in which the androgen-tethered DNA adduct retained high affinity for the AR, then selectivity toxicity by an "alkylate-then-bind" mechanism becomes more likely.

Although additional mechanistic questions remain, the findings presented are both intriguing and promising. Considering the current limitations in the effectiveness of both hormonal and cytotoxic chemotherapy of prostate cancer, the results also represent a solid step toward fulfilling the promise of hybrid toxins as more selective cancer chemotherapeutic agents. Of equal importance, this study nicely illustrates the careful thought that needs to be given to the design of hybrid toxins and the careful controls that are required to interpret mechanistically their activities in cell and animal model systems. This work will serve both as a standard and an inspiration for future studies in this area.

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Bacterial Crowd Control with Iron

Bacterial biofilms account for more than 80% of human infections. Hergenrother and coworkers report in this issue that high concentrations of iron salts can block the formation of these bacterial communities [1]; this represents an attractive new method for biofilm control.

Bacteria are not always solitary little beasts—rather, they have the ability to organize and work as a group to benefit their cause. In order to decide when to make this lifestyle switch, bacteria continually assess their population densities by using a process called quorum sensing. When a sufficient number of bacteria (a "quorum") are present, they alter gene expression to carry out processes that necessitate the cooperation of a large number of cells [2, 3]. Virulence-factor production and biofilm formation in certain pathogens, for example, are under the direct control of quorum sensing. Therefore, population density plays a critical role in pathogenesis [4]. This makes sense; the chances of a successful attack on a host are far higher with a "mob" of bacteria as opposed to a small, polite gathering.

New approaches are required to treat bacterial infections, and quorum sensing has become an attractive target [5]. Biofilms are of particular interest because these bacterial communities are involved in more than 80% of all human infections [6]. For example, chronic biofilm lung infections by the Gram-negative pathogen *Pseudomonas aeruginosa* are the primary cause of morbidity in cystic fibrosis (CF) [4, 7]. The development of small molecules that inhibit quorum sensing and biofilm formation has received much recent attention [8, 9]. In this issue of *Chemistry & Biology*, the Hergenrother lab marks an advance in this field through the discovery that high doses of simple iron salts can block biofilm formation in *P. aeruginosa* [1]. Not only does iron block biofilm formation, but the metal ion can also disrupt preexisting biofilms. This is a significant result because biofilms are notoriously resistant to both immune-system attack and antimicrobial agents [10, 11]. The main findings of this report are highlighted here.

At the outset of their work, Musk et al. screened an approximately 4000-member small-molecule library for compounds that inhibited *P. aeruginosa* biofilm formation. They utilized a novel 384-well-plate static-biofilm assay to expedite the screening process and uncovered several hits. Interestingly, the salt ferric ammonium citrate (FAC) was found to be the most active and exhibited dose-dependent inhibition of *P. aeruginosa* biofilm formation ($IC_{50} \approx 60\ \mu M$). The counter ions, ammonium citrate along with others, were found to be inactive, indicating that iron itself [most likely Fe(III)] was the active inhibitor. This was a surprising result for two reasons: (1) FAC was found in a library composed almost entirely of drug-like organic compounds, and (2) this result contrasts directly with previous data indicating that very *low* iron concentrations (i.e., iron starvation) can inhibit biofilm formation [12]. This discovery underscores the value of screening-based approaches

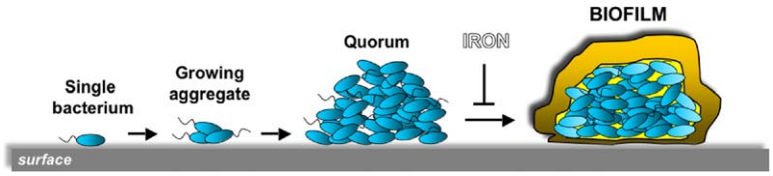

Figure 1. Schematic of the Bacterial-Biofilm Formation Process and Its Inhibition by High Concentrations of Iron

The biofilm is depicted as a cut-away image.

for the discovery of new biofilm inhibitors [13] and requires more critical examination. Importantly, high levels of FAC were not found to affect bacterial growth.

A briefing on biofilms is useful here. Biofilms are defined as sessile communities of bacteria encased in a self-generated exopolysaccharide matrix [10]—formation of this slimy coat is under the control of quorum sensing (Figure 1). These communities commonly form on solid surfaces exposed to a continuous flow of nutrients. For example, biofilms persistently colonize in-dwelling medical devices, such as catheters, stents, and ventilators. They are also a primary cause of biofouling in industrial and marine settings [14]. The compact nature of biofilms, the reduced metabolic rates of their outer strata, and the physical protection conferred by their polymeric coats render these colonies resistant to most antibiotics and, therefore, difficult to eradicate [10].

With this latter challenge in mind, Musk et al. astutely probed the antibiotic resistance of 2-day-old *P. aeruginosa* biofilms in the presence of FAC and found that the sensitivity of the biofilm to the antibiotic tobramycin increased with increasing FAC concentration. Next, the authors introduced FAC into flow-chamber devices containing 5-day-old *P. aeruginosa* biofilms and observed the complete clearance of the biofilms after an additional 5 days. This is a major finding because it means iron could be used to treat previously formed biofilms, not just prevent their formation. Indeed, few chemical treatments to mitigate biofilm formation, at any stage of growth, are known [15].

Musk et al. then used multiple techniques, including fluorescence microscopy, assays of twitching motility, and detailed time-course experiments, to collect substantial additional data that supported FAC's ability to inhibit biofilm formation. To investigate the effects of FAC on different and clinically relevant *P. aeruginosa* strains, the authors used FAC to treat clinical isolates of *P. aeruginosa* from CF patients. They found that FAC strongly inhibited biofilm formation in the majority of these strains. In view of the prominent role of *P. aeruginosa* infections in the progression of CF (see above), this last set of data is exceptional. Of note, the high FAC concentration (ca. 250 μM) required for inhibition in these experiments does raise some concerns for clinical usage. Additional studies of the effects of pH and counter ions on iron (III) solubility and activity could be beneficial in this arena.

The next challenge is to determine just how iron is inhibiting biofilm formation. The author's data, along with those of others [12], have revealed that biofilm formation in *P. aeruginosa* can occur over a range of iron concentrations (ca. 1–100 μM), above and below which the organism can exist in only a planktonic state. A minimum level of iron is required simply for bacterial growth, however. Transcriptional profiling studies have shown that low and high iron concentrations induce or repress a number of genes involved in iron acquisition, respectively [16, 17]. The bulk of these genes are involved in the biosynthesis of two siderophores (metal-ion chelators), pyochelin and pyoverdin, that are implicated in the scavenging of iron. Intriguingly, pyoverdin also signals for the production of virulence factors that are critical for pathogenesis. In addition, low levels of iron have been connected to enhanced *P. aeruginosa* virulence in CF [18]. And, to bring this story full circle, *high levels* of iron have been shown to repress genes involved in quorum sensing in *P. aeruginosa* [16].

In light of these data, the authors thus speculate that iron-replete conditions could be suppressing quorum sensing in *P. aeruginosa* and that this suppression in turn inhibits the production and/or upkeep of bacterial biofilms (Figure 1). Clearly, further studies are required to elucidate the complex roles of iron in biofilm formation and pathogenesis. In the short term, however, it would be interesting to examine the effects of iron on other Gram-negative bacteria prone to biofilm formation because quorum sensing circuits in these species are highly homologous [3].

The work of Hergenrother and coworkers provides an effective new method to eradicate bacterial biofilms. It also shows, in combination with other recent work [8, 13, 19], that chemists are poised to play an important role in this research area. The ease with which iron can be applied to and released from different surfaces suggests many immediate clinical and environmental applications for this research. As for the bacteria dwelling in biofilms, however, these research findings surely will be no crowd pleaser.

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