

# Elucidation of protein encapsulation by fluorinated ionic liquids by SAXS

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Medical treatments with therapeutic proteins have increased dramatically over the past decades. However, it is still necessary to develop effective approaches for protein drug delivery in order to overcome problems such as dosage, protein instability and degradation. Ionic liquids (ILs), a new class of environmentally benign and tailor-made solvents, have been increasingly exploited in the pharmaceutical field. Fluorinated ILs (FILs) spontaneously self-assemble at concentrations above their critical aggregation concentration (CAC) [1].

Our previous studies show that the addition of ionic liquids had no significant effect on the stability, structure and activity of lysozyme [2]. A distinct behaviour was observed in DLS experiments for non-surfactant and surfactant ILs, with the latter encapsulating the protein at concentrations above the CAC [2].

We are further investigating by SAXS the structure of micelles formed by FILs (Fig. 1) and FIL+protein and analyzing their impact at concentrations above and below its CAC. This is an important step to assess the potential use of FILs as drug delivery systems for therapeutic proteins.

Preliminary SAXS data of lysozyme show an increase in several parameters, e.g.,  $R_g$ ,  $D_{max}$  and hydration shell, suggesting larger volumes in the presence of higher FIL concentrations. However, a similar study using bovine serum albumin (BSA) seems to indicate that smaller volumes occur in the presence of the FILs. Further studies are needed to clarify the interaction between the surface of different proteins and FILs.

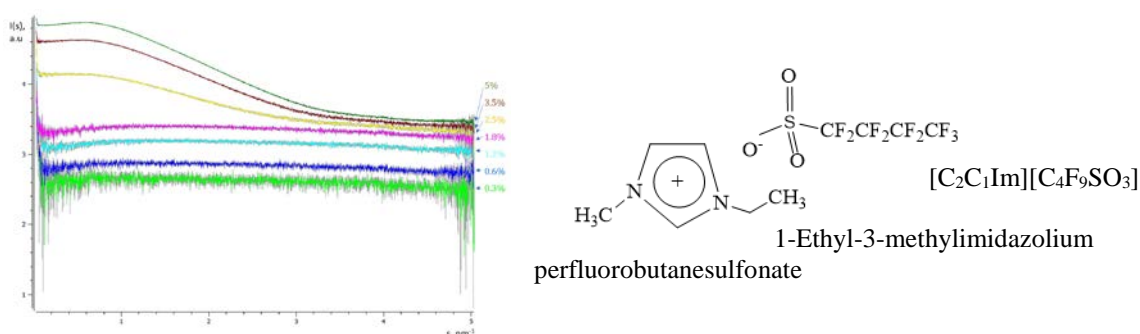


Figure 1: SAXS experiment with concentration series of  $[C_2C_1Im][C_4F_9SO_3]$  FIL, from 0.3 to 5% (v/v).

## References

- [1] - A.B. Pereiro et al., 2015. Aggregation behavior and total miscibility of fluorinated ionic liquids in water. *Langmuir* 31, 1283-1295.
- [2] - M. Alves et al., 2017. Fluorinated ionic liquids for protein drug delivery systems: investigating their impact on the structure and function of lysozyme. *Int J Pharm* 526, 309-320.