

Development of “standard” urine sample for comparability and standardization in clinical proteomics

Harald Mischak, Walter Kolch, Michalis Aivaliotis, David Bouyssié, Magali Court, Hassan Dihazi, Gry H. Dihazi, Julia Franke, Jérôme Garin, Anne Gonzalez de Peredo, Alex Iphöfer, Lothar Jänsch, Chrystelle Lacroix, Manousos Makridakis, Christophe Masselon, Jochen Metzger, Bernard Monsarrat, Michal Mrug, Jan Novak, Andreas Pich, Andrew Pitt, Joost P. Schanstra, Erik Bongcam-Rudloff, Justyna Siwy, Hitoshi Suzuki, Visith Thongboonkerd, Li-Shun Wang, Jérôme Zoidakis, Petra Züribig, and Antonia Vlahou



Development of “standard” urine sample

Background/Problem statement

Lack of inter-lab and inter-platform comparability in urine proteomics

Lack of appropriate quality controls (platform validation relies on purified proteins)

In depth characterization of urine proteome requires concerted “multi-platform” analysis

Objectives of the initiative

Generate a “real life” reference sample to be freely available to urine proteomics investigators

Characterize in depth this sample by different platforms and methods

Make data freely available: Set the foundation for comprehensive compilation of urine proteome

Outline

Collection protocol for “standard” urine sample

Characterization of proteome and peptidome by multiple techniques

Evaluation of inter-lab and inter-platform comparability

Future plans

Collection protocol: Midstream morning urine
7 male 8 female volunteers

Clinical characterization

Analyte or Parameter	male	female
Age	36 ± 6	28 ± 6
BMI	22.5 ± 1.1	22.4 ± 2.8
GFR (CG)	108 ± 6	104 ± 9
Diastolic BP	77 ± 4	71 ± 6
Systolic BP	126 ± 4	112 ± 7
Creatinine	879000,00	787000,00
total protein	14600,00	18400,00
Albumin	3500,00	4600,00
IgA	557,70	258,20
HAA-IgA	0,22	0,19
CD14	10,15	16,37
Ngal	2,20	8,89

Freeze collected samples
Thaw on ice, sonicate, combine per gender (>2,500mls)
Aliquot in 1, 10 and 50mls

Distribution of samples to multiple laboratories



Analysis by multiple proteomics technologies

CE-MS
LC-MS/MS (Orbitrap MS) → Analysis of peptidome

2DE-MALDI TOF MS
1DE-LC MS/MS (LTQ-FT Ultra; GeLCMS) → Analysis of proteome

In depth characterization of proteome and peptidome

Compile lists of identified proteins and peptides

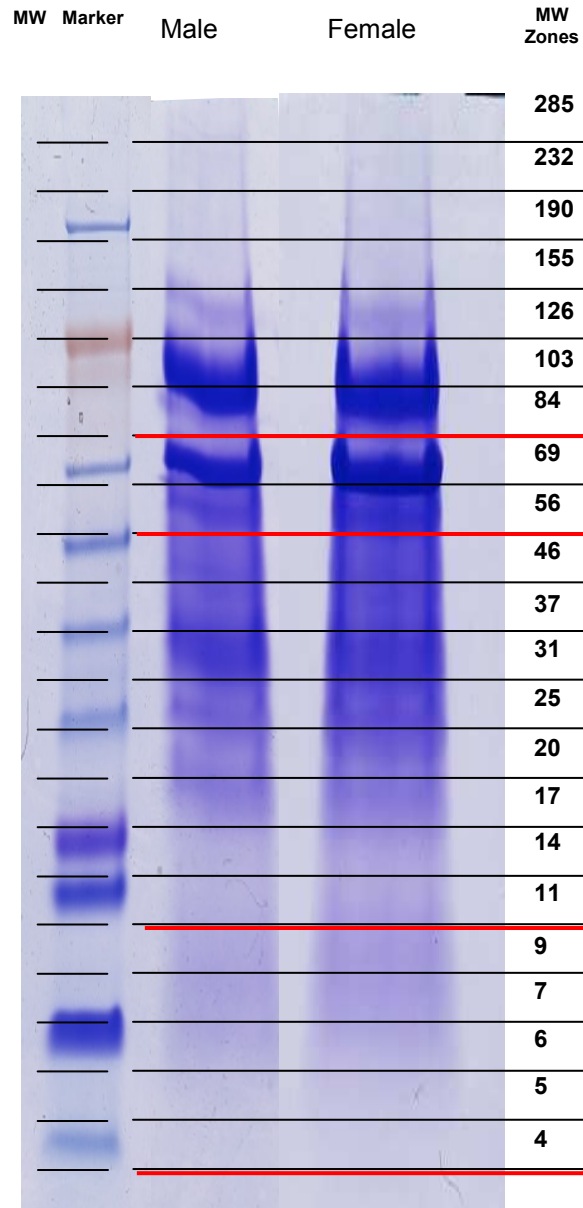
Collect data on protein PTMs and relative quantities

Absolute quantification of peptides

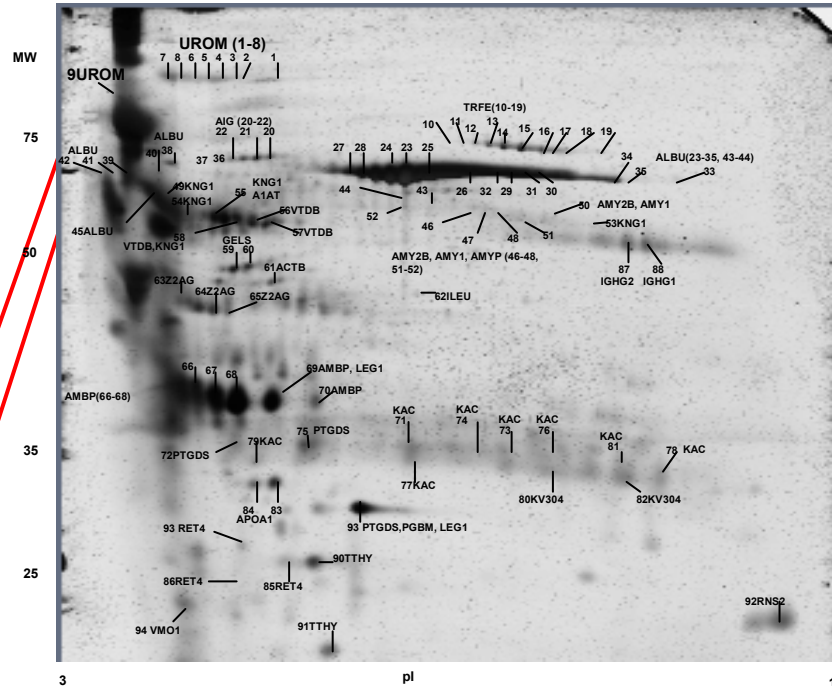
GeLC-MS

2-DE-MS

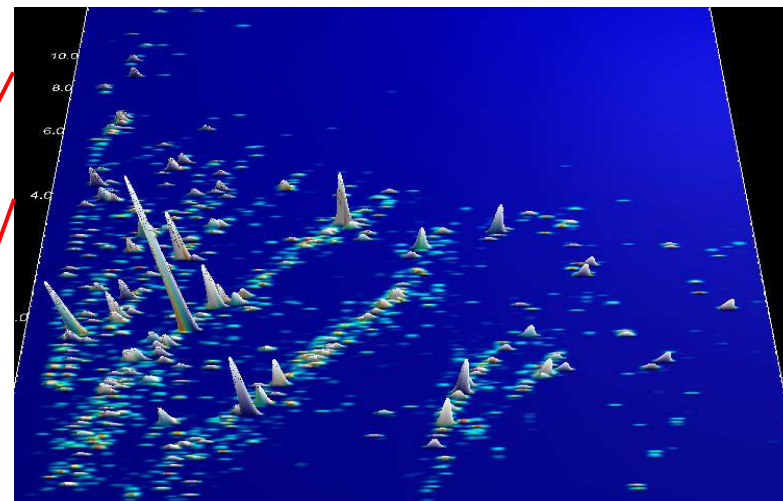
a



b



c

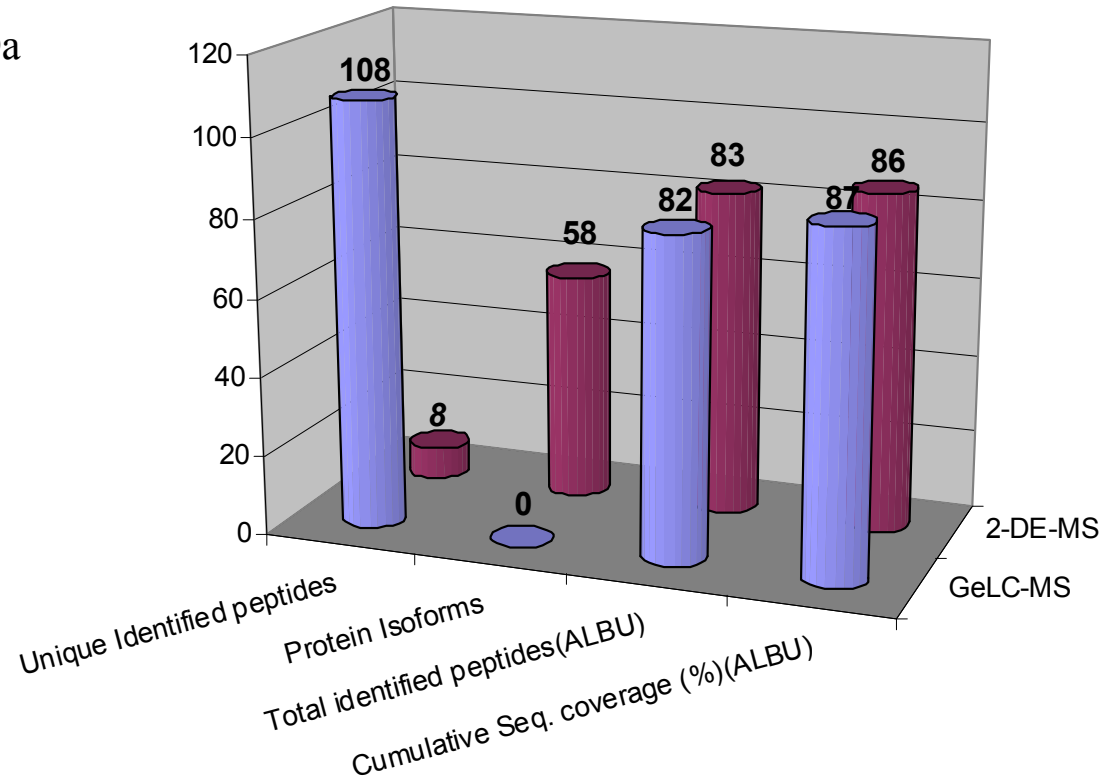


CE-MS

a

Analysis of Proteome: Platform Complementarity

Mass Zone 56-69 kDa



I

ALBU: GeLC-MS

ALBU: 2-DE-MS

II

1 MKWVTFISLL FLFSSAYSRG VFRRDAHKSE VAHRFKDLGE ENFKALVLIA
 51 FAQYLQQCPF EDHVKLVNEV TEFAKTCVAD ESAENC DKSL HTLFGDKLCT
 101 VATLRETYGE MADCCAKQEP ERNECFLOHK DDNP NLPRLV RPEVDVMCTA
 151 FHDNEETFLK KYLYEIARRH PYFYAPELLF FAKRYKAAFT ECCQAADKAA
 201 CLLPKLDEL R DEGKASSAKQ RLKCASLQKF GERAFKAWAV ARLSQRFPKA
 251 EFAEVS KLVT DLTKVHTECC HGDLL ECADD RADLAKYICE NQDSISSK LK
 301 ECCEKPLLEK SHCIAEVEND EMPADLPSLA ADFVESKDVC KNYAEAKDVF
 351 LGMFLY EYAR RHPDYSVLL LRLAKTYETT LEKCCAAADP HECYAKVFDE
 401 FKPLVEEPQN LIKQNC ELFQNA LLVRYTKKVP QVSTPTLVEV
 451 SRNLGKVGSK CCKHPEAKRM PCAEDYLSV LNQLCVLHEK TPVSDRVTKC
 501 CTESLVNRRP CFSALEVDET YVPKEFNAET FTFHADICTL SEKERQIKKQ
 551 TALVELVKHK PKATKEQLKA VMDDFAAFVE KCKKADDKET CFAEEGK LVLV
 601 AASQAALGL

1 MKWVTFISLL FLFSSAYSRG VFRRDAHKSE VAHRFKDLGE ENFKALVLIA
 51 FAQYLQQCPF EDHVKLVNEV TEFAKTCVAD ESAENC DKSL HTLFGDKLCT
 101 VATLRETYGE MADCCAKQEP ERNECFLOHK DDNP NLPRLV RPEVDVMCTA
 151 FHDNEETFLK KYLYEIARRH PYFYAPELLF FAKRYKAAFT ECCQAADKAA
 201 CLLPKLDEL R DEGKASSAKQ RLKCASLQKF GERAFKAWAV ARLSQRFPKA
 251 EFAEVS KLVT DLTKVHTECC HGDLL ECADD RADLAKYICE NQDSISSK LK
 301 ECCEKPLLEK SHCIAEVEND EMPADLPSLA ADFVESKDVC KNYAEAKDVF
 351 LGMFLY EYAR RHPDYSVLL LRLAKTYETT LEKCCAAADP HECYAKVFDE
 401 FKPLVEEPQN LIKQNC ELFQNA LLVRYTKKVP QVSTPTLVEV
 451 SRNLGKVGSK CCKHPEAKRM PCAEDYLSV LNQLCVLHEK TPVSDRVTKC
 501 CTESLVNRRP CFSALEVDET YVPKEFNAET FTFHADICTL SEKERQIKKQ
 551 TALVELVKHK PKATKEQLKA VMDDFAAFVE KCKKADDKET CFAEEGK LVLV
 601 AASQAALGL

X: unique identifications

Analysis of peptidome: Absolute quantification of peptides

Analyte or Parameter	male	female
MGPRGPpGPpG	1,82	2,84
ApGDRGEpGpp	23,86	29,19
GDPGPPGpPGpPGpPAI	18,01	22,17
SpGSpGPDGKTGPPGpAG	3,75	6,29
PpGEAGKpGEQGVPGDLG	0,79	1,39
EAIPMSIPPEVKFNKPF	n.d.	n.d.
NGDDGEAGKpGRpGERGPpGP	8,24	21,47
DAGApGApGGKGDAGApGERGPpG	23,01	31,65
AGPpGEAGKpGEQGVpGDLGAPGP	1,59	3,28
AGPpGEAGKpGEQGVpGDLGApGP	5,18	12,45
ADGQpGAKGEpGDAGAKGDAGPpGPA	8,70	16,44
GKNGDDGEAGKpGRpGERGPpGPQ	3,45	11,09
TGPIGPpGPAGApGDKGESGPSGPAGPTG	1,19	3,01
PpGESGREGApGAEGSpGRDGSpGAKGDRGETGP	56,04	193,30
MIEQNTKSPLFMGKVVNPTQK	n.d.	n.d.

Analysis of peptidome: Platform complementarity

CE-MS data						LC-MS data						Sequence
mass [Da]	migration time [min]	Intensity G_male	Intensity G_female	Intensity H_male	Intensity H_female	mass [Da]	retention time [min]	Intensity T_male	mass [Da]	retention time [min]	Intensity T_female	
1096,48	26,12	3222,70	6186,95	5267,33	1512,99							ApGDRGEpGpP
1114,49	25,76	4207,06	7762,92	2282,59	1009,35	1114,47	27,47	139,89	1114,47	27,29	110,36	SpGERGETGpP
1157,53	37,24	94,56	25,67	0,00	204,33	1157,54	26,05	4125,95	1157,54	25,78	3017,76	GPPGppGpPGPPS
1194,56	26,88	2334,03	6474,10	828,30	565,29	1194,55	22,38	3012,18	1194,55	22,26	3528,21	SpGPDGKTGPPGp
1250,56	27,89	53228,90	62674,86	35374,77	31893,60	1250,55	22,14	22474,75	1250,55	21,95	21825,56	ApGDRGEpGpPpGP
1265,59	27,47	4819,87	6345,99	2093,28	2509,39							SpGPDGKTGpPpGPA
1268,58	27,47	3911,93	7353,51	1034,12	1814,78	1268,57	24,03	192,88	1268,57	23,78	144,15	SpGERGETGpPpGP
1321,60	28,40	18758,49	15679,25	7969,92	10134,75	1321,59	21,98	15288,79	1321,59	21,79	11129,49	ApGDRGEpGpPpGPA
1424,66	39,18	789,85	730,31	2413,99	1412,06	1424,66	27,47	4434,35	1424,66	27,21	3048,19	GLPGPpGpPpGSFLSN
1451,66	29,07	11247,06	17134,60	4422,73	14454,28	1451,65	22,62	21303,69	1451,65	22,42	22529,19	SpGSpGPDGKTGPPGp
1523,74	40,13	3415,37	4396,44	13013,82	8792,82	1523,73	29,29	11292,01	1523,73	29,10	9216,13	GDPGPPGpPpGpPGpPAI
1580,89	24,87	6802,85	7289,53	863,00	2012,77	1580,90	41,91	718,20	1580,90	41,77	608,14	IDQSRVLNLGPITR
1623,72	24,12	3202,27	5147,34	4324,81	2611,99							DGApGKNGERGGpGGpGP
1679,96	23,98	2627,55	4165,70	2046,61	5049,14	1679,97	39,92	2381,61	1679,97	39,88	1564,46	VIDQSRVLNLGPITR
1737,77	23,82	3193,46	3103,71	9681,73	4323,84	1737,78	22,38	4849,79	1737,78	22,19	5597,58	NDGApGKNGERGGpGGpGp
1767,00	24,25	5477,74	4386,09	4372,93	6181,23	1767,00	41,93	931,61	1767,00	41,83	1295,56	SVIDQSRVLNLGPITR
1825,78	20,13	4454,72	3140,62	2150,74	1869,42	1825,79	18,88	938,34	1825,79	18,96	2000,48	DEAGSEADHEGTHSTKR
1876,87	22,13	4255,03	1950,89	1585,01	1379,75	1876,87	21,75	700,14	1876,87	21,56	647,11	DDGEAGKpGRpGERGppGP
1882,80	20,25	36589,33	11130,89	20292,31	25746,63	1882,80	18,88	11496,67	1882,80	18,96	22132,43	DEAGSEADHEGTHSTKRG
1911,06	25,02	106071,62	85636,90	48360,08	64980,78	1911,05	41,91	22242,49	1911,05	41,77	21038,95	SGSVIDQSRVLNLGPITR
2014,90	21,87	3317,50	3808,57	2426,47	3971,53	2014,90	19,90	1333,45	2014,90	19,86	5556,10	EGSpGRDGSpGAKGDRGETGP
2039,14	21,87	9115,14	9383,93	2200,40	3727,48	2039,16	39,11	255,78	2039,15	39,17	580,50	SGSVIDQSRVLNLGPITRK
2047,93	22,01	24338,02	16223,64	19971,26	9979,82	2047,93	21,67	15221,09	2047,93	21,56	12904,57	NGDDGEAGKpGRpGERGpPGP
2063,93	21,95	6283,48	6485,99	3164,72	24,26	2063,93	21,03	2277,41	2063,93	20,91	2175,79	NGDDGEAGKpGRpGERGpPpGP
2078,94	26,62	3970,83	2127,22	5708,97	0,00	2078,93	21,19	10084,72	2078,93	21,08	9210,22	DAGApGApGGKGDAGApGERGpPpG
2085,93	22,54	0,00	0,00	736,46	2703,67	2085,93	19,98	10384,07	2085,93	19,94	10940,59	EGSpGRDGSpGAKGDRGETGPA

Summary

A urine sample has been generated to be used as reference in clinical proteomics

Characterized in depth by a variety of proteomics platforms

Samples are freely available

Data will be become available in the EuroKUP website

Current-Future Work

Continuous collection of proteomics data on this sample

Need to integrate available results (in collaboration with WG4)

Need to establish in the EuroKUP website systems for protocol, data reporting, processing, comparison, mining (in collaboration with WG4)



Thank you...