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Fluorescence high-throughput screening for inhibitors of TonB action

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Gram (-) bacteria acquire ferric siderophores through TonB-dependent transporters (TBDT) in their outer membrane. By fluorescence spectroscopic high-throughput screening (FLHTS) we identified inhibitors of TonB-dependent ferric enterobactin (FeEnt) uptake through *E. coli* FepA (EcoFepA). Among 165 inhibitors found in a primary screen of 17,441 compounds, we evaluated 20 candidates in secondary tests of TonB activity, including ferric siderophore uptake and colicin killing. 6 of the 20 primary hits inhibited TonB action in all the tests. Further analysis of the inhibitors in [^{59}Fe] Ent and [^{14}C]-lactose accumulation experiments suggested several as proton ionophores, but two chemicals, ebselen and ST0082990, did not behave like proton ionophores and may inhibit TonB-ExbBD. The success of FLHTS against *E. coli* led us to adapt it to the ESKAPE pathogen *Acinetobacter baumannii*. We identified its FepA ortholog (*AbaFepA*), confirmed its involvement in FeEnt uptake by deleting the structural gene, cloned *AbafepA*, genetically engineered 8 Cys substitutions in its surface loops, modified them with fluorescein and made fluorescence spectroscopic observations of FeEnt uptake in *A. baumannii*. Among the Cys substitutions in *AbaFepA*, several (S279C, T562C, S665C) were well labeled by fluorescein and suitable for measurements of FeEnt transport. As in *E. coli*, the test monitored TonB-dependent FeEnt uptake by *AbaFepA*. In micro titer format FLHTS with *A. baumannii* produced Z' factors from 0.6-0.8. Overall these experiments both identified agents that block TonB action, and revealed the potential of FLHTS for larger screens of bigger libraries to find novel antimicrobial compounds against Gram (-) bacteria, including the CRE/ESKAPE pathogens.

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