

## Supporting Information

### Trypsin coatings on electrospun and alcohol-dispersed polymer nanofibers for trypsin digestion column

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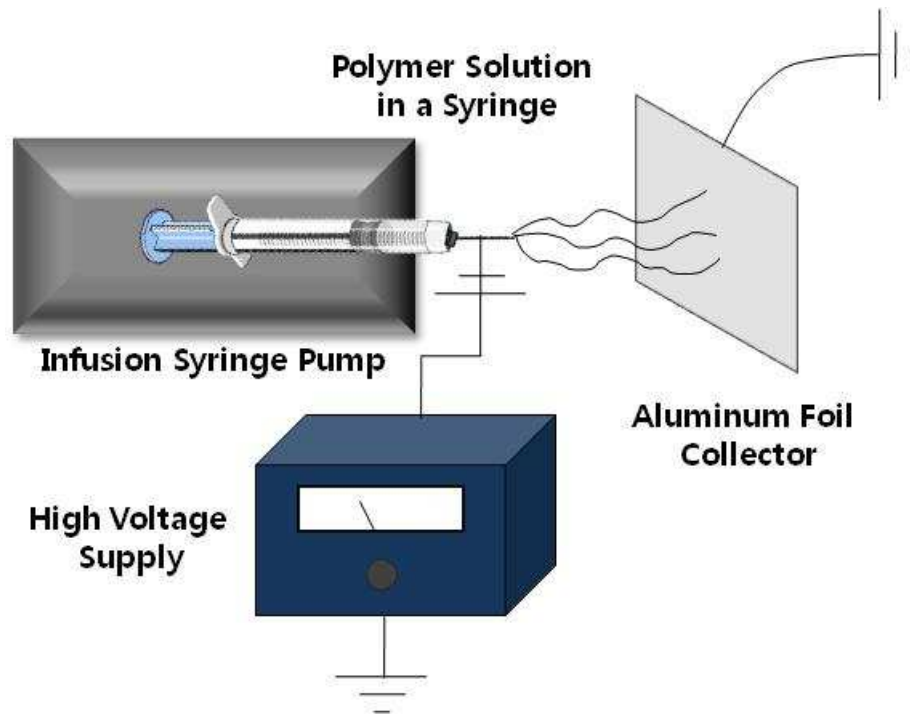
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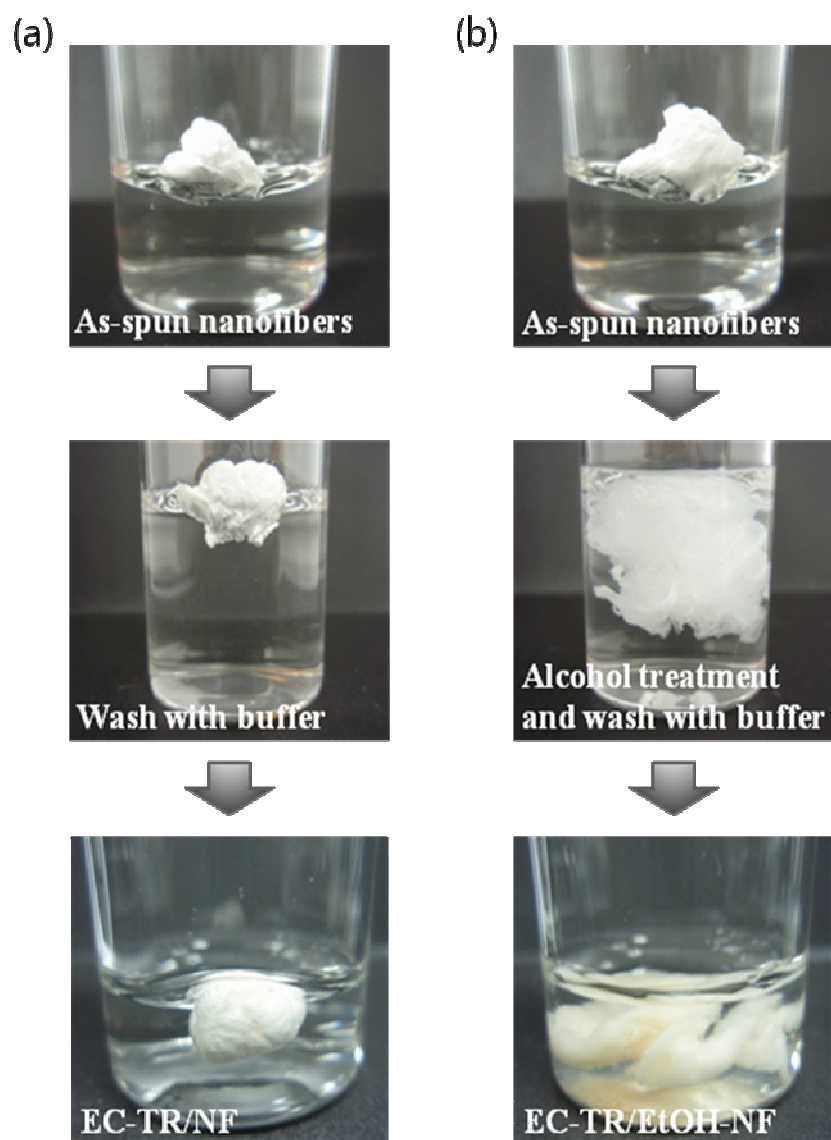
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**Table S-1.** The kinetic constants of trypsin coatings (EC-TR) on as-spun (NF) and alcohol-dispersed nanofibers (EtOH-NF).

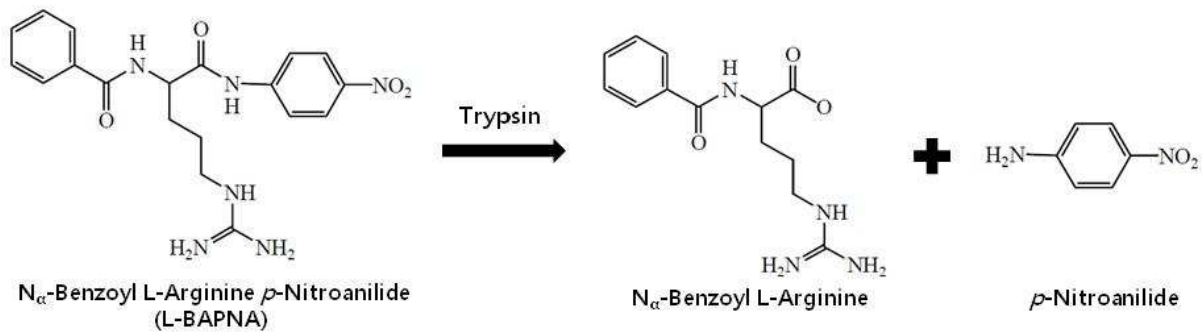
	EC-TR/NF	EC-TR/EtOH-NF	<u>Ratio</u> EC-TR/EtOH-NF to EC-TR/-NF
$V_{\max}$ ( $\times 10^3$ $\mu\text{M/s}$ )	<b>67 <math>\pm</math> 3</b>	<b>1240 <math>\pm</math> 90</b>	<b>19</b>
$K_m$ ( $\mu\text{M}$ )	<b>156 <math>\pm</math> 26</b>	<b>896 <math>\pm</math> 150</b>	<b>5.8</b>
$V_{\max}/K_m$ ( $\times 10^6$ $\text{s}^{-1}$ )	<b>430 <math>\pm</math> 75</b>	<b>1380 <math>\pm</math> 252</b>	<b>3.2</b>



**Figure S-1.** Schematic of electrospinning system [1].



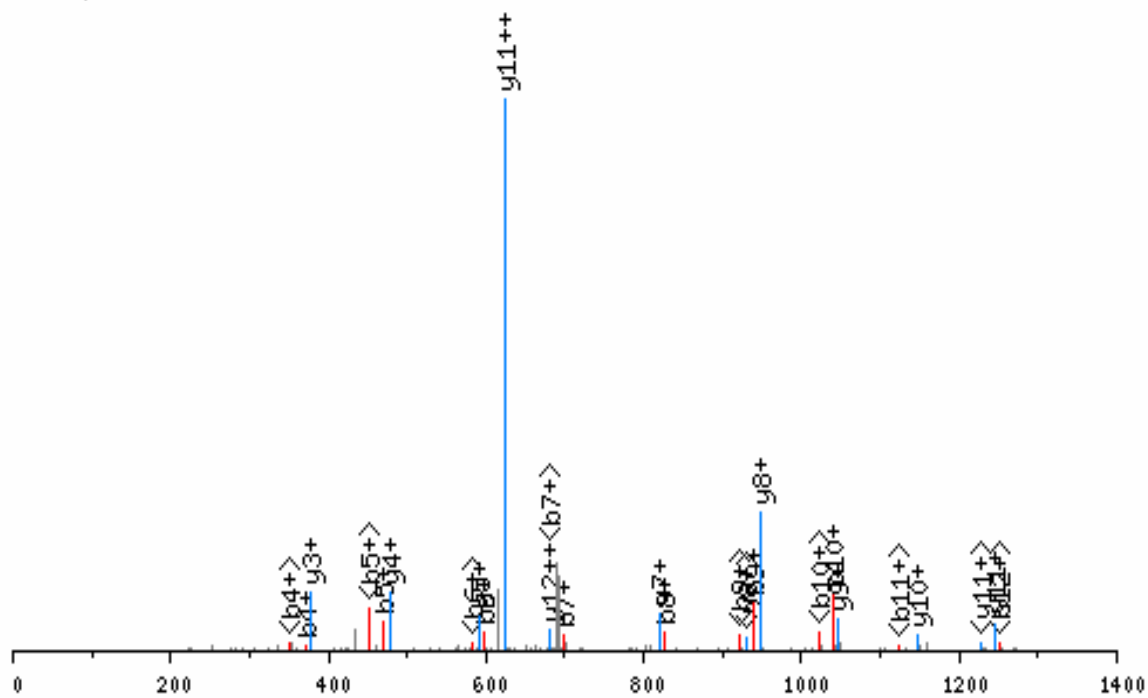
**Figure S-2.** Morphology of polymer nanofibers and trypsin-coated nanofibers; (a) trypsin coating on as-spun nanofibers and (b) trypsin coating on alcohol-dispersed nanofibers.



**Figure S-3.** Schematic for the trypsin-catalyzed hydrolysis of L-BAPNA [2].

GNPTVEVELTTEK, MH+ 1416.7222, m/z 708.8647  
KJB\_JSH\_EC\_A\_2uLmin\_080509.2621.2621.2.dta

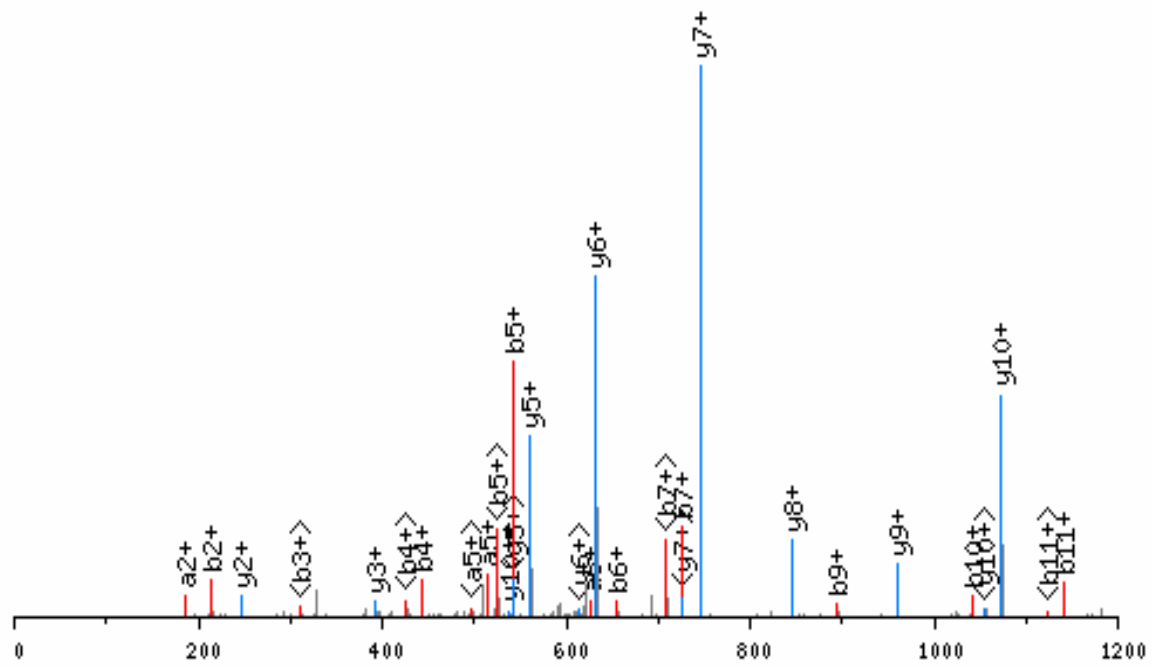
scan 0; 1.4e+004



**Figure S-4.** (a) MS/MS spectrum of fully tryptic peptide peak ( $m/z=708.87$ ) in Figures 3 and 4.

NVNDVIAPAFVK, MH+ 1286,7109, m/z 643,8591  
KJB\_JSH\_EC\_A\_2uLmin\_080509,3355,3355,2,dta

scan 0; 4.0e+004



**Figure S-4.** (b) MS/MS spectrum of fully tryptic peptide peak (m/z=643.86) in Figures 3 and 4.

TAGIQIVADDLTVTNPK, MH+ 1755.9493, m/z 878.4783  
KJB\_JSH\_EC\_A\_2uLmin\_080509,3569,3569,2,dta

scan 0; 8.6e+002

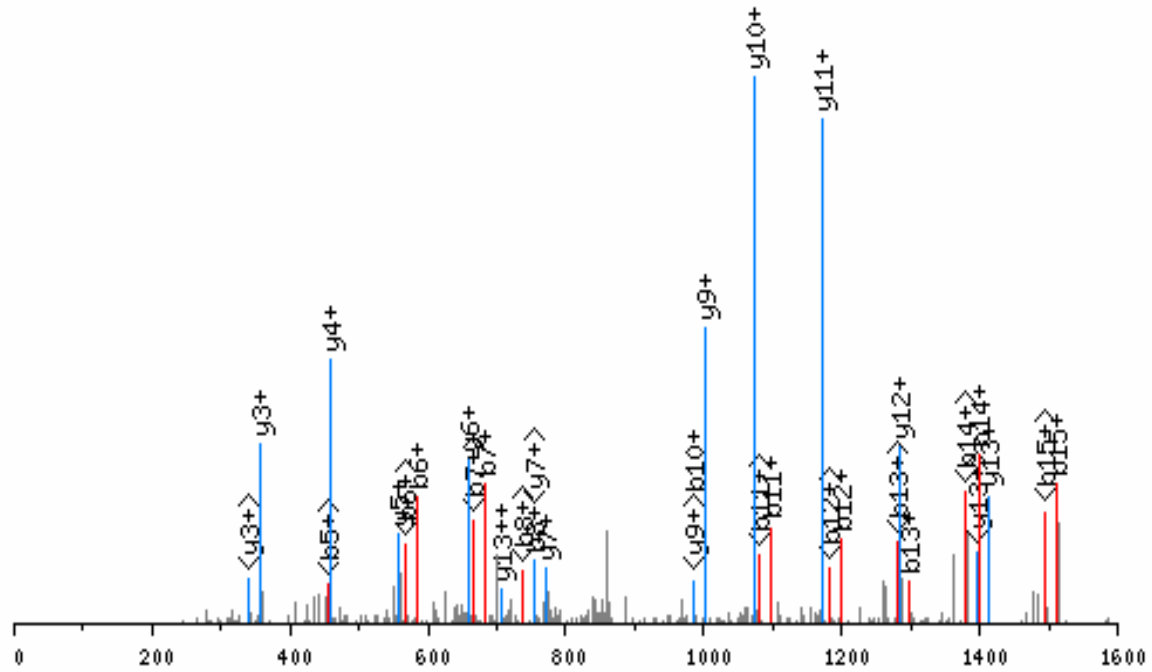
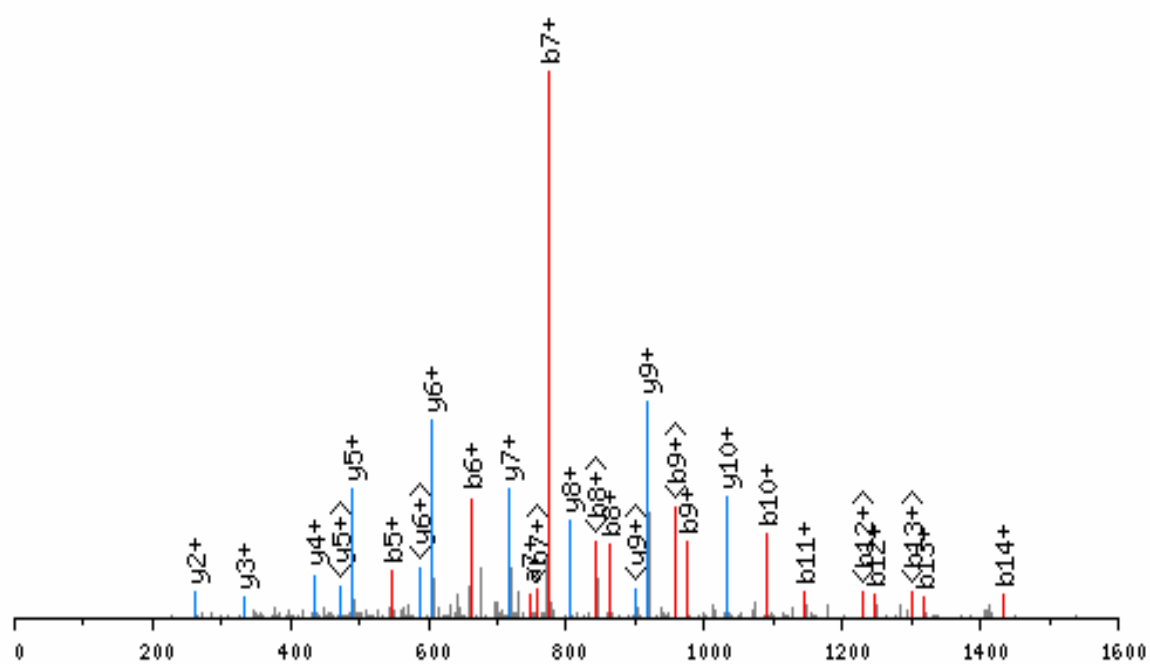


Figure S-4. (c) MS/MS spectrum of fully tryptic peptide peak (m/z=878.48) in Figures 3 and 4.



AVDDFLLSLDGTANK, MH+ 1578.8015, m/z 789.9044  
KJB\_JSH\_EC\_A\_2uLmin\_080509\_3983\_3983\_2.dta

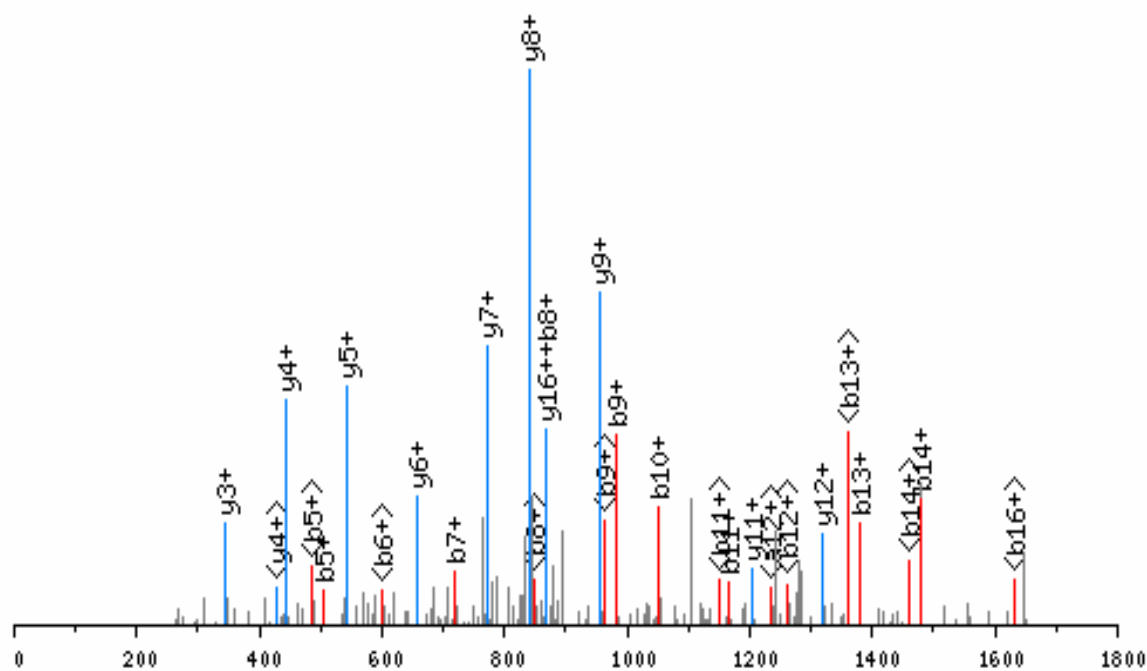
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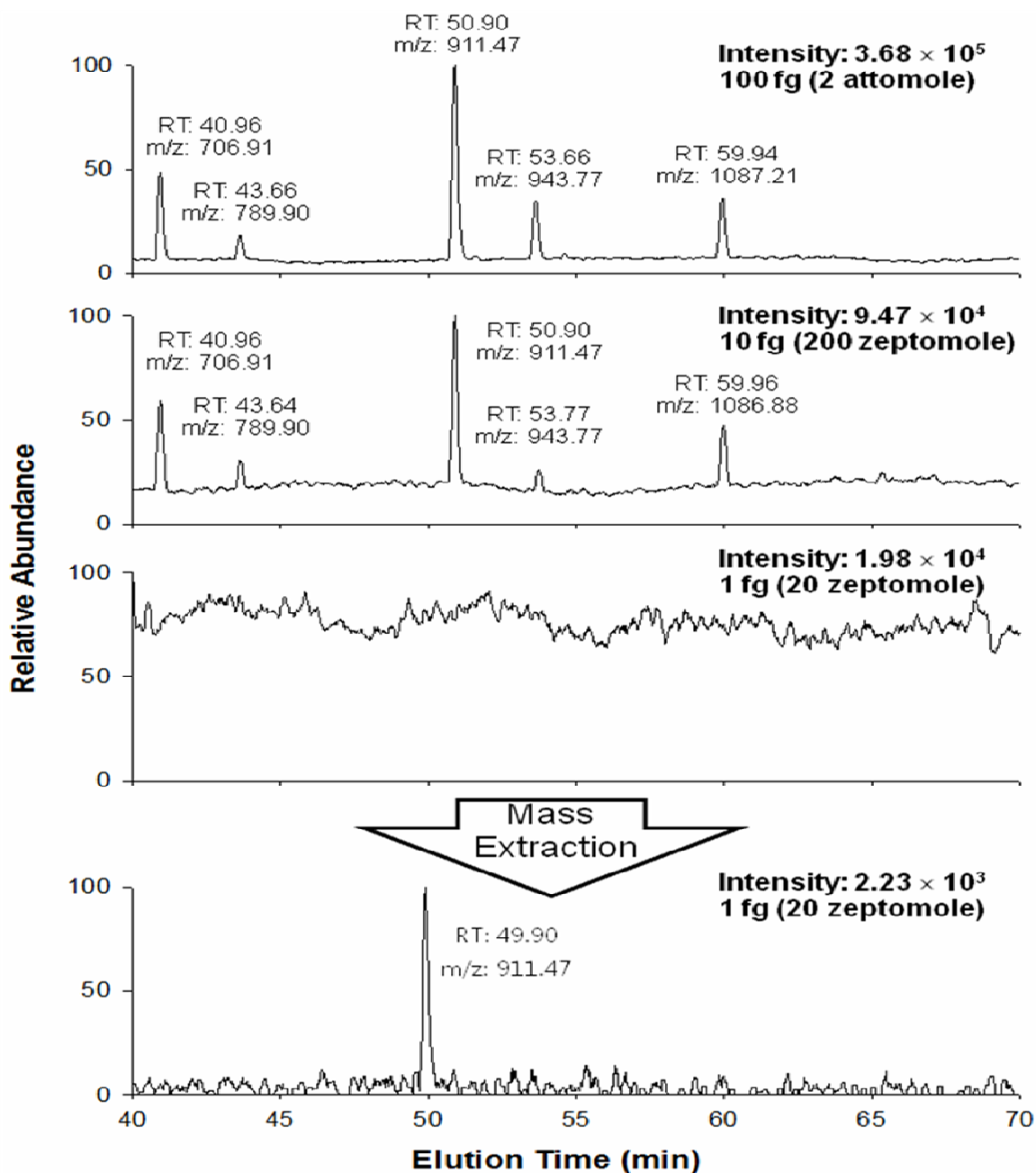
**Figure S-4.** (d) MS/MS spectrum of fully tryptic peptide peak (m/z=789.90) in Figures 3 and 4.

SGETEDTFIADLVVGLR, MH+ 1821.9234, m/z 911.4654  
KJB\_JSH\_EC\_A\_2uLmin\_080509\_4775\_4775\_2.dta

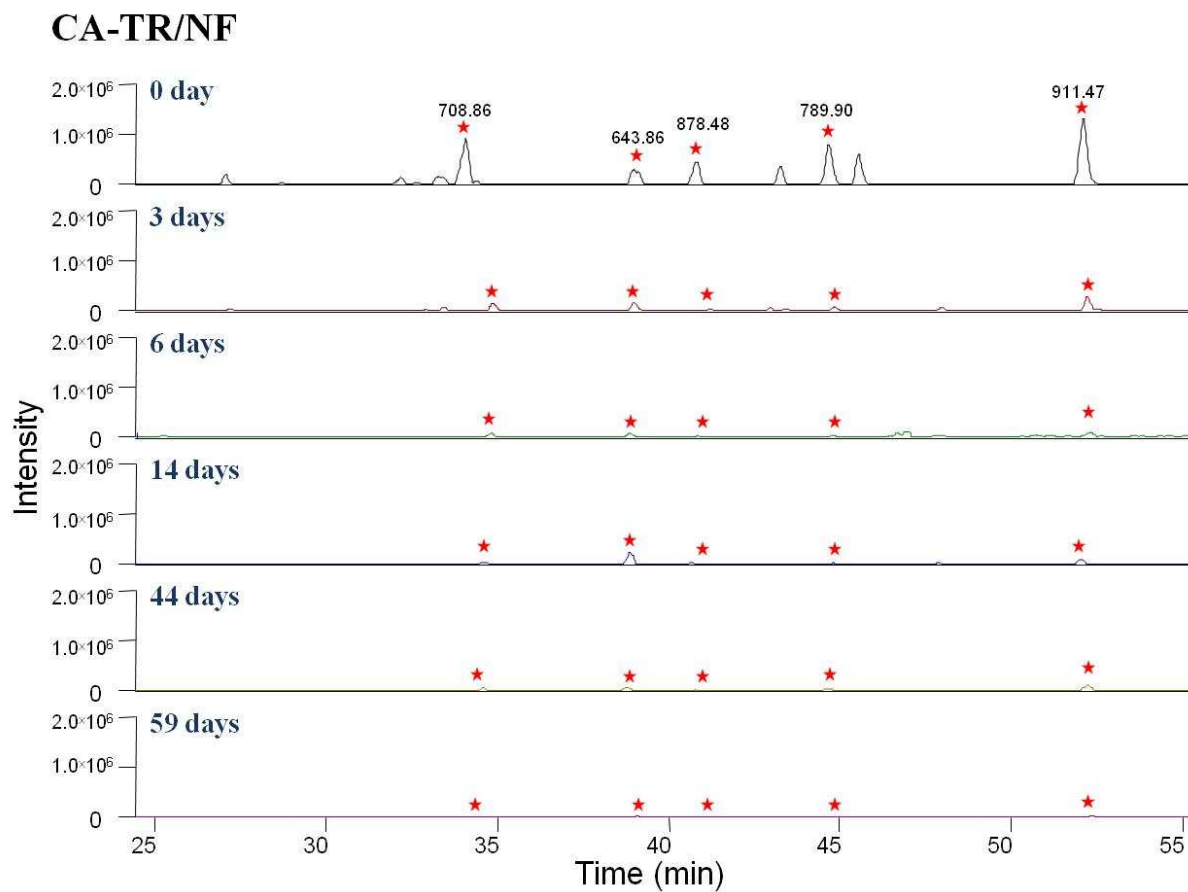
scan 0; 3.3e+002



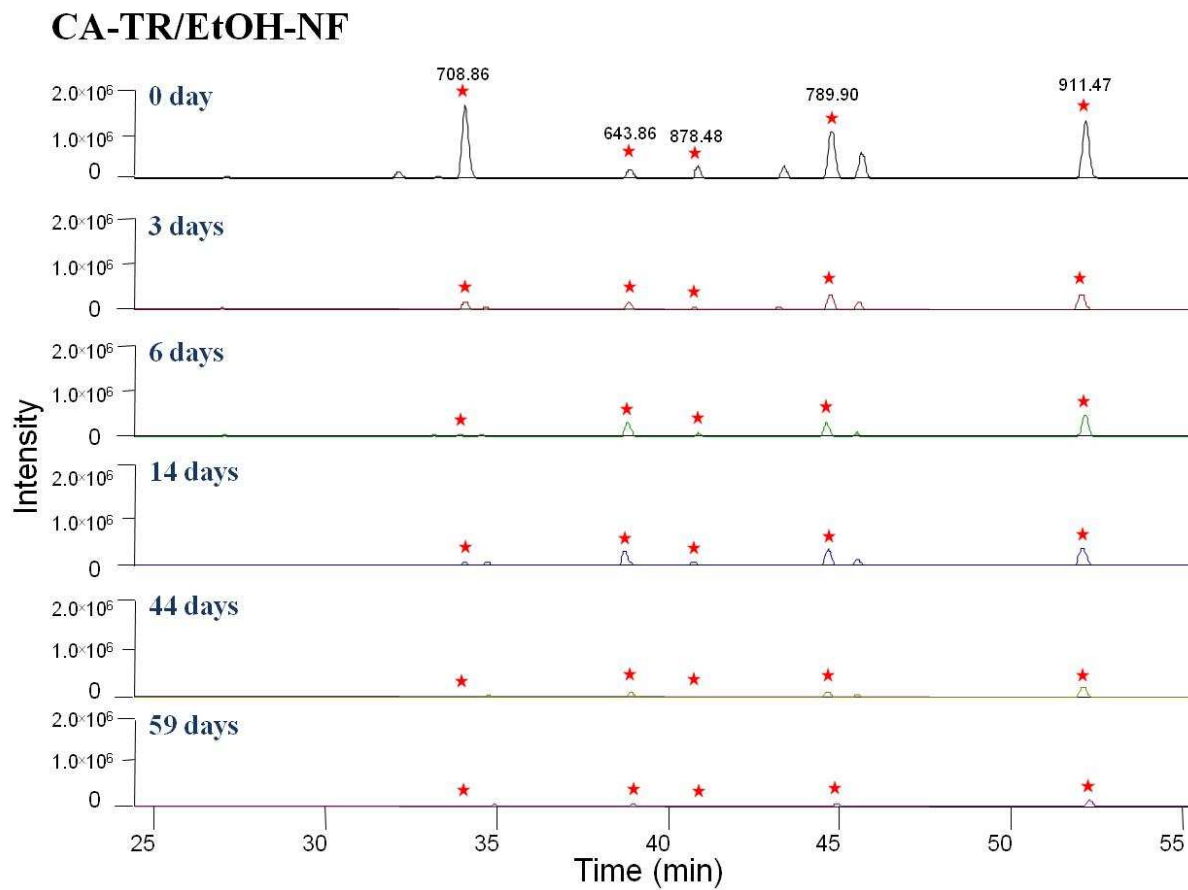
**Figure S-4.** (e) MS/MS spectrum of fully tryptic peptide peak (m/z=911.47) in Figures 3 and 4.



**Figure S-5.** Sensitivity test of the LC/MS/MS system described in experimental section. Using a tryptic enolase peptides as a standard sample, the current LC/MS/MS system exhibited sub-attomole sensitivity.

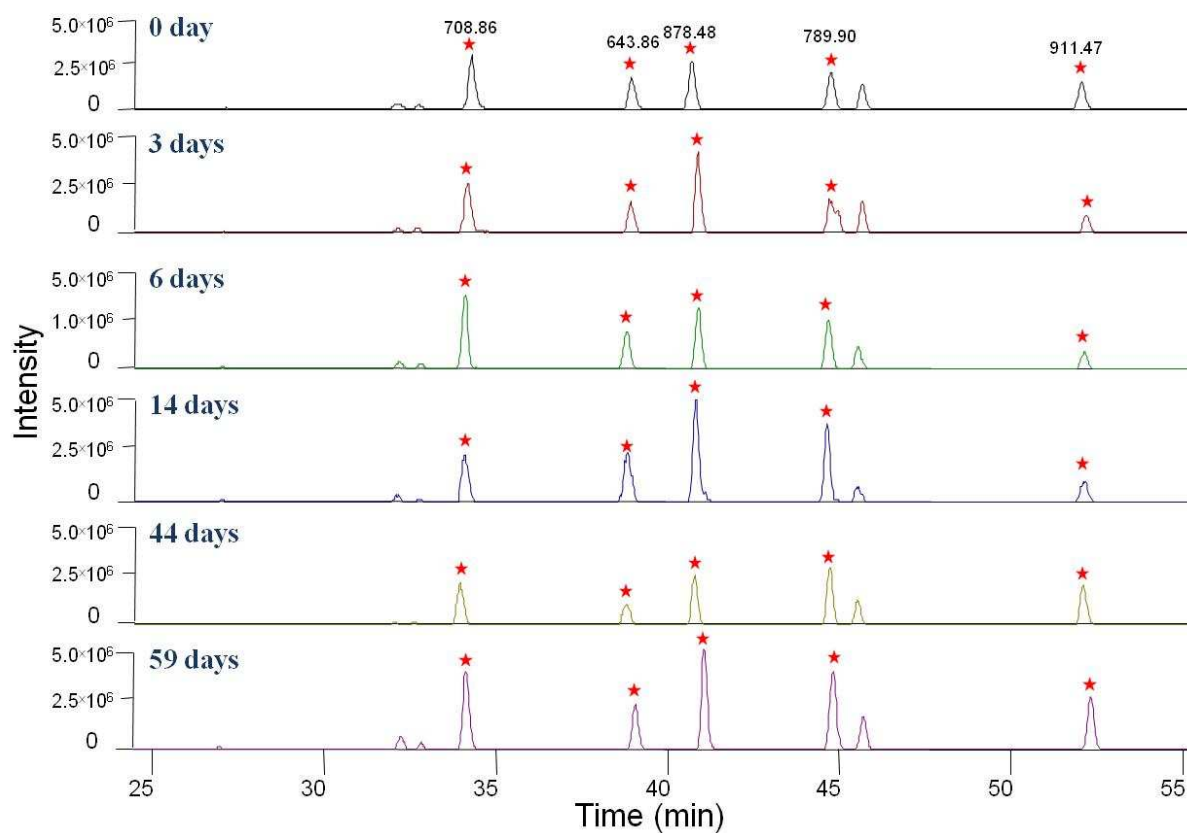


**Figure S-6.** (a) Performance stability of CA-TR/NF in the enolase digestion. Five enolase-specific peptides from extracted chromatogram were checked after enolase digestion using CA-TR/NF at each time point.



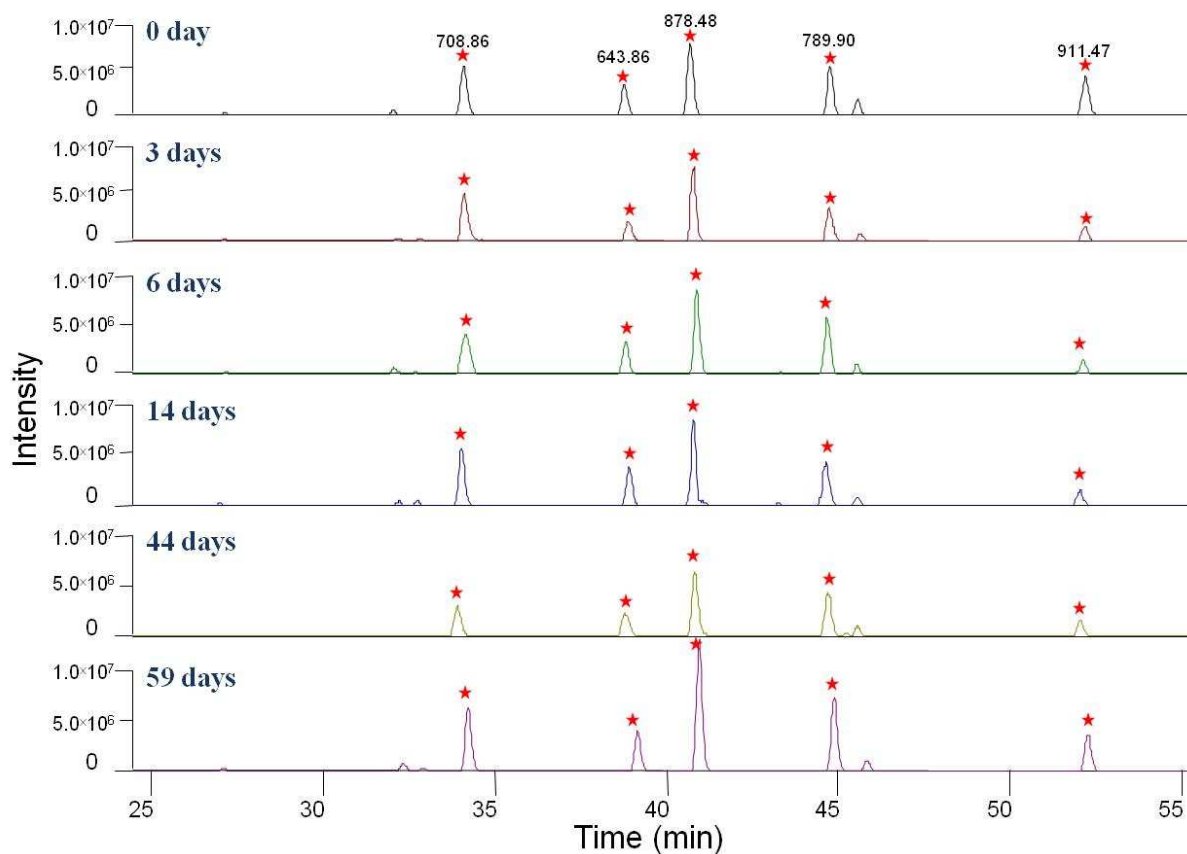
**Figure S-6.** (b) Performance stability of CA-TR/EtOH-NF in the enolase digestion. Five enolase-specific peptides from extracted chromatogram were checked after enolase digestion using CA-TR/EtOH-NF at each time point.

## EC-TR/NF

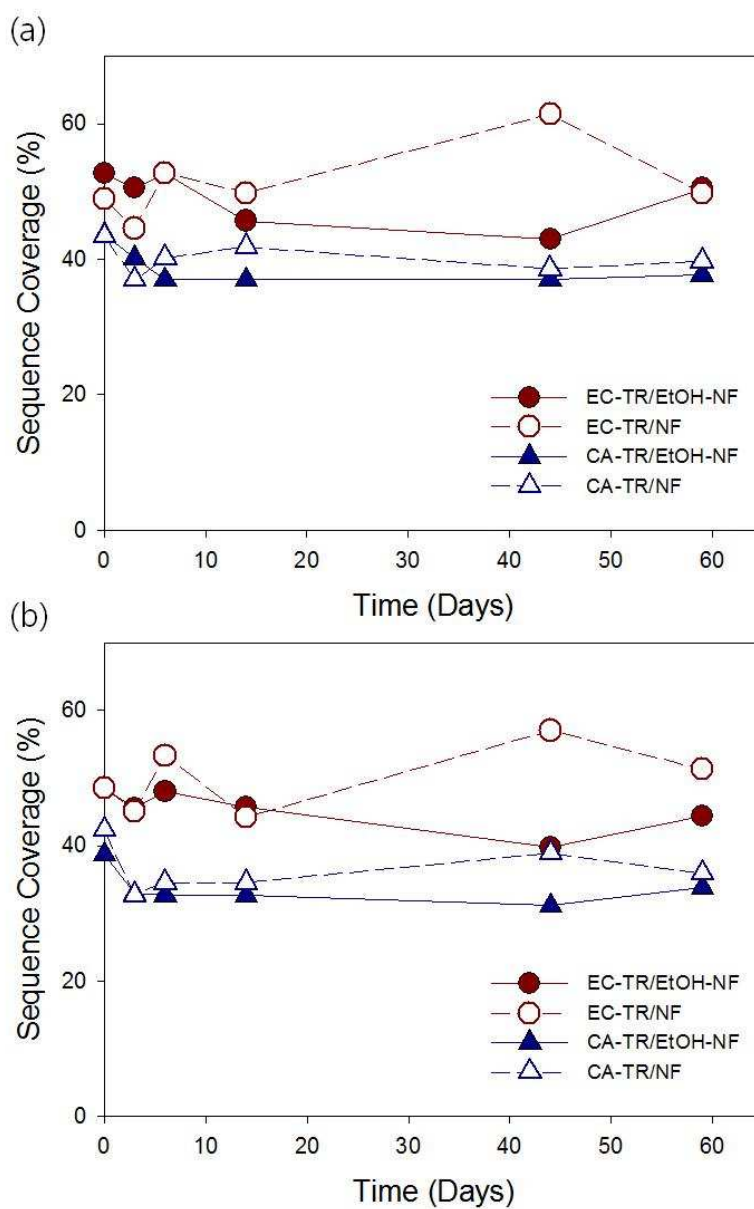


**Figure S-6.** (c) Performance stability of EC-TR/NF in the enolase digestion. Five enolase-specific peptides from extracted chromatogram were checked after enolase digestion using EC-TR/NF at each time point.

### EC-TR/EtOH-NF

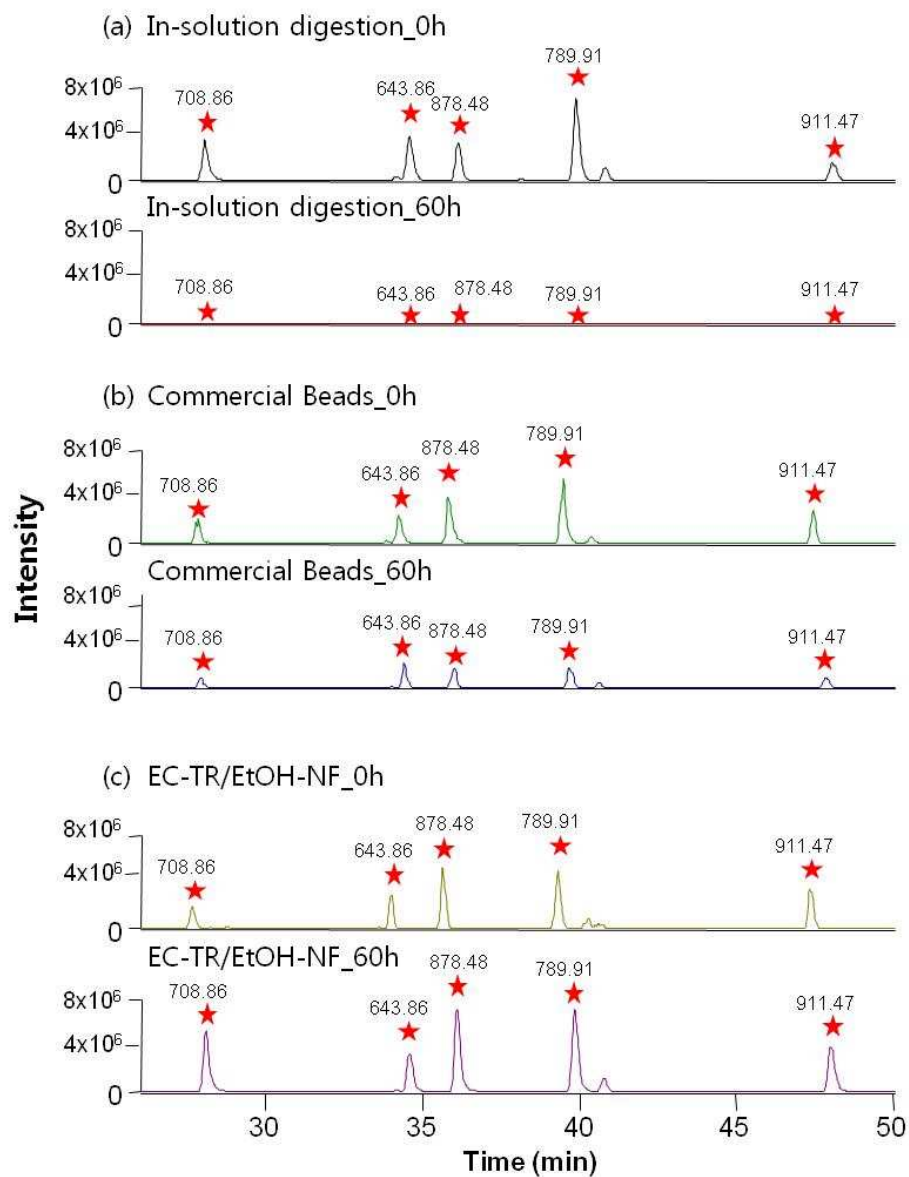


**Figure S-6.** (d) Performance stability of EC-TR/EtOH-NF in the enolase digestion. Five enolase-specific peptides from extracted chromatogram were checked after enolase digestion using EC-TR/EtOH-NF at each time point.



**Figure S-7.** The time-course of protein sequence coverage for enolase 1 (a) and enolase 2 (b) by using trypsin nanofibers under recycled uses. Figure 3 shows the same results by using the total peak area of five enolase-specific peptides instead of the protein sequence coverage.





**Figure S-8.** Digestion performance of (a) in-solution digestion, (b) commercial trypsin beads, and (c) EC-TR/EtOH-NF. In-vial enolase digestion was performed at 50°C. Five enolase-specific peptides from extracted chromatogram were checked after enolase digestion using each sample at each time point.

## REFERENCES

- [1] Kim, B. C.; Nair, S.; Kim, J.; Kwak, J. H.; Grate, J. W.; Kim, S. H.; Gu, M. B. *Nanotechnology* **2005**, *16*, S382-S388.
- [2] Kim, B. C.; Lopez-Ferrer, D.; Lee, S. M.; Ahn, H. K.; Nair, S.; Kim, S. H.; Kim, B. S.; Petritis, K.; Camp, D. G.; Grate, J. W.; Smith, R. D.; Koo, Y. M.; Gu, M. B.; Kim, J. *Proteomics* **2009**, *9*, 1893-1900.