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Supplemental Information

How Honeybees Defy Gravity

with Royal Jelly to Raise Queens

Anja Buttstedt, Carmen I. Mureşan, Hauke Lilie, Gerd Hause, Christian H. Ihling, Stefan-H. Schulze, Markus Pietzsch, and Robin F.A. Moritz

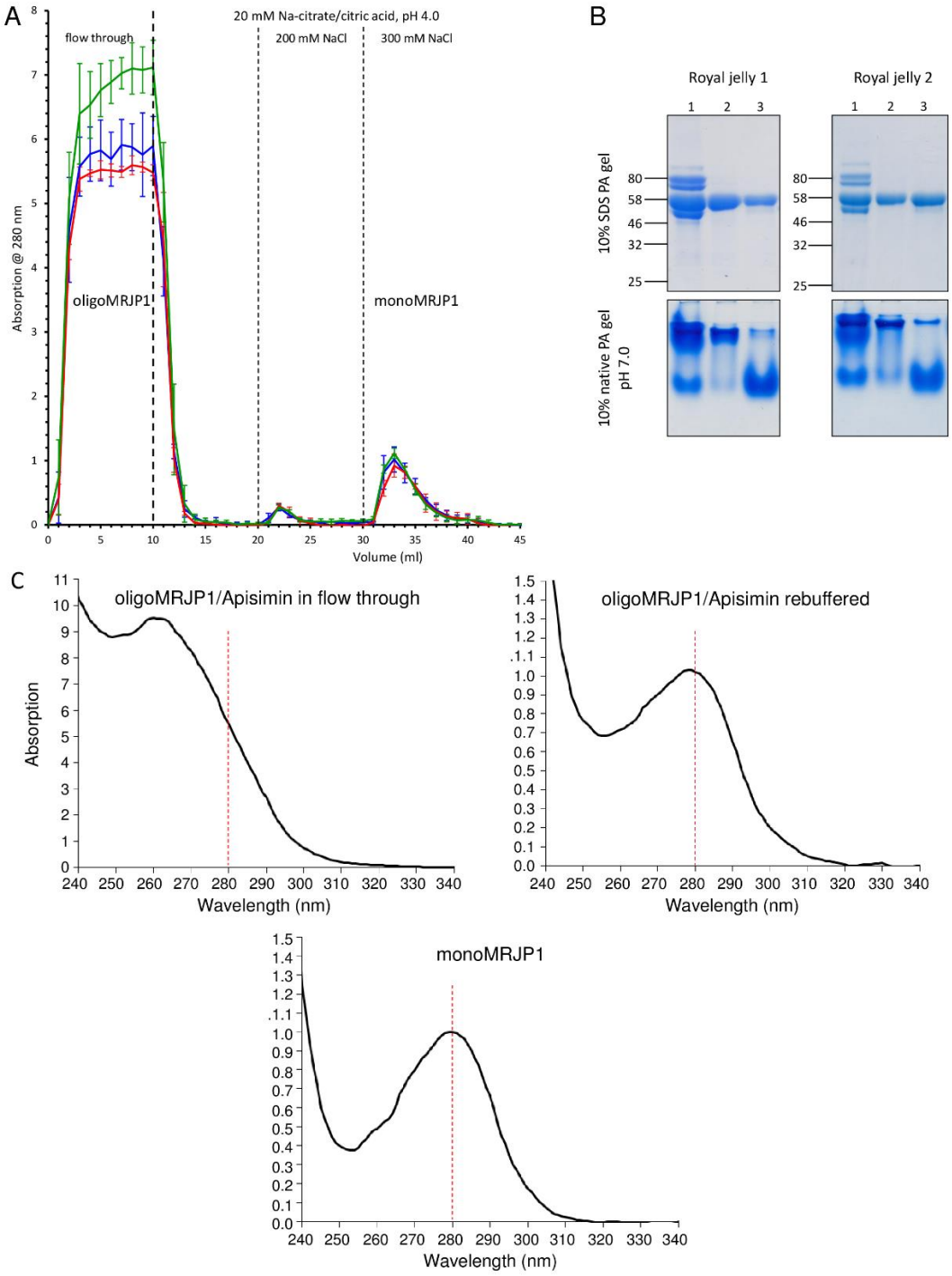

Figure S1. Protein purification and identification. Related to Figure 1. (A) Chromatogram showing the purification of oligo- and monoMRJP1. Blue line - RJ1, red line - RJ2, green line, RJ3. All lines represent the means of three purifications ± SD. **(B)** Purification of oligoMRJP1 and monoMRJP1 from RJ1 and RJ2; 1, protein extract; 2, oligoMRJP1; 3, monoMRJP1. **(C)** UV spectra of the purified oligoMRJP1 and monoMRJP1. The flow through of the SP sepharose containing oligoMRJP1 in 20 mM Na-citrate/citric acid, pH 4.0 showed an absorption maximum at 260 nm. Thus, interfering low molecular weight substances were removed either by dialysis or gel filtration with PD10-columns before further characterization of oligoMRJP1. The spectrum of monoMRJP1 is shown in in 20 mM Na-citrate/citric acid, pH 4.0, 300 mM NaCl.

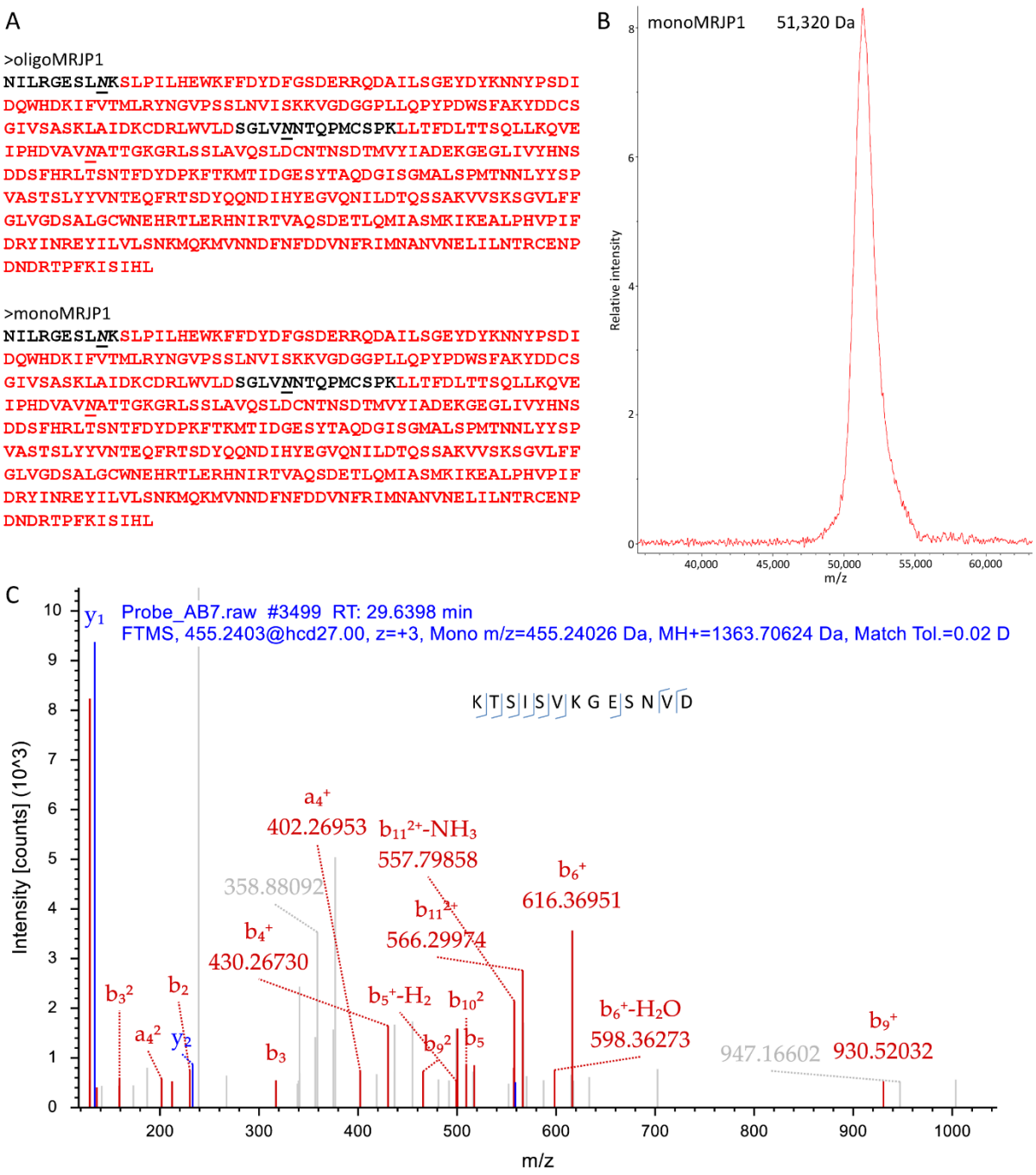

Figure S2. Mass spectrometric identification. Related to Figure 1. (A) oligoMRJP1 and monoMRJP1 have been identified by mass spectrometry with 94.2 %. Underlined and italic asparagines have previously been found to be glycosylated. More information on identified peptides can be found in Data S1. **(B)** MALDI mass spectrum of monoMRJP1. **(C)** Spectrum showing the identification of apisimin.

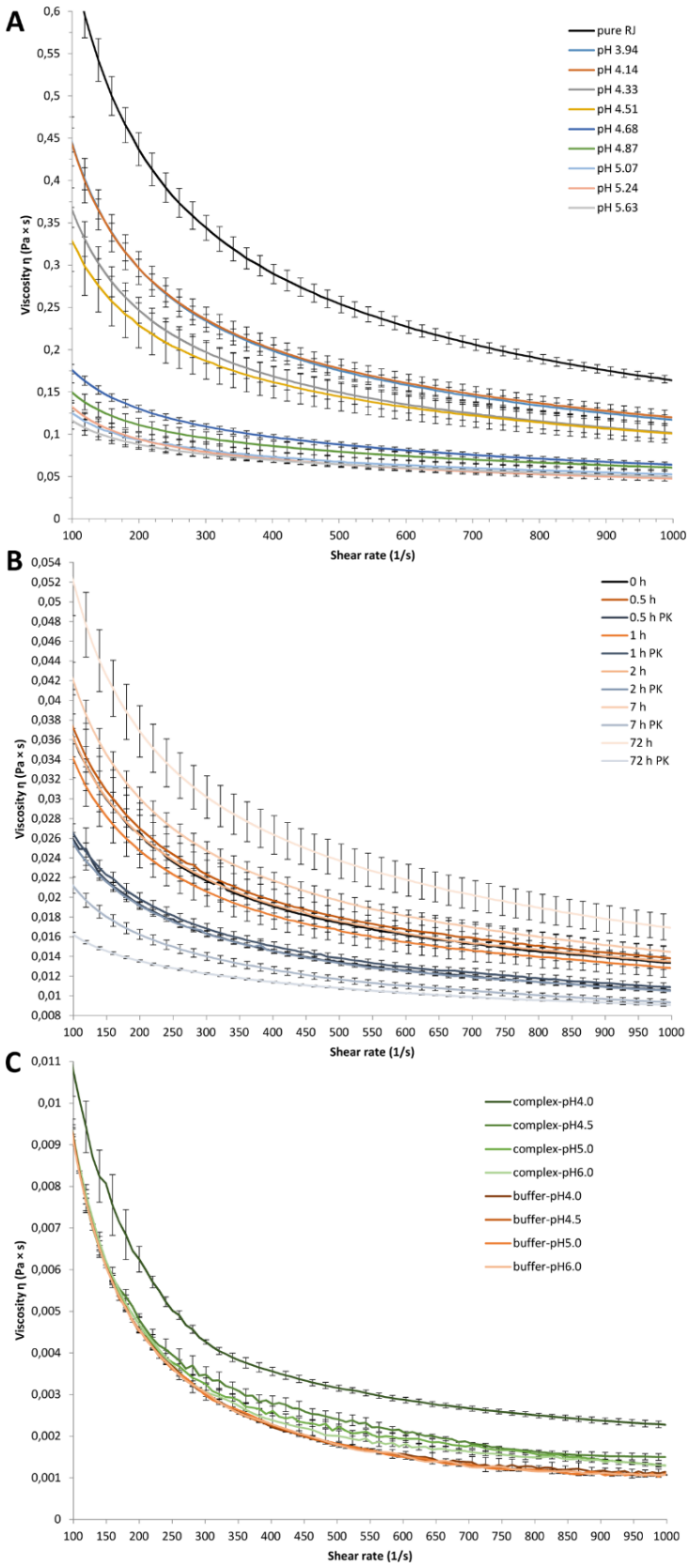

Figure S3. Viscosity in dependence of shear rate. Related to Figure 3A and Figure 4. (A) pH-changed RJ. Error bars show standard deviations of three measurements. **(B)** RJ treated with proteinase K. Error bars show standard deviations of four measurements comprising duplicate viscosity measurements of duplicate proteinase K experiments. **(C)** Complex of oligoMRJP1/apisimin (~8.5 mg/ml) at different pHs compared to buffer controls. Error bars show standard deviations of six measurements comprising triplicate viscosity measurements for the complex purified from two different RJ samples.

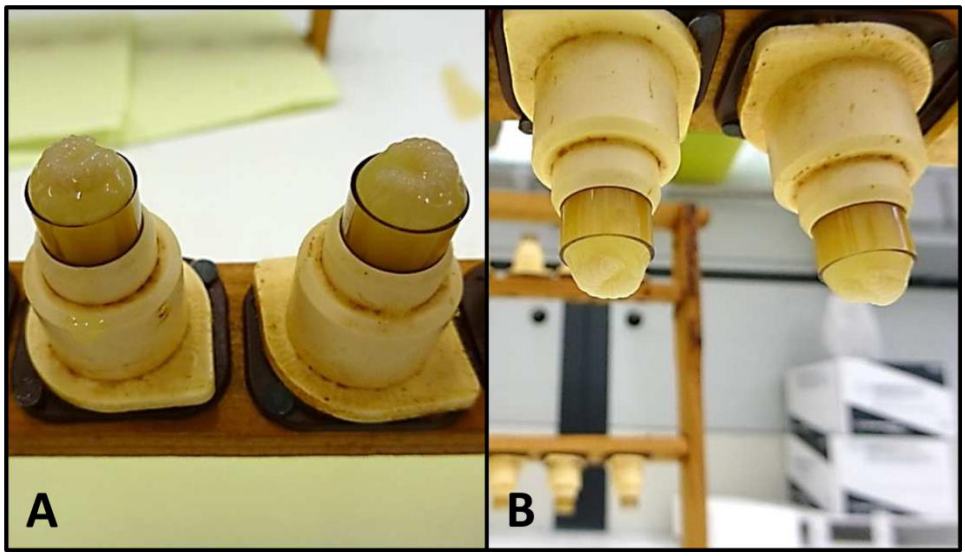

Figure S4. Experimental procedure for testing the effect of RJ pH on honeybee larvae. Related to Figure 3B. (A) Larvae were placed on top of queen cell cups filled with RJ. **(B)** At pH 3.94 ± 0.01 larvae were hold in place by the RJ when queen cell cups were turned around.

	RJ1			RJ2			RJ3		
pH	*s*(app)1 (S)	*s*(app)2 (S)	*s*(app)2 (%)	*s*(app)1 (S)	*s*(app)2 (S)	*s*(app)2 (%)	*s*(app)1 (S)	*s*(app)2 (S)	*s*(app)2 (%)
4.0	> 50			> 50			> 50		
4.5	18.00	32.00	83	12.00	31.00	93	25.00	42.00	62
4.75	10.00	18.00	58	10.00	16.00	52	12.00	21.00	66
5.0	9.16	-	-	9.13	-	-	9.50	15.00	5
6.0	8.60	-	-	8.65	-	-	8.70	15.00	2
7.0	8.50	3.7	6	8.40	3.7	6	8.70	-	-

Table S1. Apparent sedimentation coefficients (*s*(app)) determined at 20°C for the complex of oligoMRJP1/apisimin purified from three different RJs. Related to Figure 2A.