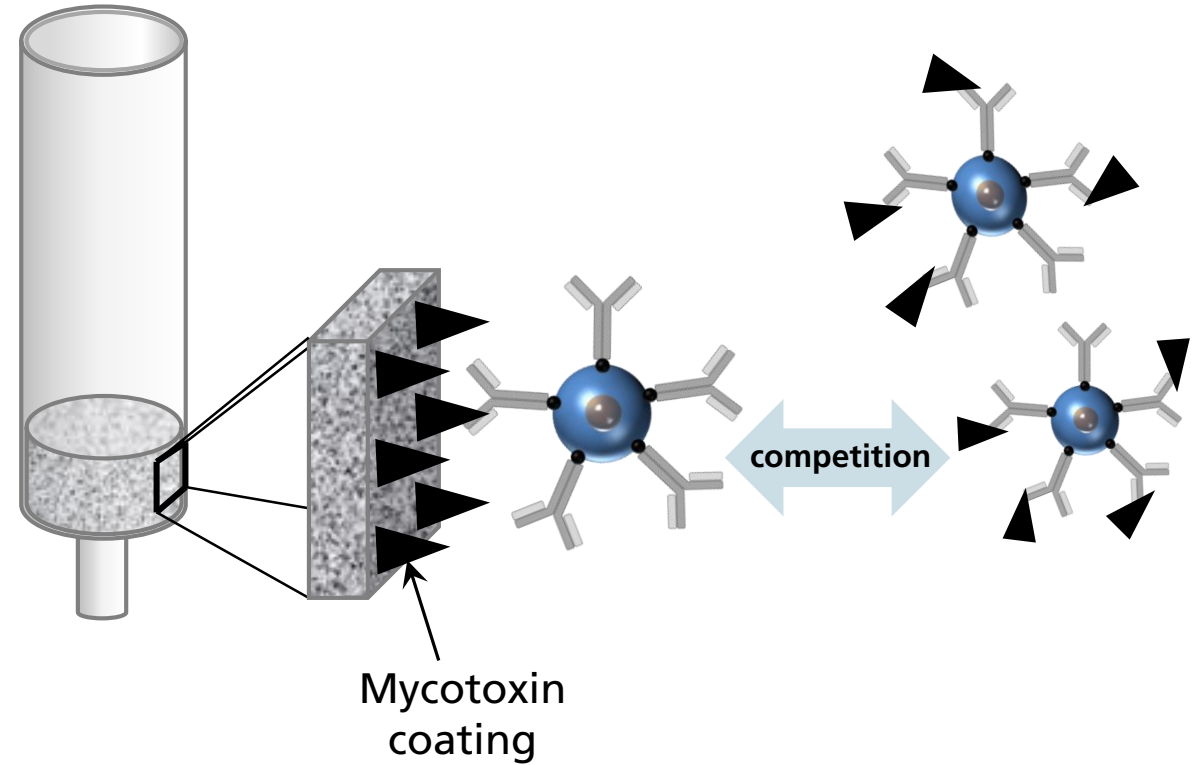
Application on immunofiltration column

Competitive binding of nanoprob

...



Immunofiltration Column



Competitive Magnetic Immunodetection

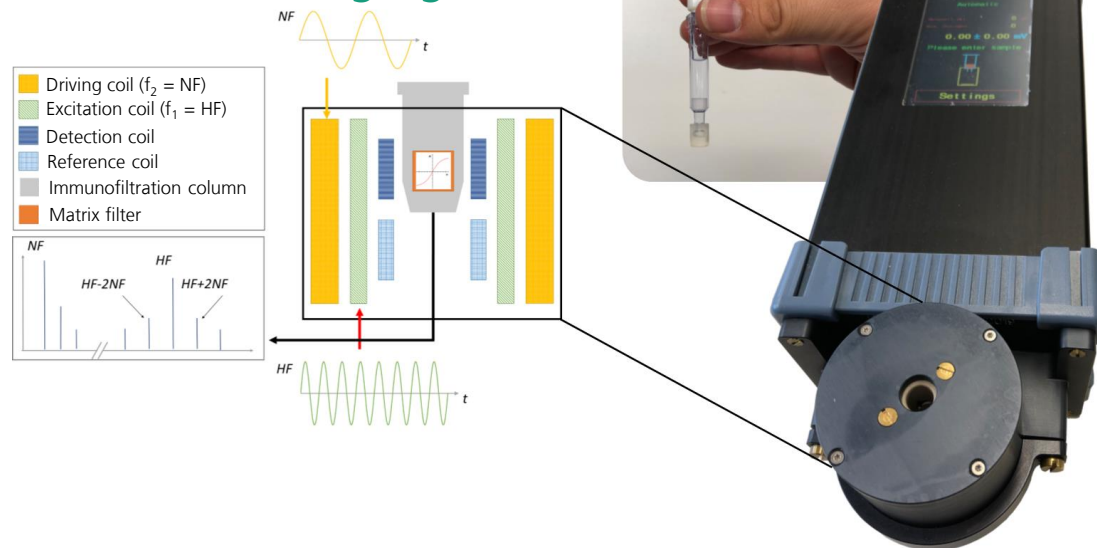
How it works

For mycotoxins:

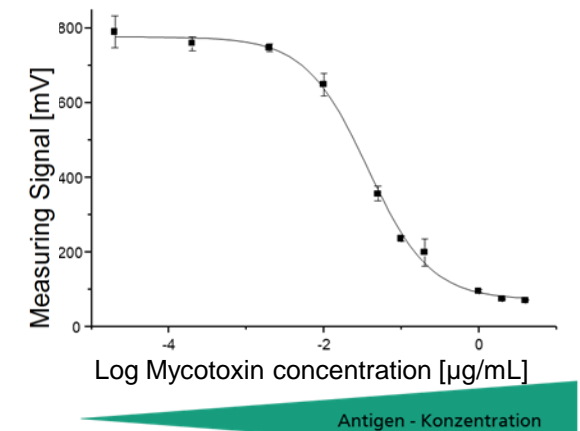
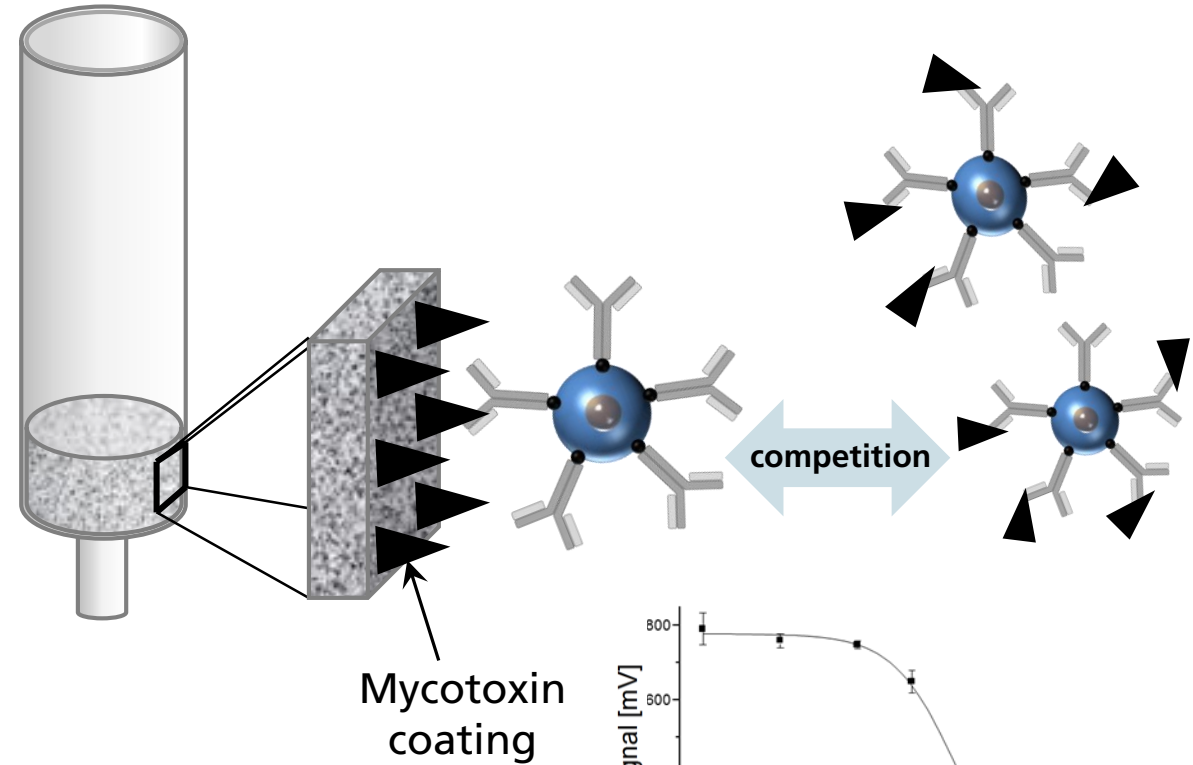
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Magnetic readout

Quantitative measuring signal



Immunofiltration Column



MykoNano Project

Generation of monoclonal antibodies

Immunization of mice

Cell fusion with myeloma cells → hybridoma cells

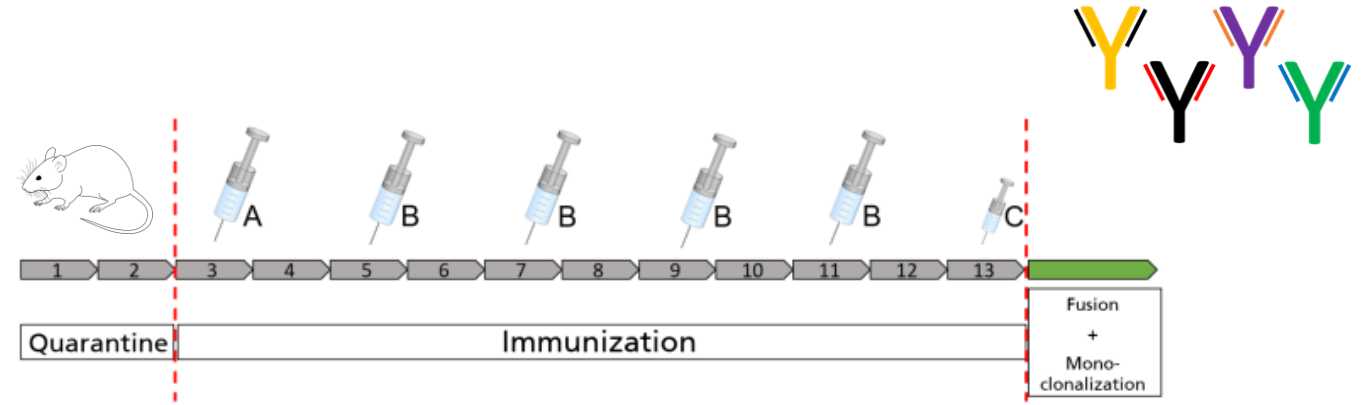
Monoclonalization and screening

Production in CELLLine™ bioreactor

Purification via FPLC and MEP

Characterization and quantification

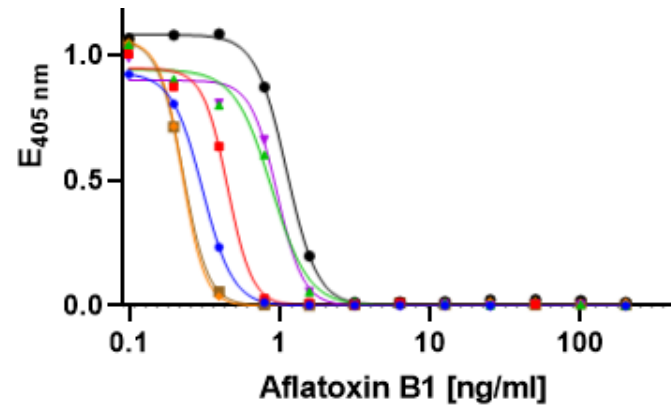
- ≈290 mg Aflatoxin B1 specific mAb
- ≈75 mg Ochratoxin A specific mAb



Result of immunization:

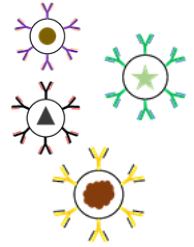
- 2 mice Ochratoxin A → 7 monoclonals adapted to H5000
- 1 mouse Aflatoxin B1 → 4 monoclonals adapted to H5000

Testing for best-performer..



MykoNano Project

Nanoprobes



Hydrodynamic size < 1 μm

Superparamagnetic core

Strong magnetic signal using FMMD

Good magnetic separation properties

Shell for biofunctionalization

Suitable for multiplex detection



Particles size between 50 nm und 1 μm

Synomag D (70 nm)

Custom Synomag/Nanomag Hybrid-Particles (700 nm)

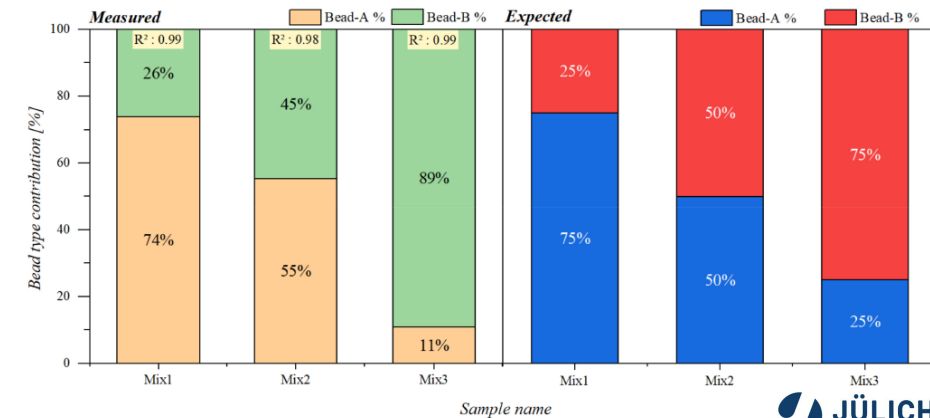
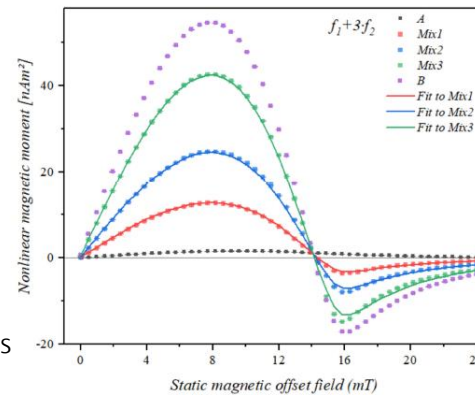
Nanomag CLD (700 nm)

Streptavidin surface functionalization

Binding of biotinylated antibodies

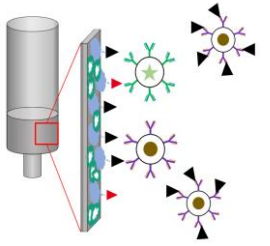
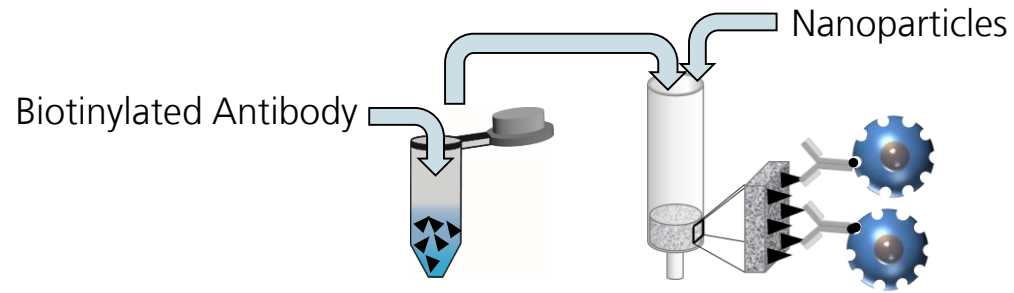
Particle A: 1 μm SynomagD

Particle B: 1 μm Synomag/Nanomag Hybrid-Particles



MykoNano Project

Assay development: cMID



Dilutions of mycotoxins in PBS buffer

Sequential assay setup

Reproducibility

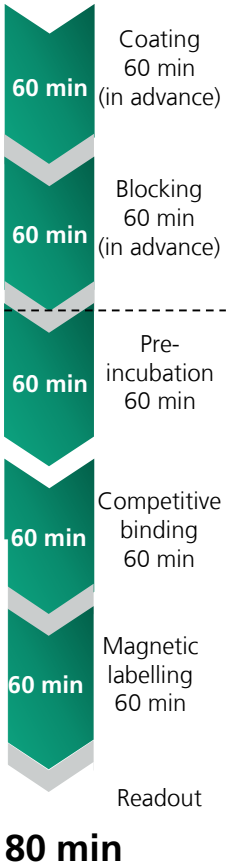
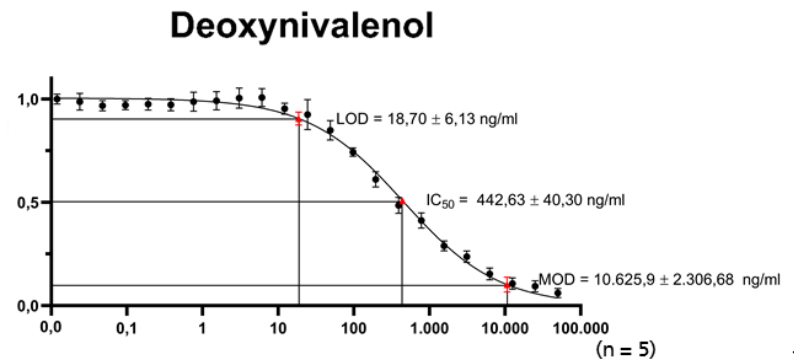
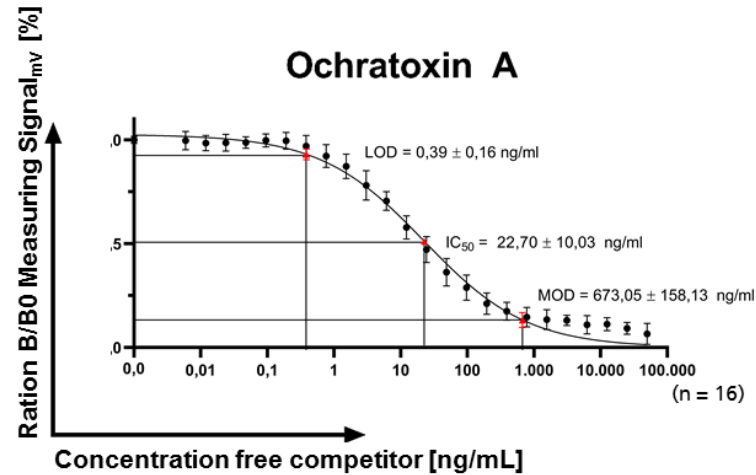
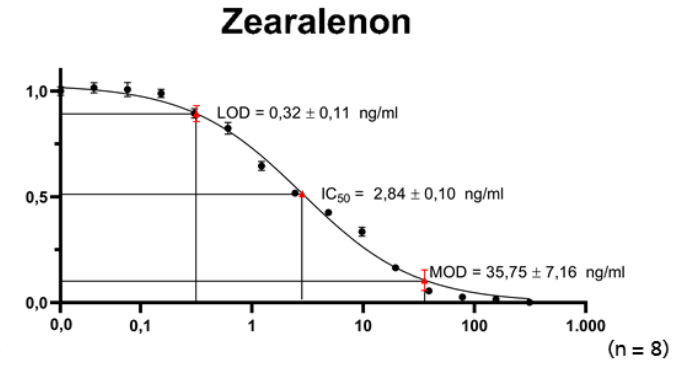
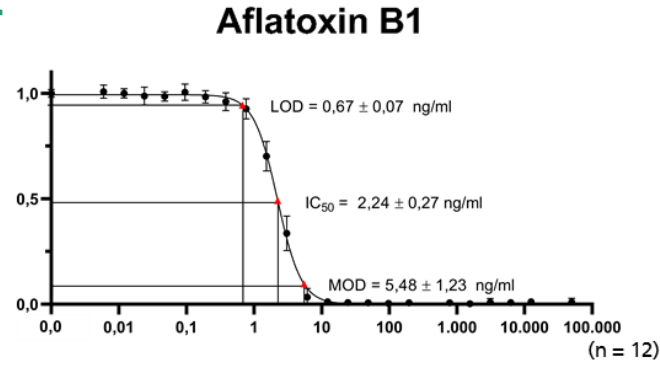
Sensitivity

Comparison to cELISA formats

Mykotoxin	MRL ¹ [ppb*]	cELISA LOD [ng/mL]	cMID LOD [ng/mL]
Aflatoxin B1	2 – 8	0.17	0.67
Zearalenon	20-200	0.02	0.32
Ochratoxin A	0.5 – 10	0.14	0.39
Deoxynivalenol	200 – 1750	12.05	18.7

¹ (EG) Nr. 1881/2006

*based on dry weight or finished product (± ng/g or ng/mL)



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Assay optimization

Assay handling

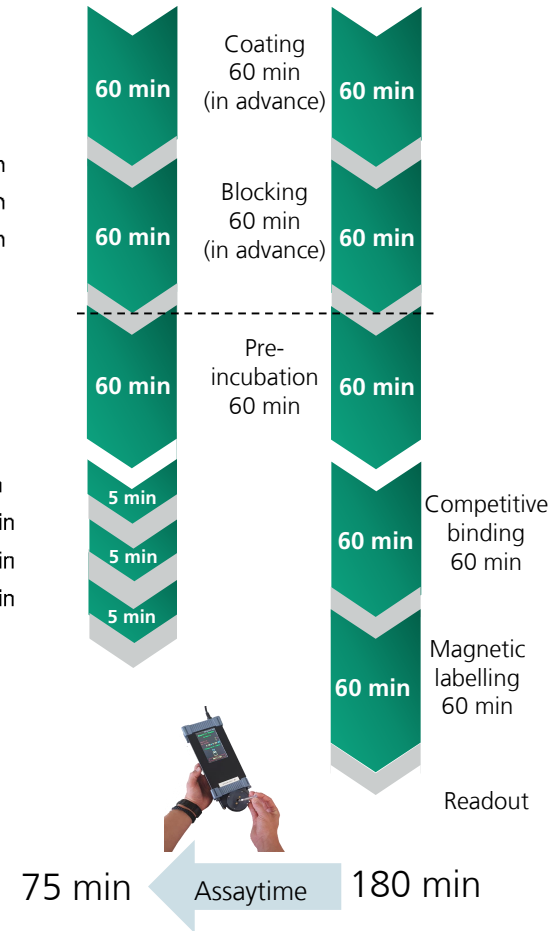
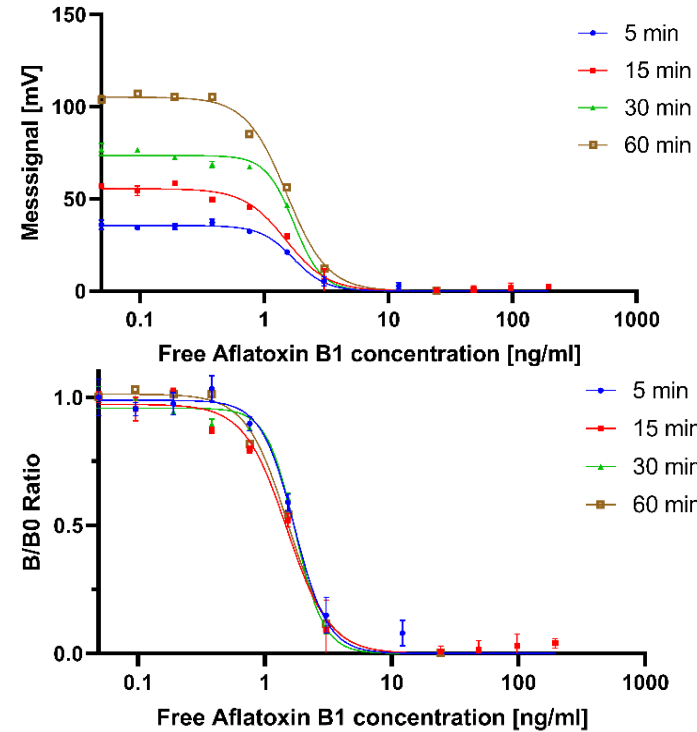
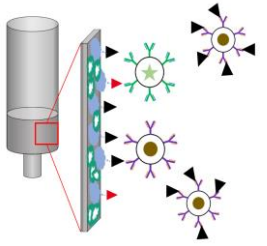
Storage of pre-coated columns

Parallelization

Assay time



Sensitivities			
	LOD [ng/ml]	IC ₅₀ [ng/ml]	MOD [ng/ml]
60 min	0,52	1,53	5,09
30 min	1,16	1,72	3,87
15 min	1,01	1,48	3,98
5 min	1,12	1,72	2,40



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MagneticReader optimization

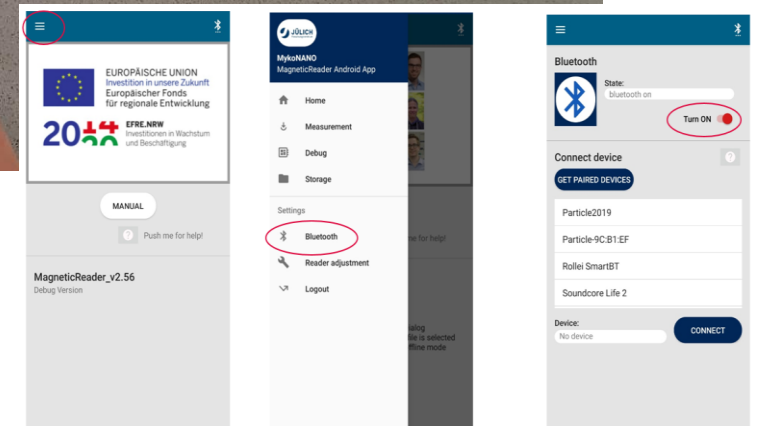
Implementation of calibration data

Automatic sample measurement / barcode reader

SD Card for data storage and USB port for connection to computer

Bluetooth connection to tablet/smartphone + android app for sample measurement and data processing

Improved on-site detection using optional battery mode



MykoNano Project

Extraction

Extraction of spiked mycotoxins from wheat

Extraction protocol suitable for all mycotoxins

Minimized matrix effects

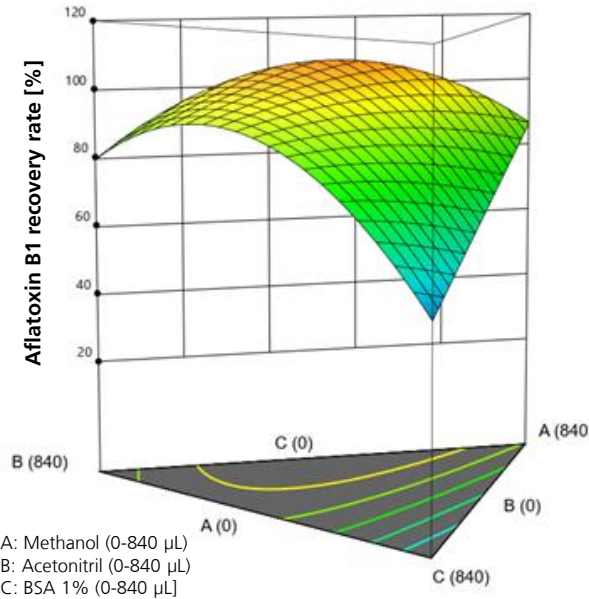
Maximum recovery rate

DoE experiments

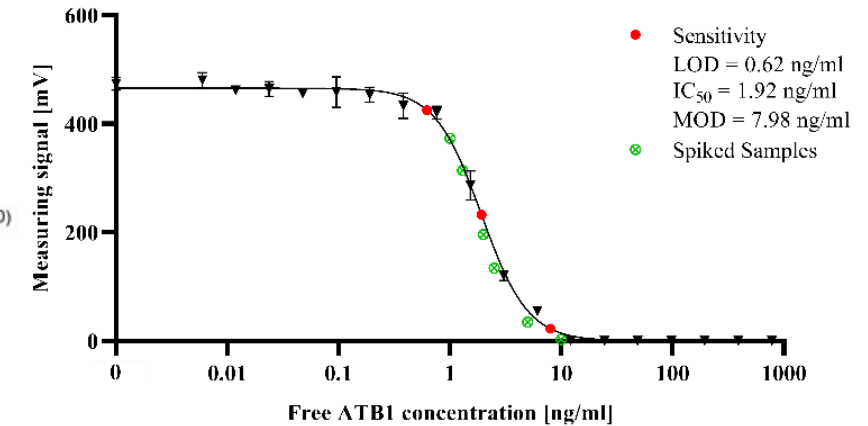


Testing 30 extraction buffer compositions with:

- Methanol
- Acetonitrile
- BSA (1%)
- Water



Aflatoxin B1



	Mixtur 4	Mixtur 11	Mixtur 15
	Mittelwert ± SD	Mittelwert ± SD	Mittelwert ± SD
Aflatoxin B1	89,4 ± 1,9	83,0 ± 6,2	92,9 ± 1,1
Zearalenon	85,4 ± 1,8	100,0 ± 3,7	91,6 ± 3,4
Ochratoxin A	80,4 ± 2,2	108,8 ± 1,0	93,7 ± 4,3
Deoxynivalenol	90,2 ± 2,7	94,0 ± 3,1	94,0 ± 3,1

0% Methanol
 36.9% Acetonitril
 10.1% BSA
 53.0% Water

0% Methanol
 46.2% Acetonitril
 0.2% BSA
 53.6% Water

0% Methanol
 38.0% Acetonitril
 13.9% BSA
 48.1% Water

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Evaluation

Single spiking experiments

Multiplex spiking experiments

- Spiking of different mycotoxin concentrations in wheat
- Simultaneous spiking with all four mycotoxins
- Extraction using extraction buffer 15
- Appropriate dilution and pre-incubation
- cMID in separate immunofiltration columns
- Recovery rates 84 – 112%



	Spiked concentration [ng/mL]	Detected \pm SD [ng/mL]	Recovery rate \pm SD [%]	n
Aflatoxin B1	2,5	2,72 \pm 0,04	108,86 \pm 1,42	6
	2,0	1,84 \pm 0,02	92,19 \pm 1,21	6
	1,5	1,56 \pm 0,04	103,71 \pm 2,60	6
	1,0	1,03 \pm 0,02	102,63 \pm 2,27	5
	Unspiked		<LOD	6
Zearalenon	10,0	8,47 \pm 0,39	84,69 \pm 3,87	6
	5,0	4,90 \pm 0,20	97,95 \pm 4,05	6
	2,5	2,32 \pm 0,10	92,72 \pm 3,86	6
	0,5	0,27 \pm 0,02	53,15 \pm 4,47	6
	Unspiked		<LOD	6
Ochratoxin A	100,0	92,66 \pm 4,04	92,66 \pm 4,04	6
	50,0	40,14 \pm 1,33	80,29 \pm 2,66	6
	20,0	17,47 \pm 0,62	87,35 \pm 3,12	6
	10,0	8,43 \pm 0,28	84,26 \pm 2,76	6
	Unspiked		<LOD	4
Deoxynivalenol	1.000,0	1.064,94 \pm 79,96	106,49 \pm 8,00	6
	500,0	518,79 \pm 19,71	103,76 \pm 3,94	6
	200,0	224,76 \pm 43,35	112,38 \pm 21,68	6
	100,0	94,04 \pm 13,78	94,04 \pm 13,78	6
	Unspiked		<LOD	6

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Outlook

Implementation of magnetic separation step

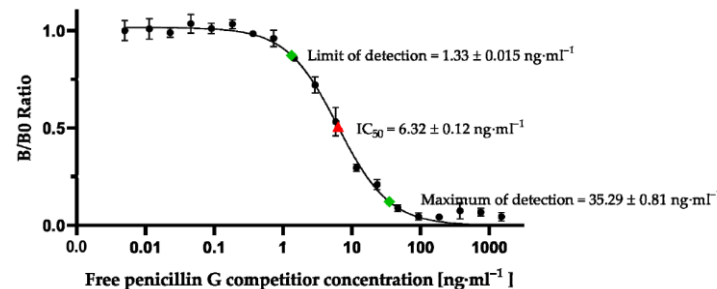
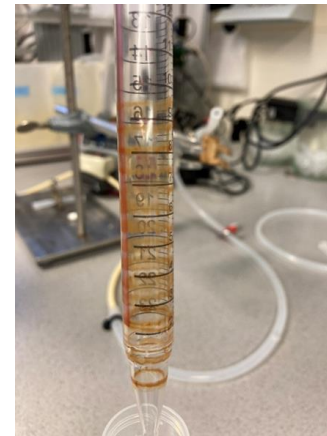
- Optimized hybrid particles
- Pre-incubation with extraction buffer
- Separation efficiencies >90%
- Separation device

Multiplex detection using different nanoprob

- Pre-incubation with different nanoprob
- Multiplex-detection in a single column

Detection of further small molecular analytes

- Antibiotics (e.g. in milk)
- Hormones
- (bacterial) signaling molecules



Sample	Signal [mV]	Separation efficiency [%]
before separation	1847,8	-
After separation	1561,5	88,3%

Thank you for your attention



Fraunhofer Institute for Molecular
Biology and Applied Ecology IME

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