

How do bacteria take up DNA?

Transporting DNA molecules across cell membranes is one of the biggest challenges bacterial cells face, but surprisingly little is known about how this is accomplished. This proposal addresses the physical obstacles to DNA uptake by naturally competent bacteria: the need for a specific initiation mechanism to overcome DNA's stiffness and charge, and the difficulty of taking up DNA fragments many times longer than the cell. Our experiments will take advantage of the well-defined competence genes and strong sequence bias of *Haemophilus influenzae*, a major human pathogen. *H. influenzae* is the model system for studies of DNA uptake in the γ -proteobacteria, and the results will have broad implications for mechanisms of DNA uptake.

Competent cells first bind dsDNA at the cell surface and transport it through the secretin pore also used by type IV pili, with the uptake force thought to arise from ATP-dependent retraction of a short pseudopilus. This uptake step is followed by a translocation step that passes ssDNA into the cytoplasm. The questions I propose to answer address two critical gaps: (1) the role of sequence biases is not understood, and (2) the proteins and forces that act on DNA during uptake have not been characterized.

Q1. What are the uptake biases of *H. influenzae* and *A. pleuropneumoniae*? We will fully characterize the diverged uptake biases of these related species, using our new 'USS-seq' strategy (Illumina sequencing of pools of degenerate DNA fragments before and after uptake). The results are needed for the analyses in Q2 and will define the specific contributions of these sequences to DNA uptake.

Q2. Which proteins mediate uptake bias? Cross-species complementation experiments will test our prediction that initiation of DNA uptake utilizes sequence-specific interactions of DNA with the secretin pore and the pseudopilus tip.

Q3. How do the physical properties of USSs contribute to DNA uptake? Assays using fragments with preferred uptake sequences will reveal whether these sequences modify DNA's flexibility or intrinsic bending, and whether ethylation, nicking or supercoiling can replace them. These will test the hypothesis that sequence biases reflect physical constraints on the initiation of DNA uptake.

Q4. Is DNA uptake biased in species without genomic uptake sequences? If uptake biases indeed have mechanistic functions, they should be present in species that lack genomic uptake sequences. We will test *Gallibacterium anatis*, *Campylobacter jejuni*, *Acinetobacter baylyii* and *Thermus thermophilus*.

Q5. How is DNA pulled into and restrained in the periplasm? *H. influenzae* and its relatives lack a homolog to the classical retraction motor PilT, so our mutant collection will be screened for retraction-defective phenotypes.

ComE1 is the best candidate for a protein that restrains DNA during uptake, and the results will distinguish between two very different models of DNA transport.

Q6. What forces do cells exert on DNA during uptake? In collaboration with a biophysicist, we will use an optical-tweezers system to characterize the forces that pull DNA into cells. This will integrate the separate investigations proposed above, testing many of our model's predictions.

My research group has the resources and expertise to address these questions. The success of our initial uptake-bias study confirms the practicality of its innovative sequencing approach, used in Q1, Q2 and Q4. Q2's powerful cross-complementation assays are made possible by the diverged uptake motifs we discovered, and by our analysis of competence regulation in both species. Q3 and Q5 are straightforward molecular genetics. Q4 applies our USS-seq strategy to new species, supported by expert collaborators. The optical tweezers work in Q6 is a new undertaking, but we have preliminary work and an expert collaborator.

The multi-pronged approach proposed here includes both conventional and innovative methods and an appropriate mix of safe and risky strategies. The result will be a much more explicit understanding of a fundamental process in many bacteria.