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Supporting Information

Self-assembled Branched Polypeptides as Amelogenin Mimics for Enamel Repair

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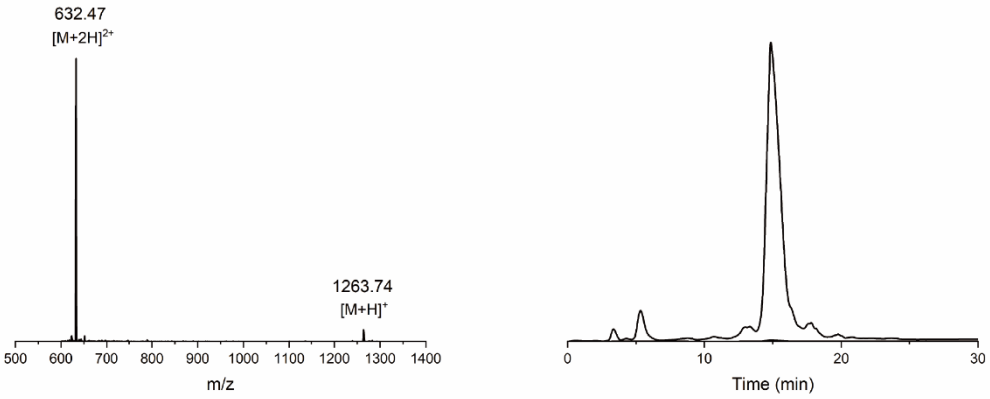
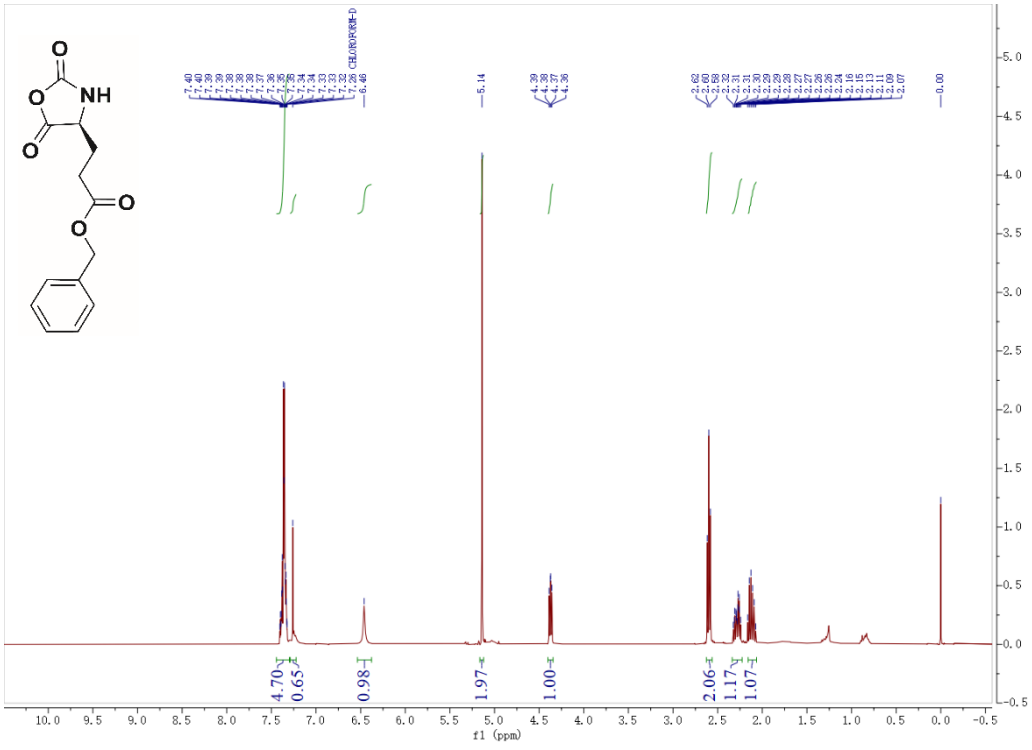
a


632.47
[M+2H]2+
1263.74
[M+H]+
500 600 700 800 900 1000 1100 1200 1300 1400
m/z
0 10 20 30
Time (min)

b


O
NH
O
O
O
O
4.70
0.65
0.98
1.97
1.00
2.06
1.17
1.07
f1 (ppm)

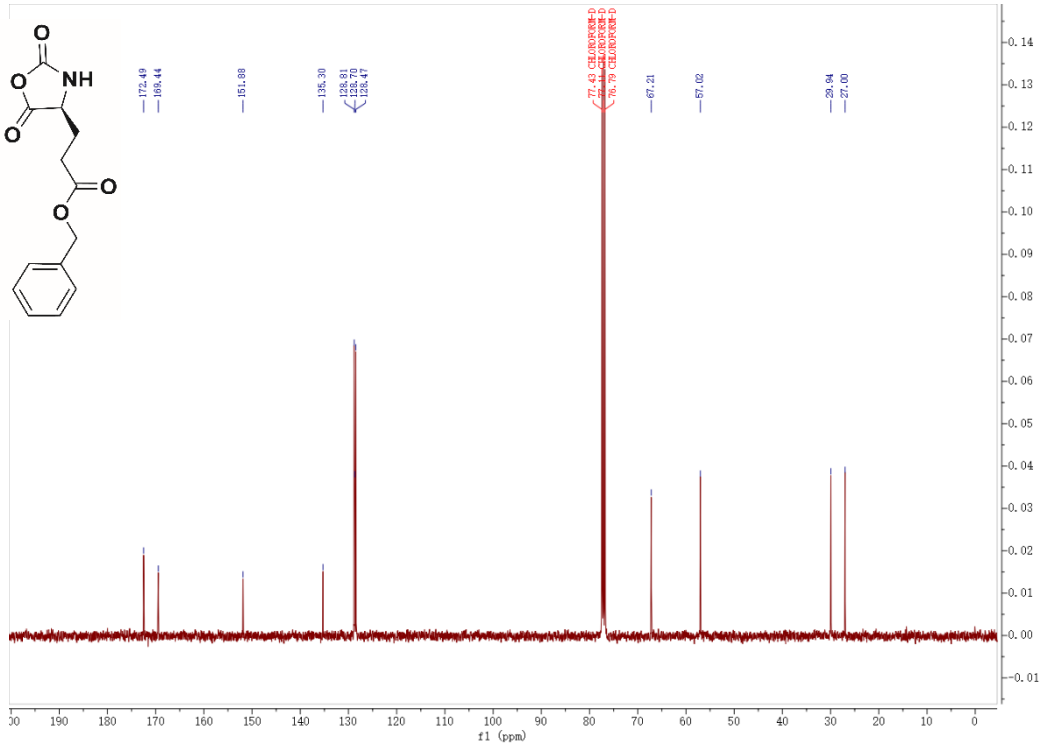
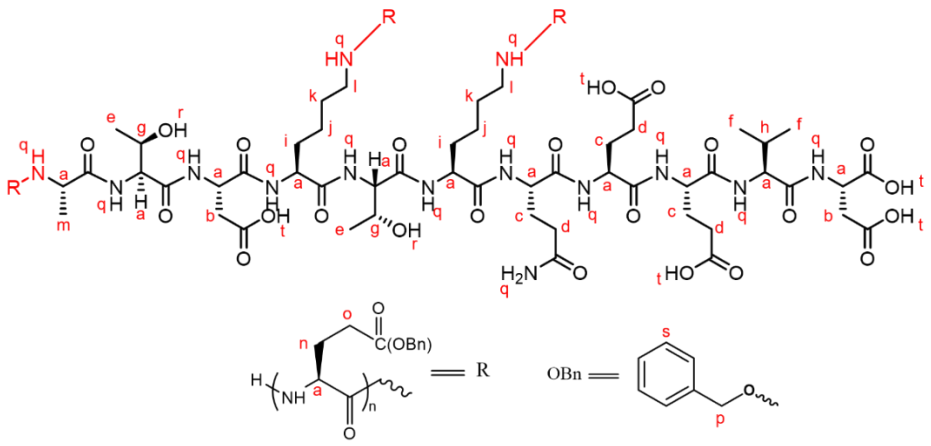
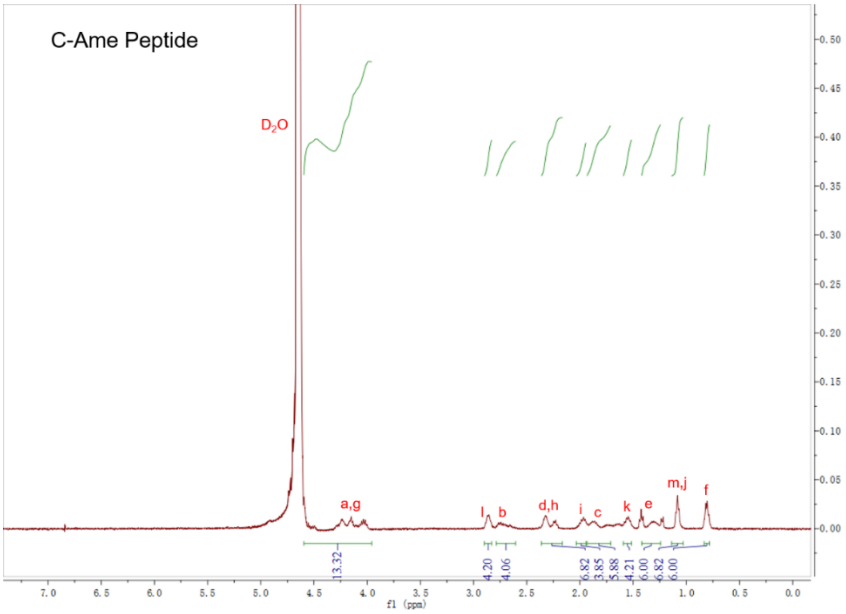


Fig. S1 a) Electrospray ionization mass spectrometry (ESI-MS) and high-performance liquid chromatography (HPLC) analysis of C-Ame Peptide. Retention time is about 14.87 min in a linear gradient of 5-45% B for 35 min (HPLC solvent A: water, 0.06% TFA; B: 80% CH_3CN/water, 0.06% TFA). b) ^{1}H NMR and ^{13}C NMR spectra of BLG-NCA monomer in $CDCl_3$.

a



b

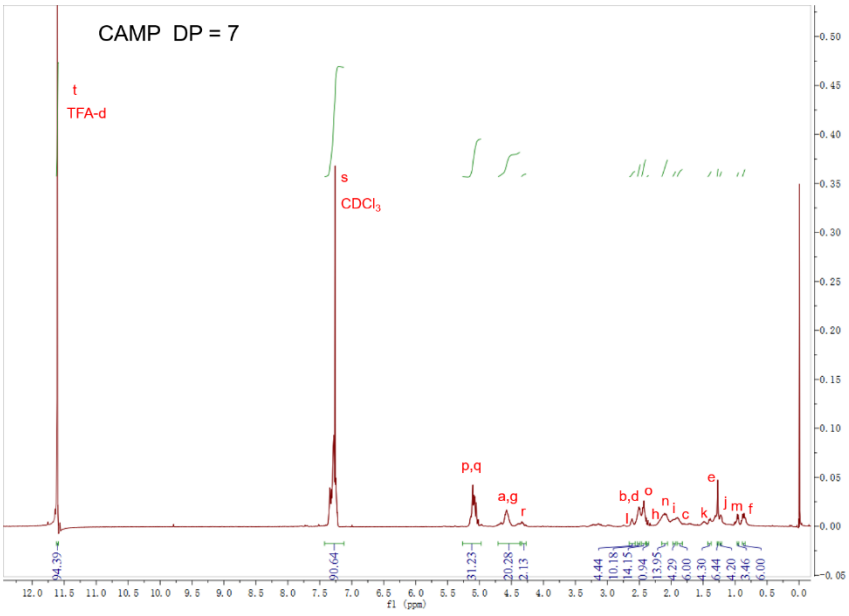


	a	b	c	d	e	f	g	h	i	j	k	l	m
Chemical Shift	4.25	2.75	1.87	2.32	1.46	0.85	4.17	2.25	1.99	1.09	1.58	2.82	1.11
Integration	11	4	6	6	6	6	2	1	4	4	4	4	3

Chemical Formula: $C_{51}H_{86}N_{14}O_{23}$

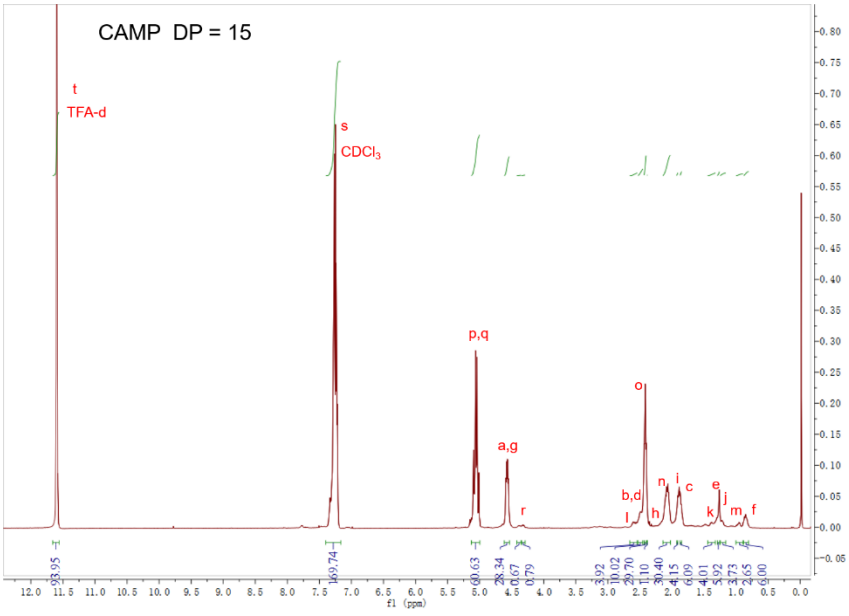
Exact Mass: 1262.60

Molecular Weight: 1263.32



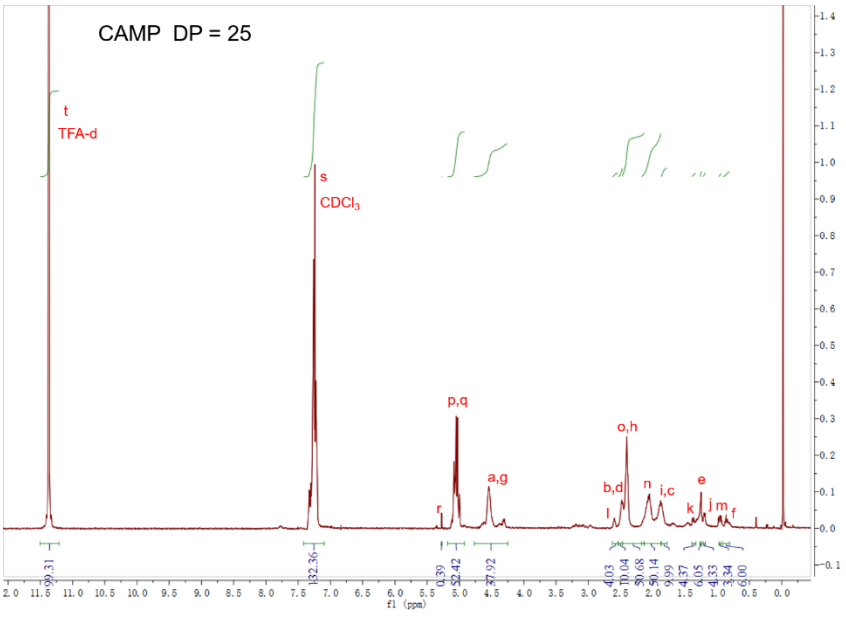
	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	r
Chemical Shift	**4.62**	**2.54**	**1.85**	**2.54**	**1.26**	**0.85**	**4.62**	**2.39**	**2.01**	**1.25**	**1.47**	**2.63**	**0.95**	**2.18**	**2.48**	**4.28**
Integration	**18**	**4**	**6**	**6**	**6**	**6**	**2**	**1**	**4**	**4**	**4**	**4**	**3**	**14**	**14**	**2**

Molecular Weight: 1263.32+(18-11)×(237.25-18.02) = 2797.93



	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	r
Chemical Shift	**4.62**	**2.54**	**1.85**	**2.54**	**1.26**	**0.85**	**4.62**	**2.39**	**2.01**	**1.25**	**1.47**	**2.63**	**0.95**	**2.18**	**2.48**	**4.28**
Integration	**26**	**4**	**6**	**6**	**6**	**6**	**2**	**1**	**4**	**4**	**4**	**4**	**3**	**30**	**30**	**2**

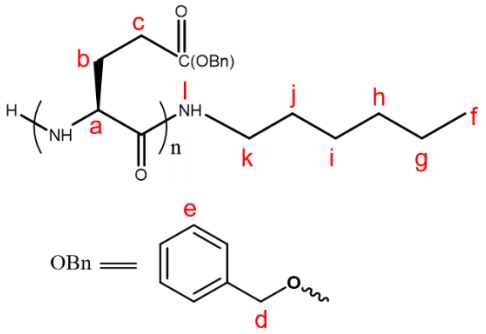
Molecular Weight: 1263.32+(26-11)×(237.25-18.02) = 4551.77



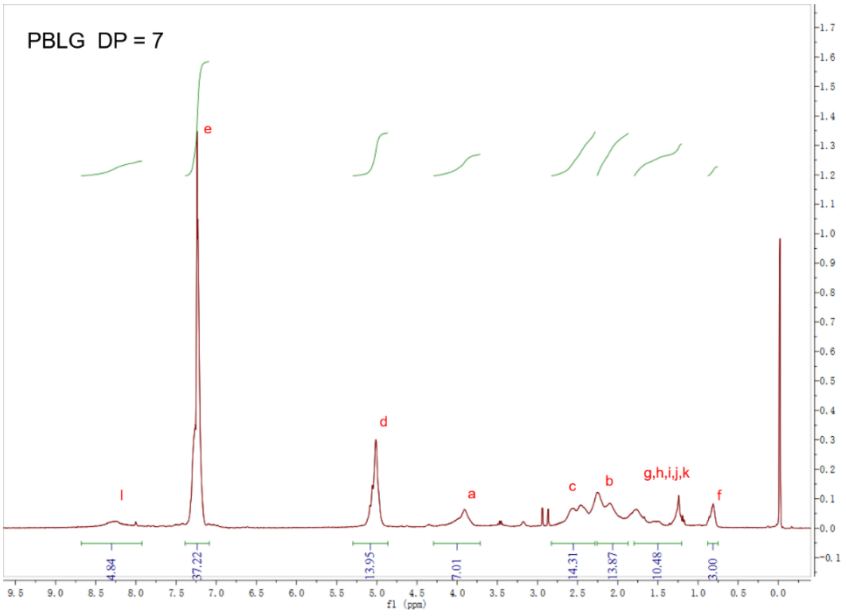
	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	r
Chemical Shift	**4.62**	**2.54**	**1.85**	**2.54**	**1.26**	**0.85**	**4.62**	**2.39**	**2.01**	**1.25**	**1.47**	**2.63**	**0.95**	**2.18**	**2.48**	**4.28**
Integration	**36**	**4**	**6**	**6**	**6**	**6**	**2**	**1**	**4**	**4**	**4**	**4**	**3**	**50**	**50**	**2**

Molecular Weight: 1263.32+(36-11)×(237.25-18.02) = 6744.07

c

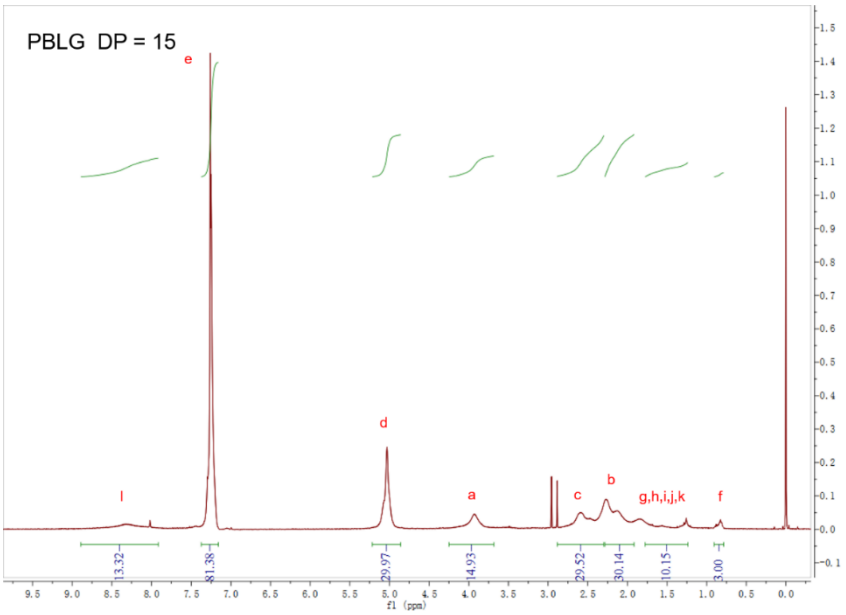


d



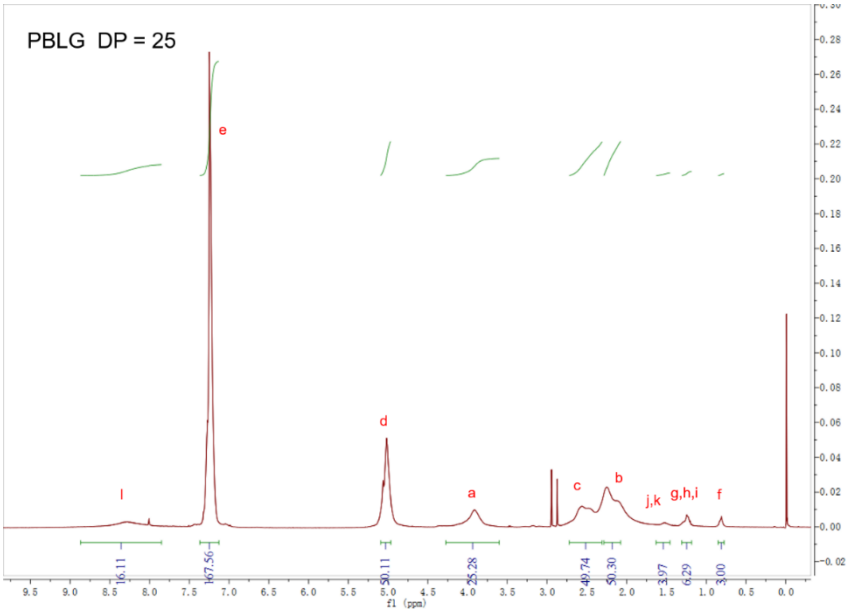
	a	b	c	d	f	g	h	i	j	k	l
Chemical Shift	**3.96**	**2.25**	**2.52**	**5.02**	**0.85**	**1.25-1.78**					**8.25**
Integration	**7**	**14**	**14**	**14**	**3**	**10**					**5**

Molecular Weight: 101.19+7×(237.25-18.02) = 1635.8



	a	b	c	d	f	g	h	i	j	k	l
Chemical Shift	**3.96**	**2.25**	**2.52**	**5.02**	**0.85**	**1.25-1.78**					**8.25**
Integration	**15**	**30**	**30**	**30**	**3**	**10**					**13**

Molecular Weight: 101.19+15×(237.25-18.02) = 3389.64



	a	b	c	d	f	g	h	i	j	k	l
Chemical Shift	**3.96**	**2.25**	**2.52**	**5.02**	**0.85**	**1.25-1.78**					**8.25**
Integration	**25**	**50**	**50**	**50**	**3**	**10**					**16**

Molecular Weight: 101.19+25×(237.25-18.02) = 5581.94

Fig. S2 a) The structure of C-Ame Peptide and CAMPs. b) ^{1}H NMR spectra of C-Ame Peptide in D_2O, $CAMP_7$, $CAMP_{15}$ and $CAMP_{25}$ in $CDCl_3$ (15% TFA-d). c) The structure of hexylamine and PBLGs. d) ^{1}H NMR spectra of $PBLG_7$, $PBLG_{15}$, $PBLG_{25}$ in $CDCl_3$.

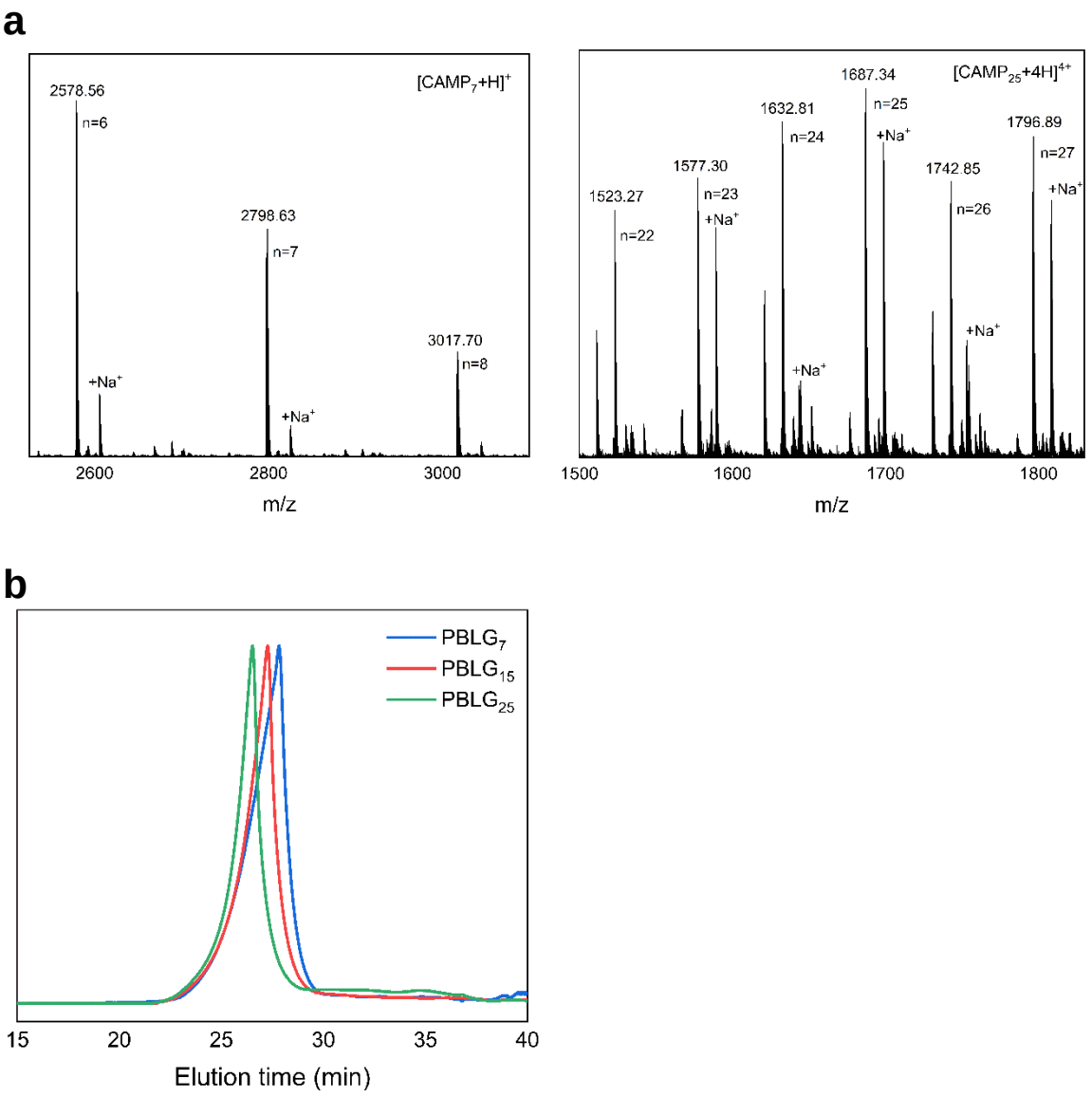


Fig. S3 a) Q-TOF-MS analysis of $CAMP_7$ and $CAMP_{25}$. b) GPC analysis of $PBLG_7$, $PBLG_{15}$ and $PBLG_{25}$.

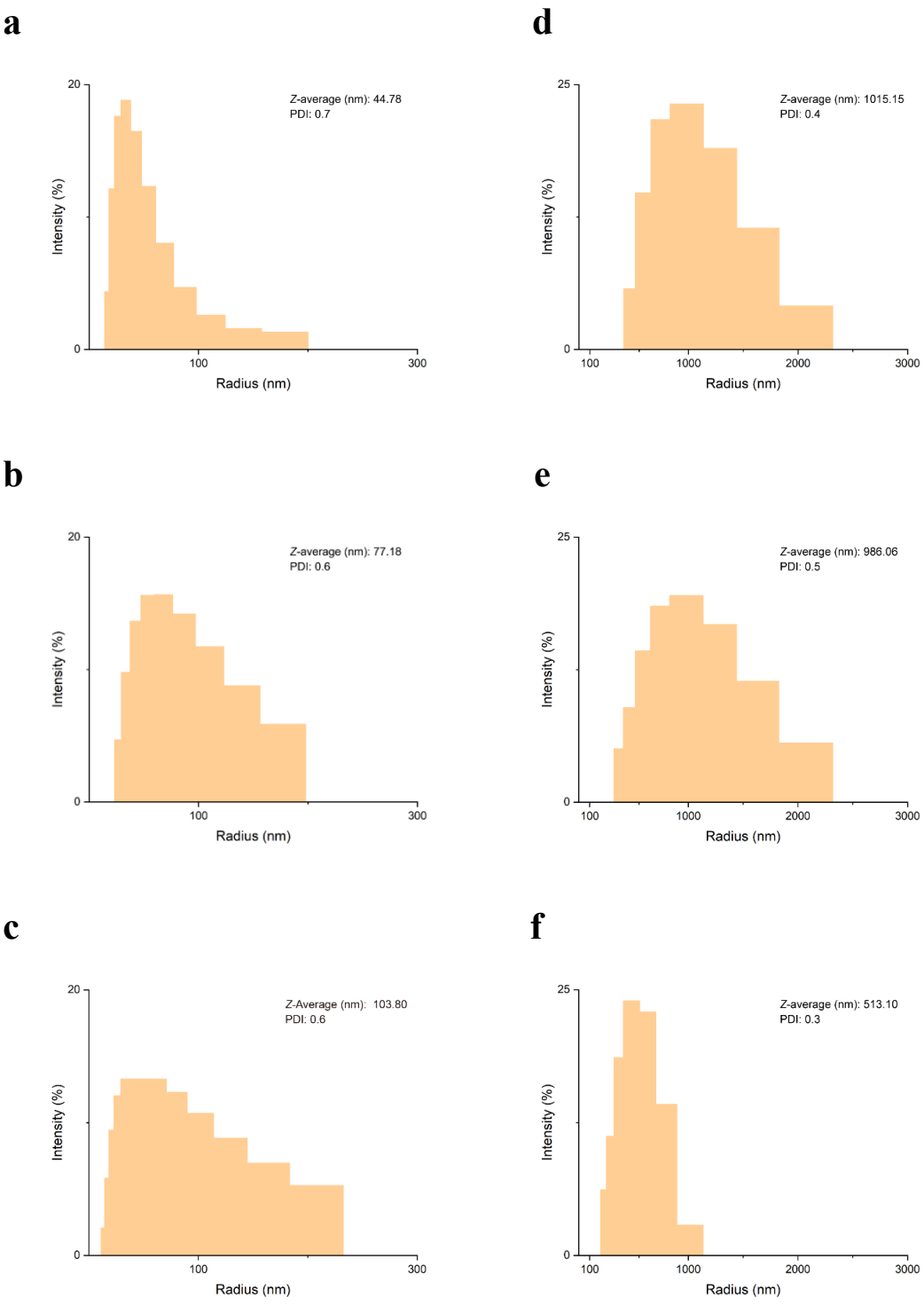
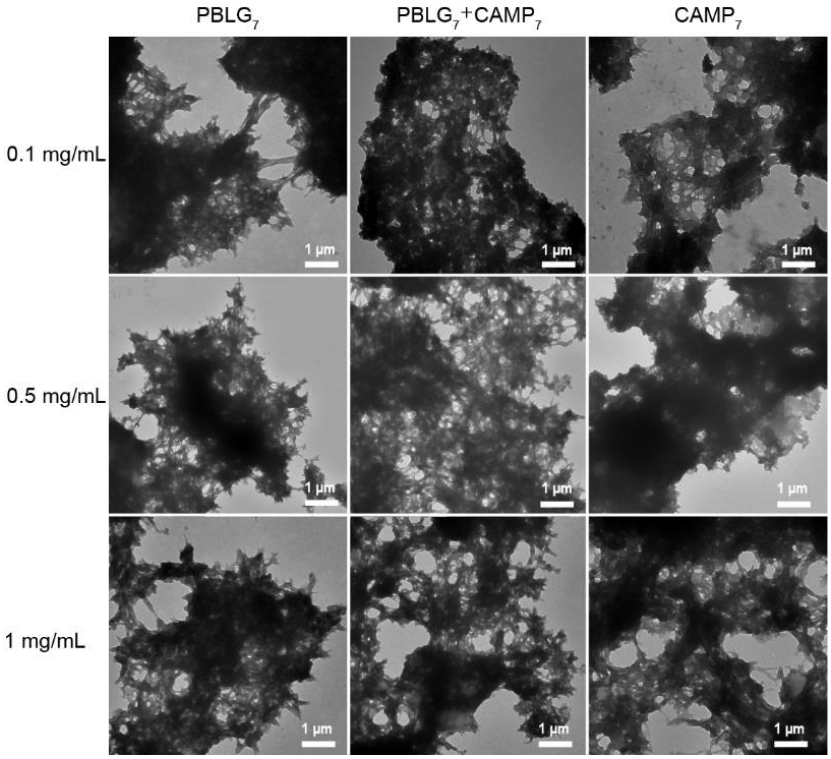
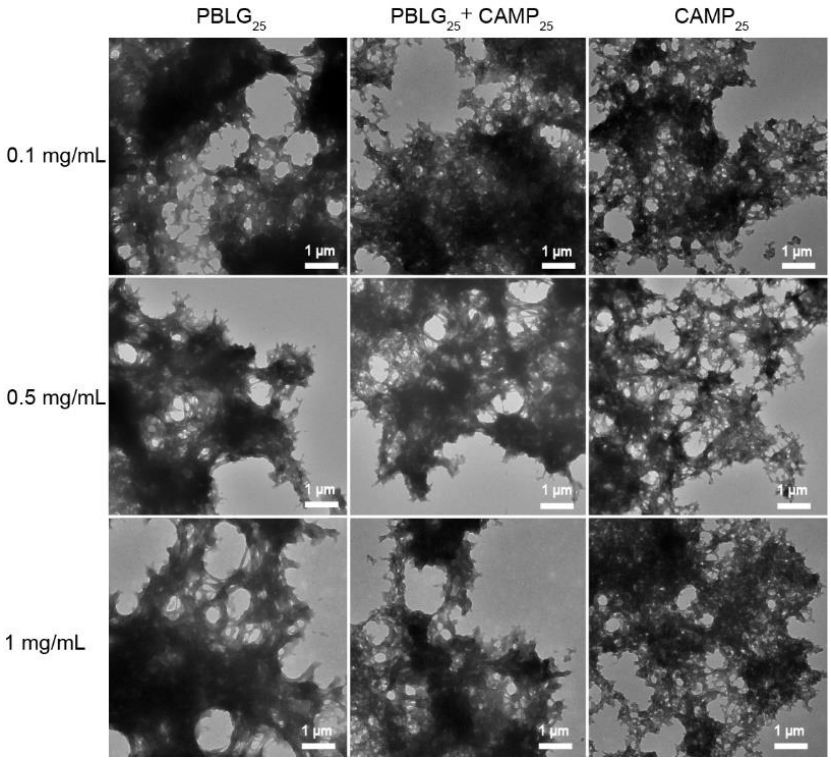


Fig. S4 Size distribution of $PBLG_7$ (a), $PBLG_7$ + $CAMP_7$ (1/1, n/n) (b), $CAMP_7$ (c), $PBLG_{25}$ (d), $PBLG_{25}$ + $CAMP_{25}$ (1/1, n/n) (e) and $CAMP_{25}$ (f) assemblies characterized by DLS.

a


PBLG7
PBLG7+CAMP7
CAMP7
0.1 mg/mL
0.5 mg/mL
1 mg/mL
1 μm

b


PBLG25
PBLG25+ CAMP25
CAMP25
0.1 mg/mL
0.5 mg/mL
1 mg/mL
1 μm

c

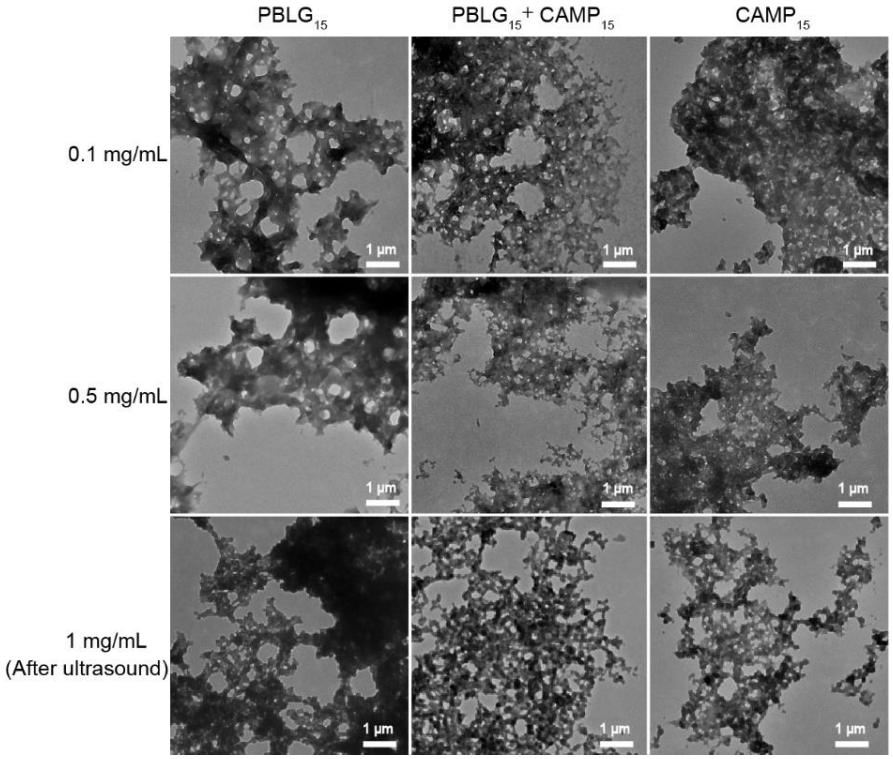


Fig. S5 a) TEM images of $PBLG_7$, $PBLG_7$ + $CAMP_7$ (1/1, n/n), $CAMP_7$ assemblies at different concentrations of 0.1 mg mL^{-1}, 0.5 mg mL^{-1} and 1 mg mL^{-1} b) TEM images of $PBLG_{25}$, $PBLG_{25}$ + $CAMP_{25}$ (1/1, n/n), $CAMP_{25}$ assemblies at different concentrations of 0.1 mg mL^{-1}, 0.5 mg mL^{-1} and 1 mg mL^{-1}. c) TEM images of $PBLG_{15}$, $PBLG_{15}$ + $CAMP_{15}$ (1/1, n/n), $CAMP_{15}$ assemblies at different concentrations of 0.1 mg mL^{-1}, 0.5 mg mL^{-1} and 1 mg mL^{-1} after 20 minutes of ultrasound. Scale bar: 1 µm.

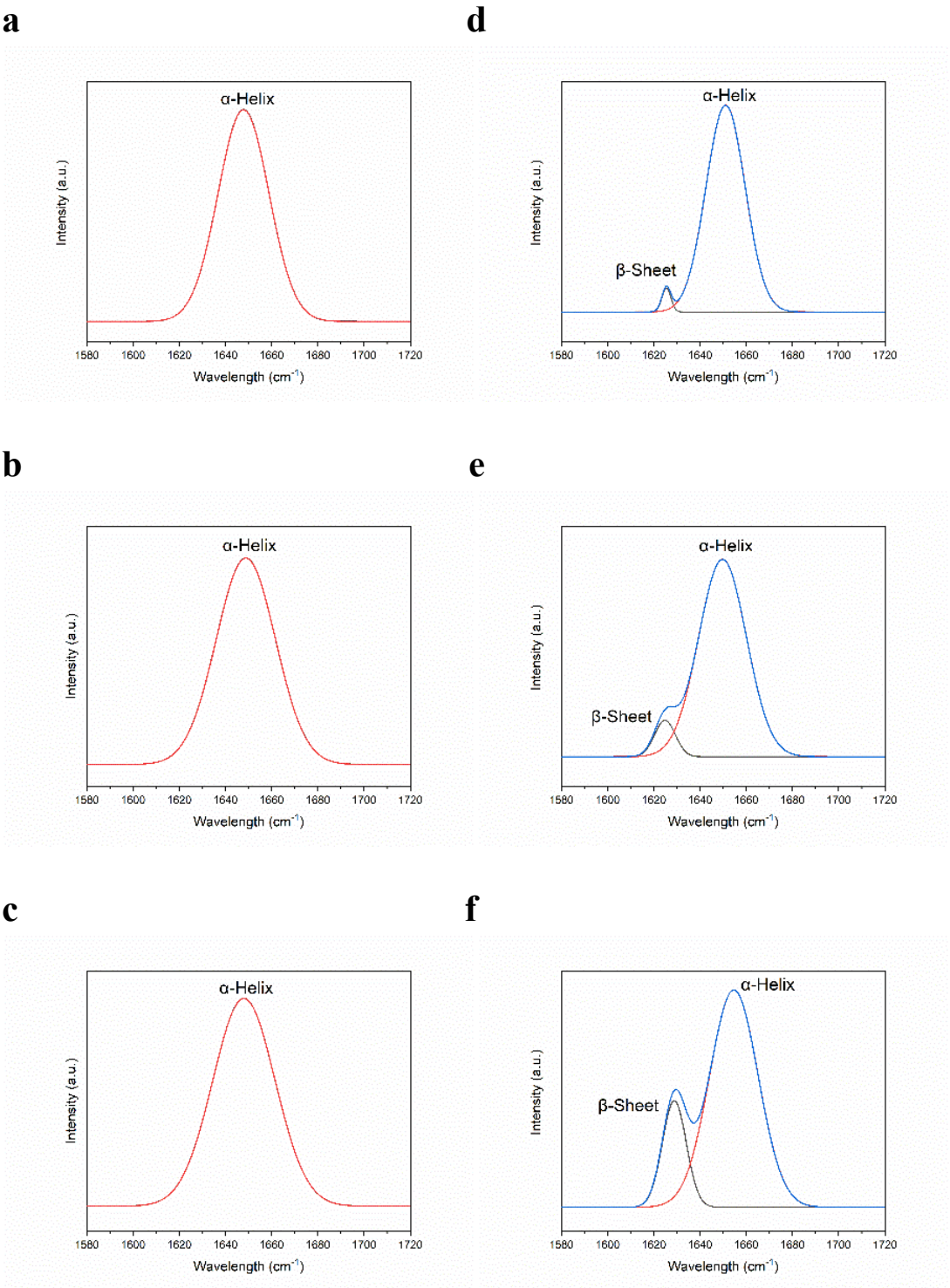


Fig. S6 FTIR spectroscopy and deconvolution of $PBLG_7$ (a), $PBLG_7$ + $CAMP_7$ (1/1, n/n) (b), $CAMP_7$ (c), $PBLG_{25}$ (d), $PBLG_{25}$ + $CAMP_{25}$ (1/1, n/n) (e) and $CAMP_{25}$ (f) assemblies at amide I spectral region.

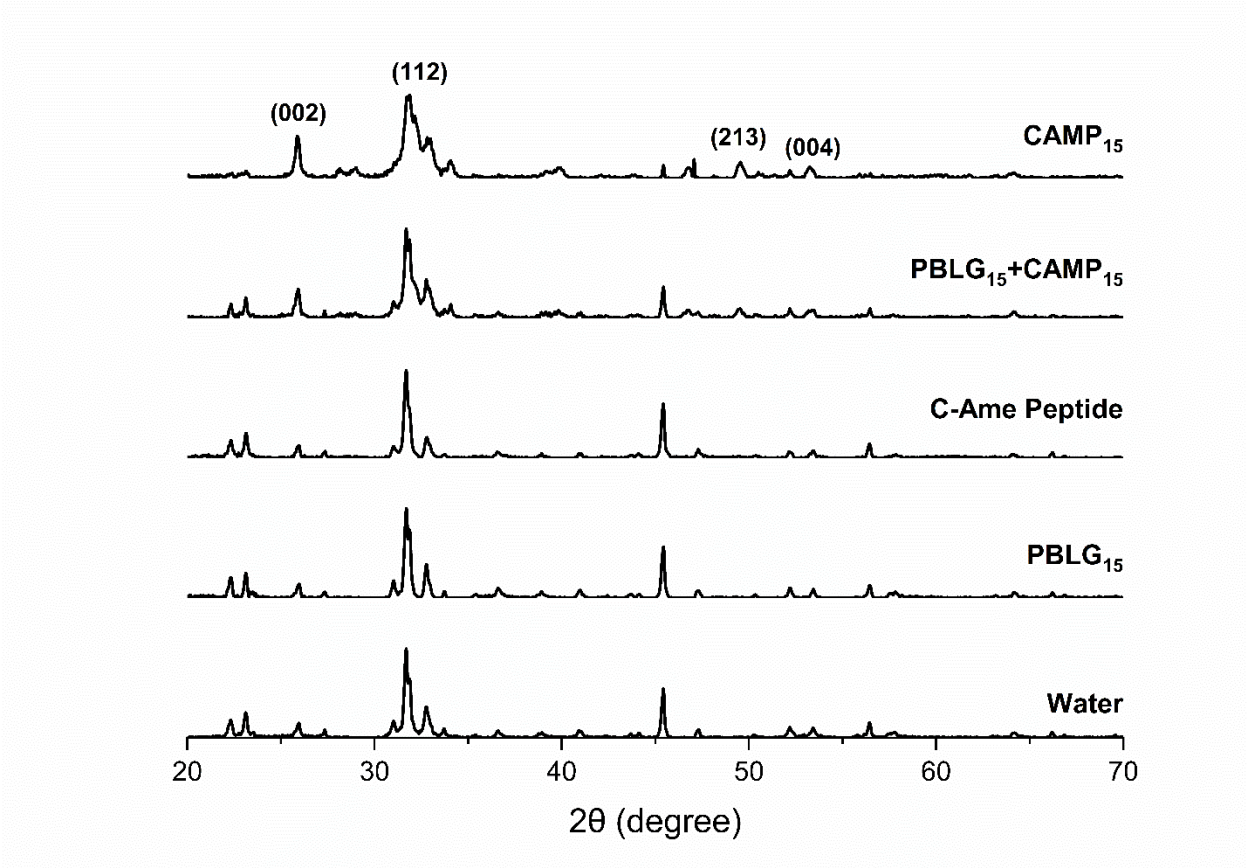
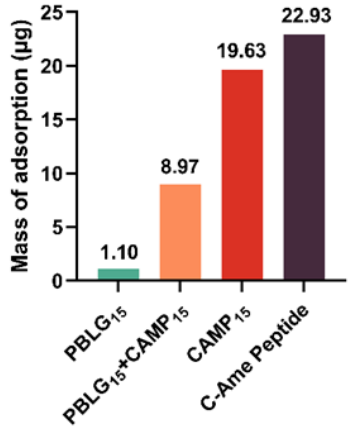
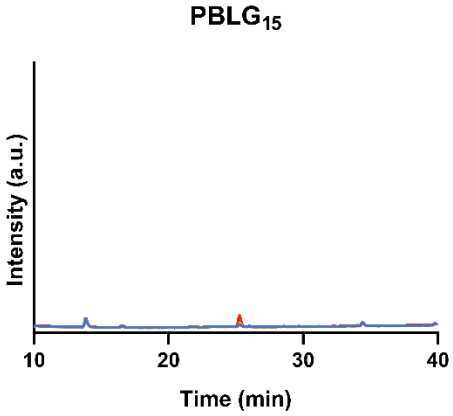


Fig. S7 XRD spectra of the mineralized products of Water, $PBLG_{15}$, C-Ame Peptide, $PBLG_{15}$ + $CAMP_{15}$ (1/1, n/n) and $CAMP_{15}$. NaCl was removed from the mineralization solution and the mineralized products were immediately frozen with liquid nitrogen and lyophilized for getting the powder sample to be tested.

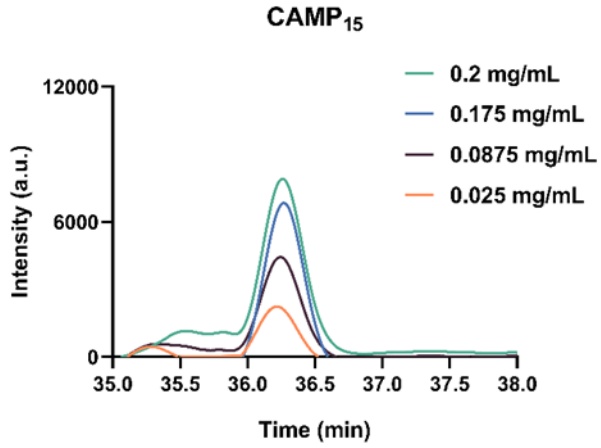
a

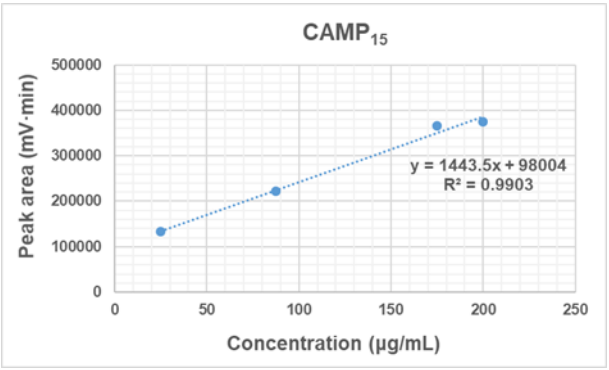

Mass of adsorption (μg)
25
20
15
10
5
0
1.10
8.97
19.63
22.93
PBLG15
PBLG15+CAMP15
CAMP15
C-Ame Peptide

b

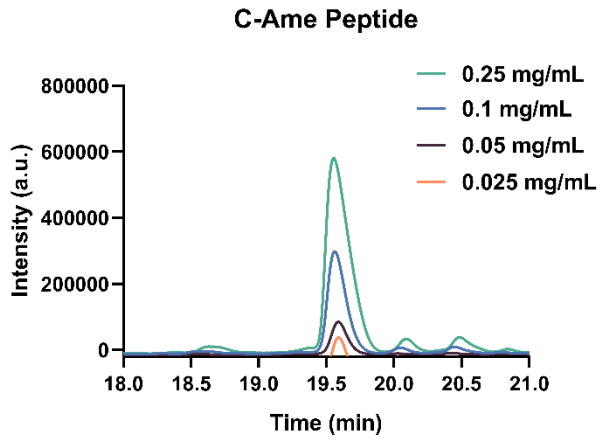

PBLG15
Intensity (a.u.)
10
20
30
40
Time (min)

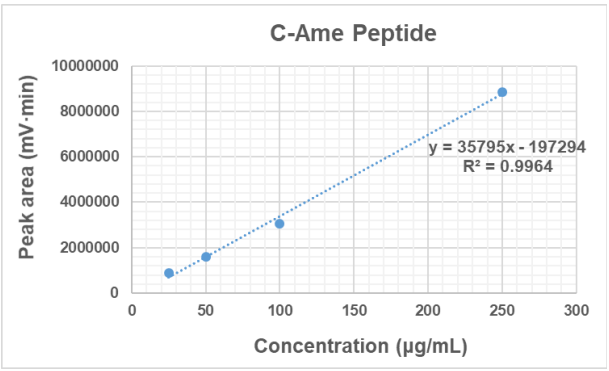
c


CAMP15
Intensity (a.u.)
12000
6000
0
0.2 mg/mL
0.175 mg/mL
0.0875 mg/mL
0.025 mg/mL
35.0
35.5
36.0
36.5
37.0
37.5
38.0
Time (min)

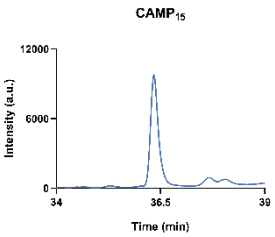
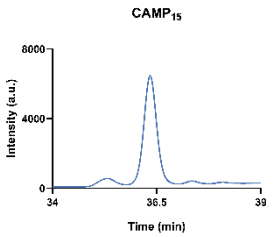
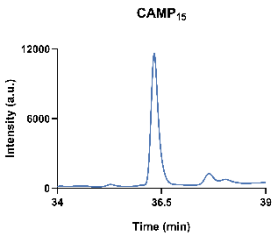

CAMP15
Peak area (mV·min)
500000
400000
300000
200000
100000
0
y = 1443.5x + 98004
R² = 0.9903
0
50
100
150
200
250
Concentration (μg/mL)

d


C-Ame Peptide
Intensity (a.u.)
800000
600000
400000
200000
0
0.25 mg/mL
0.1 mg/mL
0.05 mg/mL
0.025 mg/mL
18.0
18.5
19.0
19.5
20.0
20.5
21.0
Time (min)


C-Ame Peptide
Peak area (mV·min)
10000000
8000000
6000000
4000000
2000000
0
y = 35795x - 197294
R² = 0.9964
0
50
100
150
200
250
300
Concentration (μg/mL)

e

			
Peak area (mV·min)	129577	138558	146056
Concentration (μg/mL)	21.87	28.09	33.29
Mass of adsorption (μg)	10.94	14.05	16.64
Average mass of adsorption (μg)	13.88 ± 2.84		

f

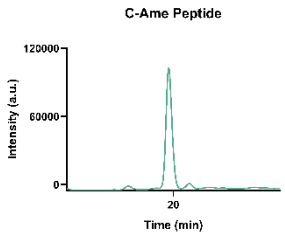
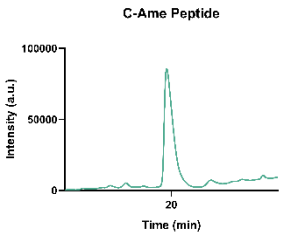
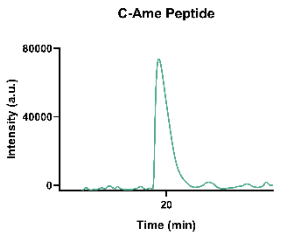
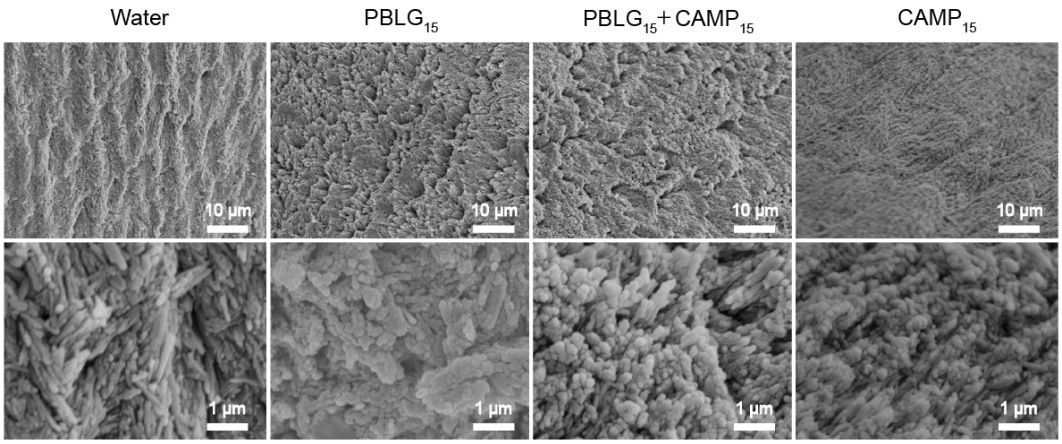
			
Peak area (mV·min)	859125	973297	1056289
Concentration (μg/mL)	29.51	32.70	35.02
Mass of adsorption (μg)	14.76	16.35	17.51
Average mass of adsorption (μg)	16.21 ± 1.38		

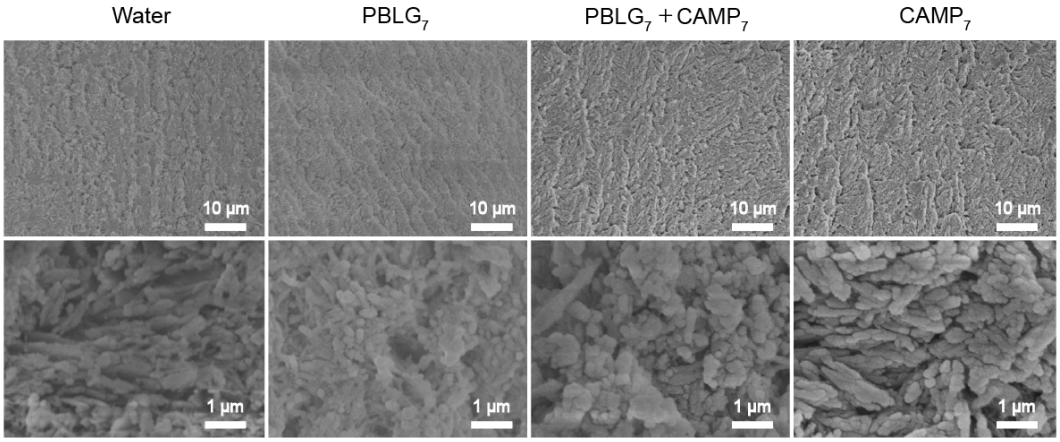
Fig. S8 Characterization of long-term adsorption capacity of polypeptides on enamel in artificial saliva. a) Average mass of $PBLG_{15}$, $PBLG_{15}$ + $CAMP_{15}$ (1/1, n/n), $CAMP_{15}$ and C-Ame Peptide coated on enamel slices using the BCA Protein Assay Kit. These data are the means (n=3). b) HPLC analysis of $PBLG_{15}$ after adding 500 μL 37% phosphoric acid and ultrasonic treatment. The analysis was conducted for three parallel determinations. Retention time of $PBLG_{15}$ is about 30.49 min in a linear gradient of 50-90% B for 40 min. c-d) Peak area-concentration standard curve of $CAMP_{15}$ (c) and C-Ame Peptide (d). e-f) HPLC analysis and average mass calculation of $CAMP_{15}$ (e) and C-Ame Peptide (f) after adding 500 μL 37% phosphoric acid and ultrasonic treatment. The

analysis was conducted for three parallel determinations. Retention time of $CAMP_{15}$ is about 36.41 min in a linear gradient of 30-80% B for 50 min. Retention time of C-Ame Peptide is about 19.96 min in a linear gradient of 5-45% B for 45 min. (HPLC solvent A: water, 0.06% TFA; B: 80% CH_3CN/water, 0.06% TFA)

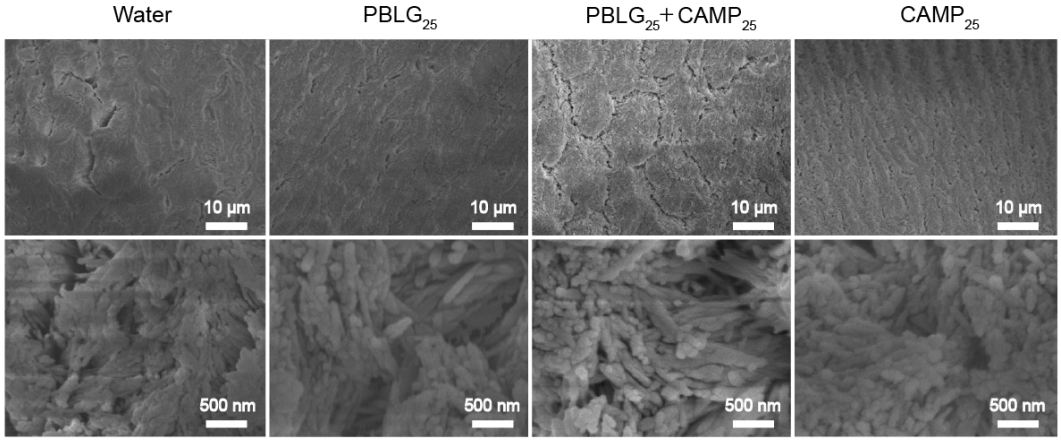
a


Water
PBLG15
PBLG15+CAMP15
CAMP15
10 μm
1 μm

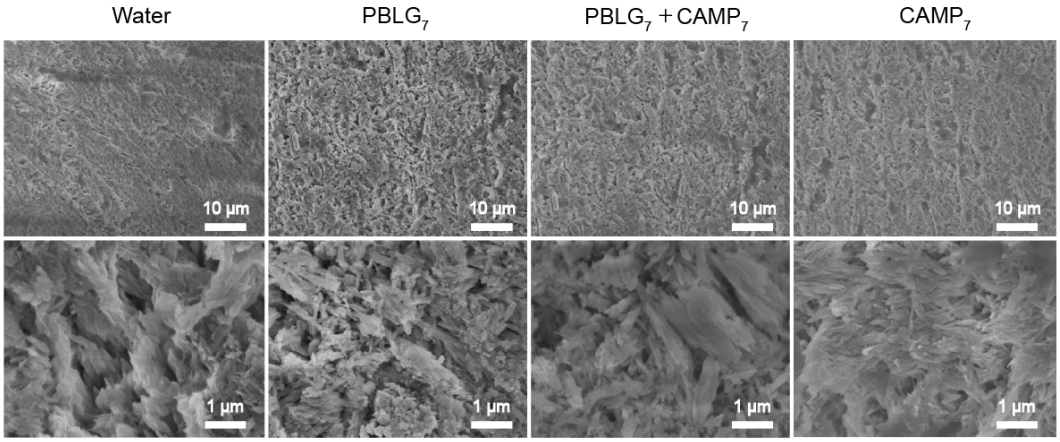
b


Water
PBLG7
PBLG7 + CAMP7
CAMP7
10 μm
1 μm

c


Water
PBLG25
PBLG25+CAMP25
CAMP25
10 μm
500 nm

d



e

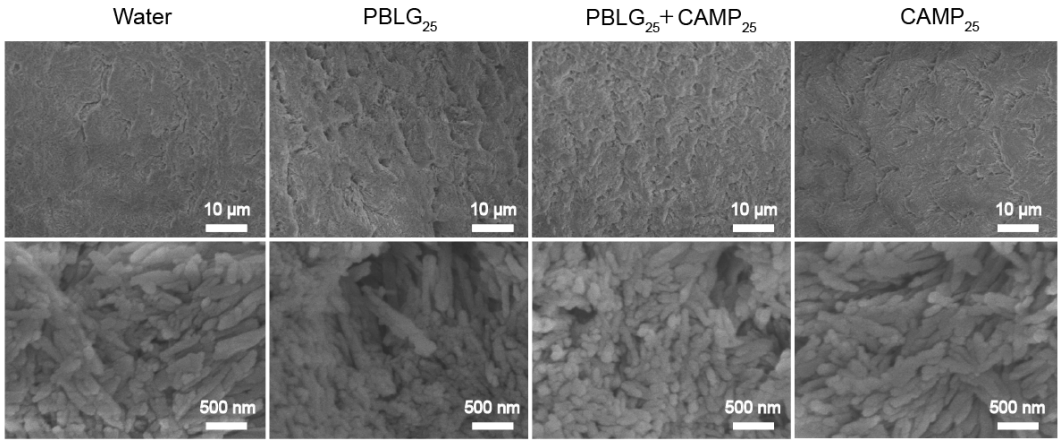
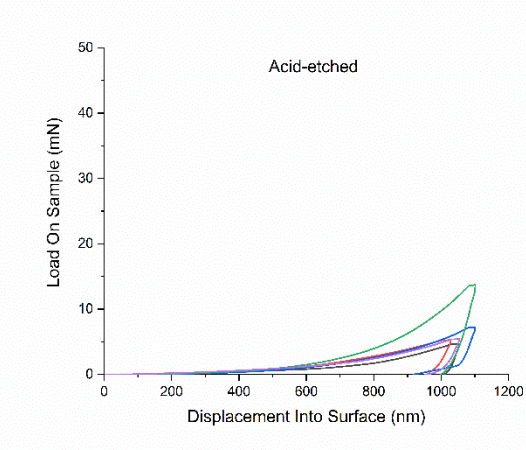
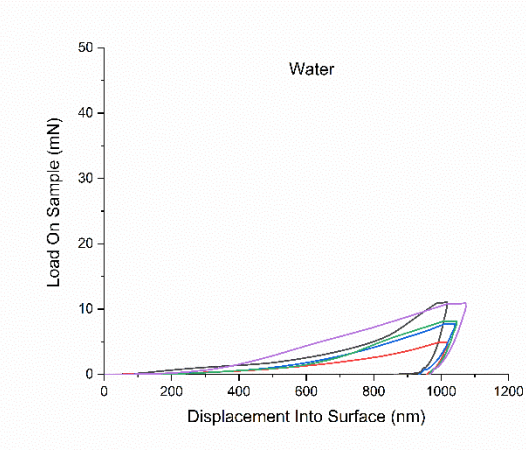
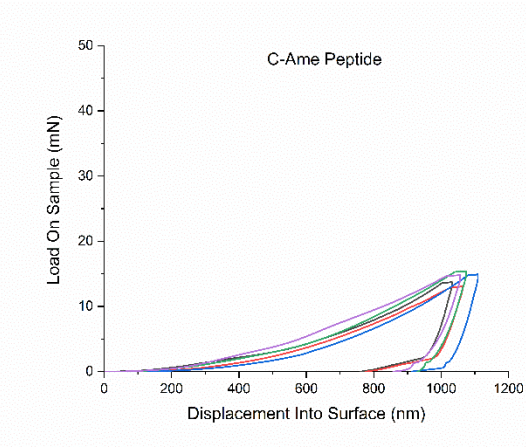


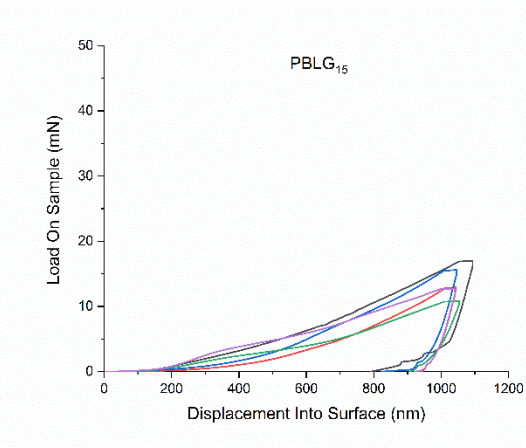
Fig. S9 a) SEM low-magnification images and high-magnification images of Water-coated enamel, $PBLG_{15}$-coated enamel, $PBLG_{15}$ + $CAMP_{15}$ (1/1, n/n)-coated enamel, and $CAMP_{15}$-coated enamel after 3 days of incubation in artificial saliva. b) SEM low-magnification images and high-magnification images of Water-coated enamel, $PBLG_7$-coated enamel, $PBLG_7$ + $CAMP_7$ (1/1, n/n)-coated enamel, and $CAMP_7$-coated enamel after 3 days of incubation in artificial saliva. c) SEM low-magnification images and high-magnification images of Water-coated enamel, $PBLG_{25}$-coated enamel, $PBLG_{25}$ + $CAMP_{25}$ (1/1, n/n)-coated enamel, and $CAMP_{25}$-coated enamel after 3 days of incubation in artificial saliva. d) SEM low-magnification images and high-magnification images of Water-coated enamel, $PBLG_7$-coated enamel, $PBLG_7$ + $CAMP_7$ (1/1, n/n)-coated enamel, and $CAMP_7$-coated enamel after 6 days of incubation in artificial saliva. e) SEM low-magnification images and high-magnification images of Water-coated enamel, $PBLG_{25}$-coated enamel, $PBLG_{25}$ + $CAMP_{25}$ (1/1, n/n)-coated enamel, and $CAMP_{25}$-coated enamel after 6 days of

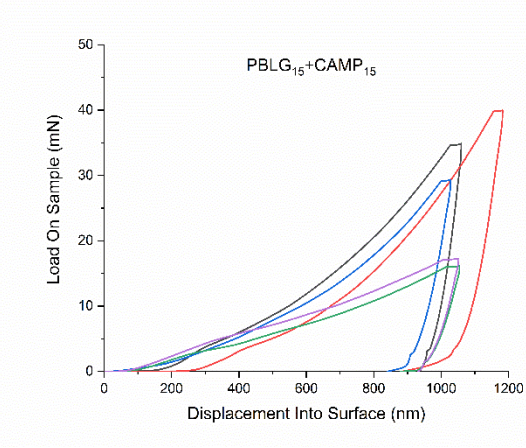
incubation in artificial saliva. Scale bars: 10 μm (a-e (top)), 1 μm (a, b, d (bottom)), and 500 nm (c, e (bottom)).

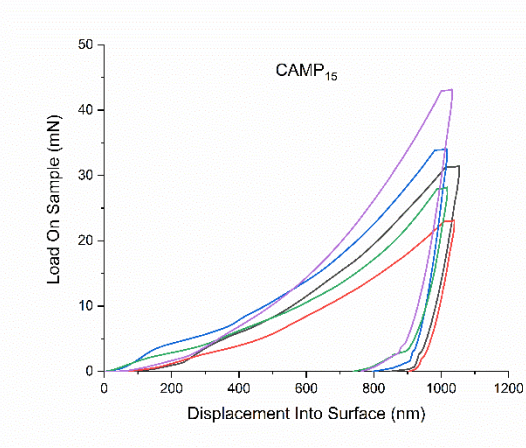

Acid-etched
Load On Sample (mN)
Displacement Into Surface (nm)
0
10
20
30
40
50
0
200
400
600
800
1000
1200


Water
Load On Sample (mN)
Displacement Into Surface (nm)
0
10
20
30
40
50
0
200
400
600
800
1000
1200


C-Ame Peptide
Load On Sample (mN)
Displacement Into Surface (nm)
0
10
20
30
40
50
0
200
400
600
800
1000
1200


PBLG15
Load On Sample (mN)
Displacement Into Surface (nm)
0
10
20
30
40
50
0
200
400
600
800
1000
1200


PBLG15+CAMP15
Load On Sample (mN)
Displacement Into Surface (nm)
0
10
20
30
40
50
0
200
400
600
800
1000
1200


CAMP15
Load On Sample (mN)
Displacement Into Surface (nm)
0
10
20
30
40
50
0
200
400
600
800
1000
1200

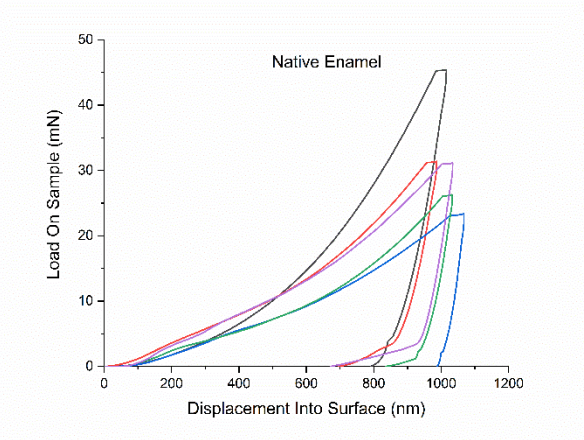
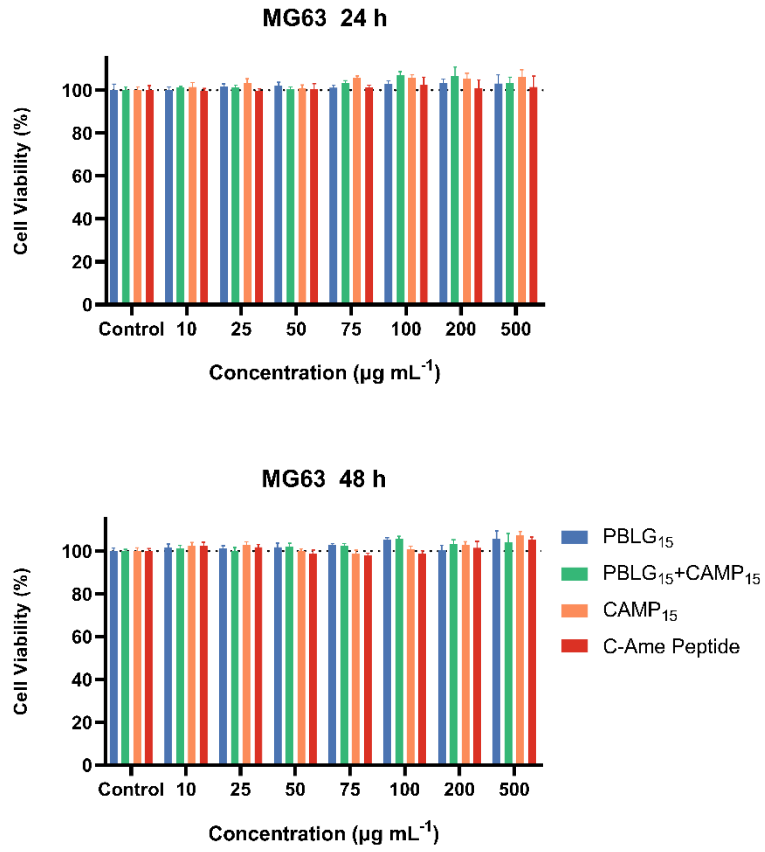
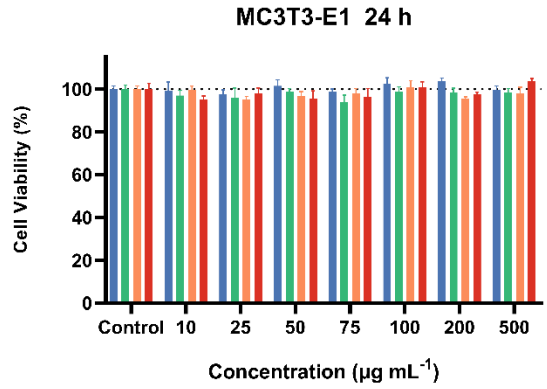


Fig. S10 Load-displacement curves in nano-indentation tests including Acid-etched enamel, Water-coated enamel, C-Ame Peptide-coated enamel, $PBLG_{15}$-coated enamel, $PBLG_{15}$ + $CAMP_{15}$ (1/1, n/n)-coated enamel, $CAMP_{15}$-coated enamel and Native enamel.

a


MG63 24 h
Cell Viability (%)
0
20
40
60
80
100
Control 10 25 50 75 100 200 500
Concentration (μg mL-1)
MG63 48 h
Cell Viability (%)
0
20
40
60
80
100
Control 10 25 50 75 100 200 500
Concentration (μg mL-1)
PBLG15
PBLG15+CAMP15
CAMP15
C-Ame Peptide

b


MC3T3-E1 24 h
Cell Viability (%)
0
20
40
60
80
100
Control 10 25 50 75 100 200 500
Concentration (μg mL-1)

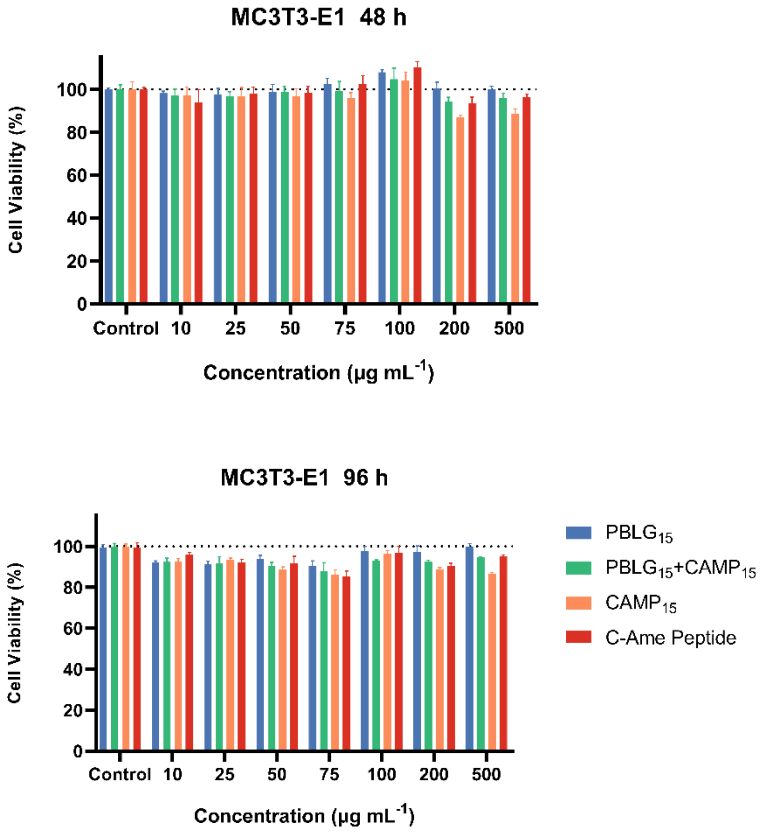
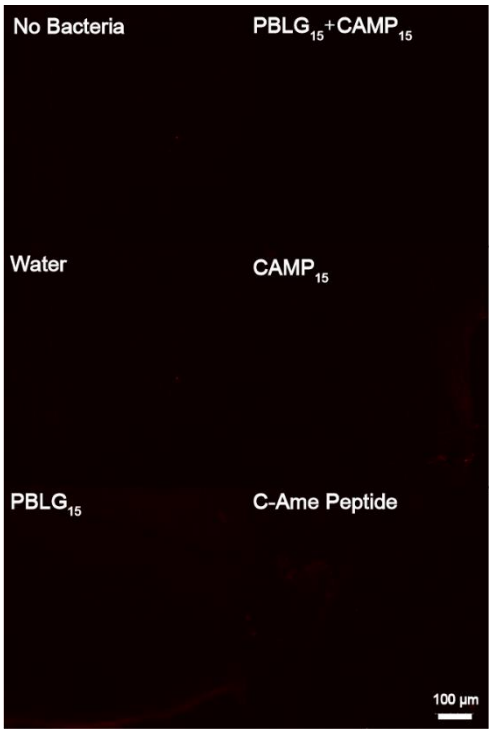
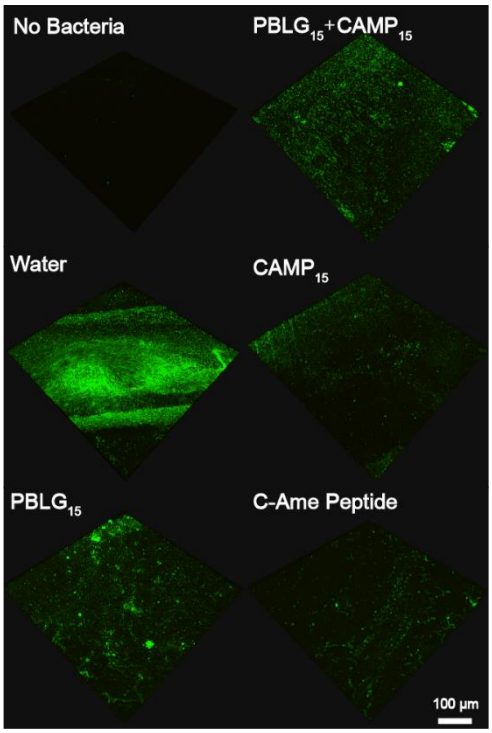


Fig. S11 Cell viability of MG63 cell for 24 h and 48 h (a), MC3T3-E1 cell for 24 h, 48 h and 96 h (b) at different concentrations (0, 10, 25, 50, 75, 100, 200, 500 μg mL^{-1}) of $PBLG_{15}$, $PBLG_{15}$ + $CAMP_{15}$ (1/1, n/n), $CAMP_{15}$ and C-Ame Peptide. These data are the means ± SD (n=5).

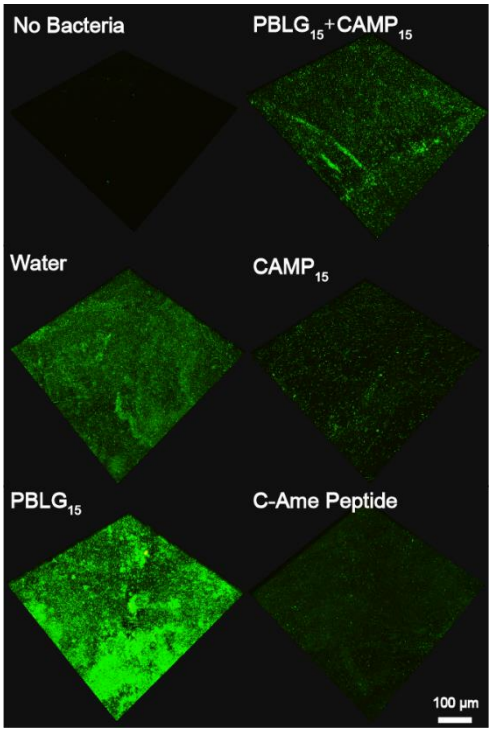
a


No Bacteria
PBLG15+CAMP15
Water
CAMP15
PBLG15
C-Ame Peptide
100 µm

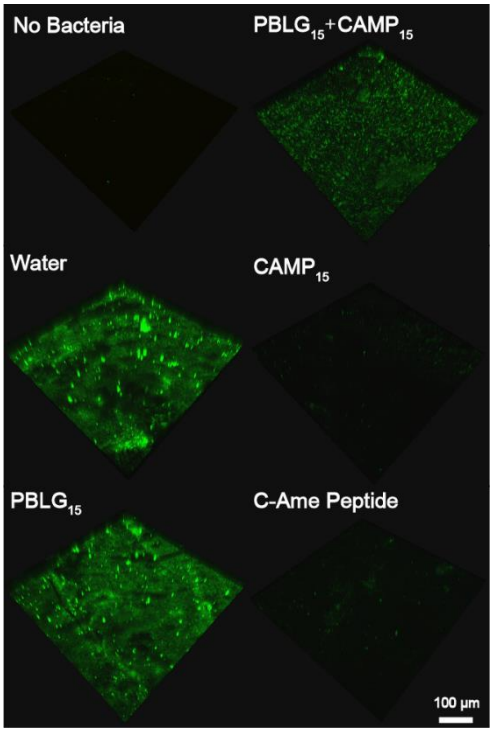
b


No Bacteria
PBLG15+CAMP15
Water
CAMP15
PBLG15
C-Ame Peptide
100 µm

c


No Bacteria
PBLG15+CAMP15
Water
CAMP15
PBLG15
C-Ame Peptide
100 µm

d


No Bacteria
PBLG15+CAMP15
Water
CAMP15
PBLG15
C-Ame Peptide
100 µm

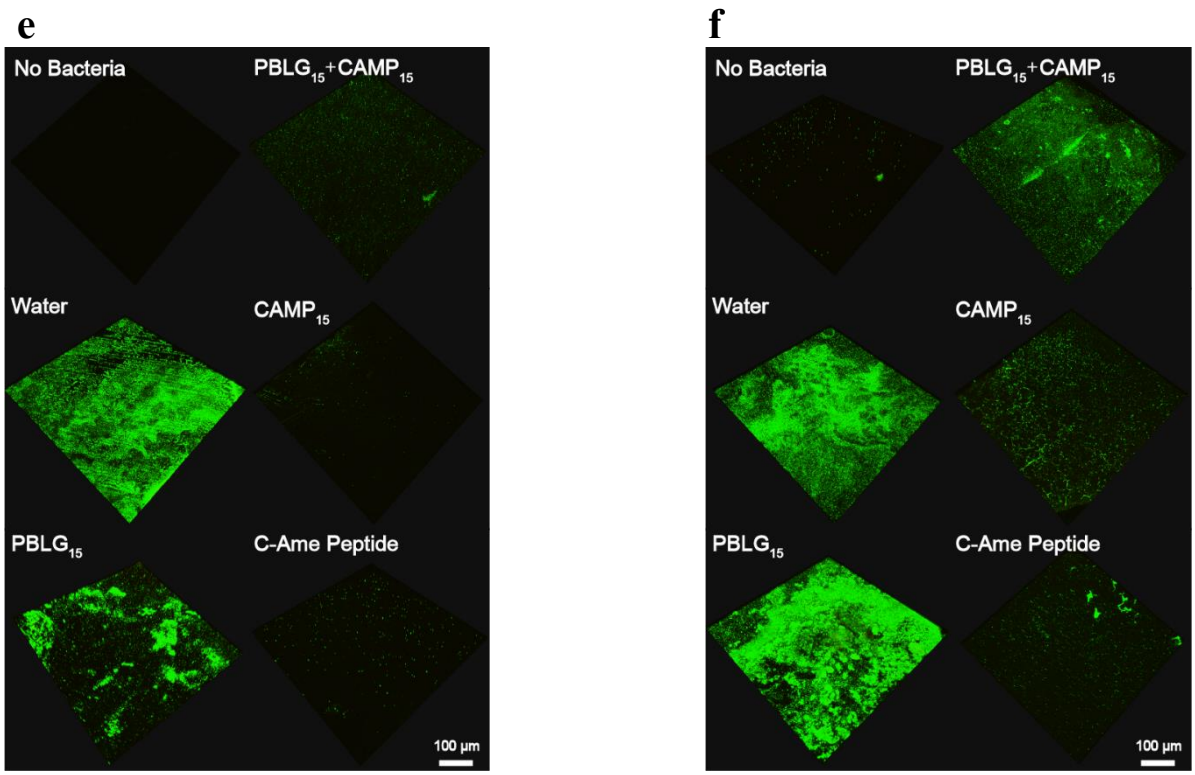


Fig. S12 CLSM images of dead *Streptococcus mutans* biofilm distribution after 24 hours (a), CLSM 3D images of *Streptococcus mutans* biofilm distribution after 48 hours (b) and 72 hours (c) on the different enamels coated with No Bacteria, Water, $PBLG_{15}$, $PBLG_{15}$ + $CAMP_{15}$ (1/1, n/n), $CAMP_{15}$ and C-Ame Peptide. CLSM 3D images of *Staphylococcus aureus* biofilm distribution after 24 hours (d), 48 hours (e) and 72 hours (f) on the different enamels coated with No Bacteria, Water, $PBLG_{15}$, $PBLG_{15}$ + $CAMP_{15}$ (1/1, n/n), $CAMP_{15}$ and C-Ame Peptide. Scale bar: 100 μm.