UNIVERSITÀ DEGLI STUDI DI SALERNO

UNIVERSITÀ DEGLI STUDI DI SALERNO
Dipartimento di Farmacia

PhD Program
in **Drug Discovery and Development**
XXXVIII Cycle-Academic year 2024/2025

PhD Thesis in

Research and development through automated laboratory procedures

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Abstract

Model membrane systems, such as lipid monolayers and Giant Unilamellar Vesicles (GUVs), are indispensable tools in pharmaceutical sciences for investigating drug-membrane interactions. However, the adoption of the technologies needed for these studies is often hindered by the prohibitive cost of commercial instrumentation, the lack of customization, and data fragmentation across proprietary formats. This doctoral thesis addresses these technological barriers by developing an integrated, open-source ecosystem of hardware and software tools designed to democratize and automate membrane biophysics research.

First, a modular Langmuir-Blodgett trough was engineered using the Arduino platform. The system, controlled via a custom Python interface (LanGUImuir) and an automated protocol generator (ScriptMaker), provides precise control over barrier compression and surface pressure acquisition. Validation tests with anionic surfactants demonstrated a high signal-to-noise ratio (42.1 dB), and the device successfully resolved the thermodynamic phase transitions of complex biological monolayers (egg yolk phospholipids) with high linearity.

Second, the production of GUVs was optimized through the design of novel stainless steel mesh electrodes. A systematic evaluation of chamber geometries identified a specific configuration (Chamber D, Mesh 30) capable of achieving a lipid incorporation yield of 0.20% of unilamellar vesicles, significantly outperforming traditional indium tin oxide (ITO) methods in terms of cost, durability, and efficiency. The platform proved robust for handling complex ternary mixtures (POPC:SM:Chol) and was successfully applied to investigate membrane rigidity via Generalized Polarization (GP) and to validate the passive translocation of Cell-Penetrating Peptides (CPPs).

Finally, to bridge the gap between raw data and biological insight, a comprehensive computational framework was developed. This suite includes utilities for cross-platform spectral data standardization (DX2CSV) and an advanced deconvolution tool (Extrapolator). The latter significantly enhances the sensitivity of membrane fluidity measurements by resolving overlapping spectral components, offering a more robust metric than traditional intensity-based methods.

Collectively, this work demonstrates that high-quality biophysical data can be obtained using accessible, custom-built instrumentation. The developed platforms offer a scalable and flexible workflow for high-throughput membrane research, paving the way for more efficient drug screening and formulation development.

Graphical Abstract