Quantification of antibiotic uptake through outer-membrane protein

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Multidrug resistant bacteria or the so-called superbugs are one of the most challenging problems faced by modern medicine. Decreased drug accumulation due to modulation of outer membrane proteins, specifically porins of Gram-negative bacteria, is a common cause of antibiotic resistance. Notably, there are not many label free techniques available which are capable of quantifying drug uptake through membrane proteins. Here, we present a semi-quantitative fluorescence based assay (using UV excitation) for studying the uptake of auto-fluorescent drug molecules in single giant protein reconstituted liposome in a microfluidic chip. Patching the proteo-liposomes in patch clamp and measuring ion current through membrane patches reveals an estimate of porin density for each liposome. Combining the two measurements enables the estimation of antibiotic translocation rate through single protein in the proteo-liposomes. We also performed single channel electrophysiology measurements that revealed an electric field driven uptake of antibiotic through porins in addition to concentration driven diffusion. Using this single molecule biophysical technique, we measured the translocation rate of a fluoroquinolone, norfloxacin through outer membrane protein F (OmpF) from *Escherichia coli*. Measurements were performed at pH 5 and pH 7, corresponding to two different charge states of norfloxacin that bacteria are likely to encounter in the human gastrointestinal tract. Based on our results, we demonstrate a physical mechanism for the pH mediated change in bacterial susceptibility to fluoroquinolone antibiotics. Moreover, this in vitro assay is transferable to different channel proteins like transporters or ion channels and analytes which exhibits auto-fluorescence.

References

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