could then be screened in pre-incubation settings (Silverman, 1995).

The results of Veith et al. (2009) can be used to predict inhibition by drug candidates, but a number of other factors are involved (Food and Drug Administration, 2006) and ultimately the true test for inhibition is an in vivo human experiment. The results can also be used to predict if compounds are substrates, and the methods for following these up are relatively straightforward. Finally, the information, if available, could be coupled with similar screens of other libraries—perhaps even if run on different platforms—to expand the results. The database used in this analysis is available in the online supplemental information.

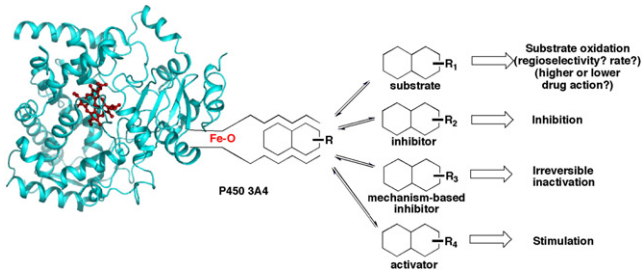

Figure 1. Outcomes of Interaction of a Ligand with a P450
The interaction of a compound with a P450 (P450 3A4 structure is shown, pdb code 1TQN) can be related to the chemical being a substrate, inhibitor, irreversible inhibitor, or stimulator—or combinations thereof. Also note that many P450 ligands can interact via multiple binding modes, leading to multiple products in the case of substrates (Guengerich, 2005).

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Targeting Multiple Biofilm Pathways

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Cegelski et al. (2009) demonstrate the importance of amyloid production for biofilm formation and host colonization using several mutant strains of pathogenic *E. coli* and small molecule inhibitors. This work reveals a path forward for studying the role of bacterial amyloids in vivo and suggests the potential for small molecules to target multiple biofilm formation pathways.

The increased prevalence of antibiotic resistant bacteria heralds a need for new drugs and novel strategies for identifying better drug targets. One such strategy is to target microbial virulence factors, which are important for causing pathology but are not required for the microbe to survive in vitro. This strategy avoids targeting essential gene functions, which may result in strong evolutionary selection for resistant strains. While this idea remains theoretical, efforts have been increased to develop new antibiotics based on this principle. One virulence process of particular interest to target is biofilm formation because of the associated antibiotic insensitivity of bacteria surviving within biofilms. Cegelski et al. (2009) have recently generated tools to allow researchers to address the relative importance of different bacterial attachment strategies during biofilm formation in vivo in a model for urinary tract infection.

Many pathogenic bacteria elaborate virulence factors in order to cause disease. Examples of factors include secretion systems to inject effector molecules into host cells, secreted toxins that manipulate host cell processes or outright kill host cells, quorum-sensing systems that

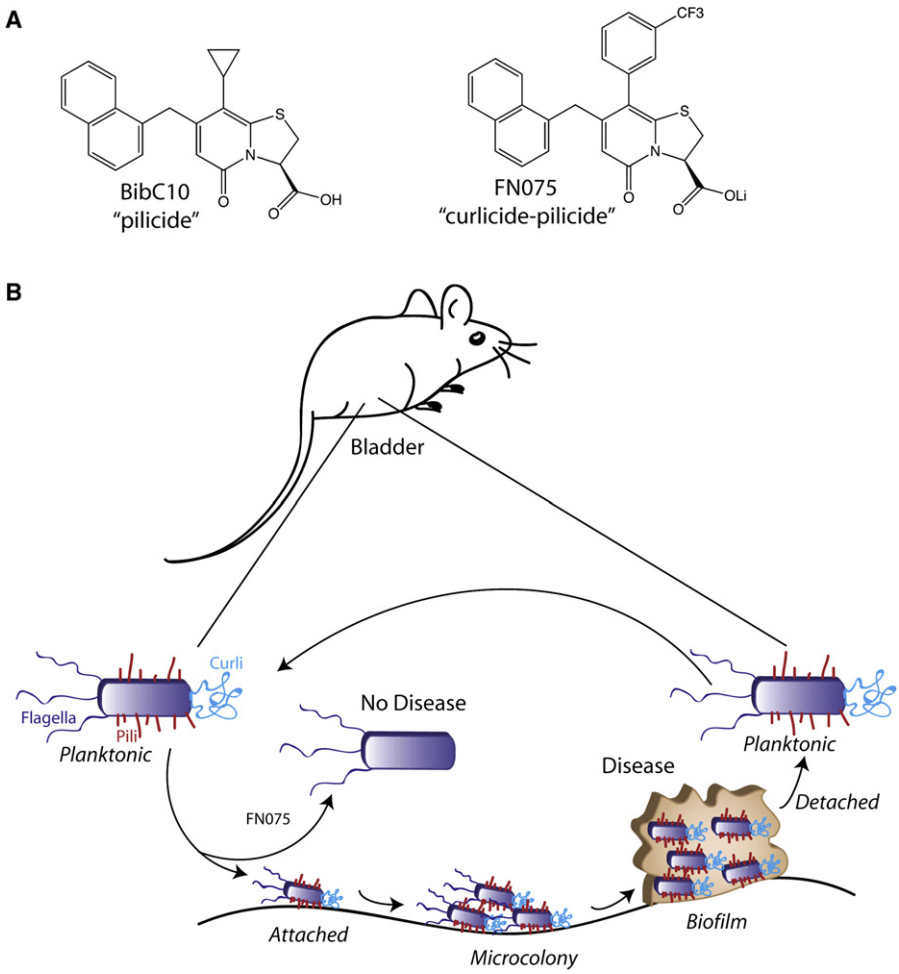

Figure 1. Inhibition of In Vivo Biofilm Formation
(A) Structures of BibC10 and FN075.
(B) The stages of a biofilm life cycle involve attachment of free-swimming planktonic cells to a surface, microcolony formation, exopolymeric material production, mature biofilm development, and the detachment of cells to start the process over as planktonic cells. FN075, used in the study, inhibits both UPEC pili and curli production, preventing bacterial attachment and resulting in attenuation in an in vivo mouse bladder infection model.

determine bacterial density and subsequently coordinate behavior, and biofilm formation. Biofilms are complex, three-dimensional bacterial communities embedded in exopolymeric material that is made up of polysaccharides, proteins, and nucleic acids. Bacteria within these communities are relatively insensitive to antibiotics and host-immune responses, allowing the development of persistent infections and significant morbidity (Costerton et al., 1999). Several of the mechanisms resulting in antibiotic tolerance in biofilms include slower bacterial growth, alterations in antibiotic targets, expression of drug pumps and antibiotic-degrading enzymes, and development of persister cells, a population of cells that neither grows nor dies when exposed to antibiotics. Biofilms complicate many clinical infections, including endocarditis, periodontal diseases, ear infections, pneumonia, and urinary tract infection (UTI). They also form on implanted devices such as catheters, artificial joints, stents, and heart valves, which provide a surface for bacteria to attach and biofilms to develop within the host (Parsek and Singh, 2003). Thus, there is intense interest in developing therapeutic agents to disrupt and prevent biofilm development.

Biofilm formation is thought to occur in response to environmental conditions through a series of reversible stages (Watnick and Kolter, 2000), depicted in Figure 1. First, free-swimming planktonic bacteria encounter a surface and form loose attachments. Next, the bacteria migrate across the surface to congregate into a microcolony, where they lose motility and often communicate through quorum-sensing systems. Then, the colony produces exopolymeric material, resulting in a complex, three-dimensional matrix in which the bacteria are antibiotic tolerant (O'Toole et al., 2000). Finally, when the environmental conditions change, some of the bacteria can become motile, detach, and disperse to more favorable locations.

Because the attachment of bacteria to a surface is an early and critical step in biofilm formation, it is an attractive target for inhibition by small molecules. Uropathogenic *E. coli* (UPEC) assembles several different structures on its cell surface to enhance adherence to different types of

surfaces. One of these structures, known as curli, is composed of extracellular amyloid fibers. These fibers are the major proteinaceous component of a complex extracellular matrix and play a role in bacterial cell aggregation, host-cell adhesion, adherence to surfaces of medical and food-handling devices, and biofilm formation. Curli are long protein fibers that bind to many host proteins, including fibronectin, fibrinogin, and laminin, and do not provide motility to the organism. In contrast, another structure that plays a role in bacterial surface adhesion is type-1 pili, which are shorter rod-like structures that provide motility through their contractile nature. Type-1 pili can bind specifically to mannosylated host receptors that are often found on epithelial cells in the bladder. Despite their structural and functional differences, curli and pili appear to be able to play similar and/or complementary roles in promoting surface attachment and subsequent biofilm formation.

Cegelski et al. (2009) have recently reported efforts to develop small molecule inhibitors of both curli and pili production as an approach to inhibiting biofilm formation. Previously, they had reported a series of inhibitors of pili formation that were rationally designed based on the protein crystal structure of the chaperone PapD (Pinkner et al., 2006). One of these "pilicides," BipC10, selectively disrupts the chaperon-usher interaction essential for pili formation in UPEC (Pinkner et al., 2006). Cegelski et al. (2009) expanded upon these initial findings by synthesizing structural analogs of BipC10, including FN075 (Figure 1A). Interestingly, slight chemical modifications of BipC10 resulted in a gain of "curlicide" activity in FN075 without loss in pilicide function. The authors show the curlicide activity of FN075 by demonstrating reduced polymerization of the main curli subunit (CsgA) and the loss of curli structures typically visible on UPEC by electron microscopy in the presence of the compound. FN075 also inhibited formation of pellicles, which are biofilms formed at the air-liquid interface. None of the compounds affected bacterial growth in vitro, supporting the conclusion that curli and pili are not essential in vitro.

Using *fim* and *csgA* mutants defective in either pili or curli production, respectively, the authors found that UPEC grown in vitro in Luria broth required pili for biofilm formation on polyvinyl chloride plastic, in contrast to UPEC grown in YESCA broth (yeast extract and casamino acids), which required curli formation for biofilm formation. These results illustrate that different mechanisms of attachment are likely required for biofilm formation under different environmental growth conditions. This observation highlights one of the major challenges of studying pathogenesis: the ability to recreate the relevant in vivo environmental conditions under in vitro conditions, and the importance of understanding the in vivo microenvironment. Unfortunately, there is currently insufficient knowledge regarding the microenvironments important for most infections, making it difficult to assign conditions for in vitro studies. Combining this uncertainty with the fact that UPEC can use either curli or pili to promote surface attachment and subsequent biofilm formation depending on the conditions, it becomes important, from a therapeutic standpoint, to inhibit both mechanisms.

The authors show that FN075 is able to inhibit biofilm formation under both growth conditions. They then test FN075 in a mouse UTI model to demonstrate that targeting biofilm production with a chemical inhibitor could be an effective route for reducing bacterial loads in the bladder. The authors test *E. coli* grown in pili-inducing conditions in the presence of FN075 prior to challenge in a mouse UTI bladder model. These pretreated *E. coli* have decreased fitness similar to the *csgA* mutant, as demonstrated by modest reductions in both colony-forming units and intracellular bacterial communities. While this experiment potentially addresses the role of pili inhibition in decreasing infection, it would have been interesting to perform the converse experiment, where *E. coli* are pretreated in curli-inducing conditions with FN075 prior to challenge in a mouse UTI model to test the importance of curli for in vivo virulence. Further, the test ultimately will be whether infected mice can be treated directly with the inhibitor to protect from infection, rather than pretreating the bacteria, as performed here.

There remains work to be done to confirm the role of curli in pathogenesis separate from the role of pili. Cegelski et al. (2009) provide mutant UPEC strains as well as pilicide-only and curlicide-pilicide compounds that will open up research on the role of amyloid production in UPEC attachment in UTI. Aside from their potential as therapeutics, it is important to recognize the value in having these types of chemical tools that can be used for probing the in vivo biology of biofilm formation required for virulence under different in vivo conditions. Researchers can now combine pili or curli mutants with pilicides or curlicide-pilicides in different mouse models to investigate the relevance of each component and potentially use them to define the microenvironments within the host.

This article represents a proof of principle for the concept of using small molecules that target multiple biofilm formation pathways. It will be interesting to see if these compounds limit the virulence of other bacteria that similarly use curli and/or pili for biofilm formation, such as *Salmonella*, to determine how broad spectrum such compounds or approaches may be. Finally, while this work is a step toward understanding the methods for targeting the formation of biofilms, it may ultimately be important, in order to have significant clinical impact, to identify methods that not only inhibit their formation, but are also capable of disassembling pre-existing biofilms (Boles and Horswill, 2008; Junker and Clardy, 2007). Efforts toward this end will be needed in order to ultimately obtain the therapeutics useful for treating infections where biofilms play a major role in the pathology.

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