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An integrated approach to target deconvolution, target engagement and compound characterization in live cells

The benefits posed by phenotypic screening are often encumbered by difficulties in identifying the underlying cellular targets. This requires using tethered derivatives of the phenotypically active compounds to selectively isolate targets from the highly complex intracellular matrix. We have developed a novel chloroquine capture moiety designed to minimally affect compound potency and permeability in cultured cells, thus allowing binding interactions with the targets to occur under conditions relevant to the desired cellular phenotype. Maintenance of potency ensures that the modified compound retains the capacity to engage with relevant pharmacological targets prior to capture and analysis by mass spectrometry. Binding of compounds to the putative targets identified by mass spectrometry, can then be further verified by resonance energy transfer. This is achieved by generating genetic fusions of putative targets with a small luciferase (NanoLuc, 19 KD), and by replacing the chloroquine moiety on the compound with the fluorophore. Direct binding of compounds to the putative targets is revealed through proximity based transfer of energy from the NanoLuc donor to the fluorophore receptor. The ability to quantify compound binding by energy transfer also permits characterization of target engagement within living cells. This can allow precise correlation of target engagement with phenotypic activity, and kinetic analysis of intracellular residence time. The assays are readily adaptable to laboratory automation, allowing application of these principles for high throughput screens.

Biography

Marjeta received her Ph.D. from the Department of Genetics, University of Wisconsin-Madison and completed her postdoctoral studies at the at Genetique Microbienne, INRA, Paris, France. She is a Director of Research at Promega Corporation, a global leader in providing innovative solutions to life scientists in academic, industrial and government settings. Prior to joining Promega, she worked in the Department of Anti-Infectives, Research and Development at GlaxoSmithKline. She has published more than 25 papers in reputed journals and has been serving on the Board of Visitors for the College of Agriculture and Life Science (CALS) at the UW-Madison, she is now a member of Scientific Working Group at the CSGID/ SSGCID and a Member of Editorial Board of the Molecular Biology Journal-Open-access.

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