Peptide-stabilized emulsions and gels from an arginine-rich surfactant-like peptide with antimicrobial activity

Article

Supplemental Material

Supporting Information

Peptide-Stabilized Emulsions and Gels from an Arginine-Rich Surfactant-Like Peptide with Antimicrobial Activity

Valeria Castelletto,* Charlotte J. C. Edwards-Gayle, Ian W. Hamley,*

Dept. of Chemistry, University of Reading, Reading RG6 6AD, United Kingdom

Glyn Barrett,

School of Biological Sciences, University of Reading, Reading RG6 6AD, United Kingdom

Jani Seitsonen and Janne Ruokolainen,

Nanomicroscopy Center , Aalto University, Puumiehenkuja 2, FIN-02150 Espoo, Finland.

* Authors for Correspondence. I.W.Hamley@reading.ac.uk, V.Castelletto@reading.ac.uk

SAXS models. The SAXS intensity from a scattering object without a particular orientation can be approximated as the following equation:[1-2]

$$I(q) \propto \left\langle F^2(q)S(q) \right\rangle \qquad (1)$$

where $F^2(q)$ is the scattering particle form factor and $S(q)$ is the interparticle structure factor.

The SAXS curves for samples containing only A_9R were fitted using a form factor for random oriented cylindrical shell with circular cross section.[3] The fitting parameters were the core radius R, the shell thickness D_r the scattering length density of the core, shell and solvent η_{core}, η_{shel} and η_{core} and the background BG.

The SAXS curves for vesicle were fitted using a form factor for a Gaussian lipid bilayer together with a structure factor for a multilayer structure influenced by thermal fluctuations.[4-5] The details of the Gaussian lipid bilayer model are given elsewhere.[6] Briefly, the model assumes an electron density profile comprising Gaussian functions for the head groups on either side of the bilayer and another Gaussian for the hydrocarbon chain interior. The midpoint of the bilayer is defined as $z = 0 = z_C$. In our model we assumed a Gaussian distribution of inter-head group thicknesses $2z_H$, with an associated degree of polydispersity Δ_{2z_H}. The fitting parameters of the model are the electron densities of the head group (ρ_H), the layer thickness z_H, the electron density of the hydrocarbon chains (ρ_C), the standard deviation of the position of the Gaussian peak at z_H (σ_H), the standard deviation of the position of the Gaussian peak at z_C (σ_C), and Δ_{2z_H}.

The structure factor was modelled using the modified Caillé theory.[4-5] *S(q)* depends on the total number of layers (*N*) within a scattering domain, the diffuse background of uncorrelated bilayers (N_{diff}) the bilayer spacing (*d*) and the Caillé parameter (η):

$$\eta = \pi k_B T / 2d^2 (BK_c)^{1/2} \qquad (2)$$

which is a measure for the bilayer fluctuations and depends on the bilayer rigidity K_c and the bulk modulus of compression *B*. All fitting was done using the software SASfit.[7]

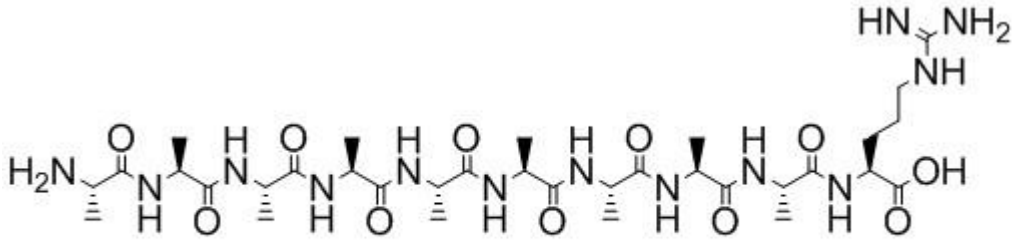
Table S1. Parameters extracted from the fitting of the SAXS curve in Figures 1e,2c,S3: core radius *R*, shell thickness D_r and scattering length density of the core, shell and solvent η_{core}, η_{shel} and η_{core} and background BG.

	0.08 wt% A_9R	0.5 wt% A_9R	1 wt% A_9R	9.5 wt% A_9R
$R \pm \Delta R$ (Å)	18 ± 10	18 ± 10	20.3± 10.8	15 ± 10
D_r (Å)	4	4	3	2
η_{core} (a.u.)	$7.17x10^{-8}$	$1.39x10^{-6}$	$1.7x10^{-6}$	$4.3x10^{-2}$
η_{shell} (a.u.)	$8.25x10^{-9}$	$1.75x10^{-7}$	$-5.0x10^{-7}$	$8.5x10^{-2}$
η_{solv} (a.u.)	0	0	0	0
BG	$2x10^{-4}$	$1.1x10^{-3}$	$1.4x10^{-3}$	10

Table S2. Parameters extracted from the fitting of the SAXS in Figure S2.

Sample / Parameter	$*\phi_{POPG}$= 0.2	ϕ_{POPG}= 0.2+ 0.08wt% A_9R	$*\phi_{DOPC}$= 0.2	ϕ_{DOPC}= 0.2+ 0.08 wt% A_9R	
N1 [arb. units]	1	--	0.25	--	--
Δ_{2zH} [Å]	5	--	5	--	--
$2z_H$ [Å]	44	49	44	38.5	38.5
σ_H [Å]	3	2.3	3.1	2.2	2.8
$l_H = 2z_H + \sigma_H$	47	51.3	47.1	40.7	41.3
ρ_H [rel. units]	2.2×10^{-3}	3.25×10^{-6}	1×10^{-3}	3.6×10^{-6}	1×10^{-6}
σ_C [Å]	1	1	1	1.37	1
ρ_C [rel. units]	-8.5×10^{-3}	-3.93×10^{-5}	-5.5×10^{-3}	-1.58×10^{-5}	-1×10^{-5}
N	--	2	8	2	6
d_o	--	81	64	65	65
$l_w = d_o - 2z_H$	--	29.7	17	24.3	23.7
N_{diff}	--	0.3	0.3	0.25	0.2
η	--	0.5	0.5	3.1	0.5
BG	0.1	--	--	0.2	0.2

Key: Gaussian bilayer form factor: scale factor N1, Gaussian half-width at half-maximum for polydispersity Δ_{2zH}, inter-head group thicknesses $2z_H$, Gaussian half-width for outer layer surface σ_H, electron density for headgroup ρ_H, Gaussian half-width for inner layer σ_C, relative electron density for inner layer ρ_C. **Caillé structure factor:** total number of layers N; layer thickness d_o; Caillé parameter η; diffuse background N_{diff}. *data extracted from ref.[8] are displayed here only as a reference. l_w and l_H are the thickness of the water and the lipid bilayer respectively.



A_9R

Scheme S1. Molecular structure of A_9R

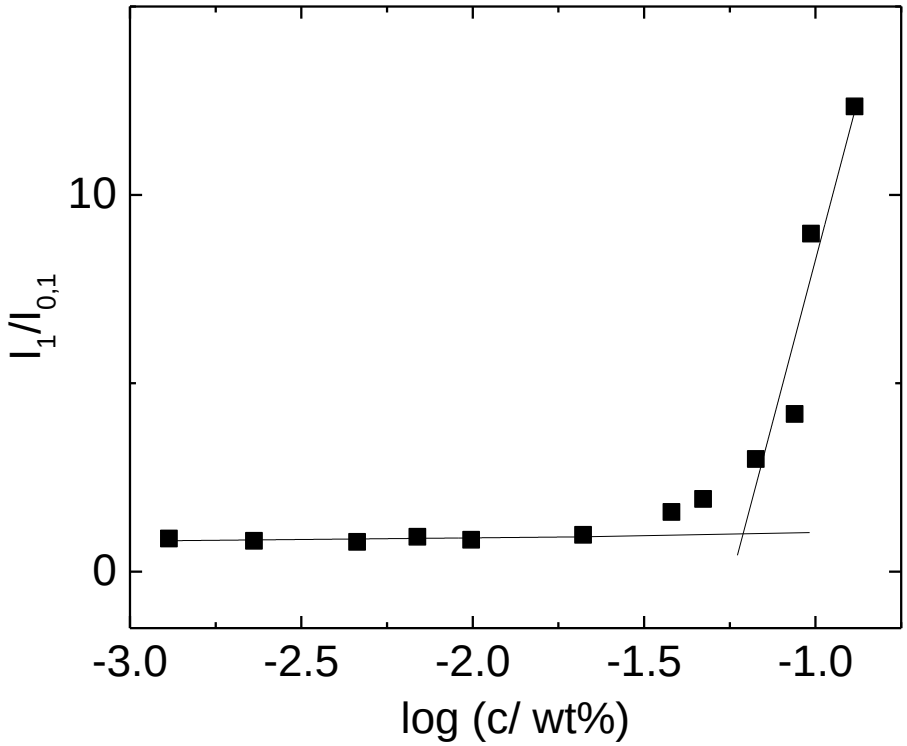


Figure S1. Pyrene assay for A_9R .

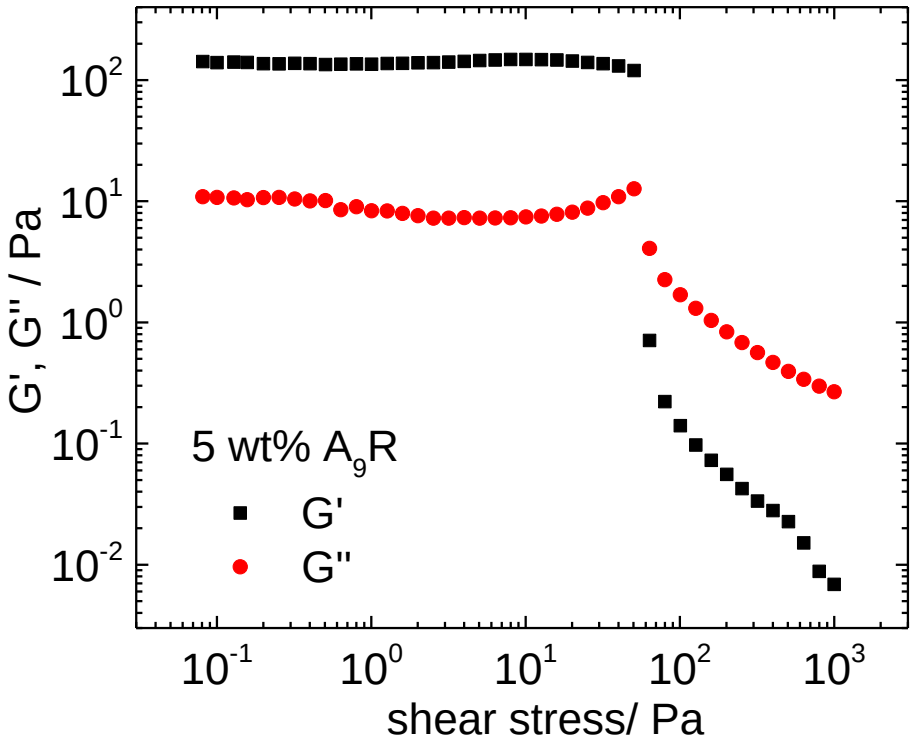


Figure S2. Plot of dynamic shear moduli versus shear stress to determine the linear viscoelastic regime for 5 wt% A_9R hydrogel.

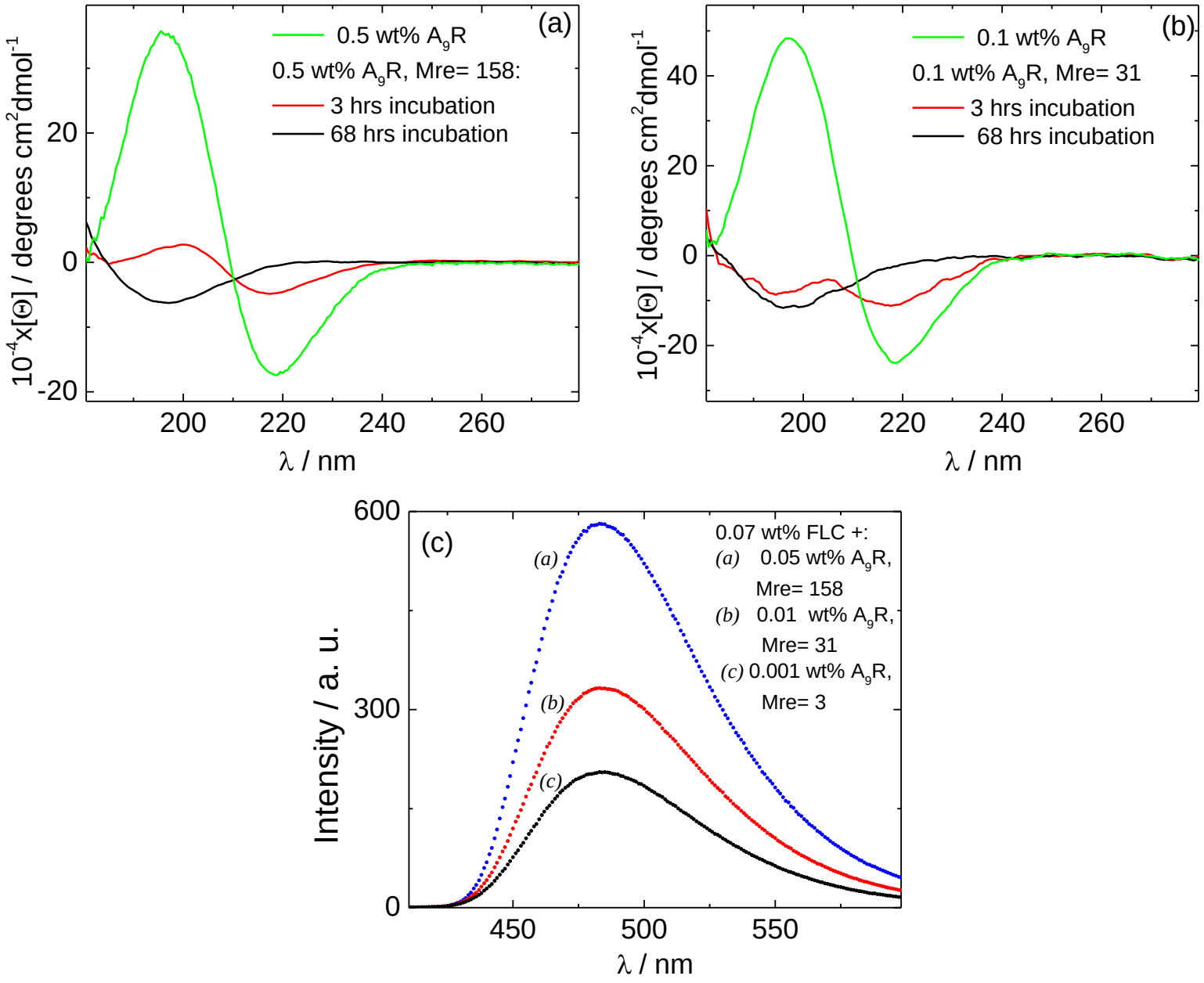


Figure S3. Elastase enzymatic assay for A_9R: CD data for (a) 0.5 wt% A_9R, Mre= 158 and (b) 0.1 wt% A_9R, Mre= 31. CD controls for pure A_9R solutions are displayed in (a-b). (c) FLC assay results at 0.07 wt% FLC and A_9R/elastase ratios used in Figures (a-b). Data for 0.001 wt% A_9R (Mre= 3) is included to confirm the trend of the fluorescence intensity.

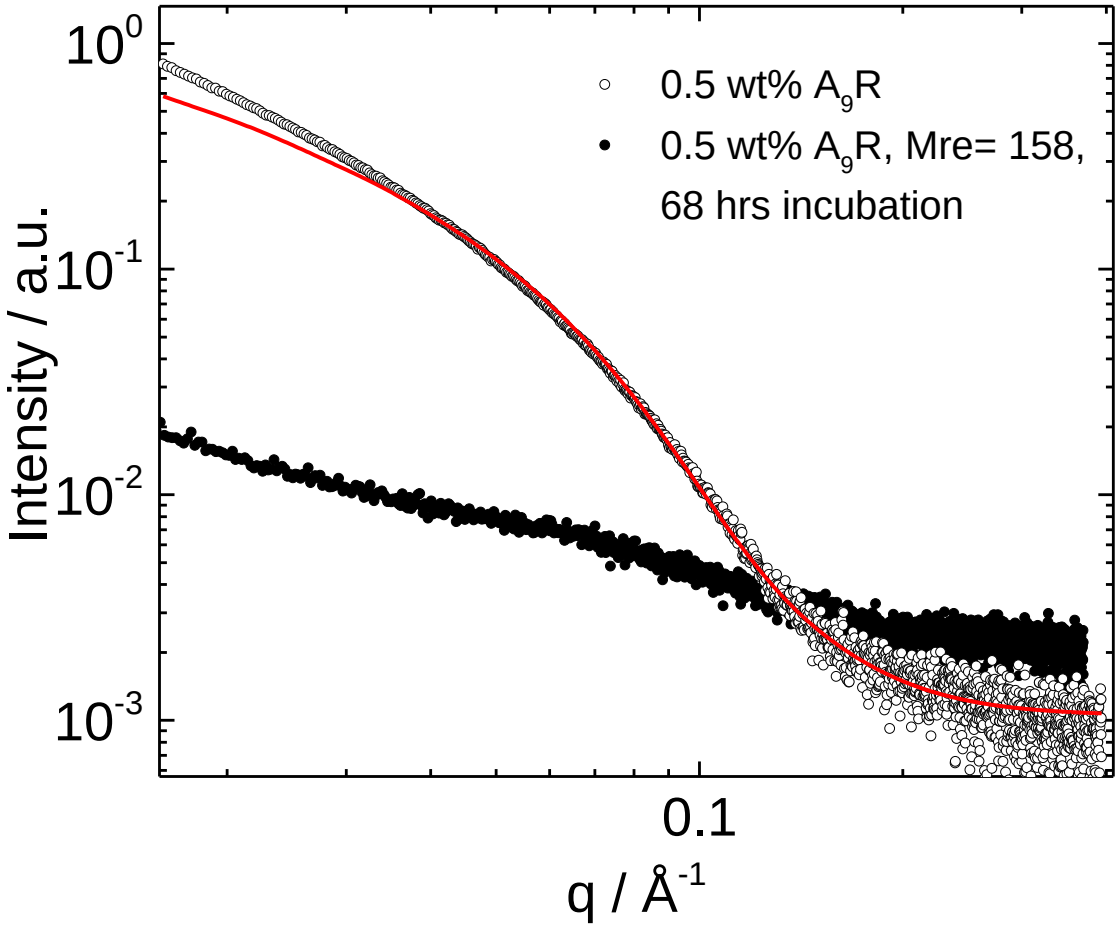


Figure S4. SAXS data for 0.5 wt% A_9R in water, and 0.5 wt% A_9R incubated with elastase at Mre = 158 for 68 hrs (same sample as in Figure S3a). The full red line is a model form factor fit to the experimental data.

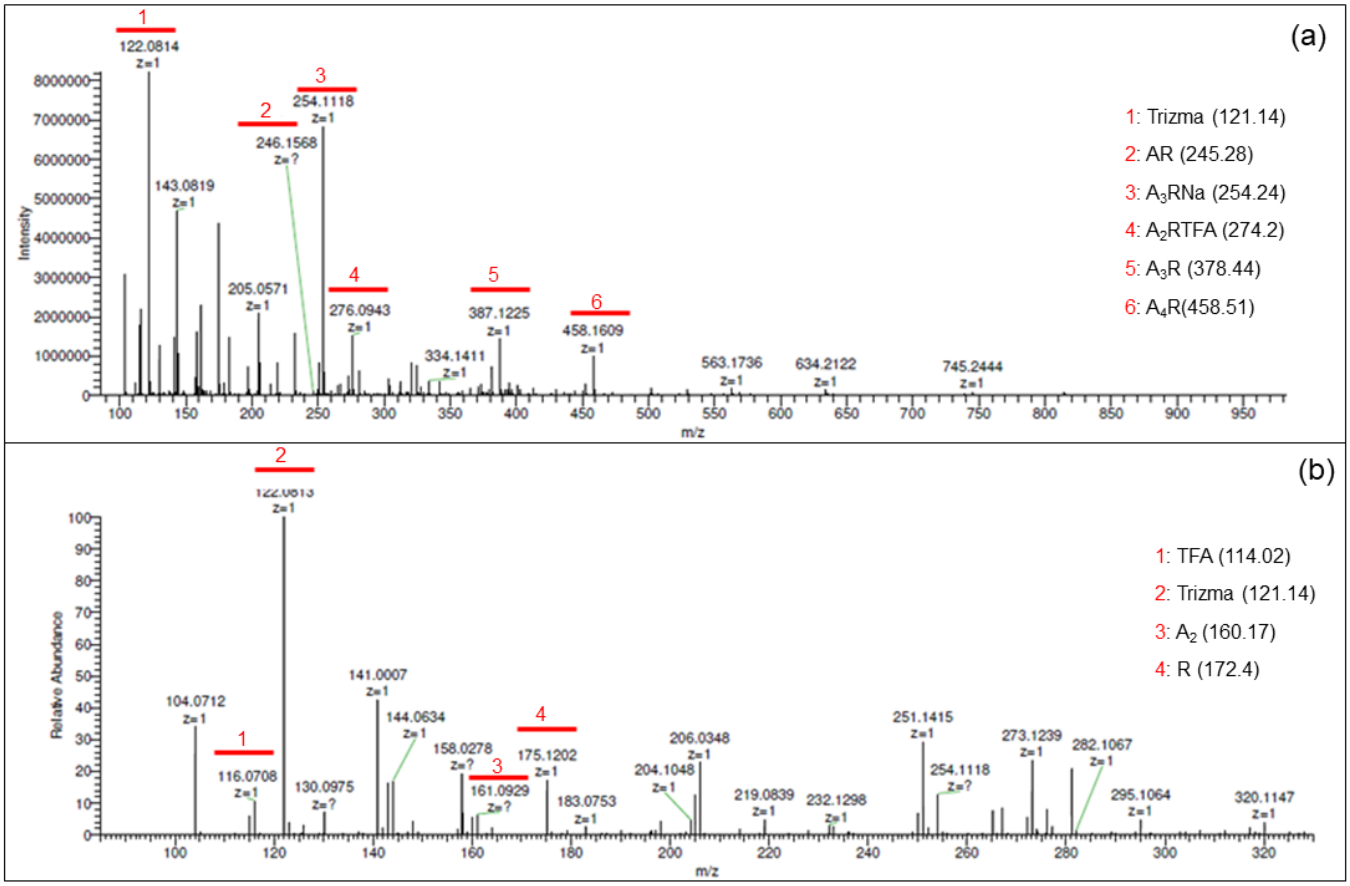


Figure S5. (a-b) ESI-MS for a sample containing 0.1 wt% A_9R and Mre= 30.8. Experiment performed for the same sample for which data is presented in Figure S2b (68 hrs of incubation). The band for the A_9R molecular weight (814.9 g mol^{-1}) is missing in the ESI spectra, due to elastase enzymatic degradation.

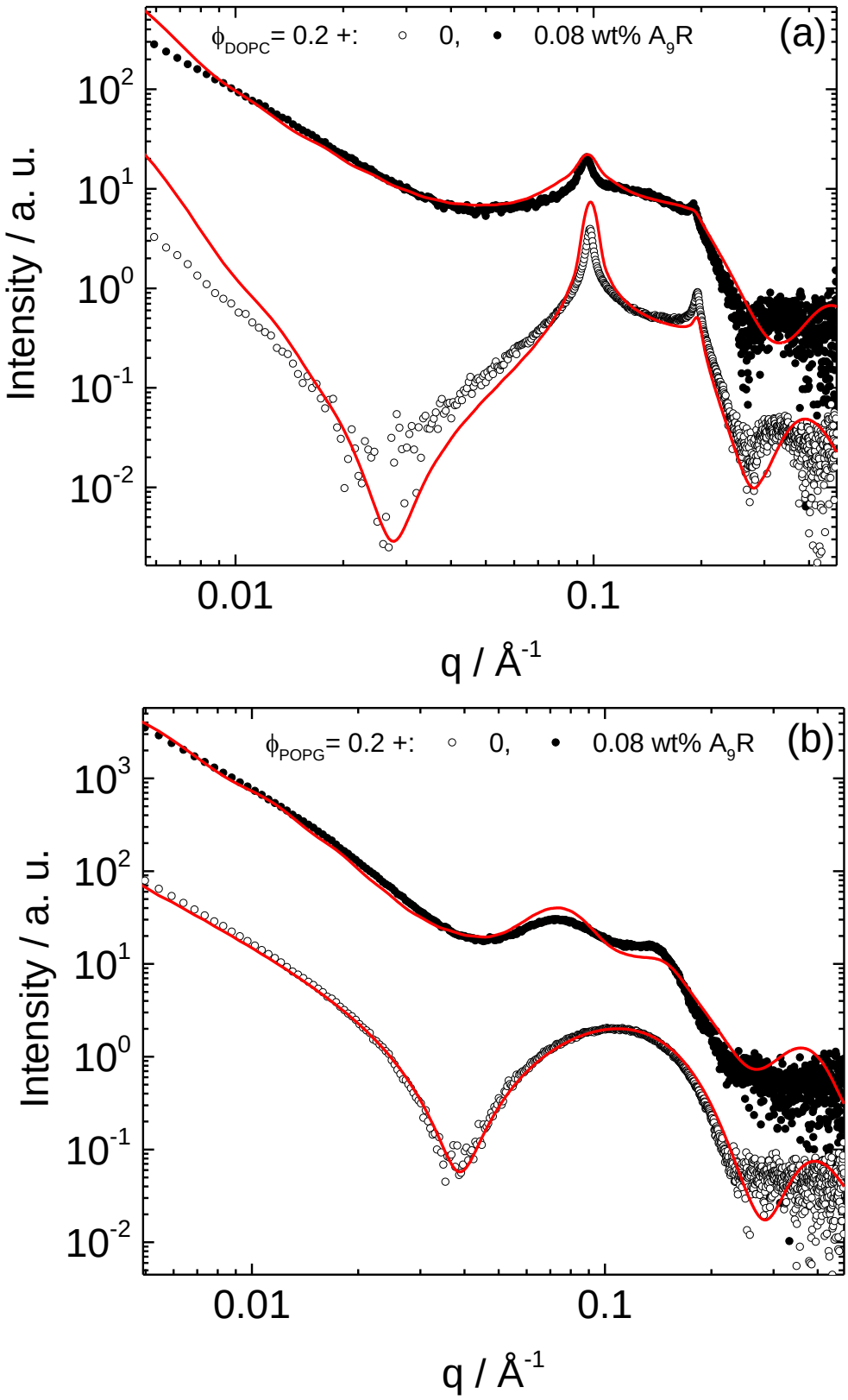


Figure S6. SAXS data for vesicles containing (a) ϕ_{DOPC}=0.2 + 0 or 0.08 wt% A_9R and (b) ϕ_{POPG}=0.2 + 0 or 0.08 wt% A_9R. The full red lines are fitted form factor profiles. Parameters from the fittings are displayed in Table S2. Results for 0 wt% A_9R are displayed as a reference.[9]

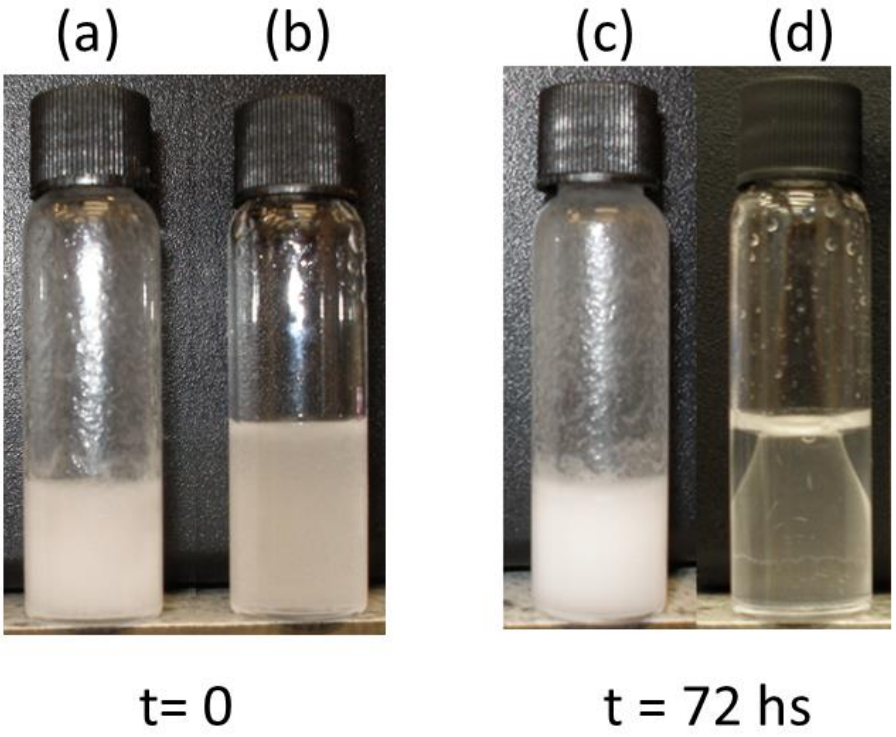


Figure S7. Stability of (a,c) 0.05 wt% A_9R emulsion compared to (b,d) water/1-bromohexadecane 30/70 v/v mixture: samples immediately after mixing (a,b) and 72 hrs after mixing (c,d).

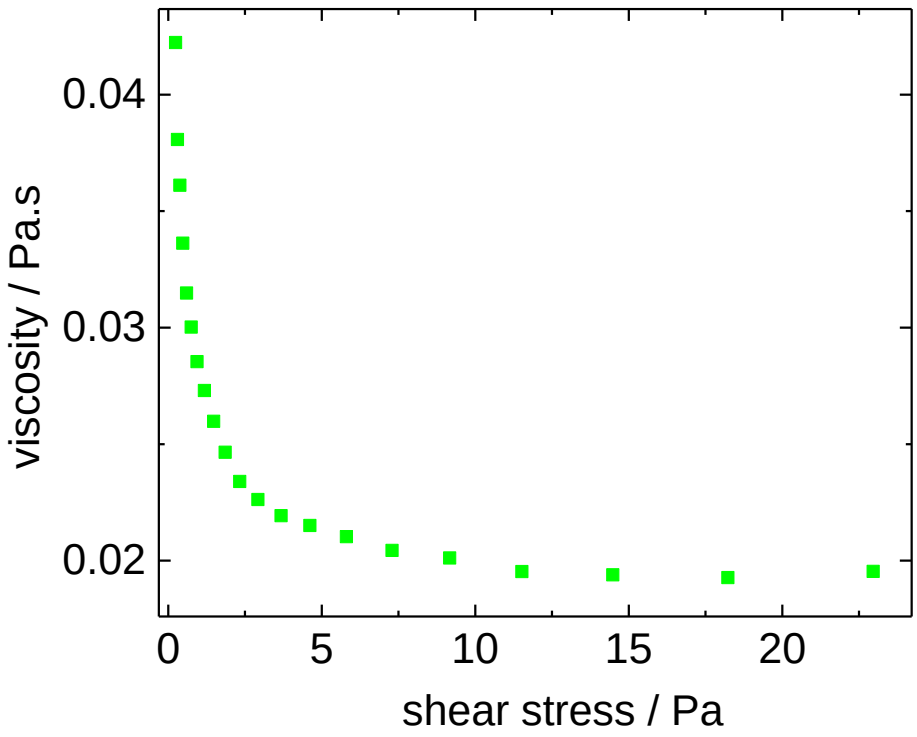


Figure S8. Viscosity versus shear stress for 0.05 wt% A_9R emulsion.

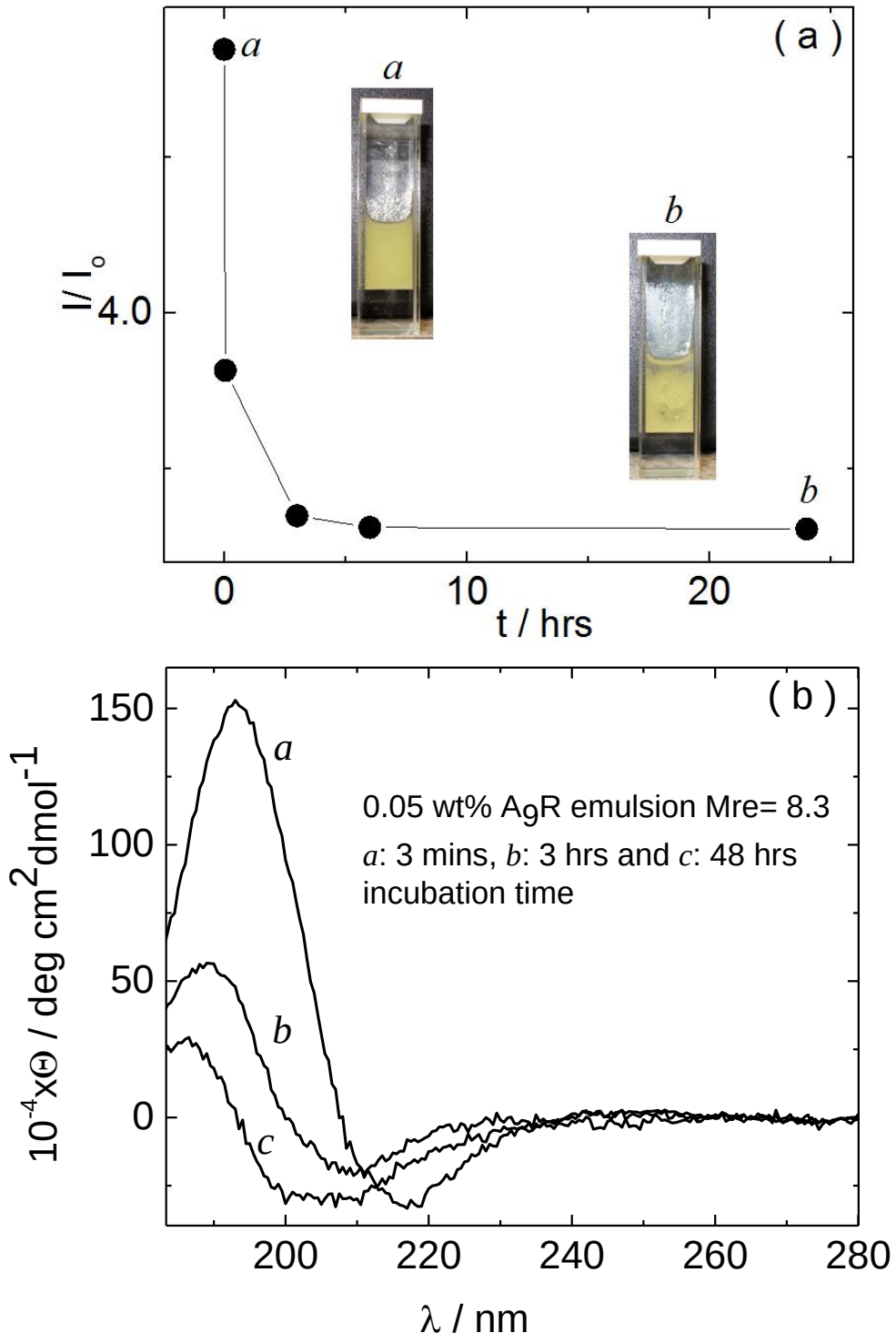


Figure S9. 0.05 wt% A_9R emulsion treated with elastase at Mre= 8.3. (a) Time evolution of ThT fluorescence intensity ratio (I/I_o) together with Images of the emulsion stained with ThT *(i)* immediately and *(ii)* after 24 hrs incubation time. (b) CD profiles as a function of the incubation time.

References

1. Pedersen, J. S.; Svaneborg, C., Scattering from block copolymer micelles. *Curr. Opin. Colloid Interface Sci.* **2002,** *7*, 158-166.
2. Castelletto, V.; Hamley, I. W., Modelling small-angle scattering data from micelles. *Curr. Opin. Colloid Interface Sci.* **2002,** *7*, 167-172.
3. Glatter, O.; Kratky, O., *Small Angle X-ray Scattering*. Academic: London, 1982.
4. Zhang, R.; Suter, R. M.; Nagle, J. F., Theory of the structure factor of lipid bilayers. *Phys. Rev. E* **1994,** *50*, 5047-5060.
5. Caillé, M., Remarques sur la Diffusion des Rayons X dans les Smetiques. *C. R. Acad. Sci. Paris* **1972,** *274*, 891-893.
6. Pabst, G.; Rappolt, M.; Amenitsch, H.; Laggner, P., Structural information from multilamellar liposomes at full hydration: Full q-range fitting with high quality x-ray data. *Phys. Rev. E* **2000,** *62*, 4000-4009.
7. Breßler, I.; Kohlbrecher, J.; Thünemann, A. F., SASfit: a tool for small-angle scattering data analysis using a library of analytical expressions. *J. Appl. Cryst.* **2015,** *48*, 1587-1598.
8. Castelletto, V.; Barnes, R. H.; Karatsas, K.-A.; Edwards-Gayle, C. J. C.; Greco, F.; Hamley, I. W.; Seitsonen, J.; Ruokolainen, J., Restructuring of Lipid Membranes by an Arginine-Capped Peptide Bolaamphiphile. *Langmuir* **2019**, *35*, 1302-1311.
9. Castelletto, V.; Barnes, R. H.; Karatzas, K.-A.; Edwards-Gayle, C. J. C.; Greco, F.; Hamley, I. W.; Rambo, R.; Seitsonen, J.; Ruokolainen, J., Arginine-Containing Surfactant-Like Peptides: Interaction with Lipid Membranes and Antimicrobial Activity. *Biomacromolecules* **2018,** *19* 2782–2794.