Assessing the cellular permeability of peptidic macrocycles in high-throughput

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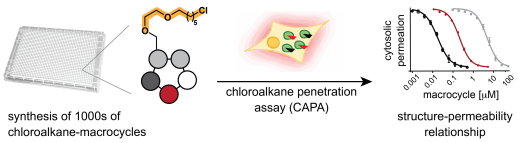
Small cyclic peptides provide an attractive modality for drug development due to their ability to bind challenging targets and their potential to cross membranes for reaching intracellular proteins. In our laboratory, we have recently developed methods to synthesize and screen large combinatorial libraries of small cyclic peptides.[1-3] For example, "*m*" short linear peptides containing thiol groups at both ends were combinatorially cyclized with "*n*" bis-electrophilic linker reagents to obtain *m* × *n* cyclic peptides that were screened in microwell plates as crude products. While the approaches yielded ligands to several disease targets, not all of them were membrane permeable. A full picture of the membrane permeability of the newly developed format of peptidic macrocycles, and factors that determine their permeability, was lacking.

In this work in progress, we have taken advantage of the chloroalkane penetration assay (CAPA), that has recently emerged as a robust method to determine cytosolic permeability of chloroalkane-tagged biomolecules.[4] We have established a method to synthesize thousands of diverse chloroalkane-tagged peptidic macrocycles to determine their cytosolic permeability using CAPA. This has given us a new insight into the structure-permeability relationships of an unprecedented number of macrocycles and provides a clearer picture of what features govern permeability of macrocyclic compounds in cellular systems.



[1] Kale, S.S. *et al. Sci. Adv.* **2019**, *5,* eaaw2851.

[2] Habeshian, S. *et al. Nat. Commun*. **2022**, *13,* 3823.

[3] Bognar, Z. *et al. Org. Biomol. Chem.* **2022**, *20,* 5699–5703.

[4] Peraro, L. *et al. J. Am. Chem. Soc.* **2018**, *140,* 11360–11369.