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Title: Improvement of microbial assays in water-in-oil droplets

Over the past 20 years, many microbial assays have been demonstrated in droplet-microfluidic format. Tools made possible with microfluidic techniques opened up new possibilities in the rapid detection of infections, determination of antimicrobial resistance, single-cell bacterial population characterization, increasing the taxonomic richness by avoiding species competition or studying bacterial responses in complex consortia.

The fundamental premise of droplet microfluidics is that each droplet can be treated as an independent and isolated bio-reactor. The ease of generation of many microreactors in a short time allowed for efficient operations on many separate reactions simultaneously. This feature is especially useful when studying bacterial populations, as numerous reactors provide excellent statistics and allow the analysis on a single bacterial cell level.

One strict technical requirement for microreactors is that the droplet interfaces must present a barrier to transporting chemical ingredients between droplets. It is essential that the dyes used to detect the growth of microbes cannot migrate between droplets to allow reliable assessment of bacterial growth in a single droplet. Similarly, tested antimicrobial agents whose effect on the growth of bacteria must be remained inside the droplets with a constant concentration not to interrupt the experiment results.

Nevertheless, recent studies report molecular transport between droplets raising highly relevant questions about the compatibility of droplet-based systems for biological applications. This work presents a problem of the molecular transfer in monodispersed emulsions on the example of tracking the leakage of fluorescent metabolic dyes and non-fluorescent antibiotics.

Metabolic markers are commonly used to rapidly visualize viable bacteria in droplets. One of the most popular fluorescent compounds – resorufin, has a significant limitation related to its fast leakage from droplets, interrupting the experiment's accurate read-out. Dodecylresorufin (C12R) is a promising alternative as a marker of droplet-encapsulated bacteria as it exhibits less leakage between the droplets. The fluorescent signal from C12R is more stable over time, and the signal-to-noise ratio is higher. The C12R assay accuracy is improved because the true positive and true negative rates are higher than in the case of standard resorufin. As a result of their higher precision, C12R droplet-based assays present exciting new opportunities for high-throughput screening and study in microbiology.

Molecular transport between droplets is relatively easy to track with the help of model fluorophores. However, determination of the leakage of antimicrobial agents is hard to perform, primarily due to their weak fluorescence. The second part of this work attempt to find the chemical factors that accelerate the escape of antimicrobials from droplets. The physicochemical model proposed in this study predicts antibiotic retention in droplets according to their partition coefficient and fractional polar surface area. The model was verified by monitoring growth inhibition by antibiotic-loaded neighboring droplets. As a result of this study, a better understanding of how tiny compounds are retained in water-in-oil emulsions will help design droplet-based antibiotic assays in the future.