Université d'Orléans

Institut de Chimie Organique et Analytique

'Intensity ion fading-SALDI MS approach for searching inhibitors of tyrosinase in complex mixtures'

Aleksander Salwiński

Prof. Benoit Maunit, Prof. Raphaël Delépée

29èmes Journées Françaises de Spectrométrie de Masse, *17-20 sept. 2012, Orléans (France)*

General outlook of the new approach

extraction

1st step: finding prospective inhibitors

PLANT EXTRACT (PE)

No pre-treatment

Enzyme coupled nanoparticle-assisted LDI (**ENALDI**) experiment

active enzyme

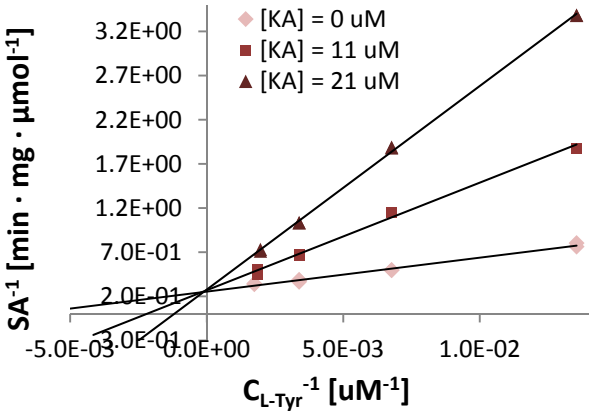
comparison

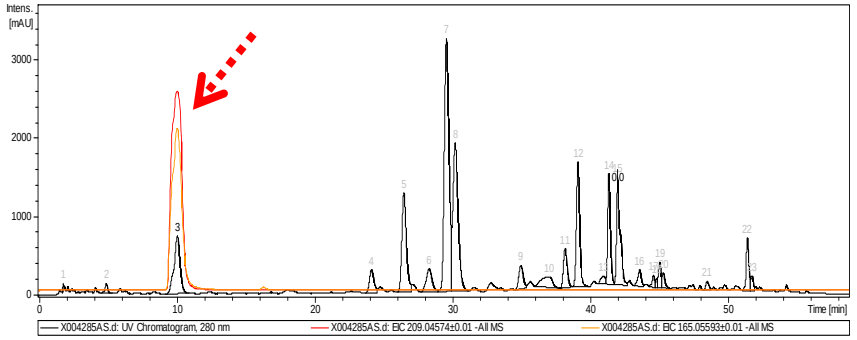
denaturated enzyme

Finding the inhibitor candidate

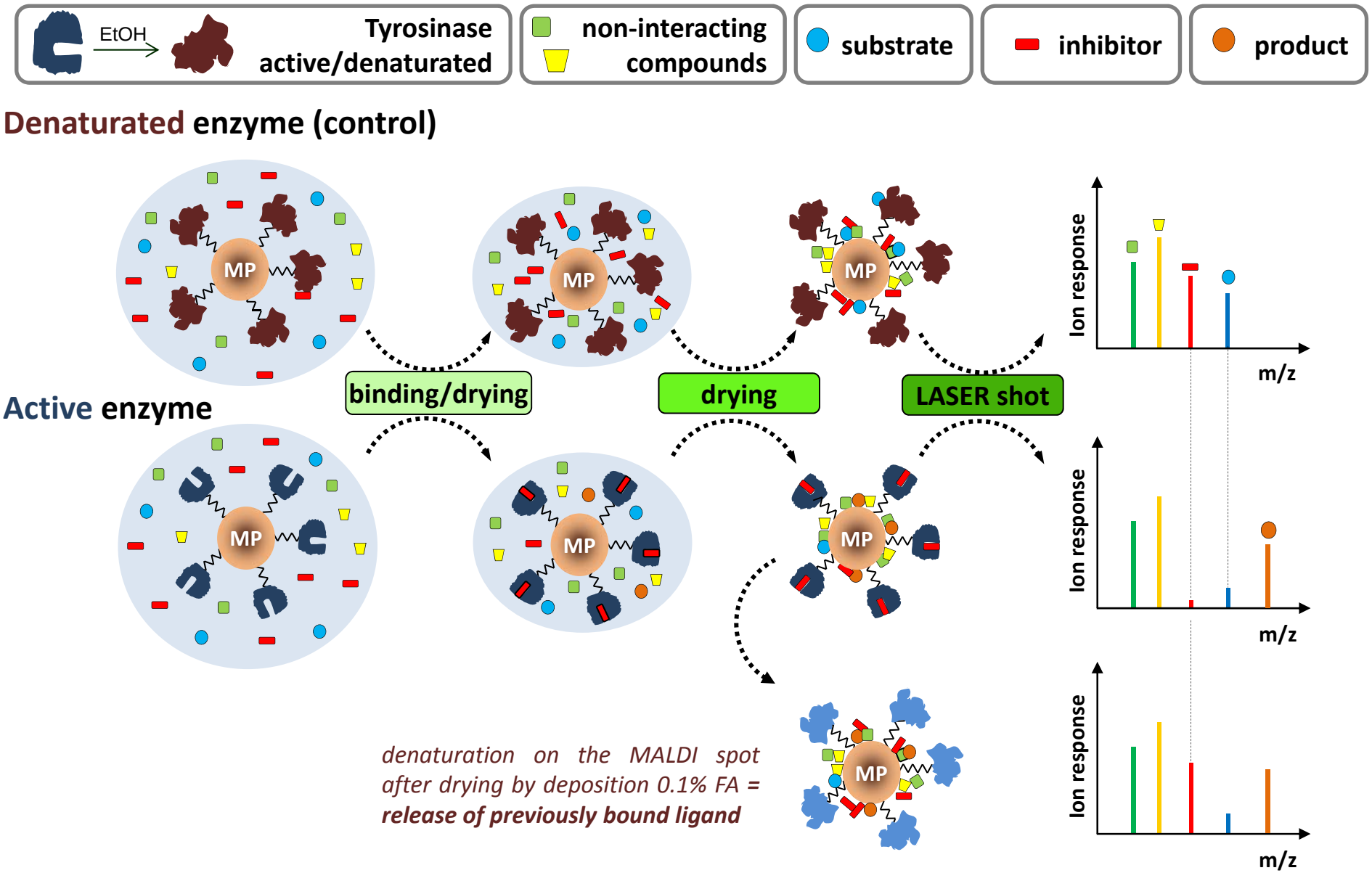
2nd step: PE separation in LC/MS mode; searching for the peak of prospective inhibitor on the chromatogram and its purification

3rd step: characterisation of the inhibitor: determination its structure, enzymatic test: K_I, type of inhibition…



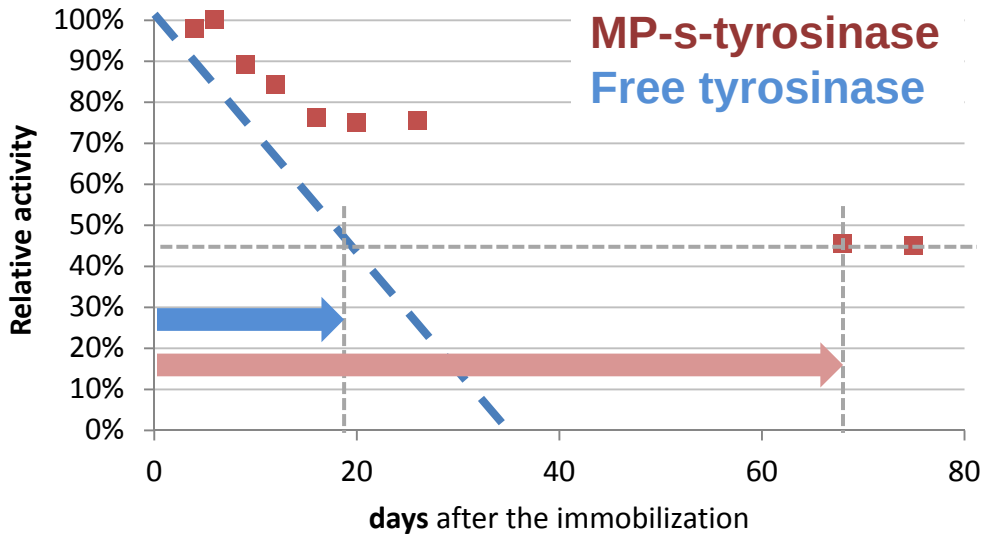


Enzyme coupled nanoparticle-assisted LDI (**ENALDI**) principle



MPs characterisation

- **Stability:**



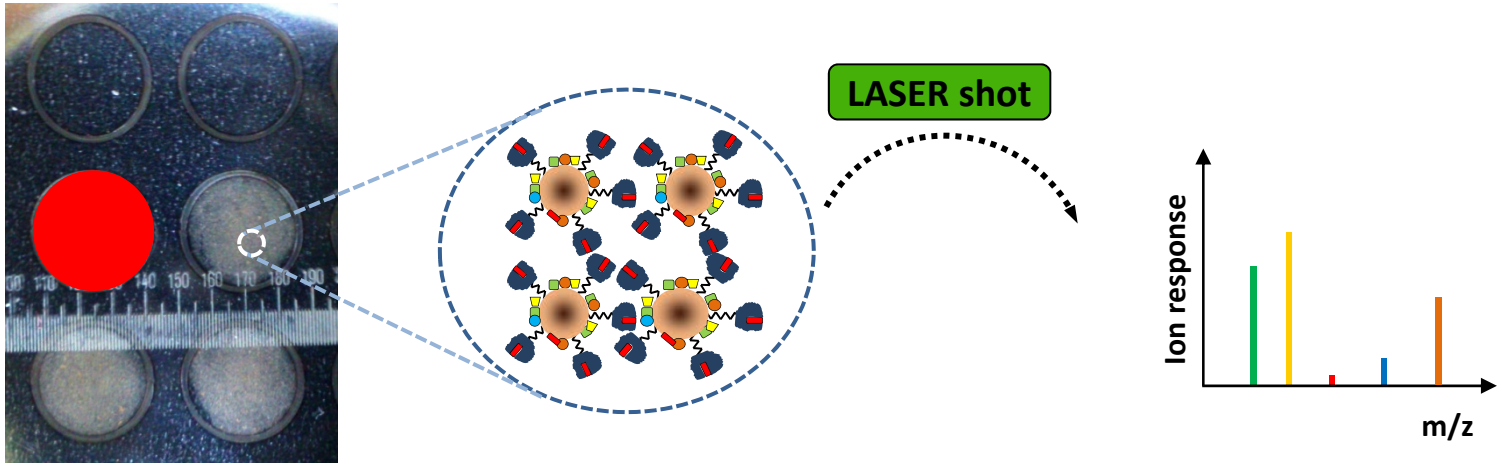
Free tyrosinase looses **55% of the initial activity** within approx. 17-20 days [1,2] when stored at 4°C. MPs-immobilized tyrosinase looses 55% of initial activity up to 70-75 days.

- **Protein binding capacity:**

245 mg of BSA/g of MPs

[1] A.M. Girelli, E. Mattei, A. Messina, D. Papaleo, *Sensors and Actuators B,* **2007**,*125*, 48–54
[2] M. Y. Arıca, G. Bayramoglu, N. Bıçak, *Process Biochemistry,* **2004***, 39*, 2007–2017

Enzyme coupled nanoparticle-assisted LDI (**ENALDI**) **highligts**



On a single spot:

- Total amount of MPs: **3.47 µg**;
- amount of tyrosinase enzyme bound to MPs: approx. **800 ng (6.5 pmol)**;
- amount of plant extract (equivalent of dry mass of plant powder): **300-450 ng**;

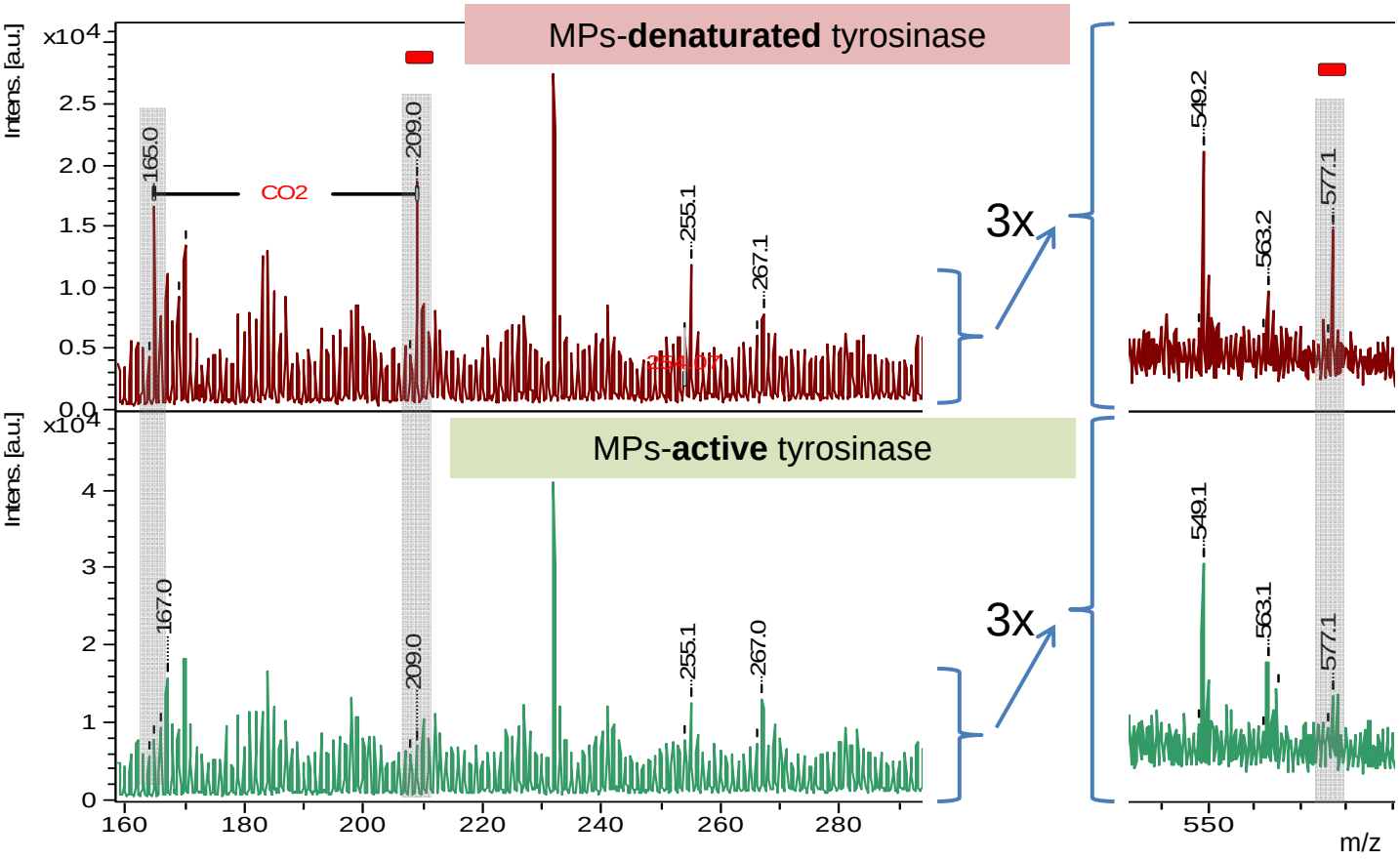
Dual function of magnetic nanoparticles:

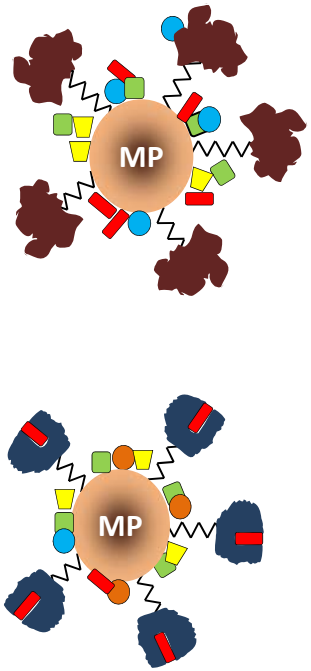
- enzyme carrier;
- energy absorber: nanoparticle-assisted ionization is believed to be generated by heat released upon the laser shot

Experimental:

- Bruker Autoflex with MTP 384 standard target plate (polished steel)
- number of LASER shots: 300
- MS spectra collected in a negative mode

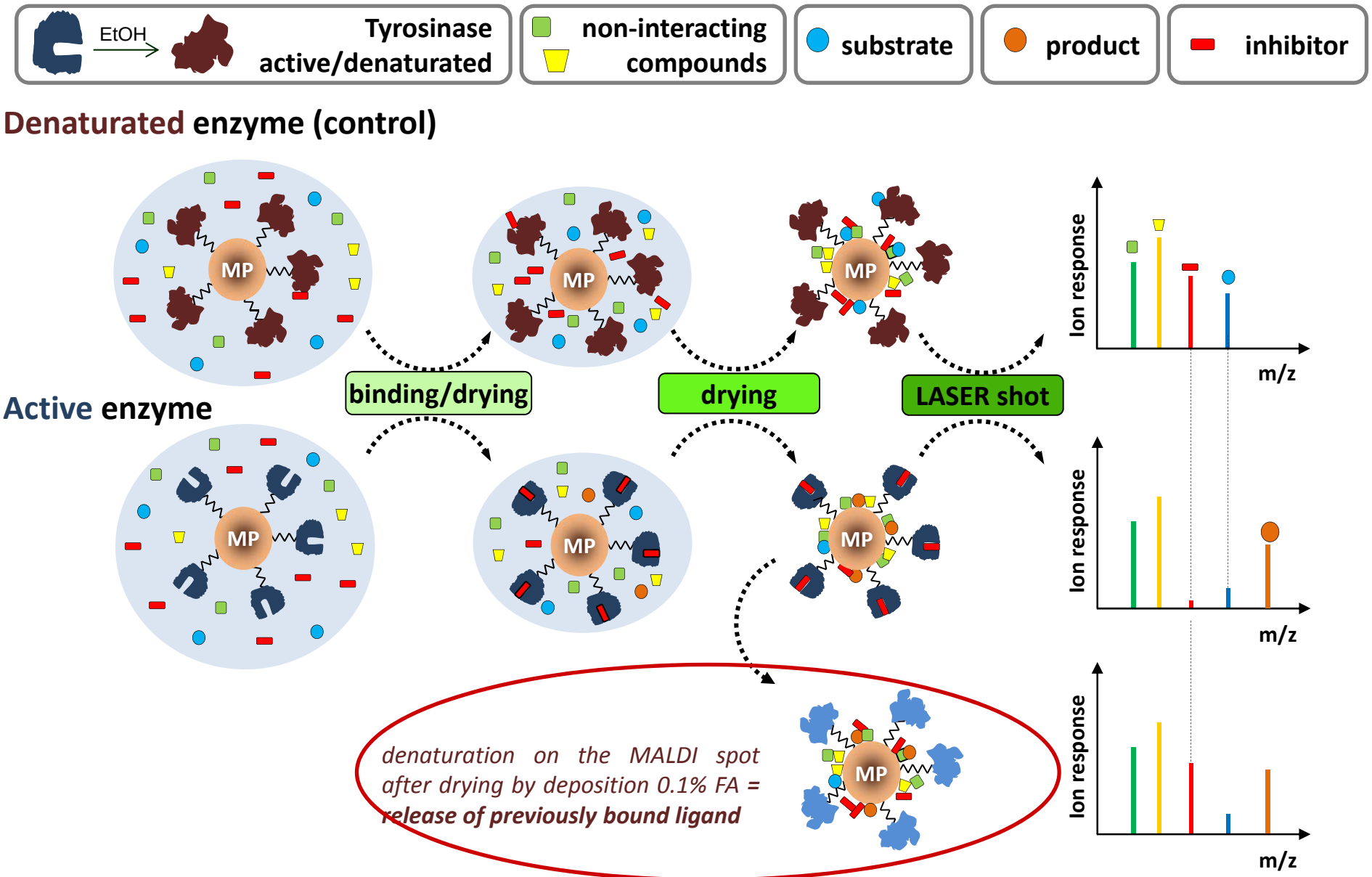
Exemplary ENALDI spectra of raw **aqueous** extract of licorice (*Glycyrrhiza glabra*) root



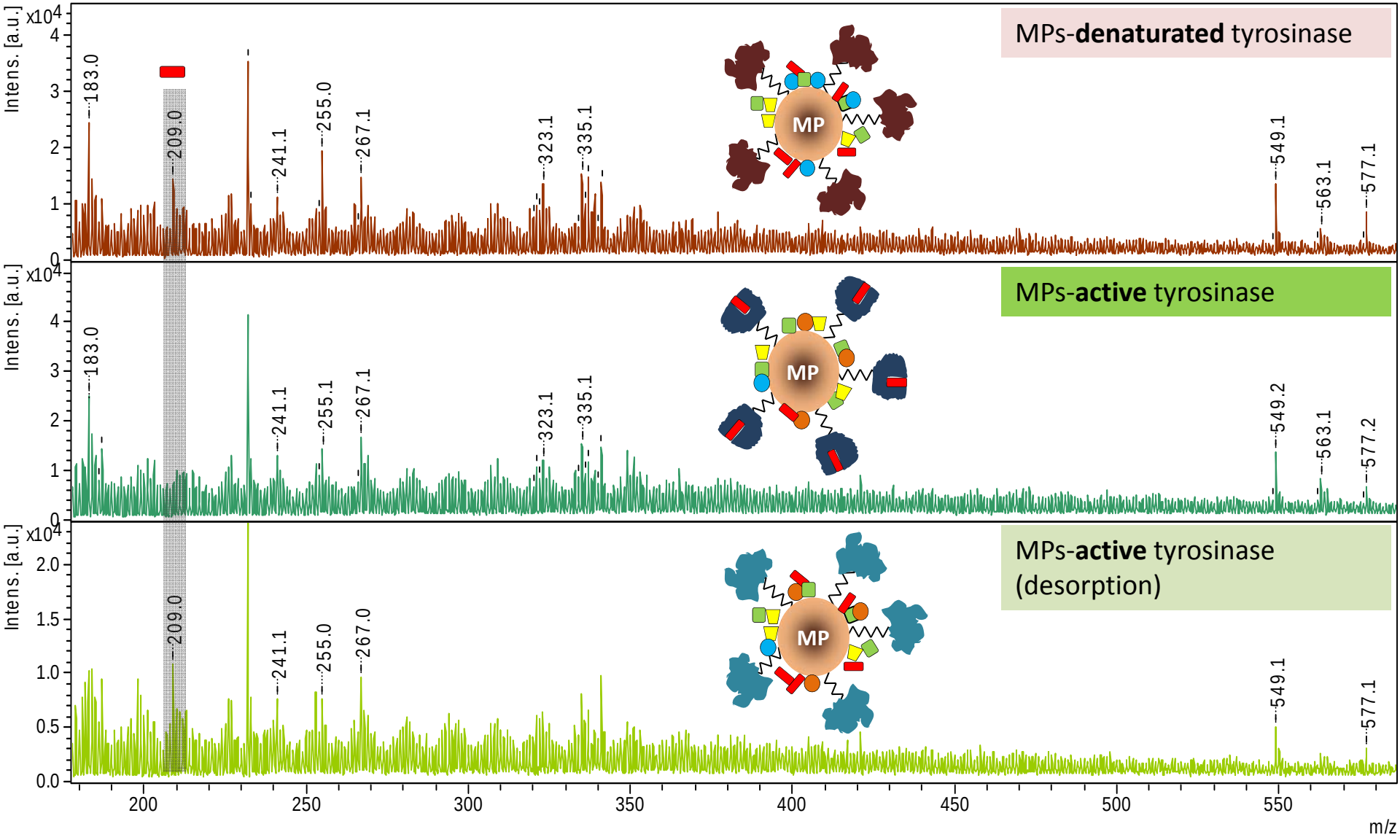


- significant drop of the ion response for 209.0 and 577.1 m/z ions

Enzyme coupled nanoparticle-assisted LDI (**ENALDI**) principle



Exemplary ENALDI spectra of raw **aqueous** extract of licorice (*Glycyrrhiza glabra*) root

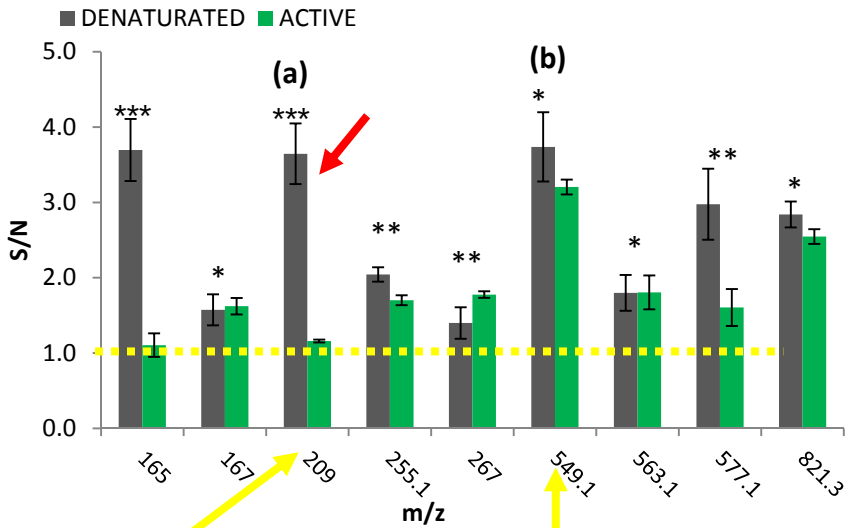


• re-appearance of the ion $[M-H]^-$: 209.0 m/z after treatment of MPs with 0.1% solution of formic acid **directly on the spot**

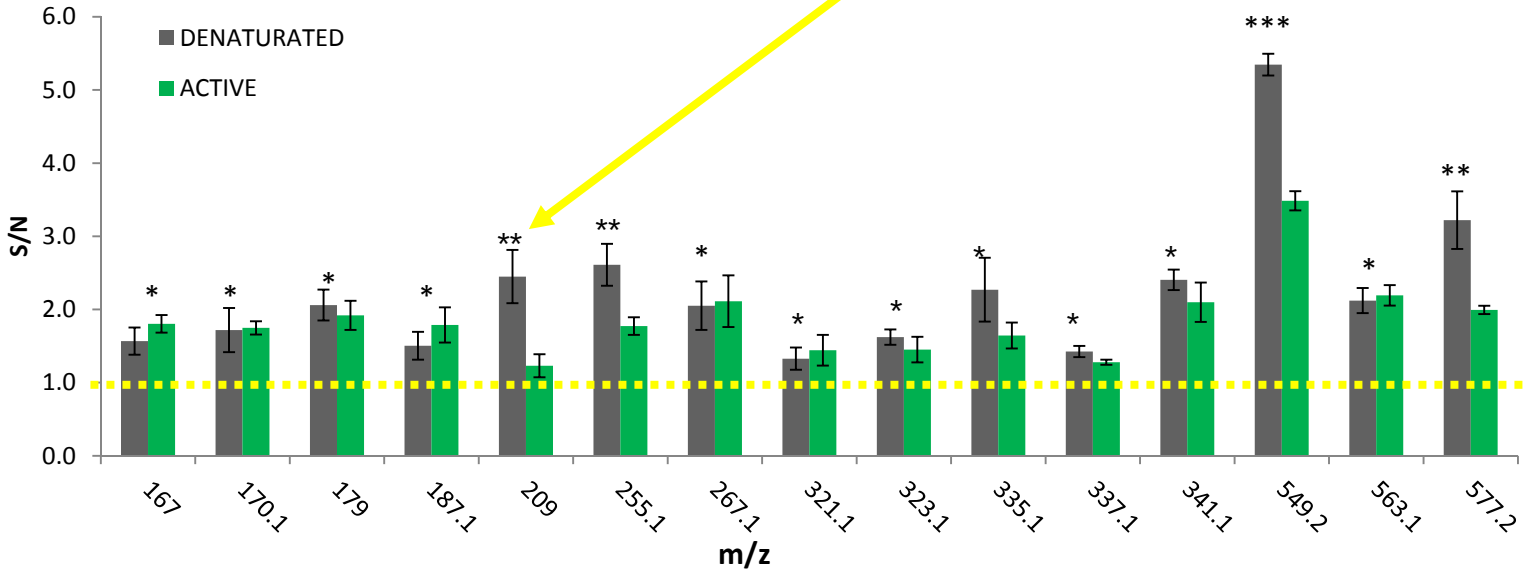
Statistical description of the data

S/N is calculated as the ratio of the intensity of given peak and the adjacent baseline

Student's t-test
*** - p >= 5%** (S/N not significantly different)
**** - p < 5% and p >= 1%** (S/N significantly different)
***** - p < 1%** (S/N highly significantly different)

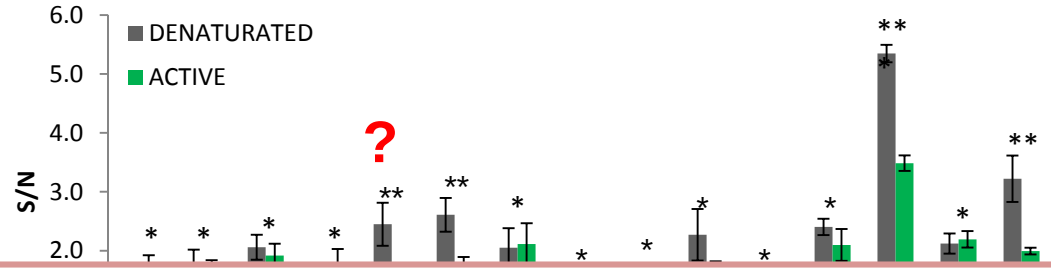


Raw aqueous extract of licorice



Raw methanol extract of licorice

Searching for the inhibitor

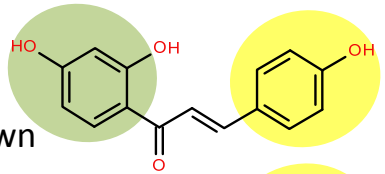


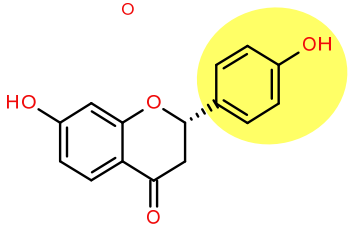
M.A. Farag et al. / Phytochemistry 76 (2012) 60–72

Table 2
Compounds assigned in *Glycyrrhiza* species methanol extract by LC–MS.

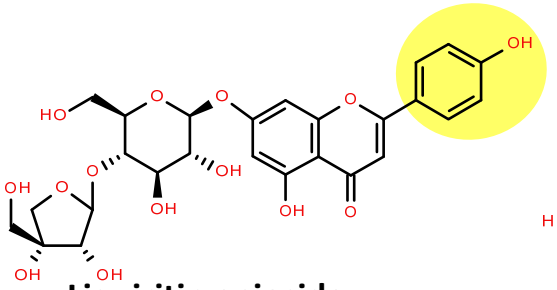
No.	rt (min)	UV max	Identification	Aglycone class	$[M-H]^-$ (m/z)	Error(ppm)	El. Comp.	MS^n ions
1	5.58	275	Unknown		209.0453	+1.14	$C_{10}H_9O_5$	165, 121
2	9.22	270, 315	Glucoliquiritin	Flavanone	579.1698	−1.5	$C_{27}H_{31}O_{14}$	417, 255

Isoliquiritigenin, known tyrosinase inhibitor[1]

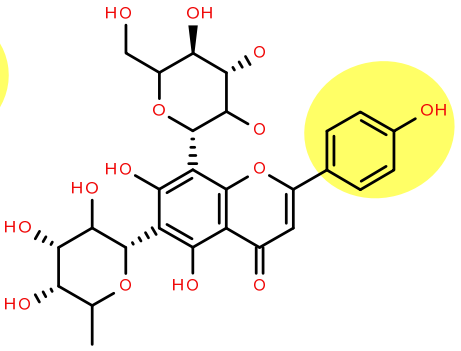




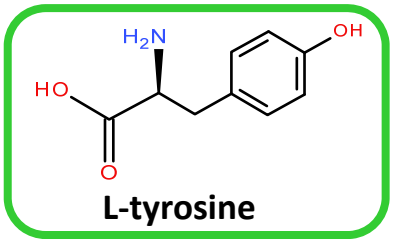
Liquiritigenin



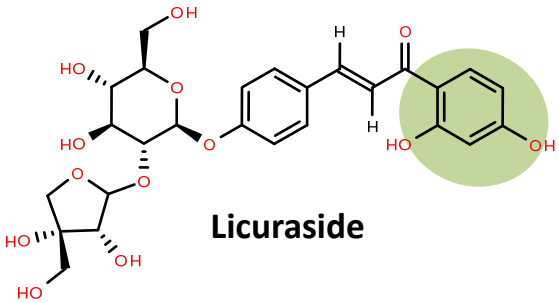
Liquiritin apioside



Isoviolanthin



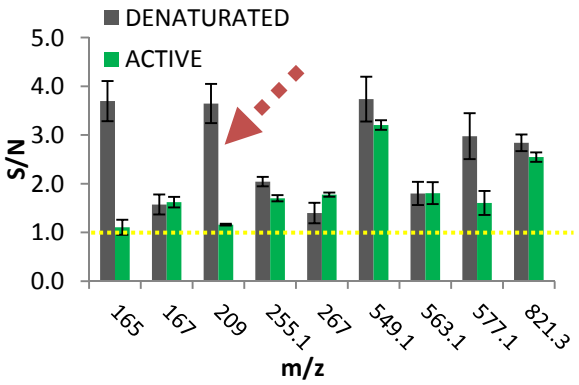
L-tyrosine



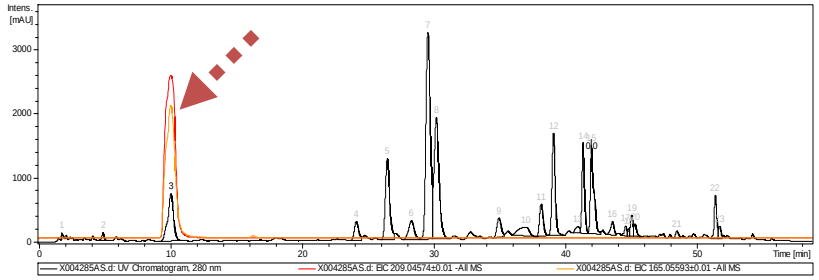
Licuraside

[1] J Agric Food Chem. **2003**, 51, 1201-1207

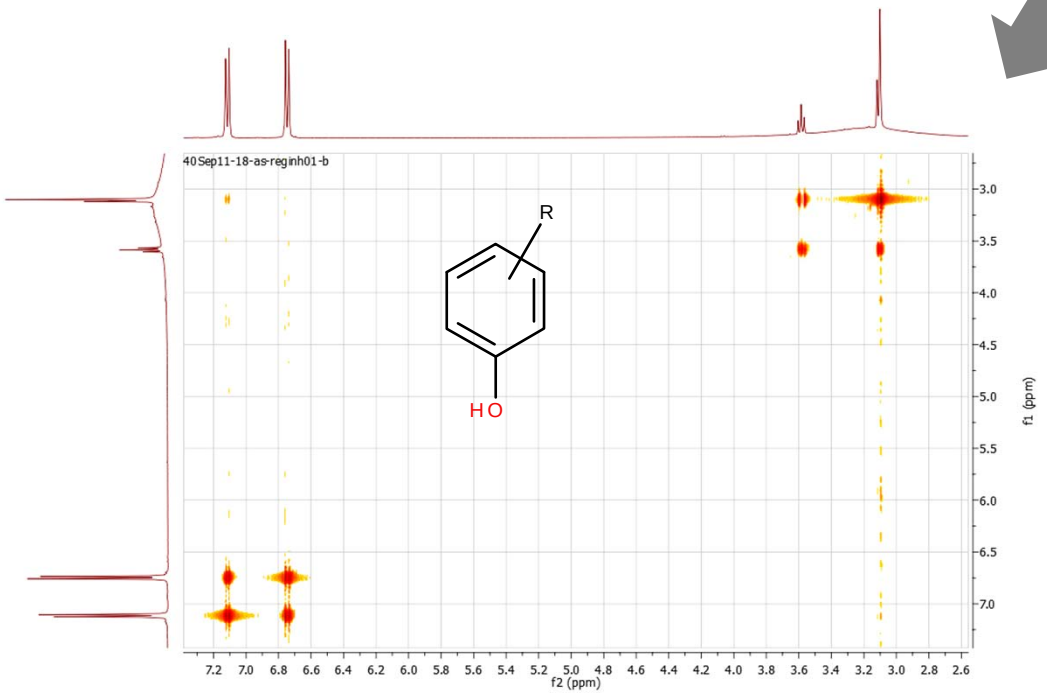
Structural identification of the inhibitor



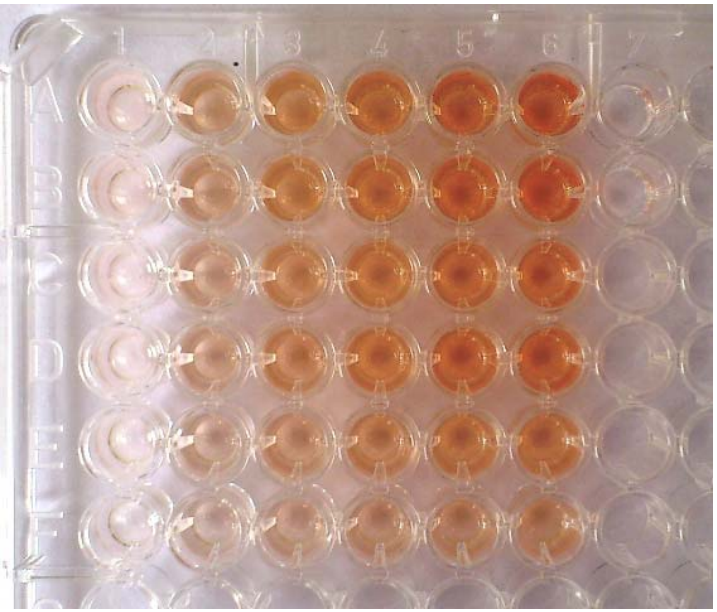
PE separation in LC/MS mode; localizing the inhibitor candidate; purification

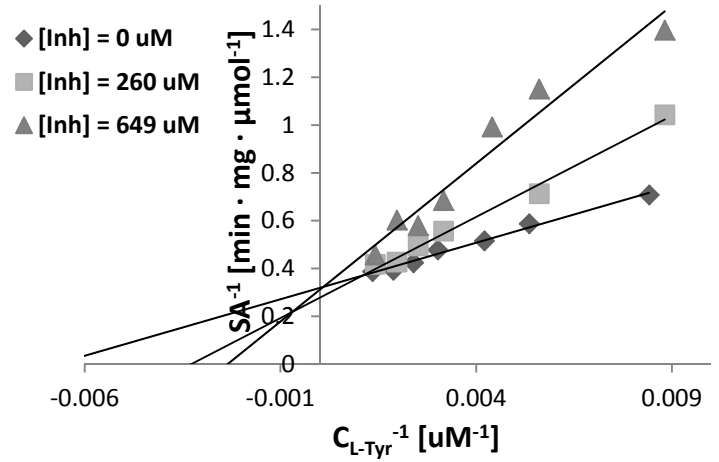


Structure identification by NMR study (^{1}H, ^{13}C, HSQC, COSY)



Kinetic test of purified compound





K_I = 291 µM

Conclusions

Advantages of ENALDI approach:

- simplified study of complex extracts (no pre-treatment, purification is limited to prospective inhibitors, no laborious fractionation required);
- high stability of MPs (45% of initial activity after 80 days of storage in 4°C);
- high homogeneity of the sample: no ‘sweet spots’ on the MALDI plate;
- possibility to distinguish substrate and inhibitor;
- magnetic properties of beads: easy purification, no centrifuge required;
- low protein consumption per analysis (approx. 6.5 pmol)

Further development

- improvement of S/N ratio in low mass range