

Chemical Biology Tools for Measuring Cytosolic Penetration

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Large-molecule therapeutics including peptides, oligonucleotides, and proteins make up a large and growing portion of the drug development pipeline. One of the greatest barriers to developing these drugs is cell penetration. Most large-molecule therapeutics enter the cell through a complex pathway involving endocytosis followed by endosomal escape. This process is so poorly understood and so difficult to study, it is challenging simply to measure how much compound has accessed the cytosol at any given point. The Kritzer Lab has developed new tools for making these and related measurements. The Chloroalkane Penetration Assay (CAPA) is a versatile assay that measures cell penetration using cellularly expressed HaloTag protein and a small chloroalkane tag on the molecule-of-interest. CAPA has been used by the Kritzer group to measure cell penetration for many classes of peptide and oligonucleotide therapeutics, to measure penetration to different subcellular compartments, and to measure relative penetration in different cell types. CAPA has also been adopted by academic and industrial groups all over the world to investigate cell penetration. In recent work, the Kritzer group has used molecular evolution to produce new HaloTag variants which work optimally with a fluorogenic benzothiadiazole dye. The resulting “BenzoTag” system allows for turn-on, no-wash cell labeling in seconds. BenzoTag is the basis of a new, a “turn-on” version of CAPA for even more precise measurements and mechanistic investigations of drug delivery and mechanisms of endosomal escape.