Supporting information

Recombinant proteins: a molecular tool to understand marine adhesion and to advance biomaterials

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Supplementary Table 1. List of recombinant marine adhesive proteins extracted from the literature, with details about their production, purification and use.

Adhesive protein [Species]	Host strain for expression	Recombinant protein	MW (kDa)	Codon optimization	Presence in the soluble or insoluble fraction	Extraction and purification strategy[e]	Yield	DOPA modification	Testing and use of recombinant protein [g]	References
Mussels										
Mfp1 [*Mytilus edulis*]	*S. cerevisiae* Yeast D8	Partial (20-80 decapeptide repeats)	from 24 to 96	No	Insoluble	Extraction with 10% formic acid; IEC	3-5 % of the total yeast cell protein	*In vitro*, bacterial tyrosinase	Surface coating analysis	[1]
Mfp1 [*M. edulis*]	*E. coli* BL21[DE3]	Partial (20 decapeptide repeats)	25	Yes	Insoluble (67% of proteins in IB)	Extraction with 2.5 M urea, 0.8 M acetic acid and 0.5% CTAB; IEC and SEC	60% of total cell proteins	No	Edman sequencing and amino acid composition	[2]
Mfp1 [*M. edulis*]	*E. coli* strain BL21 (DE3)	Partial (6 decapeptide repeats)	7	Yes	Insoluble	Extraction with 10% acetic acid, IEC	Nr	*In vitro*, mushroom tyrosinase	Structural and surface coating analysis	[3]
Mfp1 [*M. edulis*]	*E. coli* BL21 (DE3)	Partial (7 decapeptide repeats) with N-term OmpA signal peptide and C-term His-tag	~8	Nr	Both (partially soluble)	Extraction with 500 mM NaCl, 20 mM Tris-HCl, 5 mM imidazole, pH 7.9, IMAC and dialysis against 5% acetic acid	13 to 14.7 mg of total soluble protein per 100 ml of culture	*In vitro*, mushroom tyrosinase	Surface coating analysis	[4]
Mfp1 [*M. edulis*]	*S. cerevisiae*	Full-length	130	Nr	Nr	Nr	Nr	Nr	-	Cited in [5]
Mfp1 [*M. edulis]*	*E. coli* BL21 (DE3)	Partial (12 decapeptide repeats)	13.6	Yes	Insoluble	Extraction with 5% acetic acid and purified using RP-HPLC	nr	*In vitro*, mushroom tyrosinase	AFM, SFA, complex coacervation, fabrication of hydrogel, nanofibres or microparticles	[6-17]
Mfp1 [*M. edulis*]	*E. coli* BL21 (DE3)	Partial (22 decapeptide repeats)	24.9	Yes	Insoluble	Extraction with 5% acetic acid and purified using RP-HPLC	nr	*In vitro*, mushroom tyrosinase	Simple and complex coacervation	[13, 14]
Mfp1 [*M. edulis*]	*E.coli* BL21 (DE3)	Hybrid (fusion of InaKC and 12 decapeptide repeats of mfp-1 [InaKC-mfp1])	~29	nr	Insoluble	Extraction with 6 M guanidine hydrochloride, IMAC, dialysis in 5% acetic acid	66 mg/l for InaKC-mfp1 & 25 mg/l of mfp1	*In vitro*, mushroom tyrosinase	Solubility test, and magnetite microparticles agglomeration test	[18]

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Mfp2 [*M.edulis*]	*S. cerevisiae*	Full-length	42-47	nr	nr	nr	nr	nr	-	Cited in [5]
Mfp3 [*Mytilus galloprovincialis]*	*E. coli* BL21	Full-length Mfp3A with His-tag	6.96	No	Insoluble	Extraction with 8M urea, IMAC, dialysis in 5% acetic acid buffer	0.84 mg/l	*In vitro*, mushroom tyrosinase	QCM, AFM	[19]
Mfp3 [*M. galloprovincialis]*	*E.coli* BL21-CodonPlus (DE3)-RIPL	Full-length Mfp3B with or without His-tag	8.1	Yes	Insoluble	Extraction with 25% acetic acid and dialyzed again 20 mM citrate buffer	nr	*In vitro*, mushroom tyrosinase	Coacervation tests, turbidity measurements	[20]
Mfp3 [*Mytilus californianus*]	Yeast *K. lactis* (pKLAC1)	Full-length with human-influenza-virus hemagglutinin (HA) tag	~35 (aggregate)	Yes	Soluble	Affinity chromatography (HA-tag) and enterokinase cleavage of the tag	1 mg/l	No	-	[21]
Mfp3 [*Mytilus coruscus*]	*E. coli Transetta* (DE3)	Full-length with C-term His-tag	~9	No	Insoluble	Extraction 8M urea, IMAC, dialysis in 5% acetic acid buffer, and RP-HPLC	nr	*In vitro,* mushroom tyrosinase	Surface coating analysis, QCM	[22]
Mfp3 [*M. galloprovincialis*]	*E. coli* BL21 (DE3)	Full-length with His-tag	~6.8	Yes	Insoluble	Extraction 8M urea, IMAC, dialysis in 10 mM acetic acid	47 mg/l of culture (56% of the total cellular protein)	*In vitro,* mushroom tyrosinase	Measurement of bulk adhesion strength, simple coacervation, rheology, SFA	[23, 24]
Mfp3 [*M. galloprovincialis*]	*E. coli* JW2581 tyrosine auxotroph	Full-length mfp3F with His-tag	~6.8	Yes	Insoluble	Extraction 8M urea, IMAC, dialysis against 5% acetic acid with 1 mM ascorbic acid	3–5 mg/l of culture	*In vivo* by incorporation via tyrosyl-tRNA synthetase (TyrRS)	Surface coating analysis, SFA, DOPA-Fe^{3+} complexation, simple coacervation	[24-26]
Mfp3 [*M. galloprovincialis*]	*E. coli* JW2581 tyrosine auxotroph	Full-length with N-term His-tag	6.7	nr	Insoluble	Extraction 8M urea, IMAC, elution with 0.5M HCl and PD-10 desalting column to exchange the buffer condition	4.19 mg/l	*In vivo*, co-expression with *E. coli* TyrRS or *Methanococcus jannaschii* TyrRS (over 90% DOPA)	-	[27]

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Mfp3 [*M. galloprovincialis*]	*E. coli* BL21 (DE3)	Full-length Mfp3B with N-term His-tag, co-expression with various tyrosinases (TyrSc, TyrSa & TyrVs)	~10.7 or ~12.4	Yes	Both soluble and insoluble	extraction with 25% acetic acid and dialyzed with 5% acetic acid and 1 mM ascorbic acid	From 51 to 87 mg/l	*In vivo* with co-expressed tyrosinase	Surface coating analysis, adhesive shear strength measurements	[28]
Mfp3B [*M. galloprovincialis*]	*E. coli* BL21 (DE3)	Full-length with a C-term His-tag and N-term SUMO and/or TrxA tags	12	Yes	Both soluble and insoluble	Extraction with 25 mM ascorbic acid and 8 M urea, IMAC, elution with 0.5M HCl and dialysis against 5% glacial acetic acid and 1 mM ascorbic acid	From 38.1 to 47.5 mg/l	No	Surface coating analysis, and adhesive shear strength measurements	[29]
Mfp3 [nr]	*Bacillus subtilis* (PD8)	Full-length with His-tag and Spy-tag	26.6	nr	Soluble	Extraction in Tris-HCl, IMAC, tag removal with TEV protease	64 mg/l	No	Secretion assay in *B.subtilis* using different signal peptides and fusion tags, surface coating analysis	[30]
Mfp3 [*M. californianus*]	*E. coli* JW2581 tyrosine auxotroph	Full-length Mfp3F with His-tag	~6.2	Yes	Insoluble	Extraction 8M urea, IMAC	nr	*In vivo* by incorporation of tyrosyl-tRNA synthetase (TyrRS), ~97% DOPA conversion	Test of the redox effect of mfp6 on mfp3 using UV-Vis spectroscopy, SFA	[31]
Mfp3 [*M.edulis*]	*E. coli* BL21 (DE3)	Full-length with His-tag and SUMO-tag in N-term	~24	nr	Soluble	Extraction with 100 mM NaH2PO4, 10 mM TRIS, 300 mM NaCl, IMAC, SUMO cleavage using ScUlp1	250 mg/l	*In vitro*, microbial tyrosinase	QCM, tensile shear test	[32]
Mfp5 [*M. galloprovincialis*]	*E. coli* BL21	Full-length with His-tag	~18	No	Mainly soluble	Extraction with 8M urea, IMAC and dialyzed in 5% acetic acid	3 mg/l (10% of total soluble protein)	*In vitro*, mushroom tyrosinase	Surface coating analysis, QCM, AFM, testing as a cell adhesive biomaterial	[33, 34]
Mfp5 [*M. galloprovincialis*]	*E. coli* BL21 (DE3)	Full-length with N-term His-tag	10.5	Yes	Soluble (40% of total soluble proteins)	Extraction 8M urea, IMAC	50 mg/l	*In vitro*, mushroom tyrosinase	QCM, shear strength test	[35]

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Mfp5 [*M. galloprovincialis*]	*E. coli* JW2581 tyrosine auxotroph	Full-length with His-tag	~ 15	Yes	Insoluble	Extraction 8M urea, IMAC, dialysis against 5% acetic acid with 1 mM ascorbic acid	3–5 mg/l	*In vivo* by incorporation of tyrosyl-tRNA synthetase (TyrRS), 94.4% Dopa incorporation yield	Study of Dopa-Fe^{3+} complexation, SFA, coacervate formation	[24, 25]
Mfp5 [*M. galloprovincialis*]	*E. coli* BL21 (DE3)	Full-length with N-term His-tag	~ 10	Yes	Insoluble	Extraction 6M GdnCl, IMAC	100-200 mg/L of culture	*In vitro*, mushroom tyrosinase	Colloidal probe AFM, fabrication and testing of graphene oxide-mfp composites	[36, 37]
Mfp5 [*Perna viridis*]	*E. coli* BL21-Gold (DE3)	Full-length with His-tag	~ 13	Yes	Insoluble	Extraction 8M urea, IMAC	4.3 mg/g of cell pellet	No	Surface coating analysis, cell adhesion assays	[38-40]
Mfp5 [*P. viridis*]	*E. coli* BL21 (DE3)	Full-length Pvfp-5-1	nr	Yes	nr	nr	159 mg/l	No	Metal binding assay	[41]
Mfp5 [*P. viridis*]	*E. coli* BL21	Full-length Pvfp-5β with His-SUMO-tag	~ 23.5	nr	nr	IMAC	nr	*In vitro* with polyphenol oxidase	Lap shear test, NMR	[42]
Mfp5 [*P. viridis*]	*E. Coli* BL21 for Tyr-Pvfp5 and *E. coli* JW2581 tyrosine auxotroph for DOPA-Pvfp5	Full-length, unmodified (Tyr-Pvfp5) or modified (DOPA-Pvfp5)	nr	nr	nr	Resuspension of the pellet with 5% acetic acid, HPLC purification and dialysis with 5% acetic acid	nr	*In vivo* by incorporation of tyrosyl-tRNA synthetase (TyrRS)	AFM, SFA	[43, 44]
Mfp5 [nr]	*Bacillus subtilis* (PD8)	Full-length with His-tag and Spy-tag	27.4	nr	Soluble	Cell lysis in Tris-HCl, IMAC, tag removal with TEV protease	35.6 mg/l	No	Secretion assay in *B.subtilis* using different signal peptides and fusion tags, surface coating analysis	[30]
Mfp6 [*M. californianus*]	*E. coli* Rosetta 2 (DE3)	Full-length mfp-6.1 with a N-term His_6-tag	13.6	No	Insoluble	Extraction with 5% acetic acid, 50 mM TCEP and 8M urea, IMAC, dialysis in 5% acetic acid and RP-HPLC column	5-6 mg/l	No	Test of antioxidant activity using DPPH assay, SFA	[45]

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Mfp6 [*M. californianus*]	*E. coli* BL21 (DE3)	Full-length with C-term His6-tag	~12.8	Yes	Insoluble	IMAC, elution in 0.1 M hydrochloric acid & dialysis with 10 mM acetic acid	nr	No	Test of the antioxidant activity of mfp6 on mfp3f using UV-Vis spectroscopy and SFA	[31]
Mfp20 [*Mytilus coruscus*]	*E. coli* BL21 (DE3)	Full-length with C-term His6-tag	~13.5	Yes	Insoluble	Extraction in PBS buffer (pH 8.0) containing 8 M urea, IMAC, dialysis with 5% acetic acid, refolding in 20 mM Tris-HCl (pH 8.0), 0.9 mM GSH, and 0.1 mM GSSH, RP-HPLC	40 mg/l	No	Antioxidant activity assay	[46]
PreCol-D [*M. californianus*]	*E. coli* BL21 (DE3)	Partial (silk-like flanking domains) fused with baculoviral polyhedrin protein and His-tag	37	Yes	Insoluble	-	nr	No	-	[47]
PreCol-D [*M. galloprovincialis*]	*P. pastoris* strain X33	Full-length with a His_6-Tag	from ~75 to ~100	No	Soluble	IMAC, dialysis against 10 mM ammonium bicarbonate	252 mg/l	No	TEM, CD, FTIR	[48]
PreCol-NG [*M. galloprovincialis*]	*E. coli*	Partial (C-terminal flanking domain) fused with a N-term SUMO tag and C-term CBD tag	7.2	Yes	nr	Affinity purification before tag cleavage	nr	nr	CD	[49]
TMP [*M. galloprovincialis*]	*E. coli* BL21 Star cells	Partial (C-terminus of TMP) with His-tag	21.3	No	Insoluble	Extraction in a PBS buffer with 6 M GdnCl, IMAC	nr	nr	-	[50]
PTMP1 [*M. galloprovincialis*]	*E. coli* BL21(DE3)-RIPL	Full-length with SUMO tag	48.9	No	Insoluble	Extraction 8M urea, 5mM DTT, IMAC	nr	No	Crystallography, CD, SPR, FTIR, collagen-binding assay	[51-53]
PTMP1 [*M. galloprovincialis*]	*E. coli* BL21(DE3)	Full-length	48.5	Yes	Insoluble	Extraction with 10% acetic acid, RP-HPLC	~25% of total cell proteins	No	SFA (with and without preCols), mammalian cell proliferation assay	[54, 55]

Adhesive protein [Species]	Host strain for expression	Recombinant protein	MW (kDa)	Codon optimization	Presence in the soluble or insoluble fraction	Extraction and purification strategy[e]	Yield	DOPA modification	Testing and use of recombinant protein [g]	References
Hybrid proteins										
Mfp1 and mfp5 [*M. galloprovincialis*]	*E. coli* Rosetta (DE3)	Hybrid (6 mfp1 decapeptide repeats at the N- and C-termini of full-length mfp5 [fp-151], with His-tag)	~30	Yes	Insoluble	Extraction in 25% acetic acid, IMAC	~ 100 mg/l (~ 40% of total protein)	*In vitro*, mushroom tyrosinase	QCM, AFM, Tensile test, cell adhesion assays; shear strength test, complex coacervate formation, fabrication and testing of bioadhesives	[7, 56-59]
Mfp1 and mfp5 [*M. galloprovincialis*]	*E. coli* BL21(DE3)	Hybrid (fusion of fp-151 with Arg-Gly-Asp (RGD) peptide)	~28	Yes	Insoluble	Extraction with 25% acetic acid, and dialyzed in 5% (v/v) acetic acid buffer	Similar to mfp-151 (25% of total cellular protein)	*In vitro*, mushroom tyrosinase	Surface coating analysis, SFA, mammalian cell spreading, adhesion, and proliferation assays, coacervate formation	[60, 61]
Mfp1 and mfp5 [*M. galloprovincialis*]	*E. coli* BL21(DE3)	Hybrid (6 mfp1 decapeptide repeats at the N- and C-termini of full-length mfp5 [fp-151], with His-tag), co-expressed with *Vitreoscilla* hemoglobin (VHb)	~27	Yes	nr	nr	~1 g/l in fed-batch bioreactor	No	Test for improving the production of recombinant mfp-151 by co-expressing with VHb, coacervate formation for use as bioadhesive	[62] [63]
Mfp1 and mfp5 [*M. galloprovincialis*]	Insect *Sf9* cells	Hybrid (6 mfp1 decapeptide repeats at the N- and C-termini of mfp5 [fp-151] with His-tag)	23.6	No	Soluble	IMAC	23 mg/l	*In vitro,* mushroom tyrosinase	Surface coating analysis	[64]
Mfp1 and mfp5 [*M. galloprovincialis*]	*E. coli* BL21 (DE3)	Hybrid (6 mfp1 decapeptide repeats at the N- and C-termini of mfp5 [fp-151] with His-tag)	~ 24	Yes	Soluble	Extraction 8M urea, IMAC	nr	*In vivo*, co-expression of tyrosinase	Shear strength test	[65]
Mfp1 and mfp5 [*M. galloprovincialis*]	*E. coli* BL21 (DE3)	Hybrid (fusion of fp-151 and vitronectin [r-fp-151-VT], with C-terminal His-tag)	~ 27	nr	Insoluble	Extraction 8M urea, IMAC	50 mg/l	nr	Cytocompatibility, anti-inflammatory and anti-oxidant assays	[66]
Mfp1 and mfp5 [*M. galloprovincialis*]	*E. coli* BL21 (DE3)	Hybrid (fusion of fp-151 and substance P peptide [MAP-SP])	24.3		Insoluble	IB washed with 1% Triton X-114, extraction with 25% acetic acid, IEC	nr	No	Cell proliferation assay, fabrication of hydrogel for nerve repair	[67]

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Mfp1 and mfp5 [nr]	*E. coli* BL21	Hybrid (fusion of fp-151 and various antimicrobial peptides at either the N or C terminus [RAP])	~ 26	Yes	Insoluble	Extraction with acetic acid solution, acetone precipitation, IEC	nr	*In vitro,* mushroom tyrosinase	Minimum Inhibitory Concentration assay for antibacterial activity, cytotoxicity test, adhesion and coating assays, fabrication of RAP-coated skin patches	[68]
Mfp1 and mfp5 [*M. edulis*]	*E. coli* Rosetta (DE3)	Hybrid (fusion of fp-151 and various antimicrobial peptides at either the N or C terminus [MAP-FP])	~ 26	nr	Insoluble	Extraction with 25% acetic acid, and dialyzed in PBS	nr	nr	Minimum Inhibitory Concentration and Time-Kill Kinetics assays for antibacterial activity, cytotoxicity test,	[69]
Mfp1 [*M. edulis*]	*E.coli* BL21 (DE3)	Hybrid (fusion of 12 decapeptide repeats with different biofunctional peptides (MAP-VEGF, MAP-QK, MAP-FGF2, and MAP-RGD))	15.6, 15.6, 15.7 and 14.3, respectively	Yes	Insoluble	Extraction with 5% acetic acid and purified using RP-HPLC	nr	No	Fabrication of an adhesive microneedle bandage	[70]
Mfp3 and mfp5 [*M. galloprovincialis*]	*E. coli* BL21 (DE3)	Hybrid (fusion of full-length mfp3A at the N- and C-termini of full-length mfp5 [mfp-353] with His-tag)	~ 22	No	Insoluble	Extraction 6M GdnCl, IMAC	39 mg/l (21% of total protein)	*In vitro*, mushroom tyrosinase	Lap shear test, cell adhesion assay	[71]
Mfp3 [*M. galloprovincialis*] and mfp1 [*M. edulis*]	*E. coli* BL21 (DE3)	Hybrid (fusion of full-length Mgfp3B with a partial, 6-decapeptide mfp1 [mfp-31] with a C-term His-tag and N-term SUMO and TrxA tags)	~27	Yes	Mostly insoluble (85.75%)	Extraction with 25 mM ascorbic acid and 8 M urea, IMAC, elution with 0.5M HCl and dialyzed against 5% glacial acetic acid and 1 mM ascorbic acid	~40 mg/l	No	Surface coating analysis, and adhesive shear strength measurements	[29]
Mfp3 [*M. galloprovincialis*]	*E. coli* BL21 (DE3)	Hybrid (fusion of two full-length mfp3 [mfp-33] with a C-term His-tag and N-term SUMO and TrxA tags)	~26	Yes	Mostly soluble	Lyse with 25 mM ascorbic acid and 8 M urea, IMAC, elution with 0.5M HCl and dialyzed against 5% glacial acetic acid and 1 mM ascorbic acid	127.2 mg/l	No	Surface coating analysis, and adhesive shear strength measurements	[29]

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Mfp3 and mfp5 [*M. galloprovincialis*]	*E. coli* BL21 (DE3)	Hybrid (fusion of full-length Mgfp-3B with full-length Mgfp-5 [mfp-35] with a C-term His-tag and N-term SUMO and TrxA tags)	~26	Yes	Mostly soluble	Lyse with 25 mM ascorbic acid and 8 M urea, IMAC, elution with 0.5M HCl and dialyzed against 5% glacial acetic acid and 1 mM ascorbic acid	97.3 mg/l	No	Surface coating analysis, and adhesive shear strength measurements	[29]
Mfp3 [*M. galloprovincialis*]	*E. coli* NEB C3016	Hybrid (fusion of CsgA and full-length mfp3 [CsgA-mfp3] with His-tag)	~ 28.5	Yes	Insoluble	Extraction 8M GdnHCl 300 mM NaCl, IMAC	nr	*in vitro,* in the IMAC column with mushroom tyrosinase	Amyloid fibrils formation, TEM, AFM, fluorescence measurements	[72]
Mfp3 [*M. galloprovincialis*]	*E. coli* BL21 (DE3)	Hybrid (fusion of chitin-binding domain from *Bacillus circulans* chitinase, CsgA and full-length mfp3 [CBD-CsgA-mfp3] with His-tag)	~ 32	Yes	Insoluble	Extraction 8M GdnHCl 300 mM NaCl, IMAC	nr	*in vitro,* in the IMAC column with mushroom tyrosinase	Amyloid fibrils formation, AFM, ThT fluorescence measurements, CD, QCM-D, X-ray diffraction, chitin-binding assay	[73]
Mfp3 [*M. galloprovincialis*]	*E. coli* Rosetta	Hybrid (fusion of CsgA and full-length mfp3A [CsgA-mfp3] with His-tag)	~ 25	Yes?	nr	IMAC	nr	*in vitro,* overnight tyrosinase incubation	AFM, TEM	[74]
Mfp3 [*M. californianus*]	*P. pastoris* strain GS115	Hybrid (fusion of full length mfp3 and GvpA [Mfp3-GvpA] with His-tag)	~31	No	Soluble	Cell lysis in 50 mM sodium phosphate buffer, pH 7.4, with acid-washed glass beads, IMAC	1.92 g/ml	*in vitro,* tyrosinase	AFM	[75]
Mfp3 [*M.edulis*]	*E. coli* BL21 (DE3)	Hybrid (fusion of full-length mfp3 and a gel-forming protein comprising a leucine-zipper, an unstructured polyelectrolyte and a helical coiled-coil domain [A-S-Mefp3-P] with an N-terminal His-tag)	~ 30	Yes	Insoluble	extraction 8 M GdnCl, IMAC	nr	*In vitro*, mushroom tyrosinase	Fabrication of hydrogels for use as a cardiac patch, mechanical testing, in vitro cytocompatibility test, animal experiments	[76]
Mfp4 and mfp1 [*M. californianus*]	*E. coli*	Hybrid (fusion of the partial, histidine-rich domain of mfp-4 at the N- and C-termini of 12 mfp-1 decapeptide repeats [HRfp-1])	~16.2 before DOPA conversion	nr	nr	Extraction with 8 M urea, 10 mM Tris-HCl, 100 mM sodium phosphate [pH 8.0], IMAC, removal of impurities by additional sequential steps, dialysis against distilled water	nr	*in vitro*, mushroom tyrosinase	Study of the structure, rheology, adhesiveness and cytocompatibility of a HRfp-1 hydrogel	[77]

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Mfp5 [*M. galloprovincialis*]	*E. coli* BL21 (DE3)	Hybrid (two full-length mfp5 [mfp5$^{(2)}$] with a N-term His-tag)	~ 20	Yes	Insoluble	Extraction 6M GdnCl, IMAC	100-200 mg/l	*In vitro*, mushroom tyrosinase	Colloidal probe AFM, fabrication and testing of graphene oxide-mfp composites	[36, 37]
Mfp5 [*M. galloprovincialis*]	*E. coli* BL21 (DE3)	Hybrid (fusion of domains B and C of protein A with full-length mfp5 [BC-MAP] with C-term His-tag)	~23.5	No	Soluble	Extraction with 50 mM NaH_2PO_4, 300 mM NaCl (pH 8.0), IMAC	nr	No	Surface coating analysis, QCM, development of ELISA test	[78, 79]
Mfp5 [*M. galloprovincialis*]	*E. coli* NEB C3016	Hybrid (fusion of full-length mfp5 and CsgA [Mfp5-CsgA] with His-tag)	~ 28.5 unmodified and ~35 with DOPA modification	Yes	Insoluble	Extraction 8M GdnCl, IMAC	nr	*in vitro,* mushroom tyrosinase	Amyloid fibrils formation, TEM, AFM, fluorescence measurements	[72]
Mfp5 [*M. galloprovincialis*]	*E. coli* BL21 (DE3)	Hybrid (fusion of full-length mfp5, CsgA and chitin-binding domain from *Bacillus circulans* chitinase [Mfp5-CsgA-CBD] with His-tag)	~ 37	Yes	Insoluble	Extraction 8M GdnHCl 300 mM NaCl, IMAC	nr	*in vitro,* in the IMAC column with mushroom tyrosinase	Amyloid fibrils formation, AFM, ThT fluorescence measurements, CD, QCM-D, X-ray diffraction, chitin-binding assay	[73]
Mfp5 [*M. edulis*]	*E. coli* D3	Hybrid (fusion of full-length mfp5 and the C-terminus of VE-cadherin extracellular domain EC1-2 protein)	56	nr	nr	Extraction with 25% (v/v) acetic acid, IMAC purification and dialyzed against 1% (v/v) acetic acid buffer	nr	*In vitro*, mushroom tyrosinase	Test of adhesion of endothelial cells, capture of endothelial progenitor cells on vascular stents coated with VE-mfp5	[80, 81]
Mfp5 [*M. edulis*]	*E. coli* BL21 (DE3)	Hybrid (fusion of the LC domain of the transactive response (TAR) DNA binding protein of 43 kDa (TDP43 LC) and full-length mfp5 [TLC-M] with N-terminal His-tag)	nr	Yes	Soluble	Extraction with 50 mM tris-HCl and 500 mM NaCl (pH 7) with 0.2 mg/ml lysozyme, IMAC purification and elution with 50 mM tris-HCl, 500 mM NaCl, and 500 mM imidazole (pH 7)	nrC	*in vitro,* in the IMAC column with mushroom tyrosinase	LLPS and amyloid fibril formation, CR and ThT staining, AFM, TEM, QCM-D, underwater adhesion force measurements, X-ray diffraction	[82]

Adhesive protein [Species]	Host strain for expression	Recombinant protein	MW (kDa)	Codon optimization	Presence in the soluble or insoluble fraction	Extraction and purification strategy[e]	Yield	DOPA modification	Testing and use of recombinant protein [g]	References
Mfp5 [*M. galloprovincialis*]	*E. coli* NEB10β Δ*tyrA*	Hybrid (fusion of the zipper-forming domain of an amyloid protein, flexible spider silk sequences and full-length mfp5 [8xKLV-mfp-5] with C-term His-tag)	~35.9	No	Insoluble	Extraction with 6M GdnCl, IMAC then IEC, dialysis with 5% acetic acid	6-10 mg/l	*In vivo* by adding DOPA to the medium for endogenous tyrosyl tRNAs, 79-86% DOPA incorporation	Structural characterization and mechanical testing, production of an underwater adhesive protein hydrogel	[83]
Mfp5 [*M. californianus*]	*P. pastoris* strain GS115	Hybrid (fusion of full-length mfp5 and GvpA [Mfp5-GvpA] with His-tag)	~32	No	Soluble	Cell lysis with acid-washed glass beads, IMAC	1.85 g/ml	*In vitro*, mushroom tyrosinase	AFM	[75]
Mfp5 [*M.edulis*]	*E. coli* BL21 (DE3)	Hybrid (fusion of full-length mfp5 and a gel-forming protein comprising a leucine-zipper, an unstructured polyelectrolyte and a helical coiled-coil domain [A-S-Mefp5-P] with an N-terminal His-tag)	~32	Yes	Insoluble	Extraction 8M GdnCl, IMAC	nr	*In vitro*, mushroom tyrosinase	Fabrication of hydrogels for use as a cardiac patch, mechanical testing, in vitro cytocompatibility test, animal experiments	[76]
Mfp5 [*M. galloprovincialis*]	*E. coli* NEB10β	Hybrid (fusion of Mfp5 fragments to the termini of a 16-repeat of artificially-designed amyloid-silk protein 16xFGA[^{N}M-16xFGA-^{C}M]).	~60	Yes	Insoluble	Extraction in 6M GdnCl, 50mM K_2HPO_4 and 300mM NaCl (pH 7.0), IMAC, elution with 8M urea, 50mM K_2HPO_4 and 300mM NaCl (pH 7.0) containing 300mM imidazole, dialysis with 5% acetic acid	8 g/l in bioreactor production	No	Fabrication of fibres using spinning protocol, mechanical testing, SEM, polarized Raman spectroscopy	[84]
Scallops										
Sbp5-2 [*Chlamys farreri*]	*E. coli* BL21(DE3)	Partial (7 tandem repeat motifs [rTRM7])	~ 21	No	Insoluble	Extraction in a Tris-HCl buffer with 8 M urea	120 mg/l	No	Fabrication and testing of fibres, *in vitro* cytotoxicity assay	[85]
Sbp8-1 [*C. farreri*]	*E. coli* BL21(DE3)	Full-length with Trx-tag and His-tag	~ 19 (without Trx-tag)	No	Soluble	Extraction Tris-HCl buffer 500 mM NaCl, IMAC	nr	No	Polymerisation analysis based on SEC	[86]

Adhesive protein [Species]	Host strain for expression	Recombinant protein	MW (kDa)	Codon optimization	Presence in the soluble or insoluble fraction	Extraction and purification strategy[e]	Yield	DOPA modification	Testing and use of recombinant protein [g]	References
Sbp9 [*C. farreri*]	*E. coli* BL21(DE3)	Partial (1 calcium-binding and 4 EGF-like domains [CE4])	nr	Yes	Insoluble	Extraction in a Tris-HCl buffer with 8 M urea	100 mg/l	No	CD, Fabrication of hydrogels, mechanical testing, AFM, SEM, in vitro and in vivo cytocompatibility test	[87]
Sbp9 [*C. farreri*]	*E. coli* BL21(DE3)	Partial (4 EGF-like domains [Sbp9$^{\Delta}$])	nr	Yes	Insoluble	Extraction in a Tris-HCl buffer with 1 M urea and 1% triton X114, refolding by dialysis in Tris-Cl buffer	80 mg/l (Purity 80%)	No	Fabrication of coatings, AFM, SEM, FTIR, in vitro and in vivo cytocompatibility test	[88]
Hybrid proteins										
Sbp9 [*C. farreri*]	*E. coli* BL21(DE3)	Hybrid (Sbp9$^{\Delta}$ fused with antimicrobial peptide LL37)	nr	Yes	Insoluble	Extraction in a Tris-HCl buffer with 1 M urea and 1% triton X114, refolding by dialysis in Tris-Cl buffer	nr	No	Fabrication of coatings, wound-healing assays in diabetic mice	[88]
Barnacles										
Cp19k [Megab*alanus rosa*]	*E. coli* Origami (DE3)	Full-length with Trx-tag and His-tag	17.2 (without tags)	No	Soluble	Extraction Tris-HCl buffer 500 mM NaCl, IMAC	nr	N/A	SPR	[89]
Cp19k [*Fistulobalanus albicostatus*]	*E. coli* BL21(DE3)	Full-length with Trx-tag and His-tag	37	No	Soluble	Extraction phosphate buffer 300 mM NaCl, IMAC, IEC, reverse phase chromatography	10-25 mg/l	N/A	Surface coating analysis, lap-shear adhesion test, AFM	[90-92]
Cp19k [*F. albicostatus*]	*E. coli* BL21(DE3)	Full-length with His-tag and T7-tag (2 cysteine residues substituted with serine residues)	17.9	No	nr	Extraction in a Tris-HCl buffer with 8 M urea, IMAC, reverse phase chromatography	40 mg/l	N/A	ThT fluorescence, TEM, FTIR	[93]
Cp19k [*M. rosa* and *F. albicostatus*]	*E. coli* BL21	Full-length with His-tag	~ 19	Yes	Soluble	IMAC	10-20 mg/l	N/A	CD, AFM, ellipsometry	[94]
Cp19k [*Pollicipes pollicipes*]	*E. coli* BL21(DE3)	Full-length with *ompA* leader sequence and His-tag, co-expressed with GroEL-GroES chaperone	19	No	Soluble	IMAC	1-2 mg/L	N/A	SPR, AFM, ThT fluorescence	[95, 96]

Adhesive protein [Species]	Host strain for expression	Recombinant protein	MW (kDa)	Codon optimization	Presence in the soluble or insoluble fraction	Extraction and purification strategy[e]	Yield	DOPA modification	Testing and use of recombinant protein [g]	References
Cp19k [*M. rosa*]	*P. pastoris* GS115 strain	Full-length with His-tag	~ 20	Yes	Soluble	IMAC	50.4 mg/l	N/A	Fabrication of protein fibre scaffolds, CD, FTIR, ThT fluorescence, SEM, mechanical testing, QCM, cytocompatibility test	[97]
Cp20k [*M. rosa*]	*E. coli* Origami (DE3)	Full-length with His-tag	20.6	No	Soluble	Extraction Tris-HCl buffer, IMAC, IEC	nr	N/A	CD, Surface coating analysis	[98]
Cp20k [*Amphibalanus amphitrite*]	*E. coli* BL21(DE3)	Full-length with GST-tag or His-tag	nr	nr	nr	Glutathione Sepharose affinity chromatography or IMAC	nr	N/A	Antibody generation	[99]
Cp20k [*M. rosa*]	*E. coli* BL21 (DE3)	Full-length with C-terminal His-tag	21.5	Yes	Soluble	Extraction in TBS, IMAC and SEC	nr	N/A	CD, NMR, ThT assay, DLS	[100-104]
Cp20k [*F. albicostatus*]	*E. coli* Rosetta-gami (DE3)	Partial (quadruple repeat of *Bal*cp-20k fourth repetitive sequence [R4]) with N-term Trx-tag and C-term His-tag	~ 35	Yes	Soluble	PBS extraction, IMAC	1.1 mg/l	N/A	CD, AFM, QCM, XPS	[105]
Cp43k [*A. amphitrite*]	*E. coli* K-12 derived Keio strain JW1025	Full-length or partial cp43k with N-term CsgA secretion sequence	43	No	Insoluble (secreted in biofilm)	HFIP	nr	N/A	Congo red staining, SEM	[106]
Cp100k [*A. amphitrite*]	*E. coli* BL21 (DE3)	Partial (aa 10-142) with GST- or His-tag	nr	No	nr	IMAC or GST affinity chromatography	nr	N/A	Antibody generation	[107]
SIPC [*A. amphitrite*]	*Sf9* insect cells	Full-length with His-tag	~ 200	No	nr	IMAC under denaturing conditions, dialysis against PBS	nr	N/A	Chitin-binding assay, biomineralization assays	[108]

Adhesive protein [Species]	Host strain for expression	Recombinant protein	MW (kDa)	Codon optimization	Presence in the soluble or insoluble fraction	Extraction and purification strategy[e]	Yield	DOPA modification	Testing and use of recombinant protein [g]	References
lcp3_36k_3B8 [*M. rosa*]	*E. coli* BL21 (DE3)	Full-length with SUMO- and His-tag	36	Yes	Soluble	50 mM Tris, IMAC followed by SUMO protease cleavage	nr	N/A	SPR, CD, cross-linking assay	[109]
lcp2_57k_2F5 [*M. rosa*]	*E. coli* SHuffle® T7 Express	Full-length with StrepII- and His-tag	57	Yes	Soluble	50 mM Tris, IMAC, StrepII affinity chromatography	nr	N/A	SPR, CD, cross-linking assay	[109]
Hybrid proteins										
Cp19k [*F. albicostatus*]	*E. coli* BL21 (DE3) ΔCsgA	Hybrid (fusion of CsgA with full-length cp19k [CsgA-cp19k] with His-tag)	~ 30	Yes	nr	Extraction 8M GdnCl, IMAC	50 mg/L	N/A	QCM, ThT fluorescence, TEM	[110]
Cp19k [*M. rosa*]	*P. pastoris* GS115 strain	Hybrid (fusion of cp19k with *Nephila clavata* dragline silk protein[Cp19k-MaSp1] with His-tag)	~ 37	Yes	Soluble	IMAC	53.4 mg/l	N/A	Fabrication of protein fibre scaffolds, CD, FTIR, ThT fluorescence, SEM, , mechanical testing, QCM, cytocompatibility test	[97]
Tubeworms										
Sa1 [*Sabellaria alveolata*]	*E. coli*	Full-length with His-tag	~ 27	Yes	Insoluble	IMAC	50 mg/l	No	-	Cited in [111]
Sea stars										
Sfp1 [*Asterias rubens*]	*E. coli* C2566 strain	Partial (C-terminus of Sfp1β subunit) with His-tag	61	Yes	Insoluble	Extraction in TBS with 8M urea, IMAC and SEC	12 mg/l	N/A	Surface coating analysis, CD, FTIR, SPR, SEM, AFM, cytocompatibility test	[112-114]
Sfp1 [*A. rubens*]	*E. coli* C2566 strain	Partial (Sfp1δ subunit) with His-tag	67.6	Yes	Insoluble	Extraction in TBS with 8M urea, IMAC and SEC	4 mg/l	N/A	Surface coating analysis, CD, FTIR, SPR, SEM, AFM, cytocompatibility test	[112-114]

Adhesive protein [Species]	Host strain for expression	Recombinant protein	MW (kDa)	Codon optimization	Presence in the soluble or insoluble fraction	Extraction and purification strategy[e]	Yield	DOPA modification	Testing and use of recombinant protein [g]	References
Sfp1 [*A. rubens*]	*E. coli* C2566 strain	Partial (various domains of Sfp1β subunit) with His-tag	up to ~ 56	Yes	Insoluble	Extraction in TBS with 8M urea, IMAC and SEC	nr	N/A	Surface coating analysis, SEM	[113]
Ascidians										
ASP1 [*Ciona robusta*]	Insect *Sf9* cells	Full-length with His-tag	60.9	Yes	Soluble	IMAC	nr	*In vitro*, mushroom tyrosinase	Surface coating analysis, QCM	[115]
AAP1 [*C. robusta*]	High Five insect Cell System	Full-length with His-tag in C-term	58.6	Yes	Soluble	Extraction with Tris NaCl (pH 7.4), IMAC, stored in 20 mM phosphate buffer, 300 mM NaCl, pH 8.0, and 10 % glycerol	nr	N/A	Surface coating analysis, adhesion force measurement in AFM	[116]
APAP1 [*C. robusta*]	Mammalian cells HEK293	Full-length with His-tag	46.5	Yes	nr	Extraction RIPA lysis buffer, IMAC	nr	N/A	Surface coating analysis	[117]
APAP2 [*C. robusta*]	Mammalian cells HEK293	Full-length with His-tag	65	Yes	nr	Extraction RIPA lysis buffer, IMAC	nr	N/A	Surface coating analysis	[117]
Sea anemones										
TSRL [*Diadumene lineata*]	*E. coli* BL21 (DE3)	Full-length	25	No	Insoluble	Extraction 20 mM Tris-HCl (pH8.5), 8 M urea, 10 mM DTT, and 1 mM EDTA, dialysis against 20 mM Tris-HCl (pH 8.5)	500 mg/l	N/A	Surface coating analysis, SEM, AFM, hydrogel formation, cytocompatibility tests, antioxidant experiments	[118]

Abbreviations: AFM, atomic force microscopy; CBD, chitin-binding domain; CD, circular dichroism spectroscopy; CR Congo red; CTAB, cetyltrimethylammonium bromide; DLS, dynamic light scattering; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DTT, 1,4-dithiothreitol; EGF, epidermal growth factor; ELISA, enzyme-linked immunosorbent assay; FTIR, Fourier-transform infrared spectroscopy; GdnCl, guanidium hydrochloride; GSH, reduced glutathione; GSSG, oxidised glutathione; GST, glutathione S-transferase; HFIP, hexafluoroisopropanol; IEC, ion exchange chromatography; IMAC, immobilized-metal affinity chromatography; LLPS, liquid-liquid phase separation; NMR, nuclear magnetic resonance; nr, not reported; QCM, quartz crystal microbalance; RP-HPLC, reversed-phase high-performance liquid chromatography; ScUlp1, SUMO protease from *Saccharomyces cerevisiae*; SEC, size exclusion chromatography; SEM, scanning electron microscopy; SFA, surface force apparatus; SPR, surface plasmon resonance spectroscopy; SUMO, small ubiquitin-like modifier; TCEP, tris(2-carboxyethyl)phosphine; TEM, transmission electron microscopy; TEV, Tobacco Etch Virus; ThT, thioflavine-T; Trx, thioredoxin; XPS, X-ray photoelectron spectroscopy.

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The following papers were published during 2025, after the finalization of writing this review. Due to time constraints, the authors could not critically review them herein. Included here for reader convenience.

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