Microbes are present in nearly every environment that humans encounter. This ubiquity means that microbes constantly pass through the human gastrointestinal (GI) tract. During transit, physical and chemical barriers such as peristaltic action, pH gradients, and intestinal enzymes protect the gut from microbial colonization. However, select bacteria can overcome these barriers by adhering to specific niches in the GI tract using surface proteins called adhesins (*1*). Adhesins locate bacteria in environments conducive to their growth, granting them a selective advantage. Therefore, both the surface properties and environment of a microbe are essential to their survival. While structural research regarding adhesins and their targets is expanding, little is known about the parameters governing microbial binding within the GI tract; describing these parameters therefore can lead to a fundamental understanding of bacterial attachment.

Previously, complex microfluidic devices that simultaneously incorporate physiological characteristics, mechanical forces, and mammalian cell co-cultures have been used to study microbes in the GI tract (*2, 3*). While these are valuable models of *in vivo* activity, their complexity obscures analysis of how individual environmental conditions and the surface characteristics of microbes affect their attachment. To address this challenge, **I have developed a modular platform to engineer the adhesive properties of live microbes and investigate their attachment under various conditions**. This platform uses adhesive ligands conjugated to the bacterial surface as a proxy for adhesins, allowing tunable ligand density and specificity on bacteria. Using this system, the influence of bacterial surface properties on attachment can be analyzed in more detail than is possible with unmodified bacteria. Furthermore, by incorporating factors (either individually or in combination) such as bile salts, pH variation, fluid flow, and enzymatic activity, the influence of environment on bacterial attachment can be determined. **I hypothesize that bacterial adherence in the GI tract is a consequence of both bacterial surface characteristics as well as environmental factors.** By using a modular platform to control the microbe surface and its environment, the proposed approach can be used to study attachment of diverse bacterial species to biotic or abiotic surfaces under varying conditions.

Aim 1: Determine how the specificity and density of adhesive ligands on microbial surfaces affect attachment. During my first year as a graduate student, I have developed a platform based on avidin and biotin binding to mediate the attachment of bacteria to targeted surfaces. Bacteria are first functionalized with biotin using *N*-hydroxysuccinimide (NHS) chemistry, which forms an ester bond between biotin and primary amines on the bacteria surface. Adhesive ligands (such as antibodies, as shown) are conjugated to streptavidin using amine-based chemistry and are then linked to biotin groups on bacterial surfaces following mixing (**Figure 1A**). To date, I have demonstrated that biotinylation of bacteria enhances binding to an abiotic streptavidin-coated surface (**Figure 1B**) and that attachment of Intracellular Adhesion Molecule (ICAM-1) antibodies enhances binding to live Caco-2 cells, a model cell line of intestinal

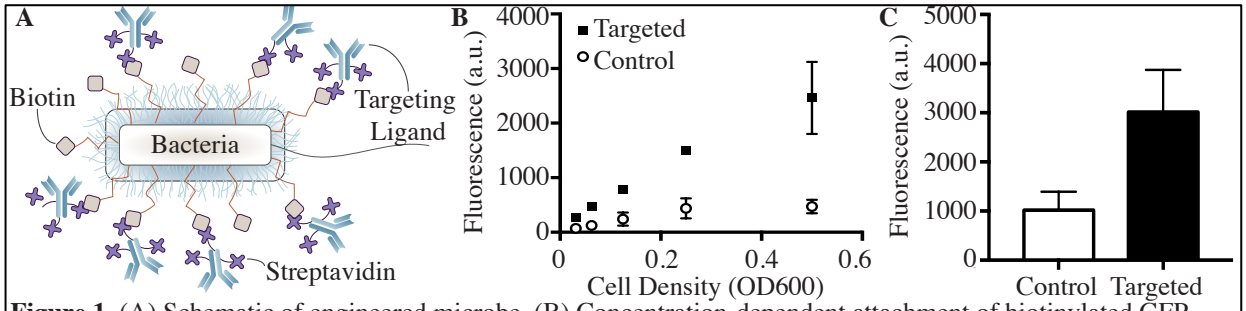

Figure 1. (A) Schematic of engineered microbe. (B) Concentration-dependent attachment of biotinylated GFP-expressing bacteria to a streptavidin-coated well plate. (C) Attachment of ICAM-1-targeted, GFP-expressing microbes to live Caco-2 cells compared to an unmodified control.

enterocytes, in comparison to a non-targeted control (**Figure 1C**). To optimize this system, I will adjust the density and specificity of antibodies on bacterial surfaces. Antibody density is controlled by the extent of bacterial-surface biotinylation and will be varied using the ratio of biotin mass to bacterial density. The density of biotin sites on the bacteria surface will be quantified following fluorescent streptavidin probe attachment using flow cytometry. Next, the effect of antibody specificity will be determined with three anti-ICAM IgG antibodies (clones R6.5, 1A6, and 1A29) with varying specificity for ICAM on Caco-2 cells. Monolayers of Caco-2 cells will be grown in tissue culture treated well plates and incubated with GFP-expressing microbes that have varying antibody density and specificity. Bacterial attachment will be quantified using the fluorescence signal from attached bacteria, measured using a plate reader and analyzed for their spatial localization using microscopy. By enabling precise control over the surface characteristics of microbes, this aim provides clear insight into how these characteristics influence microbial binding. The antibody configuration (density and specificity) that provides the highest levels of attachment in Aim 1 will be further analyzed in Aim 2.

Aim 2: Use Design of Experiments to analyze and optimize environmental parameters for microbial attachment. Design of Experiments (DOE) is a statistical approach to determine the sensitivity to and interactions between individual parameters that affect a system response (*4*). This approach will be used to screen four environmental factors for their relationship to microbial binding: bile salt concentration, pH, intestinal enzyme activity, and fluid flow rate. The factors will be tested at two levels that reflect the upper and lower limits experienced along the GI tract. Microbial attachment will be determined using a GFP-expressing strain in well plates (for static studies) or a straight-channel microfluidic chip (for fluid flow studies) that I have previously validated for targeted microbial attachment (**Figure 2**). Attachment of microbes will be quantified both through automated particle counting in ImageJ and with fluorescence intensity in the well plates or microfluidic channels. Results from the screening experiments will be used to identify which of the factors significantly influence microbial attachment. A secondary study with the identified factors will be designed to determine a response surface model and a desirability profile for microbial binding in varying environmental conditions. This will be used to estimate the conditions that optimize microbial attachment, which will be validated experimentally. By optimizing the environmental conditions, this model can determine the location of the GI tract that is most conducive for a bacteria's attachment.

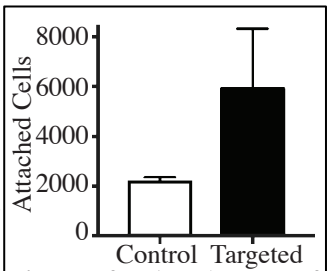

Figure 2. Attachment of biotin-conjugated bacteria to streptavidin-coated channels under flow (1μL/min)

Broader Impacts. This interdisciplinary proposal applies techniques from materials science, engineering, microbiology, and biochemistry to analyze parameters that influence microbial attachment and represents a practical, tunable system for modifying the adhesive properties of microbes. Successful completion of this project will provide valuable insight into the mechanisms of microbial colonization of the human GI tract. More broadly, due to the ubiquity of microbes in the biomedical sector, modular platforms that can be used to provide mechanistic insights into a variety of microbes on both biotic and abiotic surfaces are sorely needed. As research is most impactful when communicated directly to the public, I will teach middle school students the profound role bacteria play in our lives and our environments through Morehead Planetarium's SciMatch program. Additionally, I will broaden the reach of this research with a novel art/science collaboration designed to increase public scientific literacy in Chapel Hill.

[1] Stacy et al. *Nature reviews. Microbiology* **14**, 93-105 (2016). [2] Kim et al. *Lab on a chip* **12**, 2165-2174 (2012). [3] Bhatia and Ingber. *Nature Biotechnology* **32**, 760 (2014). [4] Anderson et al. *Productivity Press* (2007).