## Gas vesicles as acoustic force enhancers

P-17-001

V. Jazbec<sup>I</sup>, N. Varda<sup>I</sup>, A. Kežar<sup>II</sup>, M. Podobnik<sup>II</sup>, R. Jerala<sup>I</sup>, M. Benčina<sup>I</sup>

<sup>I</sup>Department of Synthetic Biology and Immunology, National Institute of Chemistry, Ljubljana, Slovenia, <sup>II</sup>Department of Molecular Biology and Nanobiotechnology, National Institute of Chemistry, Ljubljana, Slovenia

Gas vesicles represent specialized structures found in certain bacteria and archaea, facilitating control over their buoyancy within aquatic environments. Comprising proteinaceous shells predominantly constituted of structural proteins GvpA or GvpB, these vesicles are characterized by cylindrical shapes with lengths spanning 100 to 4000 nm and diameters ranging from 45 to 200 nm, and closed ends featuring two cone-shaped caps. This configuration creates an internal volume filled with gaseous phases derived from the surrounding cytosol. The distinctive properties of gas vesicles, particularly their ability to serve as contrast agents in conjunction with MRI and ultrasound, underscore their relevance in non-invasive imaging techniques.

We delved into the binding interactions between proteins within the B. megaterium cluster and isolated gas vesicles, employing flow cytometry and cryo-electron microscopy. Our results unveiled GvpJ's binding affinity to the vesicles, even in the presence of 6M urea. In order to enable binding of GVs to mammalian cells we tagged GvpJ with a peptide tag RGD which enables interaction with integrins. As GvpJ is not present on all isolated vesicles we introduced the accessory protein GvpC from the Anabaena gas vesicle cluster to gas vesicles originating from B. megaterium. Using GVs with RGD tags we were able to confer gas vesicle binding to integrins, which are cell-specific molecules that allow us to target particular cells using specially designed RGD sequences. After binding GVs to HEK293 cells we were able to improve ultrasound susceptibility of mammalian cells and production of proteins under Ca2+ dependent transcription factors.

Our research highlights the potential applications of gas vesicles in biomedical and biotechnological fields. By understanding the binding properties and incorporating accessory proteins, we can harness the unique characteristics of gas vesicles for targeted cell manipulation and enhanced therapeutic approaches.