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What is the gain from hyphenated HPTLC?

G. Morlock, JLU Giessen, Germany

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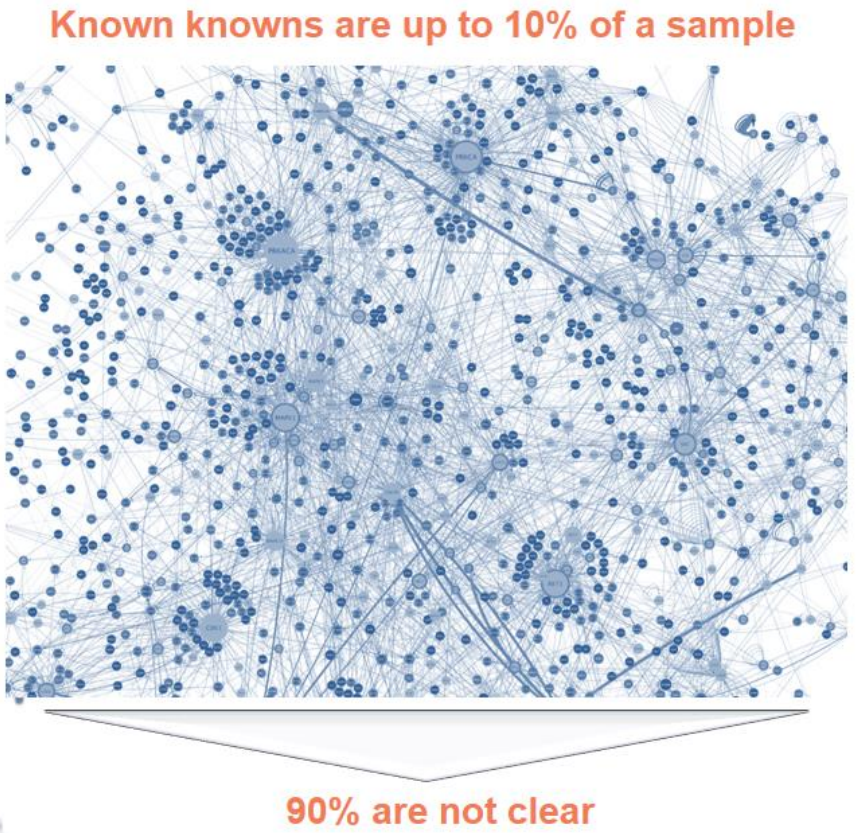
Superhyphenations as disruptive approach

- The majority of signals remains unclear.
- Low amounts can have high effect.
- We can not elucidate each substance signal.
- Toxicological assessment for each not possible.



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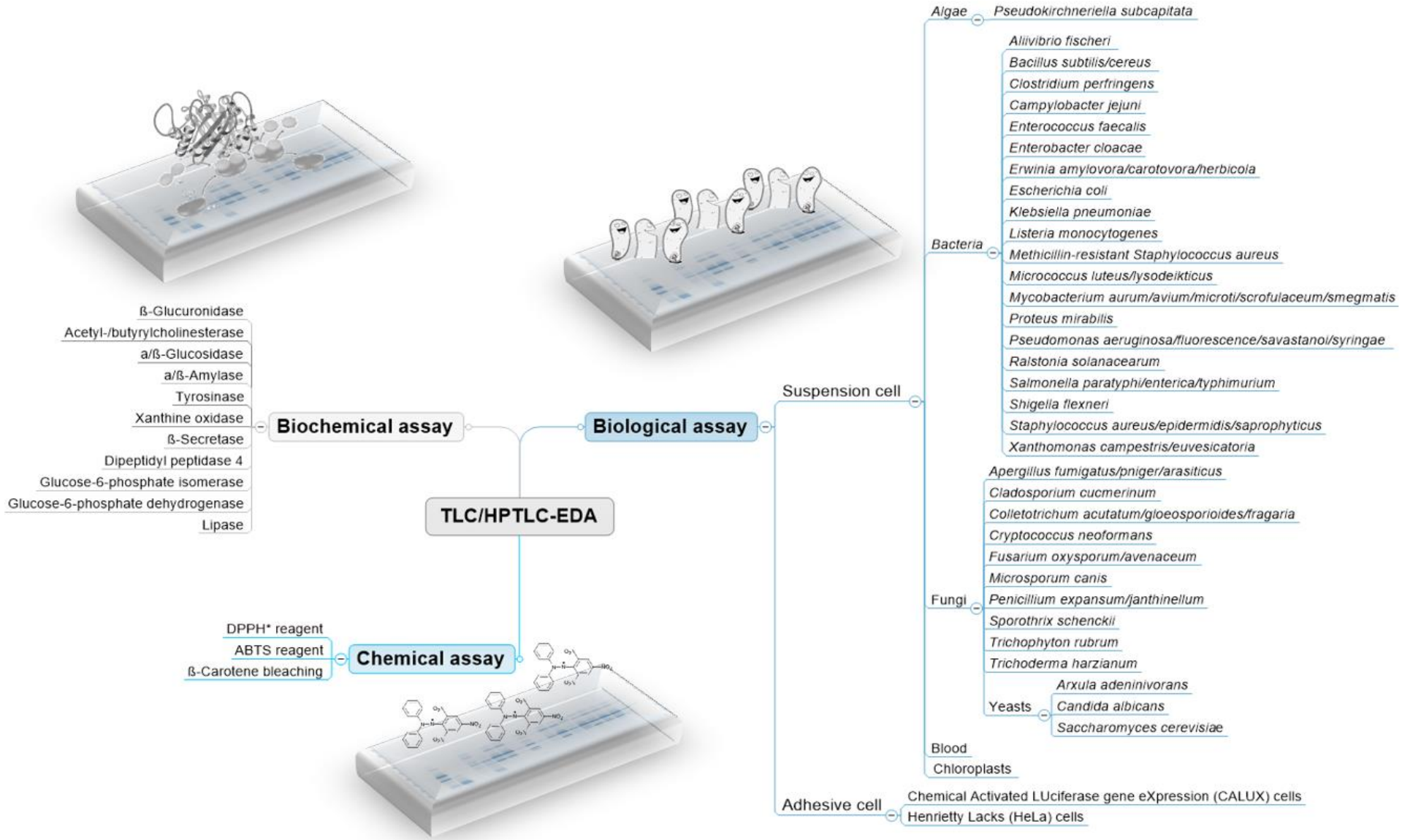
Hyphenation: Chromatography + Effect detection



G. Morlock *Anal. Chim. Acta* 1180 (2021) 338644

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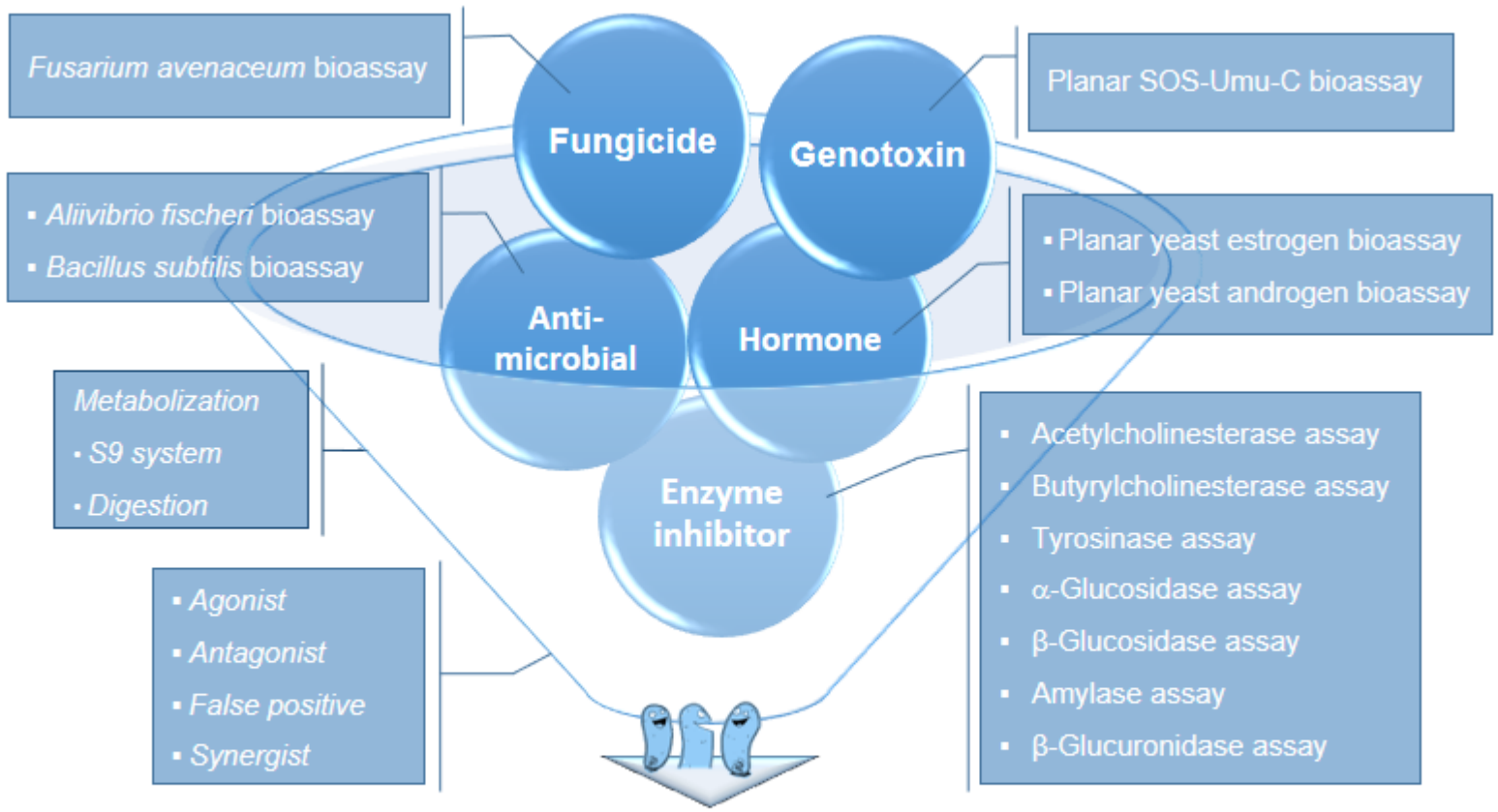
Prioritization strategy



Tousands of compounds reduced to few bioactive ones

Prioritization of important compounds

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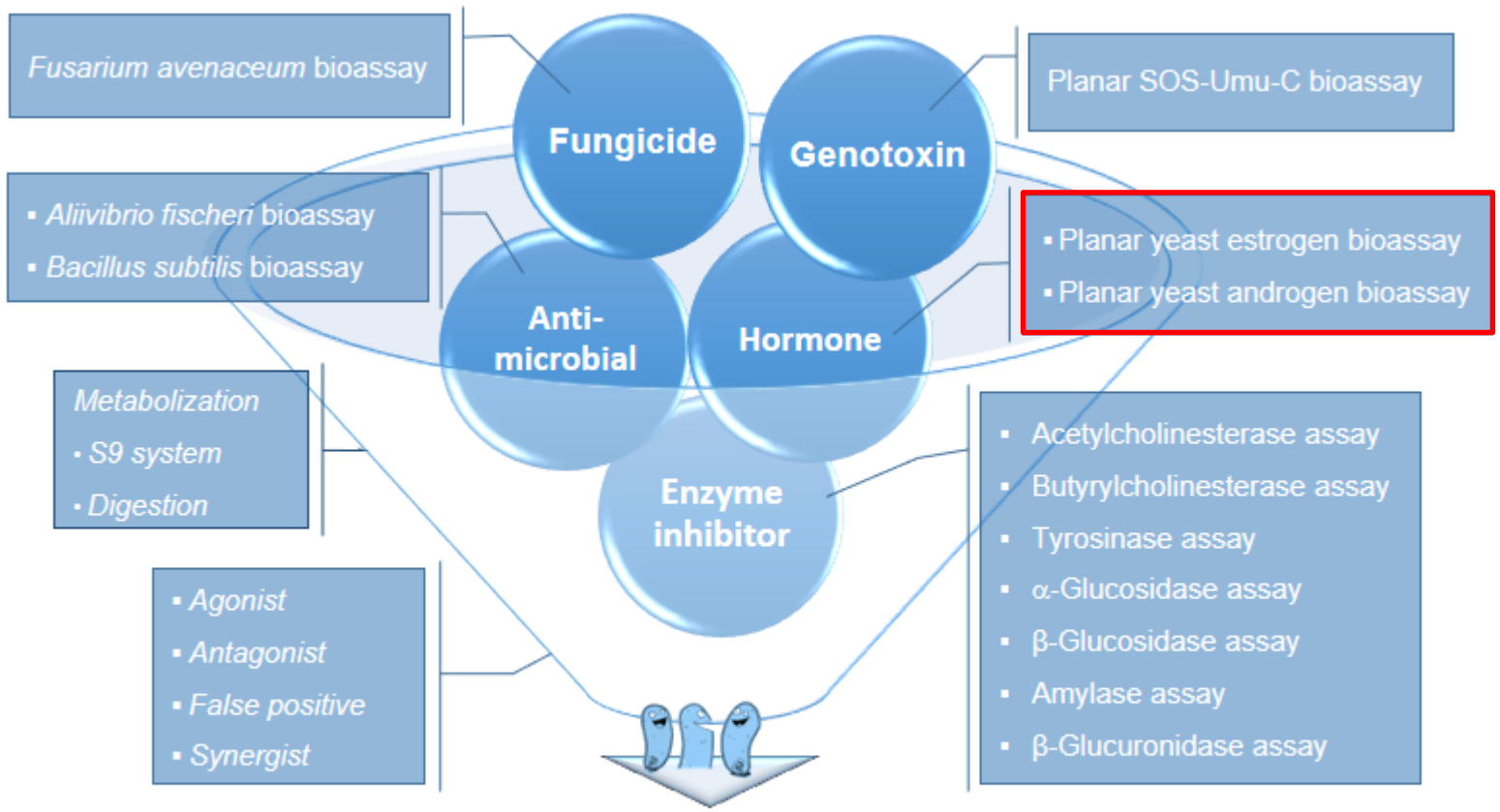
Prioritization strategy



Tousands of compounds reduced to few bioactive ones

Prioritization of important compounds

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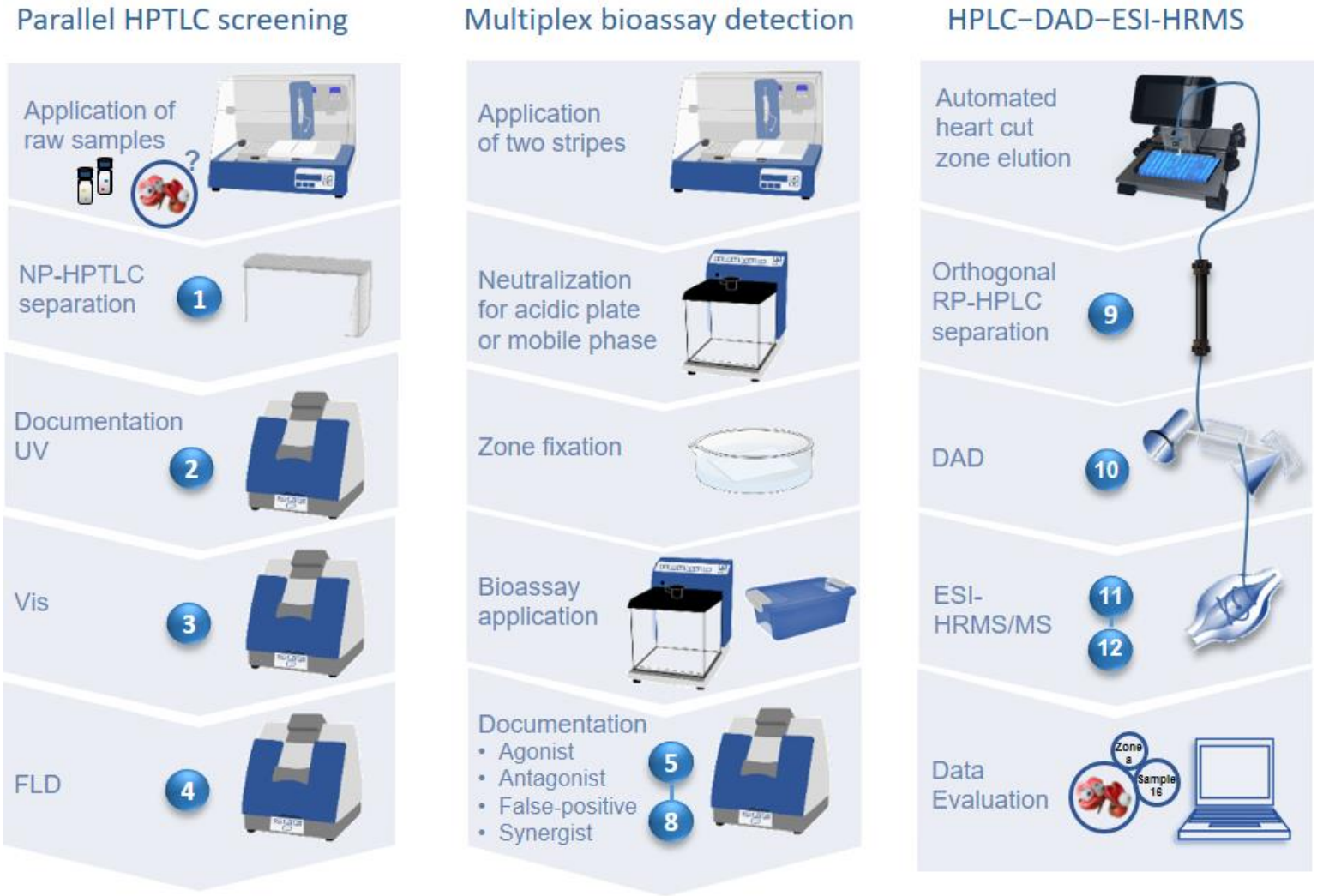
12 D hyphenation 5−25 min/sample 0.5−0.9 Euro/sample



G. Morlock *Phytochem. Reviews*, in submission

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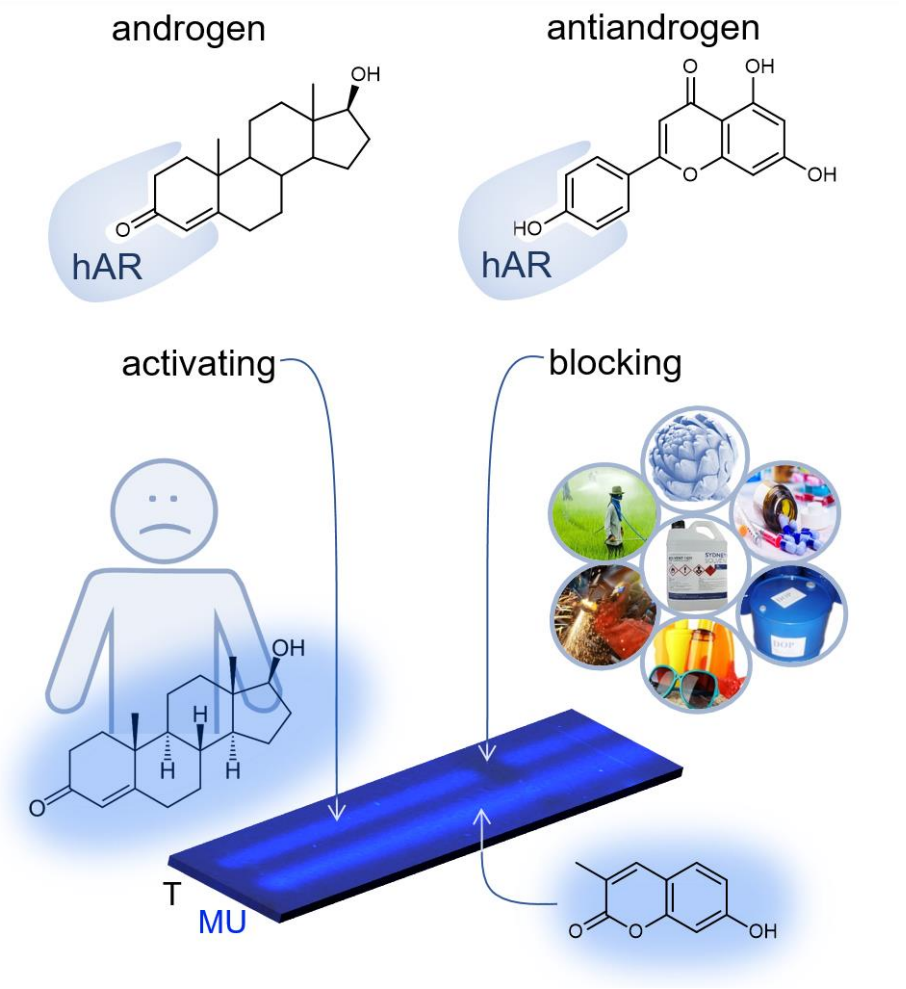
Multiplex bioassay



Schreiner, T., Ronzheimer, A.,Friz, M., Morlock, G.E., Food Chem. in print doi:10.1016/j.foodchem.2022.133610

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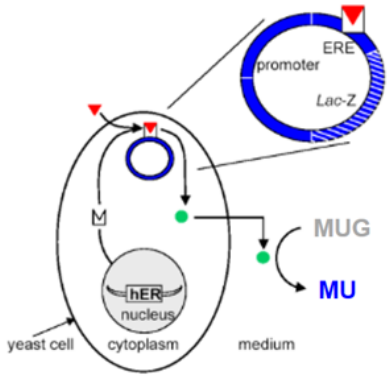
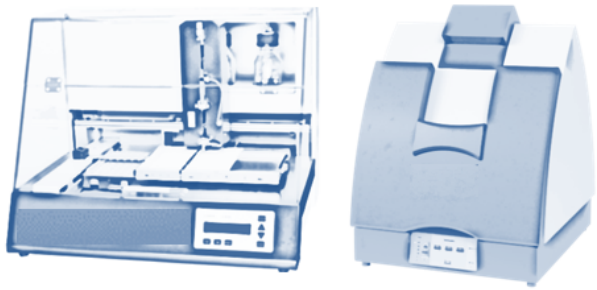
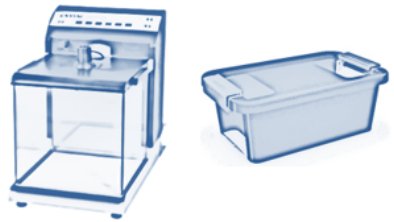
Multiplex bioassay detection



Application of stripes

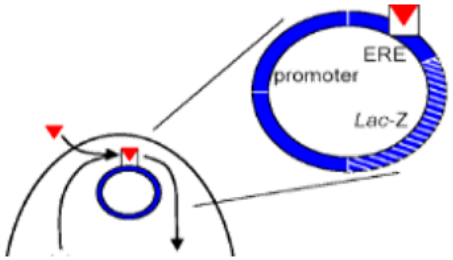


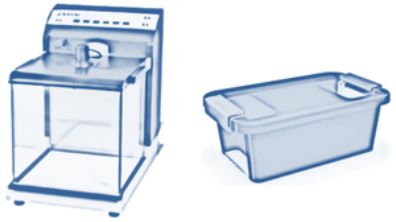
Planar yeast estrogen screen



Human estrogen receptor

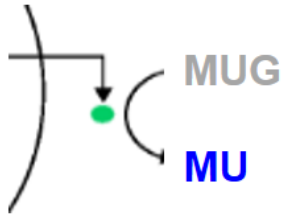
in *Saccharomyces cerevisiae*





4-Methylumbelliferone

galactoside (MUG)



Blue fluorescent

4-methylumbelliferone (MU)

Biological detection

Signal produced by yeast

detected at FLD 366 nm

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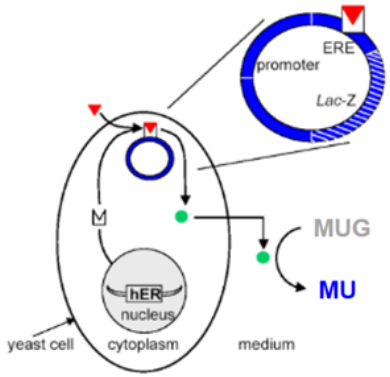
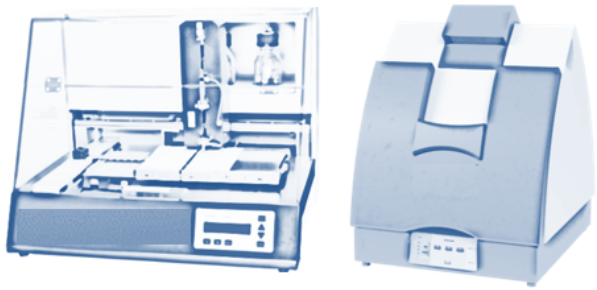
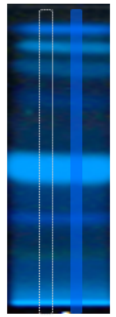
Multiplex bioassay detection



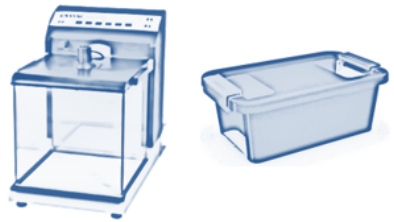
Application of stripes



E2 MU



Planar yeast estrogen screen



Human estrogen receptor

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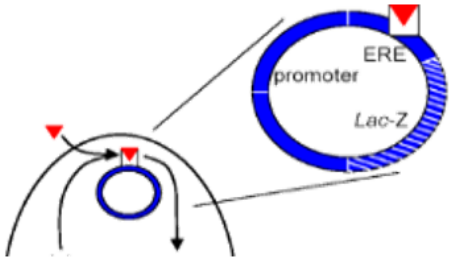
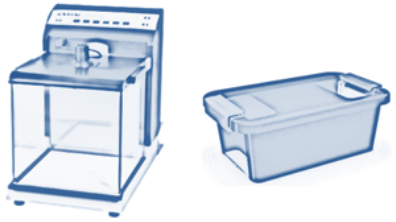
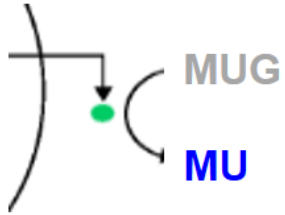




4-Methylumbelliferone

galactoside (MUG)



Blue fluorescent

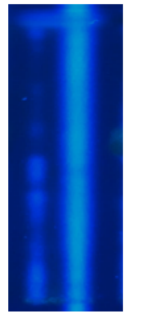
4-methylumbelliferone (MU)

Biological detection

Signal produced by yeast

detected at FLD 366 nm

E2 MU



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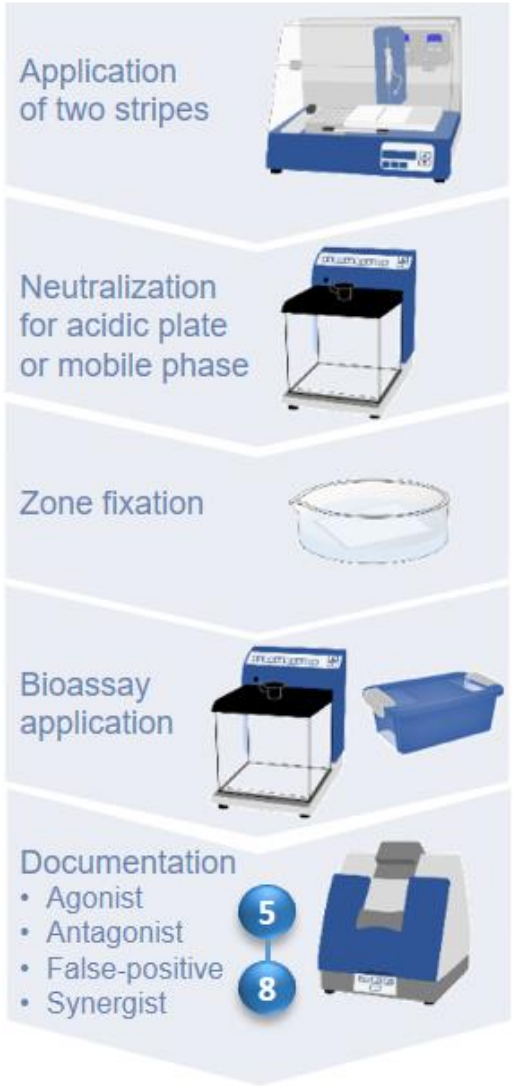
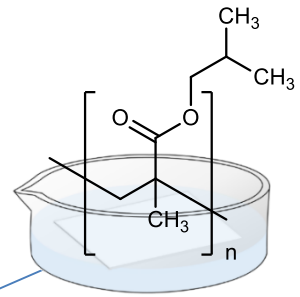
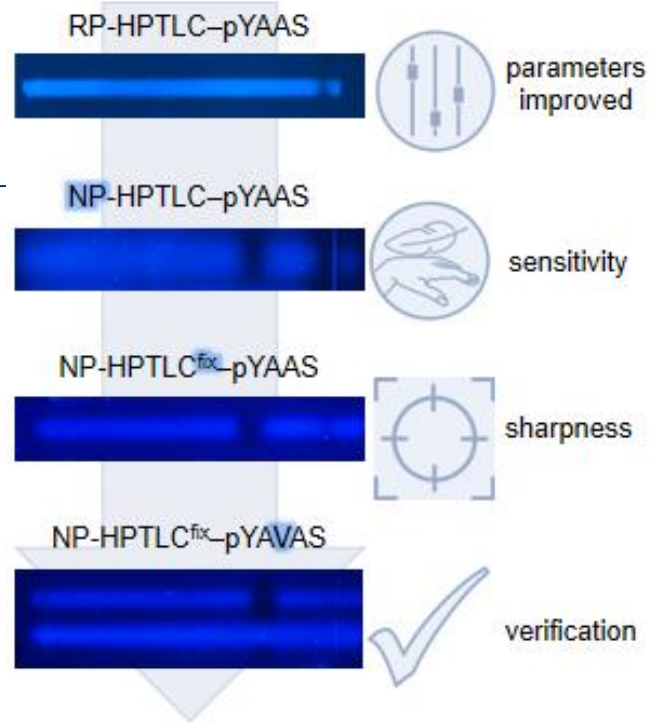
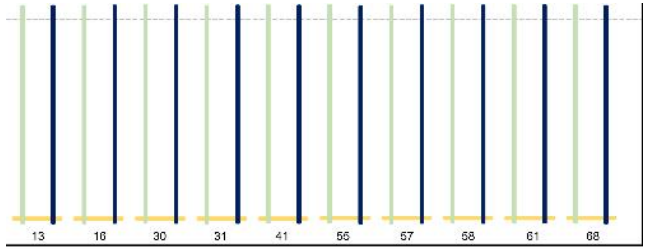
Multiplex bioassay detection



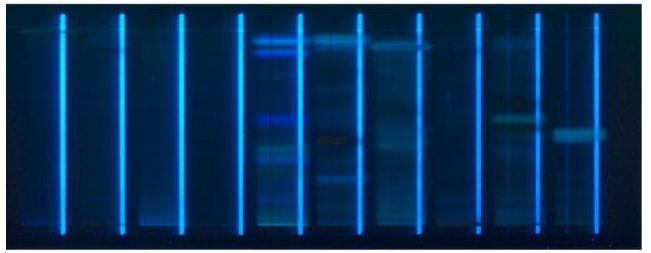




Scheme



FLD 366 nm



T. Schreiner, J. Heil, D. Sauter, M. Friz, G. Morlock *Front. Pharmacol.* 12 (2021) 755941

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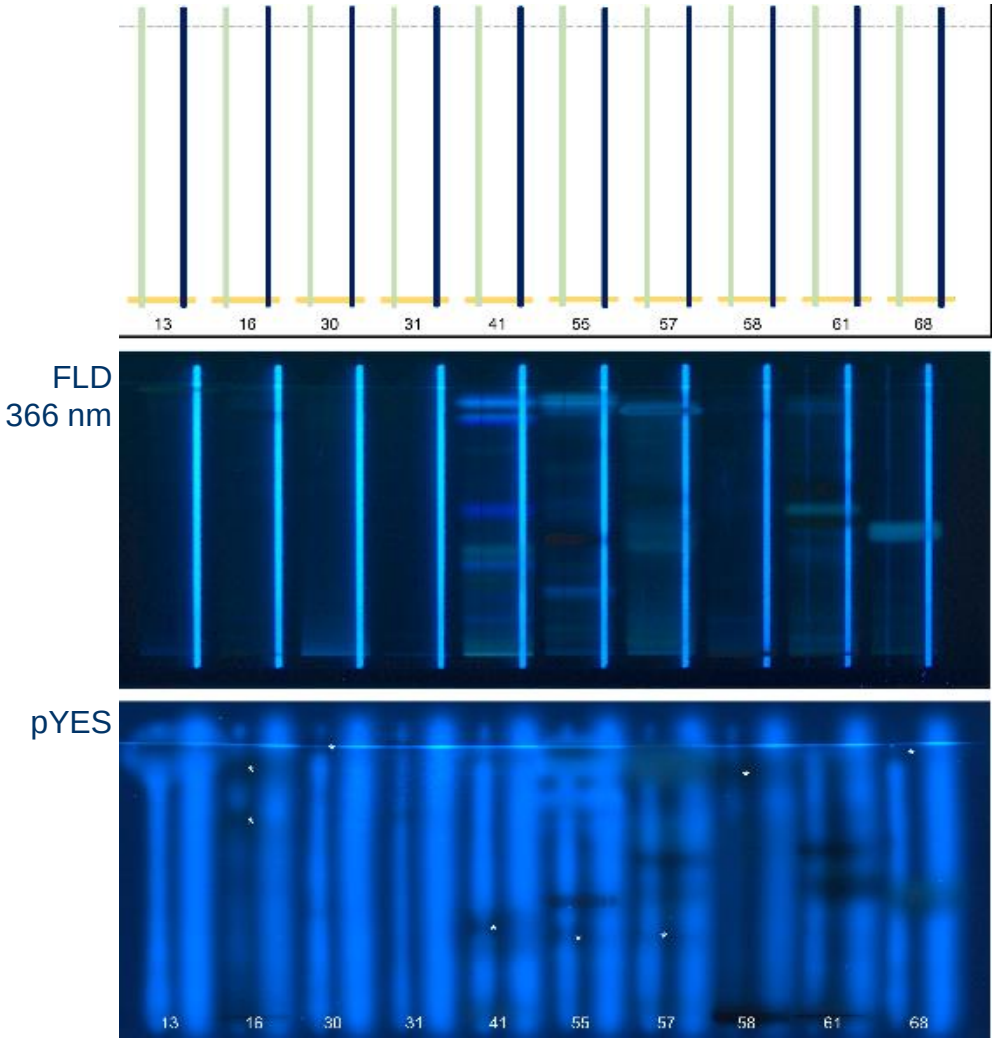
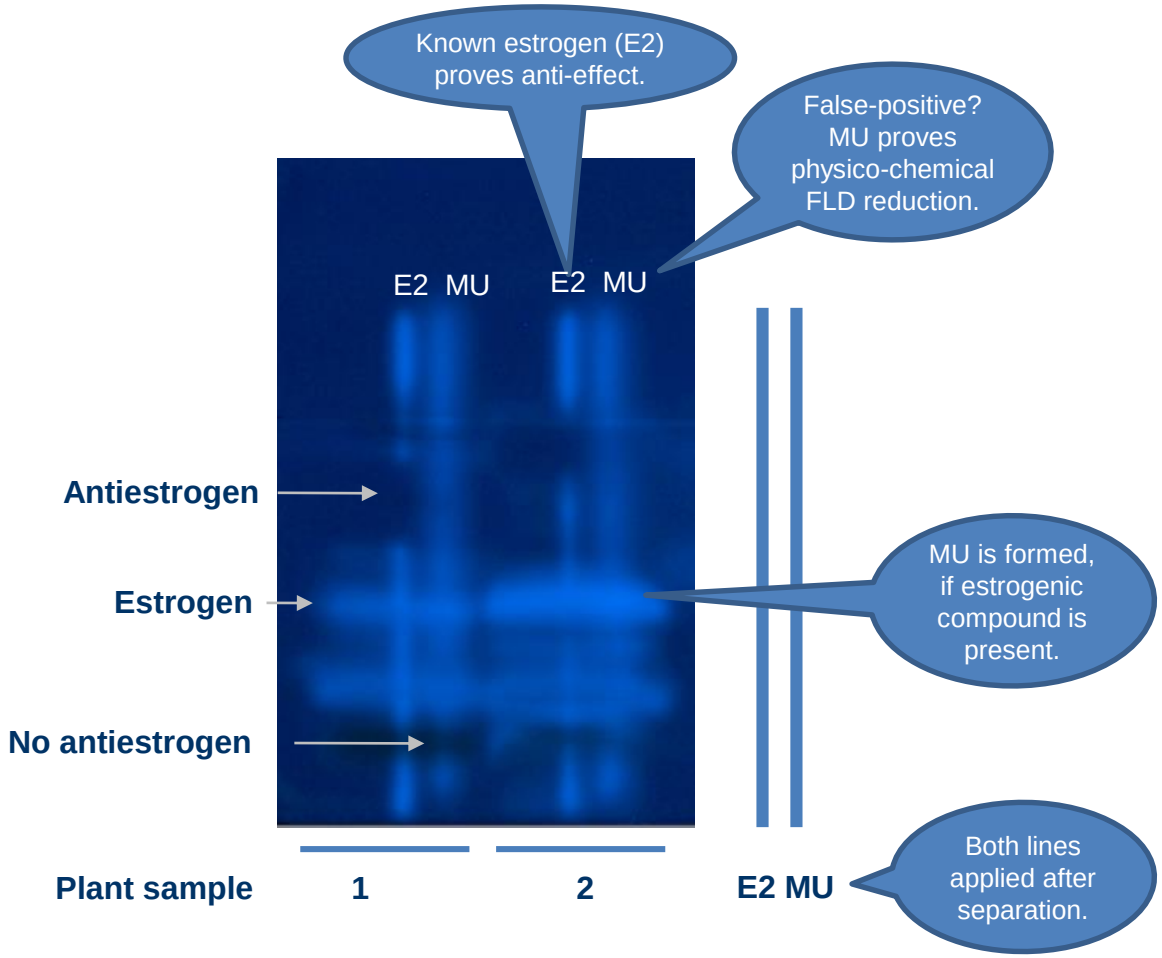
Agonistic, antagonistic and false-positive effects





T. Schreiner, J. Heil, D. Sauter, M. Friz, G. Morlock *Front. Pharmacol.* 12 (2021) 755941

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Terms used for hormonal bioassays

HPTLC^{fix} plus zone **fix**ation step

-	**pYES**	**p**lanar **y**east **e**strogen **s**creen
| E2	**pY<u>A</u>ES**	plus stripe: **a**gonist^{estrogene} → antagonist
|| E2 MU	**pYA<u>V</u>ES**	plus stripe: endproduct$^{methyl\ umbelliferone}$ → false-positives or **v**erified antiestrogen

-	**pYAS**	**p**lanar **y**east **a**ndrogen **s**creen
| T	**pY<u>A</u>AS**	plus stripe: **a**gonist^{testosterone} → antagonist
|| T MU	**pYA<u>V</u>AS**	plus stripe: endproduct$^{methyl\ umbelliferone}$ → false-positives or **v**erified antiandrogen

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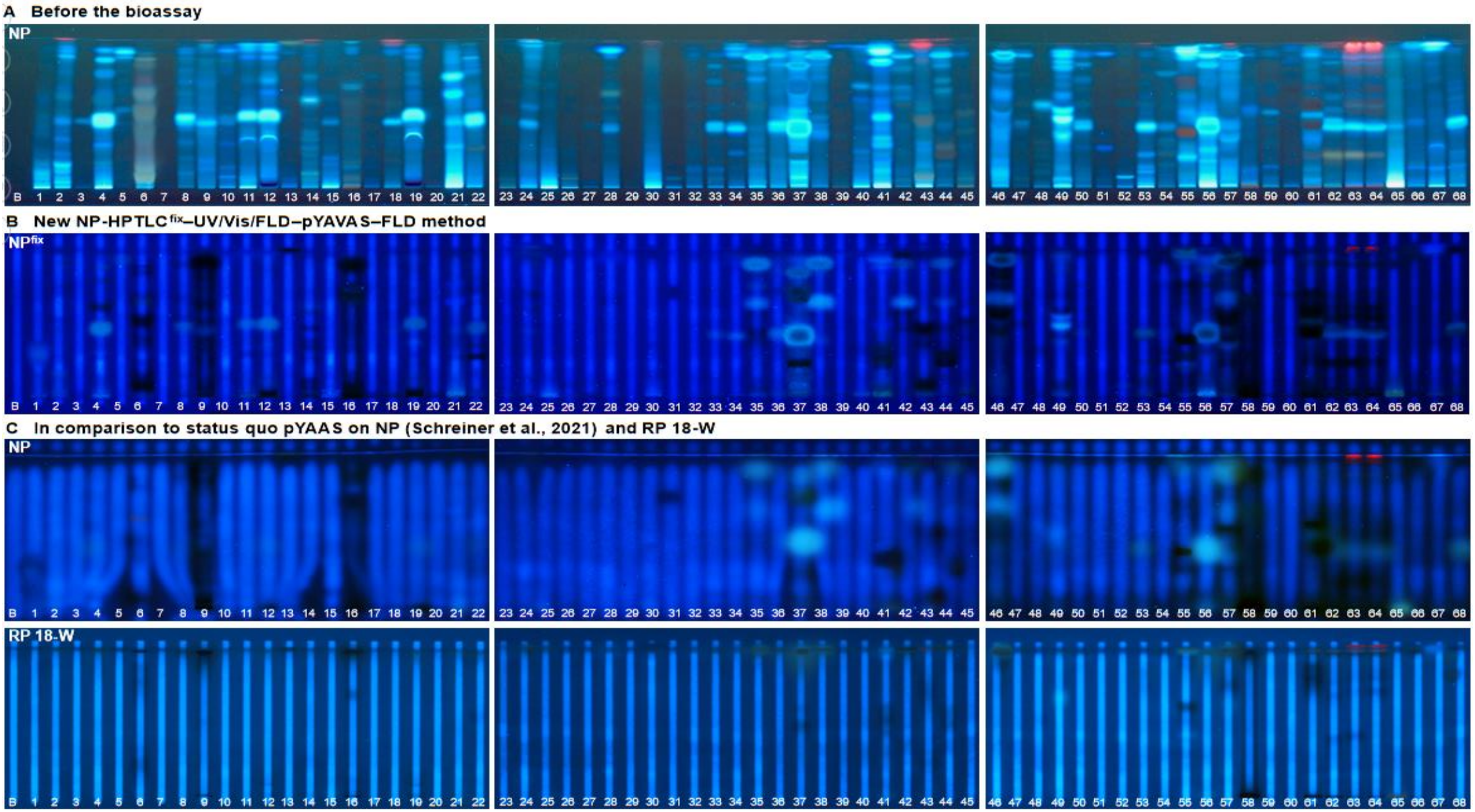
NP-HPTLC^{fix}–pYAAS for screening ….*versus* status quo on NP and RP



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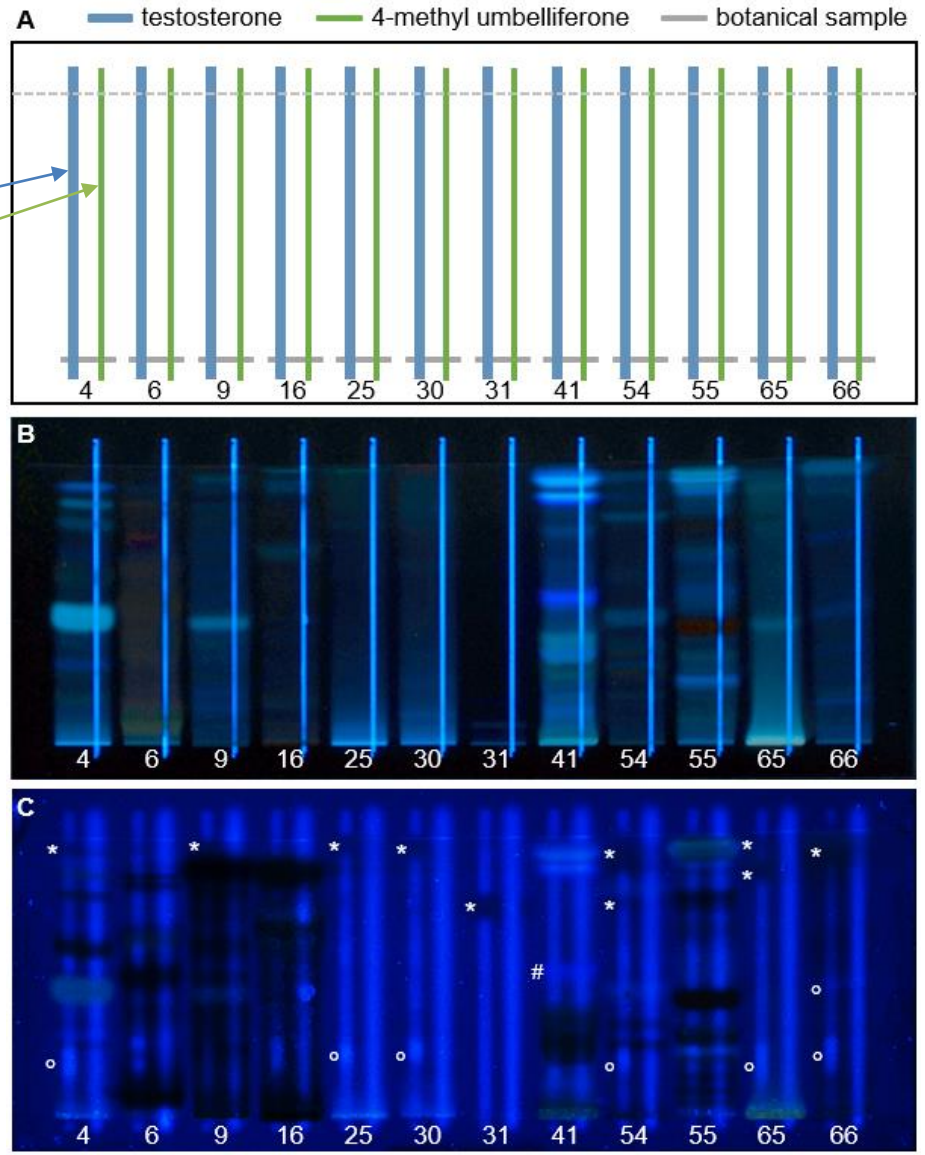
NP-HPTLC^{fix}–pYAVAS for confirmation

- **p**lanar
- **Y**east
- **A**ntagonist
- **V**erified
- **A**ndrogen
- **S**creen



Zone fixation: new zone sharpening protocol!

Schreiner, T., Ronzheimer, A.,Friz, M., Morlock, G.E., Food Chem. in print doi:10.1016/j.foodchem.2022.133610

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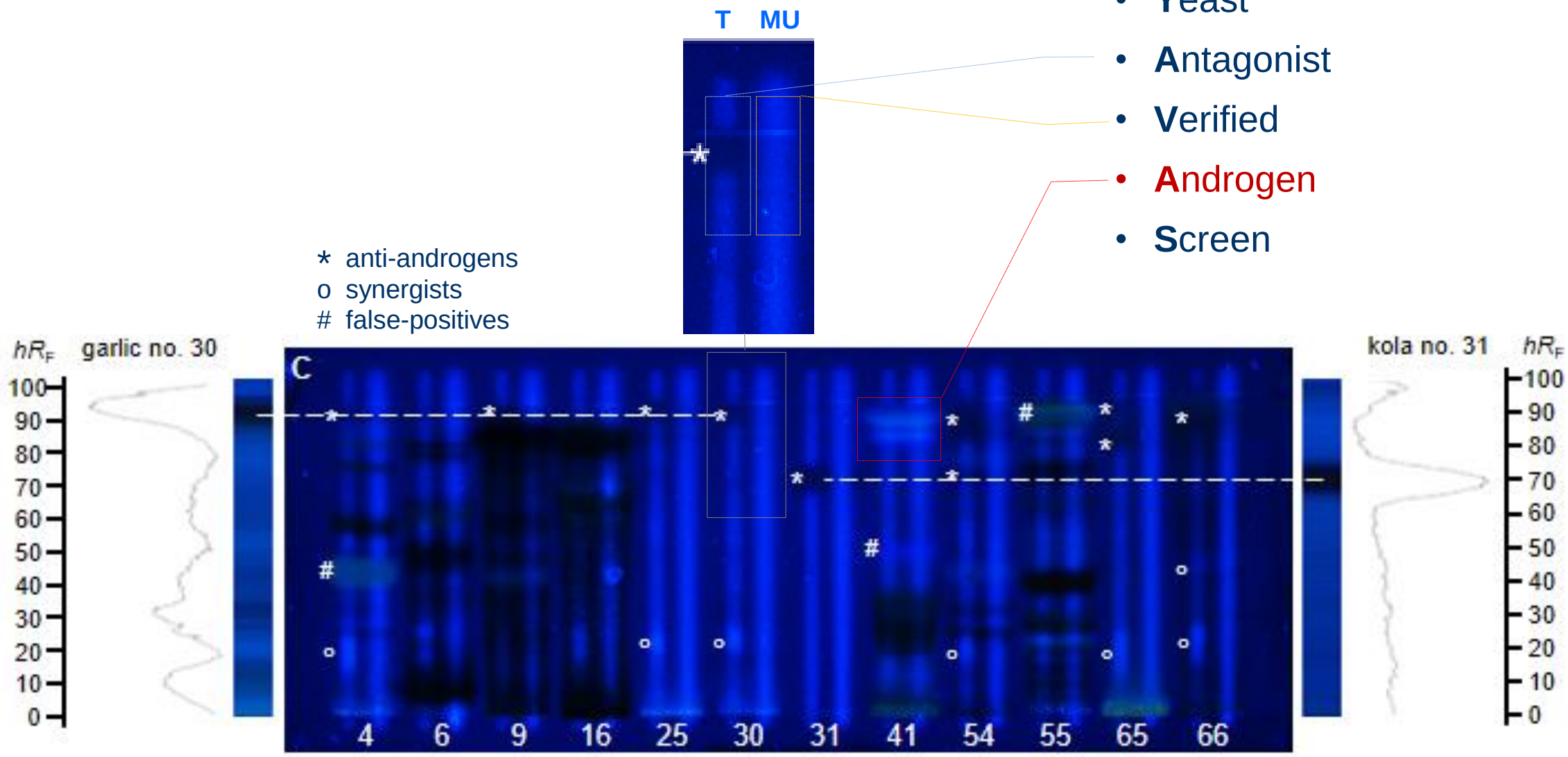
NP-HPTLC^{fix}–pYAVAS–FLD

- planar
- Yeast
- Antagonist
- Verified
- Androgen
- Screen



Schreiner, T., Ronzheimer, A.,Friz, M., Morlock, G.E., Food Chem. in print doi:10.1016/j.foodchem.2022.133610

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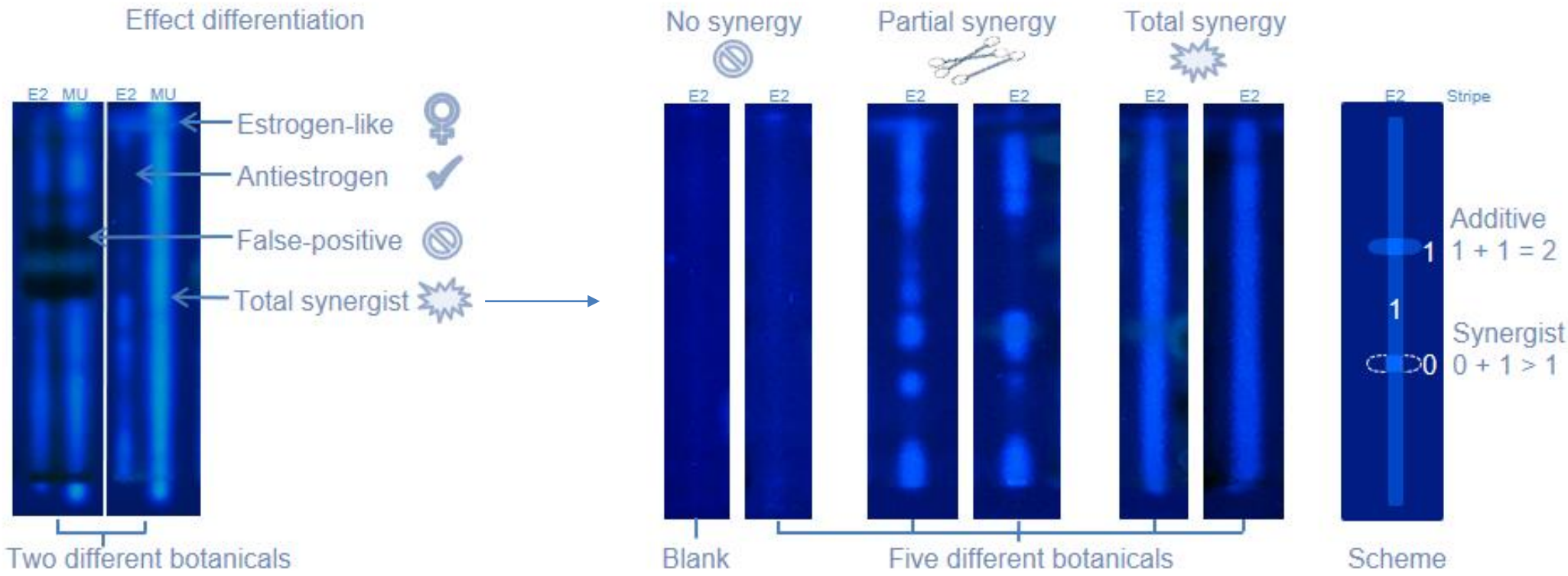
Effect differentiation and synergy



T. Schreiner, J. Heil, D. Sauter, M. Friz, G. Morlock *Front. Pharmacol.* 12 (2021) 755941

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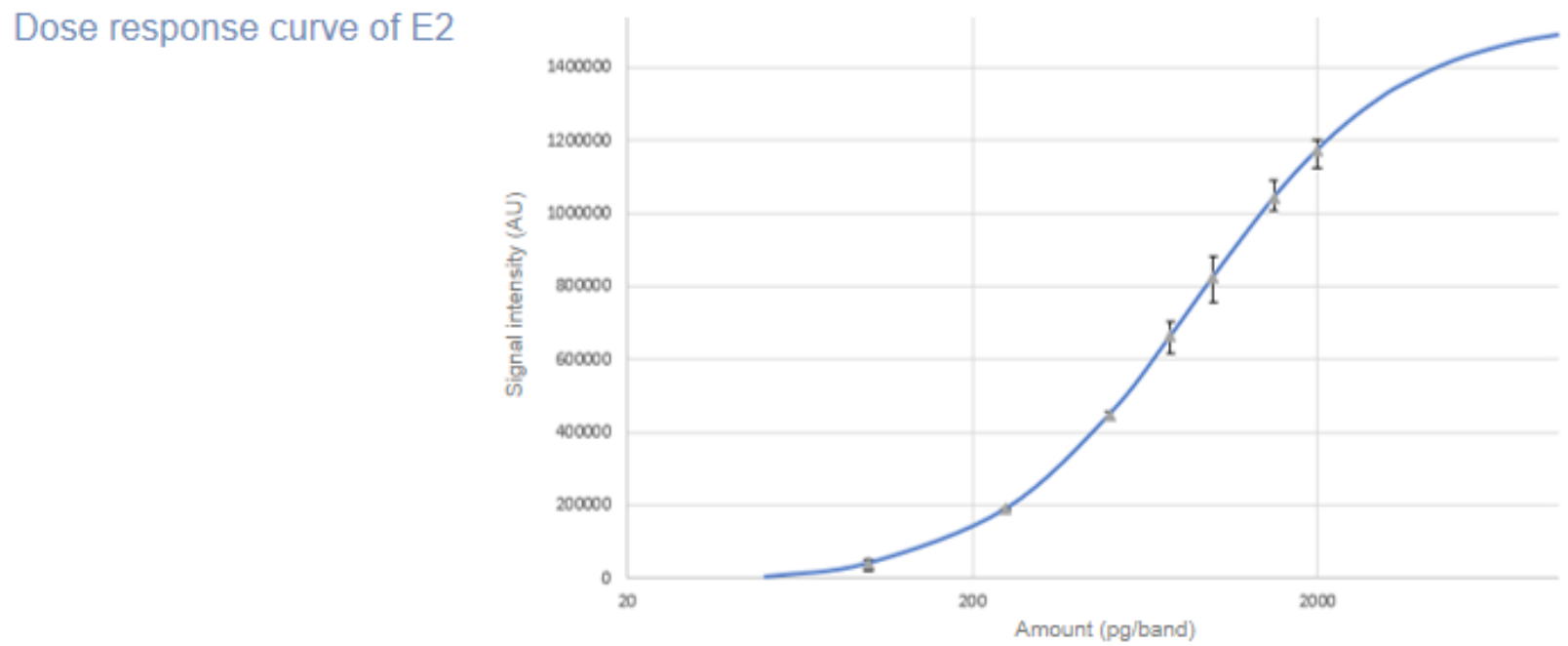
Effect quantification EC_{50}



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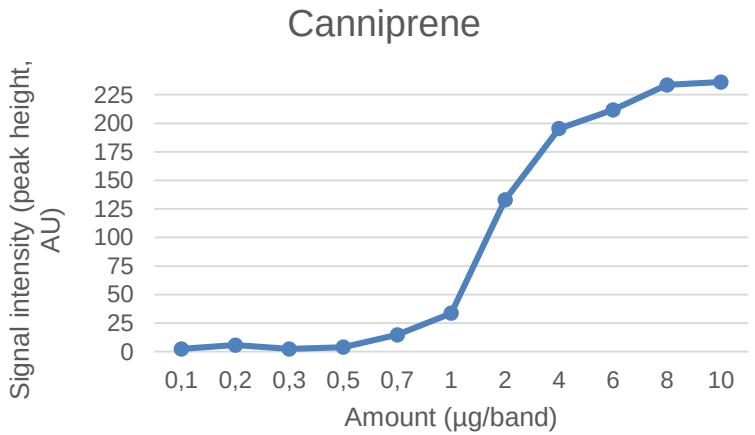
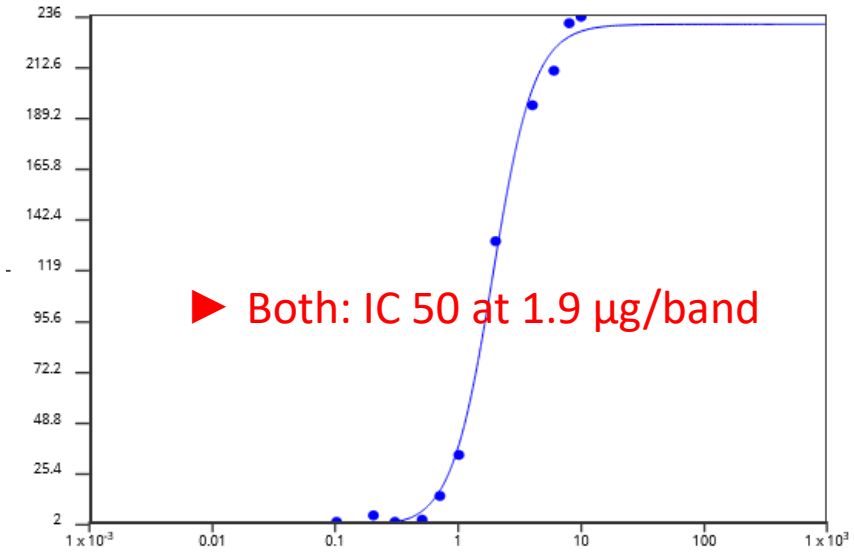
How to calculate IC_{50} calculation on surface?



0.1	2,4011
0.2	5,7537
0.3	2,4351
0.5	3,979
0.7	14,738
1	33,5967
2	132,8351
4	195,2849
6	211,7194
8	233,5187
10	236,1207

www.aatbio.com/tools/ic50-calculator



► Both: IC 50 at 1.9 µg/band

IC_{50}	1.8941
Equation	$Y = -0.5091 + \frac{232.5253 - 0.5091}{1 + (\frac{X}{1.8941})^{-2.5547}}$
Equation Form	$Y = Min + \frac{Max - Min}{1 + (\frac{X}{IC_{50}})^{Hill\ coefficient}}$

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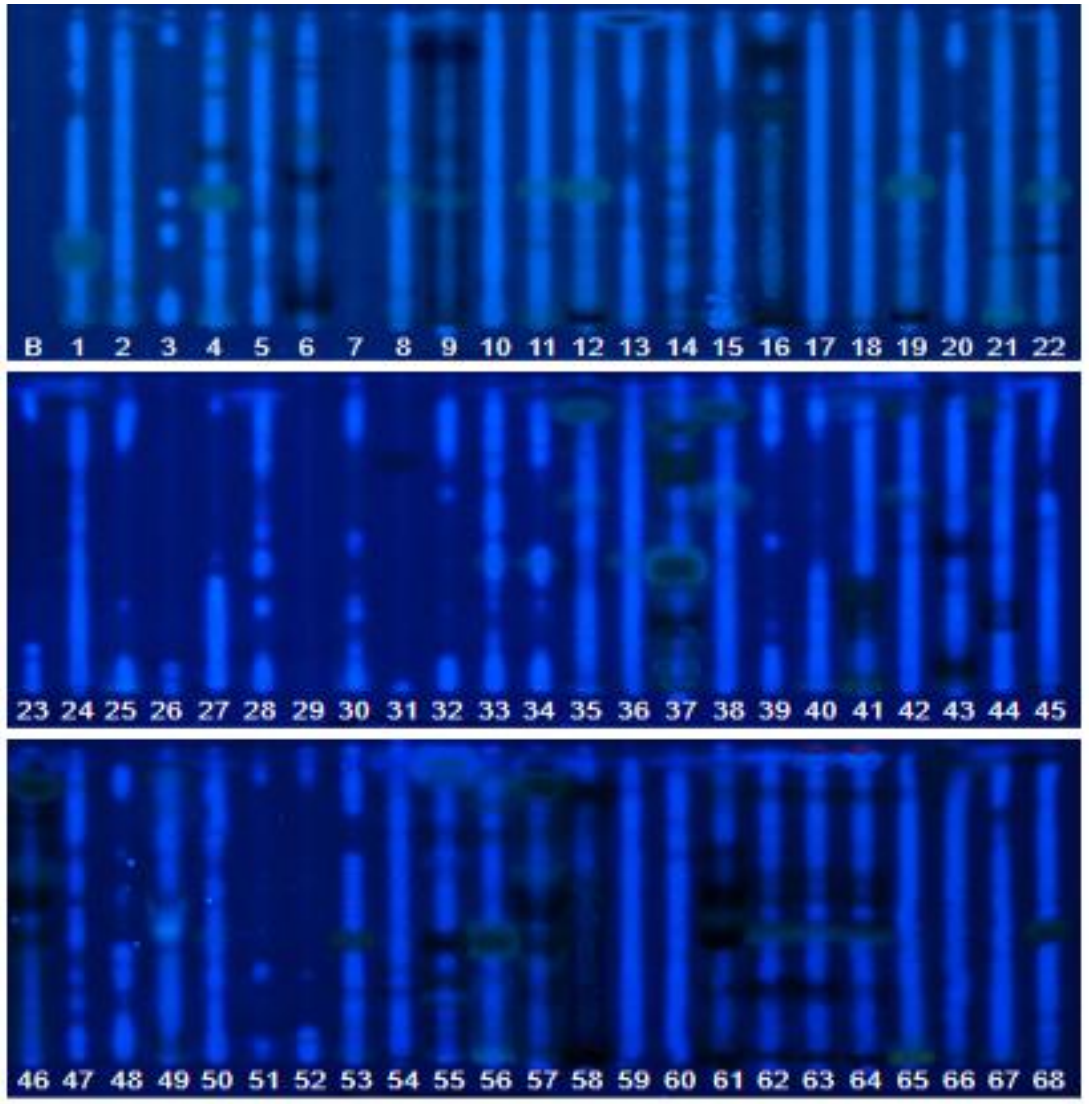
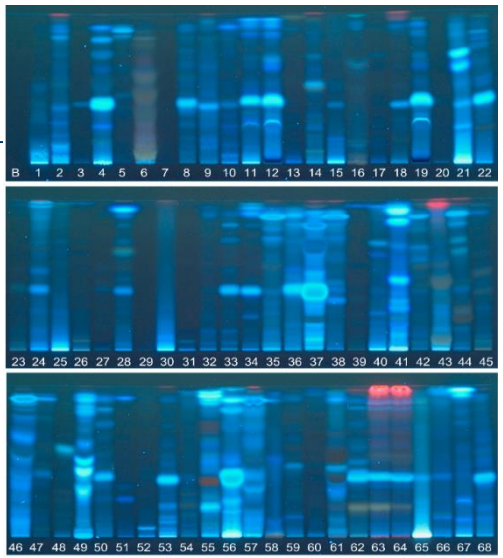
Phytochemicals → synergy for estrogenic effects!

- ✓ Few agonists
- ✓ Many antagonists
- ✓ Many synergists
- ✓ One day





FLD 366 nm

A. Ronzheimer, T. Schreiner, G. Morlock, *Phytomed*. 103 (2022) 154230

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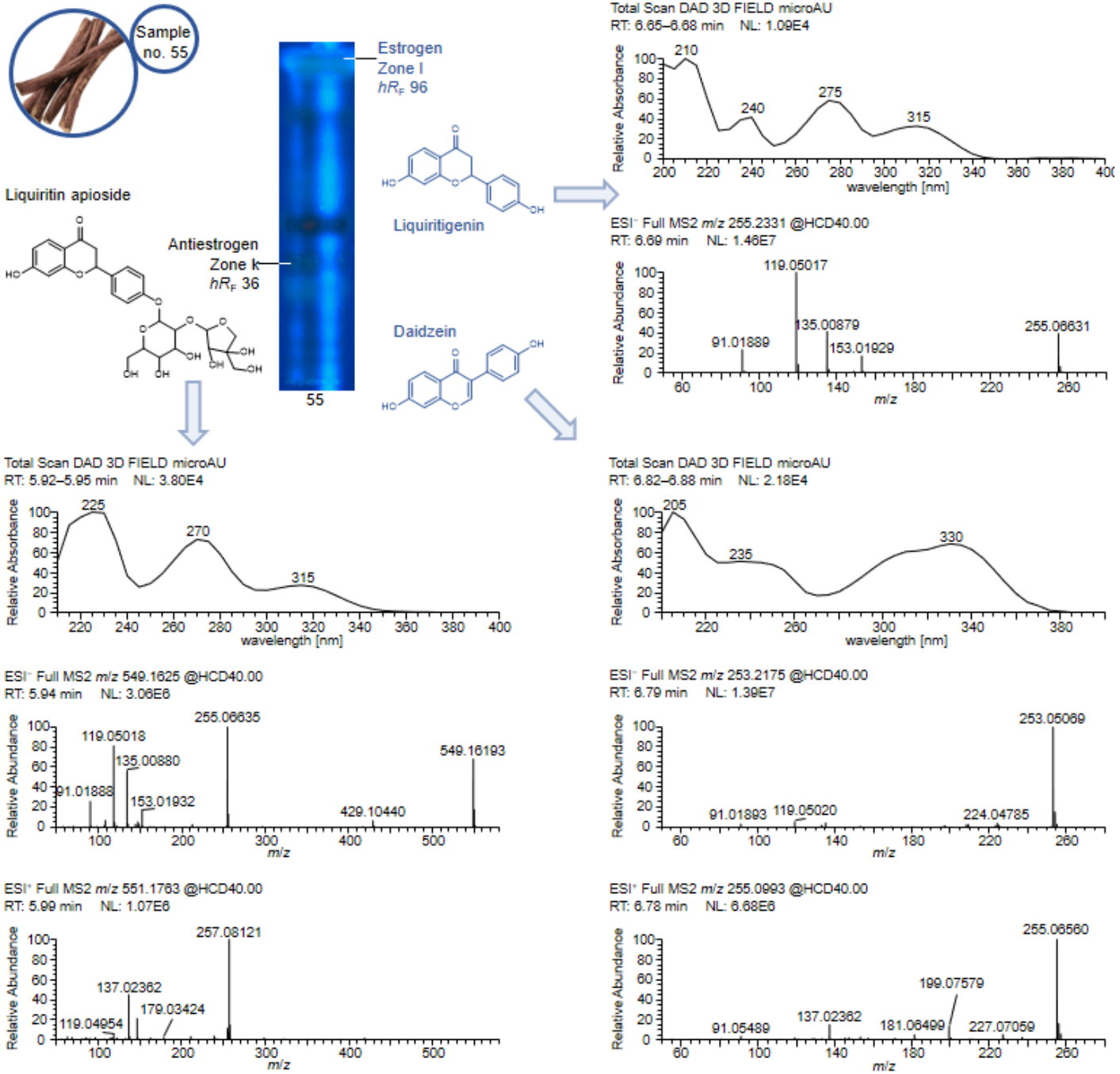
Identification



A. Ronzheimer, T. Schreiner, G. Morlock, *Phytomed*. 103 (2022) 154230

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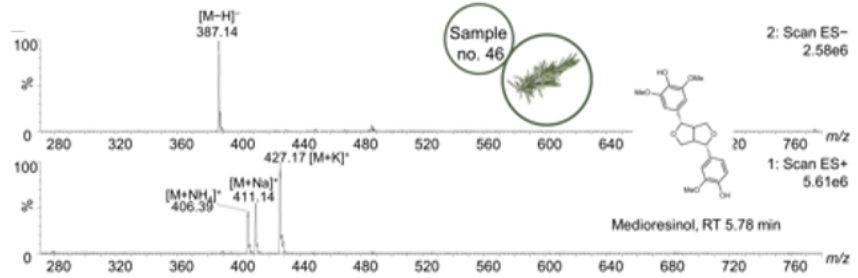
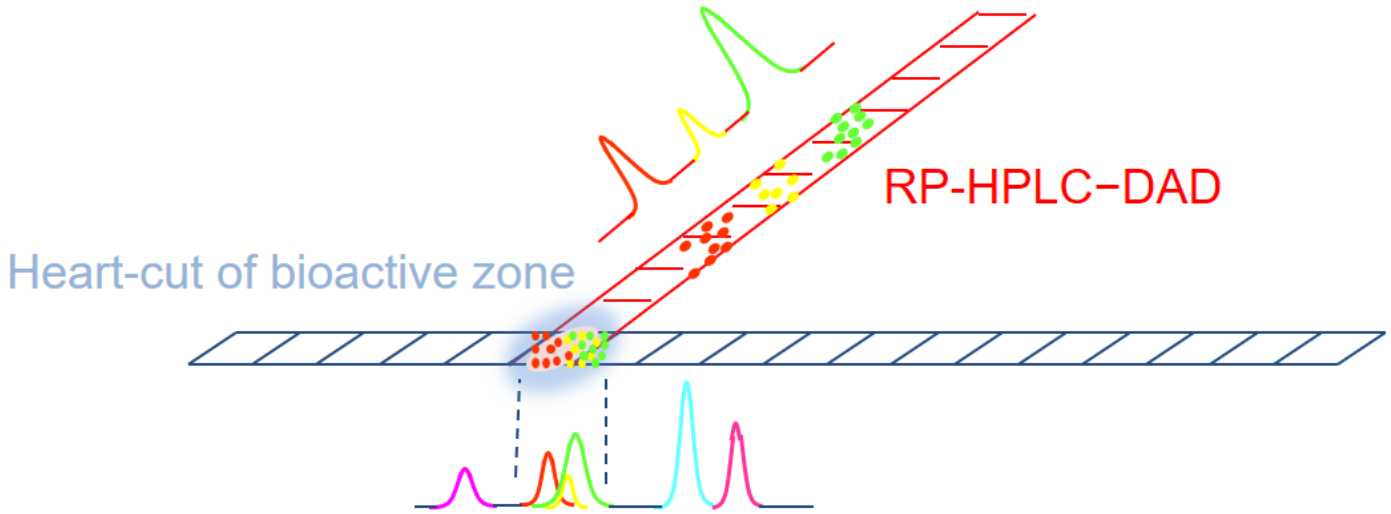
NP-HPTLC–UV/vis/FLD–EDA–RP-HPLC–DAD–MS

Orthogonal hyphenation

► HRMS directly from active zone



ESI-HRMS



NP-HPTLC–UV/vis/FLD–bioassay

A. Ronzheimer, T. Schreiner, G. Morlock, *Phytomed*. 103 (2022) 154230

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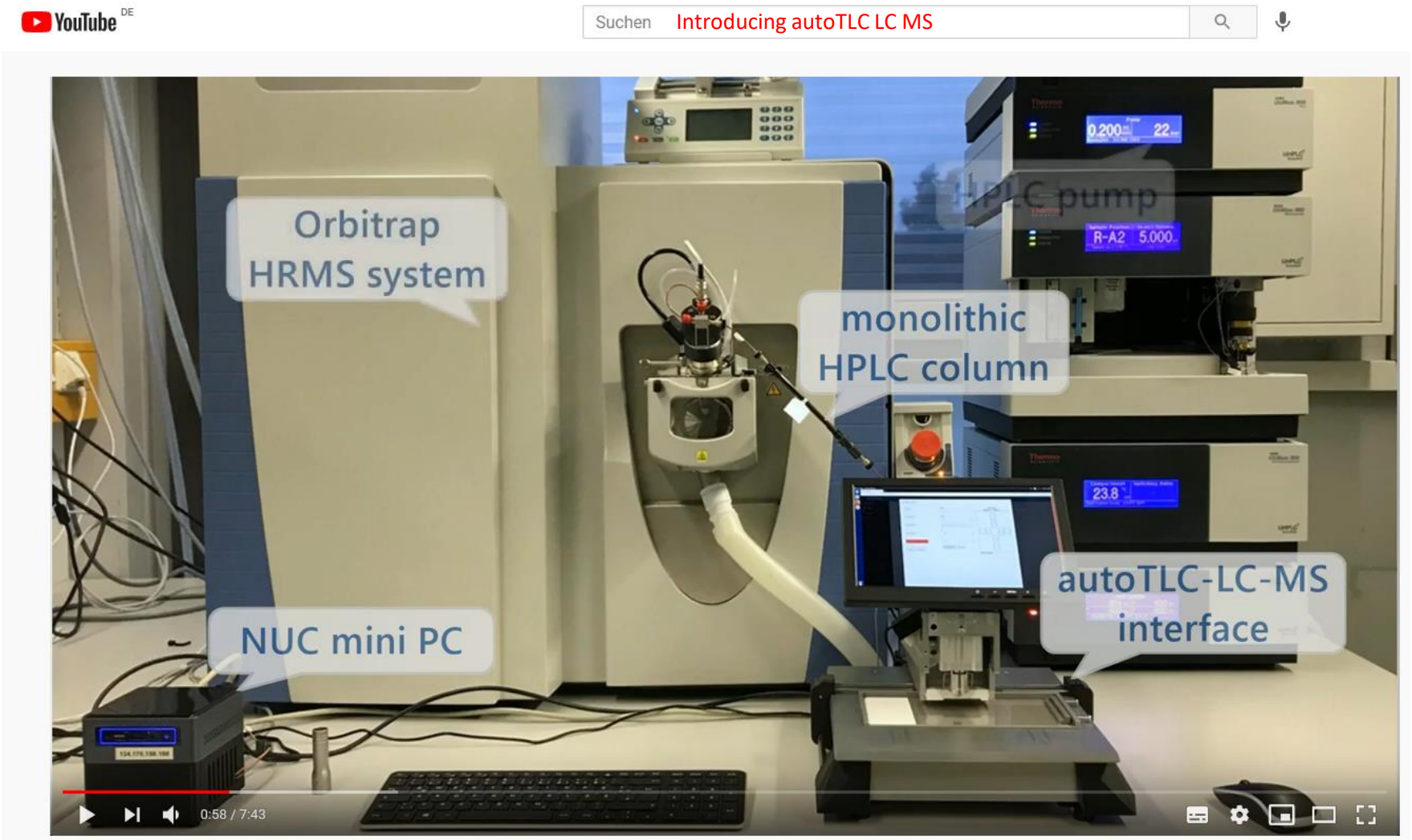
NP-HPTLC–UV/Vis/FLD–EDA–RP-HPLC–DAD–MS



A. Mehl, W. Schwack, G. Morlock *J. Chromatogr. A* 1647 (2021) 462154

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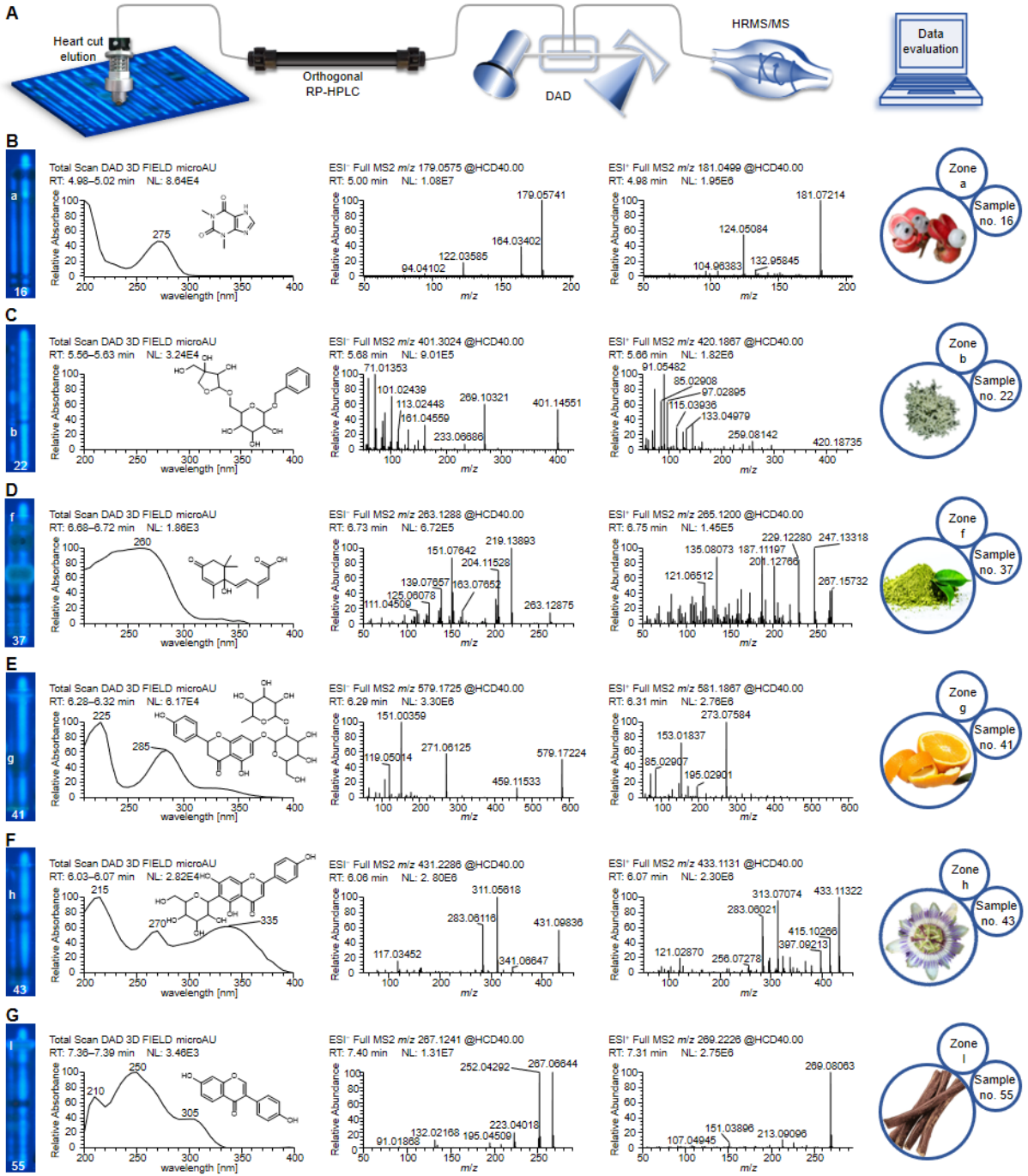

T. Schreiner, J. Heil, D. Sauter, M. Friz, G. Morlock *Front. Pharmacol.* 12 (2021) 755941

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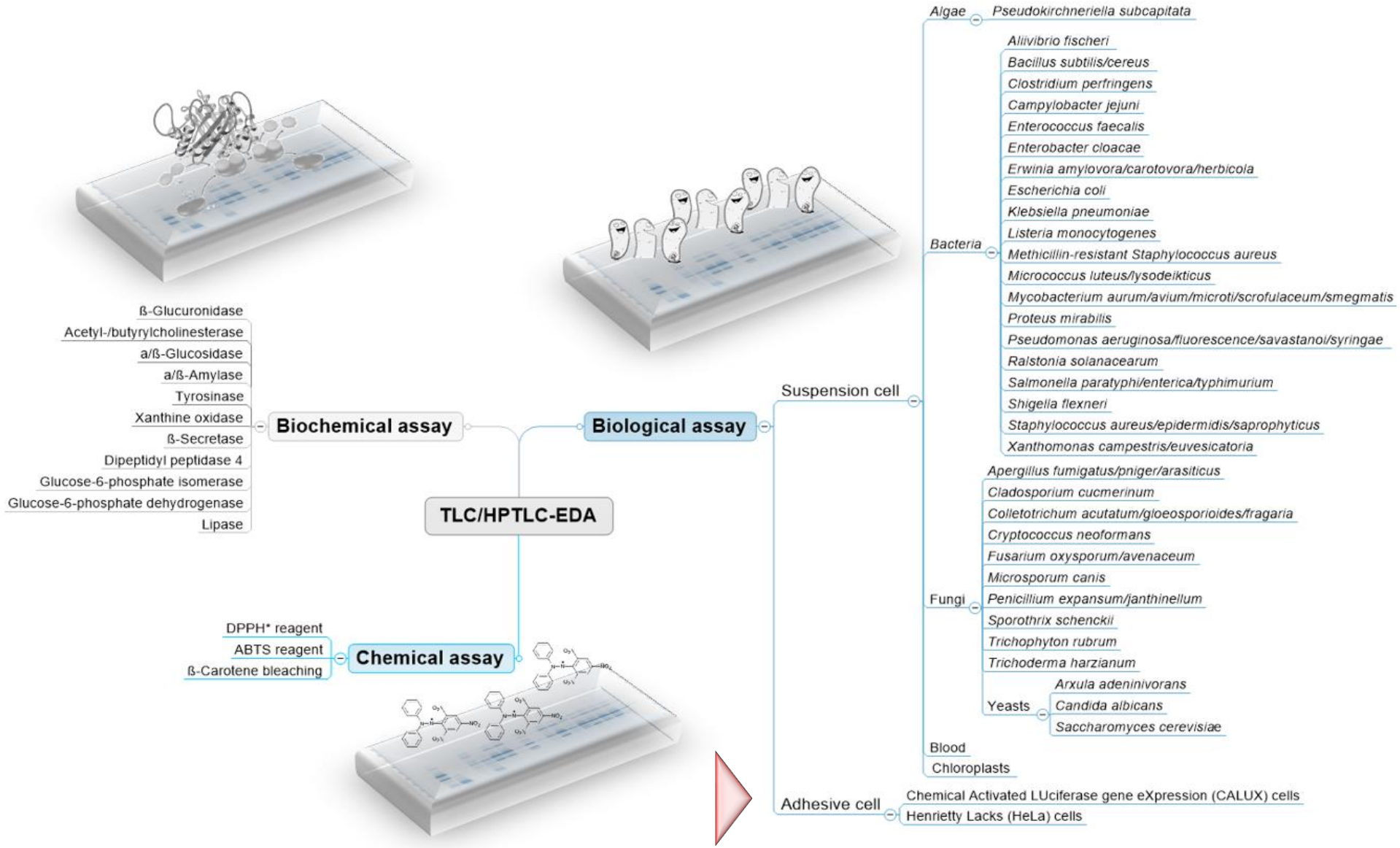
Hyphenation: Chromatography + Effect detection



G. Morlock *Anal. Chim. Acta* 1180 (2021) 338644

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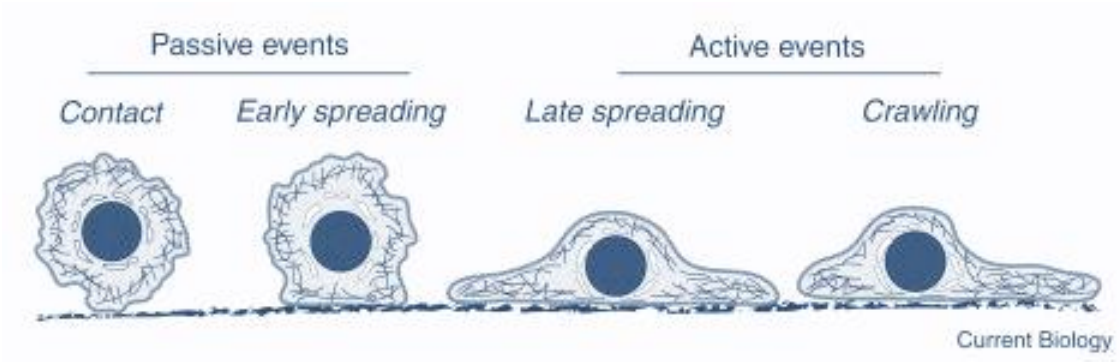
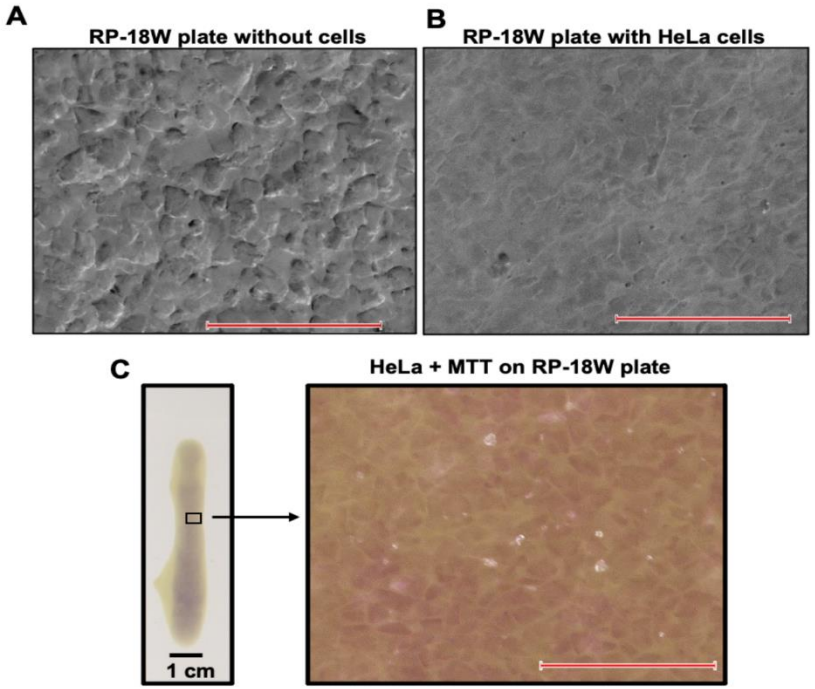
Adherent human cells as on-surface biodetector

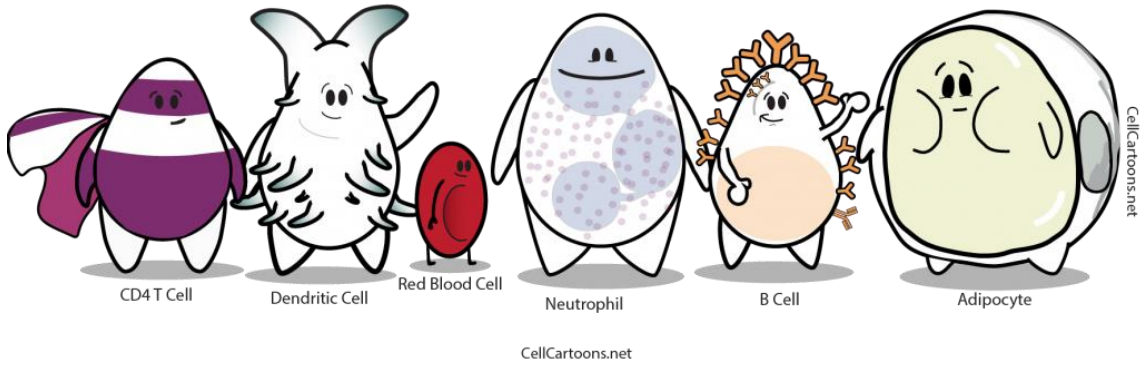
Suspension cells

Adherent cells





Mügge, F., Morlock, G.E. in submission



Similar use of the stripe technique….

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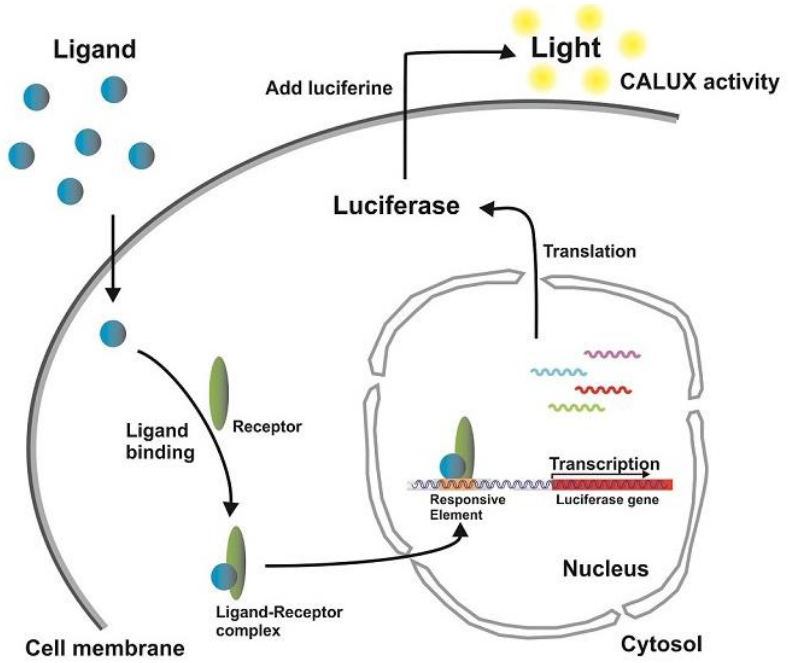
HPTLC combined with human cultured reporter cells

- First report of adherent human cells as on-surface biodetector
- Adherent human cells grow on planar chromatogram
- Non-target screening of cytotoxic substances in natural samples



https://biodetectionsystems.com

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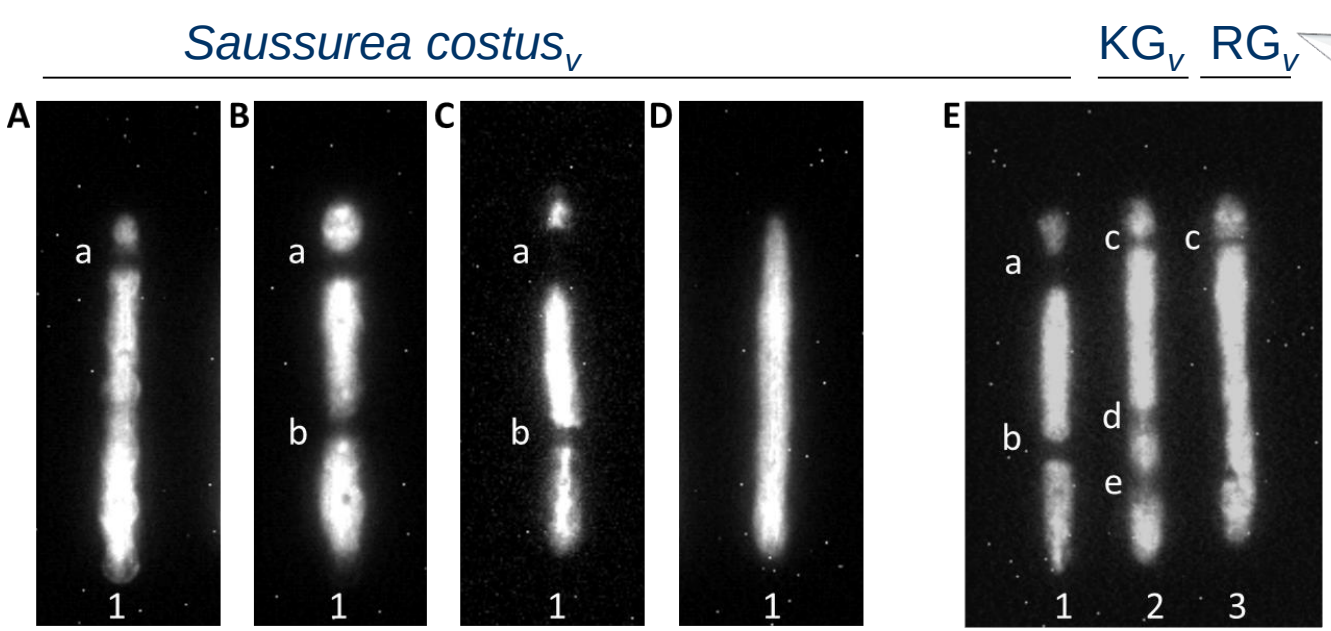
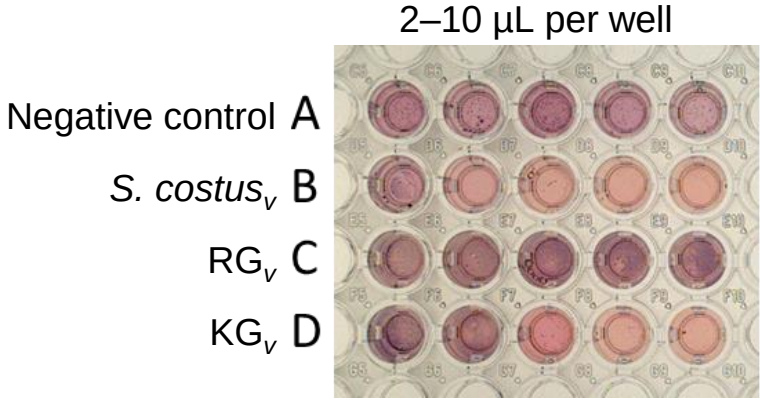
RP-HPTLC-Cytotox CALUX cell bioautograms



Cytotoxicity is lost during steam-treatment and drying (**2** *versus* **3**), making RG$_v$ less cytotoxic.

Proof of Cytotox CALUX® cell viability by MTT in microtiter plate

2–10 µL per well



Klingelhöfer, I., Pham Ngoc, L., Burg, B.v.d., Morlock, G.E. *Anal. Chim. Acta* 1183 (2021) 338956

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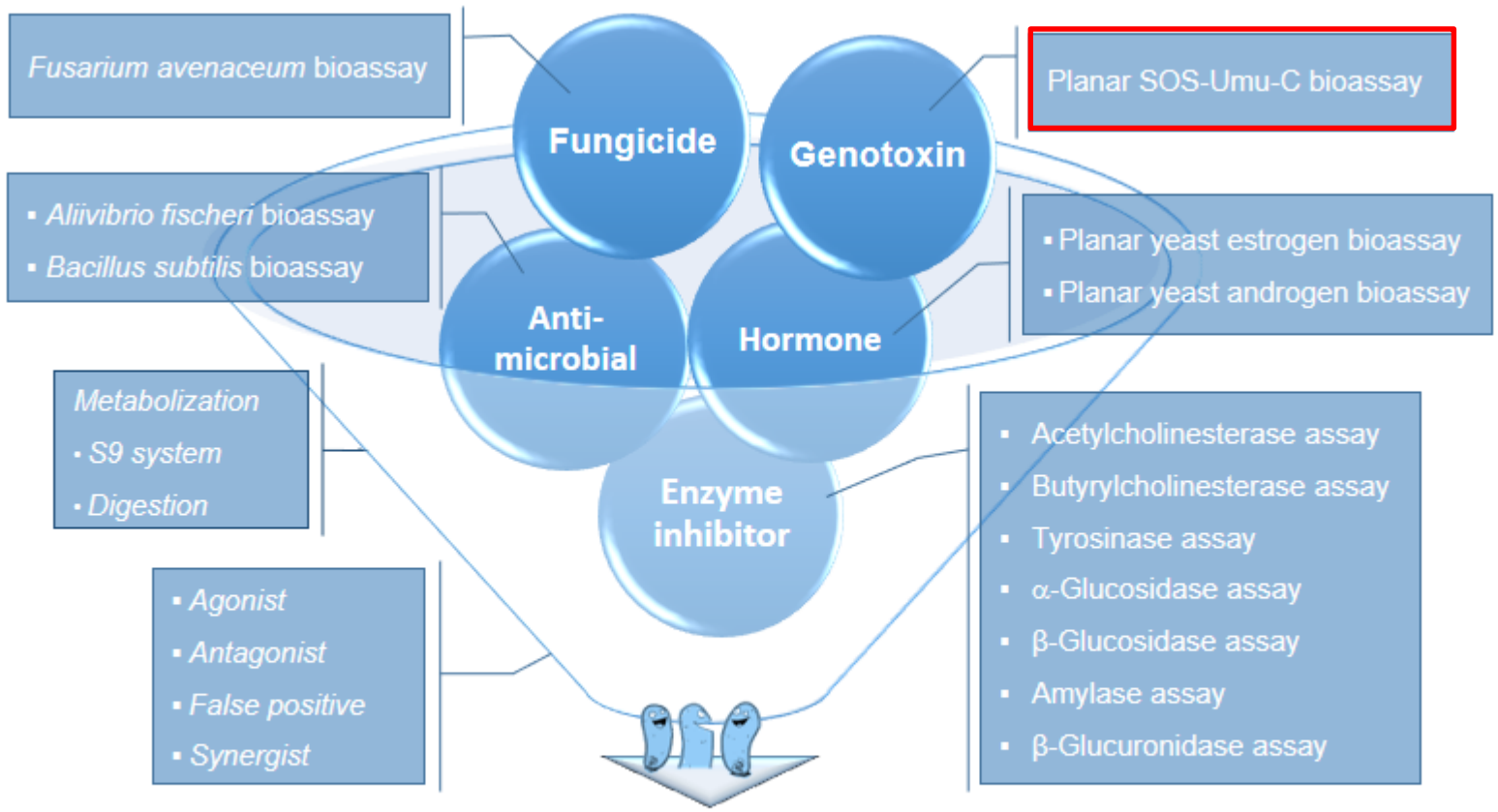
Prioritization strategy



Tousands of compounds reduced to few bioactive ones

Prioritization of important compounds

G. Morlock *Anal. Chim. Acta* 1180 (2021) 338644

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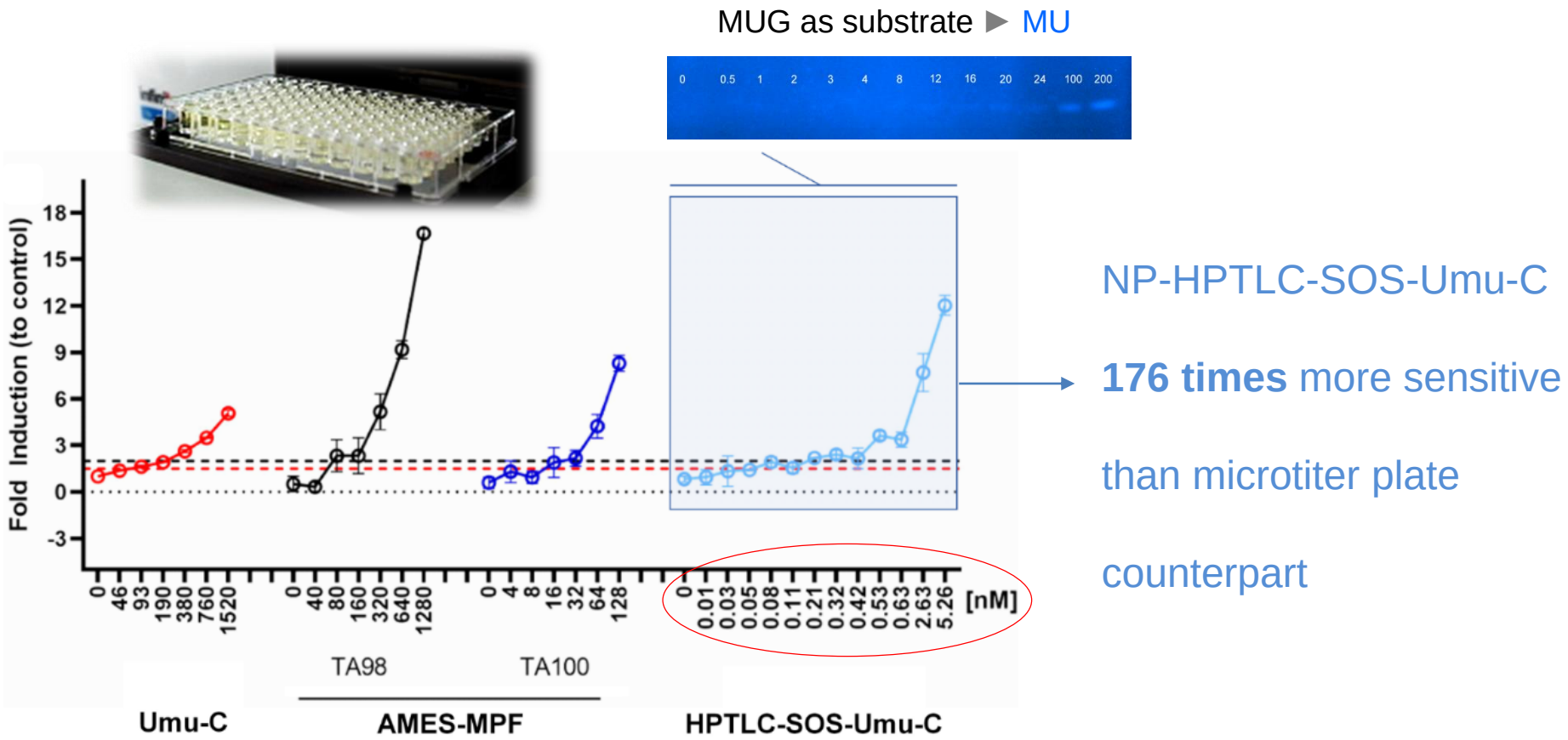
Comparison of genotoxicity assays

Genotoxin 4-nitroquinoline 1-oxide (4-NQO, 0.53 nM, LOBD 20 pg/band)



NP-HPTLC-SOS-Umu-C

176 times more sensitive

than microtiter plate

counterpart

Meyer, D., Marin-Kuan, M., Debon, E., Serrant, P., Cottet-Fontannaz, C., Schilter, B., Morlock G.E., *Alternatives to animal experimentation* 38 (2021) 387-397

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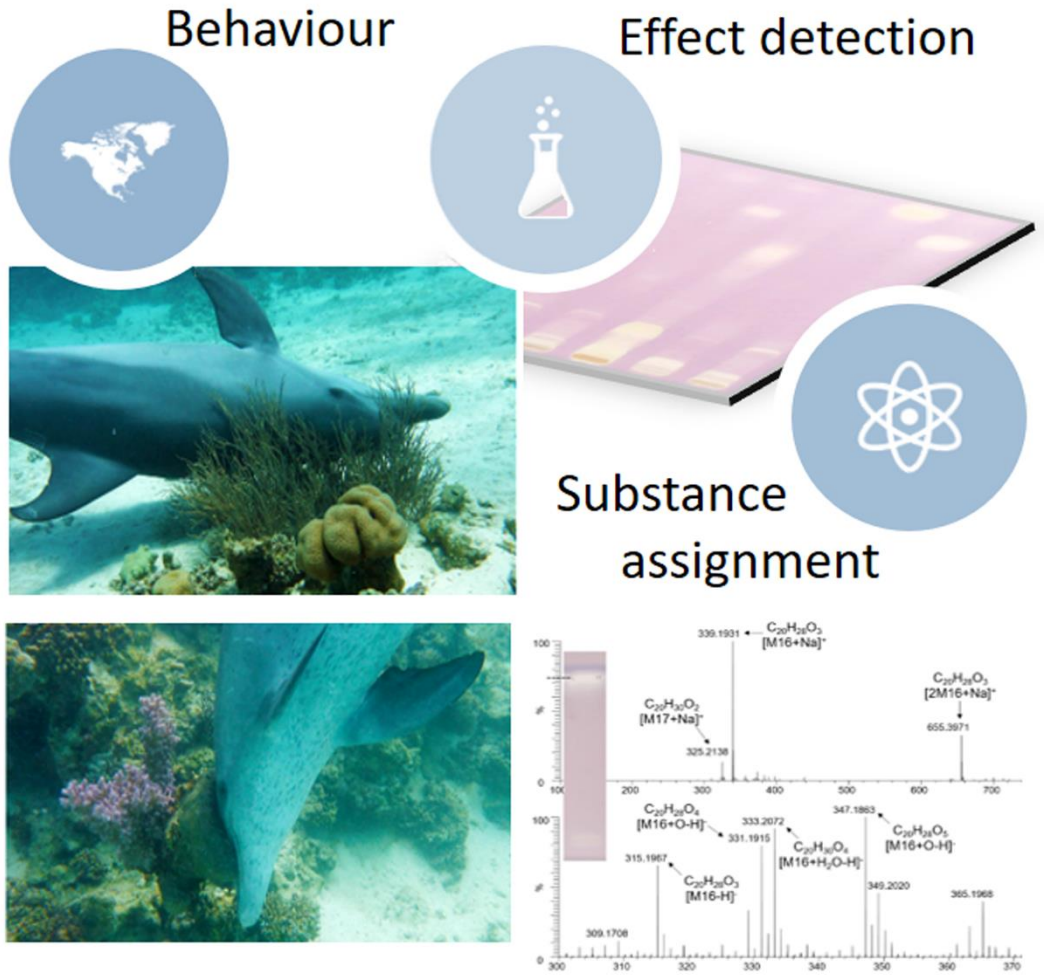
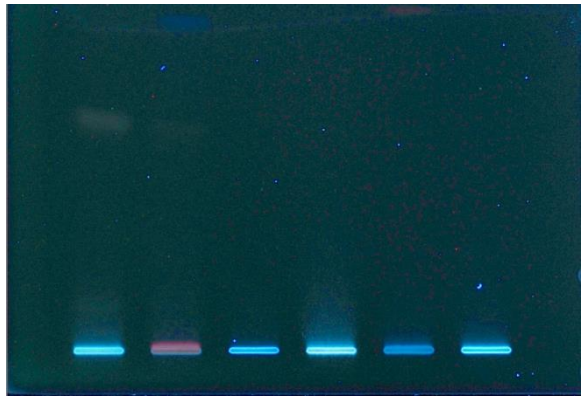
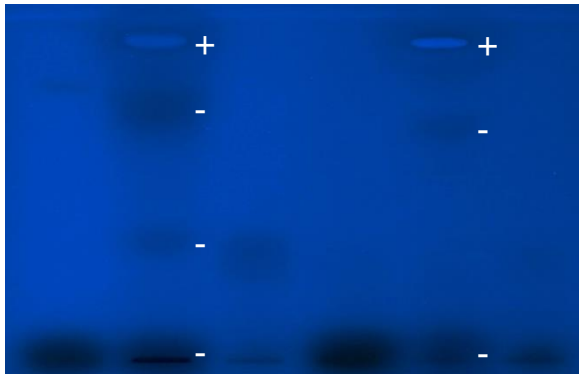
Effect differentiation in complex samples



FLD 366 nm



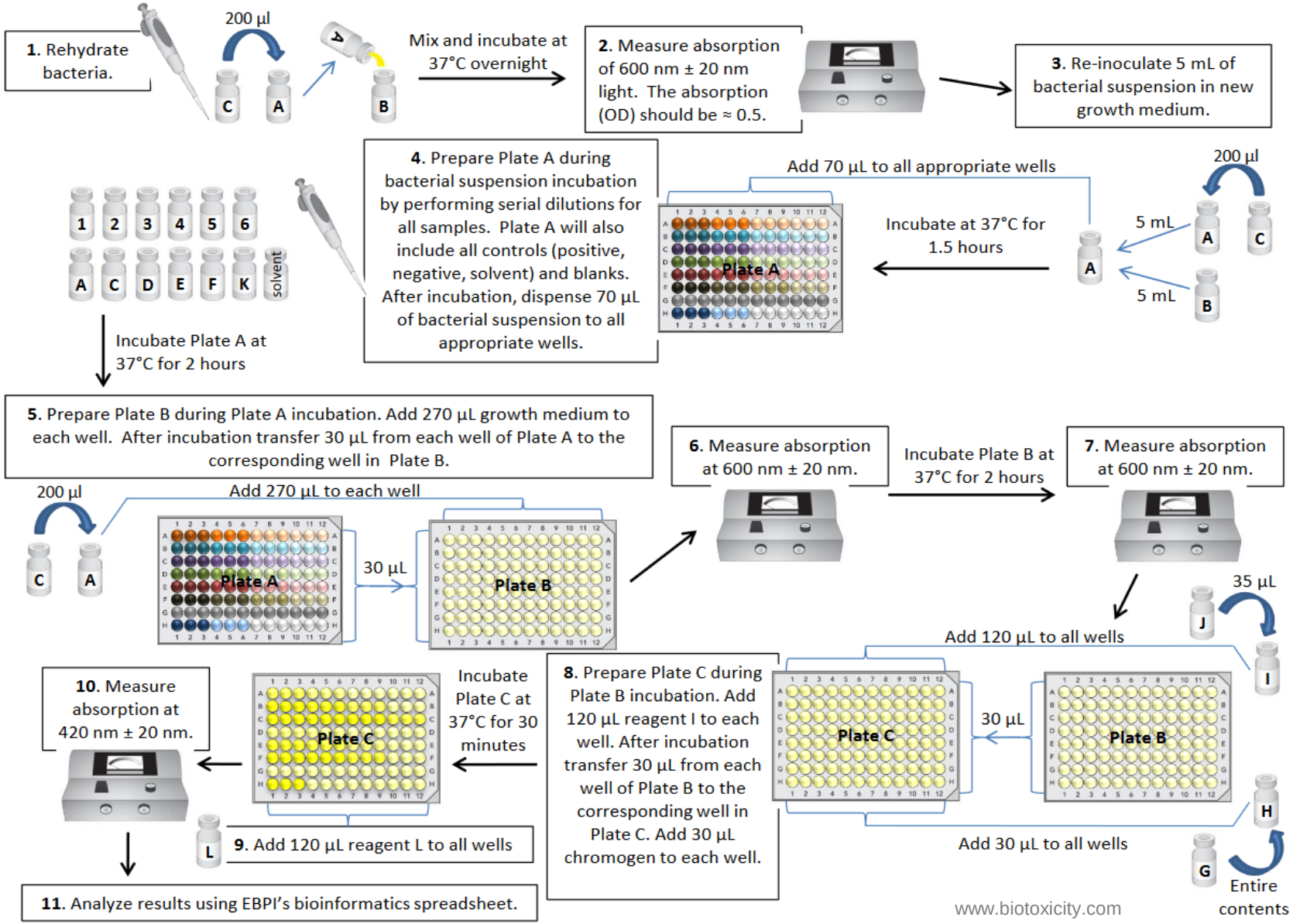
Bioassay with MUG as substrate
► MU



G10 L8 S10 G11 L13 S13

Morlock, G.E., Ziltener, A., Geyer, S., Mehl, A., Schreiner, T., Kamel, T., Tersteegen, J., Brümmer, *iScience* (2022) 104271

Impossible via microtiter plate assay ► sum parameter only



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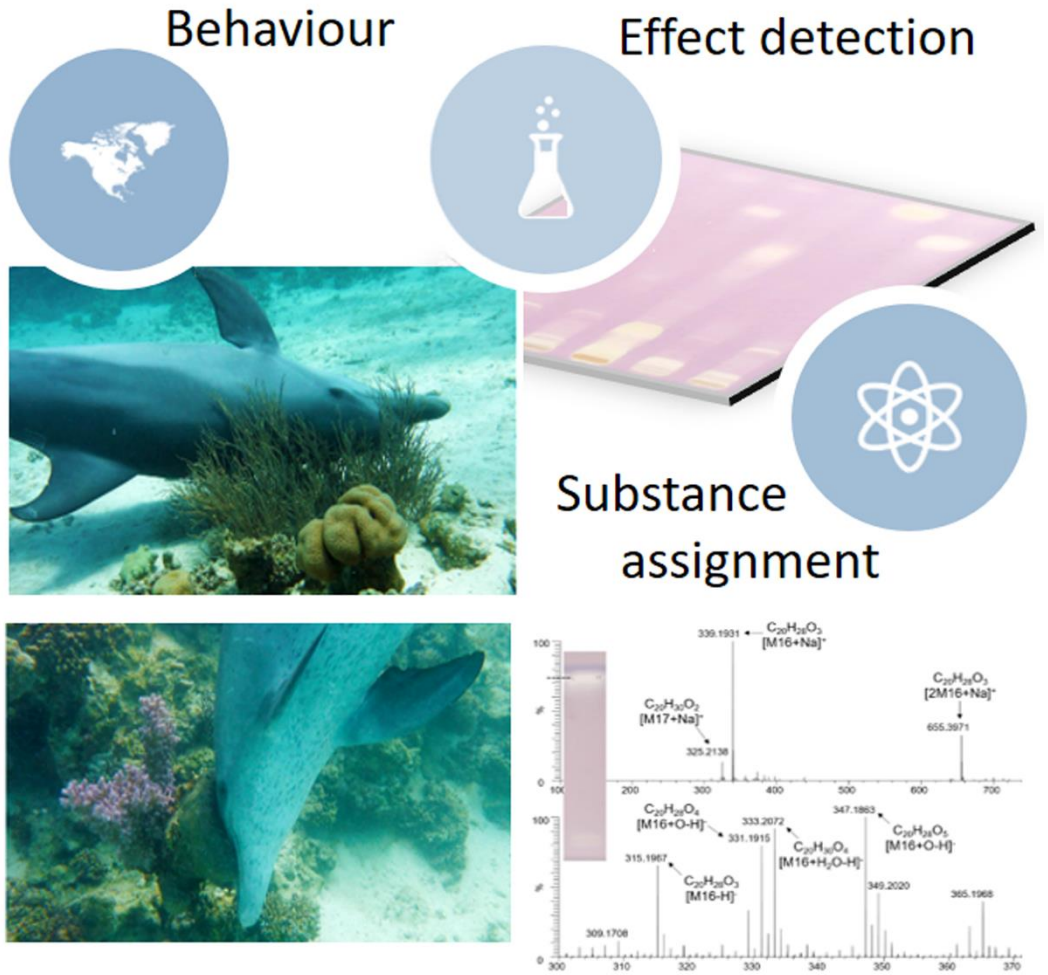
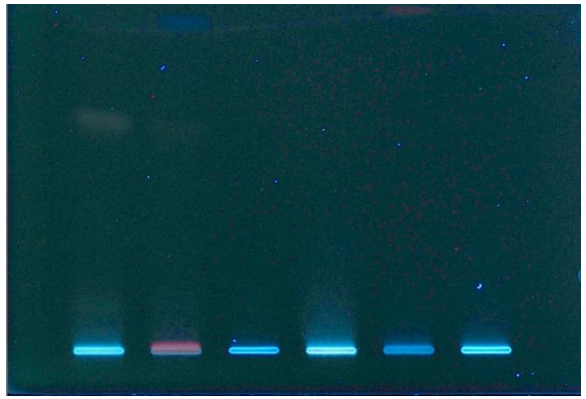
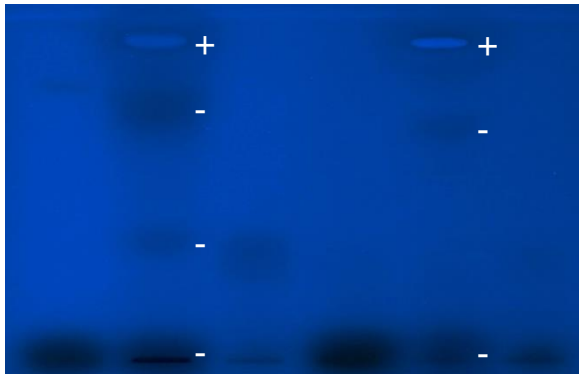
Effect differentiation in complex samples



FLD 366 nm



Bioassay with MUG as substrate
► MU



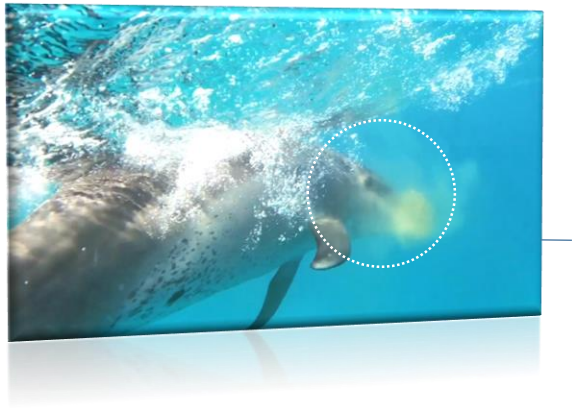
G10 L8 S10 G11 L13 S13

Morlock, G.E., Ziltener, A., Geyer, S., Mehl, A., Schreiner, T., Kamel, T., Tersteegen, J., Brümmer, *iScience* (2022) 104271

G. Morlock, A. Ziltener, S. Geyer, A. Mehl, T. Schreiner, T. Kamel, J. Tersteegen, F. Brümmer in *iScience* 2022, 104271

DOLPHIN WATCH Alliance

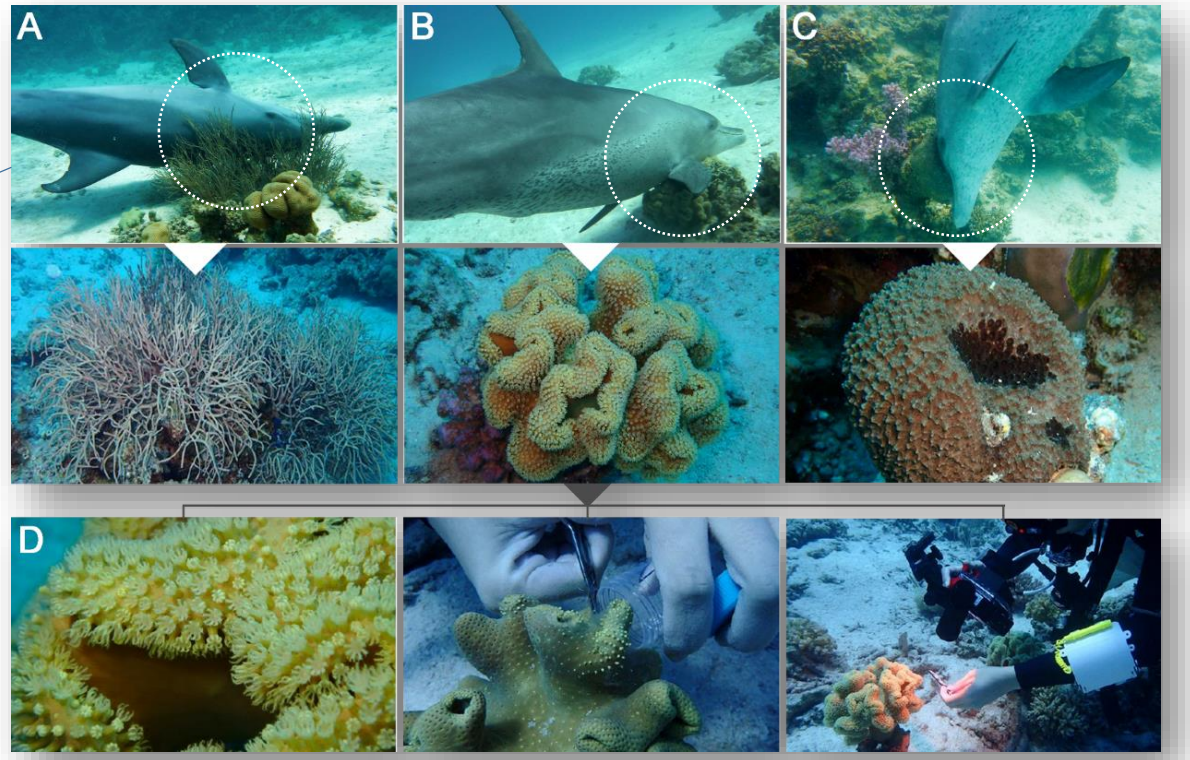
Dolphins' beauty secrets



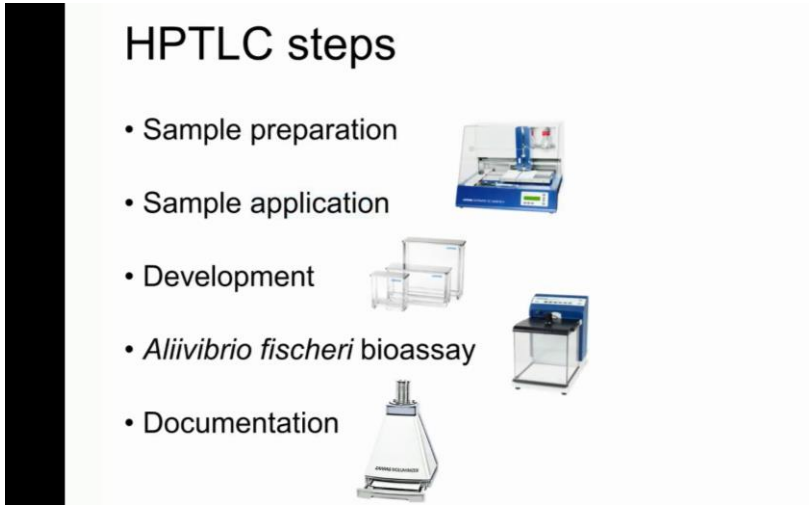
No hands, no arms, no creams?

How do you treat your skin?

Be as intelligent as dolphins!



How do you discover the secrets of nature?



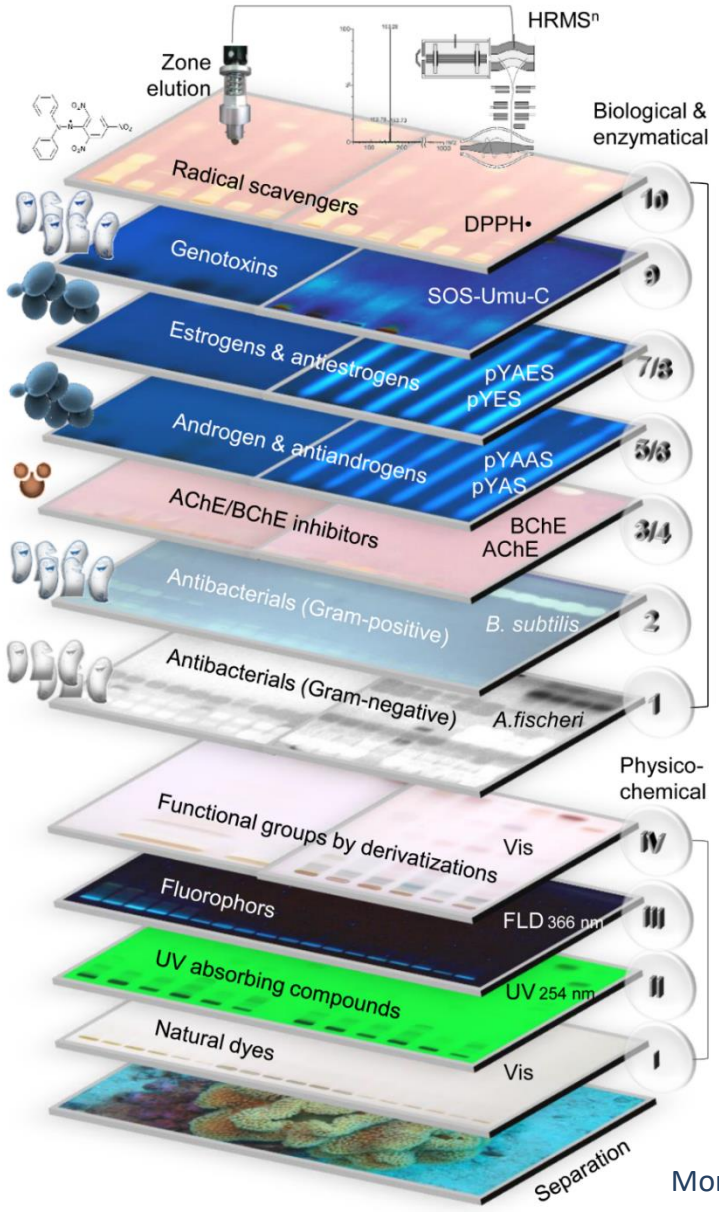
Maximum output…

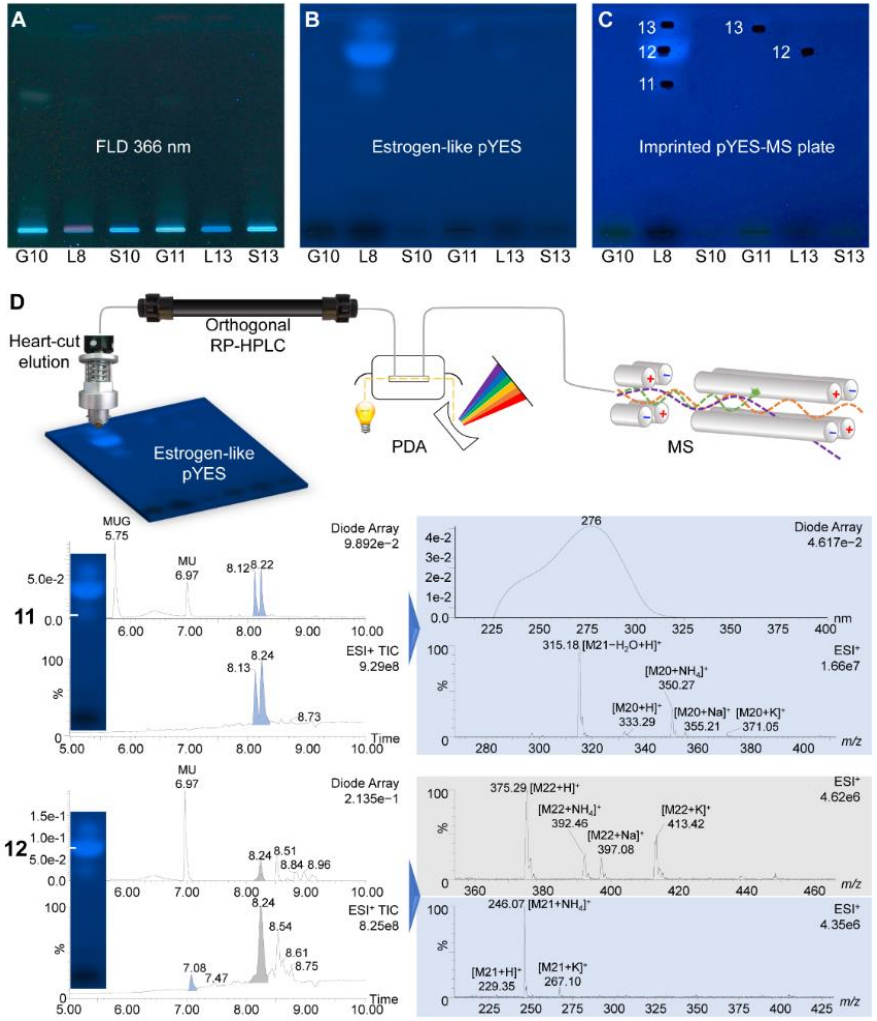
Lab tests for bioactive compound screening via straightforward hyphenated HPTLC

Minimum input…

The gorgonian coral *Rumphella aggregata*, leather coral *Sarcophyton* sp. and sponge *Ircinia* sp. were specifically accessed by the dolphins → small quantities were collected not to disturb nature.

17 active compounds found via bioprofiling





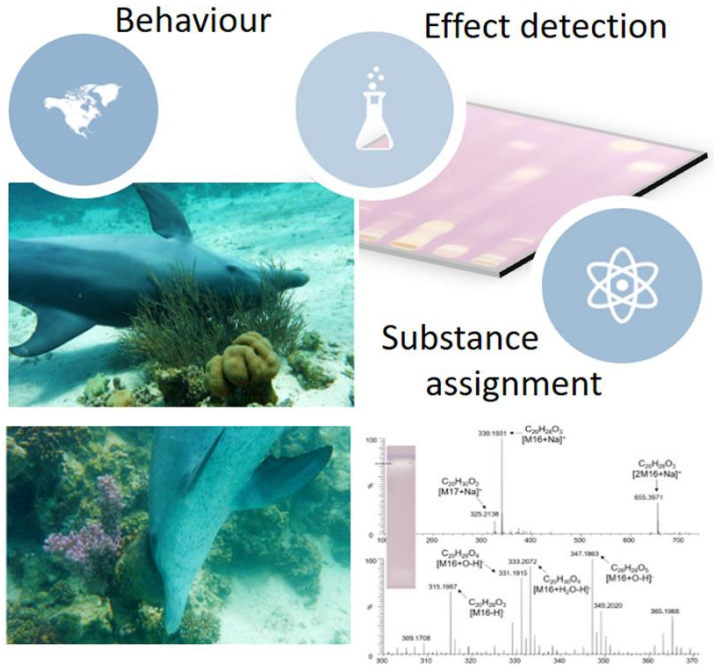
Assignment to potential candidates via (HR)MS

Take home…

Just rub and benefit!

Self-medication maintains skin health → marine pharmacy?

Let it be for the dolphins!



Morlock, G.E., Ziltener, A., Geyer, S., Mehl, A., Schreiner, T., Kamel, T., Tersteegen, J., Brümmer, *iScience* (2022) 104271

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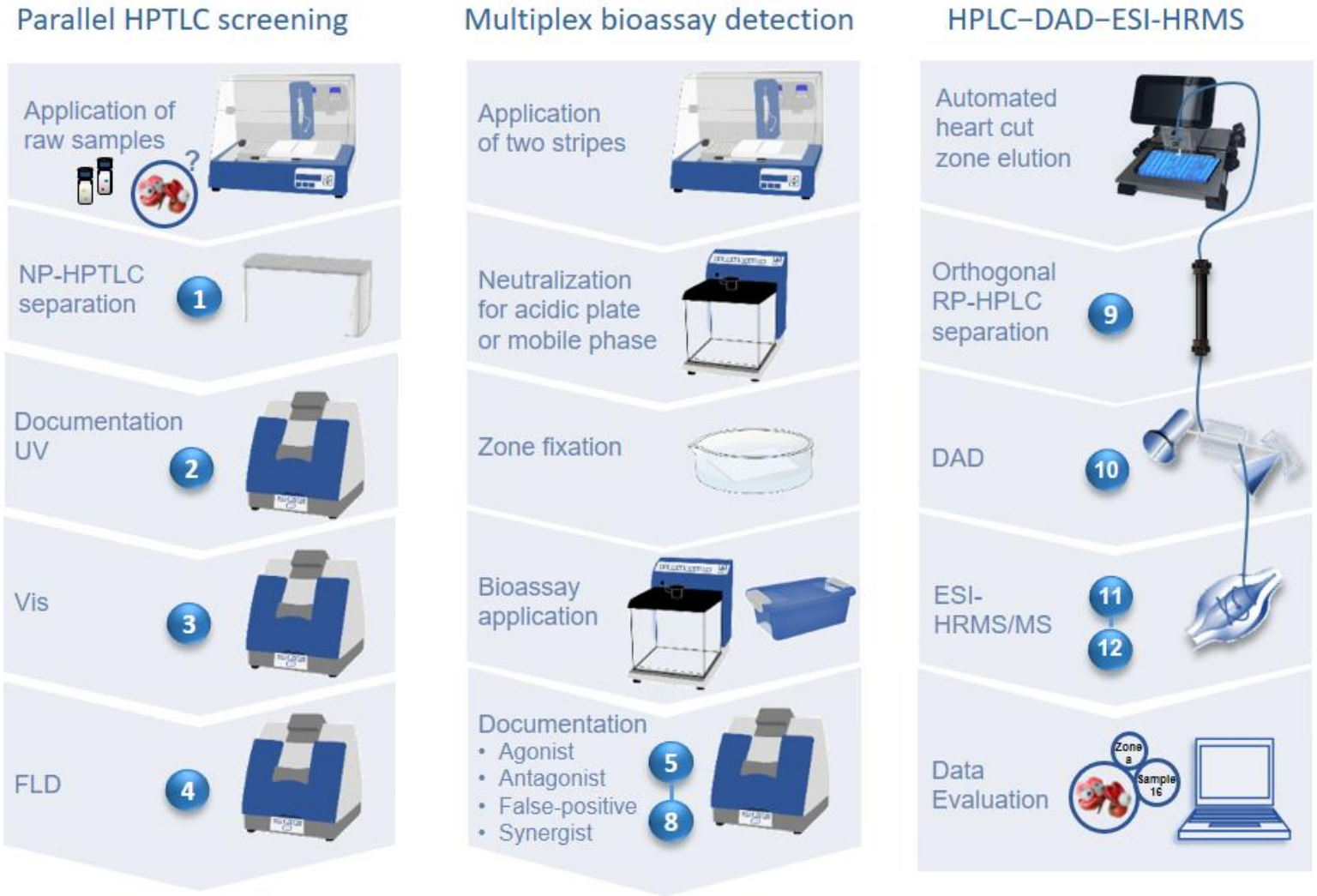
12 D hyphenation 5−25 min/sample 0.5−0.9 Euro/sample



G. Morlock *Phytochem. Reviews*, in submission

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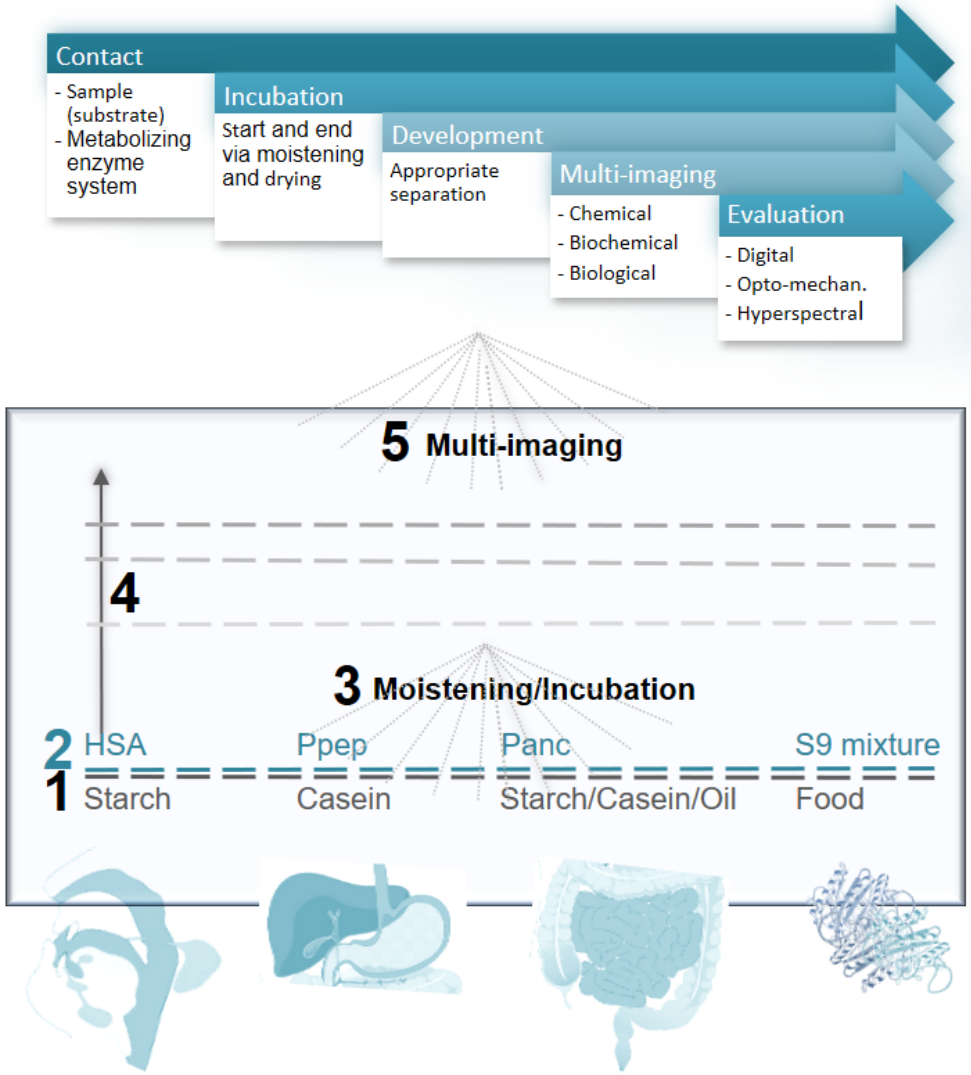
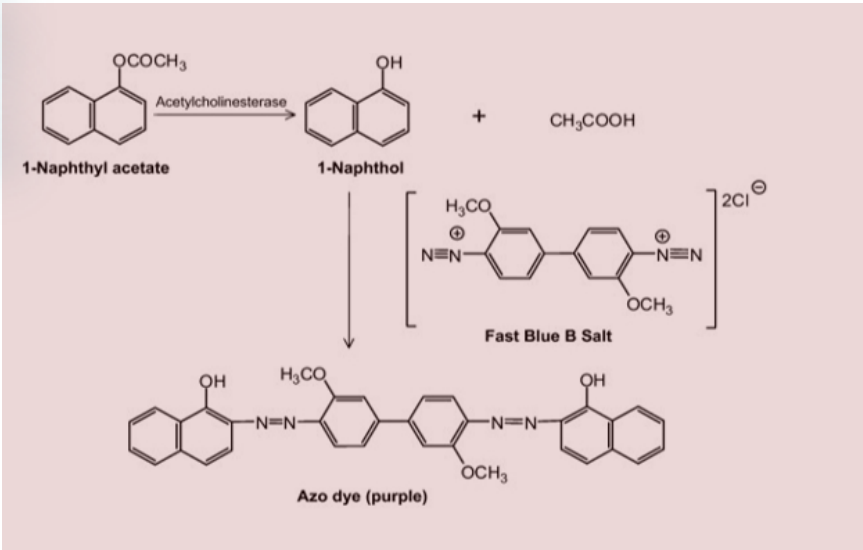
13 D hyphenation: *in situ* metabolization added



AChE assay for detection



G. Morlock, L. Drotleff, S. Brinkmann *Anal. Chim. Acta* 1154 (2021) 338307

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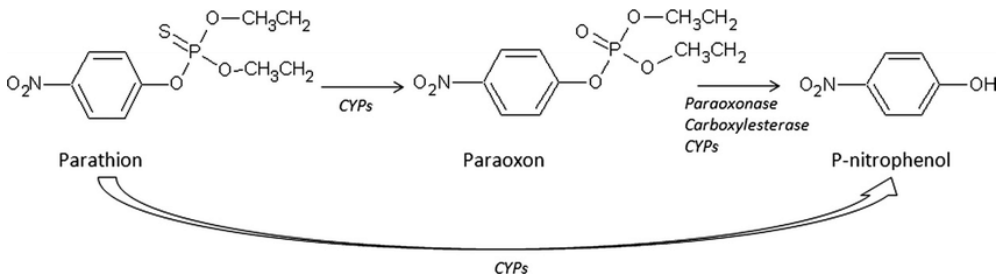
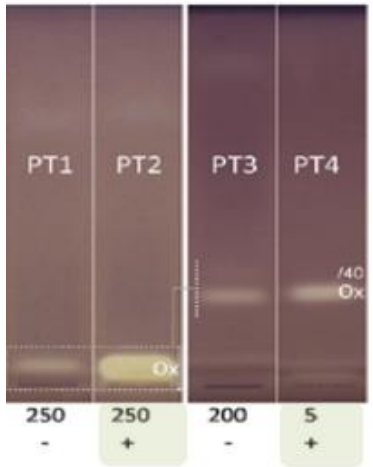
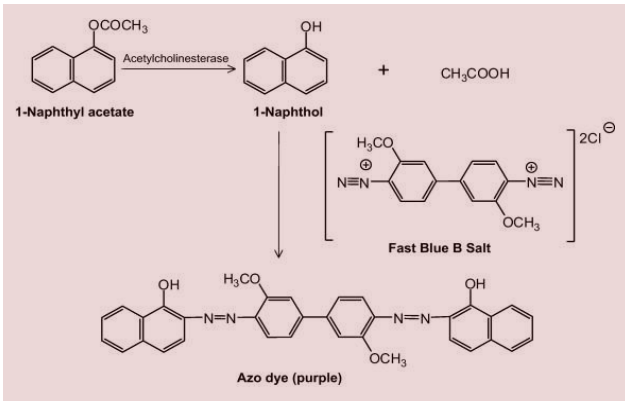
Any neurotoxins in food packaging migrates or extracts?

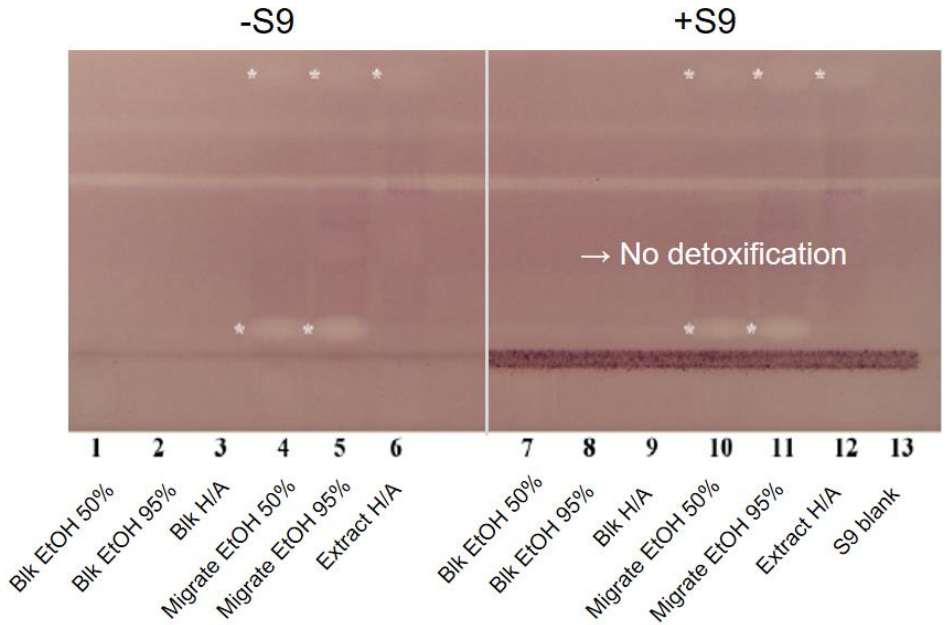
HPTLC-S9-UV/Vis/FLD-AChE assay: nanoGIT+active



AChE assay







E. Azadniya, J. Mollergues, T. Stroheker, K. Billerbeck, G. Morlock *Anal. Chim. Acta* 1129 (2020) 76-84

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Does it make a difference what kind of bread you eat?

- Amylolysis: major energy-providing source for the human diet

  Polymeric carbohydrate consisting of numerous glucose units

  → cleavage products: maltotriose, maltose, glucose, dextrins
- Catalyzed by the salivary and pancreatic α-amylase
- Susceptibility dependent on several factors, *e. g.*,
  - Botanical origin (particle structure)
  - Food processes (milling of grains, gelatinization of starch)
- Intake of starchy foods leads to rise in blood glucose

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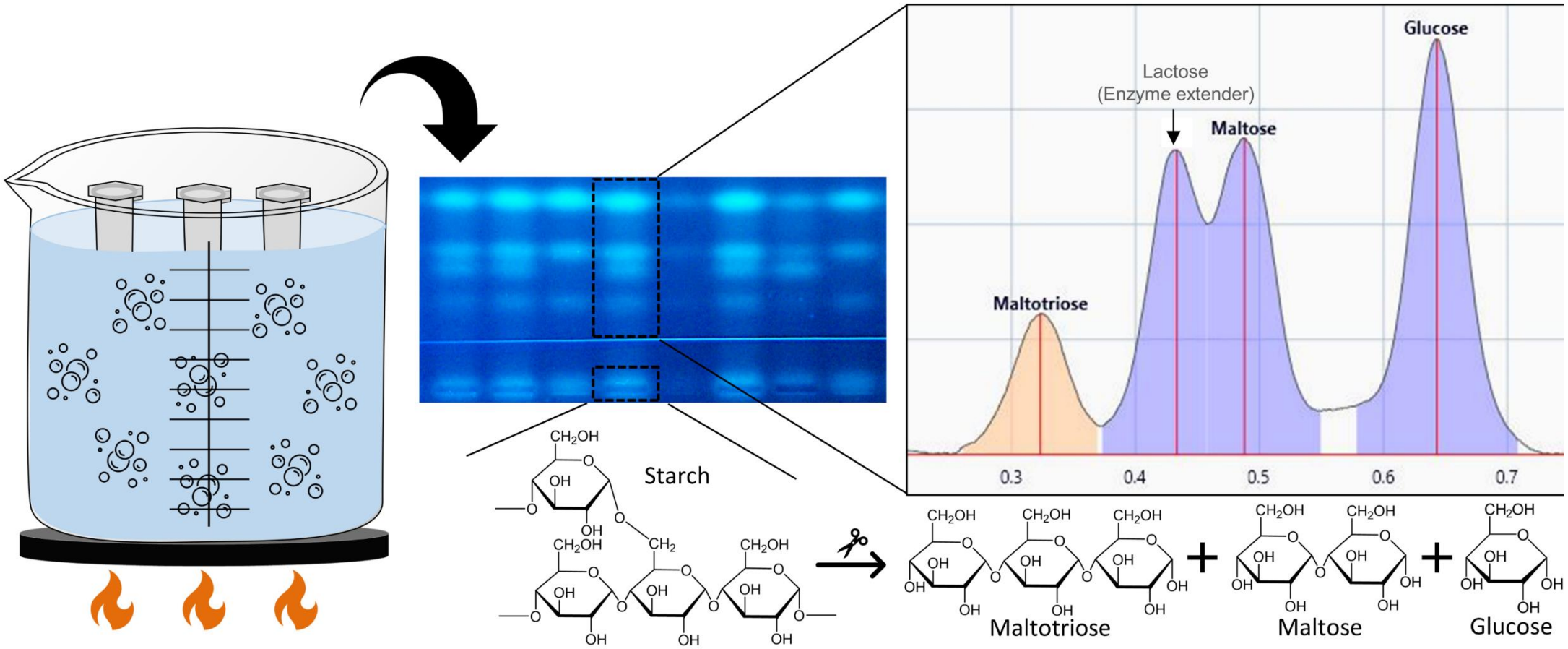
HPTLC on-surface digestion of starch: nanoGIT$^{+active}$



Müller, I., Morlock, G.E., in submission

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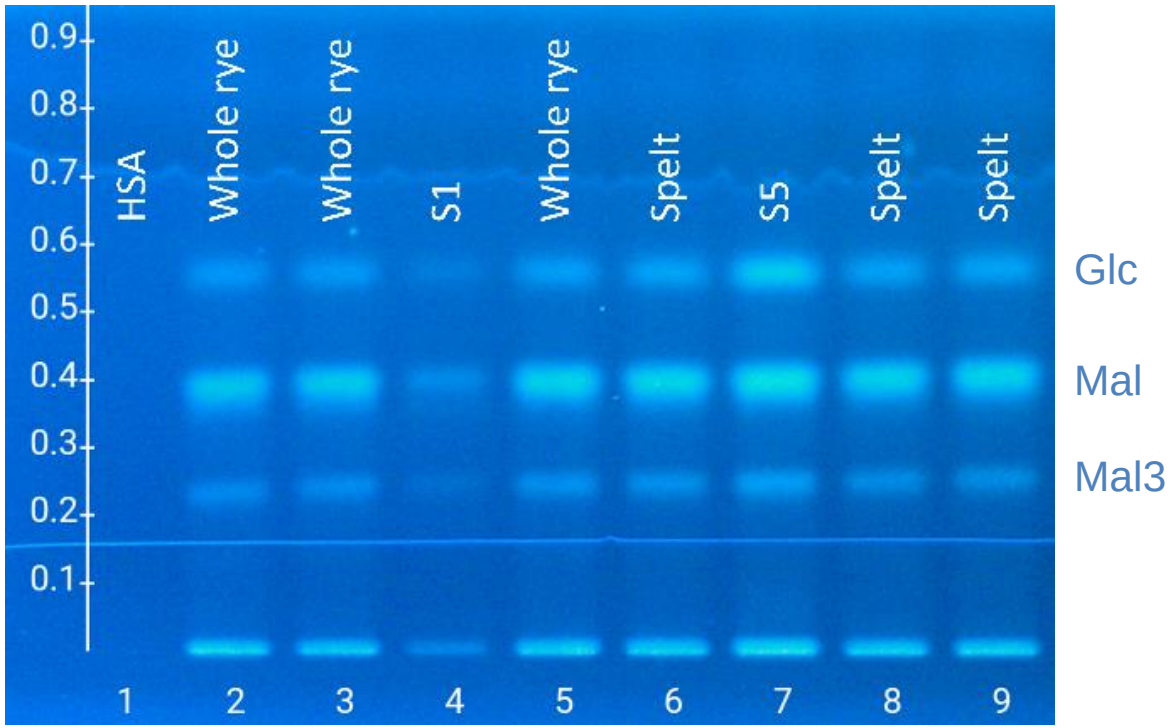
HPTLC on-surface digestion of starch: nanoGIT$^{+active}$

Why transferring amylolysis to a highly adsorbing HPTLC plate? → Because we can! ☺

- Enzymatic reaction directly on plate!
- Analysis of all products
- Very low LOD/LOQ
- 17 samples per plate



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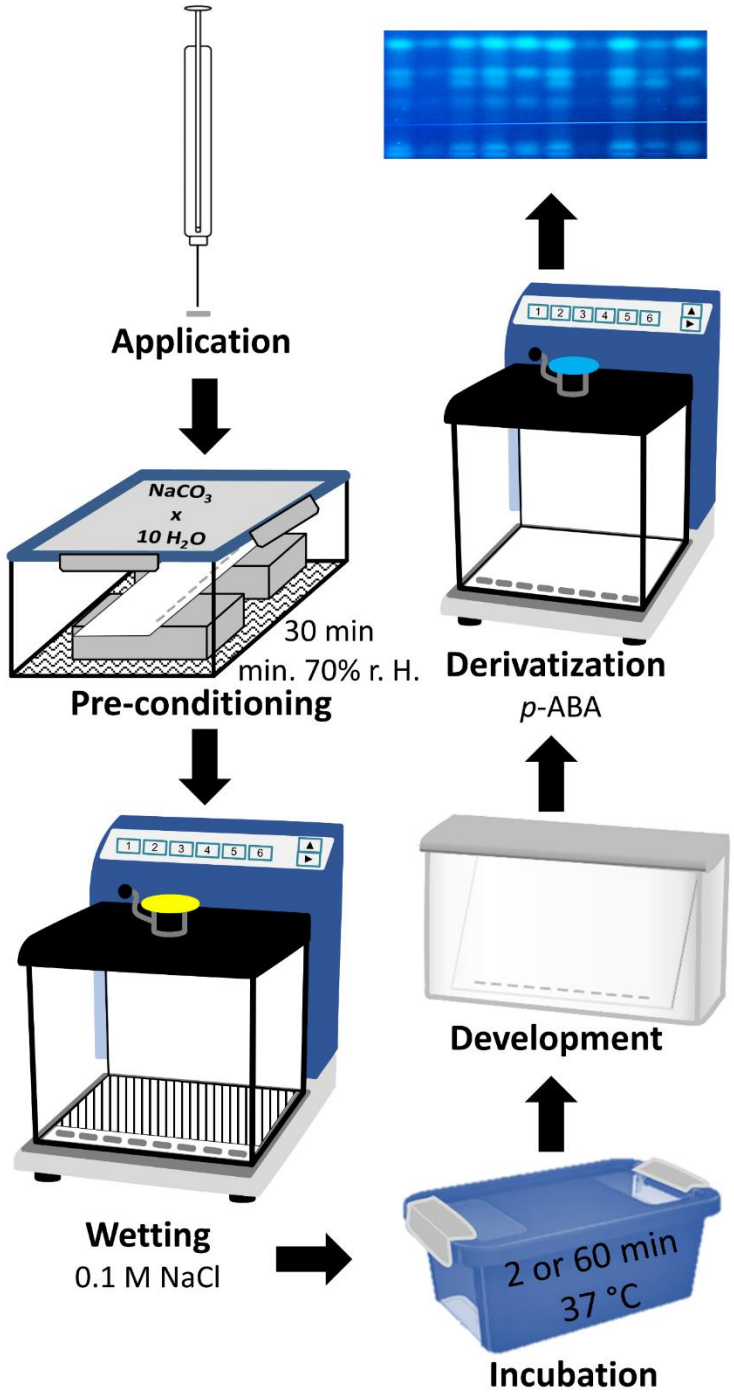

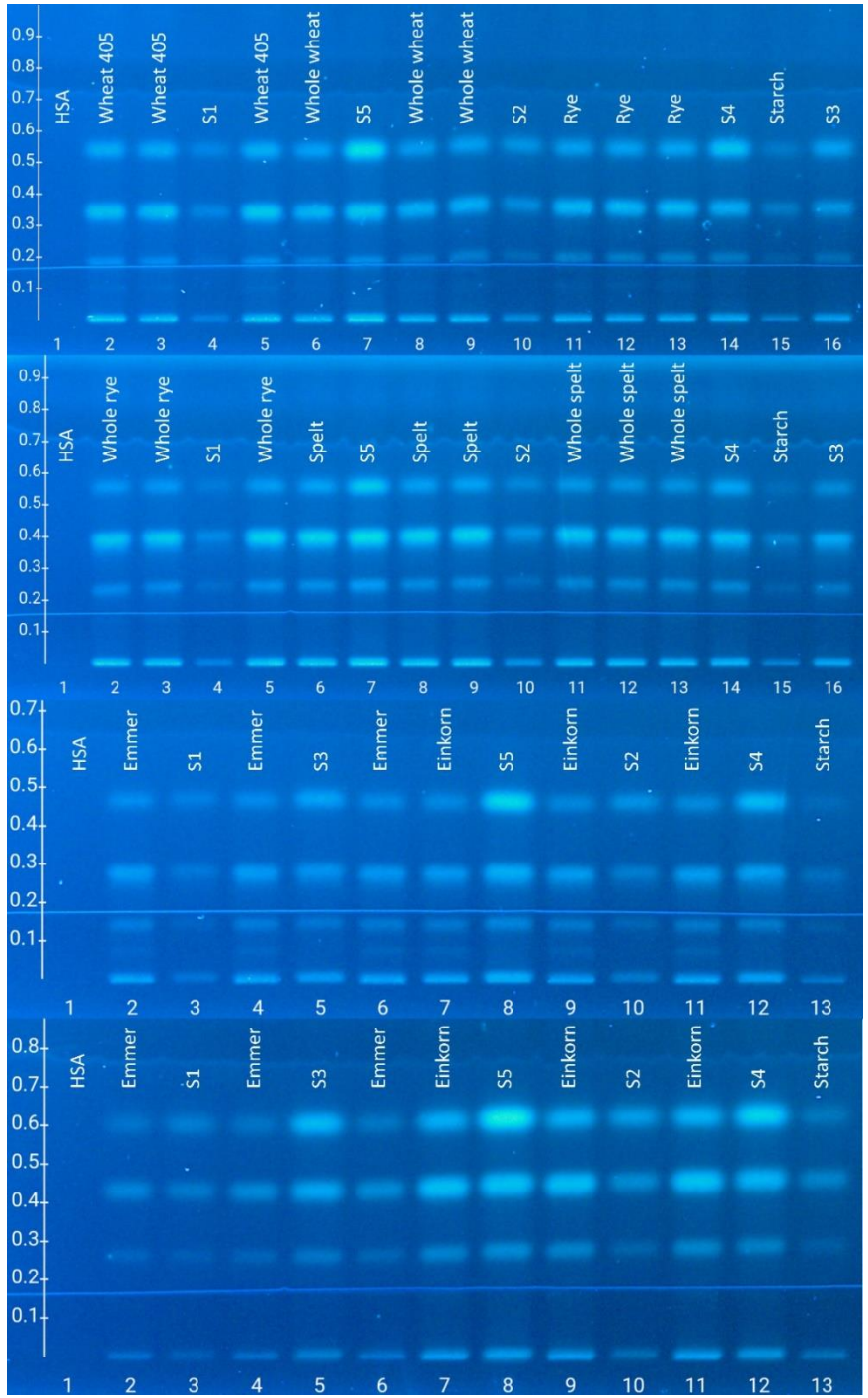

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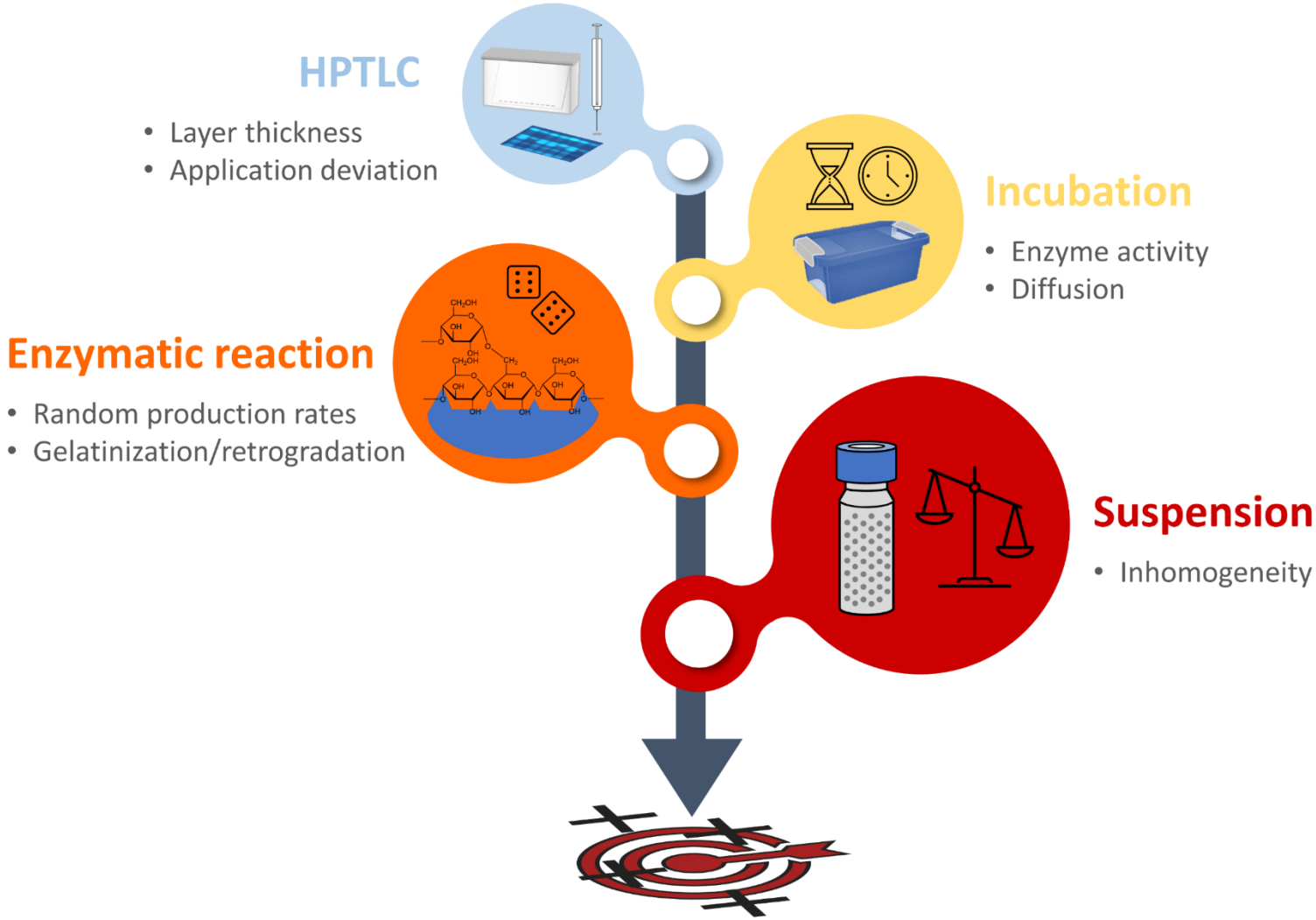
Validation of nanoGIT$^{+active}$ method



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Validation of nanoGIT$^{+active}$ method

Standardization

- Sample preparation procedure → adjust cooking time, evaluate storage conditions
- Environmental conditions → precise relative humidity control
- Wetting and incubation procedure → ensure enzyme activity, while not plate floating or drying out
- Development conditions → mobile phases are prone to relative humidity

	LOD [ng]	LOQ [ng]	Inter-day precision starch [RSD%]	Inter-day precision wheat [RSD%]	Recovery starch [%]	Recovery wheat [%]
Glucose	5	10	≤ 10	≤ 21	111–112	80–98
Maltose	38	48	≤ 10	≤ 16	106–115	62–94
Maltotriose	47	188	≤ 10	≤ 25	-	-

Müller, I., Morlock, G.E., in submission

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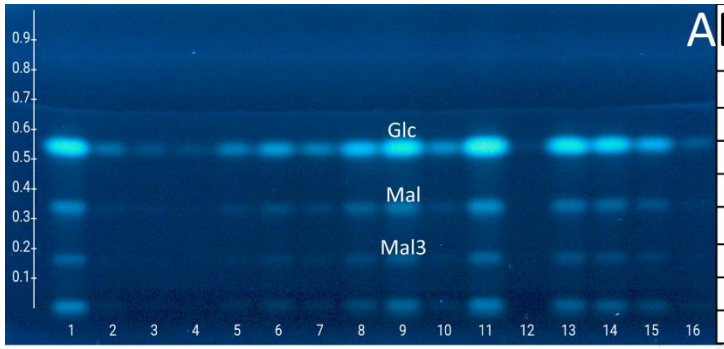
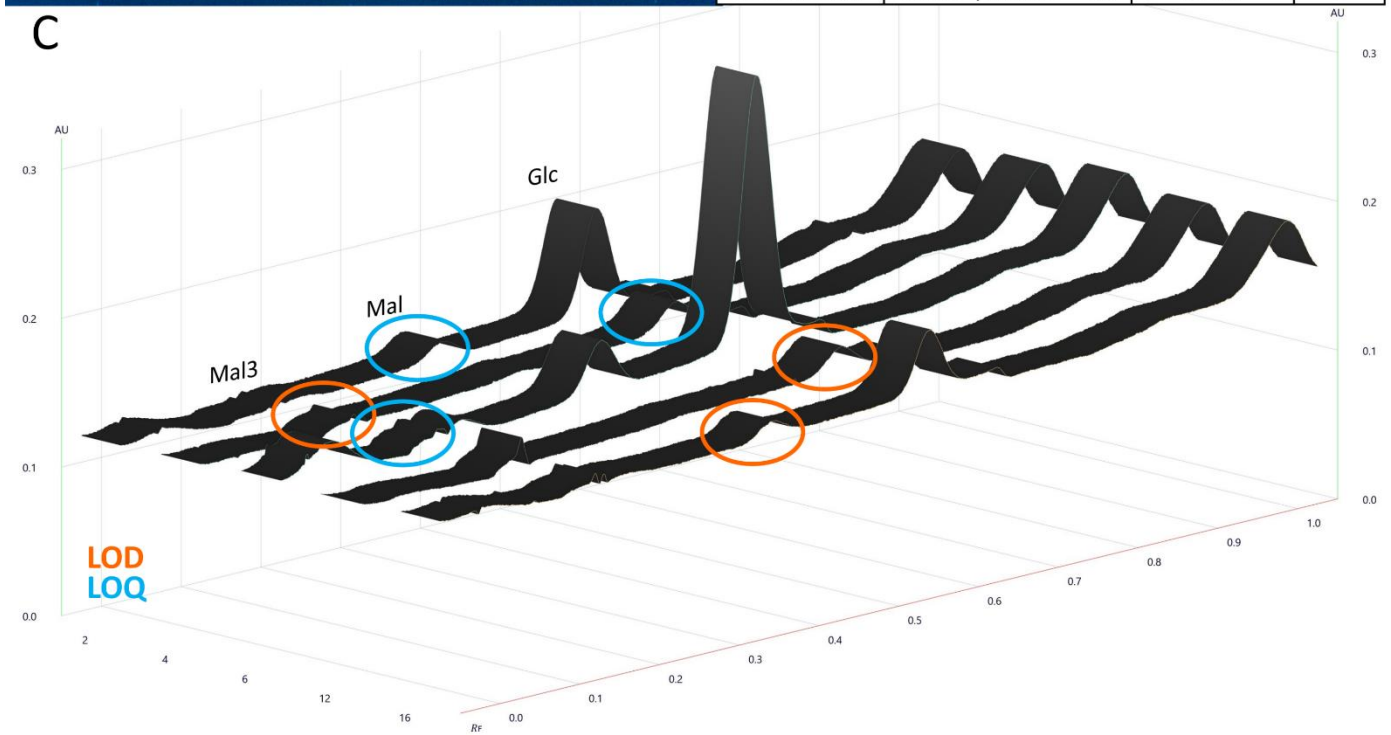
Validation of nanoGIT$^{+active}$ method



B Saccharide	S/N Height	Amount [ng]	Track
LOD			
Glucose	0.0171/0.0023 = 7.4	5.000	12
Maltose	0.0092/0.0022 = 4.2	38.00	16
Maltotriose	0.0042/0.0011 = 3.8	47.05	4
LOQ			
Glucose	0.0178/0.0015 = 12	10.00	4
Maltose	0.0113/0.0011 = 10	47.50	2
Maltotriose	0.0124/0.0012 = 10	188.2	6



Müller, I., Morlock, G.E., in submission

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Outcome of the amylolysis of starches

- Differences observed for botanically different flours, whole grain & refined flours
- Overall saccharide release of salivary amylolysis
  - Lowest: **Amaranth**, emmer and einkorn
  - Highest: **Spelt**
- Overall saccharide release of pancreatic amylolysis
  - Lowest: **Amaranth**, whole wheat and wheat
  - Highest: **Spelt** and whole rye
- Possible benefit of whole grain products due to their lower saccharide release
- It does make a difference what kind of bread you eat!

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2Labs2Go – Chromatography System for Citizen Science

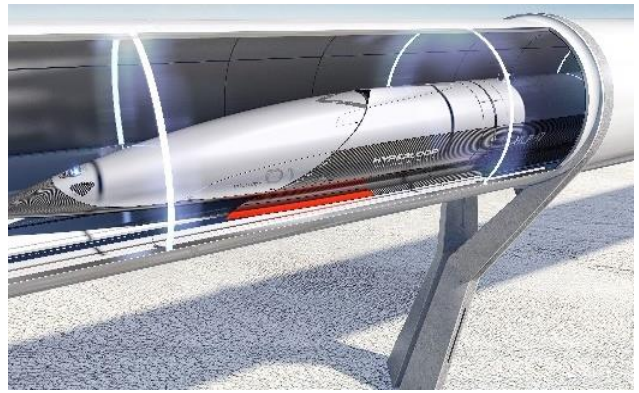
UHPLC is like…

http://ams.bg/images

HPTLC is like…

G. Morlock, q-more.chemie.de

OCLab3 is like…



https://railway-technology.com

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Simplicity is the ultimate sophistication.

Leonardo da Vinci

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2Labs2Go – Chromatography System for Citizen Science

UHPLC is like…

http://ams.bg/images

HPTLC is like…

G. Morlock, q-more.chemie.de

OCLab3 is like…

https://howldb.com

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Simplicity is the ultimate sophistication.

Leonardo da Vinci

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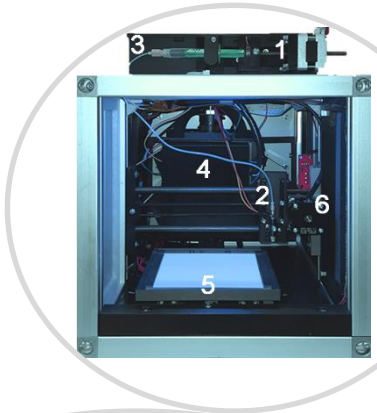
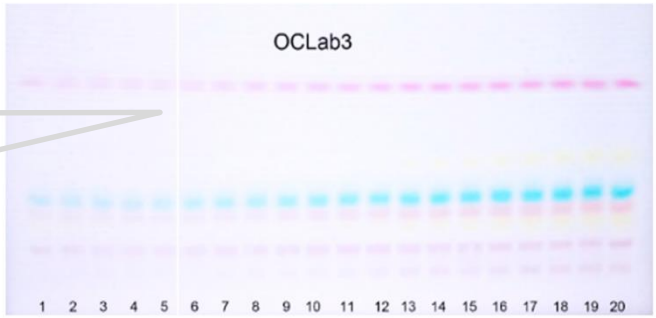
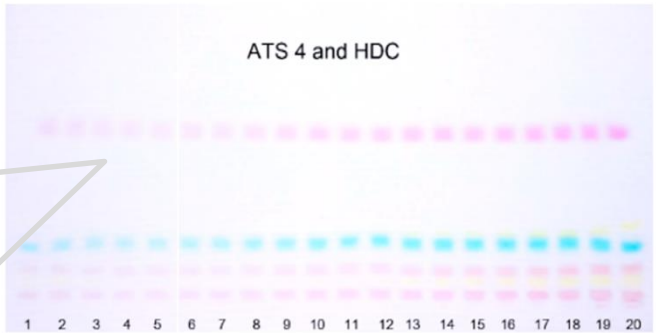
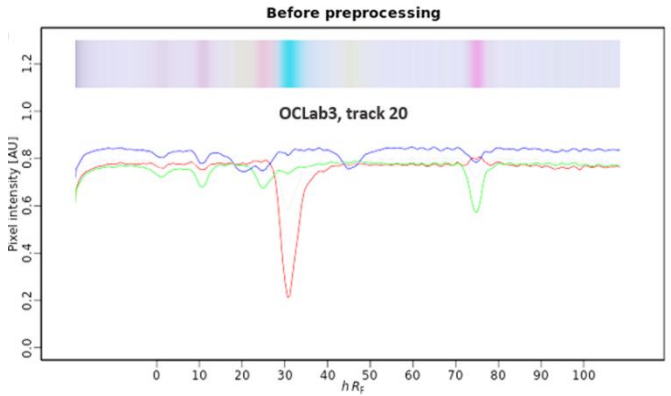
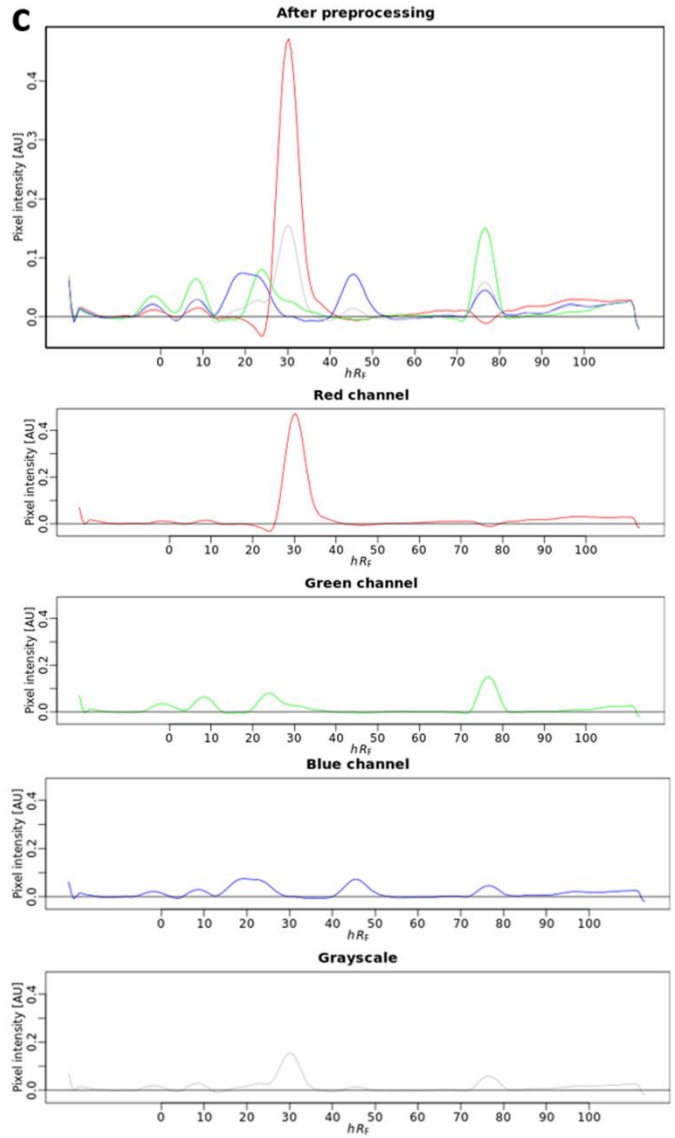
Comparison to status quo











L. Sing, W. Schwack, R. Götsche, G. Morlock, in submission

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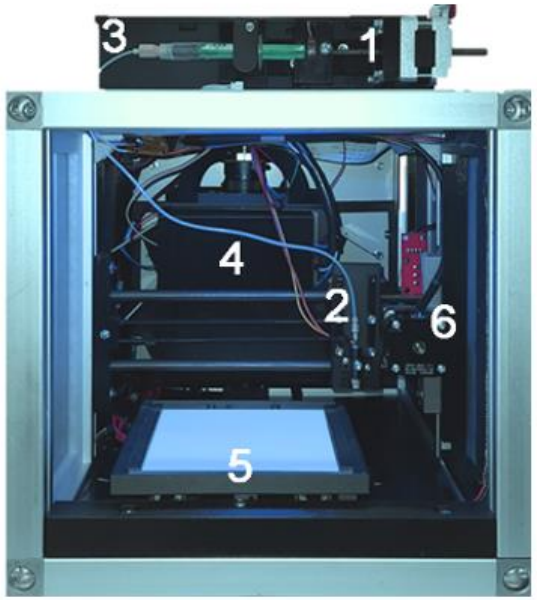
2Labs2Go system

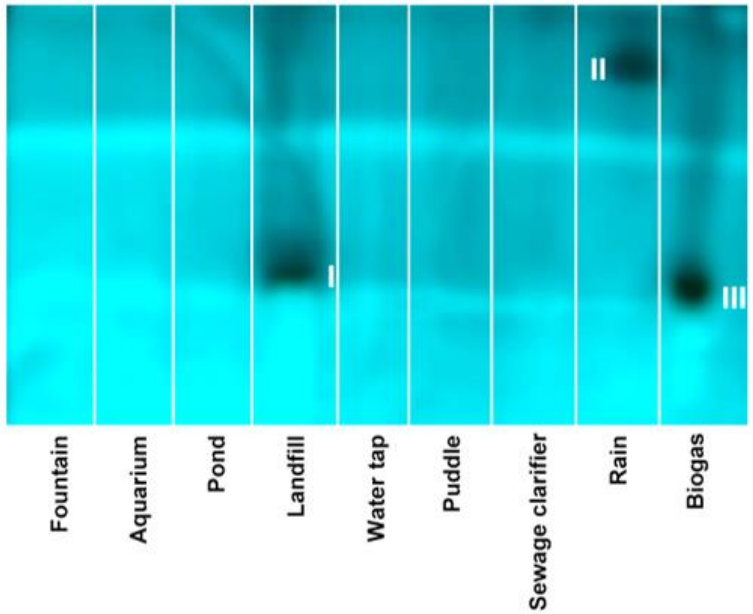
Compact: 26×31×34 cm³

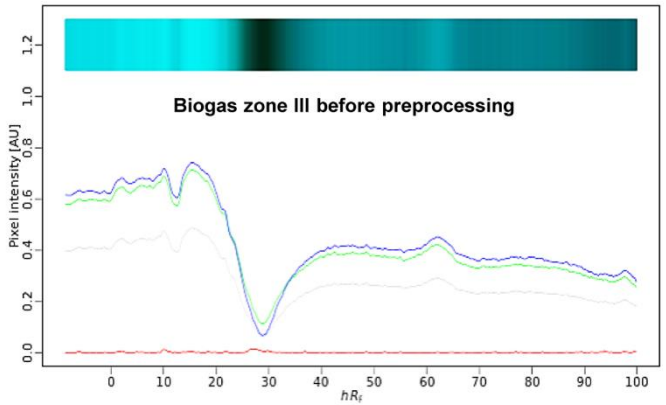
Light: 6.8 kg

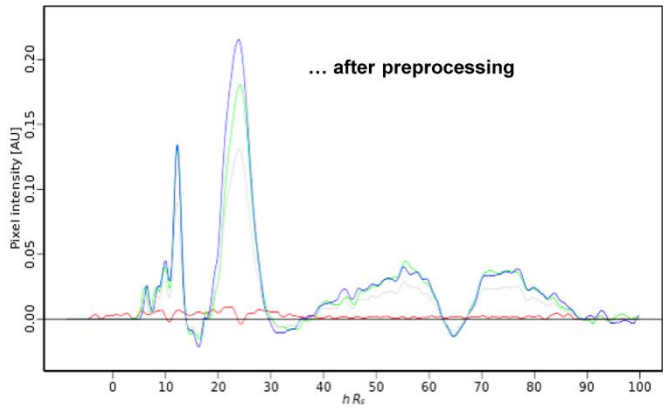
Costs: 1717 €



26 cm × 31 cm × 34 cm, 6.8 kg







Morlock, G., Morlock, J., Morlock, L., Chapter 15. In C. Poole (Ed.), Instrumental Thin-Layer Chromatography, Handbooks in Separation Science, Elsevier Science, Amsterdam, in print

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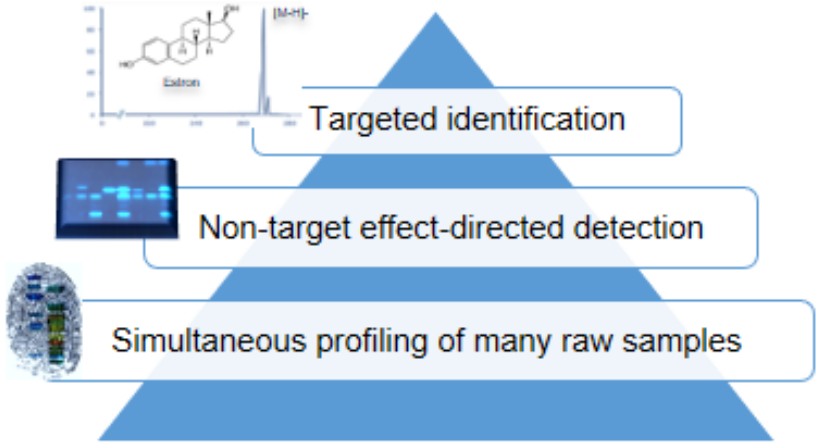
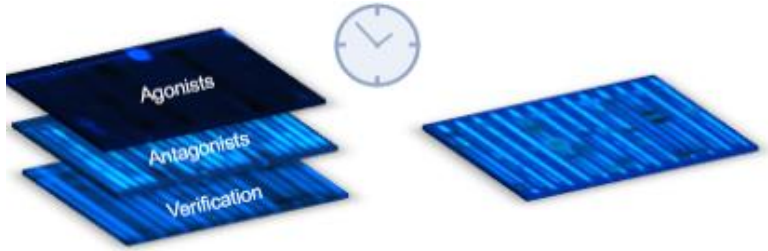
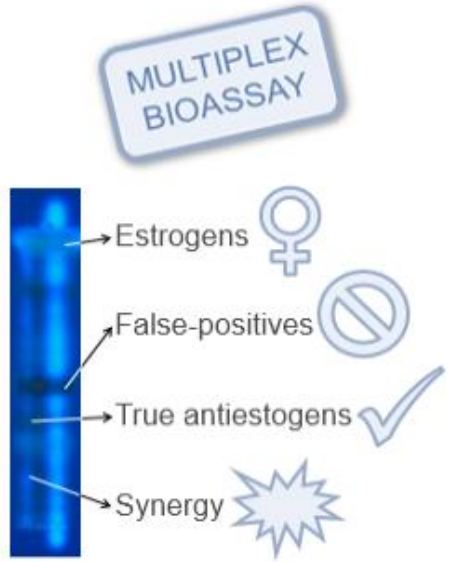
Summary I Prioritization strategy ► 12 D hyphenation

- Fast profiling of samples
- Samples as native as possible
- Minimalistic sample preparation
- Maximal view on sample
- Prioritization of compounds
- Molecular formula obtained
- Minimalisitic data storage

HPTLC-UV/Vis/FLD-assay-HRMS/MS







G. Morlock *Anal. Chim. Acta* 1180 (2021) 338644

A. Ronzheimer, T. Schreiner, G. Morlock, *Phytomed*. 103 (2022) 154230

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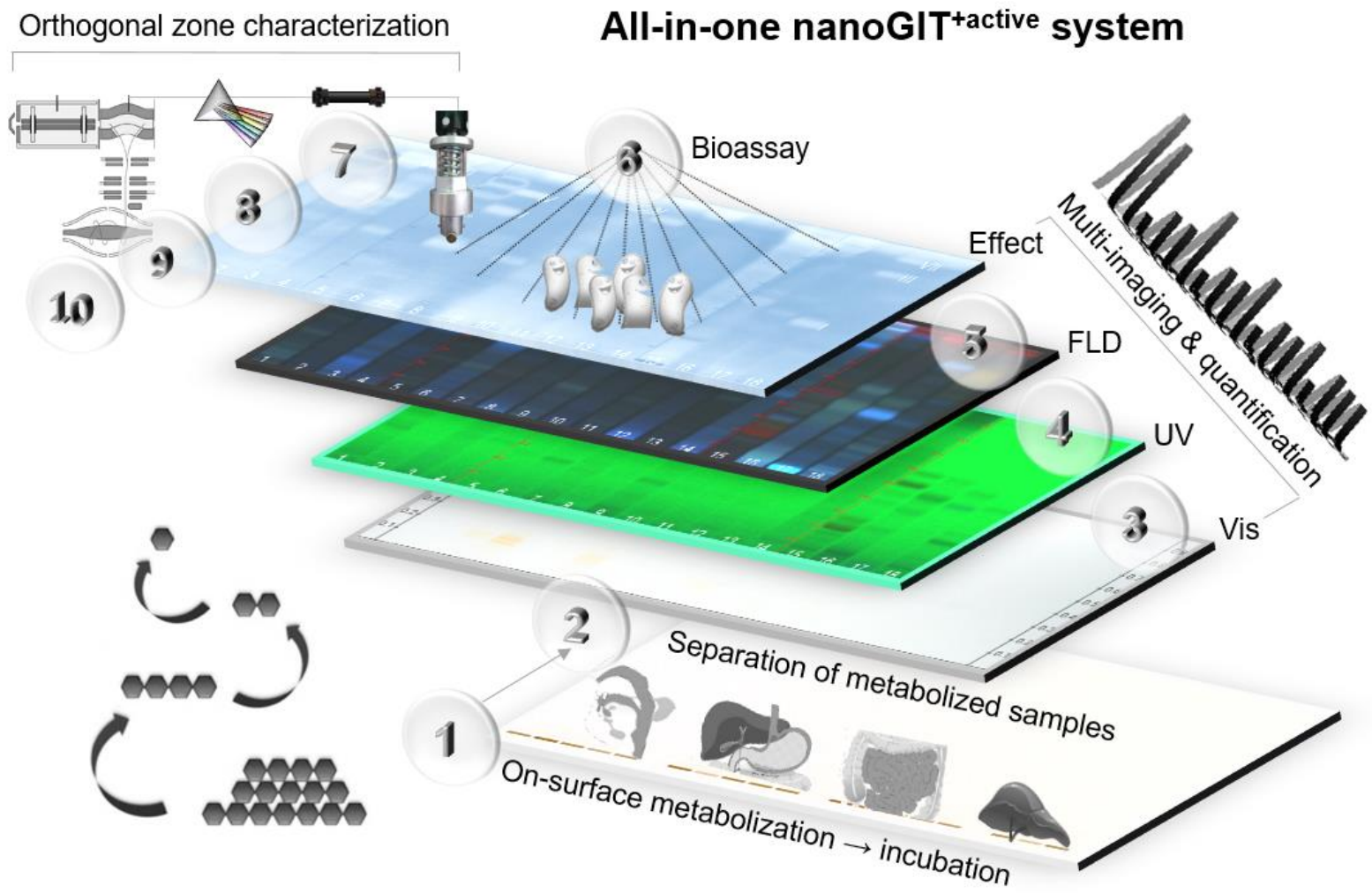
Summary II Metabolization included ► 13 D hyphenation



G. Morlock, L. Drotleff, S. Brinkmann *Anal. Chim. Acta* 1154 (2021) 338307

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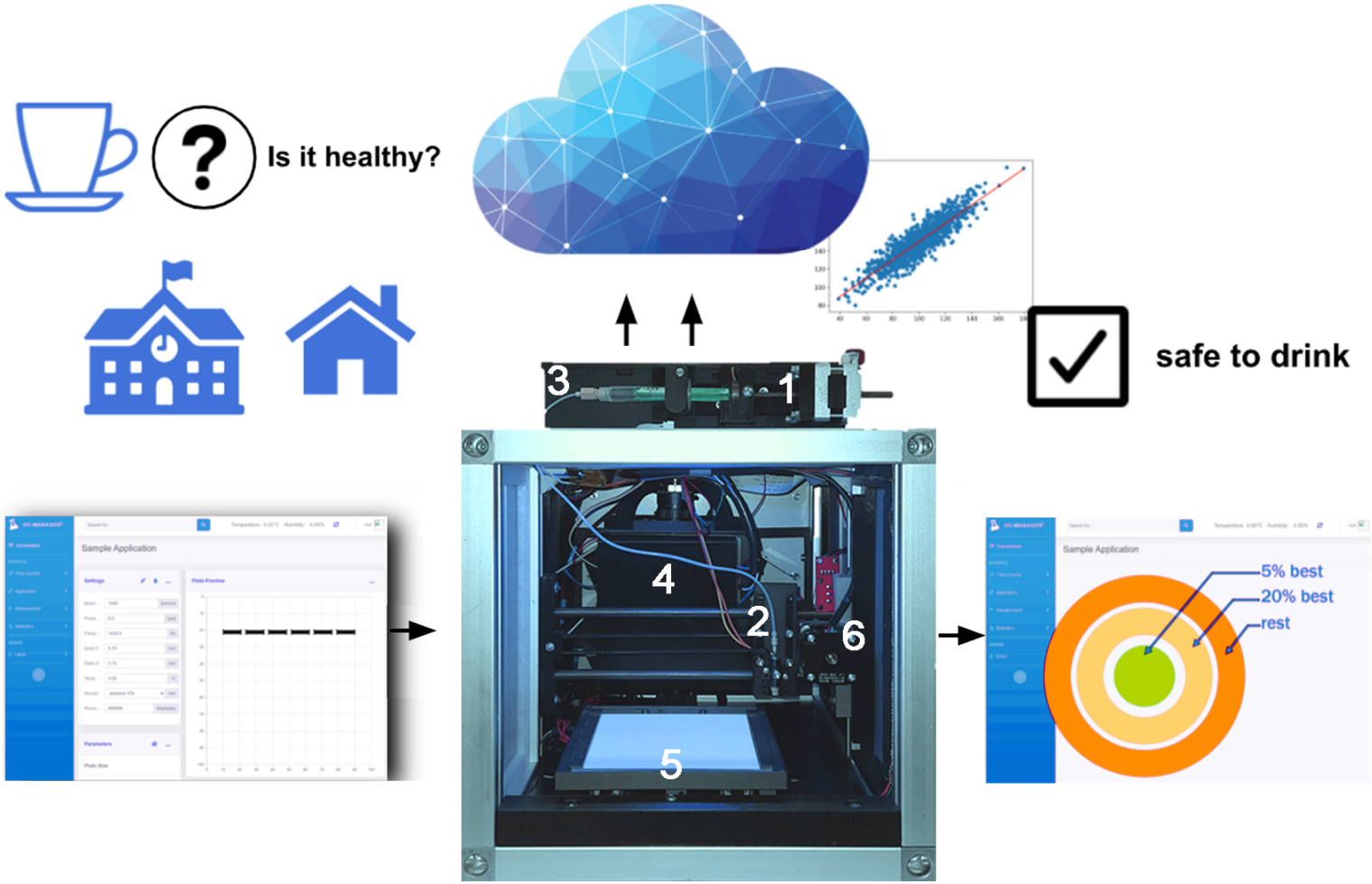
Summary III 2Labs2Go system



L. Sing, W. Schwack, R. Götsche, G. Morlock, in submission

DFG Deutsche Forschungsgemeinschaft

ADISSEO A Bluestar Company Adisseo, Antony, France

Nestlé Nestle, Lausanne, Switzerland

Merck, Darmstadt, Germany

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Thank you!