Supplementary figures for: Buttstedt A. 2022: The role of 10-hydroxy-Δ^2-decenoic acid in the formation of fibrils of the major royal jelly protein 1/apisimin/24-methylenecholesterol complex isolated from honey bee (*Apis mellifera*) royal jelly. — *Eur. J. Entomol.* **119**: 448–453.

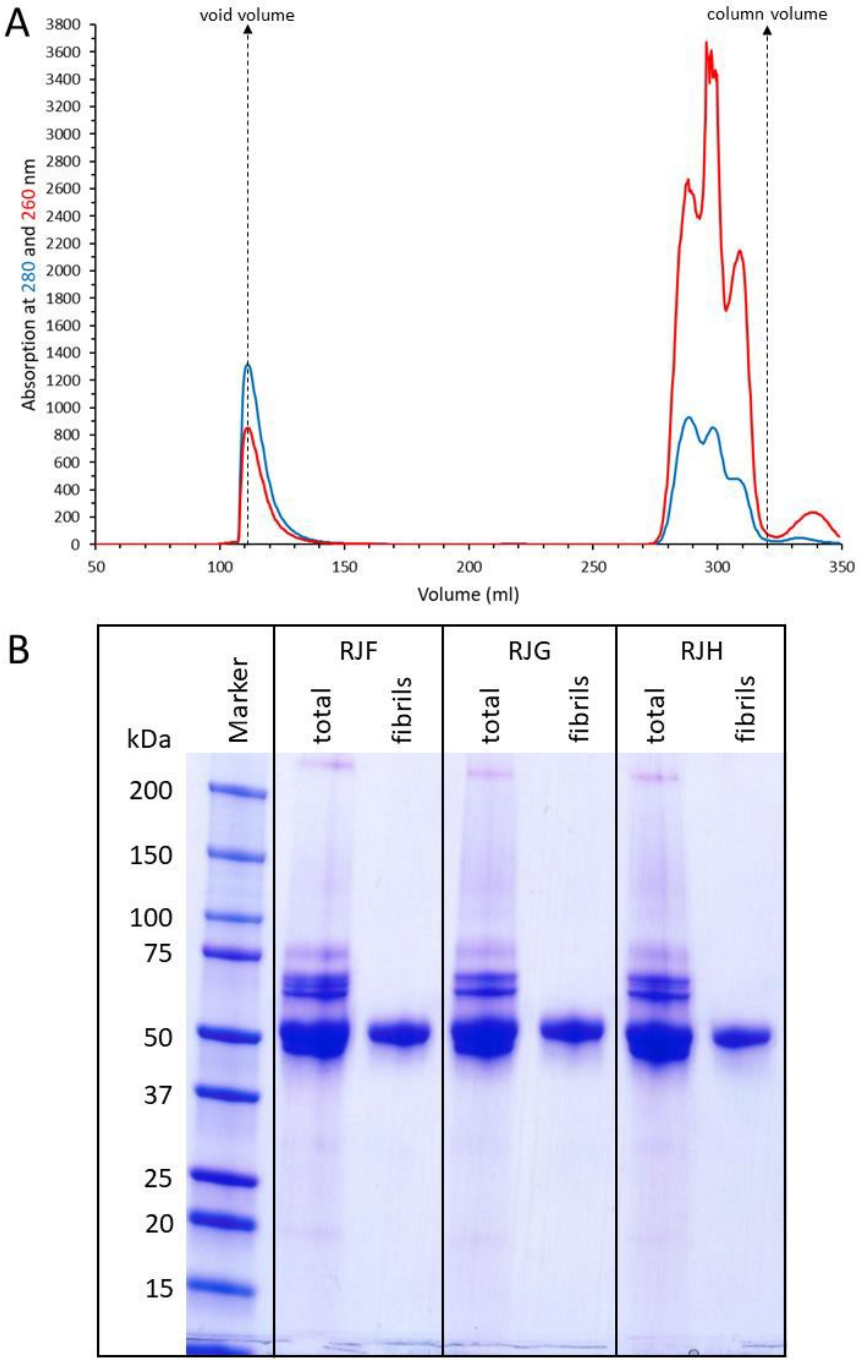

Fig. S1. Purification of fibrillar MRJP1/apisimin/24MC. **A)** Gel filtration exemplarily shown for the protein extract from RJF (column: HiLoad 26/600 Superdex 200 pg; buffer: 50 mM sodium citrate/citric acid, 150 mM NaCl, pH 4.0; flow rate: 2.0 ml/min; injection volume: 10 ml). Fibrillar $MRJP1_4$/$apisimin_4$/$24MC_8$ elutes within the void volume (V_0 = 112 ml) of the column and has, typical for a protein, a higher absorption at 280 (blue line) than at 260 nm (red line). The gel filtration step was necessary to separate the fibrillar protein from non-proteinaceous small molecular weight substances eluting shortly before the column volume (V_t = 320 ml) which had a higher absorption at 260 nm than at 280 nm and could not be removed using dialysis. **B)** SDS polyacrylamide gel (4-20% MiniProtean TGX stain-free (BioRad), separated at 175 V for 1 h, stained with Coomassie R-250) showing for all three RJ samples the protein components before (total) and after purification of the fibrils. The fibrillar fraction shows at around 50 kDa only MRJP1. Apisimin with 5 kDa is too small to be resolved within this gel.

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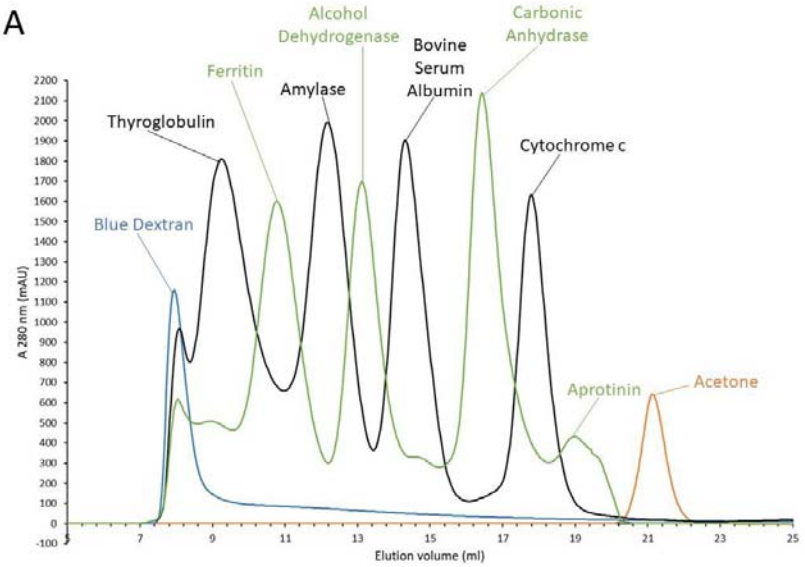
A



B

Analyte	Molecular weight (Da)	V_e (ml)	K_d
Blue Dextran	2,000,000	7.96 (V_0)	-
Thyroglobulin	669,000	9.25	0.0978
Ferritin	443,000	10.78	0.2138
Amylase	200,000	12.14	0.3169
Alcohol Dehydrogenase	150,000	13.11	0.3905
Bovine Serum Albumin	66,000	14.31	0.4814
Carbonic Anhydrase	29,000	16.44	0.6429
Cytochrome c	12,400	17.78	0.7445
Aprotinin	6,500	18.98	0.8355
Acetone	58	21.25 (V_t)	-

Da, Dalton; V_e, elution volume; V_0, void volume; V_t, total liquid volume; K_d, distribution coefficient $=(V_e-V_0)/(V_t-V_0)$.

C

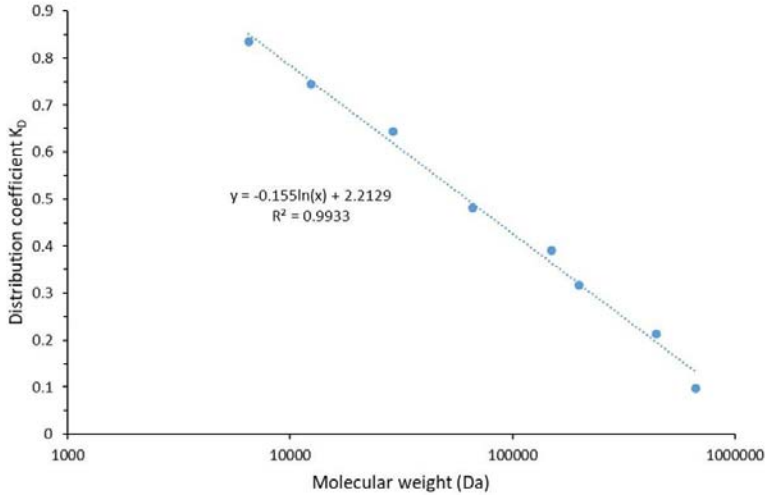


Fig. S2. Calibration of the Superdex 200 Increase 10/300 GL gel filtration column. **A)** Four separate gel filtration runs (buffer: 50 mM Tris/HCl, 150 mM NaCl, pH 7.5; flow rate: 0.7 ml/min; injection volume: 500 µl) using Blue Dextran to determine the void volume (V_0) (blue line), acetone to determine the total liquid volume (V_t) (orange line) of the column and two runs using different marker proteins (green and black lines). **B)** Elution volumes (V_e) of Blue Dextran, acetone and the different marker proteins as well as calculated distribution coefficients (K_d) for the marker proteins. **C)** Plot showing the calculated distribution coefficents depending on molecular weight. The fitted logarithmic curve was used to calculate distribution coefficients and molecular weights during the subsequent analytical runs.

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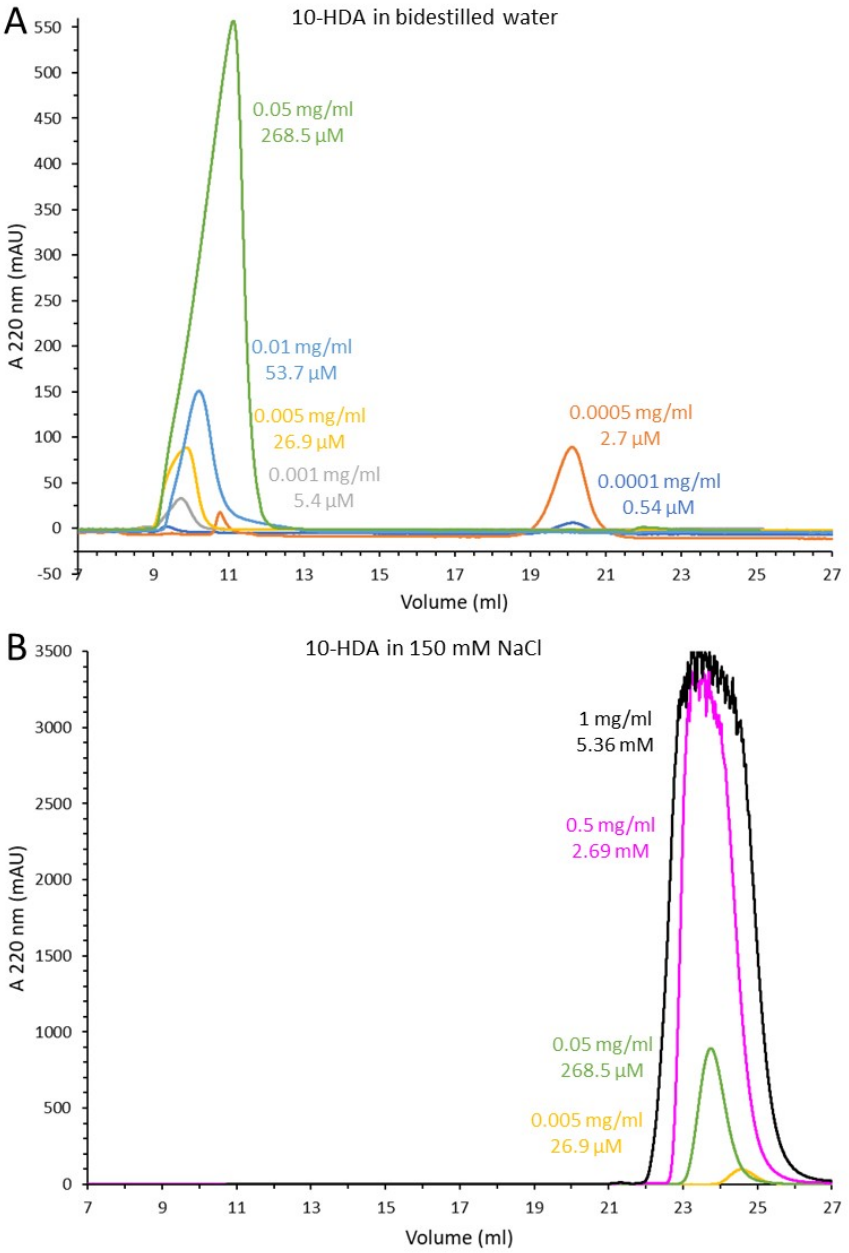

Fig. S3. Prevention of 10-HDA micelle formation by NaCl (column: Superdex 200 Increase 10/300 GL; flow rate: 0.7 ml/min; injection volume: 500 µl). 10-HDA concentrations: Dark blue, 0.54 µM; orange, 2.7 µM; grey, 5.4 µM; yellow, 26.9 µM; light blue, 53. 7 µM; green, 268.5 µM; magenta, 2.69 mM; black, 5.36 mM. Without addition of NaCl, micelles were formed from a concentration of 5.4 µM 10-HDA. **A)** 10-HDA in double distilled water. **B)** 10-HDA in 150 mM NaCl.

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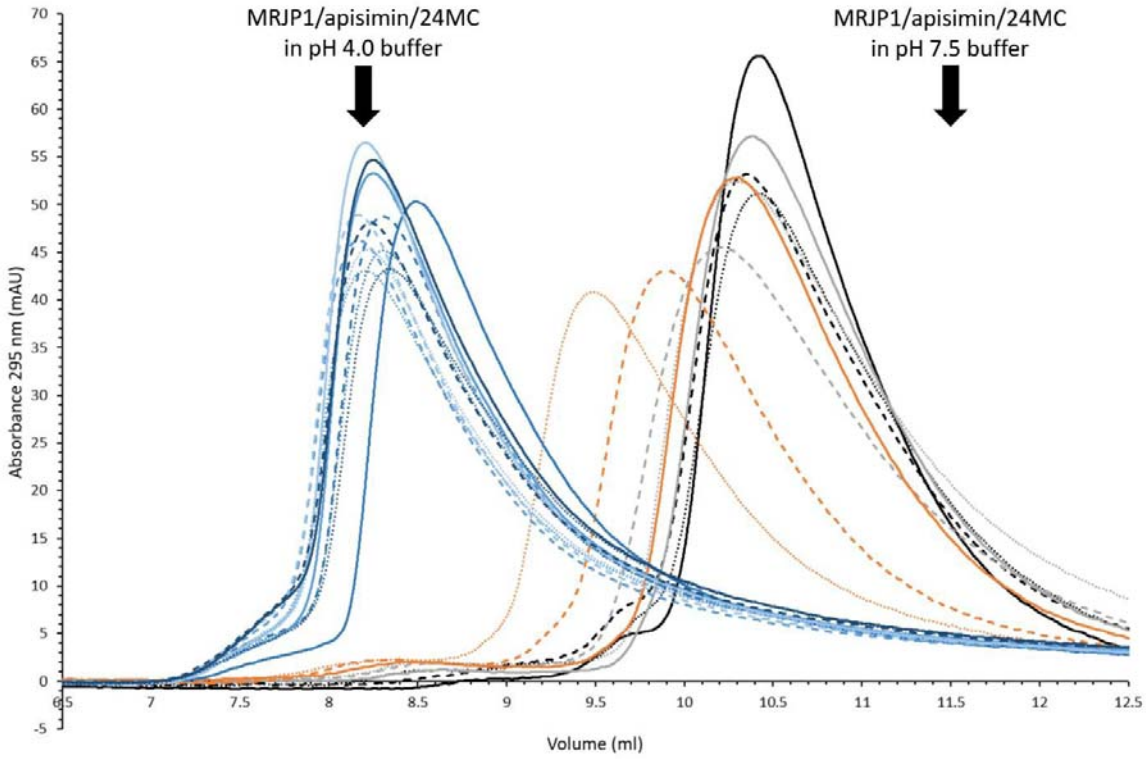

Fig. S4. Gel filtration analysis of 500 µg/ml MRJP1/apisimin/24MC in 150 mM NaCl supplemented with different concentrations of 10-HDA (0 – 805.2 µM) (column: Superdex 200 Increase 10/300 GL; flow rate: 0.7 ml/min; injection volume: 500 µl). 10-HDA concentrations: Black, 0 µM; grey, 26.8 µM; orange 134.2 µM; dark blue to light blue, 201.3 – 805.2 µM. RJF, solid line; RJG, dashed line; RJH dotted line.