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Delivery of nucleic acid mimics as a novel treatment of infections – the *Helicobacter pylori* case

The resistance of bacteria to antibiotics is driving us to the "post-antibiotic era", according to the World Health Organization. Hence, alternative therapies are of utmost importance. Nucleic acid mimics (NAMs) are promising antibacterial drugs, by hybridizing to essential bacterial genes and inhibiting their expression, while being resistant to endonucleases degradation. Bacterial infections are often associated with mucus, as mucus presents the first protective barrier against the external environment, in the gastrointestinal, respiratory, reproductive or urinary tracts. Therefore, NAMs need to be able to pass through mucus to reach their target bacteria. Afterwards, they will encounter the multi-layered cell envelope of bacteria, a challenging barrier for NAMs uptake. In this work, we focused on these two major barriers for NAMs therapy, applied to the gastric *Helicobacter pylori* (*H. pylori*) infection, the most frequent and persistent bacterial infection worldwide [1]. As model NAMs, locked nucleic acids and 2'-Omethyl RNA were used to hybridize to *H. pylori* rRNA.

We found (by fluorescence recovery after photobleaching) that the NAMs diffuse fast through native mucus collected from the stomach of pigs [2]. However, binding interactions with native mucus hampered their efficient hybridization to *H. pylori* [2]. In order to protect the NAMs from mucus interactions and to overcome the challenging bacterial envelope, the NAMs were formulated into DOTAP-DOPE liposomes and post-PEGylated with DSPE-PEG (DSPE Lp). The DSPE Lp promoted intracellular delivery of the NAMs in *H. pylori*, replacing the need of prior permeabilization treatments. In the presence of mucus, DSPE Lp showed improved diffusion, compared to cationic Lp, (as measured by single particle tracking) and enhanced the NAMs hybridization.

We conclude that NAMs hold promise to be used as novel antibacterial agents, if they are protected from interactions with mucus and delivered into the bacterial cells. We have shown that DOTAP-DOPE liposomes post-PEGylated with DSPE-PEG meet these requirements for gastric *H. pylori* infection.

References

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Figures

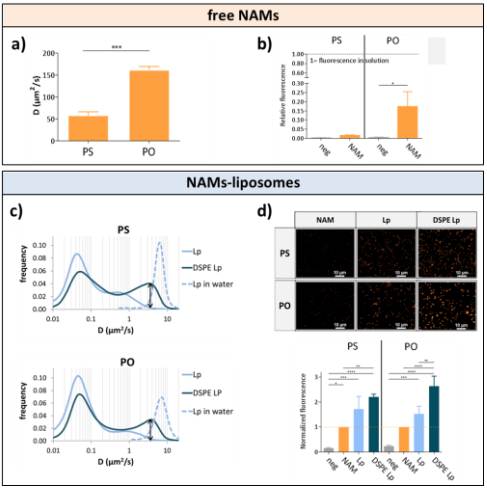

Figure 1: a) Diffusion of free NAMs in native gastric mucus; b) Hybridization fluorescence of *H. pylori* using free NAMs within native mucus, normalized to that in solution; c) Distribution of diffusion coefficients of DSPE Lp in native mucus, compared to cationic Lp (Lp) in mucus and in water; d) Hybridization fluorescence of *H. pylori* using free NAMs, Lp, or DSPE Lp within mucus – example epifluorescence images (top) and quantified fluorescence normalized to that of free NAMs within mucus (bottom).