Supplementary material for

Responses of *Acinetobacter Baumannii* Bound and Loose Extracellular Polymeric Substances to Hyperosmotic Agents Combined with or without Tobramycin: An Atomic Force Microscopy Study

Muhammedin Deliorman,[1] F. Pinar Gordesli Duatepe,[2] Emily K. Davenport,[3] Boel A. Fransson,[4] Douglas R. Call,[5] Haluk Beyenal,[3] and Nehal I. Abu-Lail[6,]*

[1] Division of Engineering, New York University Abu Dhabi, P.O. Box 129188, Abu Dhabi, UAE

[2] Faculty of Engineering, Izmir University of Economics, 35330 Izmir, Turkey

[3] Gene and Linda Voiland School of Chemical Engineering and Bioengineering, Washington State University, 99164 Pullman, WA, USA

[4] Department of Veterinary Clinical Sciences, Washington State University, 99164 Pullman, WA, USA

[5] Paul G. Allen School for Global Animal Health, Washington State University, 99164 Pullman, WA, USA

[6] Department of Biomedical Engineering, University of Texas at San Antonio, 78249 San Antonio, TX, USA

[*] E-mail: nehal.abu-lail@utsa.edu

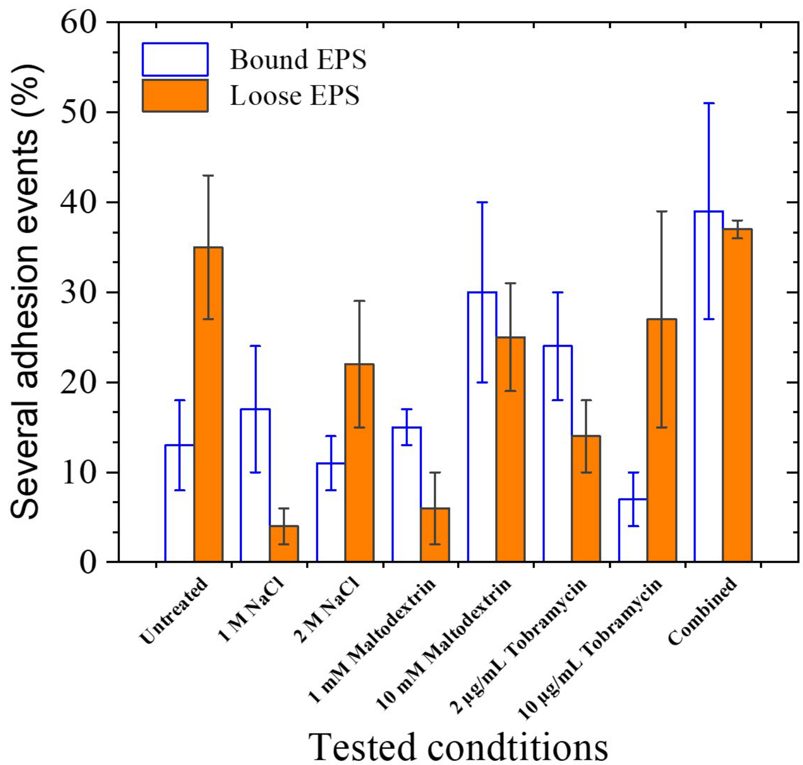

Figure S1. Percentages of curves that displayed multiple adhesion events acquired on *A. baumannii* bound (blue open bars) and loose EPS (orange full bars) in a number of tested conditions. Applied combined concentrations were 10 mM maltodextrin and 10 µg/mL tobramycin. Peak values were derived from the distribution histograms by using LogNormal probability density function. For each experiment, data were acquired from three different areas in the force-volume mode, each having 1024 pairs of force versus displacement curves. Error bars represent mean ± S.D.

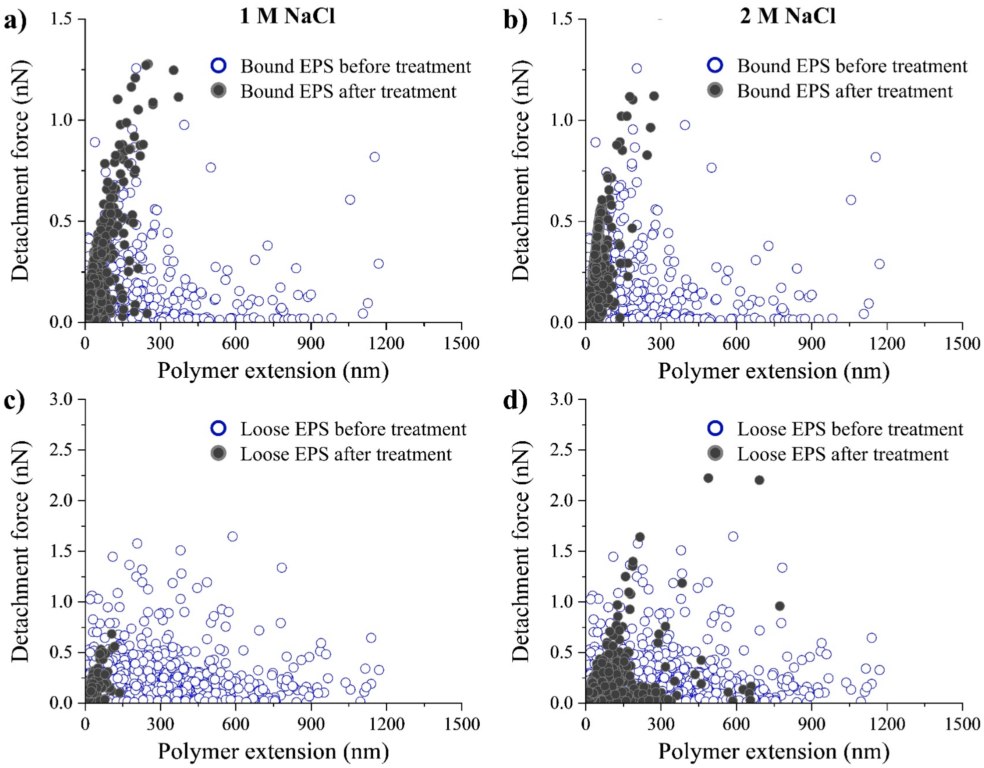

Figure S2. Detachment force versus polymer extension plots for *A. baumannii* bound and loose EPS after 1 M (*a* and *c*) and 2 M (*b* and *d*) NaCl treatments, respectively. For untreated EPS (blue open circles), the data in the plots was scatter, however with a positive trend in the 0-300 nm region. This indicates that as the length of polymers increases, higher forces are required to stretch or unravel the polymer chains. For treated EPS (grey full circles), the relative change in plots indicates that 1 M NaCl resulted in collapse of both bound and loose EPS biopolymers. In addition, the observed higher detachment forces of bound EPS compared to loose EPS suggested that bound EPS were more heterogeneous at deeper levels. 2 M NaCl did not change the overall adhesive response of bound EPS molecules compared to 1 M NaCl. This indicates that higher forces were still needed to stretch or detach shorter (<250 nm) biopolymers of bound EPS. However, it resulted in increase in the detachment forces and extensions of the loose EPS biopolymers. This suggests that 2 M NaCl led to conformational changes, such as denaturation of proteins or protonation of sugars, in loose EPS.

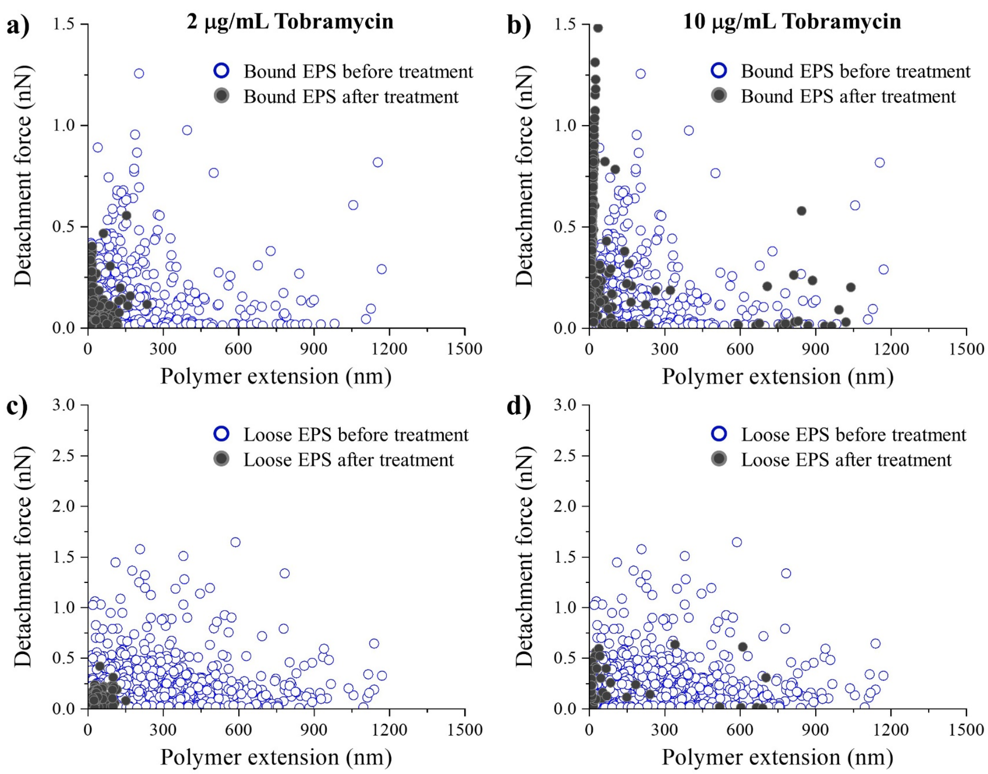

Figure S3. Detachment force versus polymer extension plots for *A. baumannii* bound and loose EPS after 2 μg/mL (*a* and *c*) and 10 μg/mL (*b* and *d*) tobramycin treatments, respectively. Compared to untreated EPS (blue open circles), the apparent effects on the reduction of the adhesion strengths and polymer extensions of treated EPS (grey full circles) were an indication of the collapse of their EPS biopolymers resulting from the changes of conformational properties of EPS.

Table S1. Mean Mass Concentrations of Bound and Loose EPS Extracted from the Biofilm Suspensions of *A. baumannii*

Type	**Protein** (μg/mL)	**Carbohydrates** (μg/mL)	**DNA** (μg/mL)
Bound EPS	536.6 ± 282.3	114.1 ± 51.0	6.4 ± 5.3
Loose EPS	352.8 ± 182.8	55.2 ± 71.4	4.7 ± 4.8

Total biofilm biomass = 237 ± 79 mg (n = 8). Data represents mean ± S.D.