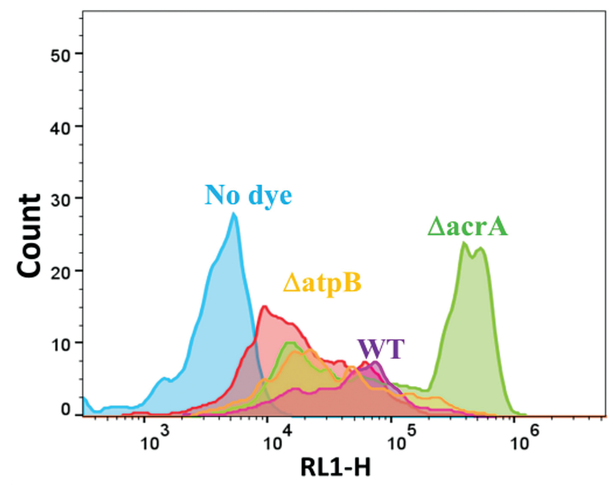
Involvement of multiple influx and efflux transporters in the accumulation of fluorescent dyes by E.coli – a high-throughput analysis using flow cytometry and PAA Automation

Srijan Jindal[1], Farah Mughal[1,3] , Lei Yang[2], Philip J. Day[1], Douglas B. Kell[1,2,3]
[1]The University of Manchester, [2]Novo Nordisk Centre for Biosustainability, Denmark, [3]University of Liverpool, UK

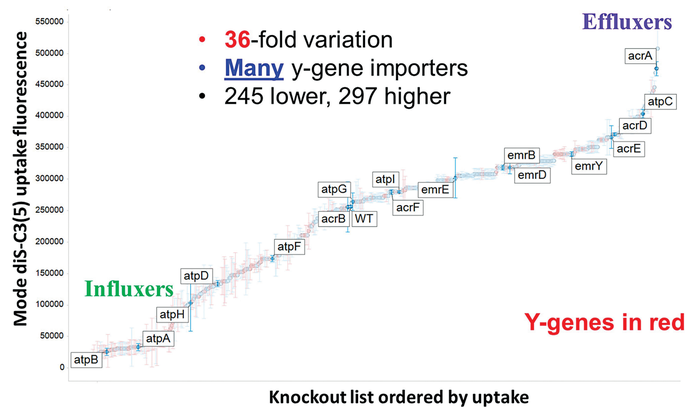
1 High-throughput flow cytometry and the PAA S-LAB™ automated plate handler was used to assess the ability of individual gene knockout strains and the wild type of E. coli to take up the membrane-permeable, cationic fluorescent dye, diS-C3(5).

Cytograms of the wild-type E. coli strain (WT) stained with diS-C3(5), along with other single-gene knockout strains. Those deleted in atpB or acrA are labelled with the relevant colours.



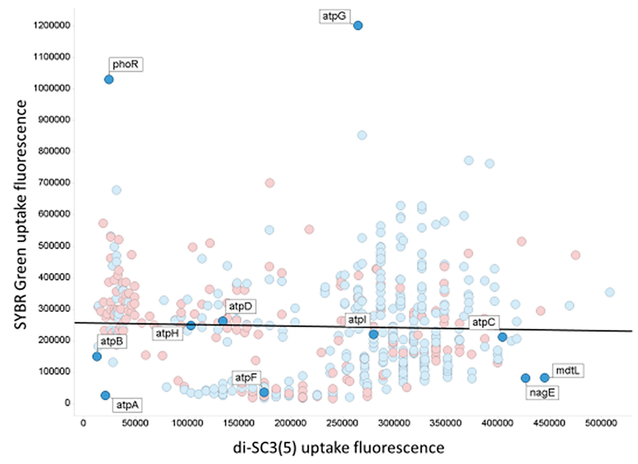
2 The range of modal steady-state uptakes for the diS-C3(5) between the different strains was 36-fold. Knockouts of the ATP synthase α and β subunits greatly inhibited uptake, implying that most uptake was ATP-driven rather than being driven by (say) a membrane potential.

Variation in the modal uptake of diS-C3(5) into different E. coli single-gene knockout strains of the Keio collection as measured by flow cytometry. Bilayer transport is negligible.



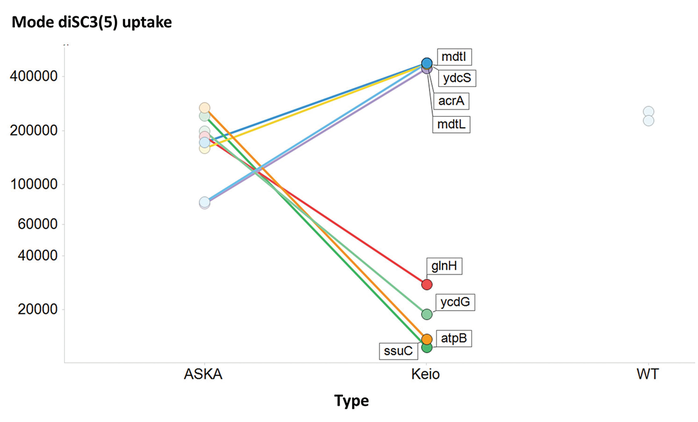
3 Similar findings were made with the cationic DNA dye SYBR Green (range 69-fold), with negligible correlation between the two (despite a shared benzimidazole motif).

Ranked order of median SYBR Green uptake into E. coli knockout strains, plotted vs that of di-S-C3(5). There is negligible correlation.



4 Several overexpression variants in the 'ASKA' collection had the anticipated, opposite effects compared to the wild type. We conclude that the uptake of these dyes may be catalysed by a great many transporters of possibly broad and presently unknown specificity. This casts serious doubt upon the use of such dyes as quantitative stains for representing either bioenergetics parameters or the amount of cellular DNA in unfixed cells (in vivo).

Effect of overexpression of a series of genes whose knockout show major changes in the modal uptake of diSC3(5) relative to that of the wild type.



1. Davey HM, Kell DB: Flow cytometry and cell sorting of heterogeneous microbial populations: Microbiol Rev 1996; 60:641-696.
2. Dobson PD, Kell DB: Carrier-mediated cellular uptake of pharmaceutical drugs: an exception or the rule? Nat Rev Drug Disc 2008; 7:205-220.
3. Iyer R, Ferrari A, Rijnbrand R, Erwin AL: A fluorescent microplate assay quantifies bacterial efflux and demonstrates two distinct compound binding sites in AcrB. Antimicrob Agents Chemother 2015; 59:2388-2397.
4. Kell DB, Oliver SG: How drugs get into cells: tested and testable predictions to help discriminate between transporter-mediated uptake and lipoidal bilayer diffusion. Front Pharmacol 2014; 5:231.
5. Jindal S, Thampy H, Day PJ, Kell DB: Very rapid flow cytometric assessment of antimicrobial susceptibility during the apparent lag phase of bacterial (re)growth. Microbiology 2019; 165, 439-454.
6. Jindal S, Yang L, Day PJ, Kell DB: Involvement of multiple influx and efflux transporters in the accumulation of cationic fluorescent dyes by Escherichia coli. BMC Microbiol 2019; 19, 195.