Supplementary Material (ESI) for Soft Matter


Supplementary Information for

# Protein, cell and bacterial fouling resistance of polypeptoid-modified surfaces: effect of side chain chemistry

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## Characterization

Purified peptidomimetic polymer fractions were analyzed by reversed-phase HPLC; spectra are shown in Figure S1. The molecular mass was confirmed using MALDI-MS; spectra are shown in Figure S2. The surface modification conditions for the peptidomimetic polymers were selected to achieve optimum polymer density by investigating the cloud point conditions. Absorbance at 500 nm was monitored with a U2010 Spectrophotometer (Hitachi, San Jose, CA) equipped with a Bath/Circulator (Neslab RTE-111; Portsmouth, NH), which was used to ramp the temperature from 25-95°C at 1°C per minute. The effect of salt content was investigated using several concentrations of NaCl. A measurable increase in optical density was indicative of polymer insolubility; the cloud point temperature was determined by linearly extrapolating the absorbance versus temperature curve to the baseline. A temperature ~10°C below the cloud point temperature was selected for substrate modification. The absorbance spectrum for PMP1 in saturated NaCl is shown in Figure S3. Optimum modification conditions for PMP1, PMP2 and PMP3 were determined to be a concentration of 1 mg $mL^{-1}$ in 3 M NaCl, 0.1 M MOPS (pH = 6) buffer at 50ºC for 24 h.

High-resolution XPS spectra of the C1s region for unmodified and polymer-modified $TiO_2$ surfaces are shown in Figure S4. The C1s spectra for the unmodified $TiO_2$ substrate contains a small peak at 284.6 eV from aliphatic hydrocarbon contamination as well as smaller signals for ether (286.3 eV) and amide (288.7 eV) carbons resulting from sample preparation and XPS chamber evacuation procedures.[1] For the polymer-modified substrates, the peak at 284.6 eV is due to the aliphatic and aromatic carbons in the peptoid side chains and the lysine and DOPA anchoring groups, as well as to some hydrocarbon contamination. The spectra for the polymer-modified substrates show a dramatic increase in the C-O and C-N peaks, compared to the unmodified $TiO_2$ surface, due to the ether carbons (286.6 eV) and carbons adjacent to amino groups (286.0 eV, 286.3 eV) in the peptoid side chains and backbone.[2] The amide groups of the peptidomimetic polymer backbone are represented by a peak at 288.0 eV.[2, 3] The differences in polypeptoid side chain compositions are reflected in the percentages of C1s components; specifically, the methoxy group in PMP1 is represented by the greater contribution from ether carbons compared to PMP2 and PMP3, while the greater contribution from aliphatic carbons for PMP3 is expected for the hydroxypropyl side chain.

## Enzyme degradation

Immobilized trypsin was used for initial solution experiments because the enzyme can be removed easily by centrifugation and separation; all mass fragments can be attributed to the polymers or buffer components, thus complicated analysis of enzyme fragments is not necessary. Experiments with PMP2 and PMP3 showed cleavage of the polymers at the carboxy side of the lysines after 4 h incubation with trypsin (Figure S5). The peaks correspond to the peptidomimetic polymers minus two DOPA residues and one lysine residue, as seen for the PMP1 sample. MALDI-MS spectra for polymer-modified substrates incubated in trypsin-containing solutions are shown in Figure S6. The MALDI-MS spectrum for the PMP2-modified $TiO_2$ substrate contains a peak at $m/z$ = 2897.9 for the $Na^+$ adduct of the immobilized polymer as well as several peaks from the MALDI matrix; PMP2 remained on the substrate after one week incubation with trypsin and was also stable to incubation with pronase. The MALDI-MS spectrum for the PMP3-modified substrate contains the expected polymer peak for the $Na^+$ adduct at $m/z$ = 3178.7 (Figure S6); for the substrates incubated with trypsin and pronase, peaks were present for the mass of the entire polymer, indicating no degradation. The ESI-MS spectra for the PMP2 and PMP3 solutions were nearly identical to the spectra for the PMP1 solutions (spectra not shown); no peaks were detected that would suggest removal of the polymers from the substrates.

Figure S7 contains MALDI-MS spectra for DOPA-Lys-DOPA-Lys-DOPA modified $TiO_2$ substrates before and after trypsin incubation. The spectra contain several peaks from the matrix as well as the expected mass peak at $m/z$ = 834.3. The MALDI-MS spectrum for a Tyr-Lys-Tyr-Lys-Tyr-(*N*-methoxyethyl glycine)$_{10}$ polymer-modified $TiO_2$ substrate contains peaks at $m/z$ = 1957.1 for the expected mass and at $m/z$ = 1980.2 for the $Na^+$ adduct (Figure S8, top). The spectrum for the Tyr-Lys-Tyr-Lys-Tyr-(*N*-methoxyethyl glycine)$_{10}$ polymer-modified $TiO_2$ substrate after 24 h incubation in U.P. $H_2O$ is shown in Figure S8 (bottom); the expected mass peaks are not present, indicating the polymer was easily removed from the $TiO_2$ substrate.

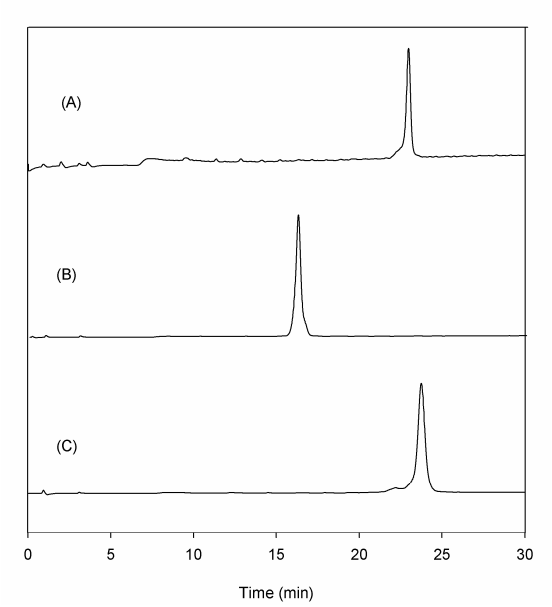


**Figure S1** Analytical RP-HPLC 215 nm spectra of (A) purified PMP1 (2-50% ACN in $H_2O$ with 0.1% v/v TFA, 1.0 mL $min^{-1}$), (B) PMP2 (2-30% ACN in $H_2O$ with 0.1% v/v TFA, 1.0 mL $min^{-1}$), and (C) PMP3 (2-50% ACN in $H_2O$ with 0.1% v/v TFA, 1.0 mL $min^{-1}$).

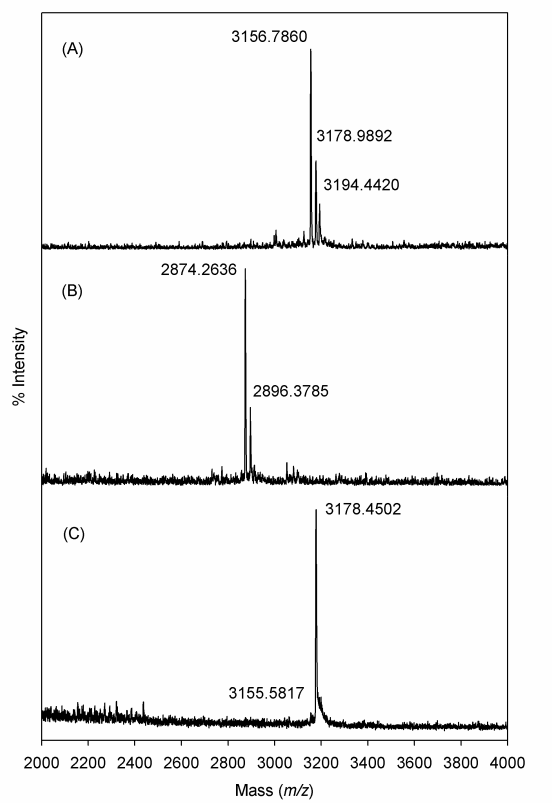


**Figure S2** MALDI-MS spectra of purified (A) PMP1 ($C_{141}H_{236}N_{28}O_{52}$), (B) PMP2 ($C_{121}H_{196}N_{28}O_{52}$) and (C) PMP3 ($C_{141}H_{236}N_{28}O_{52}$). Determined molecular weights: PMP1 *m/z* = 3156.78 (calculated molecular weight 3153.67), *m/z* = 3178.99 for $Na^+$ adduct, *m/z* = 3194.44 for $K^+$ adduct; PMP2 *m/z* = 2874.26 (calculated 2875.01), *m/z* = 2896.37 for $Na^+$ adduct; PMP3 *m/z* = 3178.45 for $Na^+$ adduct (calculated 3153.67).

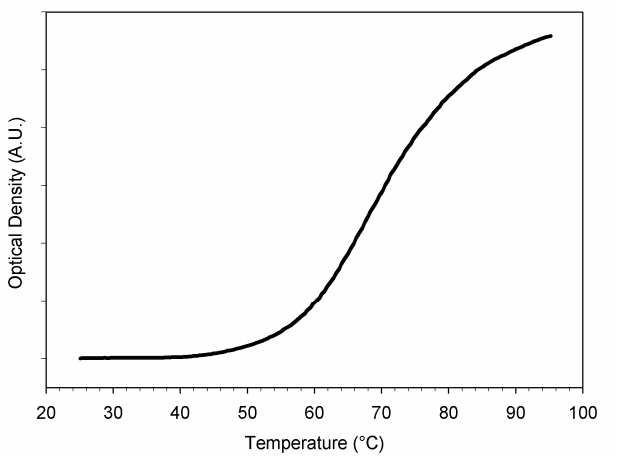


**Figure S3** Representative temperature scan for UV-Vis experiment conducted to determine the optimum modification conditions for PMP1. Plot shows scan for 1 mg $mL^{-1}$ PMP1 in sat. NaCl, 0.1 M MOPS (pH = 6) buffer.

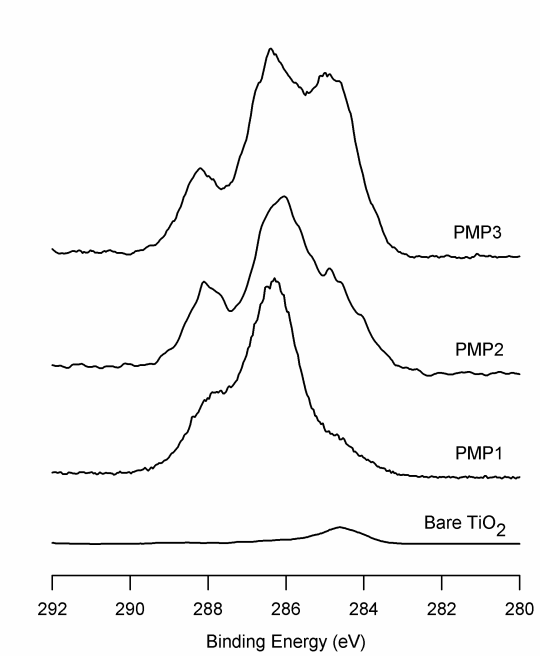


**Figure S4** High-resolution C1s XPS spectra of bare $TiO_2$, PMP1-, PMP2- and PMP3-modified $TiO_2$ substrates.

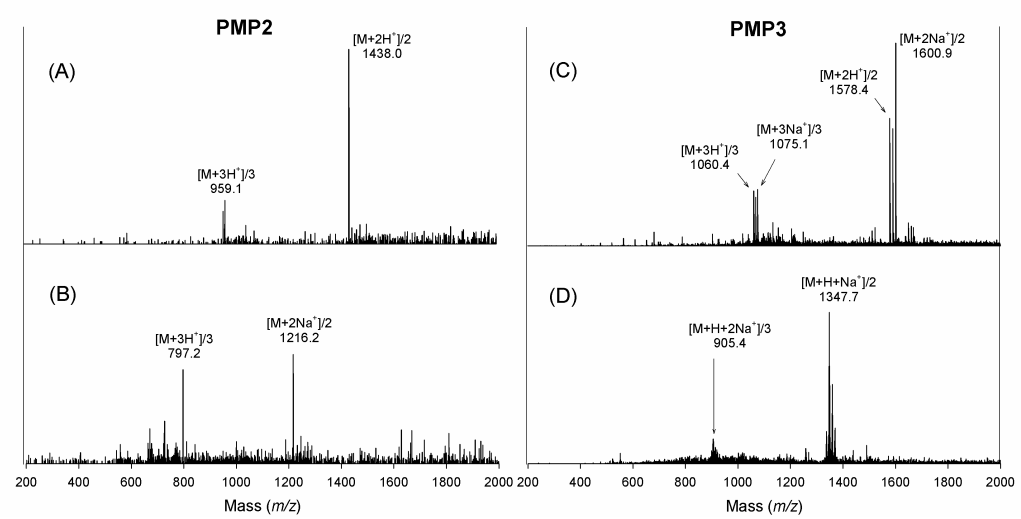


**Figure S5** ESI-MS spectra for PMP2 (left) and PMP3 (right) (A and C) before incubation and (B and D) after incubation with immobilized trypsin. Peaks in trypsin samples correspond to the polymer without DOPA-Lys-DOPA.

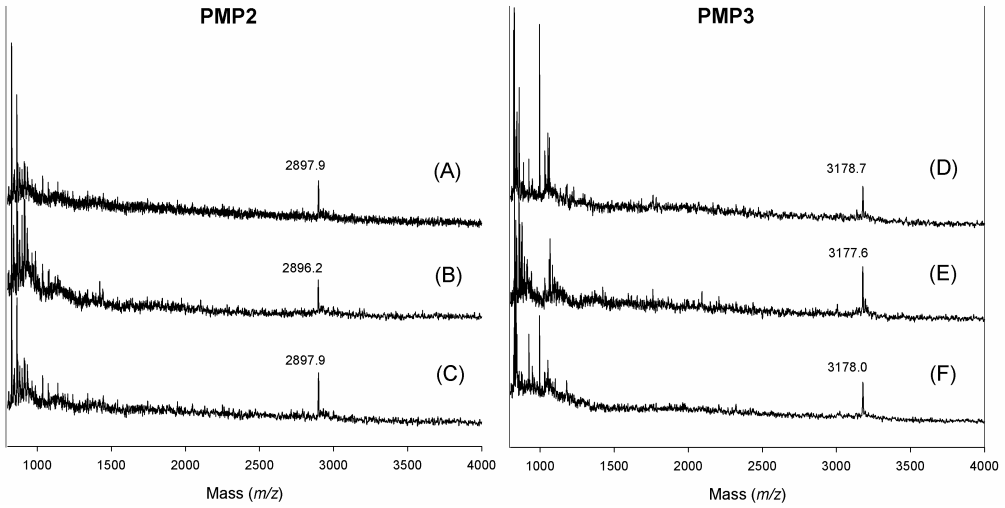


**Figure S6** MALDI-MS spectra for PMP2 (left) and PMP3 (right) substrates; modified substrates incubated with: (A and D) buffer only, (B and E) trypsin in buffer, and (C and F) pronase in buffer.

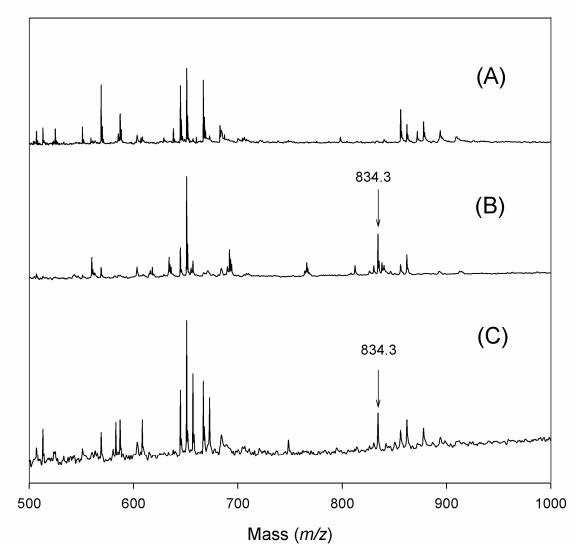


**Figure S7** MALDI-MS spectra for (A) $TiO_2$ substrate with matrix, (B) DOPA-Lys-DOPA-Lys-DOPA-modified $TiO_2$ substrate with matrix and (C) DOPA-Lys-DOPA-Lys-DOPA-modified $TiO_2$ substrate after 24 h incubation with trypsin. Peaks at *m/z* = 834.3 represent the sodium adduct for the peptide.

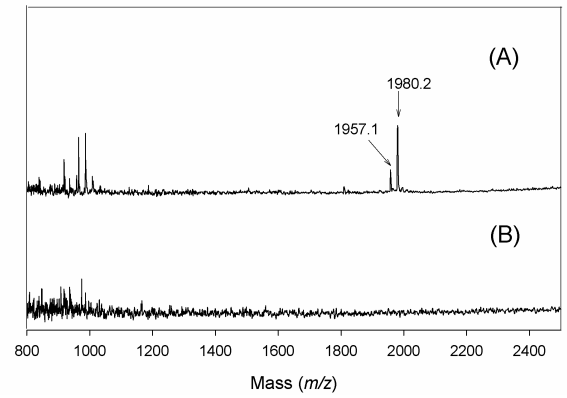


**Figure S8** MALDI-MS spectra for (A) YKYKY-$(NMEG)_{10}$-modified $TiO_2$ substrate with matrix and (B) YKYKY-$(NMEG)_{10}$-modified $TiO_2$ substrate after 24 h incubation with U.P. $H_2O$. Peaks at *m/z* = 1957.1 and 1980.2 represent the polymer mass and sodium adduct for the polymer.

## References

1. B. V. Crist, *Handbook of monochromatic XPS spectra*, Wiley, Chichester, New York, 2000.
2. N. P. Huang, R. Michel, J. Voros, M. Textor, R. Hofer, A. Rossi, D. L. Elbert, J. A. Hubbell and N. D. Spencer, *Langmuir*, 2001, **17**, 489-498.
3. G. L. Kenausis, J. Voros, D. L. Elbert, N. Huang, R. Hofer, L. Ruiz-Taylor, M. Textor, J. A. Hubbell and N. D. Spencer, *J. Phys. Chem. B*, 2000, **104**, 3298-3309.