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PROGRAM AND ABSTRACT BOOK

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CA.09 - Direct visualization of virus removal process in hollow fiber membrane using an optical microscope

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Virus removal filters developed for the decontamination of small viruses from biotherapeutic products are widely used in basic research and critical step for drug production due to their long-established quality and robust performance. A variety of imaging techniques have been employed to elucidate the mechanism(s) by which viruses are effectively captured by filter membranes, but they are limited to 'static' imaging. Here, we propose a novel method for detailed monitoring of 'dynamic process' of virus capture; specifically, direct examination of biomolecules during filtration under an ultra-stable optical microscope. Samples were fluorescently labeled and infused into a single hollow fiber membrane comprising cuprammonium regenerated-cellulose. While proteins were able to pass through the membrane, virus-like particles (VLP) accumulated stably in a defined region of the membrane. After injecting the small amount of sample into the fiber membrane, the real-time process of trapping VLP in the membrane was quantified beyond the diffraction limit. The method presented here serves as a preliminary basis for determining optimum filtration conditions, and provides new insights into the structure of novel fiber membranes. For details, please refer the following publication. https://www.nature.com/articles/s41598-020-78637-z
Keywords: molecular imaging, dynamics of virus filtration, VLP visualization

CA.10 - Imidazolium-based ionic liquids as additives to preserve green fluorescent protein activity at room-temperature and under stress

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Advances in biotechnology have allowed the development of fluorescent proteins (FP) for several industrial applications. However, there are still difficulties in their use at large scales and in novel fields due to the low stability of FP, which limit their application, distribution, and storage. The discovery of additives capable of preserving the activity of FP at room temperature and under stress conditions is needed to help expand and facilitate their commercial use. Hence, we aimed to evaluate the use of ionic liquids (ILs) as additives capable of preserving the activity of the Enhanced Green Fluorescent Protein (EGFP), an important biomarker and biosensor, at different storage times and under unfavorable conditions. We evaluated the effect of 1-alkyl-3-methylimidazolium chloride-based ILs ([Cnmim]Cl) aqueous solutions on EGFP fluorescence at short (48 h) and long-term studies (3 months), and then their ability to protect the EGFP in the presence of denaturing agents. All [Cnmim]Cl ILs (at 0.100 M) were able to preserve EGFP fluorescence for longer than the phosphate-saline buffer (PBS) and NaCl solutions, increasing from 1 to 3 months. ILs solutions with shorter to medium cation alkyl chain length were the most effective for maintaining EGFP fluorescence, as well as protectors of EGFP activity in the presence of the surfactant SDS, an acid of guanidine hydrochloride, and for H2O2. [Cnmim]Cl solutions can be added to aqueous solutions to preserve EGFP fluorescence activity at room temperature for long-storage times and to reduce the negative impact of denaturing agents on EGFP. Therefore, there is a massive potential for the application of ILs as additives to preserve FP in the long-term without refrigeration and under unfavorable conditions, which is fundamental to expanding their industrial and commercial uses.
Keywords: protein stability, ionic liquids, preservatives
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