## Synergistic Trapping and Chaotic-Mixing: A Driving Force for Assembly of Protocell-like Vesicles in Hydrothermal Pore Networks

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Identifying and quantifying the physical machinery required to drive the assembly of protocell-like vesicles is a continuing unresolved question in the origin of life. Due to their inherently rich biochemical environments, hydrothermal vents have recently gained tremendous interest as potential hotspots for these processes. Previous studies have shown the formation of protocell-like vesicles using unlimited fatty acid precursors in regions mimicking thin cracks (diameter  $\leq$  100  $\mu$ m) in the hydrothermal vents resulting in extreme thermal gradients (100-1000 °C/mm) [1-2]. However, these physical conditions do not constitute the predominant volume fraction of pores, while fatty acids are also not representative of cell membrane components. Although counterintuitive, recent studies have shown that thermally actuated chaotic flow can accelerate transport and mixing within these microenvironments [3]. Using a combination of experiments and simulations, we rationally evaluate and quantify optimally favorable 3-D chaotic flow fields with synergistic localized trapping and chaotic mixing that drive the assembly of micron-sized phosphatidylcholine vesicles, a primary cell membrane component.

We analyzed over 10,000 micropores within several crosssectional cuts of hydrothermal vents to determine the physical size ranges of microenvironments representing the greatest pore volume fraction. An ensemble of these model pores was selected for experiments in which the growth of small precursor vesicles (0.2 µm) incubated for 24 h was evaluated under variable thermal gradients. Size distribution characterization using nanoparticle tracking analysis revealed three categories of pore geometry based on the final vesicle size distribution. Computational simulations of these pore systems indicate that the pores producing the largest vesicles uniquely embed localized trapping and recirculation via micro-vortices. We extend our computational analysis across a broad range of thermal gradients and pore size to quantitatively map conditions favoring the growth of micron-sized vesicles, thus laying the foundation to pinpoint equivalent conditions within hydrothermal vents. Our studies also lay the groundwork for evaluating other physical and chemical factors, such as vibrational forces and metal ions, respectively, that could further assist in forming protocell-like vesicles.

[1] Budin, et al. (2009) JACS 131, 9628-9629

[2] Mast, et al. (2013) PNAS 110, 8030-8035

[3] Priye, et al. (2017) PNAS 114, 1275-1280