The current increased use of antifungal agents has resulted in the development of resistance to these drugs. Search for new antifungals with different mechanisms of action overcoming the multidrug resistance is thus underway. Surface-active antifungals have the advantages of minimizing host toxicity and the emergence of drug resistance.

We have developed a fluorescence method based on the use of the potentiometric fluorescent probe diS-C_3(3), substrate of two major *S. cerevisiae* MDR pumps, Pdr5p and Snq2p. It allows us to monitor with high sensitivity and in real time changes in the activities of both pumps and also in membrane potential. We present here an efficient strategy for identifying pump inhibitors with minimal side effects on membrane integrity, and compare the potencies of different inhibitors towards MDR pumps. New efficient inhibitors of MDR pumps could potentially be used in conjunction with current antimicrobials that are MDR pump substrates. The method can be also used to determine the mechanism of action of surface-active drugs and their lowest effective concentrations.