Supporting Information for

The redox capacity of an extracellular matrix protein associated with adhesion in *Mytilus californianus*[*]

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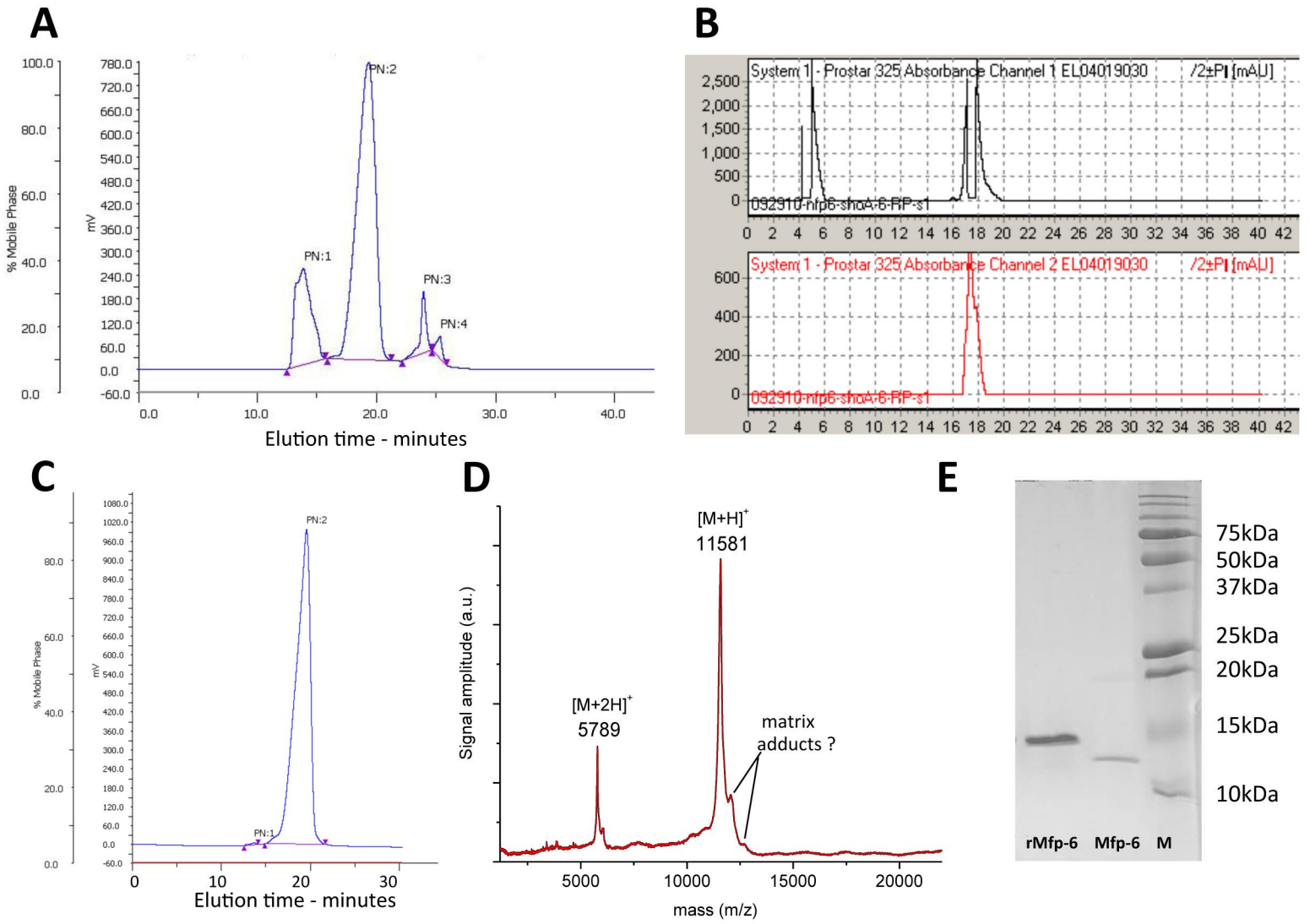

Figure S1: Purification of Mfp-6 extracted from mussel feet using sequential RP-HPLC and size exclusion chromatography. (A) Dialyzed and freeze-dried samples were reconstituted in 5% acetic acid before the application to size exclusion chromatography (Shodex KW-G pre-column and KW-803 main column with flow rate = 0.5 ml/min, time = 80 min). Mfp-6 elutes between 16-22 min. (B) Fractions "PN:2" were collected, filtered through a 0.2µm membrane filter, and subjected to C8 RP-HPLC chromatography. A linear gradient beginning with double deionized water and 0.1 % TFA (solvent #1) and ending in acetonitrile and 0.1% TFA (solvent #2) over three steps: 5-30min = 5-30% (#2), 30-60 min = 25-38% (#2), 60-80min= 38-100% (#2). Flow rate = 1 ml/min. Mfp-6 typically elutes after 16-20 min. (C) RP-HPLC fractions were collected and subjected to another SEC purification (flow rate = 0.5 ml/min, time = 30 min). Pure Mfp-6 typically eluted around 16-22 min. Final fractions selected for NMR analysis exhibited near homogeneity by MALDI-TOF (D) and 16% SDS PAGE (E). MALDI shoulders (+**Δ**200 Da) indicate adduct formation with matrix. SDS PAGE suggests slight dimerization in Mfp-6. MALDITOF conditions were: Matrix=**α**-cyano-4-hydroxycinnamic acid, Accelerating Voltage=25,000 V, Grid Voltage=93%, Guide Wire Voltage=0.3, Delay Time=300ms.

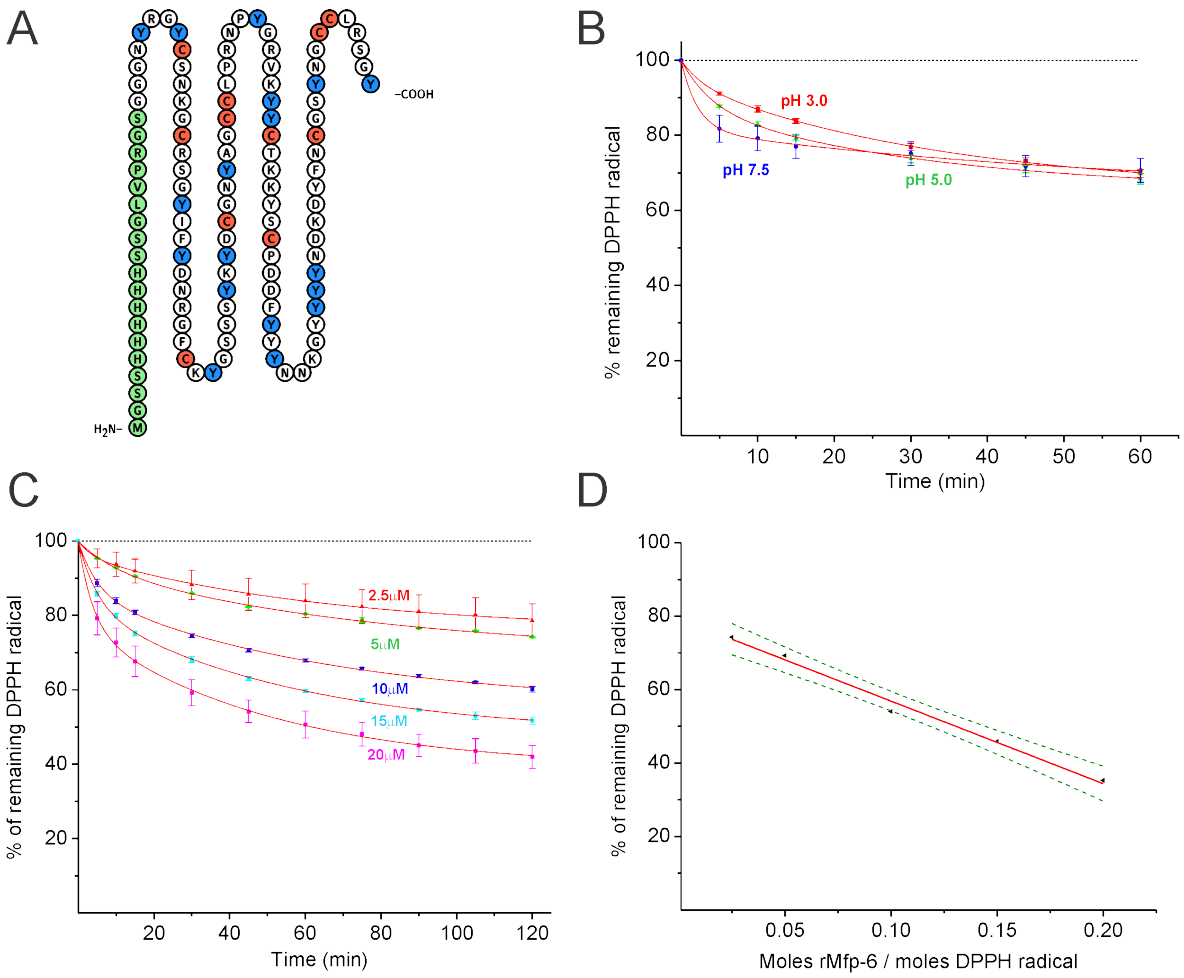

Figure S2: Reaction kinetics of DPPH radical reduction by a recombinant Mfp-6 protein. The construction of the active rMfp-6 protein (A) has been described previously (1). Time course of DPPH radical (100μM) quenching by the DOPA-less rMfp-6 protein (2.5μM) at pH 3, 5, and 7.5 (B). The lower panels show the time course of the reaction kinetics of DPPH radical reduction by rMfp-6 at varying protein concentrations at pH 3 (C) and the extrapolated fraction of remaining DPPH radical (%) at infinite time for the range of additive concentrations between 2.5 and 20μM with an initial DPPH concentration of 100μM (D). See the main text for more details.

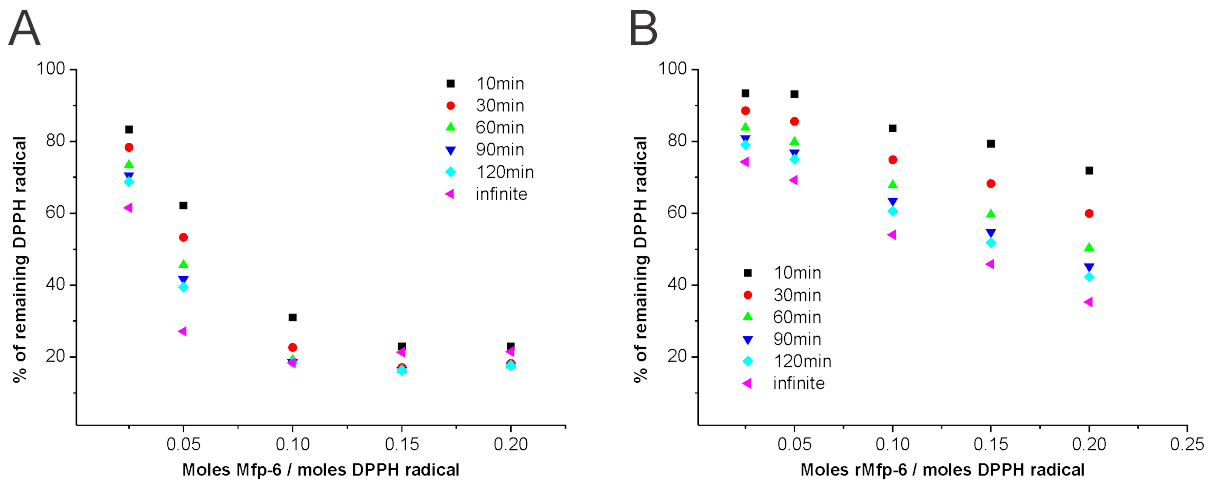

Figure S3: The reduction of DPPH radical as a function of the number of moles of Mfp-6 (A) or rMfp-6 (B) per mole DPPH radical at defined time points. To calculate the EC_{50} value for Mfp-6 and rMfp-6 interaction kinetics with the DPPH radical, the percentage of remaining DPPH radical at different time points (10min, 30min, 60min, 90min, 120min, infinite time) was evaluated as a function of the molar ratios of antioxidant to DPPH. The fraction of DPPH remaining after infinite time (F_i) was calculated using the function $F_i = (A1 + A2)/A0$, where A1 and A2 are the amplitudes and A0 is the initial absorbance of the DPPH solution prior to the addition of Mfp-6 or rMfp-6. For details, please refer to the main text and (2).

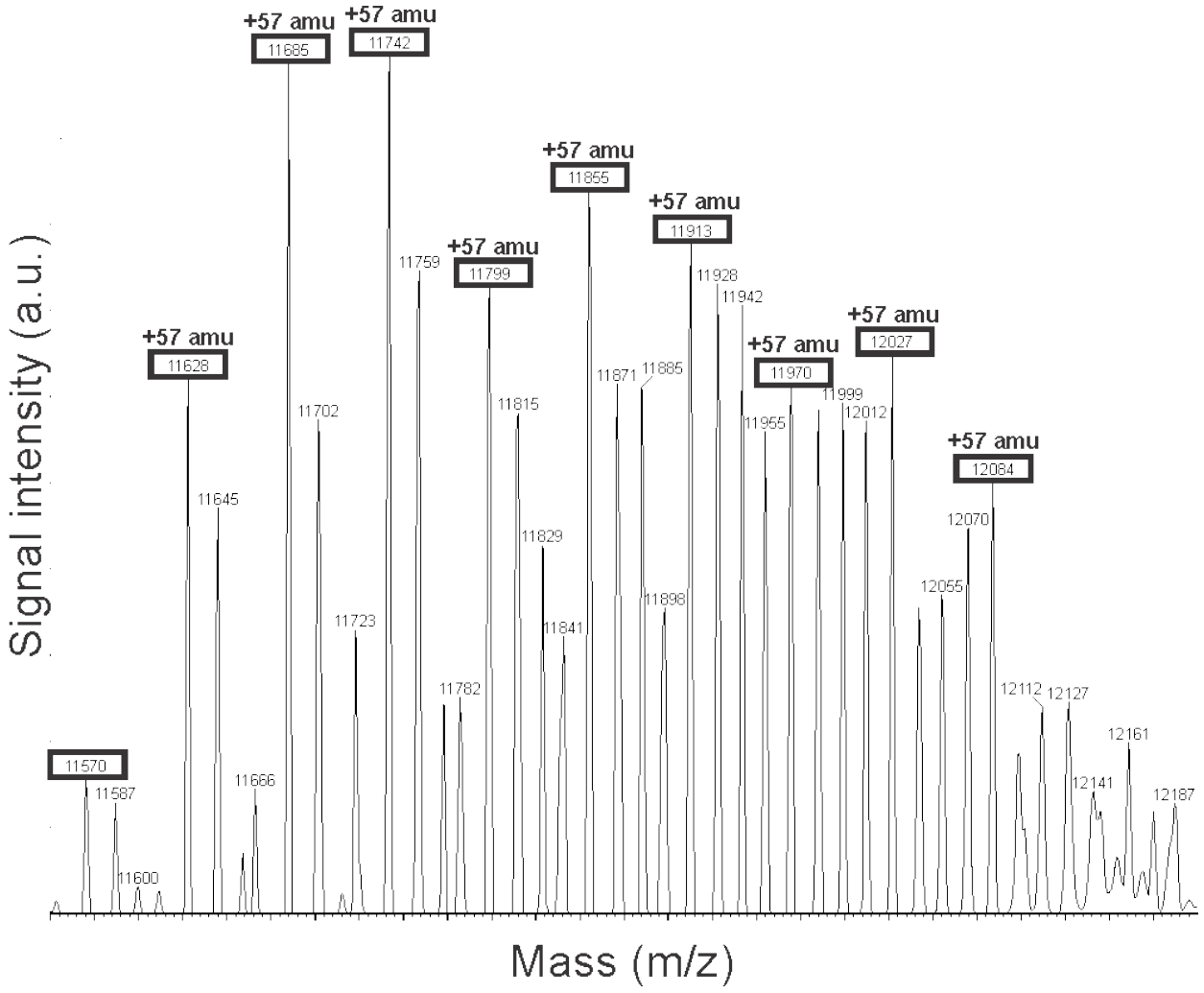

Figure S4: ESI Q TOF2 mass spectrum of IAM-labeled Mfp-6 at pH 3. S-amidomethylation of free sulfhydryl groups in Mfp-6 using 2-iodoacetamide (IAM) leads to mass shifts in increments of +57 Da per labeled residue. Unlabeled Mfp-6 protein has an apparent mass of 11,570 Da.

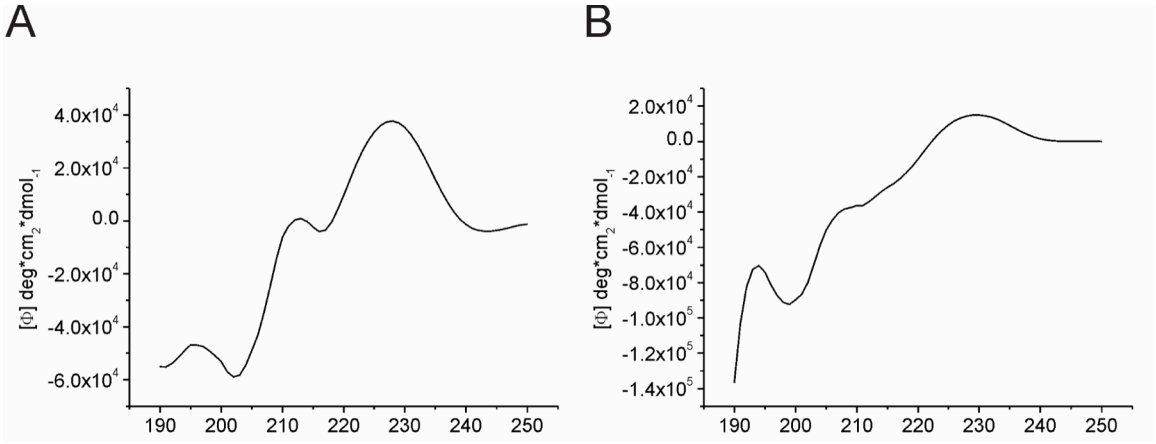

Figure S5: Far UV circular dichroism spectra of Mfp-6 (A) and rMfp-6 (B) in acetic acid buffer at pH 3. No typical signatures of secondary structure were detectable by CD in the foot-extracted and recombinant Mfp-6. The respective far-UV spectrum at RT shows no simple secondary structure elements in Mfp-6. However, less negative ellipticity at ~200 nm of foot extracted mfp-6 compared with rMfp-6 is suggestive of a beta structure whereas the strong positive ellipticity at 230 nm has been attributed to aromatic interactions among the numerous i.e. 20 tyrosines present in Mfp-6 (Pain, R. (2005) Determining the CD spectrum of a protein. *Curr. Protoc. Protein Sci.* Chapter 7, Unit B3.5 doi: 10.1002/0471140864.ps0706s38).

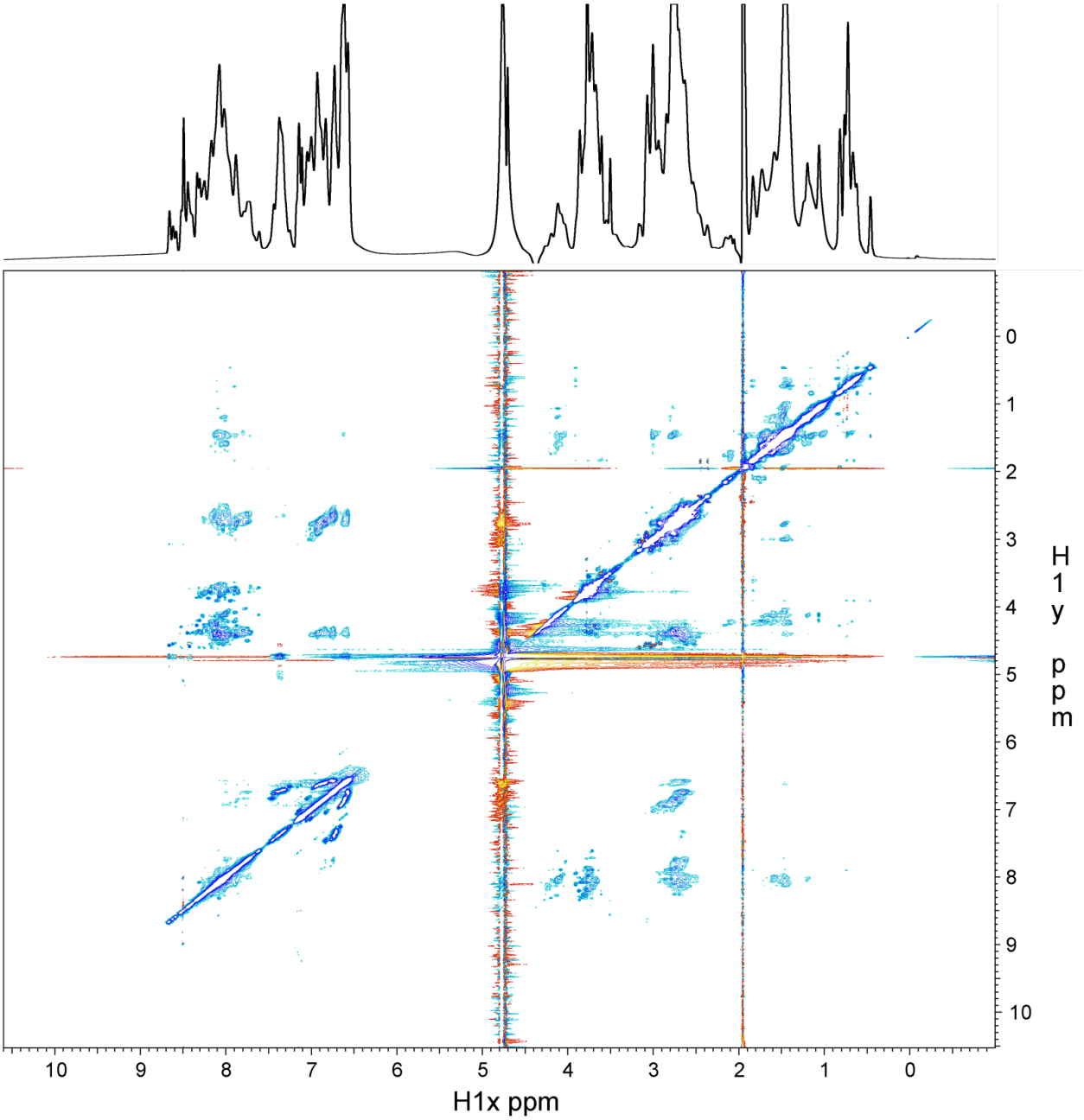

Figure S6: NMR spectra of rMfp-6. 1D and 2D NOESY proton NMR spectra of 130μM rMfp-6 in 5% d4-acetic acid at pH 3. The spectra show only a few NOEs detected in the backbone amide and aromatic proton region of 6-10ppm, indicative of a mostly unfolded or only partially folded state of the protein. In addition, no methyl peaks are visible in the upfield region of 0-1.7ppm, indicating the absence of a core structure. Collectively, rMfp-6 does not appear to have a defined globular structure and rather forms a random coil at pH 3.

Figure S7: No major structural changes occur in Mfp-6 upon DPPH saturation. Shown are the 2D proton NMR (NOESY) spectra of Mfp-6 in 5% d4-acetic acid at pH 3 alone (A) or supplemented with DPPH in a molar ratio of about 1:10 (B). Saturating Mfp-6 with DPPH (reduced to yellow hydrazine) does not change the overall folding state of the protein indicated by a comparable pattern of NOESY peaks before and after the addition. The pictures show the Mfp-6 solution in the NMR tube right after the addition of 1mM DPPH (t=0) and after the overnight incubation and NMR spectra recording (t = ~22h).

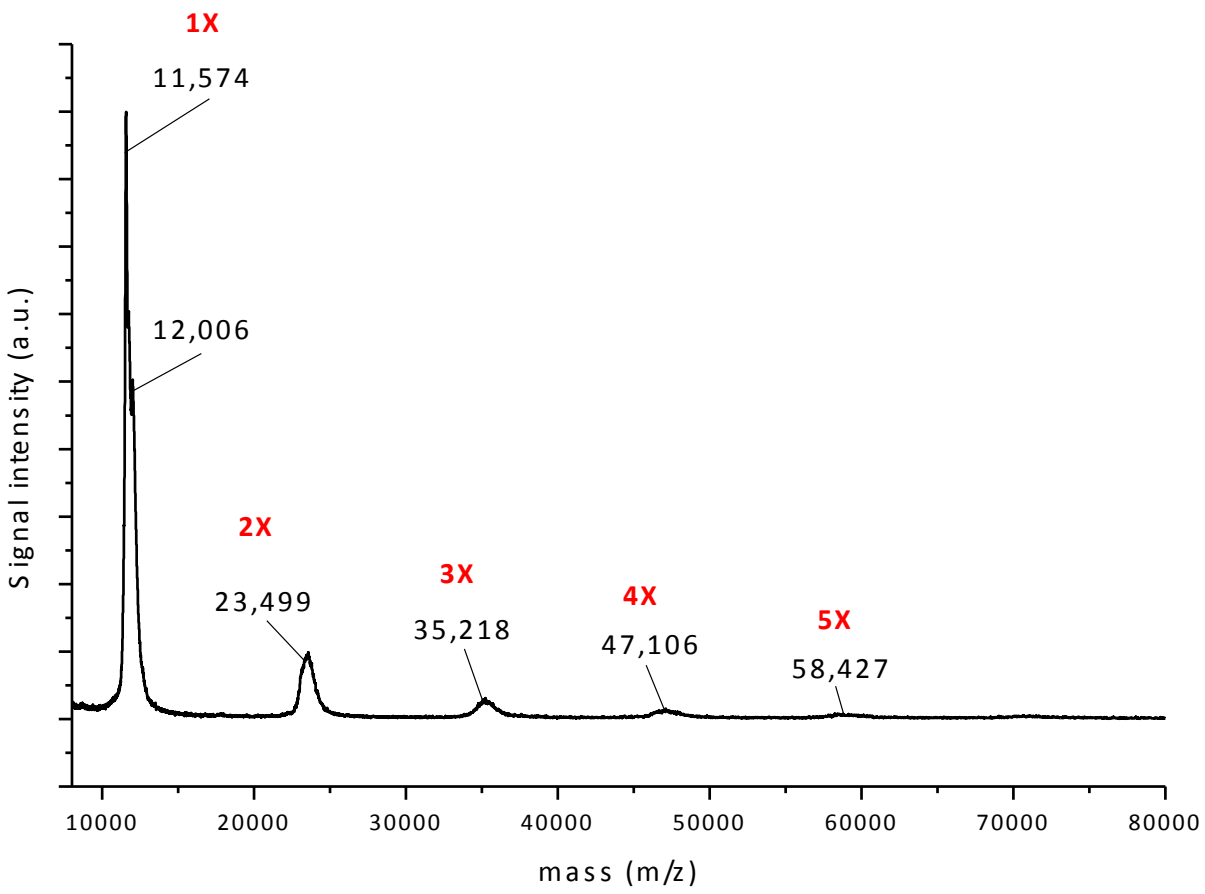

Figure S8: MALDI-TOF mass spectrum of Mfp-6 after precipitation at pH 7.5. Matrix=α-cyano-4-hydroxycinnamic acid, Accelerating Voltage=25,000 V, Grid Voltage=93%, Guide Wire Voltage=0.3, Delay Time=300ms.

Table S1: Deduced parameters of the reaction kinetics of DPPH radical reduction by the native and recombinant Mfp-6 proteins. Parameters are defined as follows: V_b bleaching rate, F_i % remaining DPPH radical at t= ∞, EC_{50} effective concentration at 50% reduction in M antioxidant/M DPPH, ARP antiradical power ($1/EC_{50}$). For details please refer to the main text and refs 2 and 3.

Mfp6	Conc, **µM**	V_b (% DPPH•/min)	F_i (% DPPH•)	EC_{50} (n)	ARP (n)	Stoichiometry (n)	red DPPH/Mfp6
native	2.5	688.2	61.5	0.03	33.9	0.06	17.0
	5	1290.9	27.1				
	10	1865.2	18.4				
	15	2497.3	21.3				
	20	2632.0	21.5				
recomb	2.5	107.2	74.3	0.13	7.7	0.26	3.8
	5	59.8	69.2				
	10	210.7	54				
	15	264.7	45.9				
	20	459.8	35.3				

RFERENCES

1. Nicklisch, S. C. T., Das, S., Martinez Rodriguez, N. R., Waite, J. H., Israelachvili, J. N. (2013) Antioxidant efficacy and adhesion rescue by a recombinant mussel foot protein-6, *Biotechnol. Prog. 29*, 1587-1593
2. Campos, A., and Duran, N. (2012) Kinetic and stoichiometric evaluation of free radicals scavengers activities based on diphenyl-picryl hydrazyyl (DPPH) consumption, *J. Chil. Chem. Soc. 4*, 1381–1384
3. Brand-Williams, W., Cuvelier, M. E., and Berset, C. (1995) Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci. Technol. 28, 25–30