SUPPORTING INFORMATION

Multi-parametric microarray for protein profiling: Simultaneous detection of 8 different cytochromes via differentially element tagged antibodies and LA-ICP-MS

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Table S-1: **Treatment of rats with xenobiotics and preparation of liver microsomes.** Male or female Sprague Dawley rats weighing about 200 g were exposed to several CYP inducing substances. Amounts, duration of treatment and application routes are indicated. *Amounts are stated as percentage in drinking water or diet (ad libitum) or as mg/kg body weight per day for the intra-peritoneal (i.p.) application of lipophilic compounds solubilized in corn oil. **For the mixture NF/Iso NF was applied on day 1 to 3 and Iso on day 4. The next day after exposure, liver microsomes were prepared by differential centrifugation from the inducer-treated rats and also of untreated male (UT m) and female (UT f) rats as described by Guengerich.[1] After the last ultracentrifugation for 1 h at 105,000 x g, the microsomal pellet was re-suspended in about 500 µl 10 mM Tris-HCl, pH 7.4, 20 % glycerol, 0.1 mM EDTA. All microsomal preparations were immediately frozen in liquid nitrogen and subsequently stored at -70°. The experiments were performed in compliance with the German Animal Protection Law (permissions 23.8720 no. 24.7 and 23.8720 no. 20.35, Bezirksregierung Arnsberg). CYP enzymes which are preferentially induced by the xenobiotics are indicated.

Xenobiotics	Code	Amount *	Days	Application	Induced CYP	Ref.
Phenobarbital	PB	0.1%	10	in drinking water	2B1	[2]
Dexamethasone	DEX	30 mg	4	i.p. in corn oil	3A	[2]
3-methylcholantrene	3MC	20 mg	3	i.p. in corn oil	1A1, 1A2	[2]
Clotrimazole	CLO	320 mg	4	i.p. in corn oil	3A	[3]
β-naphthoflavone	NF	40 mg	4	i.p. in corn oil	1A1, 1A2	[2]
β-naphthoflavone/	NF/Iso	60mg	3	i.p. in corn oil	1A1, 1A2	-
Isosafrole **		150 mg	1			[3]
Triacetyloleandomycin*	TAO	1.5%	10	in diet	3A	[4]
Isoniazide*	INH	0.1%	10	In drinking water	2E1	[2]

Table S-2: **Antibody sources.** Anti-CYP1A1 and anti-cytochrome b_5 (Cyt b_5) were produced in the lab of P.H. Roos at Ruhr-University Bochum, Bochum, Germany. Antibodies were modified with metal coded affinity tags (MeCAT, Proteome Factory, Berlin, Germany) as described by Waentig et al.[5] If the antibody stock contains additives like BSA or gelatine, a purification step was applied before tagging by using Pierce® Antibody Clean-up Kit (Thermo Fisher Scientific, Bonn, Germany). Each single antibody was tested in a LA-ICP-MS based Westernblot immunoassay. (Figure S-1 – S-3).

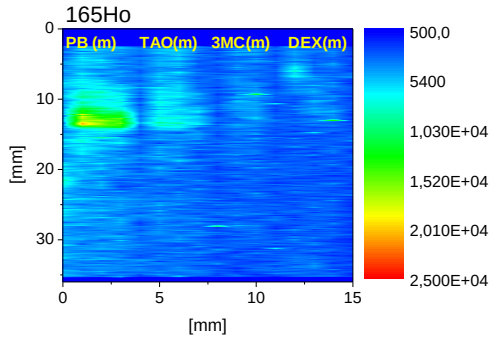
Anti-	Type	Manufacturer	MeCAT
CYP2E1	Polyclonal IgG	Abcam, Cambridge, UK	Er
CYP4A1	Monoclonal IgG_{2b}	Santa Cruz Biotechnology, Heidelberg, Germany	Eu
CYP3A	Polyclonal IgG	Santa Cruz Biotechnology, Heidelberg, Germany	Ho
CYP2C6	Monoclonal IgG1	Abcam, Cambridge, UK	Lu
CYP1A1	Monoclonal IgG1	Produced by Roos et al.	Nd
CYP1A2	Monoclonal IgG1	Abcam, Cambridge, UK	Pr
Cyt b_5	Monoclonal IgG1	Produced by Roos et al.	Tb
CYP2B1/2B2	Monoclonal IgG1	Abcam, Cambridge, UK	Tm

Table S-3: **Typical optimized operating conditions for LA-ICP-MS.** A commercial LA system (New Wave 213, ESI, Portland, USA) was coupled to an ICP sector field mass spectrometer (Element XR, Thermo Fisher Scientific, Germany). The ICP was tuned daily for maximum ion intensity, keeping the oxide ratio (ThO/Th) below 5 % using a microscopic glass slide as the control standard for ablation. The isotopes ^{127}I, ^{141}Pr, ^{153}Eu, ^{142}Nd, ^{159}Tb, ^{165}Ho, ^{166}Er, ^{169}Tm and ^{175}Lu have been selected for analysis.

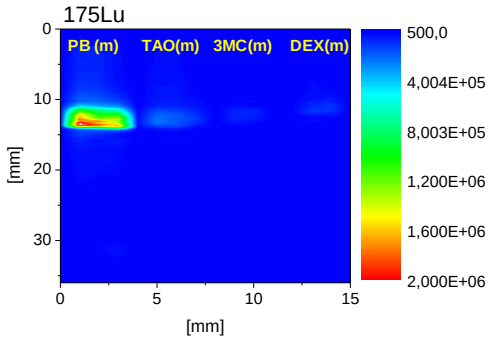
ICP-MS	
RF plasma source	1350 W
Plasma gas	15 L min^{-1}Ar
Auxiliary gas	1.0 L min^{-1}Ar
Transport gas	0.6 L min^{-1}Ar
Mass resolution (m/Δm)	300
Scanning mode	E scan
Dwell time	2 ms
LA system for microarray analysis	
Wavelength	213 nm
Helium gas flow	1 L min^{-1}
Laser energy	35 %
Laser spot size	200 µm
Scan speed	200 µm s^{-1}
Repetition frequency	20 Hz
Ablation mode	Scanning line by line next to each other
Analysis time	Approx. 2.5 h for one slide of 20 x 18 mm (including wash out delay and Laser warm-up)
LA system for Westernblot analysis	
Wavelength	213 nm
Helium gas flow	1 L min^{-1}
Laser energy	35 %
Laser spot size	250 µm
Scan speed	250 µm s^{-1}
Repetition frequency	20 Hz
Ablation mode	Scanning line by line with 750 µm space in between the lines
Analysis time	Approx. 35 min for four sample lanes (35 x 15 mm)

Figure S-1: Intensity profiles of a LA-ICP-MS based Multiplex-Westernblot immunoassay for quality control of MeCAT tagged antibodies of Table S-2. Selected liver microsomal protein (5µg each) of inducer treated rats was applied to SDS-PAGE and subsequently transferred to the nitrocellulose-membrane. A detailed description of the Westernblot procedure is given in reference [5]. The immunoassay procedure was done according to the microarray immune reaction summarized in table 1 of the main article. The blot membrane was exposed for 2 hours to the tagged antibody diluted in TBS-T at a concentration of 0.2 µg mL^{-1}. The intensity profiles of the Westernblots can't be directly compared to the expression profiles produced by the microarray (Figure 1 in the main article) because of the absence of internal standards, loading controls and data normalization.

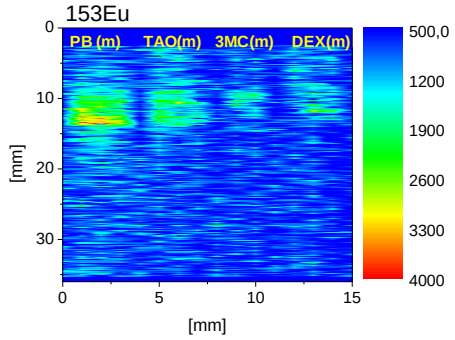
Anti-CYP3A-MECAT(Ho)



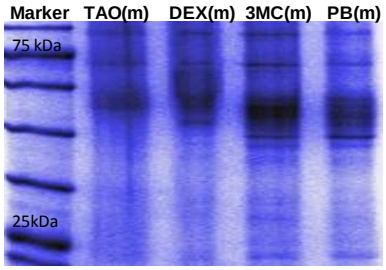
Anti-CYP2C6-MECAT(Lu)



Anti-CYP4A1-MeCAT(Eu)

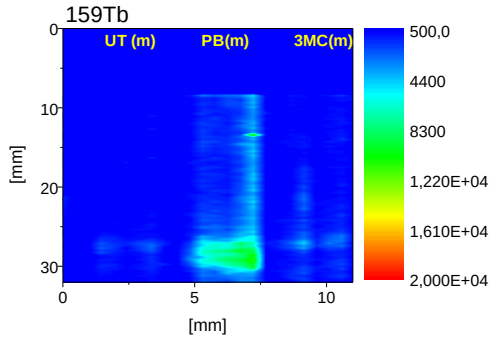


CBB stained control gel

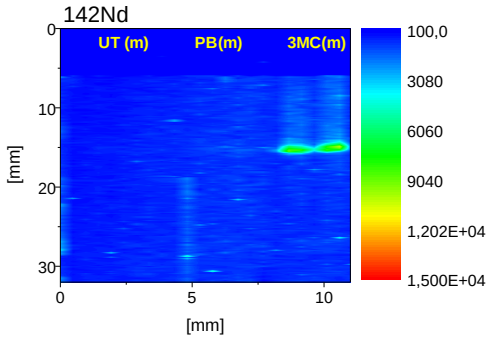


FigureS- 2: Intensity profiles of a LA-ICP-MS based Multiplex-Westernblot immunoassay for quality control of MeCAT tagged antibodies of Table S-2. Sample preparation was analog to figure S-1. Remark: Cytochrome b5 is normally not affected by the selected xenobiotics. The reason for the high intensity belonging to the protein band in lane 2 is the application of a higher amount of protein (> 5 µg) to the gel. This is also visible in the picture of the control gel where the protein bands belonging to PB(m) are stronger stained than the ones of UT(m) and 3MC(m).

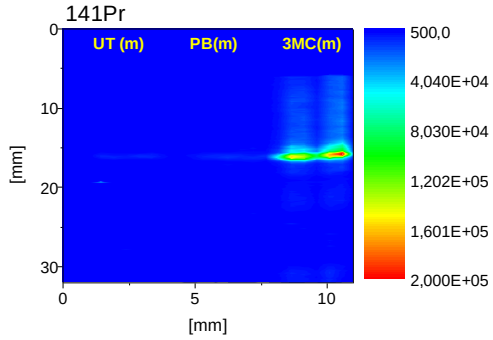
Anti-Cytochrome b5-MeCAT(Tb)



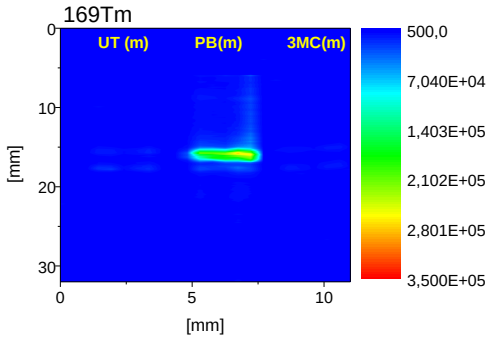
Anti-CYP1A1-MECAT(Nd)



Anti-CYP1A2-MeCAT(Pr)



Anti-CYP2B1/2B2-MeCAT(Tm)



CBB stained control gel

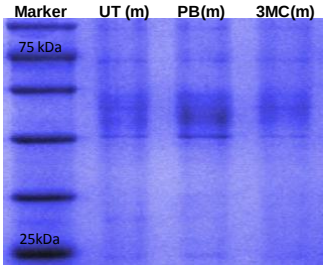
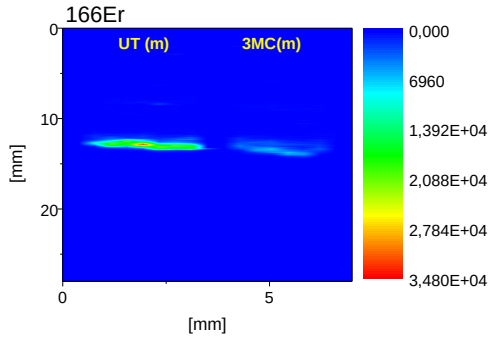


Figure S-3: Intensity profiles of a LA-ICP-MS based Westernblot immunoassay for quality control of MeCAT tagged anti-CYP2E1 antibody of Table S-2. Sample preparation was analog to Figure S-1.

Anti-CYP2E1-MeCAT(Er)



CBB stained control gel

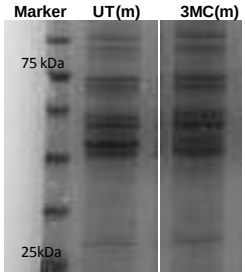


Figure S-4: **2D Intensity profiles of a multi-parametric microarray detected via LA-ICP-MS.** The antibody pool is summarized in Table S-2. The intensity profile of the iodinated BSA (internal standard) also shows the sample orientation. Each grid includes 4 replicates of each sample. Grid 1: DEX w; Grid 2: DEX m; Grid 3: UT w; Grid 4: INH m; Grid 5: PB w; Grid 6: TAO w; Grid 7: BSA-I (blank sample for taking unspecific signaling background into account); Grid 8: PB m; Grid 9: NF/Iso m; Grid 10: TAO m; Grid 11: NF w; Grid 12: UT m; Grid 13: CLO f; Grid 14: 3MC m. The seven Grids are summarized to one supergrid. In total four supergrids were spotted on a NC slide.

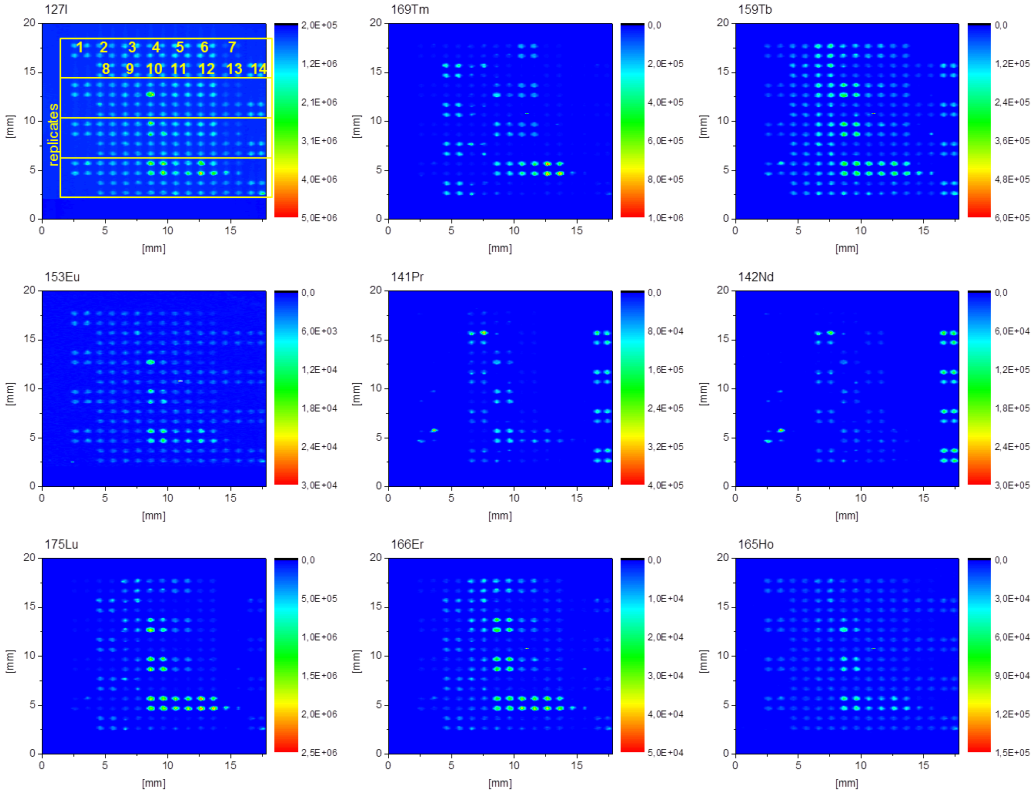


***Table S-2*: Data set used for calculation and discussion of figure 2 (main text).** Peak areas of single microarrays and multi-parametric microarrays calculated from LA-ICP-MS intensity profiles. Not analyzable samples were marked with a red arrow and were not used for data comparison.

Single microarrays

	UT m	PB m	DEX m	3MC m	NF/Iso m	TAO m	INH m	UT f	PB f	DEX f	CLO f	NF f	TAO f
CYP2E1	0,02141	0,07505	0,01688	0,02045	0,03977	0,01549	0,04596	0,05989	0,05055	0,00883	0,00532	0,00976	0,01425
CYP4A1	0,00165	0,00186	0,00187	0,00103	0,00256	0,00125	0,00127	0,00134	0,00111	0,00293	0,00137	0,00179	0,00057
CYP3A	0,00187	0,00200	0,00163	0,00289	0,00341	0,00150	0,00178	0,00524	0,00174	0,00219	0,00205	0,00208	0,00098
CYP2C6	0,05604	0,22446	0,03735	0,06032	0,09364	0,04378	0,10839	0,12437	0,15956	0,01836	0,00912	0,02610	0,05179
CYP1A1	0,00248	0,00258	0,00354	0,36377	0,18947	↗	↗	↗	↗	↗	0,00257	0,05175	↗
CYP1A2	0,00140	0,00142	0,00069	0,10358	0,11465	↗	↗	0,00840	↗	↗	0,00041	0,00663	↗
Cytb5	0,02973	0,07273	0,04402	0,07500	0,10345	0,02956	0,04797	0,11484	0,05526	0,02251	0,02182	0,02068	0,03472
CYP2B1	0,07228	0,83238	0,07417	0,03874	0,16157	0,05737	0,09622	0,06628	0,38352	0,03872	0,02435	0,02141	0,04366

Multi-parametric microarray

	UT m	PB m	DEX m	3MC m	NF/Iso m	TAO m	INH m	UT f	PB f	DEX f	CLO f	NF f	TAO f
CYP2E1	0,00862	0,02083	0,00732	0,00732	0,01378	0,00749	0,05708	0,01920	0,01935	0,00363	0,00178	0,00312	0,00659
CYP4A1	0,00157	0,00155	0,00152	0,00102	0,00164	0,00121	0,00121	0,00116	0,00099	0,00215	0,00163	0,00163	0,00038
CYP3A	0,00211	0,00166	0,00188	0,00208	0,00243	0,00168	0,00245	0,00292	0,00172	0,00282	0,00225	0,00223	0,00093
CYP2C6	0,05444	0,21984	0,04422	0,06251	0,10390	0,05762	0,23756	0,15844	0,19149	0,02094	0,00694	0,02650	0,05688
CYP1A1	0,00202	0,00126	0,00254	0,32190	0,11201	↗	↗	↗	↗	↗	0,00163	0,04826	↗
CYP1A2	0,00158	0,00127	0,00063	0,06755	0,04032	↗	↗	0,00987	↗	↗	0,00065	0,00744	↗
Cytb5	0,02765	0,05059	0,04166	0,06209	0,06665	0,03068	0,07172	0,08376	0,04872	0,02246	0,01871	0,01802	0,03452
CYP2B1	0,07476	0,70647	0,08547	0,04328	0,21638	0,07277	0,19895	0,07490	0,45875	0,04700	0,02387	0,02148	0,04352

RSD [%]

	UT m	PB m	DEX m	3MC m	NF/Iso m	TAO m	INH m	UT f	PB f	DEX f	CLO f	NF f	TAO f
CYP2E1	42,57	56,55	39,49	47,25	48,53	34,85	10,79	51,45	44,63	41,72	49,88	51,52	36,77
CYP4A1	2,66	9,03	10,31	0,47	22,05	1,64	2,39	7,37	5,61	15,45	8,80	4,66	19,35
CYP3A	5,96	9,32	7,13	16,44	16,74	5,65	15,76	28,46	0,73	12,57	4,69	3,53	2,66
CYP2C6	1,44	1,04	8,42	1,78	5,19	13,66	37,34	12,05	9,10	6,57	13,56	0,77	4,68
CYP1A1	10,20	34,26	16,51	6,11	25,69						22,44	3,49	
CYP1A2	6,07	5,22	4,41	21,06	47,96			8,03			23,45	5,78	
Cytb5	3,62	17,95	2,75	9,41	21,64	1,85	19,85	15,65	6,29	0,12	7,67	6,87	0,29
CYP2B1	1,69	8,18	7,08	5,53	14,50	11,84	34,81	6,10	8,93	9,66	1,00	0,17	0,16

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