Electronic Supplementary Material (ESI) for Analyst.


Supplementary Material

# Evaluation of Individual Aging Degree by Standard-Free, Label-Free LC-MS/MS Quantification of Formaldehyde-modified Peptides

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# Contents

# 1. Optimization of Tryptic Digestion and $^{18}O$-Labeling Conditions

In order to establish the screening model, 51 peptides (summarized in Table S1) in different retention time were selected randomly to optimize the digestion and $^{18}O$-labeling condition.

## 1.1 Optimization of Tryptic Digestion Conditions

Both of the modified and control HSA samples were digested in the different trypsin to protein ratio (w/w) and digestion time. The trypsin to HSA ratio was from 1:10 to 1:125 and the digestion time was from 0.5 to 40 h. HPLC-ESI/TOF MS analysis was performed to determinate the amount of the 51 peptides (see Table S1). The different trypsin to protein ratios and the digestion times were investigated as shown in Fig. S1 and S2.

In Fig. S1, the horizontal axis represented the different trypsin to protein ratios and the longitudinal axis represented fold change relative to trypsin to protein ratio of 1:50 (w/w). The fold change was calculated based on the peak area of peptides relative to trypsin to protein ratio of 1:50 (w/w) during HPLC-ESI/TOF MS analysis. There was no significant difference among the different ratios, so the optimized trypsin to protein ratio was 1:50 (w/w).

In Fig. S2, the horizontal axis represented the different digestion times and the longitudinal axis represented fold change relative to 24 h digestion. It can be seen that the complete digestion was finished and reached a plateau at 24 h. Finally, a 28 h digestion was chosen to digest modified HSA and control HSA in order to ensure full digestion.

### 1.2 Optimization of $^{18}O$-Labeling Conditions

The effect of urea concentration on labeling quality was needed to be detected. Urea could inhibit the $^{18}O$-labeling activity of trypsin with changing protein structure. Figure S3 measures the effects of urea concentration during the labeling reaction on the average and standard deviation of the peptide labeling percentage. The standard deviation reflects the amount of variability in the labeling percentage. It could be seen in Fig. S3 that when the urea concentration was increased from 0.5 M to 1 M, the labeling percentage has increased slightly; and then, the labeling percentage has decreased with the urea concentration was increased by 2 M; when the urea concentration was increased continually by 3 M and 4 M, the labeling percentage has decreased significantly and the SD values got larger as well. Finally, urea concentration was considered under 1 M to ensure has no negative effect on labeling efficiency.

Besides urea concentration, the pH value of the labeling buffer was also investigated in this model. Figure S4 shows the effects of the pH value of the buffer on the labeling quality. The labeling percentages were different with an increase in pH value of the 50 mM PBS buffers from 4.0 to 7.0. As a result, the optimal pH value for labeling was maintained using a PBS buffer of 50 mM at pH 4.0. A relative low concentration of buffer (50 mM) was chosen here for potentially better ionization efficiency in MS detection next.

In addition, the labeling time was investigated, and the results were shown in Fig. S5. During the first 12 h, the average on labeling percentage was increased with the

labeling time. After 12 h, almost all peptides achieved the largest labeling percentage, and the labeling efficiency would not be improved by extend the labeling time. In our study, 16 h was chosen to be the best labeling time to ensure completely labeling.

In order to realize complete inactivation of trypsin after labeling, samples were put in a boiling water bath for 10 min then 5 % (*v* %) formic acid was added into each sample. Table S2 showed the labeling efficiency of 10 randomly selected peptides before and after mixed with equal volume of $H_2{}^{16}O$.

Finally, the labeling quality under the optimized conditions was evaluated. The labeling efficiency of HSA-peptides in formaldehyde-induced aging model was 97.8 ± 0.9 %. For some peptides, their labeling efficiency was measured larger than the theoretical labeling efficiency, due to errors introduced by the software analysis. The distribution of labeling efficiency was shown in Fig. S6. It can be seen that almost all peptides achieved or closed to their labeling balance, and the efficiency of all peptides was higher than 92 %. In addition, the labeling efficiency of 51 peptides was used for calculating the precision. After digestion and labeling, the same HSA-peptides sample was injected 5 times, and the intra-day precision was 1.02 %. Subsequently, the sample was injected continually for 5 days, and the inter-day precision was 2.03 %.

## 2. Verification of the Formaldehyde-sensitive Peptides

Formaldehyde-induced modification is dependent on the concentration of formaldehyde and has an accumulation effect. As a result, the concentration of a formaldehyde-sensitive peptide in modified HSA samples will be a function of the formaldehyde concentration and the incubation duration.

Table S3-S6 showed the relative intensity (peak area ratio $^{16}$O-to-$^{18}$O of the HPLC-TOF MS data) of variations of selected peptide ions (can be steadily detected in each MS run) in modified HSA samples.

The comparing quantitative results of formaldehyde-sensitive peptides with/without internal standard and labeling have been performed to demonstrate the standard-free and label-free method. The accuracy, precision and repeatability of methodology have been investaged. All detailed information was supplied in Section 2 and Table S7 of supplementary materials. It can be seen from the comparing experimets that the developed standard-free and lable-free method can give a simiar quantitative results with using $^{18}$O-labeling technique.

## 3. OPA Assay

OPA (*o*-phthalaldehyde) assay is another common method to detect the concentration of amine group besides TNBS assay.

OPA reagent was prepared first: dissolved 40 mg OPA in 1 mL MeOH with adding 2.5 mL 20 % (w %) SDS, 25 mL 0.1 mol·$L^{-1}$ Borax ($Na_2B_4O_7 \cdot 10H_2O$) and 100 μL β-mercaptoethanol, and then the solution was diluted with distilled water to 50 mL. OPA reagent (4 mL) was added in the digested HSA smaple (the same samples as used in TNBS assay, 200 μL for each, 1 μg·$\mu L^{-1}$) to react 2 min at room temperature, and then detected at 340 nm. OPA reagent with adding 200 μL distilled water was used as a control, and *L*-leucine was used to make a standard curve. The quantitative results were given Figure S7.

Both Fig. S7 and Fig. 2 have shown the similar quantitative results of free amine group. The little difference between OPA assay and TNBS assay might be caused by different procedures. These results demonstrated that the reaction between lysine in HSA with formaldehyde was happened.

## Related Tables

Table S1. All peptides matched by Mascot searching in database.

| Entry | Peptide Location | *m/z* | z | Peptide Sequence | MASCOT Score |
|---|---|---|---|---|---|
| 1 | 25–34 | 575.3 | 2 | DAHKSEVAHR | 9 |
| 2 | 35–44 | 614.8 | 2 | FKDLGEENFK | 38 |
| 3 | 37–44 | 476.3 | 2 | DLGEENFK | 37 |
| 4 | 45–65 | 1217.2 | 2 | ALVLIAFAQYLQQCPFEDHVK | 27 |
| 5 | 45–65 | 811.9 | 3 | ALVLIAFAQYLQQCPFEDHVK | 24 |
| 6 | 66–75 | 575.3 | 2 | LVNEVTEFAK | 56 |
| 7 | 76–88 | 692.9 | 2 | TCVADESAENCDK | 99 |
| 8 | 89–105 | 938.3 | 2 | SLHTLFGDKLCTVATLR | 48 |
| 9 | 98–105 | 438.2 | 2 | LCTVATLR | 40 |
| 10 | 98–117 | 1090.1 | 2 | LCTVATLRETYGEMADCCAK | 16 |
| 11 | 106–117 | 660.9 | 2 | ETYGEMADCCAK | 39 |
| 12 | 131–138 | 470.7 | 2 | DDNPNLPR | 40 |
| 13 | 139–160 | 1297.7 | 2 | LVRPEVDVMCTAFHDNEETFLK | 27 |
| 14 | 161–168 | 528.4 | 2 | KYLYEIAR | 25 |
| 15 | 162–168 | 464.3 | 2 | YLYEIAR | 31 |
| 16 | 169–183 | 950.1 | 2 | RHPYFYAPELLFFAK | 33 |
| 17 | 185–198 | 775.0 | 2 | YKAAFTECCQAADK | 51 |
| 18 | 187–205 | 652.0 | 3 | AAFTECCQAADKAACLLPK | 29 |
| 19 | 187–205 | 977.4 | 2 | AAFTECCQAADKAACLLPK | 40 |
| 20 | 206–214 | 537.7 | 2 | LDELRDEGK | 31 |
| 21 | 243–249 | 438.2 | 2 | LSQRFPK | 25 |
| 22 | 250–257 | 440.8 | 2 | AEFAEVSK | 39 |
| 23 | 258–264 | 395.2 | 2 | LVTDLTK | 41 |
| 24 | 265–281 | 958.5 | 2 | VHTECCHGDLLECADDR | 27 |
| 25 | 265–286 | 1207.6 | 2 | VHTECCHGDLLECADDRADLAK | 12 |
| 26 | 287–298 | 694.3 | 2 | YICENQDSISSK | 60 |
| 27 | 299–310 | 717.2 | 2 | LKECCEKPLLEK | 44 |
| 28 | 301–310 | 596.3 | 2 | ECCEKPLLEK | 36 |
| 29 | 311–337 | 973.5 | 3 | SHCIAEVENDEMPADLPSLAADFVESK | 35 |
| 30 | 342–360 | 1150.6 | 2 | NYAEAKDVFLGMFLYEYAR | 58 |
| 31 | 348–360 | 812.5 | 2 | DVFLGMFLYEYAR | 60 |

| | | | | | |
|---|---|---|---|---|---|
| 32 | 376–383 | 492.8 | 2 | TYETTLEK | 32 |
| 33 | 397–413 | 1023.1 | 2 | VFDEFKPLVEEPQNLIK | 33 |
| 34 | 397–413 | 682.4 | 3 | VFDEFKPLVEEPQNLIK | 38 |
| 35 | 414–426 | 801.0 | 2 | QNCELFEQLGEYK | 70 |
| 36 | 414–434 | 848.5 | 3 | QNCELFEQLGEYKFQNALLVR | 28 |
| 37 | 427–434 | 480.8 | 2 | FQNALLVR | 53 |
| 38 | 438–452 | 820.5 | 2 | KVPQVSTPTLVEVSR | 51 |
| 39 | 438–452 | 547.5 | 3 | KVPQVSTPTLVEVSR | 40 |
| 40 | 439–452 | 756.9 | 2 | VPQVSTPTLVEVSR | 44 |
| 41 | 461–468 | 458.2 | 2 | CCKHPEAK | 20 |
| 42 | 469–490 | 854.4 | 3 | RMPCAEDYLSVVLNQLCVLHEK | 32 |
| 43 | 470–496 | 1020.7 | 3 | MPCAEDYLSVVLNQLCVLHEKTPVSDR | 26 |
| 44 | 491– 499 | 501.9 | 2 | TPVSDRVTK | 31 |
| 45 | 500–508 | 512.8 | 2 | CCTESLVNR | 53 |
| 46 | 509–524 | 927.6 | 2 | RPCFSALEVDETYVPK | 44 |
| 47 | 509–524 | 618.8 | 3 | RPCFSALEVDETYVPK | 38 |
| 48 | 549–558 | 565.2 | 2 | KQTALVELVK | 63 |
| 49 | 550–558 | 500.9 | 2 | QTALVELVK | 41 |
| 50 | 570–581 | 672.8 | 2 | AVMDDFAAFVEK | 44 |
| 51 | 599–609 | 507.4 | 2 | LVAASQAALGL | 70 |

Table S2. Labeling efficiency of randomly selected $^{18}O$-labeled peptide before/after mixed with $H_2{}^{16}O$

| Entry | $^{18}O$-Labeled Peptides (*m/z*) | Labeling Efficiency (%) | |
|---|---|---|---|
| | | Before mixed with $H_2{}^{16}O$ | After mixed with $H_2{}^{16}O$ |
| 1 | 952.1 | 97.41 | 95.65 |
| 2 | 442.8 | 98.96 | 97.07 |
| 3 | 397.2 | 96.44 | 93.05 |
| 4 | 814.5 | 98.98 | 94.80 |
| 5 | 662.9 | 97.44 | 96.59 |
| 6 | 777.0 | 90.28 | 86.68 |
| 7 | 442.8 | 96.41 | 92.71 |
| 8 | 577.3 | 92.28 | 90.71 |
| 9 | 1152.6 | 95.96 | 91.05 |
| 10 | 822.8 | 95.93 | 94.26 |
| Mean value | | 96.01 | 93.26 |
| SD value | | 2.67 | 3.16 |

Table S3. Peptide concentration variations ($^{16}$O-to-$^{18}$O ratios) for modified HSA samples after incubation with 249.0 mM formaldehyde for different days (n=6).

| Entry | *m/z* | $^{16}$O-to-$^{18}$O ratios after incubation (± SD) | | |
|---|---|---|---|---|
| | | 10 days | 20 days | 30 days |
| 1 | 575.3 | 0.463 ( ± 0.016) | 0.328 ( ± 0.021) | **0.219 ( ± 0.014)**[a]** |
| 2 | 614.8 | 0.765 ( ± 0.042) | 0.701 ( ± 0.036) | **0.555 ( ± 0.018)**** |
| 3 | 811.9 | 0.381 ( ± 0.033) | 0.201 ( ± 0.028)** | **0.181 ( ± 0.023)** |
| 4 | 438.2 | 0.280 ( ± 0.038) | 0.274 ( ± 0.026) | 0.227 ( ± 0.045) |
| 5 | 660.8 | 0.518 ( ± 0.063) | 0.445 ( ± 0.037) | 0.443 ( ± 0.051) |
| 6 | 464.3 | 0.410 ( ± 0.070) | 0.251 ( ± 0.046) * | **0.226 ( ± 0.024)** |
| 7 | 634.0 | 0.805 ( ± 0.076) | 0.439 ( ± 0.049)** | **0.434 ( ± 0.021)** |
| 8 | 537.7 | 0.574 ( ± 0.028) | 0.496 ( ± 0.047)* | **0.278 ( ± 0.015)**** |
| 9 | 440.7 | 0.519 ( ± 0.047) | 0.504 ( ± 0.036) | **0.259 ( ± 0.019)**** |
| 10 | 395.2 | 0.595 ( ± 0.073) | 0.326 ( ± 0.032)** | **0.309 ( ± 0.053)** |
| 11 | 694.3 | 0.508 ( ± 0.052) | 0.392 ( ± 0.047)* | **0.355 ( ± 0.032)** |
| 12 | 596.8 | 0.520 ( ± 0.086) | 0.399 ( ± 0.022)* | **0.244 ( ± 0.026)**** |
| 13 | 812.5 | 0.882 ( ± 0.056) | 0.542 ( ± 0.120)** | **0.462 ( ± 0.085)** |
| 14 | 490.2 | 0.304 ( ± 0.064) | 0.245 ( ± 0.042) | 0.194 ( ± 0.029)[b] |
| 15 | 492.8 | 0.337 ( ± 0.037) | 0.310 ( ± 0.025)* | 0.302 ( ± 0.024) |
| 16 | 691.8 | 0.322 ( ± 0.016) | 0.219( ± 0.036)** | 0.211 ( ± 0.051) |
| 17 | 801.0 | 0.405 ( ± 0.072) | 0.378 ( ± 0.041) | 0.246 ( ± 0.061)* |
| 18 | 820.5 | 0.723 ( ± 0.075) | 0.659 ( ± 0.042) | 0.623 ( ± 0.022) |
| 19 | 512.8 | 0.476 ( ± 0.066) | 0.392 (± 0.042) | 0.342 ( ± 0.061) |
| 20 | 500.9 | 0.329 ( ± 0.021) | 0.288 ( ± 0.037) | 0.261( ± 0.024) |
| 21 | 672.8 | 0.449 ( ± 0.060) | 0.326 ( ± 0.082) | **0.314 ( ± 0.011)** |
| 22 | 1023.1 | 0.732( ± 0.036) | 0.659 ( ± 0.039)* | 0.633 ( ± 0.061) |
| 23 | 682.4 | 0.732( ± 0.016) | 0.659 ( ± 0.021)** | **0.632 ( ± 0.027)** |
| 24 | 848.5 | 0.621 ( ± 0.077) | 0.536( ± 0.056) | 0.519 ( ± 0.038) |
| 25 | 565.2 | 0.472 ( ± 0.059) | 0.391 ( ± 0.042) | 0.359 ( ± 0.061) |
| 26 | 507.4 | 0.761 ( ± 0.039) | 0.461 ( ± 0.027)** | **0.455 ( ± 0.031)** |

* Represent values showed significant different from the previous group ($p < 0.05$);

** Represent values showed highly significant different from the previous group ($p < 0.01$);

[a] Boldface means values were highly significant different between 10/30 days groups ($p < 0.01$);

[b] Underline means values were significant different between 10/30 days groups ($p < 0.05$).

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Table S4. Peptide concentration variations ($^{16}$O-to-$^{18}$O ratios) for modified HSA samples after incubation with 49.8 mM formaldehyde for different days (n=6).

| Entry | *m/z* | $^{16}$O-to-$^{18}$O ratios after incubation (± SD) | | |
|---|---|---|---|---|
| | | 10 days | 20 days | 30 days |
| 1 | 575.3 | 0.659 ( ± 0.025) | 0.443 ( ± 0.043)** | **0.421 ( ± 0.031)**[a] |
| 2 | 614.8 | 0.772 ( ± 0.009) | 0.588 ( ± 0.021)** | **0.539 ( ± 0.042)** |
| 3 | 464.3 | 0.682 ( ± 0.020) | 0.491 ( ± 0.024)** | **0.426 ( ± 0.035)*** |
| 4 | 634.0 | 0.911 ( ± 0.106) | 0.722 ( ± 0.079)* | 0.698 ( ± 0.031)[b] |
| 5 | 537.7 | 0.681 ( ± 0.058) | 0.514 ( ± 0.027)** | **0.507 ( ± 0.020)** |
| 6 | 395.2 | 0.614 ( ± 0.035) | 0.598 ( ± 0.083) | **0.419 ( ± 0.007)*** |
| 7 | 694.3 | 0.711 ( ± 0.041) | 0.504 ( ± 0.037)** | **0.468 ( ± 0.012)** |
| 8 | 596.8 | 0.672 ( ± 0.056) | 0.503 ( ± 0.033)** | **0.477( ± 0.035)** |
| 9 | 812.5 | 0.885 ( ± 0.039) | 0.721 ( ± 0.079)* | **0.702 ( ± 0.042)** |
| 10 | 490.2 | 0.418 ( ± 0.044) | 0.319 ( ± 0.022)* | 0.294 ( ± 0.052) |
| 11 | 492.8 | 0.629 ( ± 0.052) | 0.580 ( ± 0.009) | 0.512 ( ± 0.044)* |
| 12 | 691.8 | 0.359 ( ± 0.046) | 0.187( ± 0.131)* | **0.168 ( ± 0.025)** |
| 13 | 820.5 | 0.817 ( ± 0.028) | 0.814 ( ± 0.009) | 0.656 ( ± 0.081)* |
| 14 | 672.8 | 0.619 ( ± 0.010) | 0.427 ( ± 0.028)** | **0.411 ( ± 0.051)** |
| 15 | 1023.1 | 0.912( ± 0.026) | 0.859 ( ± 0.007)* | **0.833 ( ± 0.011)*** |
| 16 | 682.4 | 0.918( ± 0.021) | 0.86 ( ± 0.004)** | **0.835 ( ± 0.007)**** |
| 17 | 565.2 | 0.783 ( ± 0.044) | 0.396 ( ± 0.034)** | **0.394 ( ± 0.076)** |
| 18 | 507.4 | 0.824 ( ± 0.033) | 0.671 ( ± 0.051)** | 0.655 ( ± 0.104) |

* Represent values showed significant different from the previous group ($p < 0.05$);

** Represent values showed highly significant different from the previous group ($p < 0.01$);

[a] Boldface means values were highly significant different between 10/30 days groups ($p < 0.01$);

[b] Underline means values were significant different between 10/30 days groups ($p < 0.05$)

Table S5. Peptide concentration variations ($^{16}$O-to-$^{18}$O ratios) for modified HSA samples after incubation with 24.9 mM formaldehyde for different days (n=6).

| Entry | *m/z* | $^{16}$O-to-$^{18}$O ratios after incubation (± SD) | | |
|---|---|---|---|---|
| | | 10 days | 20 days | 30 days |
| 1 | 575.3 | 0.662 ( ± 0.125) | 0.461 ( ± 0.113) | 0.337 ( ± 0.152)[a] |
| 2 | 614.8 | 0.972 ( ± 0.104) | 0.878 ( ± 0.121)** | **0.839 ( ± 0.142)**[b] |
| 3 | 464.3 | 0.704 ( ± 0.034) | 0.583 ( ± 0.045)* | 0.562 ( ± 0.078) |
| 4 | 634.0 | 0.939 ( ± 0.026) | 0.922 ( ± 0.039) | 0.898 ( ± 0.056) |
| 5 | 537.7 | 0.687 ( ± 0.019) | 0.634 ( ± 0.007)** | **0.524 ( ± 0.034)**** |
| 6 | 395.2 | 0.845 ( ± 0.036) | 0.715 ( ± 0.026)** | 0.619 ( ± 0.107) |
| 7 | 694.3 | 0.903 ( ± 0.022) | 0.799 ( ± 0.005)** | **0.752 ( ± 0.047)** |
| 8 | 596.8 | 0.686 ( ± 0.016) | 0.614 ( ± 0.023)** | **0.598( ± 0.015)** |
| 9 | 812.5 | 0.904 ( ± 0.131) | 0.795 ( ± 0.062) | 0.788 ( ± 0.071) |
| 10 | 492.8 | 0.705 ( ± 0.018) | 0.694 ( ± 0.027) | **0.611 ( ± 0.012)**** |
| 11 | 820.5 | 0.831 ( ± 0.026) | 0.829 ( ± 0.011) | 0.704 ( ± 0.054)** |
| 12 | 672.8 | 0.915 ( ± 0.110) | 0.655 ( ± 0.059)* | **0.507 ( ± 0.093)*** |
| 13 | 1023.1 | 0.952( ± 0.011) | 0.882 ( ± 0.010)** | **0.859 ( ± 0.022)** |
| 14 | 682.4 | 0.948( ± 0.017) | 0.884( ± 0.005)** | **0.858 ( ± 0.006)**** |

* Represent values showed significant different from the previous group ($p < 0.05$);

** Represent values showed highly significant different from the previous group ($p < 0.01$);

[a] Underline means values were significant different between 10/30 days groups ($p < 0.05$);

[b] Boldface means values were highly significant different between 10/30 days groups ($p < 0.01$).

Table S6. Peptide concentration variations ($^{16}$O-to-$^{18}$O ratios) for modified HSA samples after incubation with 6.0 mM formaldehyde for different days (n=6).

| Entry | *m/z* | $^{16}$O-to-$^{18}$O ratios after incubation (± SD) | | |
|---|---|---|---|---|
| | | 10 days | 20 days | 30 days |
| 1 | 575.3 | 0.662 ( ± 0.125) | 0.461 ( ± 0.113) | 0.337 ( ± 0.152)[a] |
| 2 | 614.8 | 0.972 ( ± 0.104) | 0.878 ( ± 0.121)** | **0.839 ( ± 0.142)**[b] |
| 3 | 811.9 | 0.381 ( ± 0.033) | 0.201 ( ± 0.028)** | **0.181 ( ± 0.023)** |
| 4 | 438.2 | 0.280 ( ± 0.038) | 0.274 ( ± 0.026) | 0.227 ( ± 0.045) |
| 5 | 660.8 | 0.518 ( ± 0.063) | 0.445 ( ± 0.037) | 0.443 ( ± 0.051) |
| 6 | 464.3 | 0.704 ( ± 0.034) | 0.583 ( ± 0.045)* | 0.562 ( ± 0.078) |
| 7 | 634.0 | 0.939 ( ± 0.026) | 0.922 ( ± 0.039) | 0.898 ( ± 0.056) |
| 8 | 537.7 | 0.785 ( ± 0.037) | 0.729 ( ± 0.011)* | **0.635 ( ± 0.008)**[a]** |
| 9 | 440.7 | 0.519 ( ± 0.047) | 0.504 ( ± 0.036) | **0.259 ( ± 0.019)**** |
| 10 | 395.2 | 0.845 ( ± 0.036) | 0.715 ( ± 0.026)** | 0.619 ( ± 0.107) |
| 11 | 694.3 | 0.991 ( ± 0.081) | 0.851 ( ± 0.045)* | 0.802 ( ± 0.077)[b] |
| 12 | 596.8 | 0.903 ( ± 0.007) | 0.854( ± 0.028)* | **0.648( ± 0.042)**** |
| 13 | 812.5 | 0.904 ( ± 0.131) | 0.795 ( ± 0.062) | 0.788 ( ± 0.071) |
| 14 | 490.2 | 0.418 ( ± 0.044) | 0.319 ( ± 0.022)* | 0.294 ( ± 0.052) |
| 15 | 492.7 | 0.847 ( ± 0.029) | 0.795 ( ± 0.062) | **0.611 ( ± 0.043)**** |
| 16 | 691.8 | 0.359 ( ± 0.046) | 0.187( ± 0.131)* | **0.168 ( ± 0.025)** |
| 17 | 801.0 | 0.405 ( ± 0.072) | 0.378 ( ± 0.041) | 0.246 ( ± 0.061)* |
| 18 | 820.5 | 0.831 ( ± 0.026) | 0.829 ( ± 0.011) | 0.704 ( ± 0.054)** |
| 19 | 512.8 | 0.476 ( ± 0.066) | 0.392 (± 0.042) | 0.342 ( ± 0.061) |
| 20 | 500.9 | 0.329 ( ± 0.021) | 0.288 ( ± 0.037) | 0.261( ± 0.024) |
| 21 | 672.8 | 0.972 ( ± 0.043) | 0.841 ( ± 0.027)** | **0.756 ( ± 0.063)*** |
| 22 | 1023.1 | 0.995( ± 0.003) | 0.985 ( ± 0.021) | **0.885 ( ± 0.018)**** |
| 23 | 682.4 | 0.997( ± 0.007) | 0.990( ± 0.017) | **0.886 ( ± 0.016)**** |
| 24 | 848.5 | 0.621 ( ± 0.077) | 0.536( ± 0.056) | 0.519 ( ± 0.038) |
| 25 | 565.2 | 0.783 ( ± 0.044) | 0.396 ( ± 0.034)** | **0.394 ( ± 0.076)** |
| 26 | 507.4 | 0.824 ( ± 0.033) | 0.671 ( ± 0.051)** | 0.655 ( ± 0.104) |

* Represent values showed significant different from the previous group ($p < 0.05$);

** Represent values showed highly significant different from the previous group ($p < 0.01$);

[a] Boldface means values were highly significant different between 10/30 days groups ($p < 0.01$);

[b] Underline means values were significant different between 10/30 days groups ($p < 0.05$).

Table S7. The comparing quantification of formaldehyde-sensitive peptides LDELRDEGK (LK) and VFDEFKPLVEEPQNLIK (VK) with/without standard and labeling (n=6).

| Peptide | Incubation condition | Conversion rate (%)[a] | | Methodology validation | | |
|---|---|---|---|---|---|---|
| | | by $^{18}$O-labeling | by IS[b] | accuracy | precision | repeatability |
| LK | 6.0 mM, 10 days | 21.5 ± 3.7 | 23.8 ± 1.1 | 0.18 | 0.84 | 0.42 |
| | 49.8 mM, 20 days | 48.6 ± 2.7 | 46.9 ± 1.8 | 0.21 | 1.27 | 0.51 |
| | 249.0 mM, 30 days | 72.2 ± 1.5 | 71.2 ± 2.8 | 0.31 | 1.89 | 0.47 |
| VK | 6.0 mM, 10 days | 0.3 ± 0.7 | 0.6 ± 0.2 | 0.26 | 0.15 | 0.07 |
| | 49.8 mM, 20 days | 14.0 ± 0.4 | 14.6 ± 0.6 | 0.13 | 0.44 | 0.20 |
| | 249.0 mM, 30 days | 36.8 ± 2.7 | 36.6 ± 1.1 | 0.45 | 0.82 | 0.38 |

[a] Conversion rate = $\frac{amount\ of\ modified\ peptide}{total\ amount\ of\ unmodifed\ peptide} \times 100\ \%$;

[b] IS: formaldehyde-insensitive peptide AAFTECCQAADKAACLLPK was served as the internal standard.

Table S8. Basic characteristics of plasma supplier.

| Characteristics | Young Group | Mid-aged Group | Elderly Group |
|---|---|---|---|
| *n* | 87 | 120 | 46 |
| Age | 23 – 40 | 41 – 60 | > 61 |
| Sexuality (M/F) | 48 / 39 | 66 / 54 | 18 / 28 |
| BMI ($kg/m^2$)[a] | | | |
| Male | 22.76 ± 2.47 | 26.5 ± 2.47‡ | 23.8 ± 2.91 |
| Female | 20.71 ± 1.89 | 25.9 ± 2.62‡ | 24.2 ± 3.20# |
| Overwight (%) | | | |
| Male | 13.9 | 46.3‡ | 20.97* |
| Female | 9.6 | 29.0‡ | 26.94# |
| SBP[b] | 117.82 ± 17.62 | 130.24 ± 15.12‡ | 136.84 ± 18.32# |
| DBP[c] | 73.79 ± 9.62 | 78.52 ± 10.04 | 82.37 ± 9.63*# |
| Dyslipidemia (%) | | | |
| Male | 7.8 | 46.5‡ | 36.7# |
| Female | 4.3 | 29.4‡ | 31.4# |
| GLU (mmol/L) | All < 6.1 | All < 6.1 | All < 6.1 |
| HB | All negative | All negative | All negative |

[a] Values were expressed as mean value;

[b] SBP = systolic blood pressure;

[c] DBP = diastolic blood pressure;

* Represent significant differences between Elderly Group and Mid-aged Group;

# Represent significant differences between Elderly Group and Young Group;

‡ Represent significant differences between Mid-aged Group and Young Group.

As shown in Table S8, the body mass index (BMI), the percentage of overweight, blood pressure and the percentage of dyslipidemia showed significant difference among the three groups.

## Related Figures

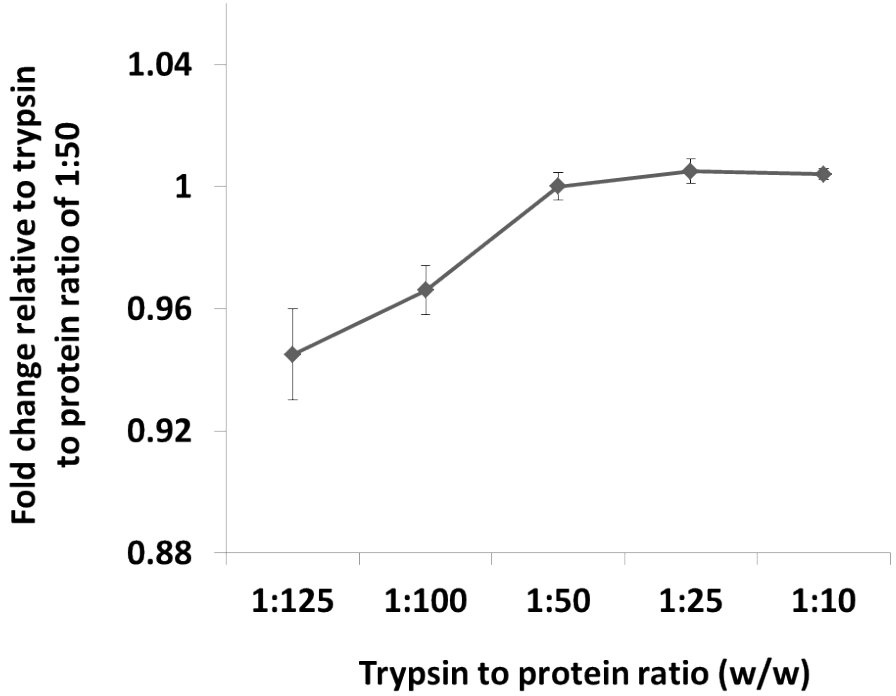


**Fig. S1** Investigation of digestion efficiency with different trypsin to protein ratio.

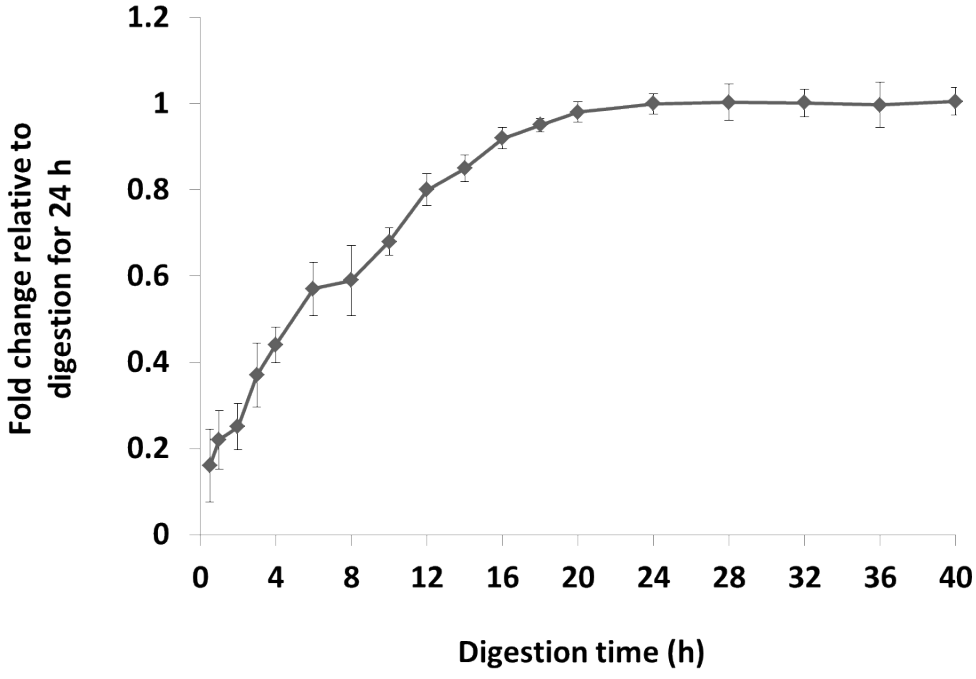


**Fig. S2** Investigation of digestion efficiency with different digestion time.

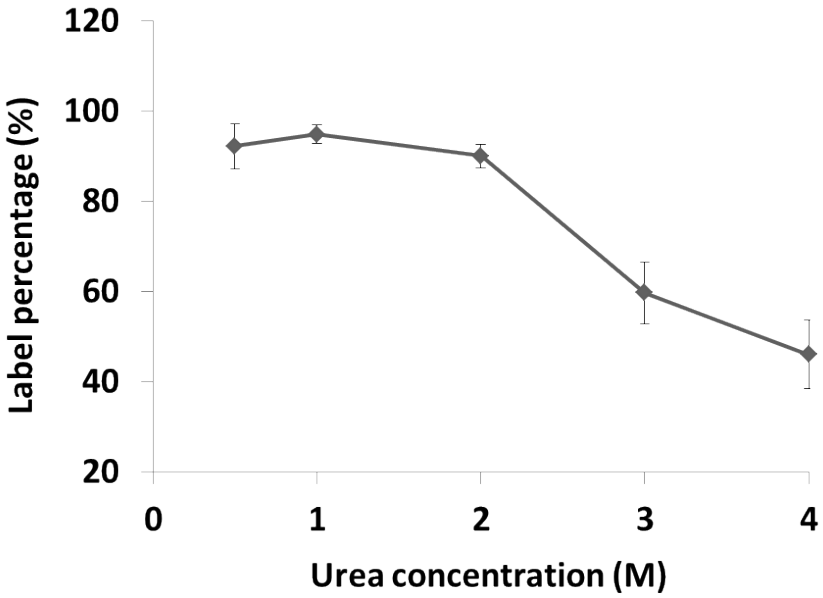


**Fig. S3** Investigation of labeling efficiency with different urea concentration.

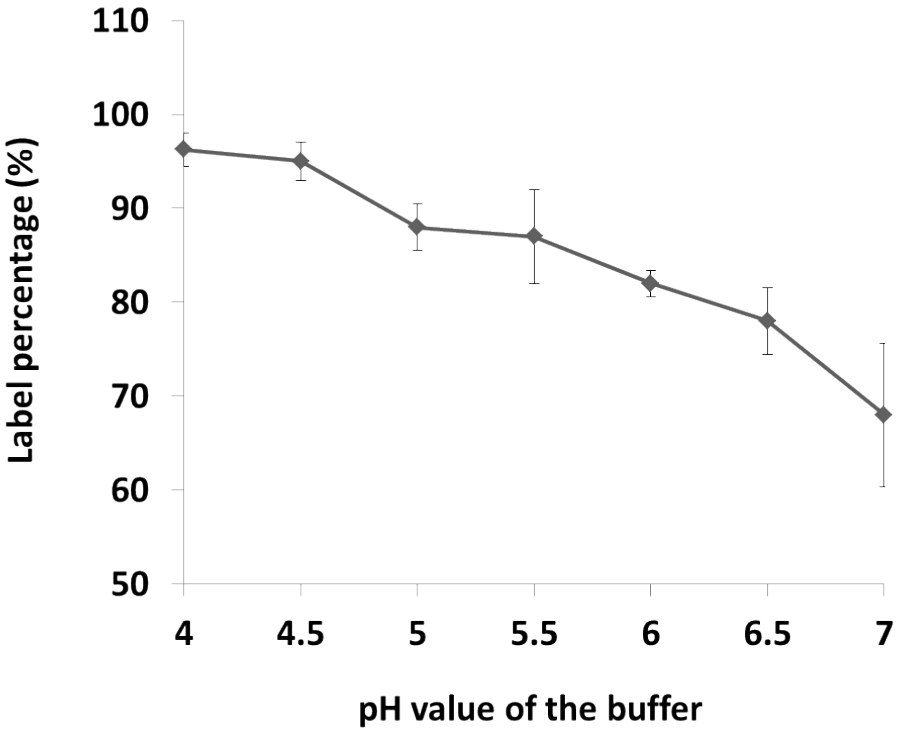


**Fig. S4** Investigation of labeling efficiency in PBS buffer with different pH value.

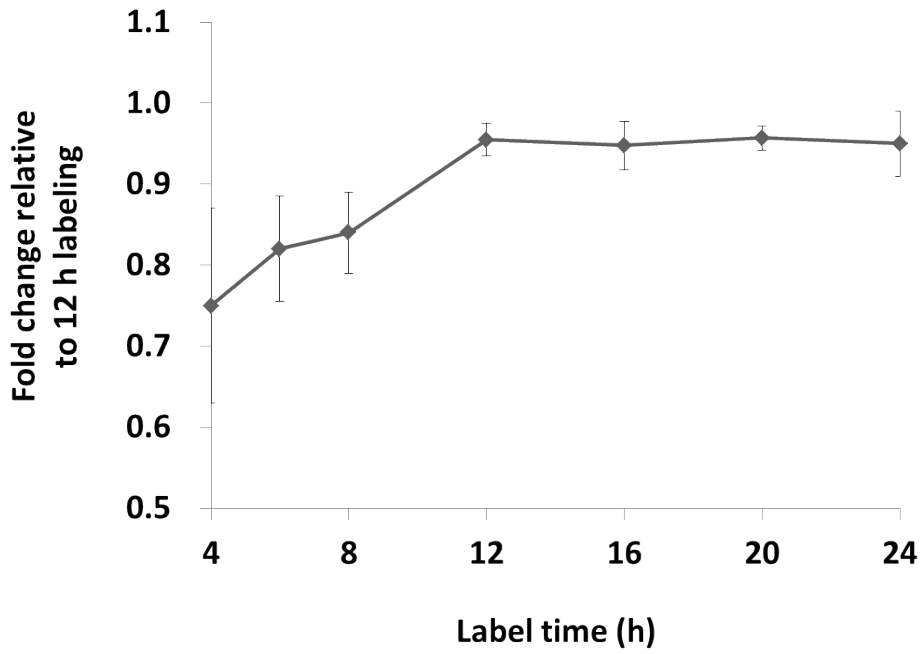


**Fig. S5** Investigation of labeling efficiency with different labeling time.

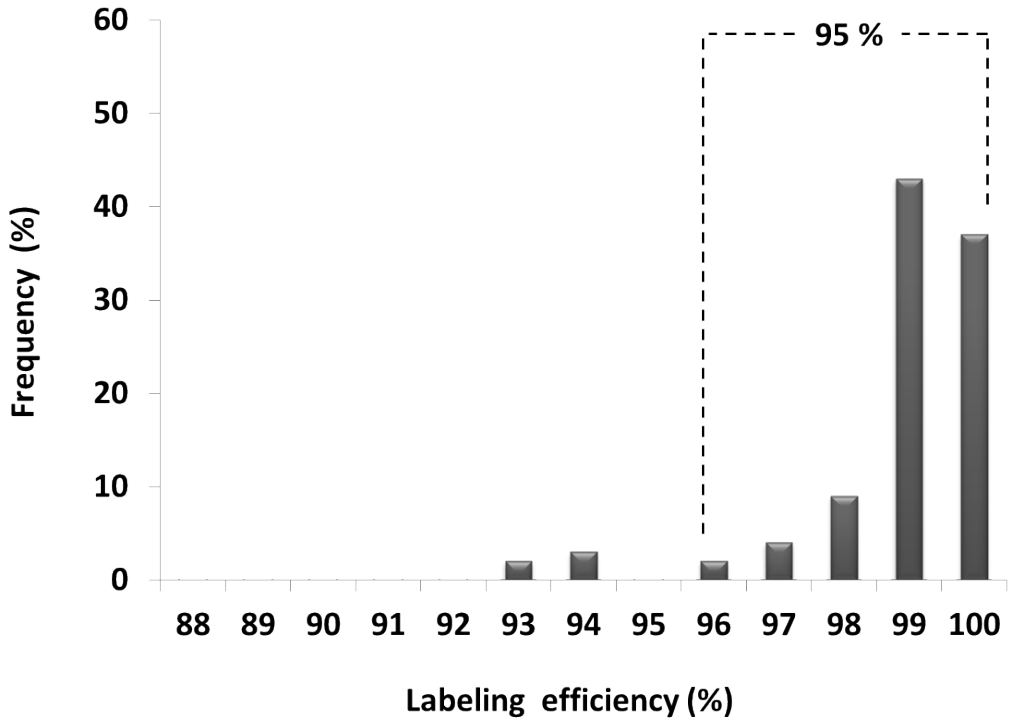


**Fig. S6** The distribution of 51 peptides' labeling efficiency under the optimized conditions.

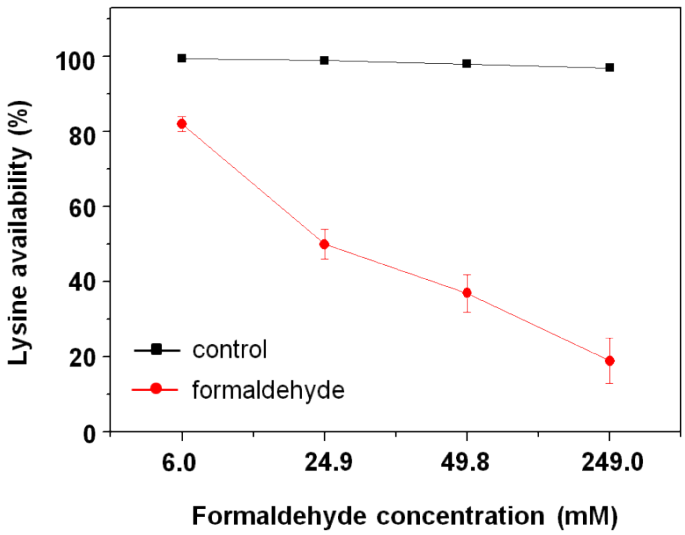


**Fig. S7** Confirmations for HSA cross-linking experiments in vitro: the measurement of free amine groups by OPA assay in HSA, which was incubated in formaldehyde solution (6.0, 24.9, 49.8 and 249.0 mM), respectively.

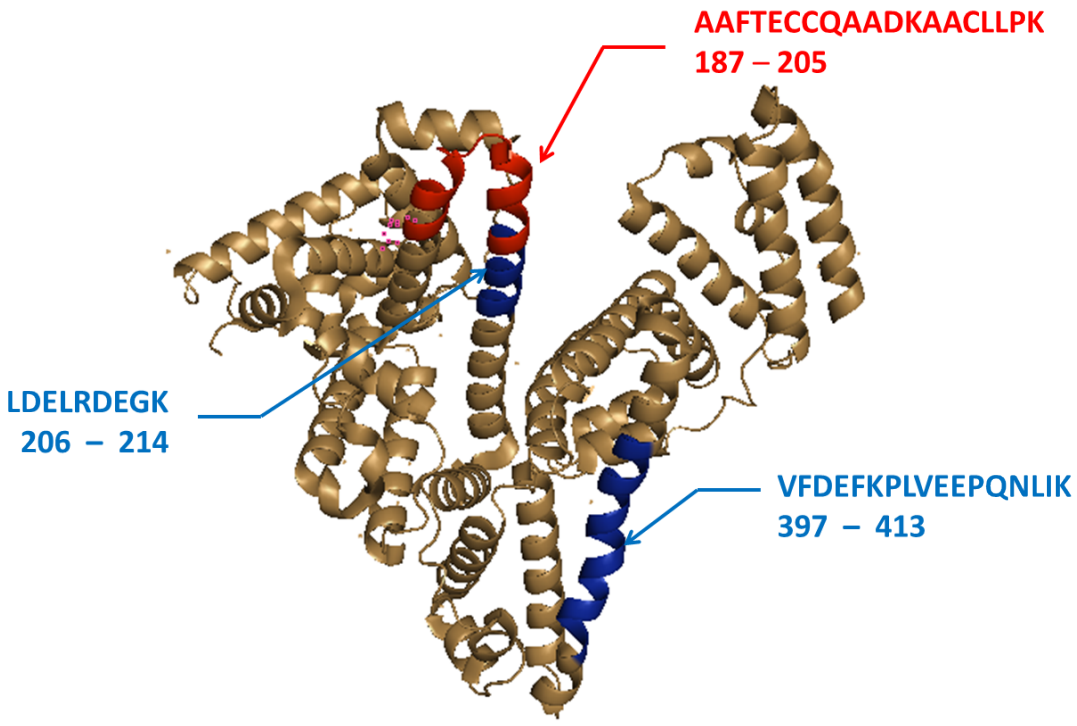


**Fig. S8** Locations of the potential peptidebiomarkers and the internal standard peptide on HSA. This image was generated by PyMOL Molecular Graphics System and crystal structure of HSA.