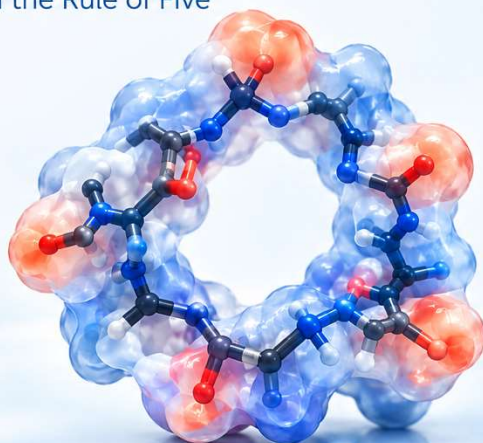


TECHNICAL DATA SHEET

Experimental Polar Surface Area (ePSA) by SFC

Characterizing exposed polarity beyond the Rule of Five



SFC



Macrocycles

Optimizing folding and permeability



Cyclic peptides

Monitoring polarity reduction



PROTACs

Tuning linker architecture and conformation



Medicinal chemistry

Comparing analogues and identifying key changes

From structure to exposed polarity | ePSA by SFC

Experimental Polar Surface Area (ePSA) by SFC

Characterizing exposed polarity beyond the Rule of Five

Overview

Predicting permeability becomes increasingly challenging as molecules move beyond conventional Rule-of-Five space. Macrocycles, cyclic peptides and PROTACs can combine high molecular weight, numerous hydrogen-bond donors and acceptors, and substantial conformational flexibility, yet some of these molecules can still achieve meaningful passive permeability and oral exposure. One reason is that molecular polarity is not necessarily static.

Computed Topological Polar Surface Area (TPSA) is a powerful and widely used molecular descriptor, but it is derived from the two-dimensional molecular structure. It therefore does not directly account for the three-dimensional conformations adopted by flexible molecules, or for the shielding of polar groups through intramolecular interactions.

Oxeltis has integrated an Experimental Polar Surface Area (ePSA) method into its drug discovery services based on supercritical fluid chromatography (SFC), providing an experimental readout of the polarity exposed by a molecule under defined low-polarity chromatographic conditions.

TPSA vs ePSA: Structure on Paper vs Exposed Polarity

TPSA estimates the amount of polar surface encoded by a molecular structure. **ePSA** provides an experimental measure related to the polarity that remains accessible when the molecule adopts conformations in a low-polarity environment. This distinction is particularly relevant for flexible molecules capable of conformational folding.

The molecular chameleon concept

A molecular chameleon can adapt its conformation to its environment. In an aqueous environment, a molecule may adopt conformations in which polar groups are exposed and stabilized by interactions with water. In a less polar environment, the same molecule may fold so that polar groups become internally satisfied or shielded.

Intramolecular hydrogen bonds (IMHBs) are one important mechanism contributing to this polarity masking. As a result, two molecules with similar TPSA values can exhibit substantially different experimentally accessible polarity, and potentially different permeability profiles. In short, TPSA describes the structure; ePSA reports how that structure behaves.

Why does this matter in drug discovery?

The ability to reduce exposed polarity without necessarily removing polar functionality can be particularly valuable in beyond-Rule-of-Five (bRo5) discovery. ePSA can therefore support:

- **Cyclic peptide design** - Monitoring polarity reduction during sequence modification, N-methylation and conformational optimization.
- **Macrocycle optimization** - Investigating the relationship between molecular folding, exposed polarity and permeability.
- **PROTAC optimization** - Characterizing the impact of linker architecture and conformational behavior on experimentally expressed polarity.
- **Medicinal chemistry** - Comparing matched analogues and identifying structural modifications associated with changes in exposed polarity.

Scientific Principle

ePSA was introduced by Goetz and co-workers at Pfizer as an SFC-based chromatographic surrogate for membrane permeability in cyclic peptides.¹ The method separates compounds on a dedicated SFC column using a calibration set of reference standards with assigned ePSA values, then converts retention behavior into an ePSA value that provides a readout of exposed polarity under the assay conditions.

ePSA and Intramolecular Hydrogen Bonding

A key feature of ePSA is its sensitivity to changes in exposed polarity caused by intramolecular interactions. For example, in cyclic peptides with established IMHB patterns, methylation of solvent-exposed N-H groups can decrease ePSA, whereas methylation of N-H groups already engaged in IMHBs may produce little additional change. This behavior illustrates how ePSA can provide indirect information about polarity masking and conformational organization. ePSA should not, however, be interpreted as a direct structural measurement of a specific hydrogen bond. Its greatest value comes from comparing related molecules within a chemical series.

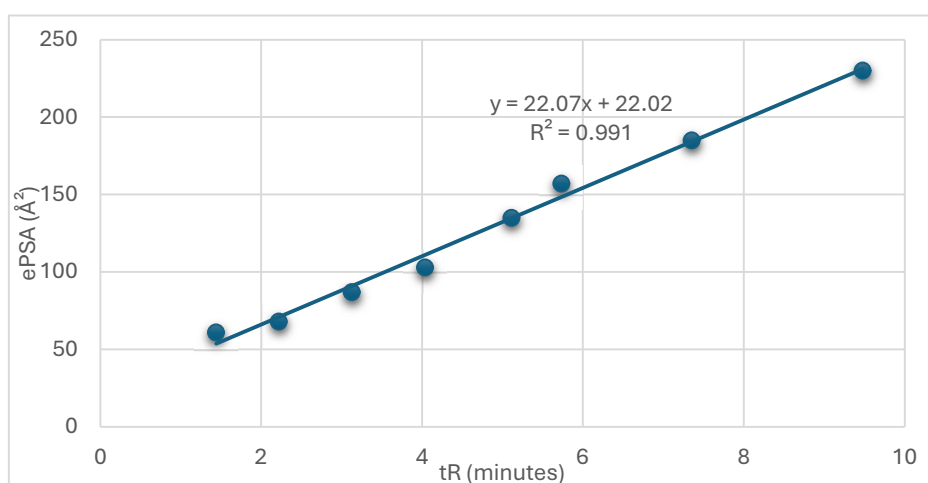
From ePSA to Passive Permeability

ePSA is not a direct permeability assay. It is an experimentally derived physicochemical descriptor that can be used as a permeability surrogate, particularly in chemical spaces where conventional descriptors become less informative. In the original Pfizer study, ePSA was determined for 814 cyclic peptides previously evaluated by RRCK permeability. Peptides with ePSA values below 80 Å² were substantially more likely to display measurable permeability, whereas compounds above 100 Å² generally showed little significant passive permeability. The authors proposed ePSA as a useful permeability filter for cyclic peptide design. These values should be regarded as context-dependent reference points rather than universal permeability thresholds. The relationship between ePSA and permeability depends on molecular class and experimental context.

Platform & Method

ePSA is run on Oxeltis's hybrid analytical/preparative SFC platform, the same system used for enantiomer separation at preparative scale.

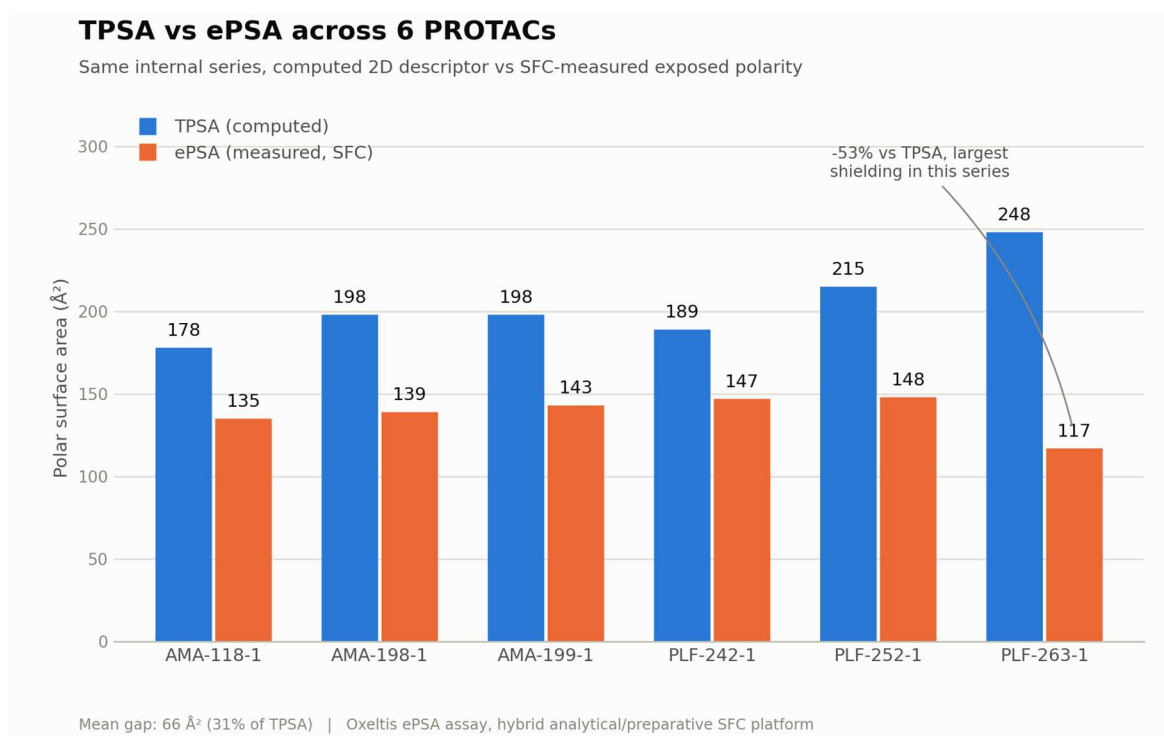
Method	Experimental Polar Surface Area (ePSA) by supercritical fluid chromatography
Platform	Hybrid analytical/preparative SFC system
Column	Chirex 3014
Calibration	Full literature standard set (ref. 1), recalibrated with each batch; typical $R^2 \geq 0.99$; In the example described below: $y = 22.07x + 22.02$, $R^2 = 0.991$, reproducing the published reference data
Complementary readout	CLogD, for combined polarity/lipophilicity interpretation
Sample requirement	~5 mg per compound
Turnaround and capacity	up to ~25 compounds per 24 h instrument run, calibration standards included



In-house ePSA calibration curve based on the full literature standard set (Goetz et al.; $R^2 = 0.991$).

Case Study: Experimental Polarity in a PROTAC Series

Six internal PROTACs ranging from 777 to 906 Da were analyzed at Oxeltis. The calculated TPSA values ranged from 178 to 248 Å², whereas measured ePSA values were substantially lower and occupied a narrower range of 117 to 148 Å². The mean absolute difference between TPSA and ePSA was 66 Å², corresponding to a mean 31% reduction relative to TPSA. For the most conformationally shielded compound, the difference reached 53%. This example illustrates how a molecule can retain a large number of polar functionalities while experimentally displaying considerably less exposed polarity than suggested by TPSA alone.



Computed TPSA vs measured ePSA for six PROTACs. ePSA values are lower, fall within a narrower range, and change the ranking: the compound with the highest TPSA shows the lowest ePSA.

ePSA and CLogD: A Complementary Read

ePSA does not replace cLogD, it complements it. cLogD captures a molecule's overall lipophilicity, while ePSA provides an experimental readout of exposed polarity under low-polarity conditions that is relevant to membrane permeation. Read together, the two measurements help flag molecular chameleon behavior, in which compounds adapt their conformation and reduce exposed polarity in low-polarity environments. This behavior is increasingly documented across PROTACs and macrocycle series. Recent work from AbbVie has highlighted the added value of ePSA over TPSA and PAMPA for the analysis and optimization of bRo5 compounds and PROTACs, using datasets of approximately 1,000 compounds with human absorption data and approximately 10,000 internal compounds, including about 1,000 PROTACs.² The same study introduced the ePSA-to-TPSA ratio (ETR) as a simple index of chameleonicity: the lower the ETR, the larger the share of a molecule's structural polarity that is shielded in a low-polarity environment.²

Compound	TPSA (Å ² , computed)	ePSA (measured)	ETR (ePSA/TPSA)	Rank (ETR)
PLF-263-1	248	117	0.47	1
PLF-252-1	215	148	0.69	2
AMA-198-1	198	139	0.70	3
AMA-199-1	198	143	0.72	4
AMA-118-1	178	135	0.76	5
PLF-242-1	189	147	0.78	6

Deliverables

Each study report includes retention times (n = 3), the batch calibration curve and R², ePSA (mean ± SD) for each compound, computed TPSA and ePSA/TPSA ratio, cLogD when requested, and a short interpretive summary. Results are typically delivered within 5 working days of sample receipt.

References

- 1) Goetz, G. H.; Farrell, W.; Shalaeva, M.; Sciabola, S.; Anderson, D.; Yan, J.; Philippe, L.; Shapiro, M. J. EPSA: A Novel Supercritical Fluid Chromatography Technique Enabling the Design of Permeable Cyclic Peptides. ACS Med. Chem. Lett. 2014, 5, 1167 to 1172. DOI: 10.1021/ml500239m
- 2) Price, E.; Weinheimer, M.; Rivkin, A.; Jenkins, G.; Nijssen, M.; Cox, P. B.; DeGoey, D. Beyond Rule of Five and PROTACs in Modern Drug Discovery: Polarity Reducers, Chameleonicity, and the Evolving Physicochemical Landscape. J. Med. Chem. 2024, 67 (7), 5683 to 5698. DOI: 10.1021/acs.jmedchem.3c02332

Talk with Us

Interested in running ePSA on your series? ePSA can be combined with cLogD and conformational modelling with our partner, Molecular Forecaster to guide bRo5 design.

Contact Oxeltis's Business Development team to discuss sample requirements and turnaround.

Christophe Parsy, Business & Strategic Development Director, parsy.christophe@oxeltis.com