

# In Vitro Characterization of Diclofenac-Loaded Lipid Nanoparticles for Neutrophil Surface Modulation

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## Introduction

### Traumatic brain injury is a devastating disease

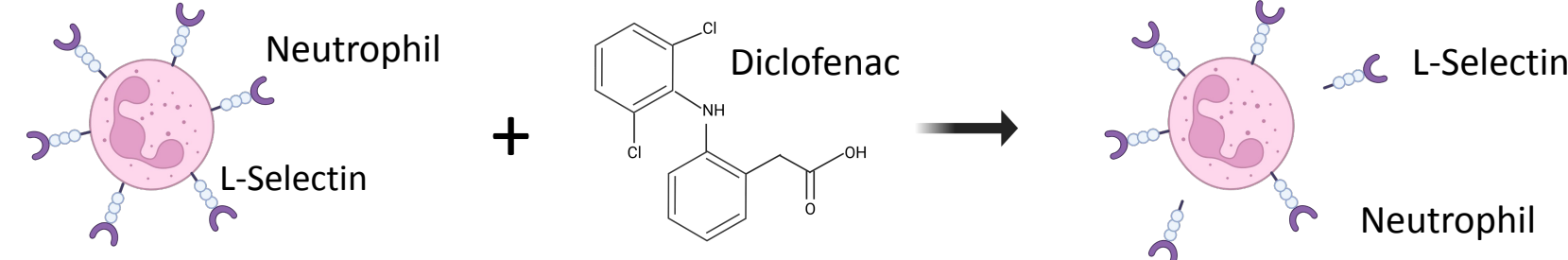
- 27 million new cases of TBI are reported globally each year.
- Current treatments are focused on managing symptoms, rather than addressing underlying disease progression.

### Blood-brain barrier breakdown leads to neutrophil infiltration

- L-selectin, an adhesion molecule, allows neutrophils to migrate to sites of inflammation.

### Lipid Nanoparticles are promising for drug delivery

- LNPs can be used to encapsulate diclofenac, an anti-inflammatory drug, which enhances L-selectin shedding in neutrophils.

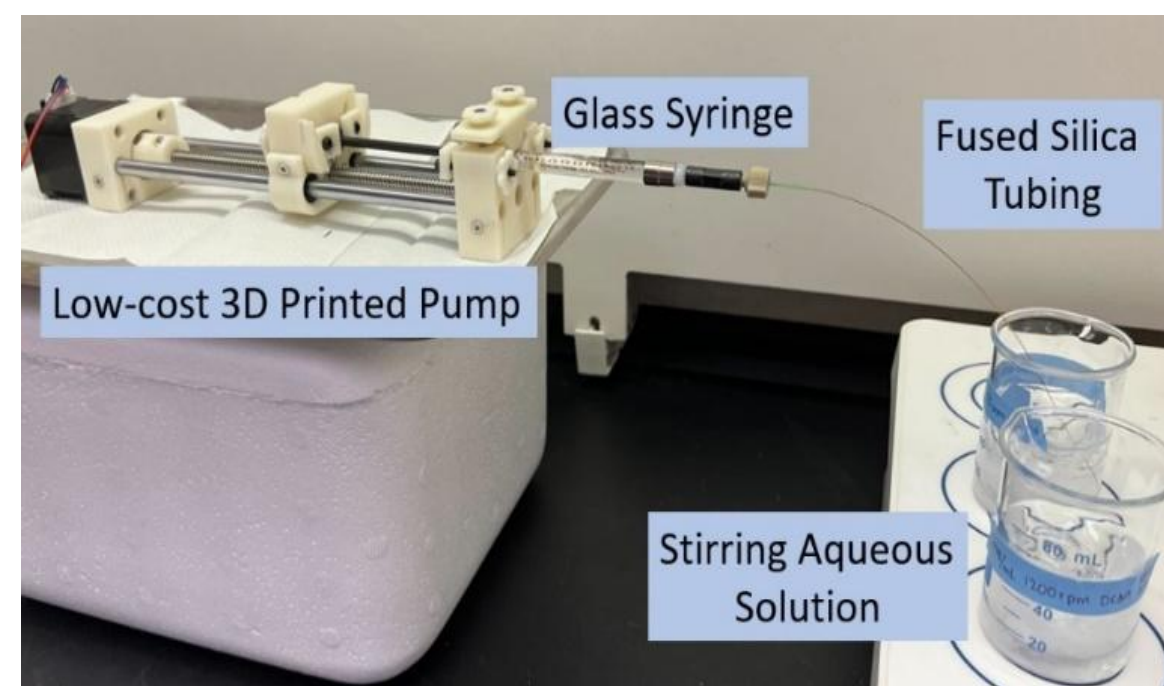


## Hypothesis

Diclofenac-loaded NPs—in comparison to free diclofenac—will have a comparable effect on enhancing the shedding of L-selectin for neutrophils in vitro.

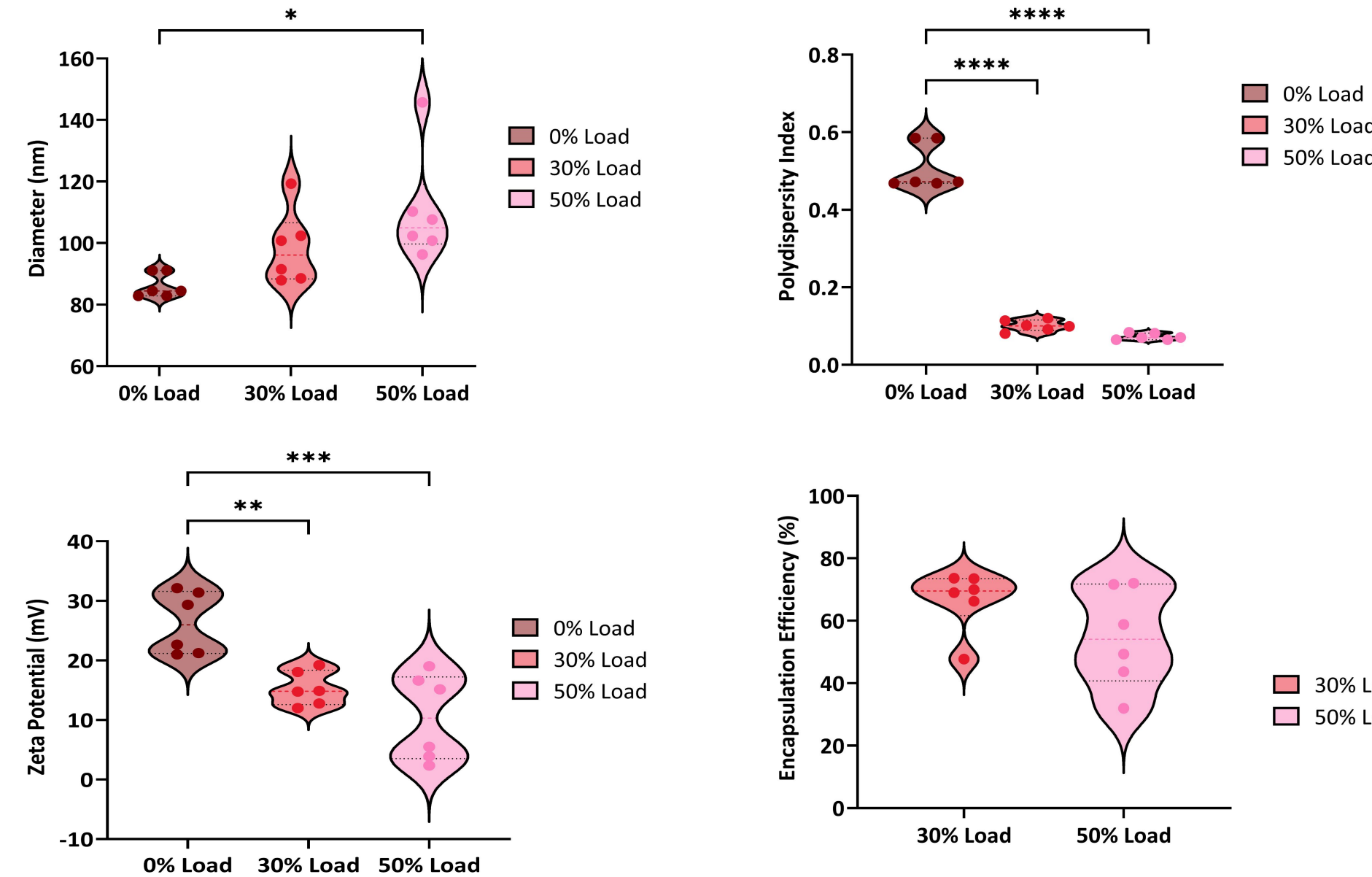
## Nanoparticle Formulation

Lipid Nanoparticles (LNP) were fabricated via nanoprecipitation utilizing a low-cost, open-source syringe pump. Fabrication variables such as: Aqueous:organic (A:O) ratio, injection rate (IR), and stirring rate were manipulated to obtain LNPs with desired physical characteristics.

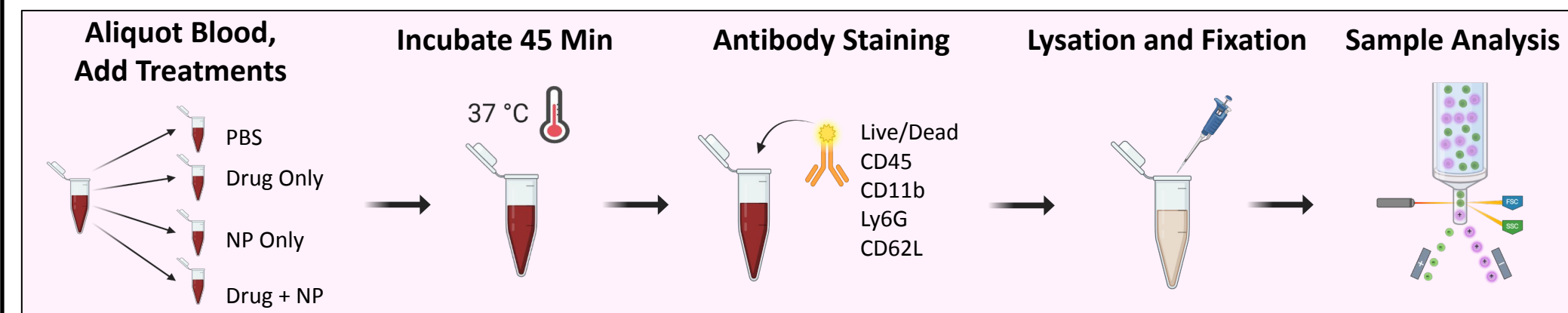


## Nanoparticle Characterization

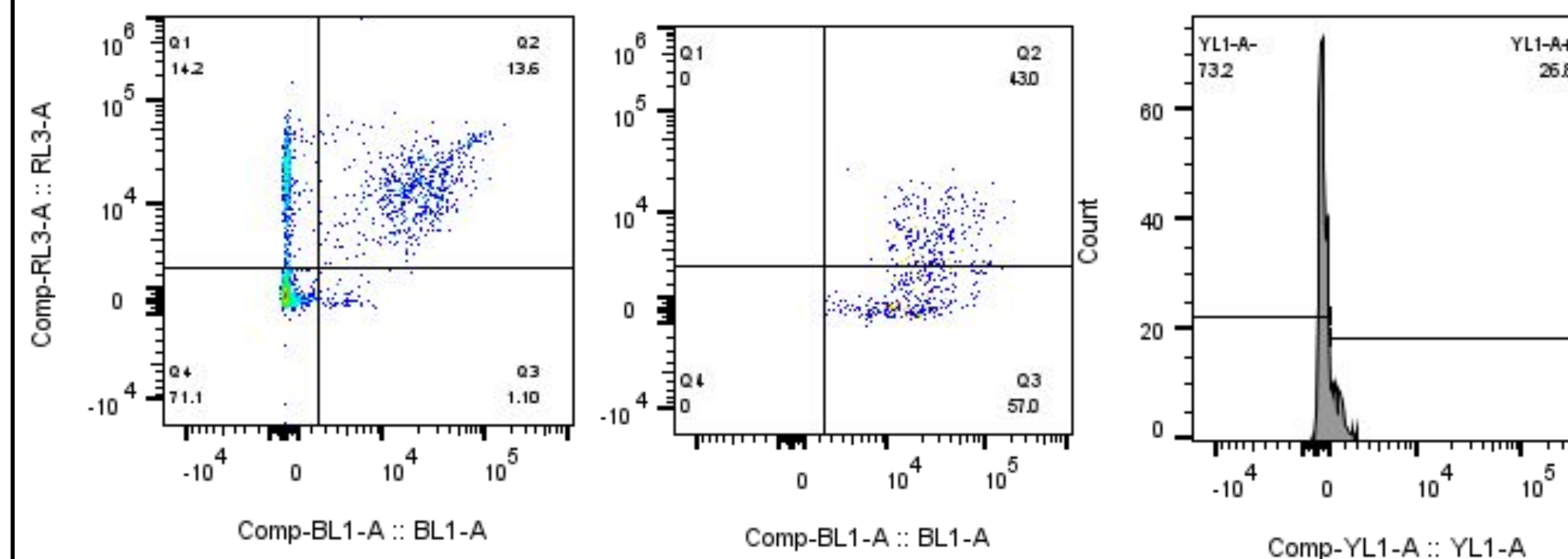
LNPs fabricated with an A:O ratio of 5:1, IR 3 ml/min, and a SR of 400 rpms yielded the smallest LNPs (55.58 nm) and were used in subsequent drug encapsulation experiments.



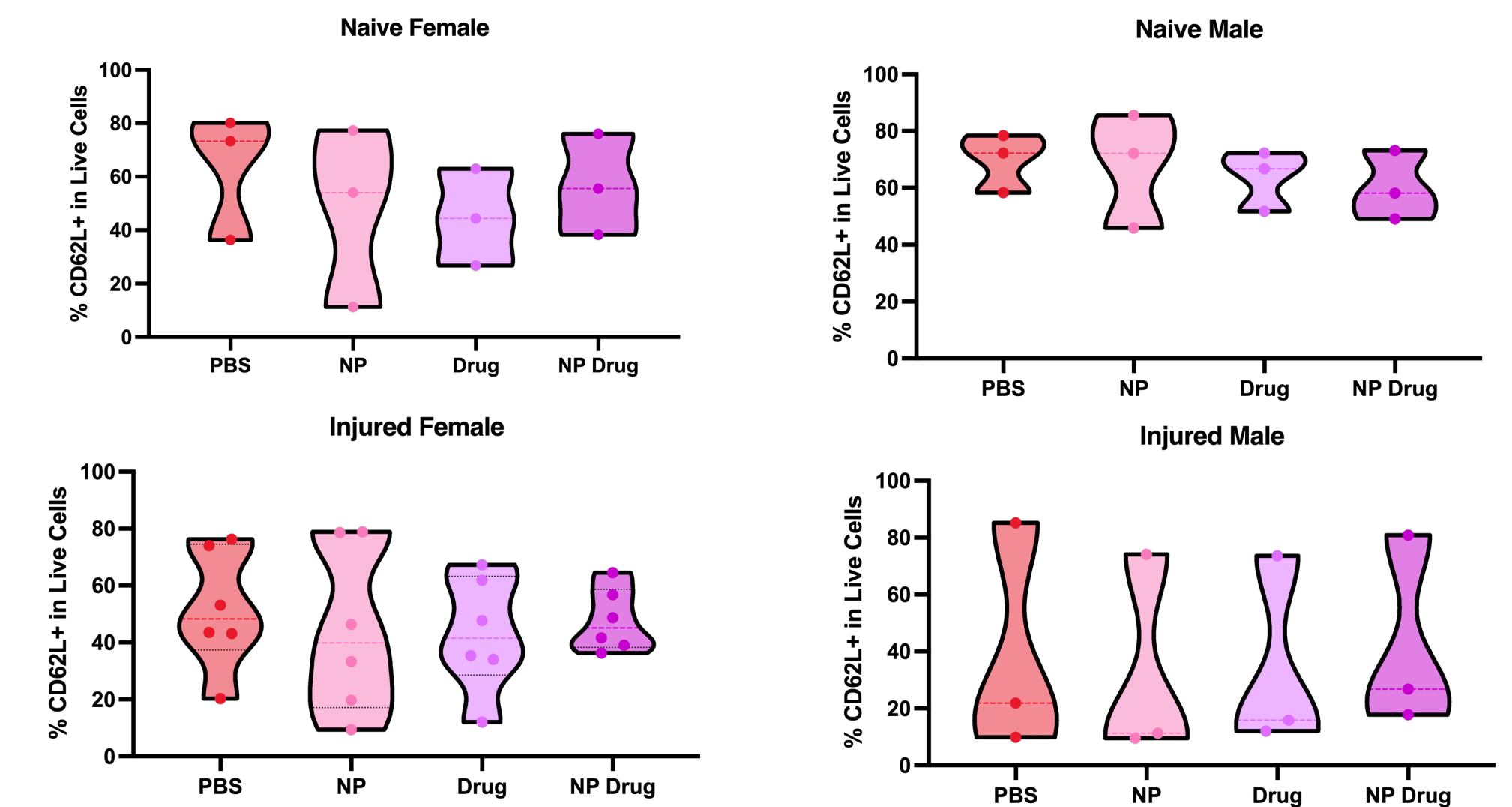
## Flow Cytometry L-Selectin Analysis



## Gating Strategy



## Quantifying CD62L Expression



## Conclusions

- LNP formula was optimized to produce sub-100 nm particles and successfully encapsulated diclofenac.
- Similar levels of L-selectin shedding was measured across experimental groups.

## Future Work

- Repeat flow cytometry analysis with lower incubation temperature for a greater amount of time. Incubation parameters, instead of treatments, may have induced L-selectin shedding.
- Measure L-selectin shedding in vivo, lack of neutrophil circulation may have caused L-selectin shedding.

## References

- [1] Bin et al. 2023 BMJ Open [3] McCreedy et al. 2018 eNeuro  
[2] Mann et al. 2015 Nature Communications

## Acknowledgements

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