Supporting Information

Electrospray micromixer chip for on-line derivatization and kinetic studies

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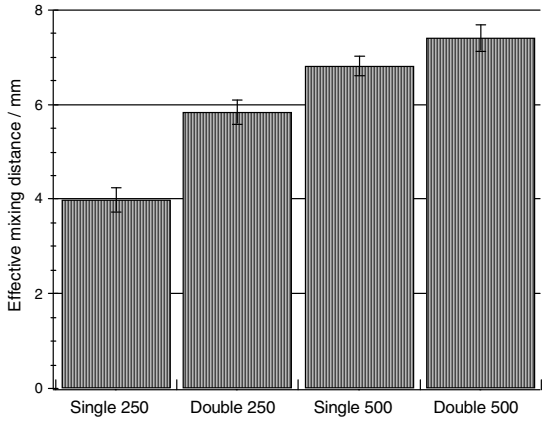


Mixing efficiency depending on groove design constituting the mixing unit. Single or double grooves were drilled and spaced by 250 or 500 µm. The effective mixing distance, *i.e.* the distance for which the fluorescence becomes homogeneous (± 10%) along the microchannel width, was averaged for fluorescein solution flow rates from 0.5, 1, 1.5 and 2 $\mu L \cdot min^{-1}$. The flow rate of the main microchannel was fixed to 4 $\mu L \cdot min^{-1}$.

Figure S1.

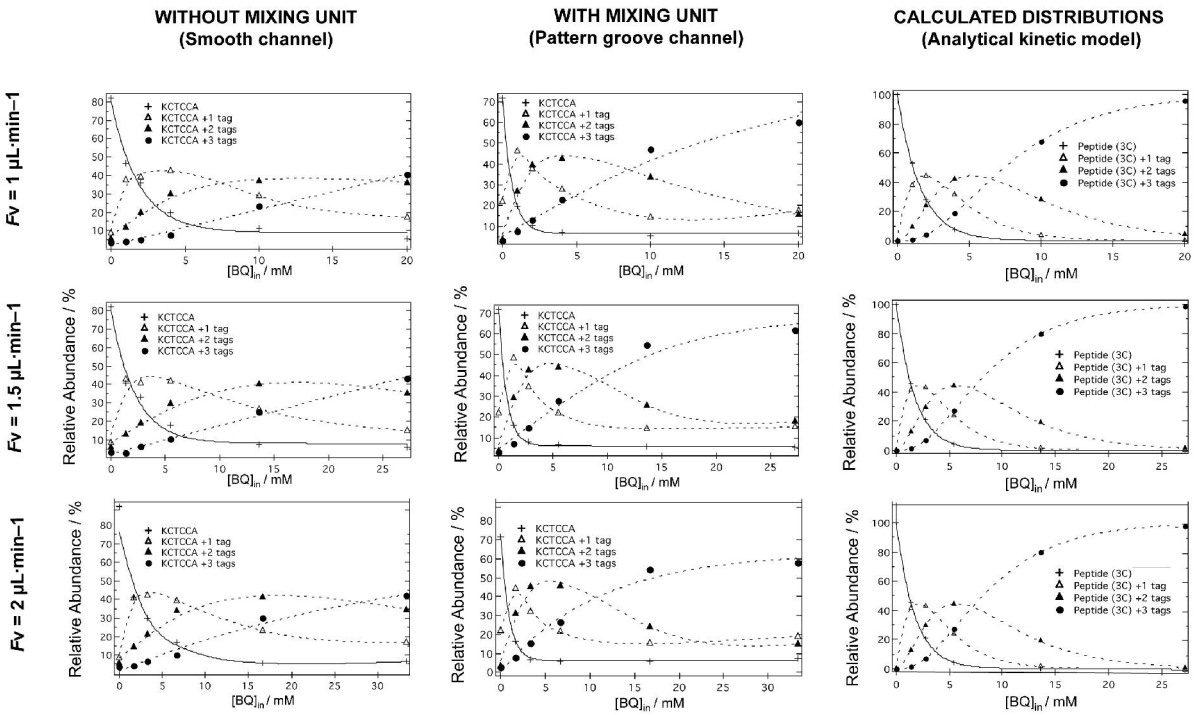

WITHOUT MIXING UNIT
(Smooth channel)
WITH MIXING UNIT
(Pattern groove channel)
CALCULATED DISTRIBUTIONS
(Analytical kinetic model)
Fv = 1 µL·min–1
Fv = 1.5 µL·min–1
Fv = 2 µL·min–1
Relative Abundance / %
[BQ]in / mM
KCTCCA
KCTCCA +1 tag
KCTCCA +2 tags
KCTCCA +3 tags
Peptide (3C)
Peptide (3C) +1 tag
Peptide (3C) +2 tags
Peptide (3C) +3 tags

Figure S2.

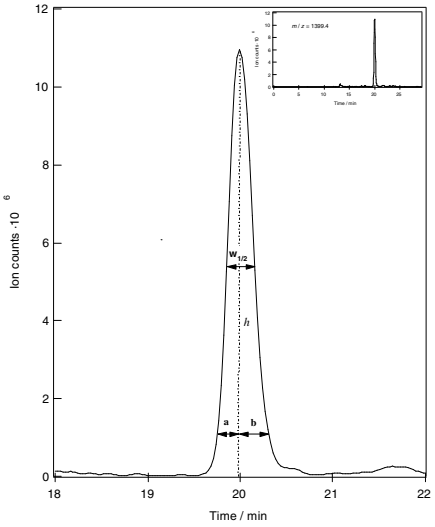
Band broadening and Peak asymmetry (Figure S3)

The effect of the HPLC-Chip-MS coupling on the separation efficiency was evaluated (band broadening, peak asymmetry) by comparing two coupling configurations:

1) HPLC-MS coupling with a classical ESI source
2) HPLC-Chip-MS coupling with a microchip made of a main microchannel with a mixing unit + a secondary microchannel

The band broadening was evaluated by taking the peak width at its half maximum (*h/2*). The peak asymmetry was calculated at 10% of its height according to the following expression:

$$A_{10\%} = \frac{\beta}{\alpha}$$



For studying the peptide peak characteristics, peak smoothing was done using a 10 point Gaussian algorithm with the ICIS™ algorithm (BioWorks™ 3.1) for peak detection.

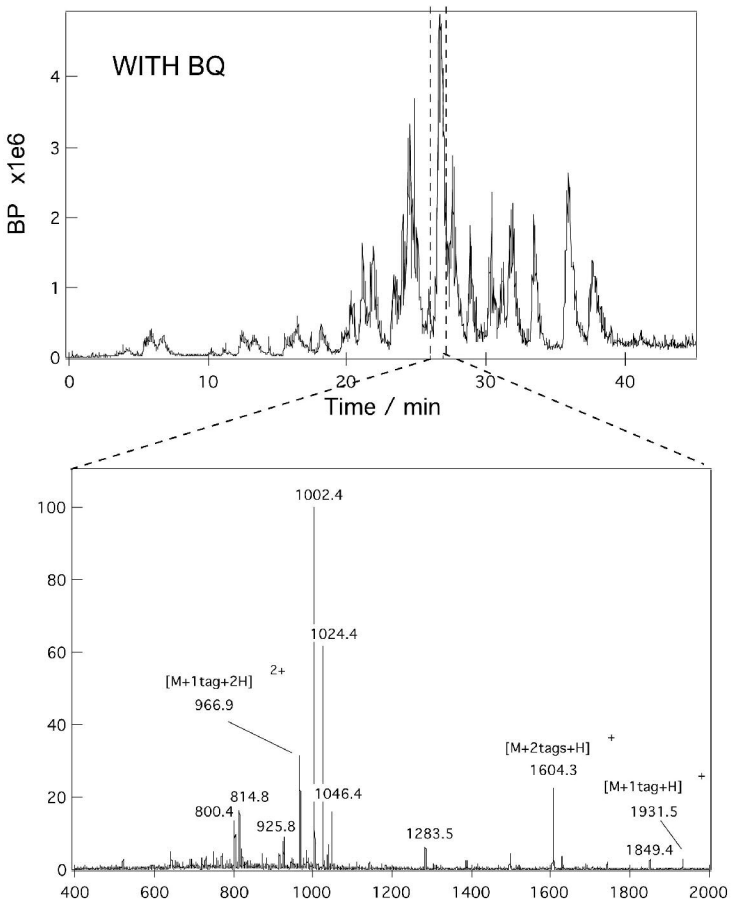
The band broadening and peak asymmetry values were averaged on 9 peaks from three experiments.

	Classical ESI source		Electrospray micromixer Chip	
m / z	**$w_{1/2}$**	**$A_{10\%}$**	**$w_{1/2}$**	**$A_{10\%}$**
776.4	0.418	2.32	0.69	1.57
789.4	0.305	1.35	0.525	2.78
820.4	0.385	1.66	0.52	2.93
1002.4	0.303	0.28	0.548	2.74
1014.5	0.345	1.2	0.496	3.02
1046.5	0.386	0.98	0.575	2.21
1163.5	0.275	1.075	0.604	2.93
1399.4	0.304	1.34	0.48	2.07
1491.5	0.303	1.35	0.439	2.18

Table 1. Example of band broadening and peak asymmetry values obtained from a single experiment with either the classical ESI source or the electrospray micromixer chip (setup 4). *m/z* values correspond to those of BSA tryptic peptides.

	$w_{1/2}$	**$A_{10\%}$**
Classical ESI source	0.373	1.58
Electrospray micromixer Chip	0.541	2.12

Table 2: Averaged band broadening and peak asymmetry values obtained from triplicate experiments on 9 selected MS peaks, within the four configurations of HPLC-MS setup.



On-line chemical derivatization of cysteinyl peptide of a BSA digest. Base peak chromatograms (top) of the HPLC-MS analysis of the BSA digest with an electrospray micromixer chip infused with a solution of $[BQ]_0$ = 100 mM. The mass spectrum (bottom) was integrated over 1 min. Sequences of the cysteinyl identified peptides: $[M+ 2tags+ H]^+$ = 1604.3 Da "EYEATLEEC*C*AK"; $[M +1tag+ 2H]^+$ = 966.9 Da and $[M +1tag+ H]^+$ =1931.5 Da "RPC*FSALTPDETYVPK". The main microchannel was connected to the HPLC capillary outlet (4 $\mu L \cdot min^{-1}$). The BQ solution provided by the liquid junction was kept at 1 $\mu L \cdot min^{-1}$.

Figure S4.

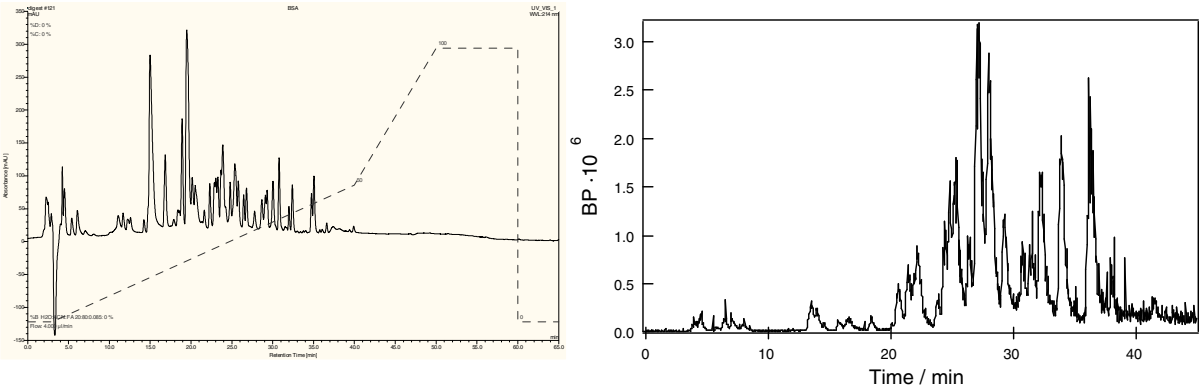
Start - End	Sequence[a]	Cysteine content	Theoretical (m/z)[b] / Th	Observed untagged (m/z)[c] /Th	Observed tagged (m/z)[d] /Th
89-100	SLHTLFGDELCK	1	1362.6722	1362.47	1470.4
89-109	SLHTLFGDELCKVASLR	1	1888.9949	1888.6 945.53	999 1052.47
106-117	ETYGDMADCCEK	2	1364.4803	1364.27	1472.2;1580.2
106-122	ETYGDMADCCEKQEPER	2	2003.7706	1002.33	-
123-130	NECFLSHK	1	977.4509	977.33	-
139-151	LKPDPNTLCDEFK	1	1519.7461	1519.47	1627.47
139-155	LKPDPNTLCDEFKADEK	1	1962.9477	1963.33	980.3; 1034.3
184-197	YNGVFQECCQAEDK	2	1633.6621	1633.33	1741.13;1849.33
198-204	GACLLPK	1	701.4015	701.4	809.4
223-228	CASIQK	1	649.3338	649.33	757.3
262-273	YICDNQDTISSK	1	1386.6206	1386.4	1494.4
298-309	LKECCDKPLLEK	2	1418.7382	1418.47	1526.47; 1634.4
300-309	ECCDKPLLEK	2	1177.5592	1177.33	1185.33;1293.33
310-318	SHCIAEVEK	1	1015.4877	1015.07 508.3	1123.2 562.9
375-386	EYEATLEECCAK	2	1388.5708	1388.4	1496.4;1604.5
387-399	DDPHACYSTVFDK	1	1497.6315	1497.2 749.5	1605.02 803.4
413-420	QNCDQFEK	1	1011.42	1011.27	1119.33
456-468	VGTRCCTKPESER	2	1465.6886	- 733.4	- 841.3; 949.3
460-468	CCTKPESER	2	1052.4499	1052.47	1160.3;1268.3
469-482	MPCTEDYLSLILNR	1	1667.8131	1667.53 834.53	1775.5 888.47
483-489	LCVLHEK	1	841.46	841.33	949.33
499-507	CCTESLVNR	2	1024.455	1024.33	1132.27;1240.33
508-523	RPCFSALTPDETYVPK	1	1823.8996	1823.4 912.53	1931.4 966.53
588-597	EACFAVEGPK	1	1050.4925	1050.53	1158.53
588-607	EACFAVEGPKLVVSTQTALA	1	2034.0503	- 1017.53	- 1125.5

Identification of BSA cysteinyl peptides by on-line HPLC-Chip-MS derivatization at $[BQ]_0$ = 50 mM. [a] Sequence of cysteinyl identified peptides. [b] m/z of predicted cysteinyl peptides. [c] m/z of the experimentally observed untagged peptides. [d] m/z of the experimentally observed cysteinyl peptides tagged with BQ.

Table S5.

On-line derivatization of tryptic cysteinyl peptides of BSA (Figures S6)

UV chromatogram (left) and base peak chromatogram (right) for on-line HPLC-MS tagging of cysteinyl peptide of BSA:



Peak list extraction:

Due to license limitations, *m*/*z* values corresponding to monoisotopic peaks were manually picked up for each LC-MS experiments when the peaks showed good signal-to-noise ratios and their relative abundances were superior to 15% on the mass spectra.

Submitted peak list (without cysteine content information):

303.4 330.87 331.4 333.4 347.47 356.8 375.4 387.47 395.27 427.4 432.47 439.4 453.4
454.33 476.33 508.3 511.33 531.47 544.47 545.4 549.47 553.6 558.4 560.53 567.33 569.4
572.4 577.33 594.47 609.33 617.47 649.33 649.33 656.4 660.47 665.47 671.3 672.33 682.4
689.4 693.47 700.4 701.4 707.47 712.4 719.4 720.53 721.5 727.47 731.27 732.47 733.4
734.33 740.53 743.4 746.47 749.5 752.33 759.4 760.84 774.27 779.33 779.53 784.4 789.4
790.27 795.0 800.47 801.4 818.33 820.53 828.33 831.27 834.53 834.85 841.33 841.4 847.2
871.4 886.27 887.4 899.4 905.33 906.47 912.53 921.47 922.33 927.4 928.33 945.53 949.53
951.4 957.47 963.33 974.33 977.33 1002.33 1011.27 1014.53 1015.07 1017.53 1024.33 1038.4 1046.27
1050.53 1052.47 1068.4 1079.33 1083.47 1088.6 1090.53 1111.4 1121.47 1125.2 142.47 1163.53 1177.33
1183 1201.47 1218.4 1249.4 1283.53 1305.53 1324.27 1364.27 1386.4 1388.4 1399.47 1439.6
1479.6 1491.47 1497.2 1511.8 1519.47 1519.53 1633.33 1639.67 1823.4 1879.27 1963.33 1362.47
1567.53 1667.53 1850 1888.60 1349.53 1418.47

Submitted peak list (with cysteine content information):

303.4 330.87 331.4 333.4 347.47 356.8 375.4 387.47 395.27 427.4 432.47 439.4 453.4
454.33 476.33 508.3 511.33 531.47 544.47 545.4 549.47 553.6 558.4 560.53 567.33 569.4
572.4 577.33 594.47 609.33 617.47 649.33 comp(1[C]) 649.33 656.4 660.47 665.47 671.3
672.33 682.4 689.4 693.47 700.4 701.4 comp(1[C]) 707.47 712.4 719.4 720.53 721.5
727.47 731.27 732.47 733.4 734.33 740.53 743.4 746.47 749.5 752.33 759.4 760.84 774.27
779.33 779.53 784.4 789.4 790.27 795 800.47 801.4 818.33 820.53 828.33 831.27 834.53
834.85comp(1[C]) 841.33 841.4 847.2comp(2[C]) 871.4 886.27 887.4 899.4 905.33
906.47912.53comp(1[C]) 921.47 922.33comp(1[C]) 927.4 928.33 945.53comp(1[C]) 949.53
951.4957.47 963.33 974.33 977.33comp(1[C]) 1002.33 1011.27comp(1[C]) 1014.53
1015.07comp(1[C]) 1017.53 1024.33comp(2[C]) 1038.4comp(1[C]) 1046.27
1050.53comp(1[C]) 1052.47comp(2[C]) 1068.4 1079.33 1083.47 1088.6 1090.53 1111.4
1121.47 1125.2 1142.47 1163.53 1177.33comp(2[C]) 1183 1201.4 comp(1[C])
1218.4 1249.4 1283.53 1305.53 1324.27 1364.27comp(2[C]) 1386.4comp(1[C])
1388.4comp(2[C]) 1399.47 1439.6 1479.6 1491.47 1497.2comp(1[C]) 1511.8
1519.47 comp(1[C]) 1519.53 1633.33comp(2[C]) 1639.67 1823.4comp(1[C]) 1879.27

1963.33 1362.47 comp(1[C]) 1567.53 1667.53comp(1[C]) 1850comp(1[C]) 1888.60comp(1[C]) 1349.53comp(1[C]) 1418.47comp(2[C])

*Mascot identification scores of BSA by RP-HPLC-MS analysis A) whitout and B) with on-chip derivatization of tryptic cysteinyl peptides. Mascot score is -10*log(P) where P is the probability that the observed match is a random event. Protein scores greater that 72 are significant (P<0.05).*