

Electronic Supporting Information

Biomimetic Vaterite Formation at Surface – Structurally Templated by

Oligo(Glutamic Acid) Peptides

Hao Lu,^a Matthew A. Hood,^{a§} Sergio Mauri,^a Joe E. Baio,^b Mischa Bonn,^a Rafael Muñoz-Espí^a
and Tobias Weidner^a

^a *Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz Germany*

^b *School of Chemical, Biological, and Environmental Engineering Oregon State University, OR,
USA.*

§ *Current address: Max Planck Institute of Colloids and Interfaces, Am Mühlenberg 1, 14476
Potsdam Germany*

▪ Experimental Section:

Materials. Dodecanethiol (DDT, $\geq 97\%$) was purchased from Fluka. Phosphate buffered saline (PBS), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ($\geq 99\%$) were purchased from Sigma. NaHCO_3 (analytical grade, 100%) was purchased from Fisher Chemicals. Potassium chloride (KCl, molecular biology grade) was purchased from Serva Electrophoresis GmbH. Potassium hydroxide (KOH, ≥ 85 pellet) was purchased from Sigma Aldrich. Absolute ethanol ($\geq 99.99\%$) was purchased from Sigma Aldrich, and used for preparation of DDT Self-Assembled Monolayers (SAMs) on Au. Milli-Q water ($18.5 \Omega \cdot \text{m}$) was used for all other experiments.

Synthesis of Glu5 precursor. The synthesis of oligo(L-glutamic acid) pentamers was performed following the protocol in Ref. 1, then 2-iminothiolane hydrochloride was used to functionalize the oligo(L-glutamic acid) with a thiol linker. The peptide (50 mg) was dissolved in phosphate buffered solution with EDTA (1 mL) to chelate divalent metals. 20-fold molar excess of 2-iminothiolane was added to the solutions and allowed to incubate for 3 hours at room temperature, under constant stirring. Under these conditions the ring of the 2-iminothiolane opens to preferentially bind with primary amines. The modified peptides were then dialyzed in a dialysis bag with a 500 MWCO for 1 day, with a minimum of 5 exchanges with distilled water. The solutions were collected from the dialysis bag, and frozen with liquid nitrogen in round bottom flasks for lyophilization. The dried powders were stored at -20°C . The obtained thiol-terminated poly glutamic acid with pentamer was abbreviated as Glu5, with structure shown in Scheme 1.

Monolayer Preparation. Silicon wafers ($1 \times 1 \text{ cm}^2$, Active Business Company GmbH, Germany) were coated with 1.2 nm Cr and 50 nm Au (99.99%) by electron beam evaporation (pressure $< 1 \times 10^{-6}$ Torr). Following established protocol,^{2,3} DDT SAMs were assembled by immersing Au coated substrates in a 1 mM ethanolic solution for 24 hours, and then rinsed with absolute ethanol. Following the rinse step, the samples were dried by N_2 and stored under inert Ar for X-ray photoelectron spectroscopy (XPS) analysis or further experiments. Glu5 monolayers were prepared by immersing Au coated substrates in a 30 μM peptide in PBS solution for 40 hours at temperature of 37°C . To avoid lifting out the Glu5 layer at the air-water interface when taking sample out, the target solution was diluted 10 times by exchanging the upper solution with pure PBS. Afterwards, the sample was taken out and washed intensively with Milli-Q water, then dried by N_2 and stored under Ar for further use.

Crystallization on Monolayer Surface. A calcium-containing aqueous solution was degassed prior to use, and prepared as follows: $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.1 M, 2 mL) and KCl (3.0 M, 0.5 mL, added to keep the ionic strength constant) were mixed with 45.5 mL Milli-Q water to maintain the final volume at 50 mL. The substrate was fixed on one Teflon holder, and immersed in the target solution with the peptide-layer side down. The pH of the solution was adjusted to 8.5 by the addition of KOH solution (0.1 M). After complexation of surface-bound Glu5 with calcium ion for 1 hour, crystallization of CaCO_3 was conducted by direct addition of NaHCO_3 (0.1 M, 2 mL) in equal molar ratio to CaCl_2 solution. The complexation and crystallization were carried out under constant stirring (450 rpm) at room temperature, and the beaker was sealed to prevent any CO_2 exchange with the environment. After crystallization, the samples were dried in air and dipped into Milli Q water for a while, above process was reproduced three times in order to remove the salts attached on the surface.

Characterization.

XPS

X-ray photoelectron spectroscopy was conducted using a Kratos Axis Ultra^{DLD} spectrometer (Kratos, Manchester, England) using an Al $K\alpha$ excitation source with a photon energy of 1487 eV. The data was acquired in the hybrid mode using a 0° take-off angle, defined as the angle between the surface normal and the axis of the analyzer lens. For calculating atomic concentration (Table 1), spectra were collected with setting analyzer pass energy at 80 eV, and a linear background was subtracted for all peak quantifications. The peak areas were normalized by the manufacturer supplied sensitivity factors and surface concentrations were calculated using the Kratos Vision software. N 1s and S 2p high-resolution spectra (Figure 1 a and b) were collected with analyzer pass energy of 20 eV. Binding energy scales were calibrated to Au 4f_{7/2} emission at 84 eV.⁴

The thickness of Glu5 peptide monolayer (d_{sample}) was calculated by evaluating the intensity ratios of the C 1s and Au 4f emissions,⁵ according to a standard expression:

$$\frac{\frac{I_{\text{C}}}{I_{\text{Au}}}(\text{sample})}{\frac{I_{\text{C}}}{I_{\text{Au}}}(\text{refer})} = \frac{1 - \exp\left(-\frac{d_{\text{sample}}}{\lambda_{\text{C1s}}}\right)}{\exp\left(-\frac{d_{\text{sample}}}{\lambda_{\text{Au4f}}}\right)} \times \frac{\exp\left(-\frac{d_{\text{refer}}}{\lambda_{\text{Au4f}}}\right)}{1 - \exp\left(-\frac{d_{\text{refer}}}{\lambda_{\text{C1s}}}\right)} \quad (1)$$

and using DDT SAM with a well-known thickness (d_{refer}) of 15 Å as a reference.⁶ This value agrees well with the theoretical estimate of the SAM thickness on the basis of the alkyl chain length (1.26 Å per CH_2 moiety),⁷ molecular inclination (30–33.5°),^{8,9} and Au–S distance (1.8 Å).¹⁰ In equation 1, the attenuation lengths of the C 1s (λ_{C1s}) and Au 4f (λ_{Au4f}) photoelectrons

were taken at 28 and 30.9 Å, respectively, corresponding to the respective kinetic energies of 1197 eV (E_C) and 1398 eV (E_{Au}).¹¹

NEXAFS

Nitrogen K-edge near edge X-ray absorption fine structure (NEXAFS) spectra were collected at the U7A beamline at the National Synchrotron Light Source (NSLS, Brookhaven National Laboratory, Upton, NY) using an elliptically p-polarized (~85%) beam. The beamline is equipped with a monochromator and a 600 L/mm grating that provides a full-width at half-maximum resolution of ~0.15 eV at the carbon K-edge (285 eV). The monochromator energy scale was calibrated using the 398 eV N 1s π^* transition on a titanium nitride grid,¹² while partial electron yields were divided by the beam flux of a gold grid during data acquisition.¹³ A detector with a bias voltage of -360 V monitored partial electron yields. All samples were mounted to allow rotation about the vertical axis, thus, allowing the NEXAFS angle, the angle between the incident X-ray beam and the sample surface, to change. Previously, we ruled out the possibility of beam damage by repeatedly scanning between the 388 to 410 eV energy range while monitoring the partial electron yield at 398 eV.¹⁴ No changes in the spectra were observed within 15 min, which exceeded the exposure time in the longest nitrogen scan.

SFG

The sum frequency generation (SFG) vibration spectra were obtained by overlapping, in time and space, visible and IR pulses of light. A Ti:Sapphire amplified system (Spitfire Ace, Spectra Physics Inc.) delivers 35 fs long pulses at a central wavelength of 800 nm and 1 KHz repetition rate. The beam is split in two parts: one it is spectrally narrowed using a Fabry-Perot etalon to achieve spectral resolution (15cm^{-1} , $\lambda=800$, $E\sim 25\text{mJ/pulse}$). The other part is used to generate tunable broadband IR pulses thanks to a parametric optical amplifier followed by a noncollinear difference frequency generation module (TOPAS Prime, LightConversion). The average power at $6\mu\text{m}$ is 2mJ/pulse. Visible and IR beams are focused onto the sample using respectively a 20cm and 5cm focal length lenses. The polarization of both beams can be controlled (s or p) with a polarizer and a half waveplate. Beams are temporally and spatially overlapped at the sample position. The SFG signal is collimated using a 20cm FL lens, further focused into a spectrograph using a 5cm FL achromatic lens, dispersed by a grating and collected by an intensified CCD camera. The polarization of the SFG signal can also be controlled. All SFG spectra are collected using ppp polarization

combination. Each spectra was acquired for 5 minutes and we averaged over 5 different spots for each sample. The spectral response of the system is measured using a fresh evaporated gold substrate, and spectra are normalized accordingly.

XRD

X-ray diffraction (XRD) patterns were recorded on a Phillips PW 1820 diffractometer with an X-ray source producing a Cu K α wavelength of 1.5406 Å.

SEM

The morphology of the crystals was investigated by scanning electron microscopy (SEM) on a Zeiss 1530 LEO Gemini microscope with an accelerating voltage of 1 kV and a working distance of ~3 mm.

CD

Circular dichroism spectroscopy were recorded on a Jasco J-815 spectropolarimeter at 25°C in a step-scan mode using 0.1-cm path length quartz cells from Hellma (Mülheim, Germany). The concentration of Glu5 peptide was 0.2 mg/mL in phosphate buffer. Data points were collected at a resolution of 1 nm, an integration time of 1 s, and a bandwidth of 1 nm. The spectrum results from at least three averaged scans from which buffer scans were subtracted.

Table S1: Summary of XPS determined atomic composition^a for Glu5 SAM on Au.

	C	N	S	O
Theor. composition	55.9	11.5	2	30.6
Exp. composition	63.1 (1)	9.8 (0.6)	2.1 (0.3)	25 (0.9)

^a Values in atomic % with experimental errors in parentheses.

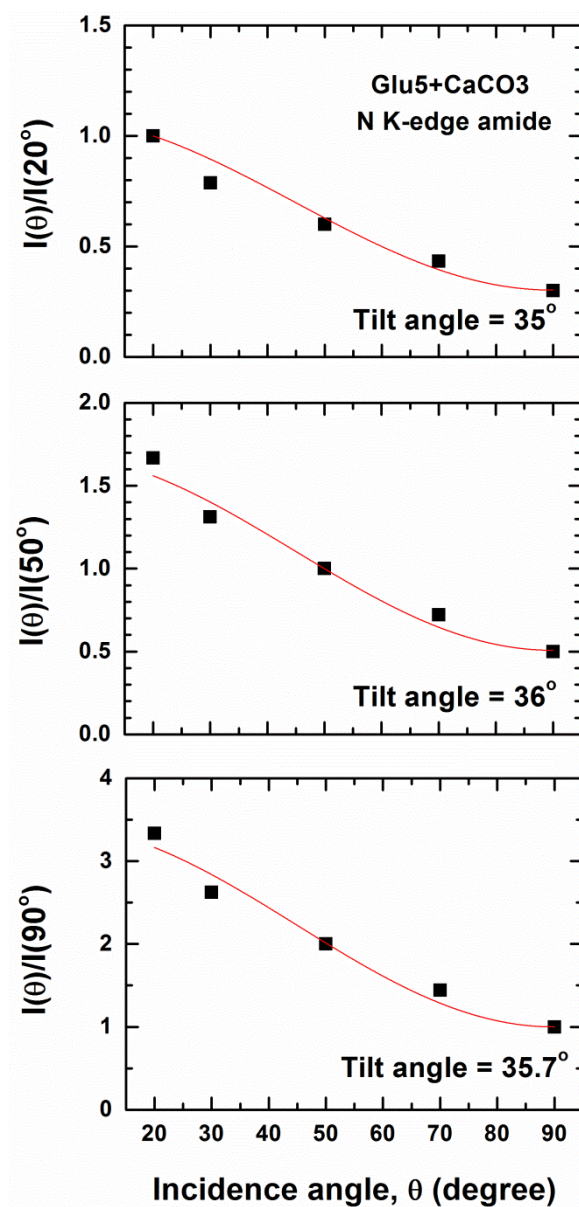


Figure S1. The angular dependence of the $I(\theta)/I(90^\circ)$ intensity ratios for the π^* resonance in Glu5 peptide layer after CaCO_3 mineralization. The best theoretical fits are shown as the red solid lines, and the derived values of the average tilt angles are given.

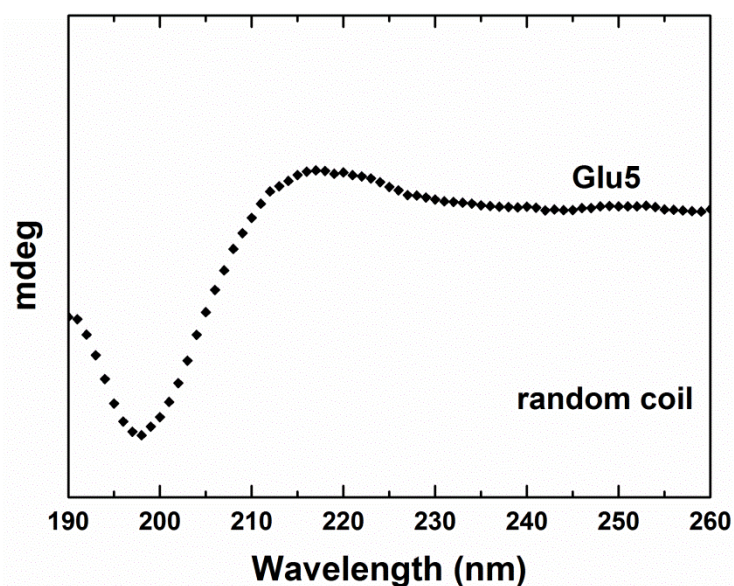


Figure S2. Circular Dichroism Spectra of Glu5 peptide in phosphate buffer solution

References

1. V. Fischer, K. Landfester and R. Muñoz-Espí, *Cryst. Growth Des.*, 2011, **11**, 1880-1890.
2. M. Zharnikov, *J. Electron Spectrosc. Relat. Phenom.*, 2010, **178**, 380-393.
3. J. C. Love, L. A. Estroff, J. K. Kriebel, R. G. Nuzzo and G. M. Whitesides, *Chem. Rev.*, 2005, **105**, 1103-1169.
4. J. F. Moulder, W. E. Stickle, P. E. Sobol and K. D. Bomben, *Handbook of X-ray Photoelectron Spectroscopy. A Reference Book of Standard Spectra for Identification and Interpretation of XPS Data*, Physical Electronics Division, Perkin-Elmer Corporation, Eden Prairie, 1992.
5. J. X. Liu, B. Schupbach, A. Bashir, O. Shekhah, A. Nefedov, M. Kind, A. Terfort and C. Wöll, *Phys. Chem. Chem. Phys.*, 2010, **12**, 4459-4472.
6. H. A. Biebuyck, C. D. Bain and G. M. Whitesides, *Langmuir*, 1994, **10**, 1825-1831.
7. M. M. Walczak, C. K. Chung, S. M. Stole, C. A. Widrig and M. D. Porter, *J. Am. Chem. Soc.*, 1991, **113**, 2370-2378.
8. U. A., in *Thin Films*, ed. U. A., Academic Press San Diego, CA, 1998.
9. A. Ulman, *Chem. Rev.*, 1996, **96**, 1533-1554.
10. H. Sellers, A. Ulman, Y. Shnidman and J. E. Eilers, *J. Am. Chem. Soc.*, 1993, **115**, 9389-9401.
11. C. L. A. Lamont and J. Wilkes, *Langmuir*, 1999, **15**, 2037-2042.
12. J. G. Chen, *Surf. Sci. Rep.*, 1997, **30**, 1-152.
13. J. Stöhr, *NEXAFS Spectroscopy*, Springer-Verlag, Berlin, 1992.
14. J. E. Baio, T. Weidner, J. Brison, D. J. Graham, L. J. Gamble and D. G. Castner, *J. Electron Spectrosc. Relat. Phenom.*, 2009, **172**, 2-8.