

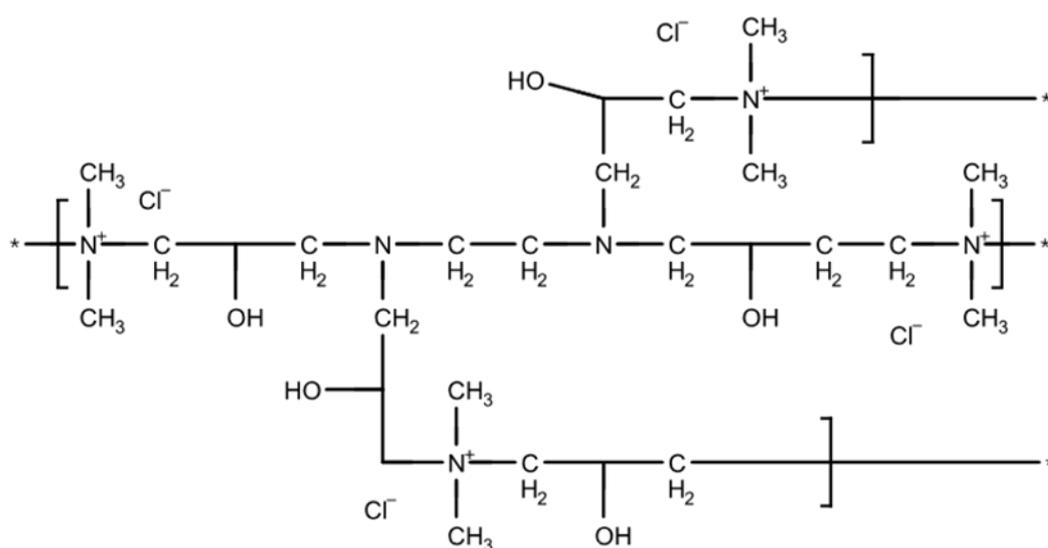
## Hyperbranched Kaustamin as an antibacterial for surface treatment

Vladislava A. Pigareva, Daria A. Stepanova, Anastasia V. Bolshakova,  
Valeria I. Marina, Ilya A. Osterman and Andrey V. Sybachin

### Materials

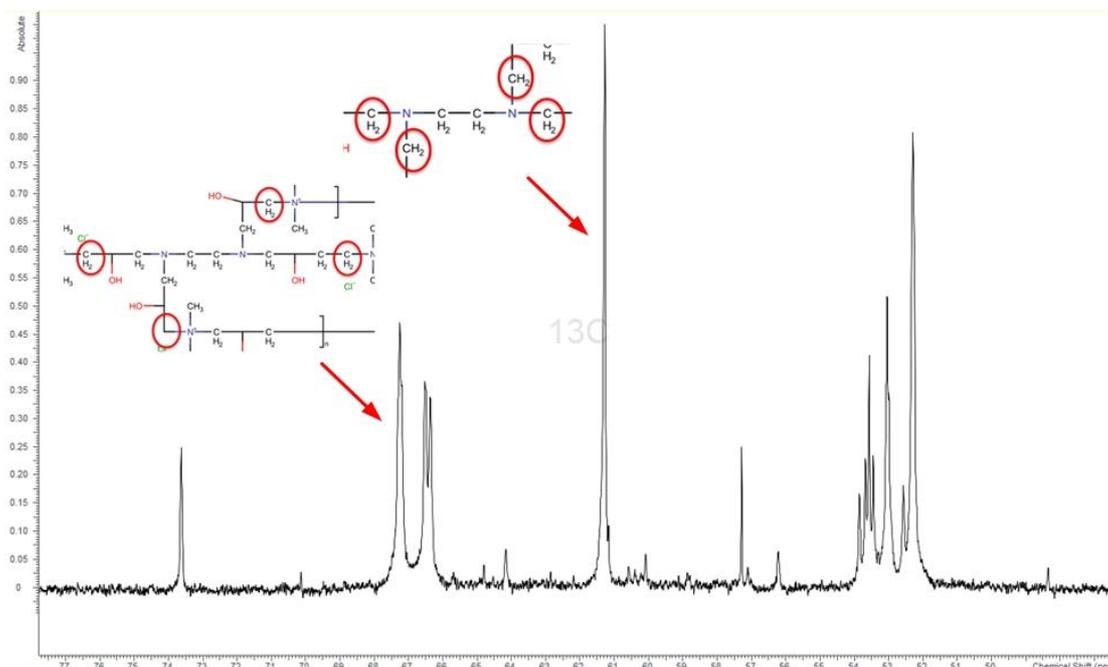
Hyperbranched kaustamin (HKAU) commercial sample FL2850 lot LCAV 11/6660 was presented by SNF Vostok (Russia) as 50 wt.% solution. Polymer was used as received.

Bidistilled water was used in the experiments.



**Figure S1.** Structure formula of monomer unit of HKAU.

The <sup>13</sup>C NMR spectrum of the HKAU is presented in Figure S2. The integral intensities of the peaks corresponding to C atoms neighbor to quaternary (67.2 ppm) and tertiary (61.28 ppm) amino groups allows one to estimate the degree of the branching of polymer. It was estimated that for the sample under investigation there are 2 branching points per 4 quaternized amino groups in other words 4 kaustamin monomer units per 1 ethylenediamine fragment.



**Figure S2.**  $^{13}\text{C}$  NMR spectrum of the HKAU in  $\text{D}_2\text{O}$ .

## Experimental

*Static light-scattering (SLS)* experiments were performed using goniometer with He-Ne laser ( $\lambda_0 = 633 \text{ nm}$ ) and photomultiplier supplied with Photocore software.

The series of HKAU solutions with concentrations from  $0.06$  to  $0.14 \text{ mg ml}^{-1}$  were prepared in  $0.15 \text{ M}$  sodium chloride. The parameter “a” in Zimm plot in the abscissa axis  $\sin(\Theta/2) + a \times c$  was calculated as reciprocal maximal concentration  $0,00014 \text{ g ml}^{-1}$  and the resulted value was  $a = 7142,86$ . The intensities of scattering of light were measured at angles ( $\Theta$ ) from  $30$  to  $120$  degrees. The data is presented in Table S1.

**Table S1.** The SLS measurement data.

$\Theta$	Concentration, $\text{mg ml}^{-1}$				
	0,06	0,08	0,1	0,12	0,14
30	21560	23489	26245	34500	78000
45	8146	9108	9412	11941	26743
60	4681	5622	6171	6777	10141
75	2893	3518	4254	5350	6505
90	2457	2782	3186	3543	5404
120	2502	2901	3173	3396	4380

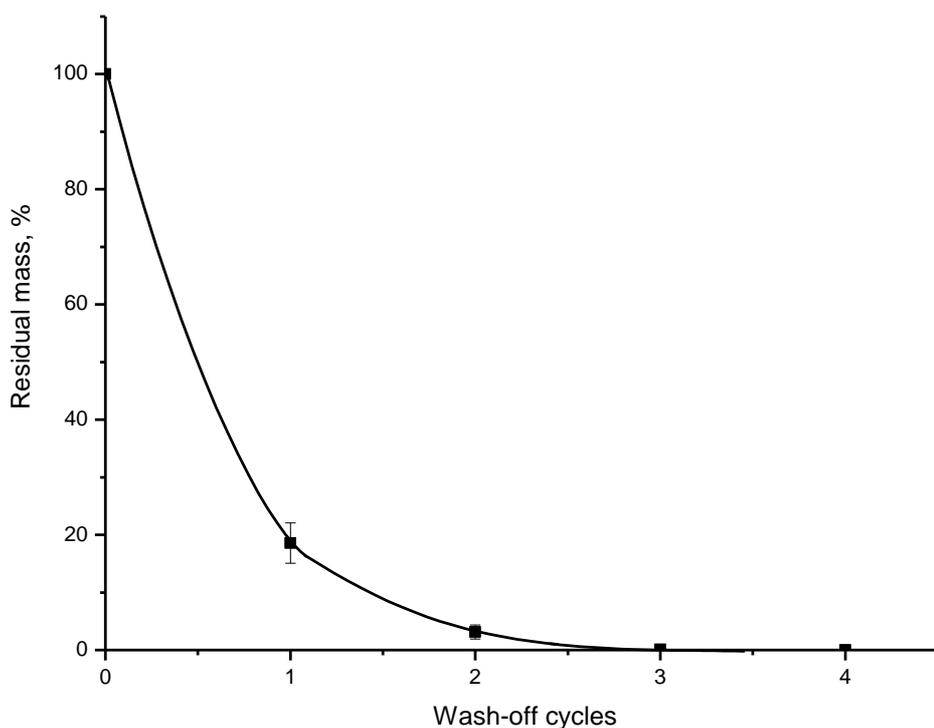
Refractive index increment  $\left(\frac{\partial n}{\partial c}\right)$  was measured with laser differential refractometer Cromatix KMX-16 and was found to be  $0,097$ . Optical constant of the solution ( $K$ ) was calculated as

$$K = \frac{4\pi^2 n_0^2}{\lambda_0^4 N_A} \left( \frac{\partial n}{\partial c} \right)^2$$

where  $n_0$  is the refractive index of solvent.

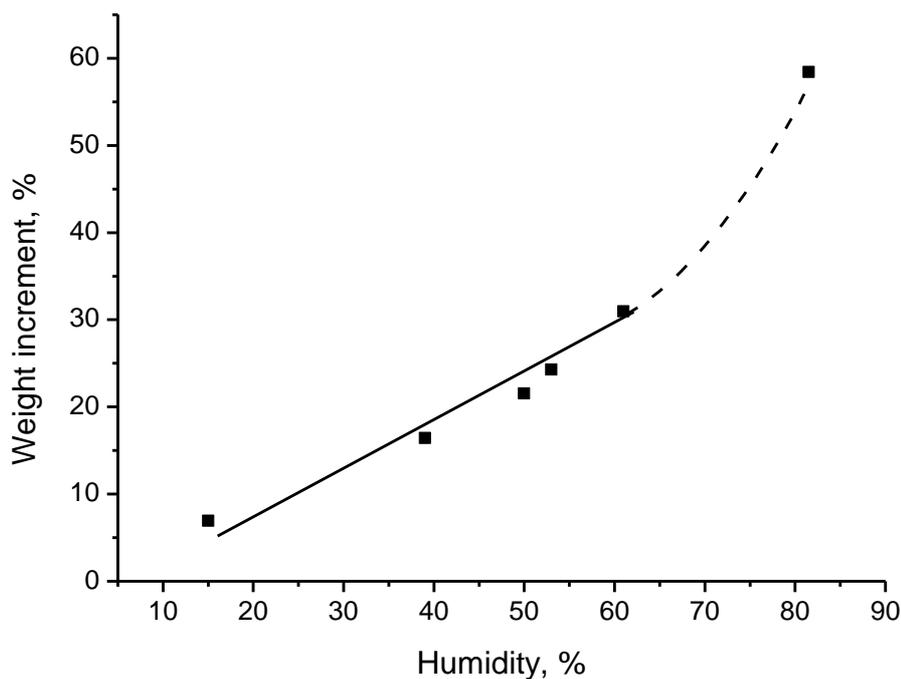
*Minimal inhibition concentrations (MIC)* for the antibiotics was determined as follows. An overnight culture of *B. subtilis* was diluted to OD=0.01 in LB medium, the 20 mg ml<sup>-1</sup> solution of HKAU was added and performed a two-fold serial dilution method (100μl/well). After addition of biocides the microplates were incubated for 16-20 hours at 37°C with shaking at 200 rpm, the optical density was measured using a multilabel plate reader Victor X5 2030 (Perkin Elmer). The MIC was considered to be the concentration, the optical density of which did not vary from the control point, where only the medium was added. The same procedures were performed for other bacteria and media.

*Wash-off control of the HKAU films.* The formation of HKAU covering on the glass surface was performed by deposition of 200 μl of the 20 mg ml<sup>-1</sup> solution to a freshly cleaned and weighed glass coverslip with an area of 2.89 cm<sup>2</sup> with consequent drying at air. The dried glass was weighed and the weight of the polymer film was determined from the mass difference. Then 200 μl of water was applied to the glass so that it completely covered the surface of the film. After 1 minute of incubation the liquid was eliminated and the sample was left to dry. After complete drying, the glass was weighed again. The resistance of the film towards wash-off with water was monitored by the weight loss of the sample. The dependence of the residual mass of the HKAU film upon the number of wash-off cycle is presented in Figure S3.



**Figure S3.** The residual mass of the HKAU film upon the number of wash-off cycle.

*Measurement of the HKAU film swelling* in conditions of the controlled relative humidity. The HKAU film was prepared by the following method: 3 ml of the  $20 \text{ mg ml}^{-1}$  solution was added to a Petri dish, then the films were dried in a laboratory oven in atmosphere with relative humidity 5%. Then the sample was weighted. The difference in masses of the coverslip with film and individual Petri dish was used to determine mass of the HKAU film. This value was used as reference. The masses of the film after incubation in environment with controlled fixed humidity were used to analyze the capacity of the film on absorbed water from the air. The dependence of the increase of the mass of the HKAU film upon the relative humidity of the environment is presented in Figure S4.



**Figure S4.** The dependence of the HKAU film weight increment upon relative humidity of the environment.

*Atomic-force microscopy (AFM) imaging* was performed using scanning probe microscope Nanoscope IIIa (USA) operating in tapping modes in air. The cantilevers, made from silicon, were used (TipsNano, Russia). The resonance frequencies were 140-150 KHz.

*Sample preparation for AFM imaging.* A freshly cleaned glass coverslip with a surface area of 2.89 cm<sup>2</sup> was dipped into an aqueous solution of HKAU with a concentration of 20 mg ml<sup>-1</sup> and kept there for 2 minutes to ensure the exhaustive adsorption of the polymer. Then the sample was placed in a beaker with distilled water and was subjected to vigorous shaking for 2 minutes to eliminate HKAU macromolecules from the surface. After that, the coverslip with HKAU was dried in air and then was analyzed with AFM.