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DIE HOCHSCHULE, DIE MEHR KANN.

Ames Sense: Analysis of plastic extracts with an improved miniaturized Ames test

Thomas Czerny, Lukas Prielinger: Migratox Industry board meeting, 21.09.2023

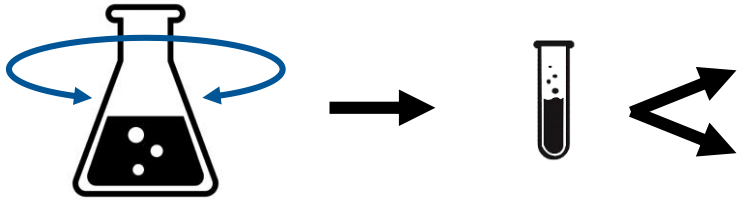
Ames Test

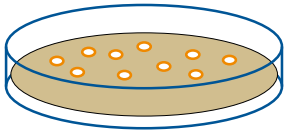
Salmonella bacteria with mutation in his pathway, strains TA100 and TA98

O/N culture with external his

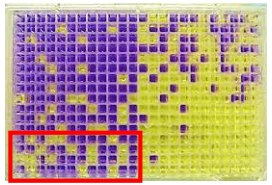
exposure -> reverse mutations

medium without his: only mutated bacteria will grow





colonies



liquid culture

Sense Ames

high reproducibility

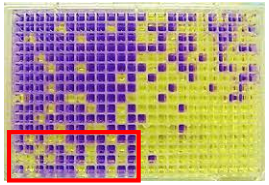
frozen bacteria -80°C

high sensitivity

4h

+ further adaptations buffers, media, ...

high performance



2 µL sample volume high throughput

Comparison of assays

	Plate Assay	SenseAmes
	colonies on agar plate	liquid culture in 384 well plates
volume exposure	650 µL	20 µL
sample volume	50 µL	2 µL
exposure time	20-60 min	4 hours
bacteria	fresh o/n culture	frozen
reproducibility	low (experienced technician)	high (fresh student)

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Performance of the experiments

	Plate Assay	SenseAmes
preparation day before	pour agar plates inoculate culture	-
experiment: samples/day/person	48 test concentrations	288 test concentrations
evaluation after 2 days	colony counting	plate reader

Improvement of detection limits

Fold improvement of detection limit with Sense Ames compared to plate assay			
		Concentration	**Amount**
2-Acetylaminofluorene	2AAF	12,1	1636
Aflatoxin B1	AFB1	7,8	1053
Benzo-α-pyrene	BaP	35,9	4844
Cisplatin	CisP	22,2	3002
2,4-Diaminotoluene	DAT	13,5	1823
7,12 Dimethylbenzanthracene	DMBA	733	98988
N-Ethyl nitrosourea	ENU	4,2	567
2-Amino-3-methyl-3H-imidazo[4,5-f]quinoline	IQ	39,8	5375
Methyl Methanosulphonate	MMS	1,8	242
4-Nitroquinoline-N-Oxide	4NQO	54,0	7289
2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine	PhIP	62,9	8486
Dimethylcarbamoyl chloride	DMC	5,5	737
Glycidol	Gly	14,7	1988
Hydrazine	Hyd	328	44294
Dimethyl Sulfate	DS	0,2	20
Acridin orange	AO	31,1	4194
2-Aminoanthracen	2AA	95,7	12919
2-Aminofluorene	2AF	3,0	402
Triglycidyl Isocyanurat	TGI	11,5	1559
2-Nitropropane	NP	3,3	440
Ethidium Bromide	EtBr	195	26320
Quercetine	Q	2,0	273
Phenylglycidylether	PGE	2,5	335
2-Nitrofluorene	2NF	23,6	3186
	Mean of all	71,0	9582

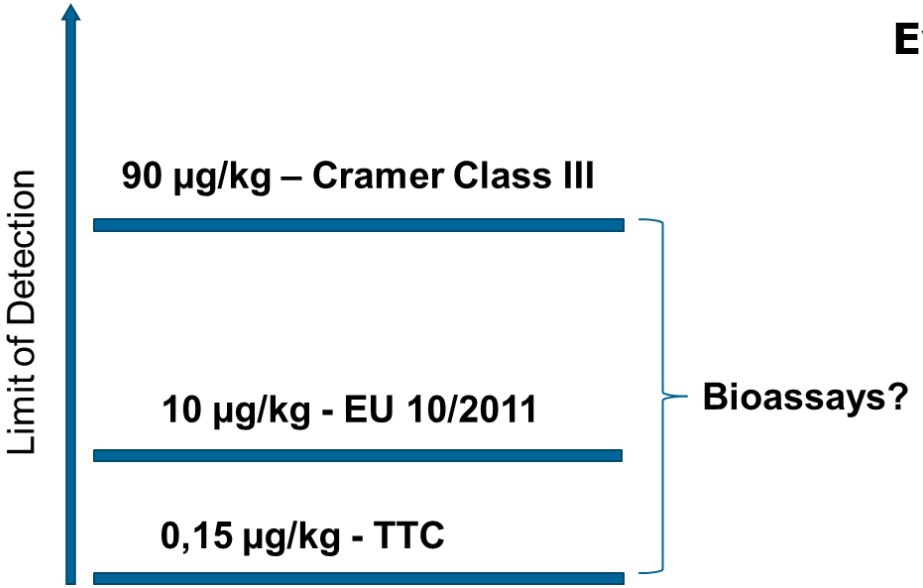
Results Sense Ames compared to plate assay

experiments with 2 strains: TA98 and TA100 +/- S9

71 x improvement for concentration

9600 x improvement for amount of substance

Strategy - Analysis of NIAS



Evaluation criteria of EFSA:

- >Lowest effect concentration (LEC) of <0.15 µg/kg is required.
- >If sufficient information is available, the use of the TTC approach is acceptable.
- >Unknown substances are classified as potentially DNA-reactive (EFSA, 2019).

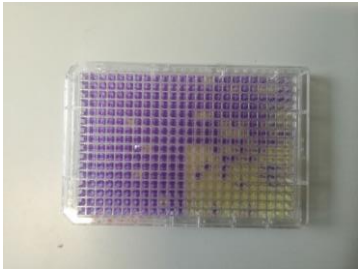
Source: EFSA (2019):Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment; https://doi.org/10.2903/j.efsa.2019.5708

Material & Methods - Sample preparation *

>Used for packaging materials especially food contact materials (FCMs)
>Migration experiments based on EU10/2011 and DIN-standards
>Achieving a sample concentration factor of 300 by rotary evaporation
>Solvent exchange to DMSO → extract is suitable for Ames test

Migration: 95% Ethanol
10 days, 60 °C

Concentration (300x)
and solvent exchange
to DMSO



Ames Test

*__Source__: Rainer, Mayrhofer et. al. (2019): Mutagenicity assessment of food contact material migrates with the Ames MPF assay. https://doi.org/10.1080/19440049.2019.1634841

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Material & Methods – Testing parameters

Materials:

>24 plastic sample extracts (SA01-SA24)
>Samples are not representative for the market
>Some worst-case samples were selected (for research purposes)

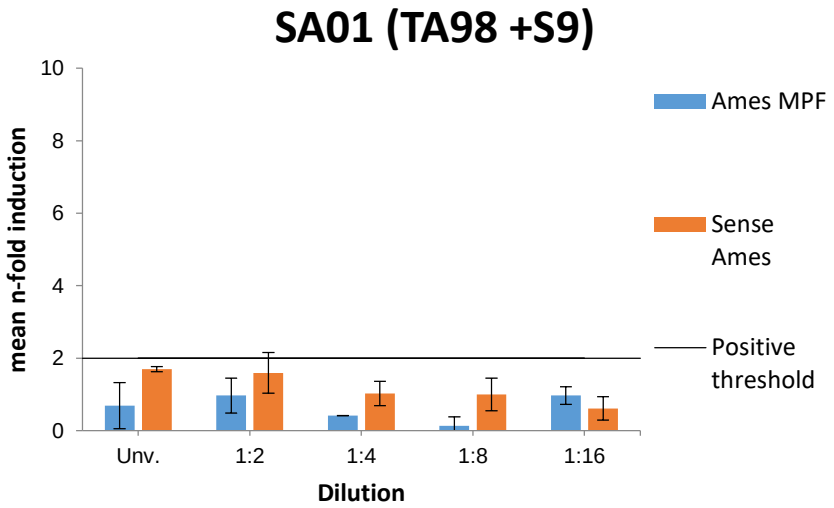
Ames test conditions:

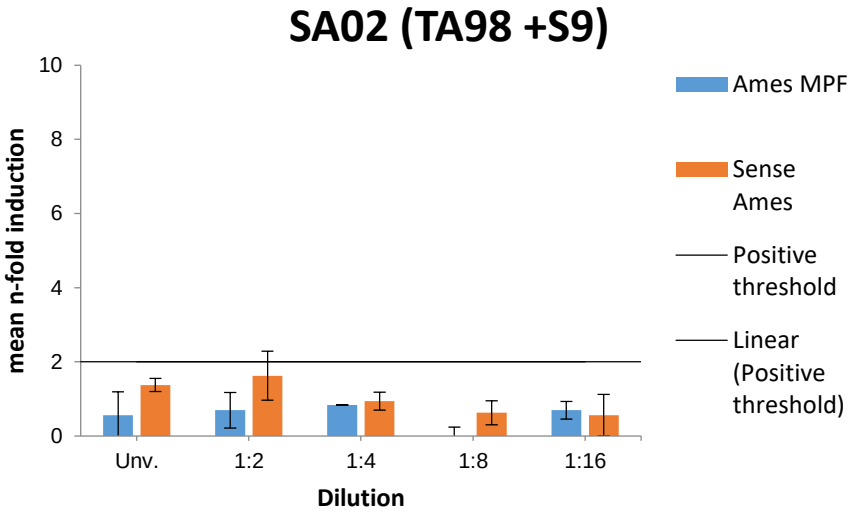
>Direct comparison of two Ames test formats (Sense Ames vs. MPF)
>TA98 with and without metabolic activation (+/- S9)
>Comparison of the LEC values
>At least two runs for each sample (duplicate determination)
>Ames MPF: 10 µl sample volume; Sense Ames: 2 µl sample volume

Ames Test - Evaluation criteria

- **Baseline:** Mean number of the negative control + Standard deviation
- **Positive threshold:** Baseline multiplied by 2
- **Fold induction:** Mean number of revertants of the tested sample divided by the baseline
- **Positive result**: Mean number of revertants surpassed the positive threshold (n-fold induction>2)
- **Lowest effect concentration (LEC):** Positive result based on the lowest dilution which exceeded the positive threshold. Comparison of both LEC values (Sense Ames vs. Ames MPF)

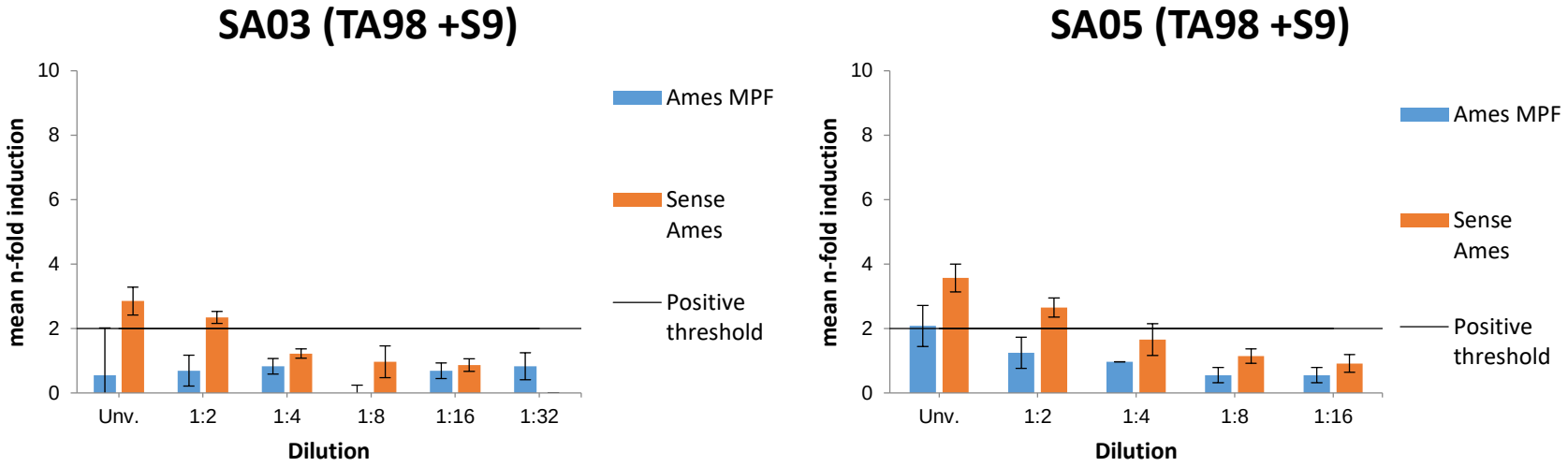
Results - Sense Ames vs. Ames MPF





> Samples SA01 and SA02 were negative in both Ames variants but the Sense Ames had a higher dose-response.

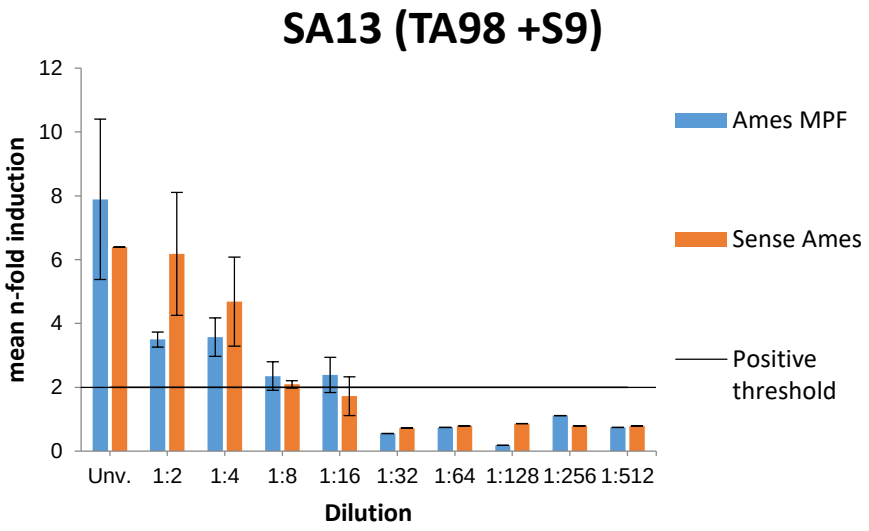
Results - Sense Ames vs. Ames MPF

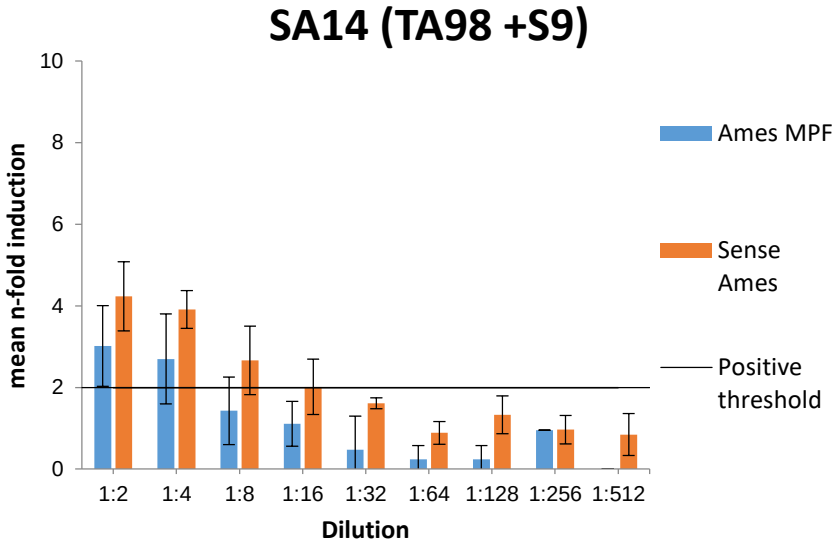


> Sense Ames (SA03) had a n-fold induction of 2.9 (Ames MPF was negative)

> Sense Ames (SA05) had a n-fold induction of 3.6 (Ames MPF: n-fold induction of 2.1)

Results - Sense Ames vs. Ames MPF

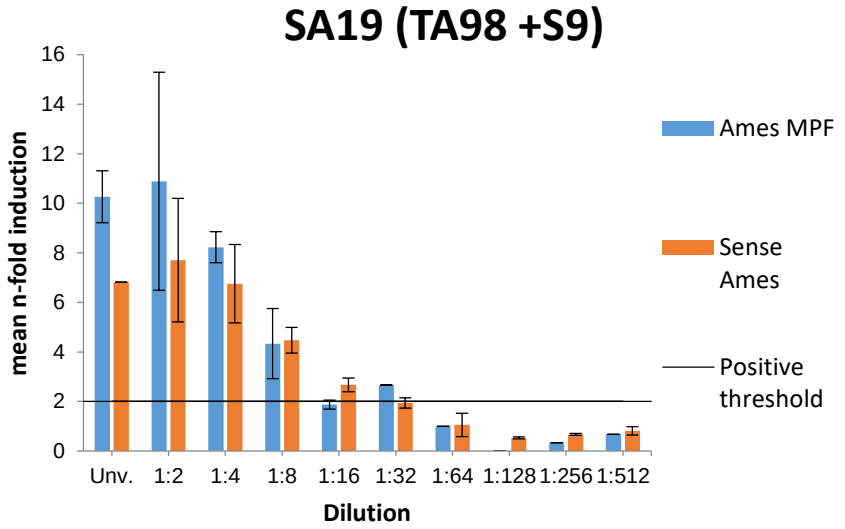


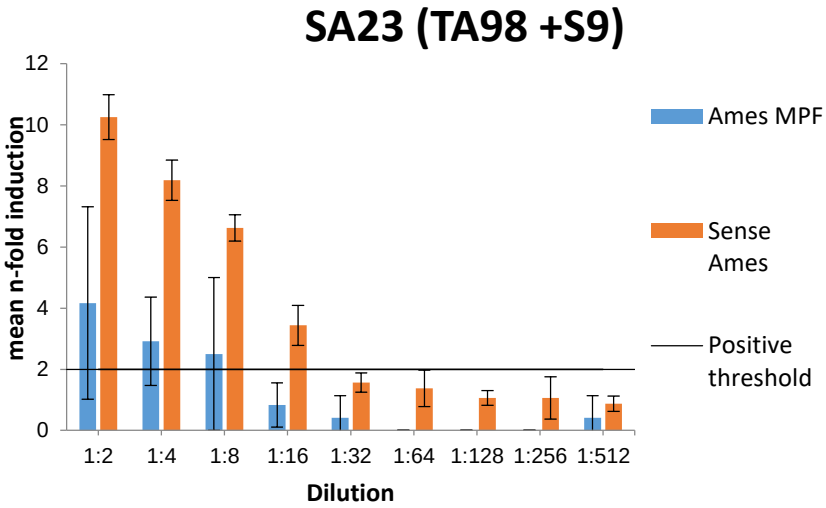


> Sample SA13 was positive up to a dilution of 1:8 (Ames MPF up to 1:16).

> Sample SA14 was positive up to a dilution of 1:16 (Ames MPF up to 1:4).

Results - Sense Ames vs. Ames MPF

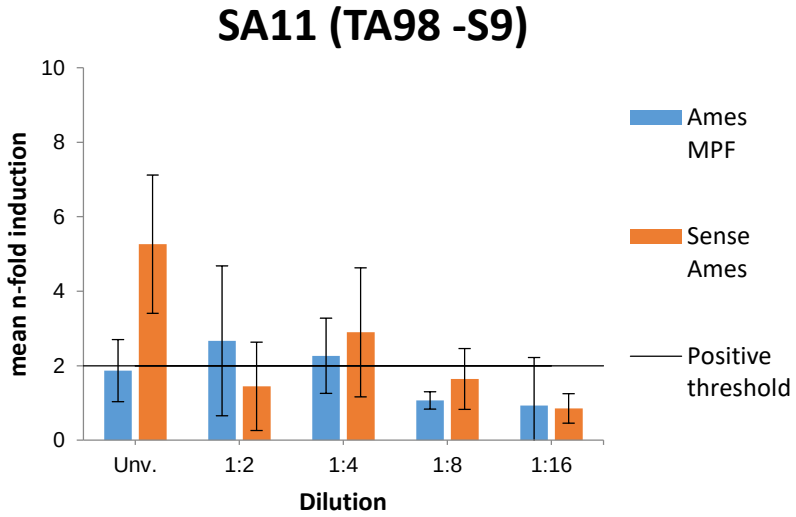


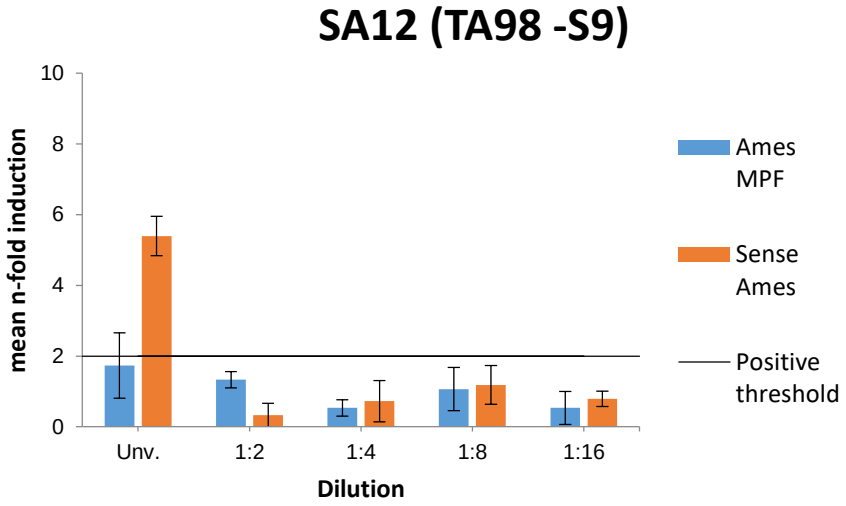


> Sample SA19 was positive up to a dilution of 1:16 (Ames MPF up to 1:32).

> Sample SA23 was positive up to a dilution of 1:16 (Ames MPF up to 1:8).

Results - Sense Ames vs. Ames MPF





> Sample SA11 was positive up to a dilution of 1:4 (Ames MPF up to 1:4).

> Sample SA12 was positive up to a dilution of 1:1 (Ames MPF was negative).

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Overview of the results – Sense vs. MPF

Sample Code	Ames MPF TA98 -S9		Sense Ames TA98 -S9		Ames MPF TA98 +S9		Sense Ames TA98 +S9	
	Result	Based on dilution	Result	Based on dilution	Result	Based on dilution	Result	Based on dilution
SA01	-	-	-	-	-	-	-	-
SA02	-	-	-	-	-	-	-	-
SA03	-	-	-	-	-	-	+	1:2
SA04	-	-	-	-	+	1:1	+	1:2
SA05	-	-	-	-	+	1:1	+	1:2
SA06	-	-	-	-	-	-	+	1:1
SA07	-	-	-	-	-	-	-	-
SA08	-	-	-	-	-	-	-	-
SA09	-	-	-	-	-	-	-	-
SA10	-	-	-	-	-	-	+	1:1
SA11	-	-	+	1:1	+	1:256	+	1:256
SA12	+	1:2	+	1:8	+	1:256	+	1:256
SA13	-	-	+	1:2	+	1:16	+	1:8
SA14	-	-	+	1:1	+	1:4	+	1:16
SA15	-	-	-	-	+	1:4	+	1:8
SA16	-	-	-	-	-	-	-	-
SA17	-	-	-	-	-	-	-	-
SA18	-	-	-	-	+	1:8	+	1:16
SA19	-	-	+	1:8	+	1:32	+	1:16
SA20	-	-	-	-	+	1:8	+	1:8
SA21	-	-	-	-	-	-	-	-
SA22	-	-	-	-	+	1:16	+	1:32
SA23	-	-	-	-	+	1:8	+	1:16
SA24	-	-	-	-	+	1:1	+	1:2

Experimental work and data by Thomas Barila, BSc

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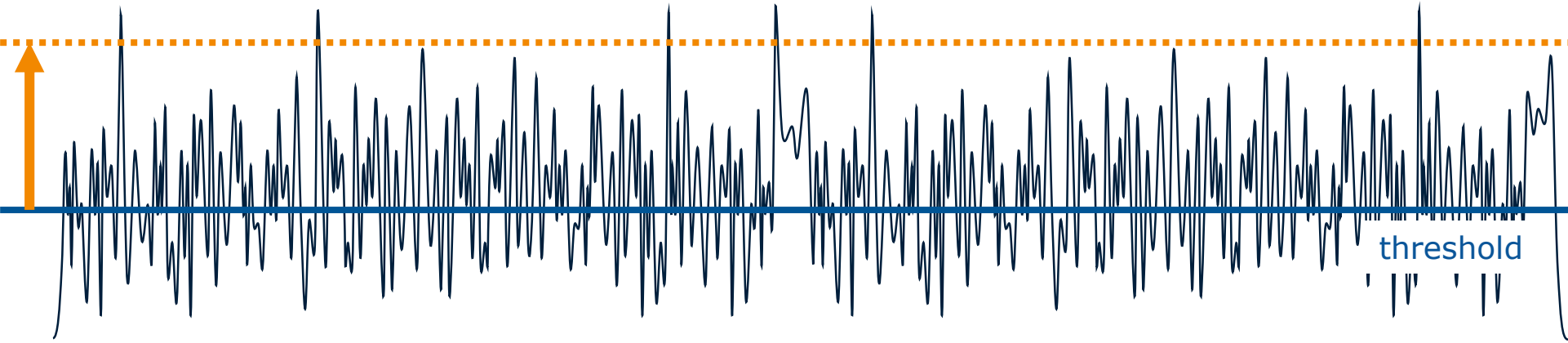
Conclusions – Sense Ames vs. MPF

- >Sense Ames could confirm all the positive Ames MPF results and detected even more
- >In many cases, the LECs of the Sense Ames were lower (1 or 2 dilution steps)
- >Sense Ames could detect more positive samples without metabolic activation (TA98 –S9) → Inhibition is not such a big issue with the Sense Ames
- >Sense Ames has many advantages compared to the Ames MPF (lower sample volume, lower detection limits especially for pure substances etc.)

Future prospects – Sense Ames

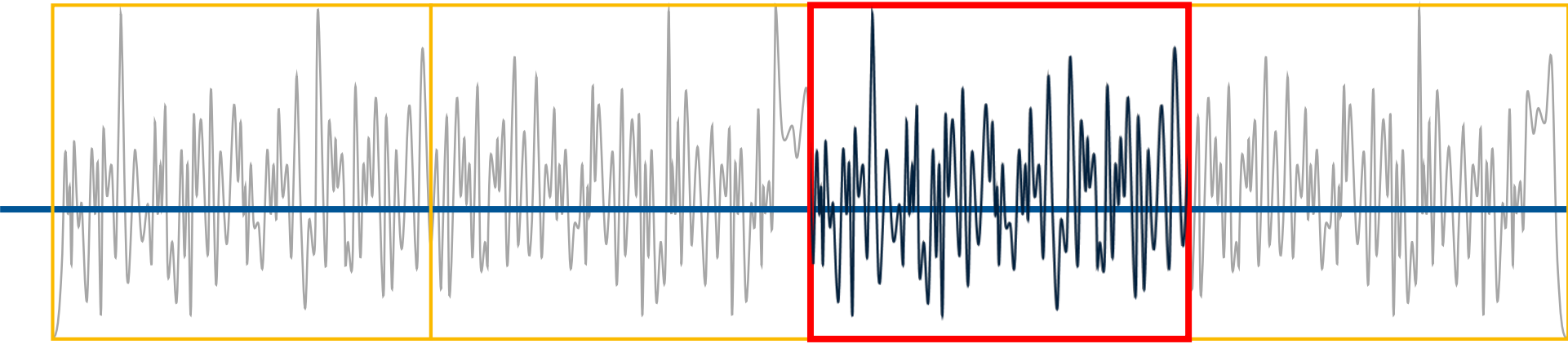
Reduction of complexity: raising the threshold according to the TTC concept



HPLC-MS → Unknowns

Future prospects – Sense Ames

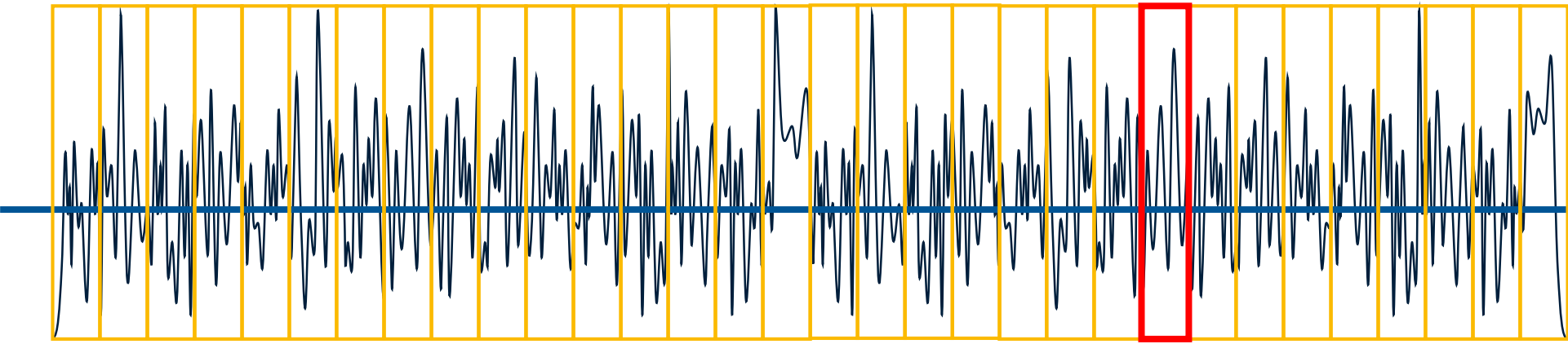
Reduction of complexity: fractionation

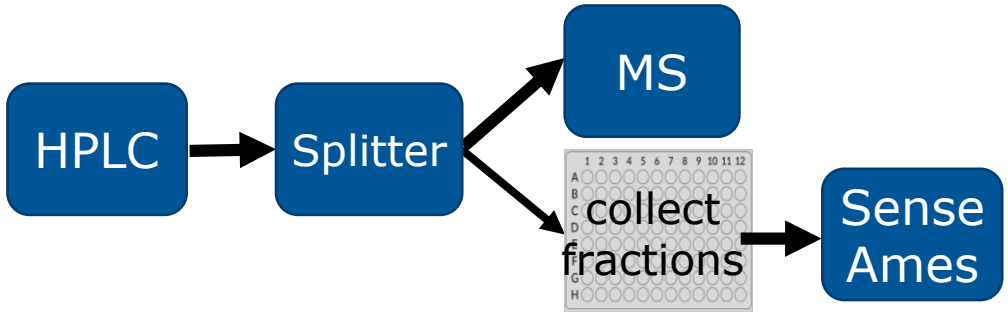


Fractionation → Ames → HPLC-MS

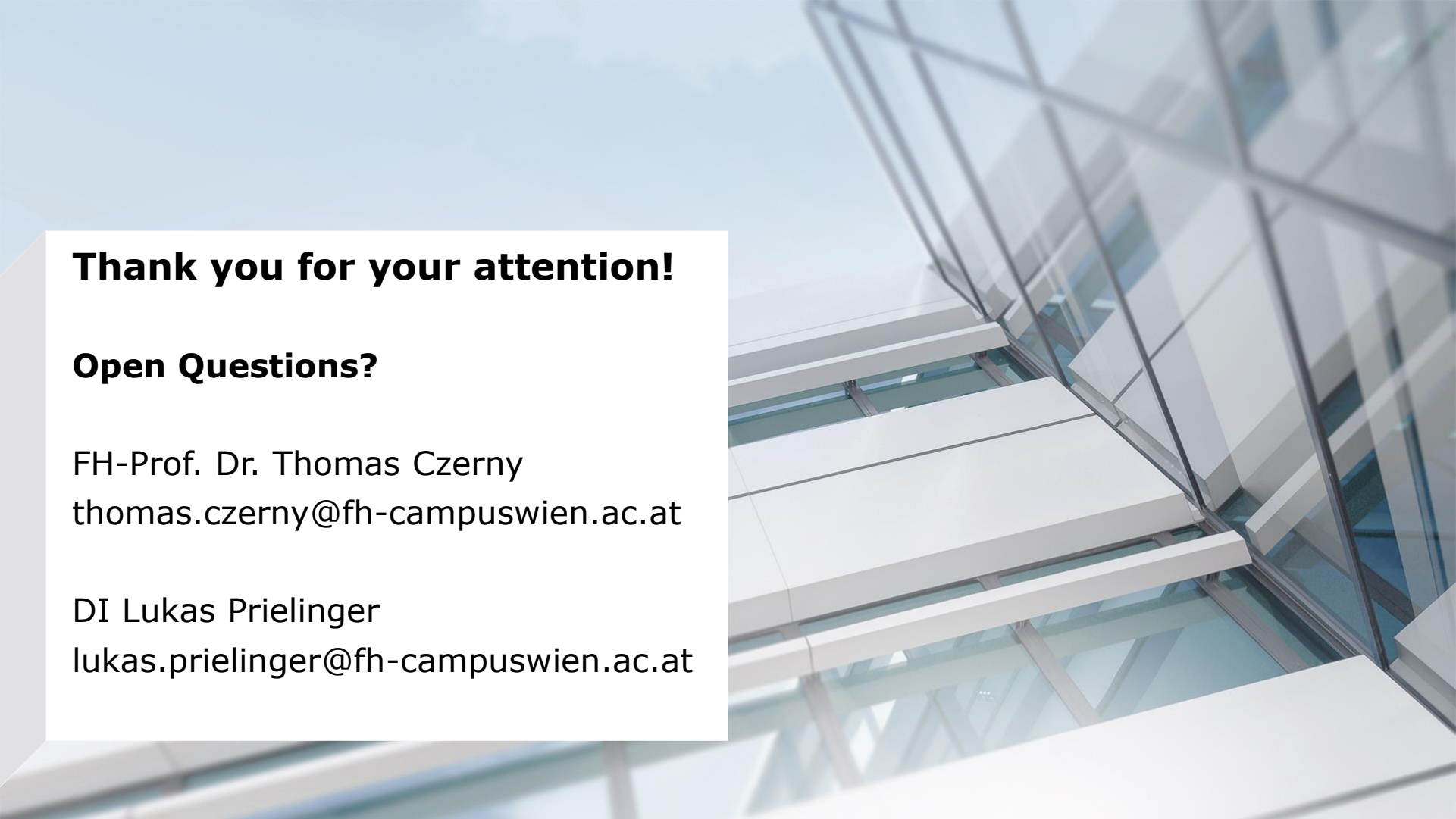
Future prospects – Sense Ames

Reduction of complexity: parallel chemical and biological analysis





> complete HPLC-MS data
> parallel data from bioassay
> low volume / high throughput of Sense Ames



Thank you for your attention!

Open Questions?

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