**Supporting Information**

# Structural optimization of cyclic peptides that efficiently detect denatured collagen

Koh K. Takita, [a] Kazunori K. Fujii, [a] Kento Ishii [a] and Takaki Koide * [a,b]

[a] Department of Chemistry and Biochemistry, School of Advanced Science and Engineering, Waseda University, Japan.

[b] Waseda Research Institute for Science and Engineering, Waseda University, Japan.

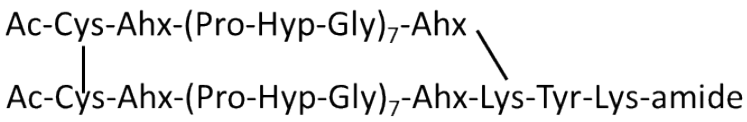



**Figure S1**. Sequence of **cCMP7.**

Ahx: 6-aminohexanoic acid.

**Table S1:** Analytical mass data of the synthesized CMPs.

| Peptide | Sequence | Found | Calcd. |
|---|---|---|---|
| **cE3** | Ac-C-Ahx-$(POGEOG)_3$-POG-Ahx-C-amide<br>Ac-C-Ahx-$(POG)_7$-Ahx————C-YK-amide | 1277.296 | 1277.318[b] |
| **cE7** | Ac-C-Ahx-$(EOG)_7$-Ahx-C-amide<br>Ac-C-Ahx-$(POG)_7$-Ahx-C-YK-amide | 1309.294 | 1309.308[b] |
| **cR3** | Ac-C-Ahx-$(POGPRG)_3$-POG-Ahx-C-amide<br>Ac-C-Ahx-$(POG)_7$-Ahx————C-YK-amide | 1285.697 | 1285.616[b] |
| **cR7** | Ac-C-Ahx-$(PRG)_7$-Ahx-C-amide<br>Ac-C-Ahx-$(POG)_7$-Ahx-C-YK-amide | 1328.645 | 1328.670[b] |
| **cE3-E3** | Ac-C-Ahx-$(POGEOG)_3$-POG-Ahx-C-amide<br>Ac-C-Ahx-$(POGEOG)_3$-POG-Ahx-C-YK-amide | 1732.758 | 1732.730[d] |
| **cR3-R3** | Ac-C-Ahx-$(POGPRG)_3$-POG-Ahx-C-amide<br>Ac-C-Ahx-$(POGPRG)_3$-POG-Ahx-C-YK-amide | 1317.884 | 1317.906[b] |
| **cR3-E3** | Ac-C-Ahx-$(POGEOG)_3$-POG-Ahx-C-amide<br>Ac-C-Ahx-$(POGPRG)_3$-POG-Ahx-C-YK-amide | 1309.592 | 1309.609[b] |
| **soCMP5-7** | Ac-C-$(POG)_5$-<br>Ac-C-$(POG)_7$-K-YK-amide | 1310.980 | 1310.928[a] |
| **soCMP6-7** | Ac-C-$(POG)_6$-<br>Ac-C-$(POG)_7$-K-YK-amide | 1400.023 | 1399.969[a] |
| **soCMP7-6** | Ac-C-$(POG)_7$-<br>Ac-C-$(POG)_6$-K-YK-amide | 1400.067 | 1399.969[a] |
| **soCMP6-8** | Ac-C-$(POG)_6$-<br>Ac-C-$(POG)_8$-K-YK-amide | 1489.060 | 1489.009[a] |
| **soCMP8-6** | Ac-C-$(POG)_8$-<br>Ac-C-$(POG)_6$-K-YK-amide | 1489.115 | 1489.009[a] |
| **soCMP7-8** | Ac-C-$(POG)_7$-<br>Ac-C-$(POG)_8$-K-YK-amide | 1578.103 | 1578.050[a] |
| **soCMP8-7** | Ac-C-$(POG)_8$-<br>Ac-C-$(POG)_7$-K-YK-amide | 1578.176 | 1578.050[a] |
| **soCMP6-7 (Glu)2** | Ac-C-$(POG)_2$-EOG-$(POG)_3$-<br>Ac-C-$(POG)_2$-EOG-$(POG)_4$-K-YK-amide | 1421.355 | 1421.295[d] |

| | | | |
|---|---|---|---|
| **soCMP6-7 (Glu)4** | Ac-C-POG-(POGEOG)$_2$-POG<br>Ac-C-POG-(POGEOG)$_2$-(POG)$_2$-K-YK-amide | 1442.678 | 1442.622[d] |
| **Bio-cE3** | Ac-C-Ahx-(POGEOG)$_3$-POG- Ahx-C-amide<br>Ac-C-Ahx-(POG)$_7$-Ahx ———— C-YK(Biotin)-amide | 1395.600 | 1395.623[b] |
| **Bio-cE7** | Ac-C-Ahx-(EOG)$_7$-Ahx-C-amide<br>Ac-C-Ahx-(POG)$_7$-Ahx-C-YK(Biotin)-amide | 1427.603 | 1427.613[b] |
| **Bio-cR3** | Ac-C-Ahx-(POGPRG)$_3$-POG-Ahx-C-amide<br>Ac-C-Ahx-(POG)$_7$-Ahx ————C-YK(Biotin)-amide | 1404.005 | 1403.921[b] |
| **Bio-cR7** | Ac-C-Ahx-(PRG)$_7$-Ahx-C-amide<br>Ac-C-Ahx-(POG)$_7$-Ahx-C-YK(Biotin)-amide | 1446.944 | 1446.974[b] |
| **Bio-cE3-E3** | Ac-C-Ahx-(POGEOG)$_3$-POG-Ahx-C-amide<br>Ac-C-Ahx-(POGEOG)$_3$-POG-Ahx-C-YK(Biotin)-amide | 1890.565 | 1890.469[d] |
| **Bio-cR3-R3** | Ac-C-Ahx-(POGPRG)$_3$-POG-Ahx-C-amide<br>Ac-C-Ahx-(POGPRG)$_3$-POG-Ahx-C-YK(Biotin)-amide | 1149.181 | 1149.170[c] |
| **Bio-cR3-E3** | Ac-C-Ahx-(POGEOG)$_3$-POG-Ahx-C-amide<br>Ac-C-Ahx-(POGPRG)$_3$-POG-Ahx-C-YK(Biotin)-amide | 1427.887 | 1427.913[b] |
| **Bio-cCMP5-7** | Ac-C-(POG)$_5$<br>Ac-C-(POG)$_7$-K-YK(Biotin)-amide | 1468.719 | 1468.668[a] |
| **Bio-soCMP6-7** | Ac-C-(POG)$_6$<br>Ac-C-(POG)$_7$-K-YK(Biotin)-amide | 1557.762 | 1557.708[a] |
| **Bio-soCMP7-6** | Ac-C-(POG)$_7$<br>Ac-C-(POG)$_6$-K-YK(Biotin)-amide | 1557.813 | 1557.708[a] |
| **Bio-soCMP6-8** | Ac-C-(POG)$_6$<br>Ac-C-(POG)$_8$-K-YK(Biotin)-amide | 1646.802 | 1646.749[a] |
| **Bio-soCMP8-6** | Ac-C-(POG)$_8$<br>Ac-C-(POG)$_6$-K-YK(Biotin)-amide | 1646.877 | 1646.749[a] |
| **Bio-soCMP7-8** | Ac-C-(POG)$_7$<br>Ac-C-(POG)$_8$-K-YK(Biotin)-amide | 1735.839 | 1735.789[a] |
| **Bio-soCMP8-7** | Ac-C-(POG)$_8$<br>Ac-C-(POG)$_7$-K-YK(Biotin)-amide | 1735.878 | 1735.789[a] |
| **Bio-soCMP6-7 (Glu)2** | Ac-C-(POG)$_2$-EOG-(POG)$_3$<br>Ac-C-(POG)$_2$-EOG-(POG)$_4$-K-YK(Biotin)-amide | 1579.100 | 1579.035[d] |

| | | | |
|---|---|---|---|
| **Bio-soCMP6-7 (Glu)4** | Ac-C-POG-(POGEOG)$_2$-POG<br>Ac-C-POG-(POGEOG)$_2$-(POG)$_2$-K-YK(Biotin)-amide | 1600.428 | 1600.361[d] |
| **FAM-soCMP6-7 (Glu)2** | Ac-C-(POG)$_2$-EOG-(POG)$_3$<br>Ac-C-(POG)$_2$-EOG-(POG)$_4$-K-YK(FAM)-amide | 1540.768 | 1540.644[a] |

Performed by ESI-MS. [a] $[M_m + 3H]^{3+}/3$. [b] $[M_m + 4H]^{4+}/4$. [c] $[M_m + 5H]^{5+}/5$. [d] $[M_m - 3H]^{3-}/3$.

O: 4-hydroxyproline; Ahx: 6-aminohexanoic acid; Biotin: biotin-PEG4; FAM: 5-carboxyfluorescein.

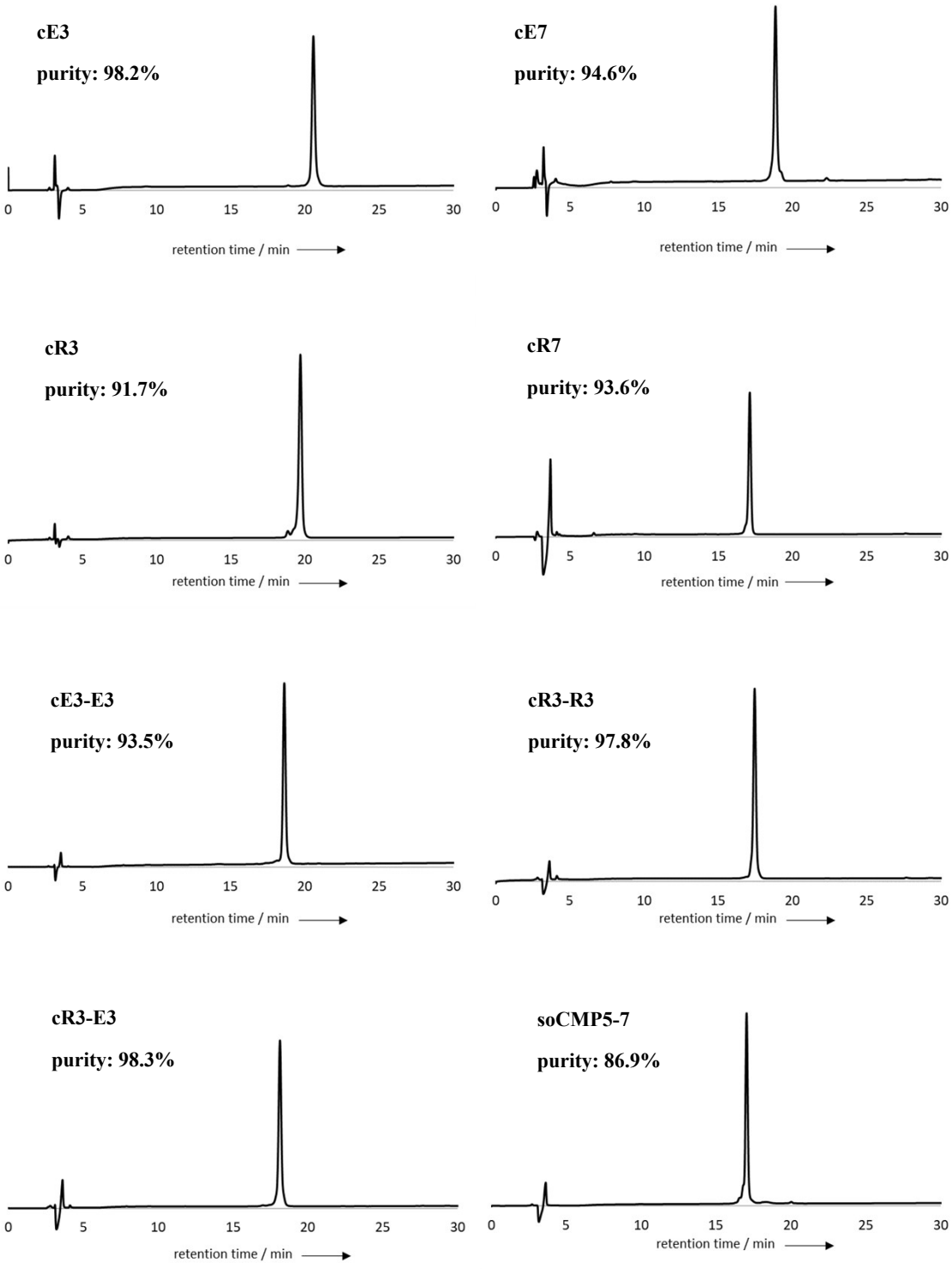



**Figure S2.** HPLC profiles of the synthesized cCMPs. Unlabelled cCMPs were purified by RP-HPLC. They were further labelled with biotin or fluorescein and they were purified by gel filtration. The purified samples were analyzed with RP-HPLC. HPLC gradient: 10%–30% $CH_3CN$ in water both containing 0.05% (v/v) TFA over 30 min at 60 °C. detection: 220 nm.

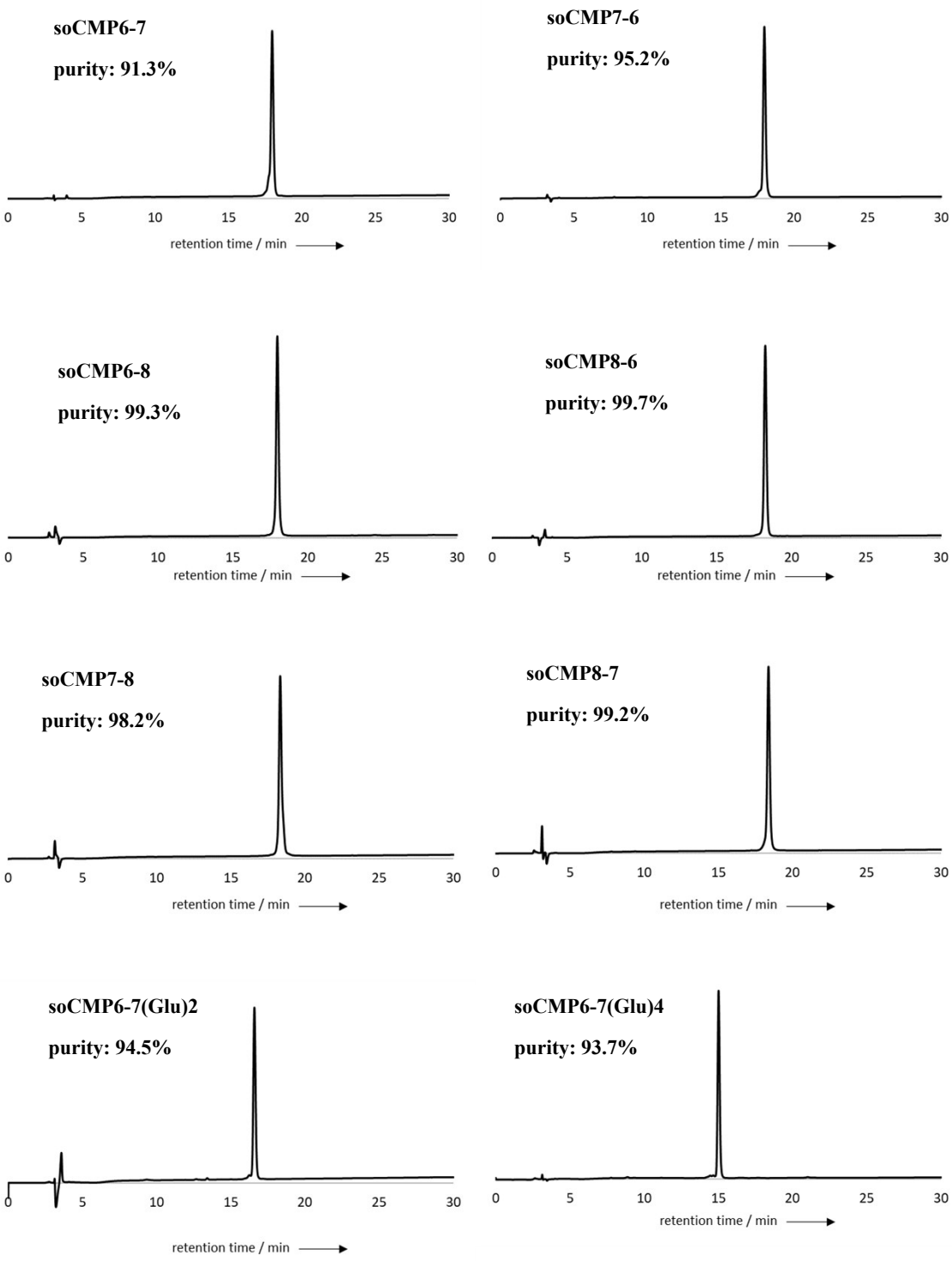



**Figure S2.** *Continued.*

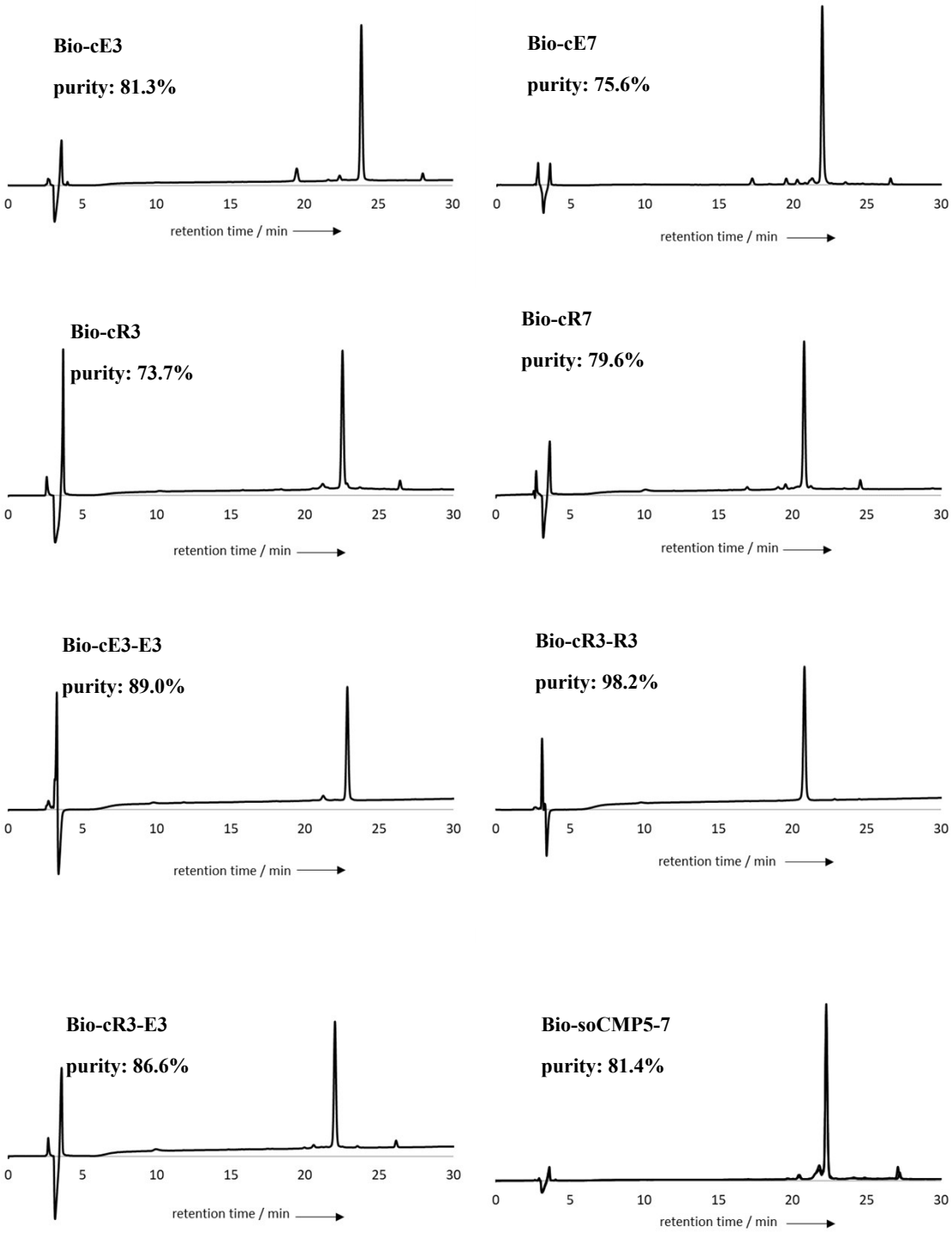



**Figure S2.** *Continued.*

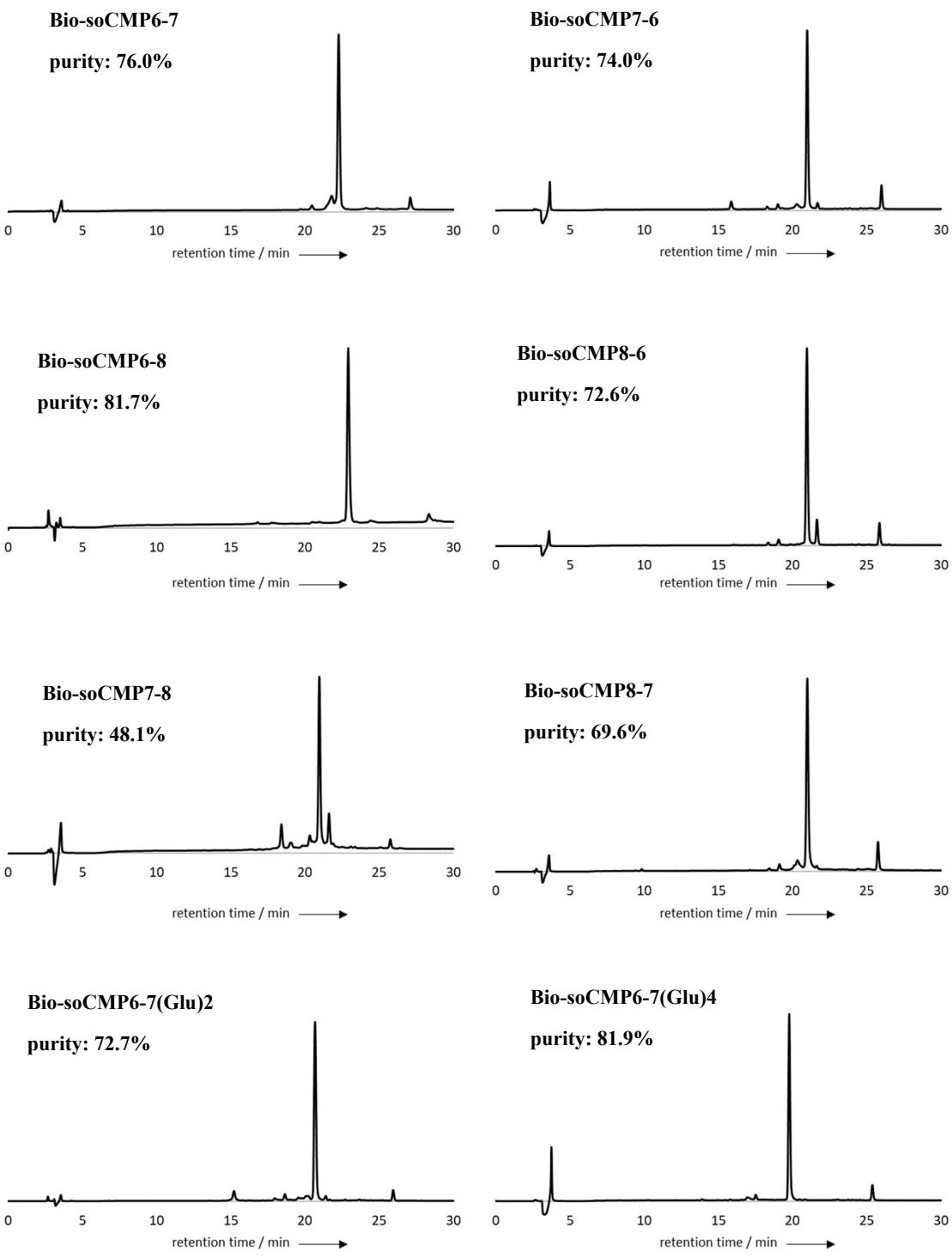



**Figure S2.** *Continued.*

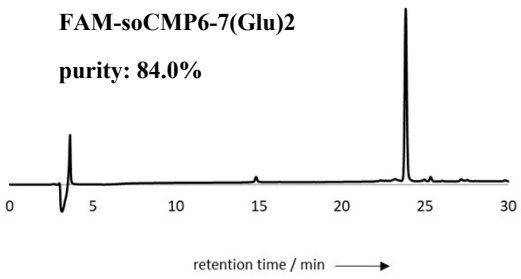
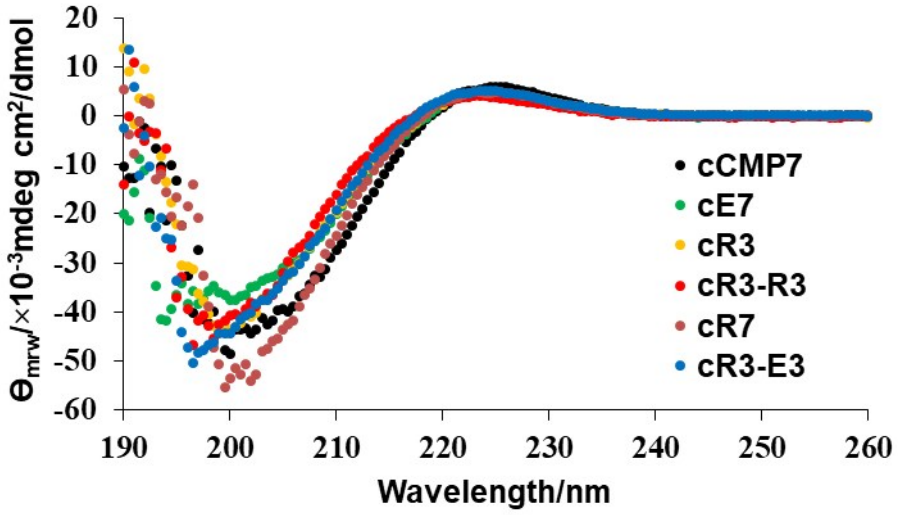



**Figure S2.** *Continued.*

(a)




(b)

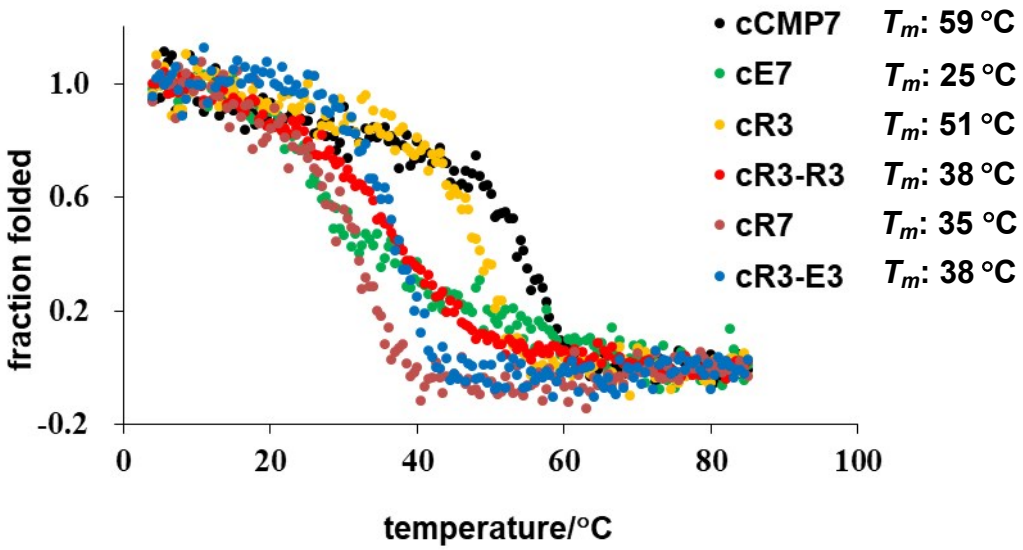



**Figure S3**. CD analysis of charged cCMPs. a) CD spectra of charged cCMPs at 4 °C. b) Thermal unfolding curves of charged cCMPs. Signals were detected at 225 nm.

**Table S2:** Components of protein marker

| protein | molecular weight (kDa) | ng/5µl |
|---|---|---|
| myosin heavy chain | 227 | - |
| β-galactosidase | 116 | 88.0 |
| phosphorylase b | 97.2 | 84.0 |
| bovine serum albumin | 66.4 | 320 |
| glutamate dehydrogenase | 55.6 | 160 |
| ovalbumin | 45.0 | 192 |
| glyceraldehyde-3-phosphate dehydrogenase | 35.7 | 160 |
| carbonic anhydrase II | 29.0 | 120 |
| soybean trypsin inhibitor A | 20.1 | 144 |
| lysozyme | 14.3 | 144 |
| aprotinin | 6.5 | 200 |

Reference

[1] J. Ottl, L. Moroder, *J. Pept. Sci.* **1999**, *5*, 103–110.