

Supporting Information

On-chip mesoporous functionalized magnetic microspheres for protein sequencing by extended bottom- up mass spectrometry

*Natalia Gasilova,^a Kristina Srzentić,^b Liang Qiao,^a Baohong Liu,^c Alain Beck,^d Yury O. Tsybin^e
and Hubert H. Girault^{a,*}*

^a Laboratory of Physical and Analytical Electrochemistry, EPFL Valais, Ecole Polytechnique Fédérale de Lausanne, 1951 Sion, Switzerland

^b Biomolecular Mass Spectrometry Laboratory, Ecole Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland

^c Department of Chemistry and Institute of Biomedical Sciences, Fudan University, Shanghai, PR China

^d Centre d'Immunologie Pierre Fabre, 74160 St. Julien-en-Genevois, France

^e Spectroswiss Sàrl, EPFL Innovation Park, 1015 Lausanne, Switzerland

*Corresponding author: Prof. Hubert H. Girault, e-mail: hubert.girault@epfl.ch; phone: +41 (0)21 693 31 51; fax: +41 (0)21 693 36 67

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SI-1. SPE-GEMS/MS microchip fabrication

SPE-GEMS/MS microchip was fabricated by scanning laser ablation. Microchannels were drilled in a polyimide (PI) substrate (125 μm thick, DuPont™Kapton® polyimide film, Dupont) by photoablation with an ArgonFluoride excimer laser (Lambda Physics LPX 210I). The laser was operated at 193 nm wave length with pulse width ~ 20 ns, energy of 0.35 J and frequency of 50 Hz. Depth and width of all microchannels were 50 and 100 μm , respectively. A round chamber (diameter of 500 μm , depth of 50 μm) for the trapping of the magnetic SPE sorbent was created using laser ablation in a static shot mode. After fabrication the quality of the photoablated substrate was inspected under a laser scanning microscope VK-8710 (Keyence Corporation). The electrode microchannel was filled with conductive carbon ink (Electrador, Electra Polymer & Chemicals Ltd.) and was cured for 30 min at 80 °C in the oven. Finally, the microchip was covered with a 25/10 μm thick polyethylene/polyethyleneterephthalate (PE/PET) layer using a lamination apparatus (Morane Senator) at 130 °C and 3 bars. For improving the lamination, the microchip was additionally cured for 1 h at 80 °C in the oven. To facilitate the electrospray formation, the tip of the microchannel A was cut in a V-shape and the microchip was ready to use.

SI-2. SEM and TEM images of $C_8-Fe_3O_4@mSiO_2$ microspheres

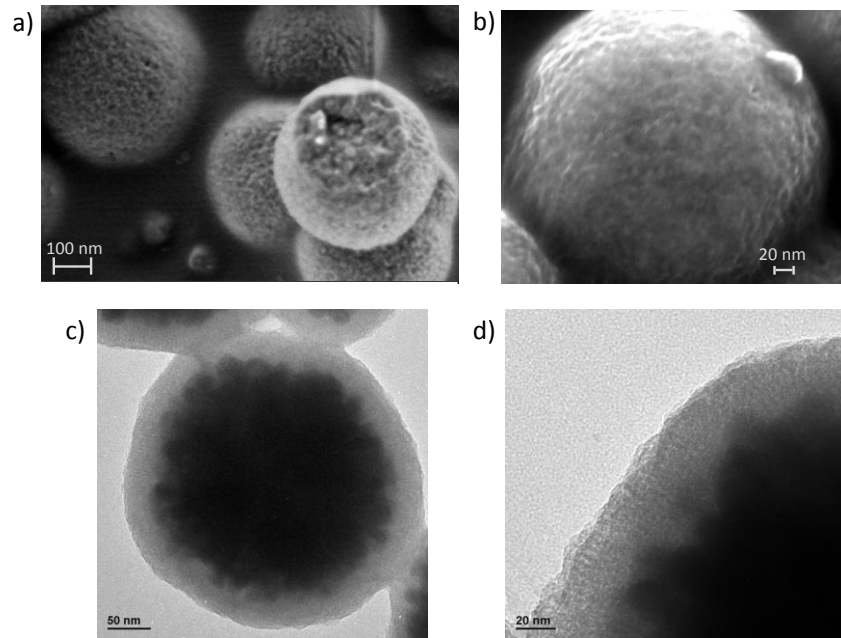


Figure SI-2. Images of the $C_8-Fe_3O_4@mSiO_2$ microspheres obtained by a) HR-SEM, 0.5 kV; b) HR-SEM, 5 kV; c) TEM at 120 kV; d) TEM at 120 kV, zoomed view.

SI-3. Model peptide mixture analysis

Table SI-3. Sensitivity and preconcentration efficiency of SPE-GEMS/MS analysis of model peptide mixture.

C₈-Fe₃O₄@mSiO₂ microspheres

Peptides in elution order	MW, Da	LOD		LOQ		LDR, pmol	EF
		nM	Amount, fmol/pg	nM	Amount, fmol/pg		
Angiotensin I	1296.48	5	50/65	15	150/195	0.15-10	50
Insulin chain B oxidized	3495.89	1	10/35	3	30/105	0.03-4	200
ACTH (1-24)	2933.44	1	10/30	3	30/90	0.03-5	240

Commercial C₈-coated magnetic beads

Peptides in elution order	MW, Da	LOD		LOQ		LDR, pmol	EF
		nM	Amount, fmol/pg	nM	Amount, fmol/pg		
Angiotensin I	1296.48	25	250/325	75	750/975	0.75-1	15
Insulin chain B oxidized	3495.89	10	100/350	30	300/1050	0.3-0.6	20
ACTH (1-24)	2933.44	50	500/1500	200	2000/6000	0.2-0.7	5

MW – molecular weight; LOD – limit of detection; LOQ – limit of quantification; LDR – estimated linear dynamic range of analyte amount; EF – enrichment factor, calculated *vs* model peptide mixture analysis using plain microchip emitter, *i.e.* microchip without SPE sorbent. Sample volume loaded: 10 μ l.

LOD is defined as 3 signal-to-noise ratio, LOQ is defined as 10 signal-to-noise ratio, while EF is defined as peptide peak signal-to-noise ratio obtained with preconcentration \times dilution factor/peptide peak signal-to-noise ratio obtained without preconcentration. LDR is estimated based on LOQ values and maximum loading capacity of the corresponding magnetic sorbent.

SI-4. Peptides identified from BSA tryptic digest

Table SI-4.1. Putative peptides[§] identified from SPE-GEMS/MS analysis using 75 fmol of the sample and C₈-Fe₃O₄@mSiO₂ microspheres

Peptide sequence	MW, Da	m/z, Th	z	Probability	Hyperscore
D.C''C'EKQEPERNEC'FLSHK.D	2232.9	559.2	4	0.9873	325
K.GLVLIAFSQYLQQ'PFDEH.V	2264.1	567.0	4	0.9892	284
L.RETYGDMADC'C'EKQEPER.N	2272.9	569.2	4	0.9874	319
L.RETYGDM''ADC'C'EKQEPER.N	2288.9	573.2	4	0.9866	301
Q.QC'PFDEHVKLVNELTEFAK.T	2303.1	576.8	4	0.9809	331
-.nM''KWVTFISLLLLFSSAYS.R.G	2319.2	580.8	4	0.9885	333
K.VGTRC'C'TKPESERM''PC'TED.Y	2327.9	582.9	4	0.9812	324
-.nMKWVTFISLLLLFSSAYS.R.G.V	2360.3	591.0	4	0.9874	322
K.AFDEKLTFHADIC'TLPDTE.K	2369.1	593.3	4	0.9872	330
E.IARRHPYFYAPELLYANK.Y	2384.2	597.1	4	0.9874	318
K.E*C'C'HGDLEEC'ADDRADLAKY.L	2391.9	599.0	4	0.9809	275
Q.NC'DQFEKLGEYGFQNALIVR.Y	2400.2	601.0	4	0.9847	288
Q.EC'C'QAEDKGAC'LLPKIETMRE	2408.1	603.0	4	0.9847	288
K.PKATEEQLKTVMENFVAFVDK.C	2423.2	606.8	4	0.9806	335
W.VTFISLLLLFSSAYS.R.G.V.FRR.D	2431.4	608.8	4	0.9807	333
A.DESHAGC'EKSLHTLFGDELCK.V	2432.1	609.0	4	0.9868	304
K.PKATEEQLKTVM''ENFVAFVDK.C	2439.2	610.8	4	0.9803	338
A.SLRETYGDMADC'C'EKQEPER.N	2473.0	619.3	4	0.9874	323
A.SLRETYGDM''ADC'C'EKQEPER.N	2489.0	623.3	4	0.9851	290
Y.E*IARRHPYFYAPELLYANK.Y	2495.3	624.8	4	0.9812	316
K.nWVTFISLLLLFSSAYS.R.G.V.FRR.R	2503.4	626.9	4	0.987	292
Y.LQQC'PFDEHVKLVNELTEFAK.T	2544.3	637.1	4	0.9857	294
A.TLEEC'C'AKDDPHAC'YSTVFDK.L	2545.1	637.3	4	0.9859	295
H.KPKATEEQLKTVMENFVAFVDK.C	2551.3	638.8	4	0.9804	337
F.QEC'C'QAEDKGAC'LLPKIETM''R.E	2552.1	639.0	4	0.9869	305
K.LVNELTEFAKTC'VADESHAGC'EK.S	2607.2	652.8	4	0.9905	285
K.WVTFISLLLLFSSAYS.R.G.V.FRR.D	2617.5	655.4	4	0.985	294
C.IAEVEKDAIPENLPLTADFAEDK.D	2624.3	657.1	4	0.9873	313
K.E*C'C'DKPLLEKSHC'IAEVEKDAIP	2625.2	657.3	4	0.9871	309
M.nKWVTFISLLLLFSSAYS.R.G.V.FRR.R	2631.5	658.8	4	0.9876	296
K.SLHTLFGDELCK'KVASLRETYGDM.A	2641.3	661.3	4	0.9867	303
F.DEKLTFHADIC'TLPDTEKQIK.K	2648.3	663.1	4	0.9869	336
R.E*TYGDMADC'C'EKQEPERNEC'F.L	2649.0	663.3	4	0.9835	283
R.E*TYGDM''ADC'C'EKQEPERNEC'F.L	2665.0	667.3	4	0.9874	319
R.MPC'TEDYLSLILNRLC'VLHEKT.P	2704.3	677.1	4	0.9847	357
K.SLHTLFGDELCK'KVASLRETYGDM.A.D	2712.3	679.1	4	0.9824	279
-.MKWVTFISLLLLFSSAYS.R.G.V.FRR.R	2720.5	681.1	4	0.9879	381
K.SLHTLFGDELCK'KVASLRETYGDM''A.D	2728.3	683.1	4	0.9851	290

Table SI-4.1. Continuation

Peptide sequence	MW, Da	m/z, Th	z	Probability	Hyperscore
R.RHPYFYAPELLYYANKYNGVFQ.E	2752.3	689.1	4	0.9873	314
E.RMPC''TEDYLSLILNRLC''VLHEK.T	2759.4	690.9	4	0.981	329
H.C''IAEVEKDAIPENLPPLTADFAEDK.D	2784.4	697.1	4	0.9855	351
K.ATEEQKLTVM''ENFVAFVDKC''AAD.D	2791.3	698.8	4	0.9811	312
A.FLGSFLYEYSRRRHEPYAVSVLLR.L	2801.5	701.4	4	0.986	296
K.LVNELTEFAKTC''VADESHAGC''EKSL.H	2807.3	702.8	4	0.9865	300
R.E''TYGDMADC''EKQEPERNEC''FLS.H	2849.1	713.3	4	0.987	333
K.AFDEKLFTFHADIC''TLPDTEKQIK.K	2866.4	717.6	4	0.9847	368
T.YGDM''ADC''EKQEPERNEC''FLSHK.D	2918.2	730.6	4	0.9874	319
K.EYEATLEEC''C''AKDDPHAC''YSTVFDK.L	3037.2	760.3	4	0.9867	319
R.RPC''FSALTPEDETYVPKAFDEKLFTFHAD.L	3301.6	826.4	4	0.9811	314
T.E''FAKTC''VADESHAGC''EKSLHTLFGDELK.V	3320.5	831.1	4	0.9809	309
R.LAKEYEATLEEC''C''AKDDPHAC''YSTVFDK.L	3349.5	838.4	4	0.9843	290
K.E''C''C''HGDLLC''ADDRADLAKYIC''DNQDTLS	3351.4	838.9	4	0.9805	378
K.C''C''TESLVNRRPC''FSALTPEDETYVPKAFDE.K	3461.6	866.4	4	0.9845	358
A.LTPDETYVPKAFDEKLFTFHADIC''TLPDTEK.Q	3640.8	911.2	4	0.9874	321
K.EC''C''DKPILLEKSHC''IAEVEKDAIPENLPPLTADF.A	3837.8	960.5	4	0.986	346
L.QQC''PFDEHVKLVNELTEFAKTC''VADESHAGC''EK.S	3875.8	969.9	4	0.9813	276
K.GLVLIAFSQYLQQC''PFDEHVKLVNELTEFAKTC''.V	3896.9	975.2	4	0.987	307

§Complete list of identified peptides including peptide isoforms with various modifications.

C' – carboxyamidomethyl cysteine residue; C'' – (R)-5-oxoperhydro-1,4-thiazine-3-carboxylic acid, product of S- carboxyamidomethyl cysteine cyclization at the N-terminus; E* – pyroglutamic acid, product of glutamic acid cyclisation at the N-terminus; M'' – methionine sulfoxide; nX – carbamylation of X at N-terminus.

Table SI-4.2. Putative peptides[§] identified from SPE-GEMS/MS analysis using 75 fmol of the sample and commercial C₈-coated magnetic beads

Peptide sequence	MW, Da	m/z, Th	z	Probability	Hyperscore
K.C''C'TESLVNR.R	1120.5	561.2	2	0.9998	504
R.C''C'TKPESER.M	1148.5	575.2	2	0.9998	451
K.LVNELTEFAK.T	1162.6	582.3	2	0.9996	574
K.EC''C'DKPLLEK.S	1290.6	646.3	2	0.9994	345
K.HLVDEPQNLK.Q	1304.7	653.4	2	0.9999	559
K.TVMENFVAFVVK.C	1398.7	700.3	2	0.9933	477
K.TVM''ENFVAFVVK.C	1414.7	708.3	2	0.9983	256
K.SLHTLFGDELK.V	1418.7	710.4	2	0.9999	634
K.YIC'DNQDTISSK.L	1442.7	722.3	2	0.9999	747
K.EYEATLEEC''C'AK.D	1501.7	751.8	2	0.9996	616
K.VPQVSTPTLVEVSR.S	1510.8	756.4	2	0.9998	567
R.KVPQVSTPTLVEVSR.S	1638.9	820.5	2	0.9999	483
K.GLVLIAFSQYLQQC'PFDEH.V	2264.1	567.0	4	0.9883	271
K.VGTRC''C'TKPESERM''PC'TED.Y	2327.9	583.0	4	0.9796	308
-nMKWVTFISLLLLFSSAYSRG.V	2360.3	591.1	4	0.9796	308
E.IARRHPYFYAPPELLYYANK.Y	2384.2	597.1	4	0.9796	307
A.DESHAGC'EKSLHTLFGDELK.V	2432.1	609.0	4	0.9796	306
R.FKDLGEEHFKGLVLIAFSQYL.Q	2453.3	614.3	4	0.9796	305
K.GLVLIAFSQYLQQC'PFDEHVK.L	2491.3	623.8	4	0.9867	625
V.HKEC''C'HGDLLEC'ADDRADLAK.Y	2512.1	629.0	4	0.9796	311
Y.LQQC'PFDEHVKLVNELTEFAK.T	2544.3	637.1	4	0.9796	302
K.HKPKATEEQKTVMENFVAFVVK.C	2688.4	673.1	4	0.981	324
-MKWVTFISLLLLFSSAYSRGVFR.R	2720.5	681.1	4	0.9816	300
-M''KWVTFISLLLLFSSAYSRGVFR.R	2736.5	685.1	4	0.9817	302
R.RHPYFYAPPELLYYANKYNGVFQ.E	2752.3	689.1	4	0.9796	304
E.ATLEEC''C'AKDDPHAC'YSTVFDKHK.H	2857.3	715.3	4	0.9796	311

[§]Complete list of identified peptides including peptide isoforms with various modifications.

C' – carboxyamidomethyl cysteine residue; C'' – (R)-5-oxoperhydro-1,4-thiazine-3-carboxylic acid, product of S- carboxyamidomethyl cysteine cyclization at the N-terminus; E* – pyroglutamic acid, product of glutamic acid cyclisation at the N-terminus; M'' – methionine sulfoxide; nX – carbamylation of X at N-terminus.

SI-5. Peptides identified from Trastuzumab digested with Sap9

Table SI-5. Putative peptides[§] identified from SPE-GEMS/MS analysis using 33 fmol of the sample and C₈-Fe₃O₄@mSiO₂ microspheres

Peptide sequence	MW, Da	m/z, Th	z	Probability	Hyper score
R.WGGDGFYAMDYWGQGLVTVSS.A	2396.0	600.0	4	0.9753	315
K.C'KVSNKALPAPIEKTISKAKGQP.R	2464.4	617.1	4	0.977	335
K.VSNKALPAPIEKTISKAKGQPREP.Q	2558.5	640.6	4	0.9676	313
R.WGGDGFYAM'DYWGQGLVTVSSAS.T	2570.1	643.5	4	0.9665	285
K.E*YKC'KVSNKALPAPIEKTISKAK.G	2584.4	647.1	4	0.9834	310
L.E*KTISKAKGQPREPQVYTLPPSR.E	2591.4	648.9	4	0.9636	303
S.Q*ESVTEQDSKDYSLSTLTLK.A	2616.2	655.1	4	0.9615	353
W.QQGNVFC'SVM'HEALHNHYTQK.S	2630.2	658.6	4	0.9781	345
G.GSLRLSC'AASGFNIKDTYIHWVR.Q	2650.3	663.6	4	0.9612	363
K.VDKKVEPKSC'DKTHTC'PPC'PAPE.L	2679.2	670.8	4	0.9686	312
K.VSNKALPAPIEKTISKAKGQPREPQ.V	2686.5	672.6	4	0.9703	323
L.NGKEYKC'KVSNKALPAPIEKTISK.A	2702.5	676.6	4	0.9645	305
L.C'NVNHKPSNTKVDKKVEPKSC'DK.T	2711.3	678.8	4	0.9602	297
P.E*DFATYYC'QHYTTPPTFGQGTK.V	2721.2	681.3	4	0.979	362
F.NIKDTYIHWVRQAPGKLEWVAR.L	2736.5	685.1	4	0.9724	328
S.KNTAYLQM'NSLRAEDTAVYYC'SR.W	2769.3	693.3	4	0.965	331
K.DYFPEPVTVSWNSGALTSVHTFPAV.L	2777.3	695.3	4	0.9817	341
R.WGGDGFYAMDYWGQGLVTVSSASTK.G	2783.3	696.8	4	0.9843	313
G.TKVEIKRTVAAPSVFIFPPSDEQLK.S	2799.5	700.9	4	0.9666	346
R.WQGNVFC'SVM'HEALHNHYTQK.S	2816.3	705.1	4	0.9875	359
K.ALPAPIEKTISKAKGQPREPQVYTL.P	2831.6	708.9	4	0.9738	341
K.VQWKVDNALQSGNSQESVTEQDSKDS.T	2878.3	720.6	4	0.9609	341
K.VDNALQSGNSQESVTEQDSKDYSL.S	2888.3	723.1	4	0.963	322
E.YKC'KVSNKALPAPIEKTISKAKGQPR.E	2911.6	728.9	4	0.9733	335
K.ALPAPIEKTISKAKGQPREPQVYTLPP.S	2928.6	733.2	4	0.9684	300
R.VVSVLTVLHQDWLNGKEYKC'KVSNK.A	2943.6	736.9	4	0.9834	336
L.HQDWLNGKEYKC'KVSNKALPAPIEK.T	2952.5	739.1	4	0.9766	331
K.C'KVSNKALPAPIEKTISKAKGQPREPQ.V	2974.6	744.7	4	0.9682	311
K.HKVYAC'EVTHQGLSSPVTKSFNRGEC'.	2990.4	748.6	4	0.9829	388
K.VEIKRTVAAPSVFIFPPSDEQLKSGTASV.V	3072.7	769.2	4	0.9664	342
A.M'DYWGQGLVTVSSASTKGPSVFPLPSSK.S	3113.5	779.4	4	0.9655	374
R.E*PQVYTLPPSREEMTKNQVSLTC'LVKGF.Y	3232.6	809.2	4	0.9727	368
T.YRVVSVLTVLHQDWLNGKEYKC'KVSNK.A	3262.7	816.7	4	0.9729	377
L.TVLHQDWLNGKEYKC'KVSNKALPAPIEK.T	3265.8	817.4	4	0.9612	288
R.SGTDFTLTISSLPEDFATYYC'QHYTT.P	3273.4	819.4	4	0.9703	300
N.KALPAPIEKTISKAKGQPREPQVYTLPPSR.E	3299.9	826.0	4	0.9718	332
P.IEKTISKAKGQPREPQVYTLPPSREEMTK.N	3340.8	836.2	4	0.962	300
R.YADSVKGRFTISADTSKNTAYLQM'NSLRAE.D	3352.6	839.2	4	0.9734	314
K.VSNKALPAPIEKTISKAKGQPREPQVYTLPP.S	3356.9	840.2	4	0.9741	347

Table SI-5.2. Continuation

Peptide sequence	MW, Da	m/z, Th	z	Probability	Hyper score
Q.ESVTEQDSKDSTYLSSTLTLSKADYEKHK.V	3376.6	845.2	4	0.9728	378
R.WQQGNVFC'SVM''HEALHNHYTQKSLSLSP.G	3400.6	851.2	4	0.9618	318
S.NKALPAIEKTISKAKGQPREPQVYTLPPSR.E	3413.9	854.5	4	0.9688	317
K.ALPAIEKTISKAKGQPREPQVYTLPPSREE.M	3429.8	858.5	4	0.9611	296
R.TPEVTC'VVVDVSHEDPEVKFNWYVDGVEVH.N	3483.6	871.9	4	0.9646	329
K.GQPREPQVYTLPPSREEMTKNQVSLTC'LVK.G	3484.8	872.2	4	0.9738	292
V.SNKALPAIEKTISKAKGQPREPQVYTLPPSR.E	3500.9	876.2	4	0.9724	328
R.AEDTAVYYC'SRWGGDGFYAM''DYWGQGLVTV.S	3553.5	889.4	4	0.9659	371
R.FTISADTSKNTAYLQMNSLRAEDTAVYYC'SR.W	3575.7	894.9	4	0.9706	312
K.C'KVSNKALPAIEKTISKAKGQPREPQVYTLPP.S	3645.0	912.3	4	0.9732	351
N.AKTKPREEQNSTYRVVSVLTVLHQDWLNGK.E	3658.9	915.7	4	0.9708	385
F.TLTISSLQPEDFATYYC'QQHYTTPPTFGQGTK.V	3679.7	920.9	4	0.973	307
K.C''KVSNKALPAIEKTISKAKGQPREPQVYTLPPS.R	3715.0	929.8	4	0.9717	387
K.E*YKC'KVSNKALPAIEKTISKAKGQPREPQVYTL.P	3853.1	964.3	4	0.9695	315
R.AEDTAVYYC'SRWGGDGFYAM''DYWGQGLVTVSSAS.T	3885.7	972.4	4	0.9648	330
R.EPQVYTLPPSREEM''TKNQVSLTC'LVKGFYPSDIA.V	3912.9	979.2	4	0.9746	394
R.SGTDFTLTISSLQPEDFATYYC'QQHYTTPPTFGQG.T	3957.8	990.4	4	0.9804	385
R.EEM''TKNQVSLTC'LVKGFYPSDIAVEWESNGQPENNY.K	4191.9	1048.9	4	0.9661	369
R.AEDTAVYYC'SRWGGDGFYAM''DYWGQGLVTVSSASTKGP.S	4268.9	1068.2	4	0.9607	369
R.FSGRSRSGTDFTLTISSLQPEDFATYYC'QQHYTTPPTFG.Q	4306.9	1077.7	4	0.9642	327
R.E*PQVYTLPPSREEM''TKNQVSLTC'LVKGFYPSDIAVEW.E	4309.1	1078.3	4	0.9737	368
K.SGTASVVC'LLNMFYPREAKVQWKVDNALQSGNSQESVTE.Q	4325.1	1082.3	4	0.9776	363
Y.VDGVEVHNAKTKPREEQNSTYRVVSVLTVLHQDWLNGK.E	4508.3	1128.1	4	0.9672	404
T.YYC'QQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK.S	4728.4	1183.1	4	0.9654	375
G.GSLRLSC'AASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTR.Y	5008.6	1253.1	4	0.9763	381
-.E*VQLVESGGGLVQPGGSLRLSC'AASGFNIKDTYIHWVRQAPGKGLEW V.A.R	5218.7	1305.7	4	0.9721	375
V.LHQDWLNGKEYKC'KVSNKALPAIEKTISKAKGQPREPQVYTLPPSR.E	5399.9	1351.0	4	0.9689	301
K.NTAYLQMNSLRAEDTAVYYC'SRWGGDGFYAMDYWGQGLVTVSSAST KG.P	5447.5	1362.9	4	0.9607	369
K.APKLLIYSASFLYSGVPSRFSGRSGTDFTLTISSLQPEDFATYYC'QQHYT TPPTFGQG.T	6542.1	1636.5	4	0.9725	329

§Complete list of identified peptides including peptide isoforms with various modifications.

C' – carboxyamidomethyl cysteine residue; C'' – (R)-5-oxoperhydro-1,4-thiazine-3-carboxylic acid, product of S- carboxyamidomethyl cysteine cyclization at the N-terminus; E* – pyroglutamic acid, product of glutamic acid cyclisation at the N-terminus; Q* – pyroglutamic acid, product of glutamine cyclisation at the N-terminus; M'' – methionine sulfoxide.

SI-6. Effect of various sample loading schemes on the SPE-GEMS/MS analysis of antibodies mixture digested with Sap9

Table SI-6. SPE-GEMS/MS analysis of IgG mixture digested with Sap9: various sample loading schemes

Name		Sequence coverage, %		
		Single sample, 10 μ L	Sample + 1 st waste, 15 μ L	Sample + 1 st & 2 nd wastes, 20 μ L
Adalimumab, IgG1	Lc	60	72	81
	Hc	37	54	69
Bevacizumab, IgG1	Lc	43	76	84
	Hc	36	61	77
Trastuzumab, IgG1	Lc	48	80	85
	Hc	51	66	79
Natalizumab, IgG4	Lc	41	69	81
	Hc	54	73	83
Panitumumab, IgG2	Lc	36	56	67
	Hc	45	51	62
Rituximab, IgG1	Lc	36	57	74
	Hc	54	60	75
Total sample loading time		10 min	15 min	20 min

Sample amount: 333 fmol (50 ng), equimolar mixture; Hc – heavy chain; Lc – light chain.

SI-7. Representative CID mass spectra obtained during SPE-GEMS/MS analysis of IgG mixture digested with Sap9

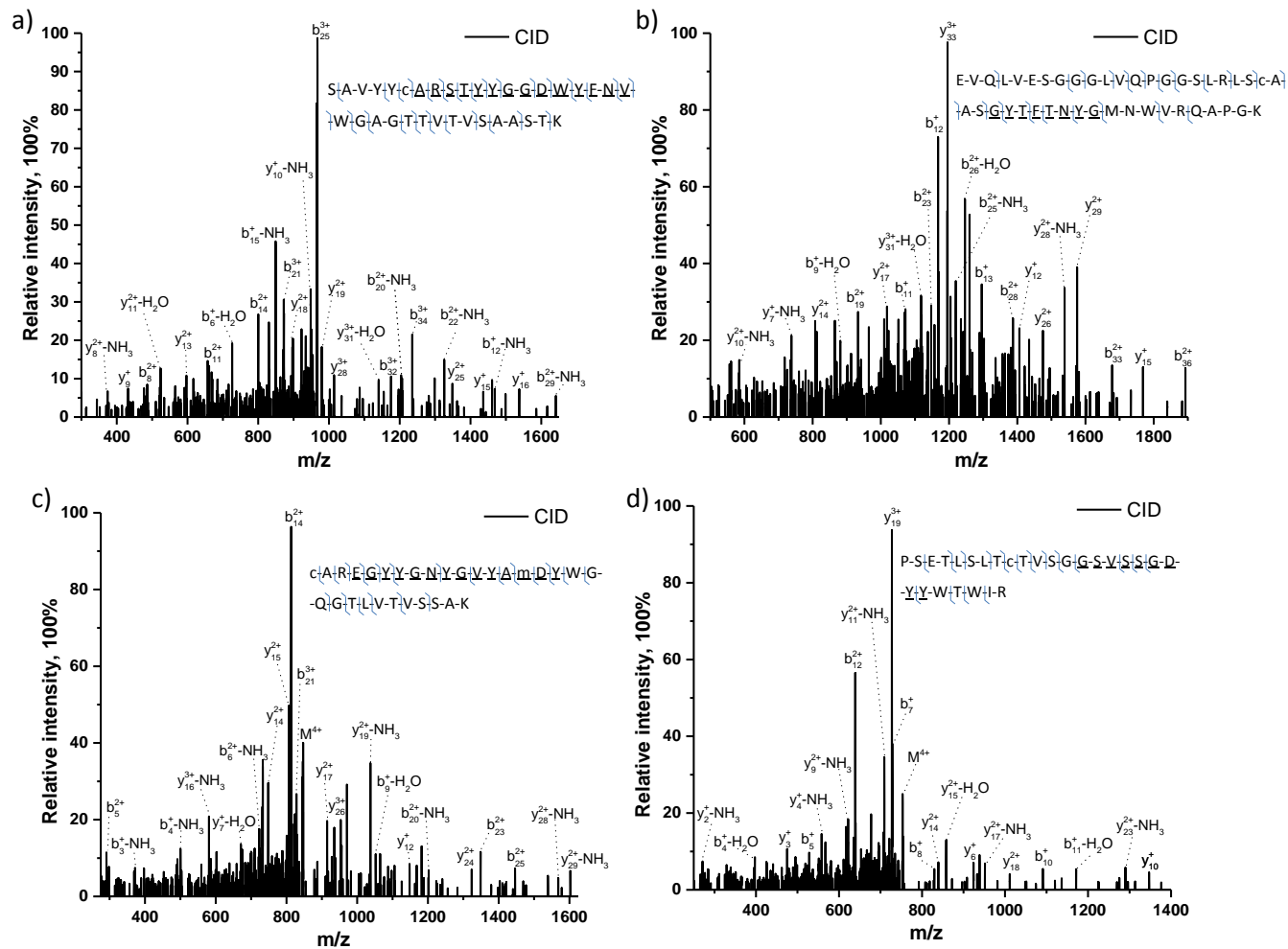


Figure SI-7. Representative CID mass spectra of CDR-containing peptides with assignments of their respective *b*, *y* product ions, identified by SPE-GEMS/MS analysis of IgG mixture digested with Sap9. Selected peptides derived from the Hc of a) Rituximab, IgG1, with CDR 3, MW=3858.8 Da; b) Bevacizumab, IgG1, with CDR 1, MW=4541.3 Da; c) Natalizumab, IgG4, with CDR 3, MW=3382.5 Da; d) Panitumumab, IgG2, with CDR 1, MW=3008.4 Da. Insets: corresponding peptide sequences with assigned peptide backbone cleavage sites and underlined CDRs. Experimental conditions: C₃-Fe₃O₄@mSiO₂ microspheres as a SPE sorbent, 333 fmol (50 ng) of loaded sample, equimolar mixture.