ChameLogK: A Rapid, High-Throughput Assay to Assess Bioavailability of Beyond Rule of Five Compounds

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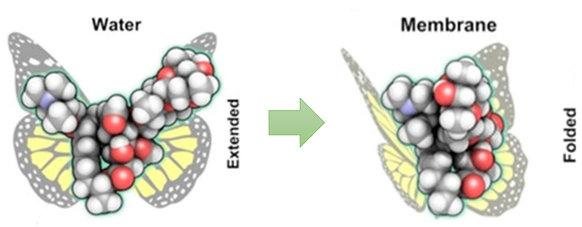
Abstract

ChameLogK is a cutting-edge method for measuring chameleonicity, a vital factor in assessing cell permeability propensity of small molecules, including 'Beyond Rule-of-Five' such as PROTACs, macrocycles and peptides. Chameleonicity is ability of molecules to conformationally adapt to their environment, driven by intramolecular hydrogen bonds; ChameLogK combines lipophilicity (BRlogD) and polarity (Δlog k_w^{IAM}) descriptors. Leveraging literature methods, o2h has developed a reliable, high-throughput platform to rapidly and inexpensively measure chameleonicity, accelerating optimization of Beyond Rule-of-Five therapeutic compounds

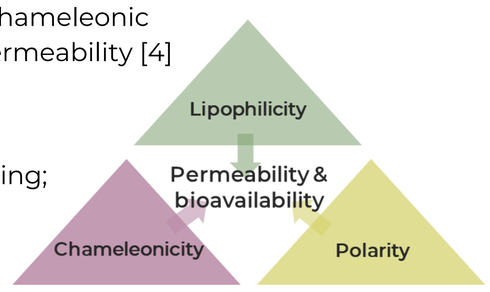
Chameleonicity: An indicator of bioavailability and cell permeability for Beyond Rule of Five molecules

- Chameleonicity is the capacity of a molecule to hide polarity in non-polar environments and expose it in water, help achieving sufficient permeability and solubility for drug molecules with high molecular weight [1]



Adapted from Danielus *et al*, Nature 2020 [2]

- For many years, it has been known that bRo5 molecules, such as macrocycles and small peptides, can exhibit chameleonic properties – conformational flexibility - driven by the formation of intramolecular hydrogen bonds [3]
- bRo5 compounds often suffer from a poor permeability and bioavailability profile. The design and optimization of compounds with chameleonic properties is central to optimizing solubility and permeability [4]
- BRlogD, Δlog k_w^{IAM} and ChameLogK are three complementary measures revealing lipophilicity, solubility, exposed polarity, and chameleonic masking; together forming a triad of drug-likeness, guiding smarter compound prioritization by explaining permeability and bioavailability [5]



- Other established phys chem measures relating to permeability, such as ePSA [6][1] are comparatively low throughput and costly, revealing a need for an easily-accessible alternative measure to support rapid design and optimization of bRo5 compounds
- The ChameLogK method was pioneered in by Giulia Caron's group in Turin [5] and has garnered significant interest in the academic setting, but had not been developed into a fully-transferable platform for routine screening in the commercial setting
- **o2h discovery is the first in the world to offer the ChameLogK assay to clients to support their drug discovery programs**

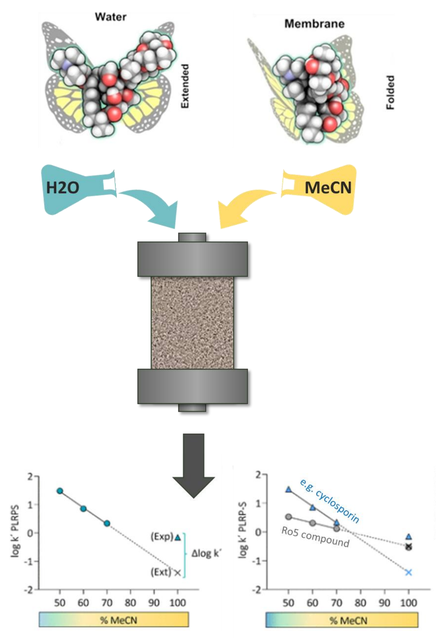
Measuring ChameLogK: How it works

ChameLogK is the difference (Δlog k') between the experimental retention at 100% MeCN on a PLRP-S column, and the extrapolated retention at 100% MeCN obtained from the linear region measured at 50/60/70% MeCN. Positive values indicate extra retention in the non-polar limit, consistent with polarity masking/folding (i.e. chameleonicity).

Method features:

- HPLC-based method, using PLRP-S column
- Record the retention time (RT) of compound in differing ratios of water:acetonitrile
- Linear regression to fit of Log k' PLRP-S and the % acetonitrile; Chameleonic properties are observed as a steeper downward slope with an up-tick
- Extrapolate to calculate at 100% acetonitrile and calculate Chamelogk using the formula:

  Chamelogk = Exp. log k' 100 - Ext. log k' 100



Graphics adapted from [2] and [5]

References

[1] David *et al* (2021) 'Prediction of Chameleonic Efficiency', ChemMedChem 16(7), pp2669-2685

[2] Danielas et al, (2020) 'Solution Conformations Explain the Chameleonic Behaviour of Macrocyclic Drugs', *Nature* 26(23), pp5231-5244

[3] Bockus and Lokey, (2017) 'Chapter 5: Bioactive and Membrane-Permeable Cyclic Peptide Natural Products', *Practical Medicinal Chemistry with Macrocycles: Design, Synthesis, and Case Studies*, John Wiley and Sons, Inc.

[4] Ramelot *et al*, (2023), 'Cell-permeable chameleonic peptides: Exploiting conformational dynamics in de novo cyclic peptide design, Current Opinion in Structural Biology 80, p102603

[5] Garcia Jimenez et al (2023), 'Chamelogk: A Chromatographic Chameleonicity Quantifier to Design Orally Bioavailable Beyond-Rule-of-5 Drugs', Journal of Medicinal Chemistry 66(15), p10681. **Seminal article by Giulia Caron's group**

[6] Goetz *et al* (2014), 'EPSA: A Novel Supercritical Fluid Chromatography Technique Enabling the Design of Permeable Cyclic Peptides', ACS Medicinal Chemistry Letters, 5(10).

[7] Apprato et al (2024), 'Exploring the chemical space of orally bioavailable PROTACs', Drug Discovery Today 29(4), p103917.

Assay Validation and Platform Development

o2h conducted a rigorous validation process, with a variety of different chemical species, underscoring the reliability of the assay as a robust and transferable platform, broadly applicable for multiple drug discovery programs.

- Validated with >20 different compounds, including macrocycles, non-macrocyclic compounds, and PROTACs
- >100 compounds examined to date confirm versatility and effectiveness in designing bioavailable compounds beyond Ro5 constraints

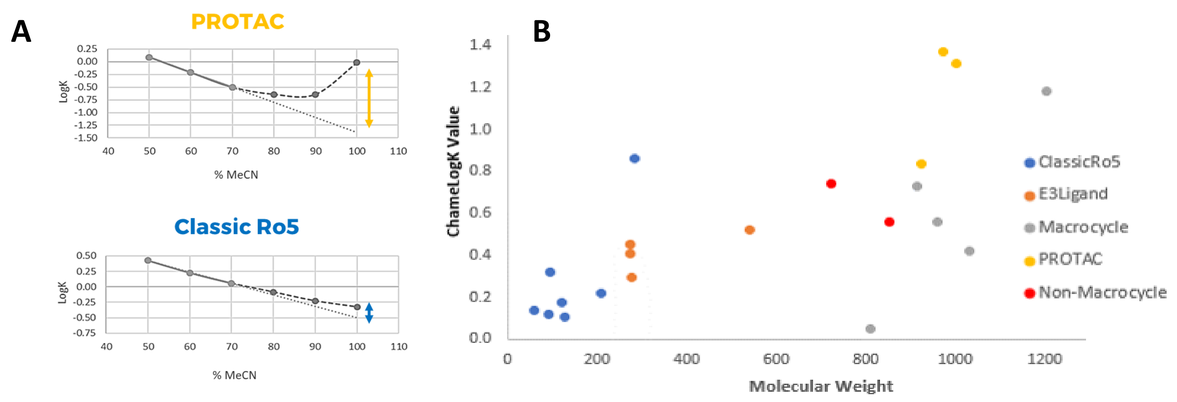


Figure 1: A. Output data showing correlation of Log K' with % acetonitrile for a PROTAC and a classic Ro5 compound; **B.** Summary of chameleonicity across a range of chemical species

Results

- PROTACs: Displayed distinct "Chameleonic" qualities.
- Non-macrocycles: Moderate chameleonicity, showing expected flexibility compared to classic Ro5 and PROTACs
- Macrocycles: Led by Cyclosporin, showed variable behaviour
- E3 Ligands: Consistent behaviour, possibly involving internal folding or interactions
- Ro5 molecules like caffeine, and diazepam defied expectation

o2h has streamlined processes for **maximal throughput and productivity**, creating a **cost-effective and easy-access** version of this ground-breaking technique

Supporting a range of modalities

- The **increasing need to explore the bRo5 space** to access novel chemistry, better mechanisms of action, and exciting new modalities, requires a way to quickly and inexpensively measure chameleonicity
- This assay represents a **significant step forward** in assessing molecules such as PROTACs and optimization of their properties [7] Enabling:

Hetero-bifunctionals

Glues

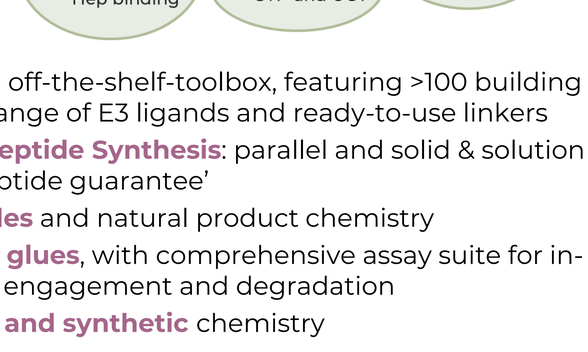
- ✓ Optimization to fit with target biology; e.g. crossing the **BBB** or efficiently accessing **intracellular targets**
- ✓ Characterization of molecules to guide **design** and create a **robust pre-clinical data package**
- ✓ Understand the **relationship between biophysical and cell-based** results

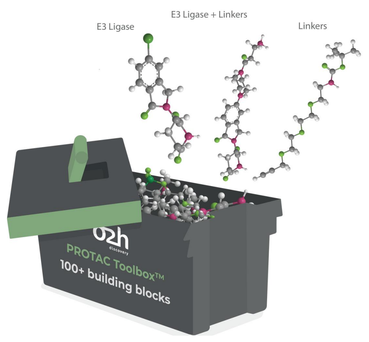
Peptides

Macrocycles

A key part of your drug discovery program

- o2h offers the ChameLogK assay as part of a comprehensive suite of ADMET and assay biology, enabling full characterisation of compounds
- o2h also offers a range of integrated services linking directly to this field:





- ✓ **PROTACs:** off-the-shelf-toolbox, featuring >100 building blocks, a range of E3 ligands and ready-to-use linkers
- ✓ **Custom Peptide Synthesis**: parallel and solid & solution phase; 'peptide guarantee'
- ✓ **Macrocycles** and natural product chemistry
- ✓ **Molecular glues**, with comprehensive assay suite for in-cell target engagement and degradation
- ✓ **Medicinal and synthetic** chemistry

Want to learn more about this assay and how o2h supports integrated drug discovery programs...?

Scan the QR code to explore our website:

Read our blog on ChameLogK:

Contact us: info@o2h.com