Supplementary materials

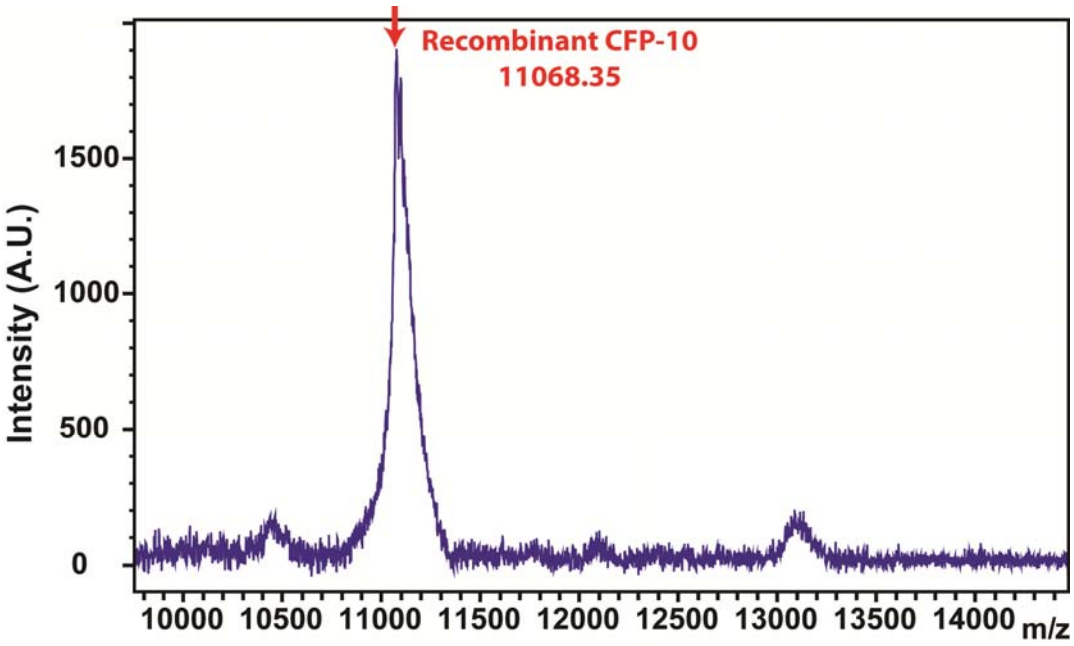

Figure S1. Mass spectrum of full length recombinant CFP-10 (5 μM) collected in linear mode of MALDI-TOF MS. The molecular weight of recombinant, His-tagged CFP-10 is 11 kDa.

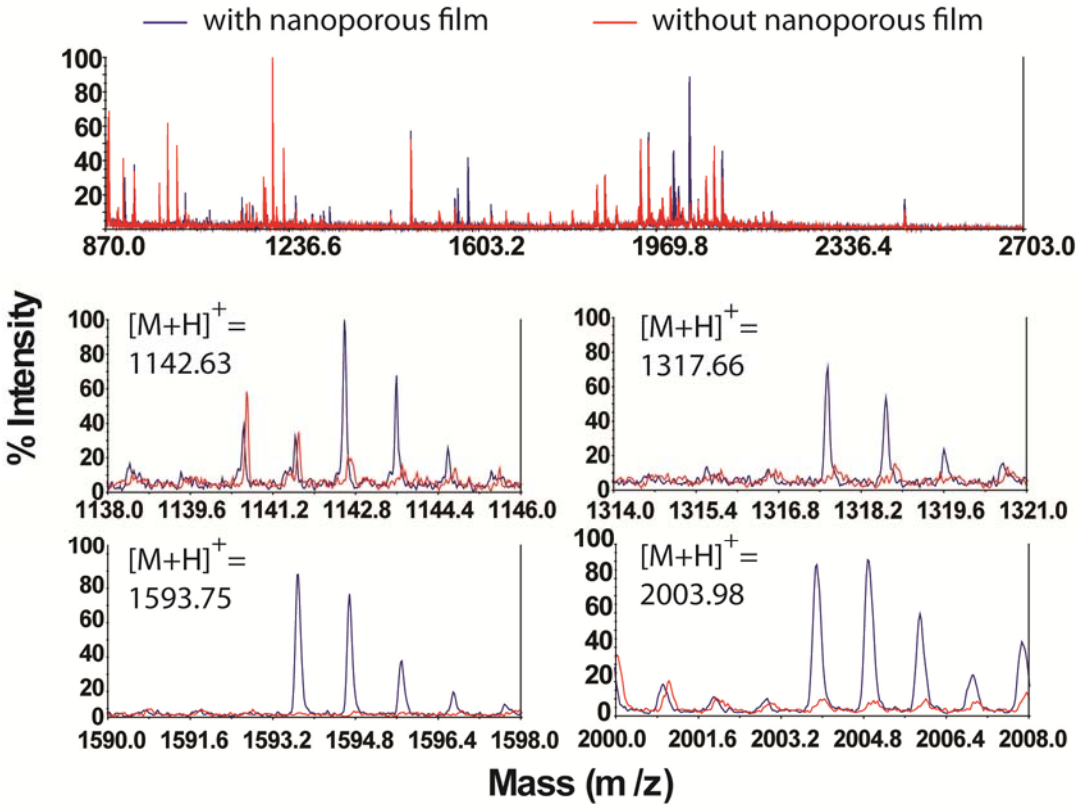

Figure S2. MALDI TOF MS analysis of culture media with or without on-chip fractionation. The top panel shows the overall mass spectra. The bottom panels represent the close-up spectra of CFP-10 fragments. Without prior on-chip processing of samples, signals from the major CFP-10 fragments were overshadowed by other abundant species in solution. With fractionation, the enriched major CFP-10 peaks became more evident and crisp in the mass spectrum. The observed multiple peaks in mass spectra with 1Da difference are caused by the isotope of carbon (^{13}C) in nature.

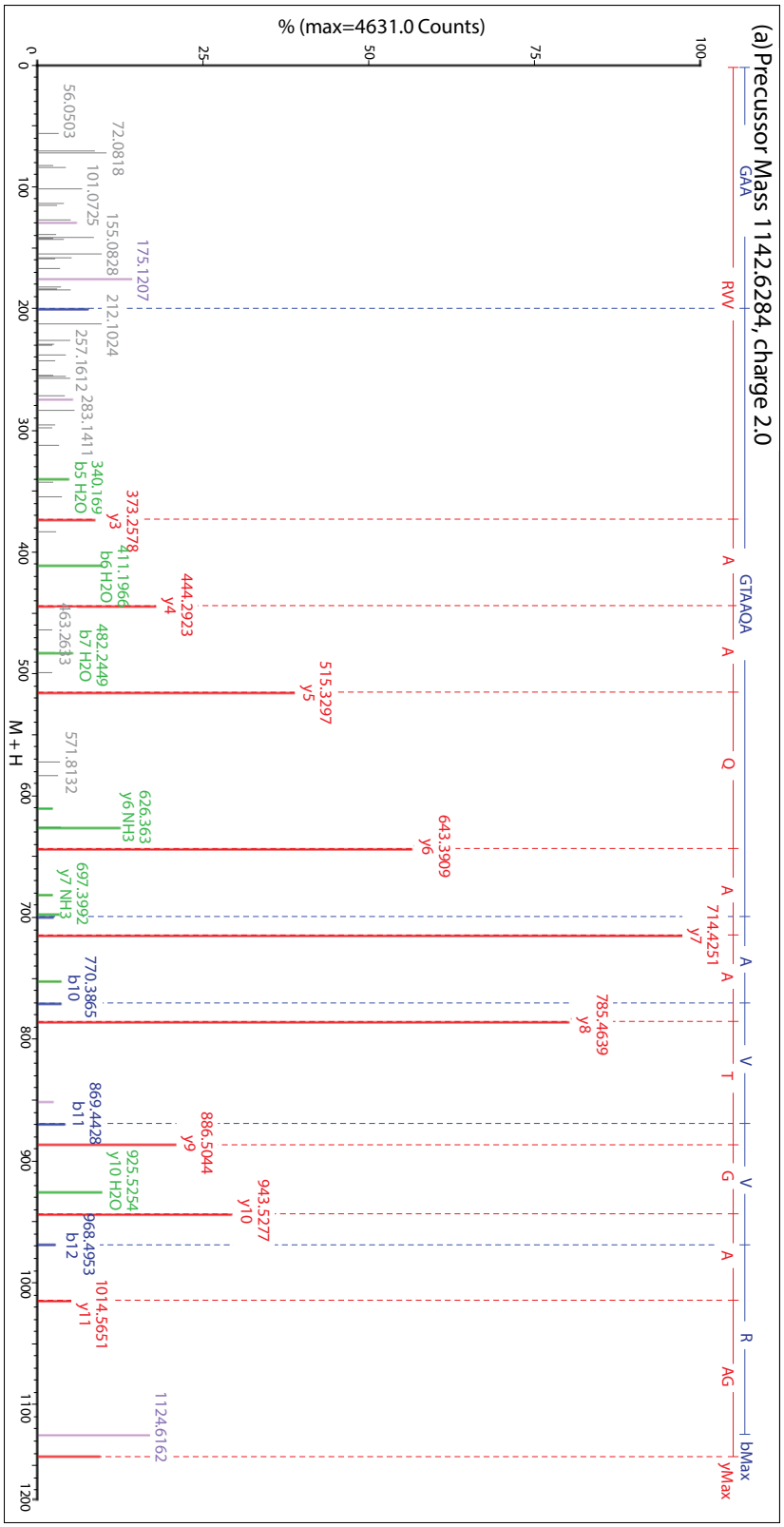

Figure S3 (a). LC-MS/MS spectra of recombinant CFP-10 fragments ($[M+H]^+$=1142.63).

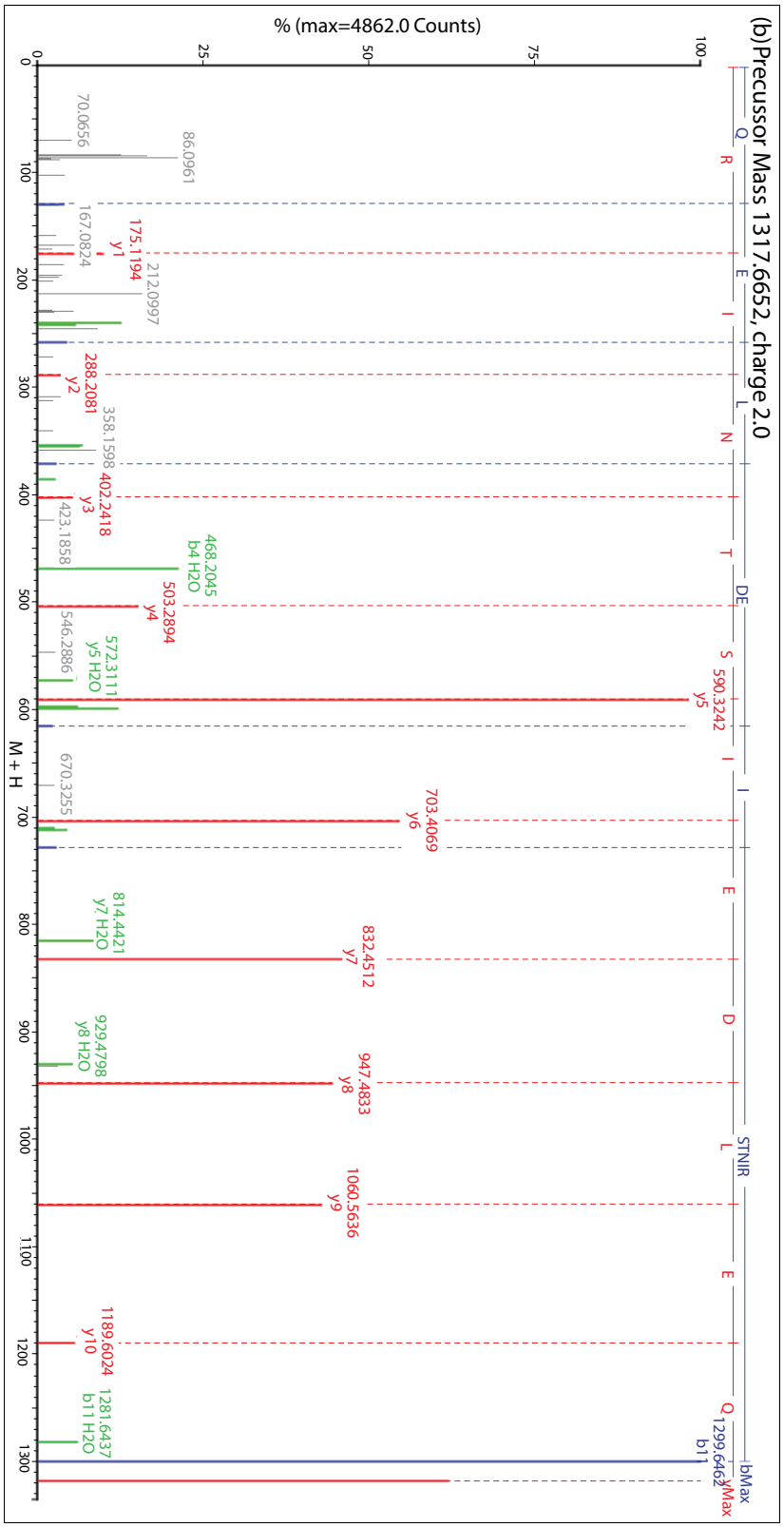

Figure S3 (b). LC-MS/MS spectra of recombinant CFP-10 fragments ($[M+H]^+$=1317.66).

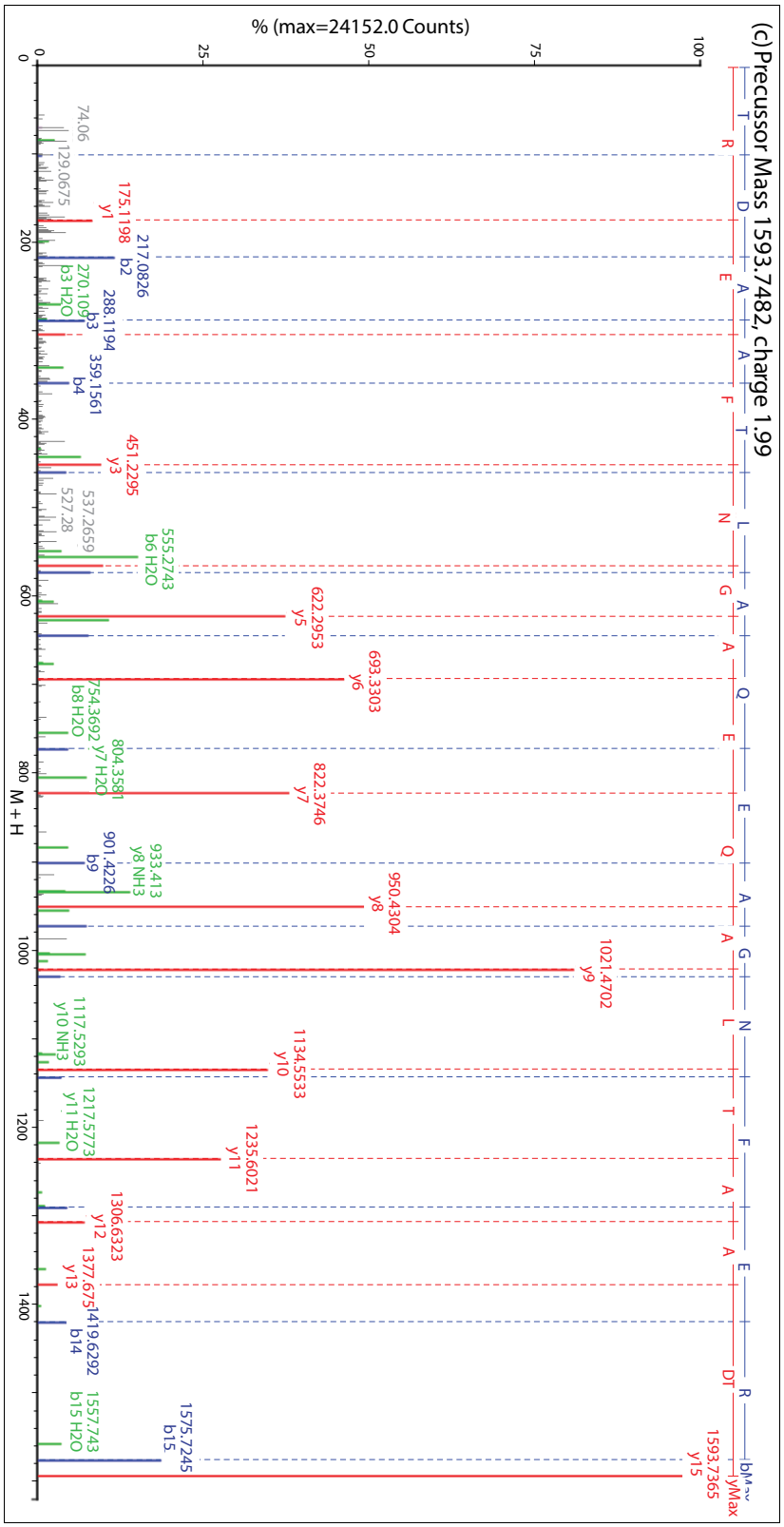

Figure S3 (c). LC-MS/MS spectra of recombinant CFP-10 fragments ($[M+H]^+$=1593.75).

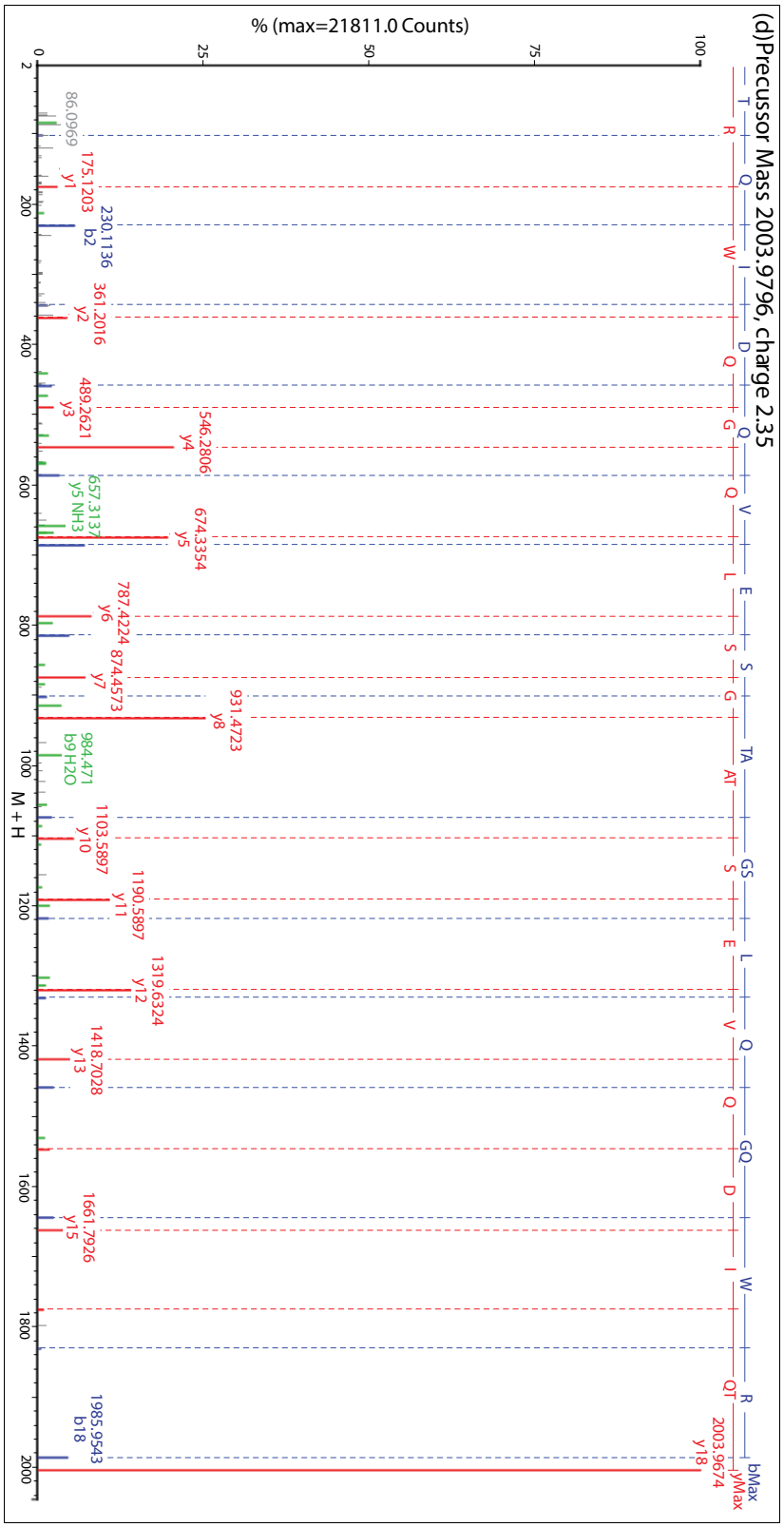

Figure S3 (d). LC-MS/MS spectra of recombinant CFP-10 fragments ($[M+H]^+$=2003.98).

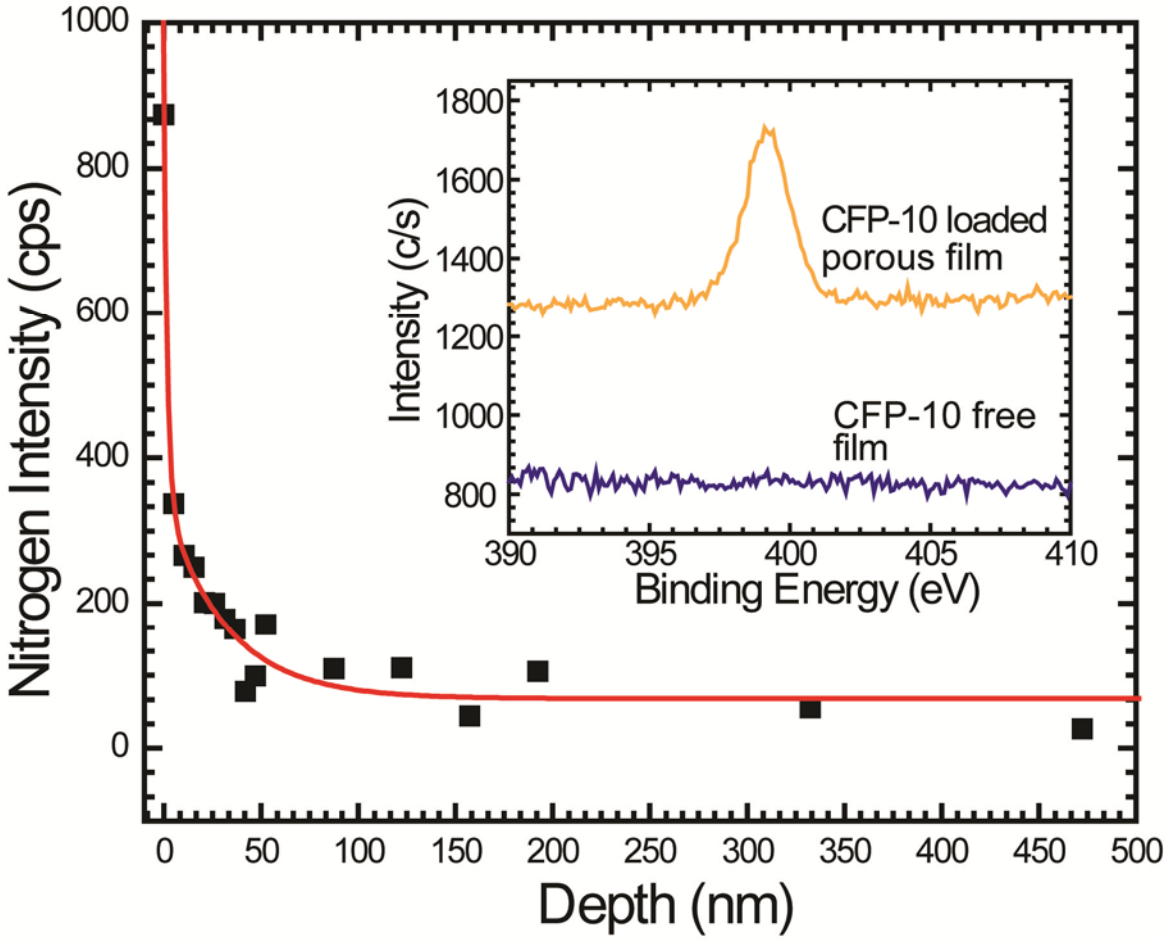

Figure S4. The depth profiles of CFP-10 enriched on L121+25% PPG, as determined from the N1s spectrum collected using XPS (described in Supporting Methods). The line represent the exponential fit of $y=y0+A\cdot\exp(-x/B)+C\cdot\exp(-x/D)$. CFP-10 could penetrate 100nm into the film. The inset shows representative XPS N1S spectra of nanoporous film with and without CFP-10.

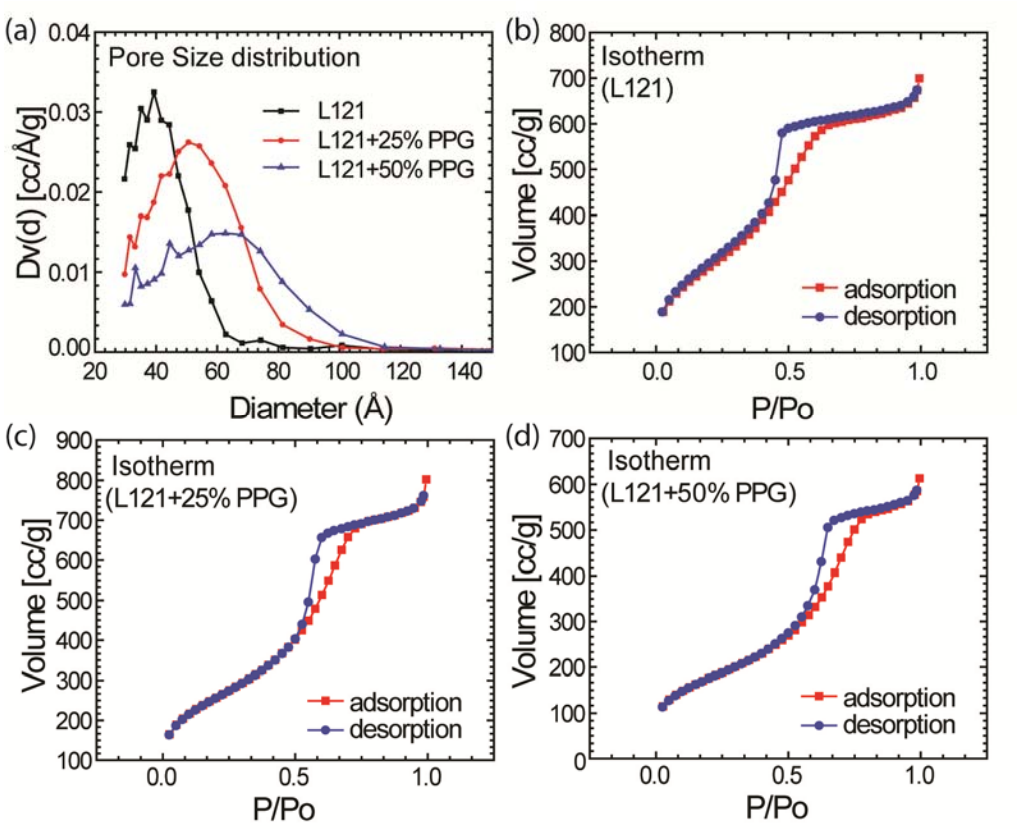

Figure S5.BET measurement. (a) The pore size distributions of different nanoporous film determined by N_2 adsorption analysis. (b)-(d) N_2 adsorption/desorption isotherms for L121, L121+25% PPG, and L121+50% PPG, respectively.

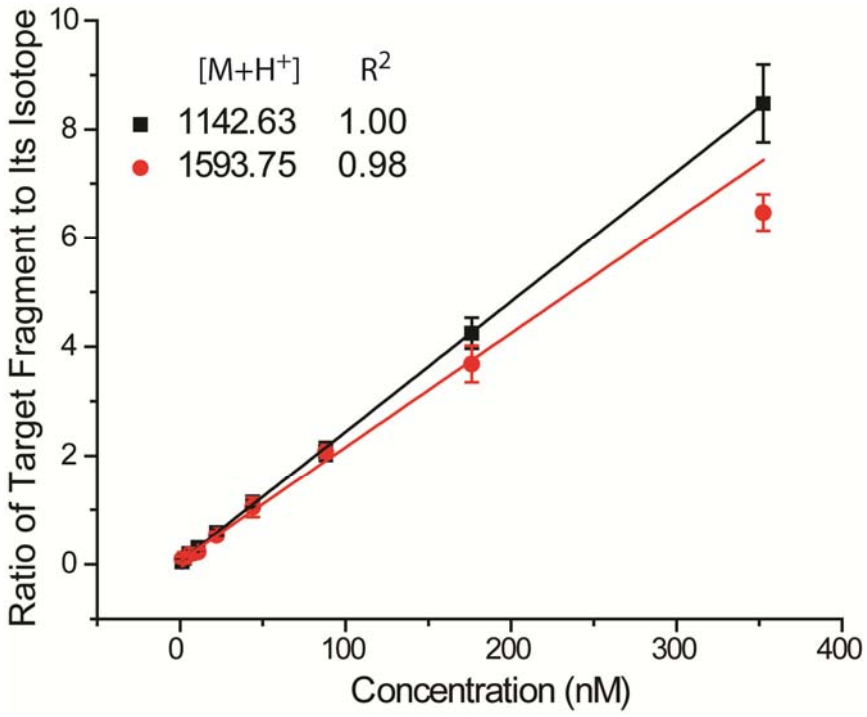

Figure S6. The relative intensity of each major CFP-10 fragment to its isotopic fragment is plotted verses the input CFP-10 concentration. The isotopic ^{18}O-labeled fragments were generated by trypsin digestion in $H_2{}^{18}O$. Isotopic CFP-10 at 42 nM of was added in equal proportion to known digested CFP-10 before spiking on MALDI MS plate. In this condition, the 1142.63 and 1593.75 fragments show good linear relation with their respective isotopic fragments below 400 nM.

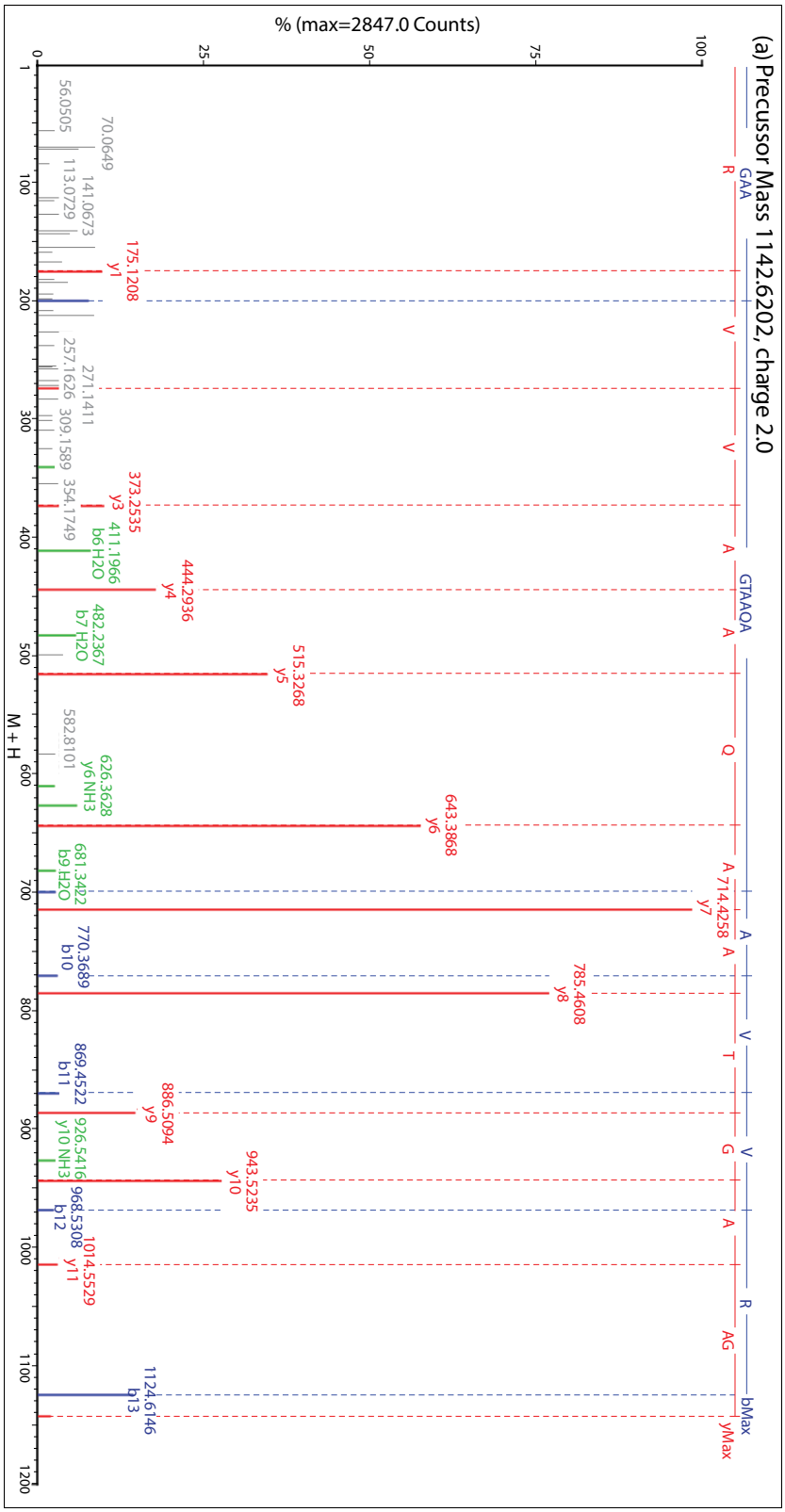

Figure S7 (a). LC-MS/MS spectra of CFP-10 fragments from MTB culture media ($[M+H]^+$=1142.63).

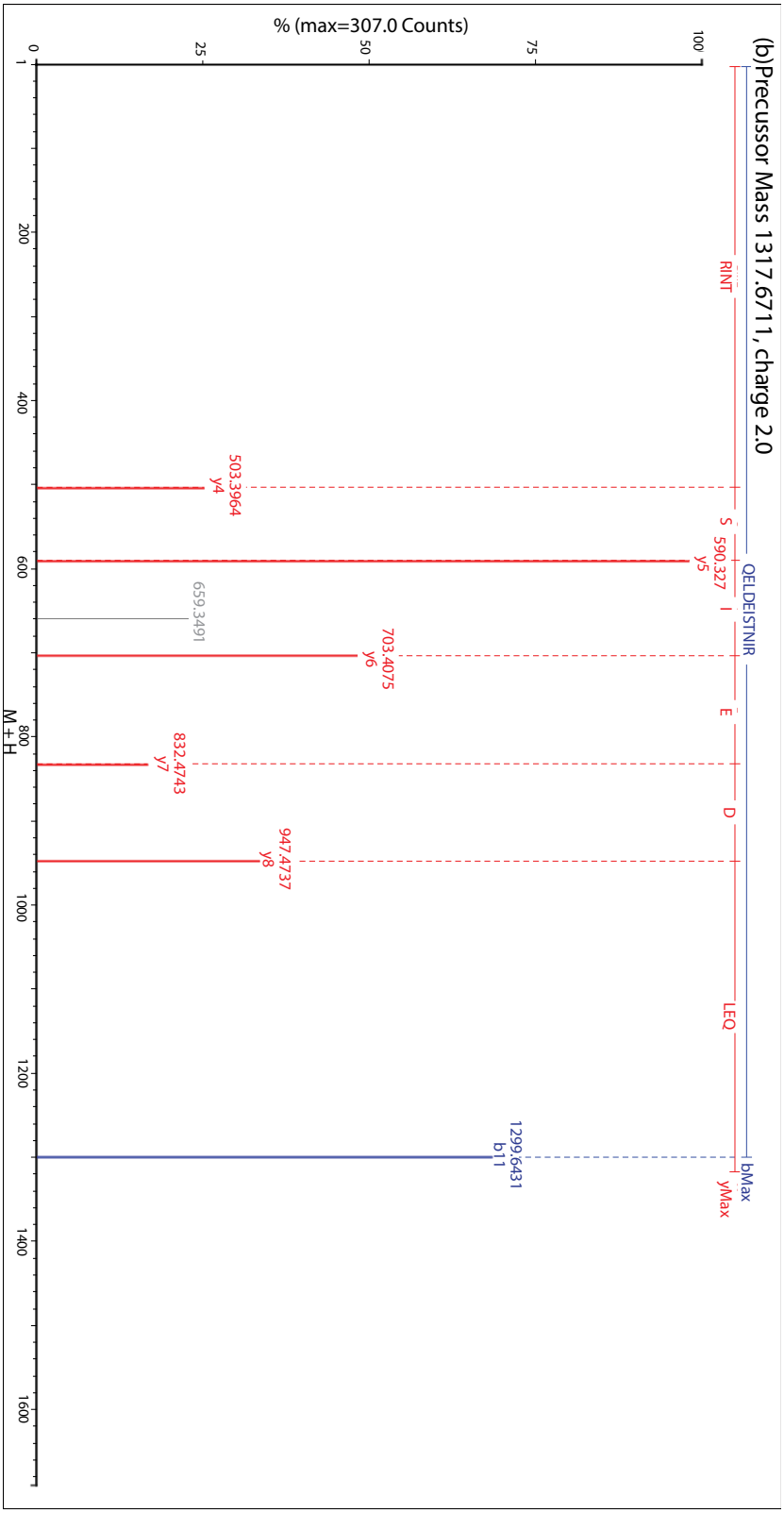

Figure S7 (b). LC-MS/MS spectra of CFP-10 fragments from MTB culture media ($[M+H]^+$=1317.66).

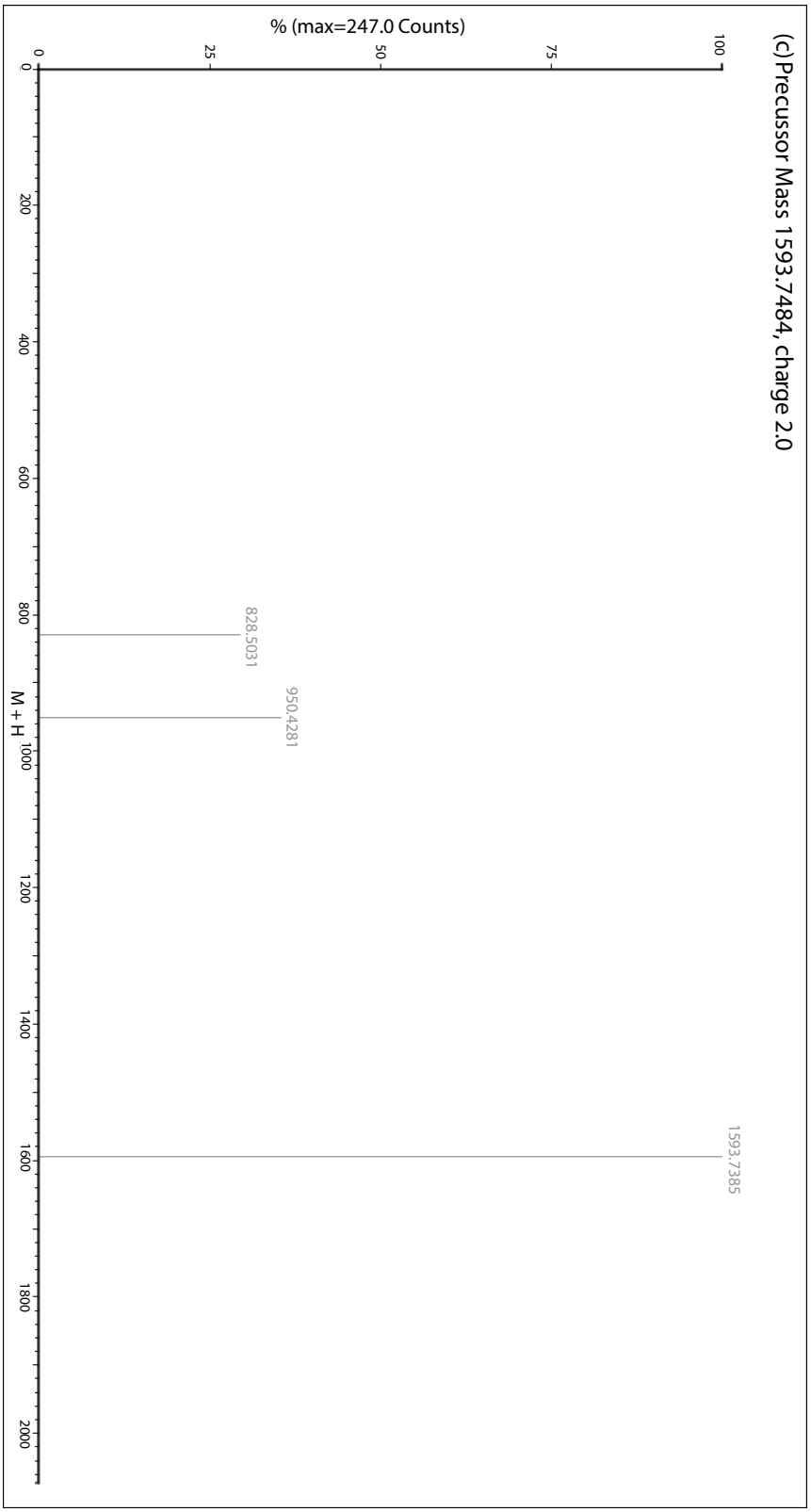

Figure S7 (c). LC-MS/MS spectra of CFP-10 fragments from MTB culture media ($[M+H]^+$=1593.75).

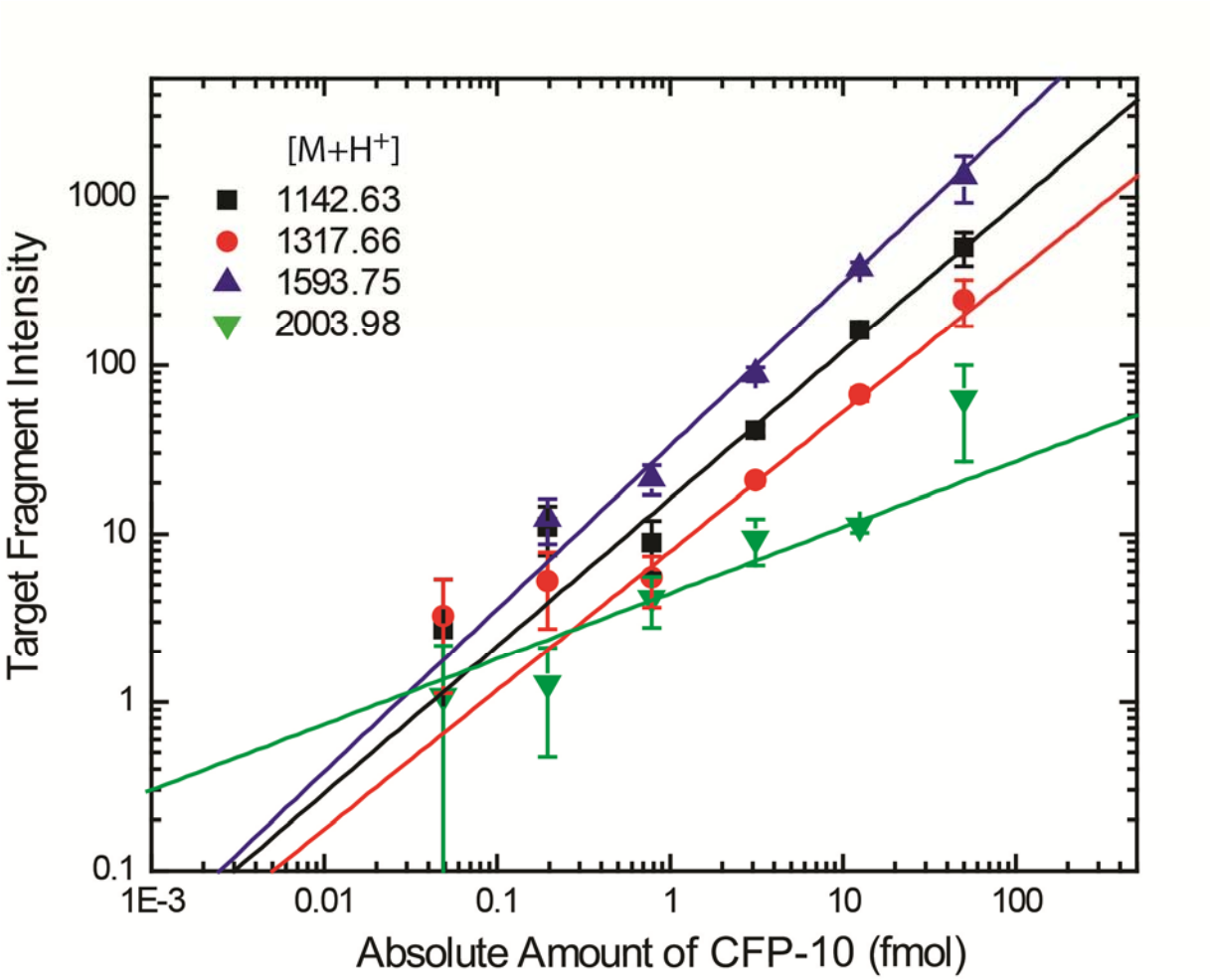

Figure S8. Titrating the detection curve for major CFP-10 fragments by MALDI TOF MS. 0.5µl of each known concentration of digested CFP-10 fragments were spotted on the MALDI TOF MS target plate. Under the operating conditions specified in Methods, we were able to detect the presence of CFP-10 at an amount as low as 0.2 fmol. [Average intensities are above background cut-off (mean of negative control + 3X standard deviation)].

Table S1: Inter-day accuracy and reproducibility of CFP-10 on-chip fractionation-MS analysis (1317.664 & 2003.978 fragments)

concentration (nM)	N	Fragments	Mean (ug/ml)	Standard Deviation	Precision (CV)	Accuracy (RE)
90.3	5	1317.664	1.7127	0.9940	58.03%	71.27%
		2003.978	0.0559	0.0351	62.66%	94.41%
11.3	5	1317.664	0.1361	0.0998	73.33%	8.91%
		2003.978	0.0559	0.0351	62.66%	55.24%
1.4	5	1317.664	0.6532	0.4959	75.91%	4080.41%
		2003.978	0.0031	0.0030	97.31%	80.20%

Table S2: Intra-day accuracy and reproducibility of CFP-10 on-chip fractionation-MS analysis. (1317.664 & 2003.978 fragments)

concentration (nM)	N	Fragments	Mean (ug/ml)	Standard Deviation	Precision (%CV)	Accuracy (%RE)
90.3	9	1317.664	1.2903	0.6716	52.05%	29.03%
		2003.978	0.0732	0.0412	56.32%	92.68%
11.3	9	1317.664	146.5446	78.1665	53.34%	17.24%
		2003.978	0.0732	0.0412	61.74%	41.44%
1.4	9	1317.664	0.4283	0.4536	105.90%	2641.13%
		2003.978	0.0029	0.0033	112.94%	81.30%

Table S3: The recipes of coating solution of various nanoporous films used in the paper.

Surfactant Polymer	**The amount of reactants in coating solution**			
	Silicate sol solution (ml)	Ethanol (ml)	polymer (g)	PPG (g)
L121	10	5	1.2	0
L121+25% PPG	10	10	1.2	0.3
L121+25% PPG (thin)	10	40	1.2	0.3
L121+50% PPG	10	10	1.2	0.6
L121+100% PPG	10	10	1.2	1.2
F127 (2D hexagonal)	7.5	10	1.8	0
L64	10	10	1.2	0

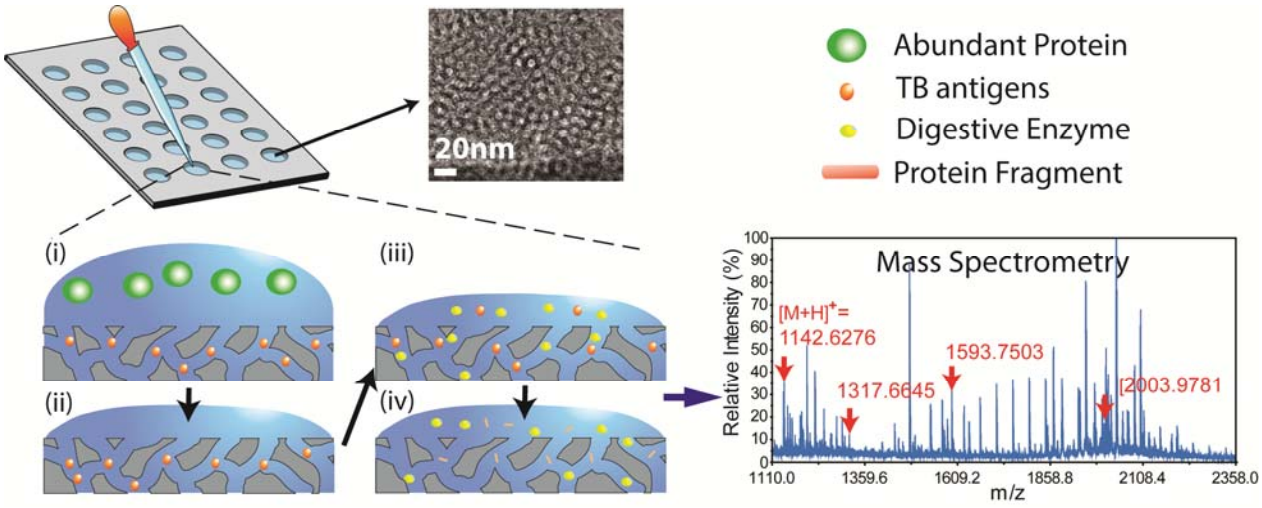

Table of Content