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**Electronic Supplementary Material**

*This supplementary material has not been peer-reviewed.*

**Supplementary Methods:**

**S1. Protein purification**

Cell pellets were resuspended in 4 mL of ice cold Tris-Sucrose EDTA buffer (0.5 M Sucrose, 1 mM EDTA, 0.2 M Tris-HCL, pH 8.0) and incubation on ice for 30 min. Ice cold dH20 (4 mL) was added and the solution was incubated for 60 min on ice prior to centrifugation at 6000 *g* for 15 min at 4°C. The supernatant was passed through a 0.45 μm filter (Sarstedt, Germany) to yield the soluble protein fraction. The remaining pellet was resuspended in an equivalent volume of PBS to form the insoluble protein fraction.

The soluble fraction was dialysed against 5 L PBS prior to purification as follows using Cobalt-IDA Agarose resin (Jena Bioscience, Germany): imidazole (5 mM) and Tween 20 (2%) were added to samples prior to application to the resin, followed by washes with 20 resin volumes of PBS containing 20 mM imidazole and elution using 10 mL PBS containing 300 mM imidazole. After dialysis of eluates against purification buffer (20 mM phosphate, 500 mM NaCl, pH 7.4), Tween 20 (2% v/v) and 30 mM imidazole were added to samples and a second purification step was carried out using a 1 mL HisTrap™HP column (GE Healthcare Life Sciences, PA, USA) prepacked with Ni-Sepharose™ resin, with a flow rate of 1 mL min-1. To remove non-specifically adsorbed materials, the column was washed with 20 mL each of purification buffer containing 30 mM and 40 mM imidazole, and 10 mL containing 50 mM imidazole. Proteins were eluted in 0.5 mL volumes of purification buffer containing 500 mM imidazole. Following buffer exchange into 50 mM Tris-HCL pH 8, 0.5 mM EDTA using Zeba™ Spin Desalting Columns (Thermo Fisher Scientific, MA, USA), the hexahistidine tag was cleaved from the recombinant protein by incubating with gentle agitation with 1 U AcTev™ protease (Invitrogen, CA, USA) per 3 µg of protein for 16 h at 4°C. To isolate tag-free protein, which we termed ‘rPpolcp19k’, the digestion products were loaded onto a HisPur™ Ni-NTA Spin Column to remove the His-tagged TEV protease, with tag-free rPpolcp19k collected in the flow through. *E. coli* trigger factor (TF*EC*) was expressed and purified as described by Robin *et al*. [1].

**S2. Preparation of SAMs**

SAMs were prepared on 12 x 12 mm2 glass chips with a 45 nm gold layer (obtained from GE Healthcare, Uppsala, Sweden) for SPR, and on 20 x 40 mm2 glass slides (pre-cleaned Nexterion glass B, Schott, Germany), coated with 25 Å Ti and 2000 Å Au in a high-vacuum electron-beam evaporation system, for SAM characterization. Gold-coated substrates were cleaned in a 5:1:1 mixture of ultrapure water (Milli-Q), 30% hydrogen peroxide and 25% ammonia, at 85°C for 5 min, and rinsed under running Milli-Q water, before being immersed in thiol solutions.

Five different thiols were used: HS(CH2)15CH3 (CH3)(Fluka), HS(CH2)16OH (OH)(gift from Biacore AB (now GE Healthcare)), HS(CH2)15COOH (COO-)(Sigma-Aldrich), HS(CH2)16N(CH3)3+ Cl- (N(CH3)3+)(Prochimia) and HS(CH2)15CONH(C2H4O)6H (OEG)(prepared as described by [2]).

Cleaned gold-coated substrates were immersed in 50 µM solutions of the thiols in ethanol (99.5%, Solveco) for at least 24h and sonicated in ethanol and dried with nitrogen immediately before use. The COO- thiol was incubated under acidic conditions by adding 10% (v/v) glacial acetic acid to the solvent, and the N(CH3)3+ thiol incubated under alkaline conditions by adding 2% (v/v) 25% ammonia to the solvent. The corresponding SAMs were also sonicated under similar acidic and alkaline conditions, respectively.

SAM thicknesses were obtained with an ellipsometer (Rudolph Research AutoELIII) with a 70° angle of incidence and a He–Ne laser (λ = 632.8 nm) as a light source. Optical properties of the gold substrates were obtained immediately after cleaning, before immersion in thiol solutions. A three-layer substrate/organic film/air model with a refractive index of 1.5 for the organic layer was used to calculate thicknesses. Data from five different spots on at least two samples of each type were automatically acquired and averaged.

The advancing contact angles were determined using a semi-automatic contact angle meter (KSV CAM 200). Sessile water droplets were manually expanded or contracted using a syringe, and contact angles were determined from edge analysis of images recorded of the droplets during expansion, using the software supplied with the instrument. Data for each SAM were obtained from averaging two samples. Two measurements were made on each sample, and each measurement consisted of at least 10 images.

**Table S1**. Ellipsometric thicknesses and results from contact angle measurements

|  |  |  |
| --- | --- | --- |
|  | Ellipsometric | Advancing contact |
| SAM | thicknesses (Å) | angles (°) |
| CH3 | 20.8 ± 0.5 | 108 ± 1 |
| OH | 23.2 ± 0.4 | 35 ± 2 |
| COO- | 22.7 ± 0.5 | 46 ± 2 |
| N(CH3)3+ | 27.5 ± 1.1 | 59 ± 2 |
| OEG | 35.4 ± 0.5 | 36 ± 1 |

Ellipsometric thicknesses and results from contact angle measurements are shown in Table S1. Infrared reflection-absorption spectra of the formed SAMs verify the crystalline nature of the aliphatic chain regions of the monolayers via the positions of the methylene C-H stretching vibrations. The symmetric C-H stretch appears at 2850 cm-1 for all SAMs except the OEG SAM, for which it is at 2851 cm-1, and the asymmetric C-H stretch is located at 2918 cm-1 for all SAMs, indicating highly oriented and all-trans chains in the SAMs.

rPpolcp19k 1 ATPNCNISSESSLGQSGRTAGNAAVSGTTSTSGSASGLCGFQSPIAKLKDNGAVNSGVTG 60

FN244142 1 ATPNCNISSESSLGQSGRTAGNAAVSGTTSTSGSASGLCGFQSPIAKLKDNGAVNSGVTG 60

rPpolcp19k 61 TVVSAGFGSAGQQANSKGAVGTTPDGTTVTTTSGGSGGSNGGGGVSQGGGANAGATKKKV 120

FN244142 61 TVVSAGFGSAGQHANSKGAVGTTPGGTTVTTTSGGSGGSNGGGGVSQGGGANAGATKKKV 120

 \* \*

rPpolcp19k 121 VVVVLANGHKVVKLEDQAEGSGTSSSGHKASSTHNGVFNIQQGGETKIKLPPPLTG 176

FN244142 121 VVVVLANGHKVVKLEDQAEGSGTSSSGHKASSTHNGVFNIQQGGETKIKLPPPLTG 176

**Figure S1.** Amino acid sequence alignment of rPpolcp19k toGenBank Accession number FN244142 [3]. Amino acid substitutions are highlighted by an asterisk.



**Figure S2**. SPR sensorgrams for the adsorption of rPpolcp19k, TF, BSA and fibrinogen on CH3, OH, N(CH3)3+, COO- and OEG SAMS in ‘seawater conditions’ i.e. at pH 8.0, 600 mM NaCl, 10 μl min-1 flow rate and 25 oC. A 3 min protein injection was followed by a 2 min dissociation phase with 0.01 M sodium phosphate buffer pH 8.0 and 600 mM NaCl.

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**Figure S3**. SPR sensorgrams for the adsorption of BSA, Cell-Tak and rPpolcp19k on on CH3, OH, N(CH3)3+, COO- and OEG SAMS in ‘gland conditions’ i.e. at pH 4.0, 150 mM NaCl, 10 μl min-1 flow rate and 25 oC. A 3 min protein injection was followed by a 2 min dissociation phase with 0.01 M sodium acetate buffer pH 4.0 and 150 mM NaCl.

**Table S2.** Amino acid composition of cp19k proteins from different barnacle species.

Six amino acids that are biased, *i.e.* over-represented, in this protein relative to other barnacle cement proteins are indicated in bold.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | *Pollicipes pollicipes* | *Megabalanus rosa* | *Balanus albicostatus* | *Balanus amphitrite* | *Balanus improvisus* |
|  | **MW:**16.559**pI:** 9.26  | **MW:**19.51**pI:** 5.45 | **MW:** 17.348**pI:** 9.80 | **MW:**20.16**pI:** 9.67 | **MW:**16.85**pI:** 9.77 |
| **Ala** | **9.7** | **12.1**  | **10.4**  | **10.3**  | **12.1** |
| Arg | 0.6  | 0.5  | 0.6  | 0.5  | 0 |
| Asn | 6.2  | 4.0  | 3.5  | 2.5  | 4 |
| Asp | 1.7  | 5.6  | 2.9  | 4.4  | 2.9 |
| Cys | 1.1  | 1.5  | 1.2  | 1.0  | 1.2 |
| Gln | 4.5  | 2.0  | 1.7  | 1.5  | 4.6 |
| Glu | 2.3  | 4.5  | 4.6  | 3.4  | 1.7 |
| **Gly** | **20.5**  | **14.1**  | **12.7**  | **14.8**  | **14.5** |
| His | 1.7  | 0.5  | 1.2  | 1.5  | 1.2 |
| Ile | 2.3 | 2.5  | 1.7  | 4.4  | 5.2 |
| Leu | 4  | 6.6  | 7.5  | 7.4  | 8.1 |
| **Lys** | **6.2**  | **8.6**  | **13.9**  | **12.8**  | **9.8** |
| Met | 0 | 0.5  | 0 | 0.5  | 0 |
| Phe | 1.7  | 3.0  | 2.3  | 1  | 1.2 |
| Pro | 3.4  | 2.5  | 2.3  | 3.4  | 2.9 |
| **Ser** | **14.2**  | **10.6**  | **8.7**  | **6.4**  | **9.8** |
| **Thr** | **10.8**  | **11.1**  | **14.5**  | **11.3**  | **11.6** |
| Trp | 0 | 0.5  | 0 | 0.5  | 0 |
| Tyr | 0 | 0 | 0 | 0 | 0 |
| **Val** | **9.1**  | **9.1**  | **10.4**  | **12.3**  | **9.2** |
| Pyl | 0 | 0 | 0 | 0 | 0 |
| Sec | 0 | 0 | 0 | 0 | 0 |

**Table S3.** Amounts of protein (ng cm-2) adsorbed onto SAMs at pH 4.0, 150 mM NaCl. Cell-Tak: commercial *Mytilus edulis* mussel foot protein (mfp).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | pI | COO- | N(CH3)3+ | CH3 | OEG | OH |
| BSA | 4.7 | 131.27 ±0.04 | 26.03 ±0.05 | 57.67 ±2.71 | 1.66 ±0.02 | 23.84 ±1.22 |
| Cell-Tak | mfp1=9.99 mfp2 =9.14 | 53.10 ±15.26 | 130.49 ±0.03 | 71.61 ±7.73 | 0.70 ±0.18 | 20.30 ±4.89 |
| rPpolcp19k | 9.26 | 24.17 ±2.41 | 17.65 ±0.01 | 39.35 ±26.59 | 2.80 ±1.99 | 4.84 ±1.09 |

**Table S4.** Amounts of protein (ng cm-2) adsorbed onto SAMs at pH 8.0, 600 mM NaCl.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | pI | COO- | N(CH3)3+ | CH3 | OEG | OH |
| BSA | 4.7 | 1.22 ±0.44 | 15.27 ±11.45 | 44.23 ±7.11 | 0.34 ±0.02 | 11.51 ±3.05 |
| Fibrinogen | 5.3-6.1 | 7.62 ±1.44 | 93.70 ±79.22 | 210.55 ±9.46 | 6.89 ±8.99 | 73.45 ±5.79 |
| Trigger factor | 4.8 | 139.75 ±2.68 | 50.63 ±31.60 | 80.12 ±15.80 | 1.10 ±0.15 | 28.22 ±9.68 |
| rPpolcp19k | 9.3 | 1.71 ±0.35 | 14.05 ±4.74 | 45.31 ±15.09 | 1.27 ±0.47 | 5.36 ±2.34 |

**Supplementary material**

**References**

[1] Robin, S., Togashi, D. M., Ryder, A. G. & Wall, J. G. 2009 Trigger Factor from the psychrophilic bacterium *Psychrobacter frigidicola* is a monomeric chaperone. *J Bacteriol* **191**, 1162-1168. (DOI: 10.1128/JB.01137-08).

[2] Svedhem, S., Hollander, C. A., Shi, J., Konradsson, P., Liedberg, B. & Svensson, S. C. 2001 Synthesis of a series of oligo(ethylene glycol)-terminated alkanethiol amides designed to address structure and stability of biosensing interfaces. *J Org Chem* **66**, 4494-4503.

[3] Meusemann, K., von Reumont, B. M., Simon, S., Roeding, F., Strauss, S., Kuck, P., Ebersberger, I., Walzl, M., Pass, G., Breuers, S., et al. 2010 A phylogenomic approach to resolve the arthropod tree of life. *Mol Biol Evol* **27**, 2451-2464. (DOI:10.1093/molbev/msq130).