

Demystifying the role of excipients in whole viral vaccines

Praveen Muralikrishnan[†], Jonathan Zajac[‡], Xianci Zeng[‡], Idris Tohidian[†], Caryn L. Heldt[†], Sarah L. Perry[‡], Sapna Sarupria[†]

[†]Department of Chemical Engineering, Michigan Technological University, [‡]Department of Chemical Engineering, University of Massachusetts Amherst, [§]Department of Chemistry, University of Minnesota – Twin Cities, [¶]Department of Chemical Engineering, University of Minnesota – Twin Cities



Funding for this work is supported by NSF DMREF Collaborative Projects: 2118788, 2118693, and 2118638. Access to computing resources was provided by the Minnesota Supercomputing Institute.

Grand Challenges in Vaccine Design

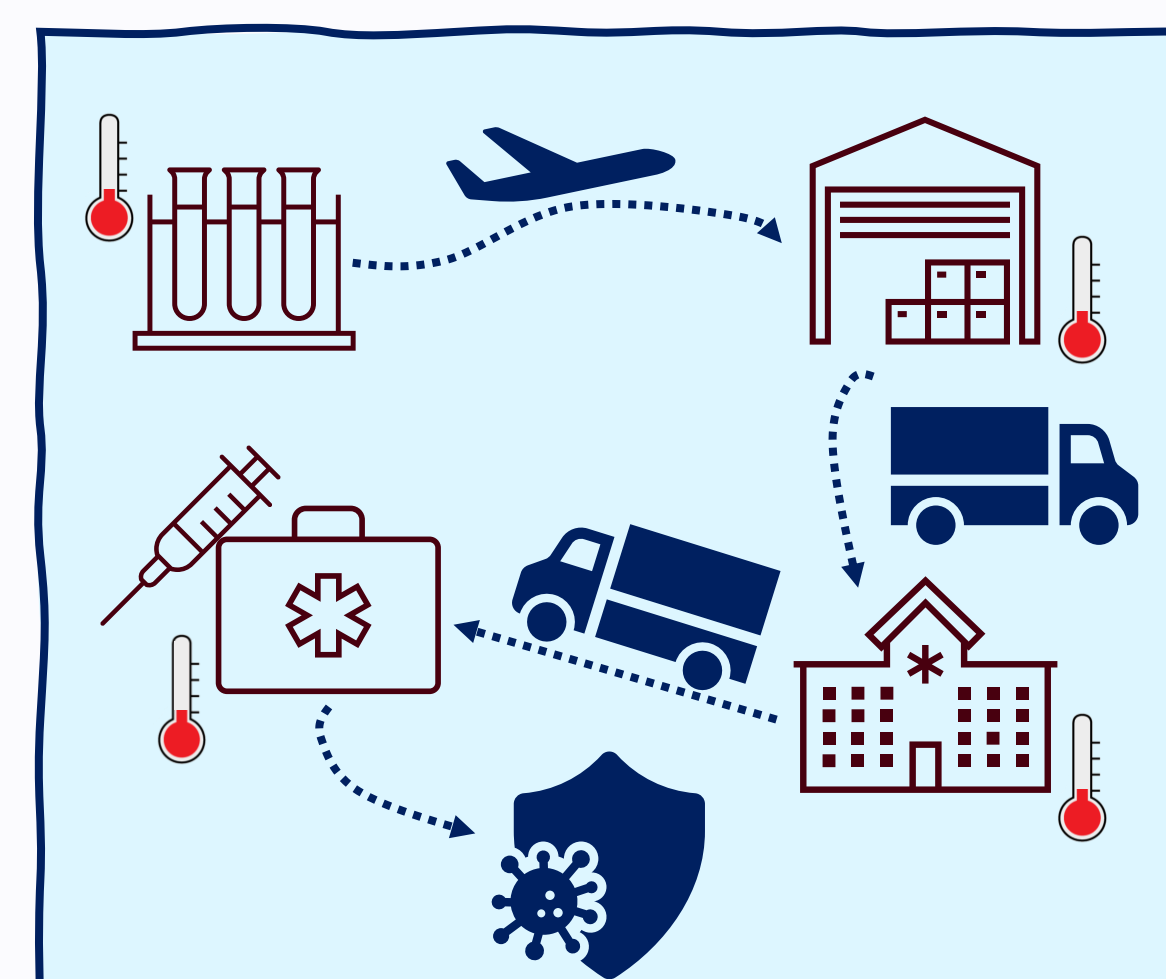


Fig. 1 The Cold Chain

- Most vaccines must be maintained at refrigerated temperatures, within a narrow 2-8 °C range
- The cold chain (Fig. 1) is a constraint on vaccine manufacturing, storage, and distribution
- Breakdowns in the cold chain disrupt outreach of vaccination programs, necessitating the design of temperature-stable formulations

Excipient innovation is a promising strategy for improving temperature stability and alleviating the cold chain

Existing strategies for excipient selection are generally iterative and Edisonian in nature (Fig. 2), lacking the mechanistic insights required to create an **information-driven design strategy**

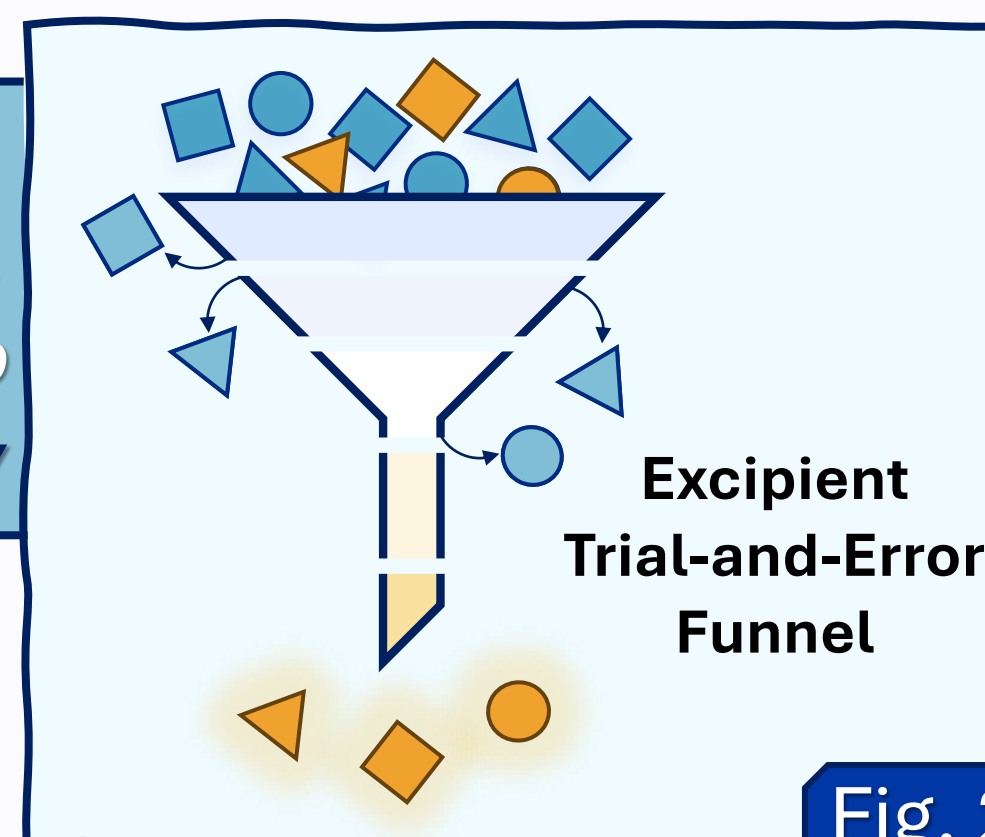


Fig. 2

Viruses are much larger than traditional stability models (Fig. 3), requiring a **multiscale approach** to investigate excipient effects at the **molecular level**

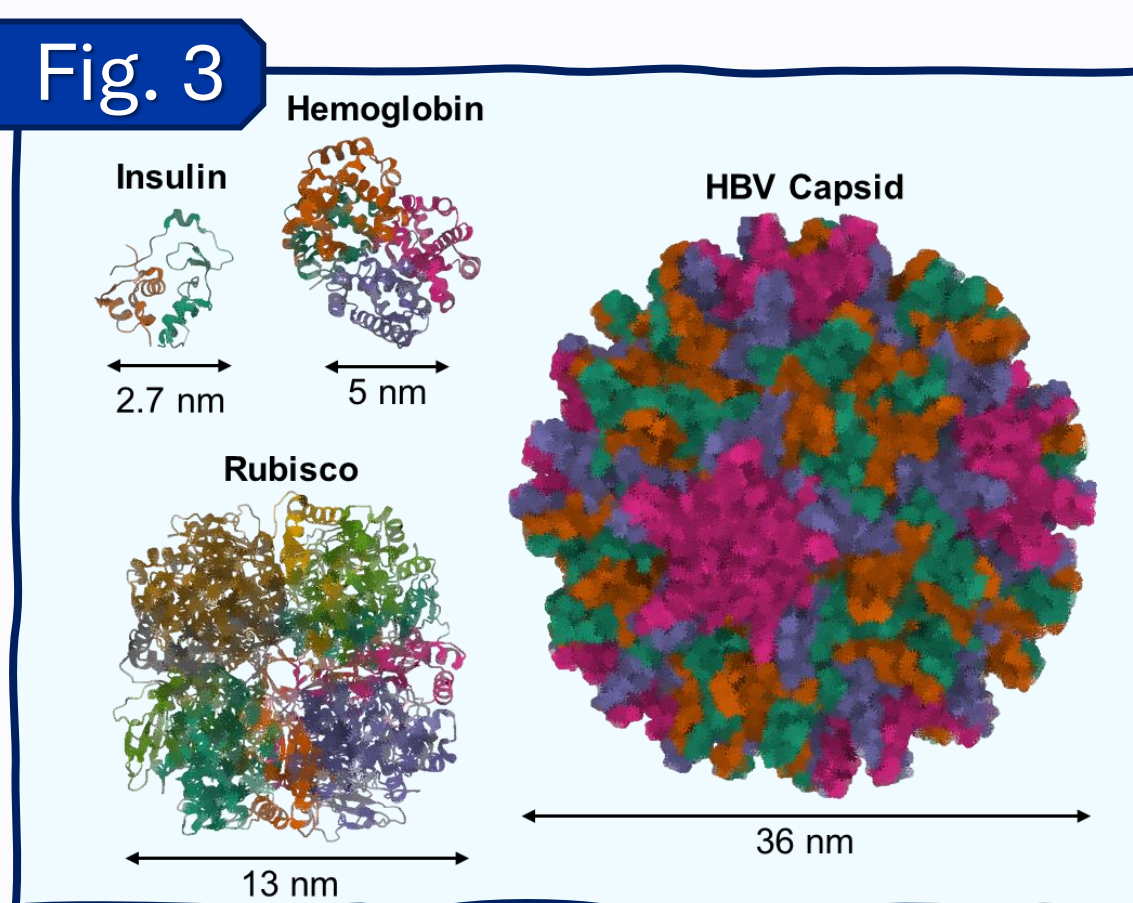
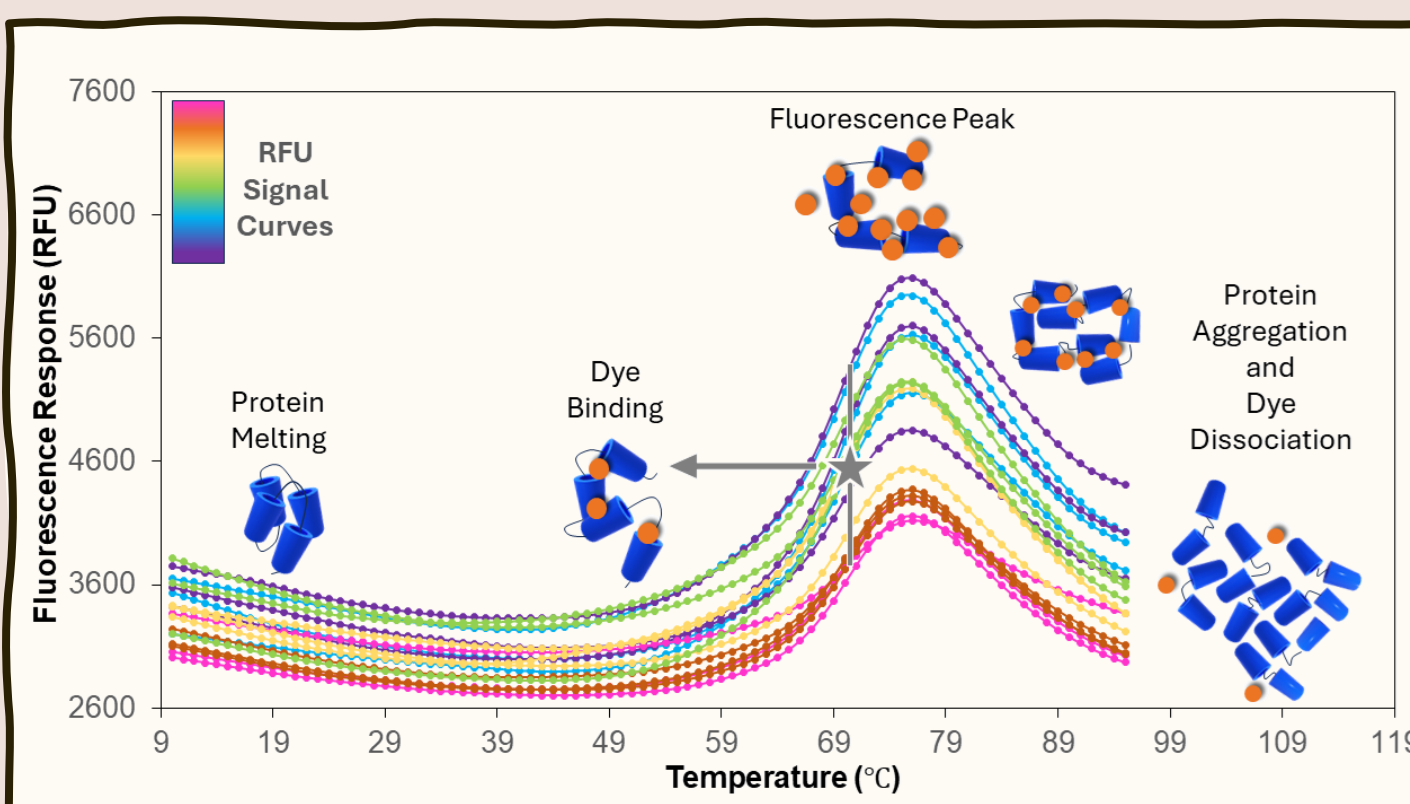


Fig. 3

Experimental Methods

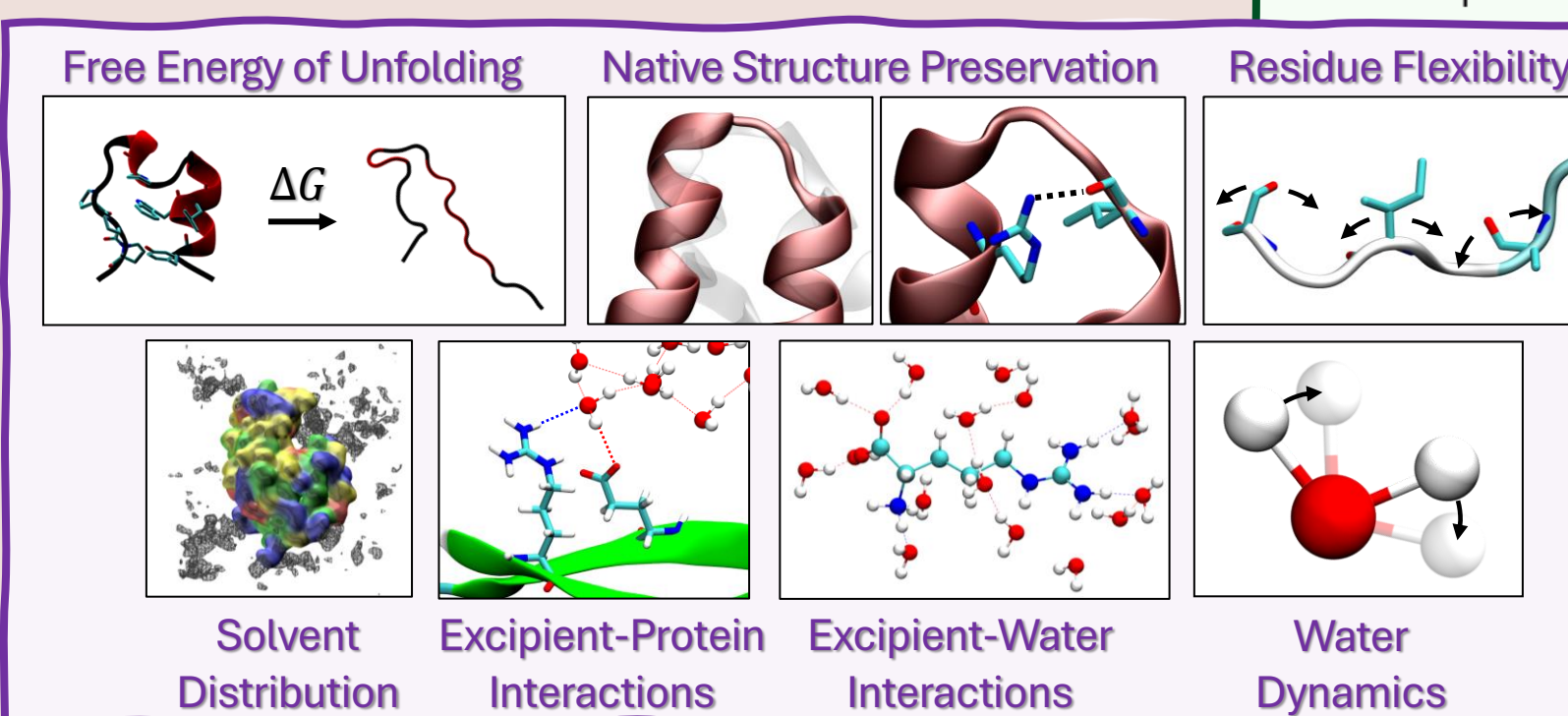
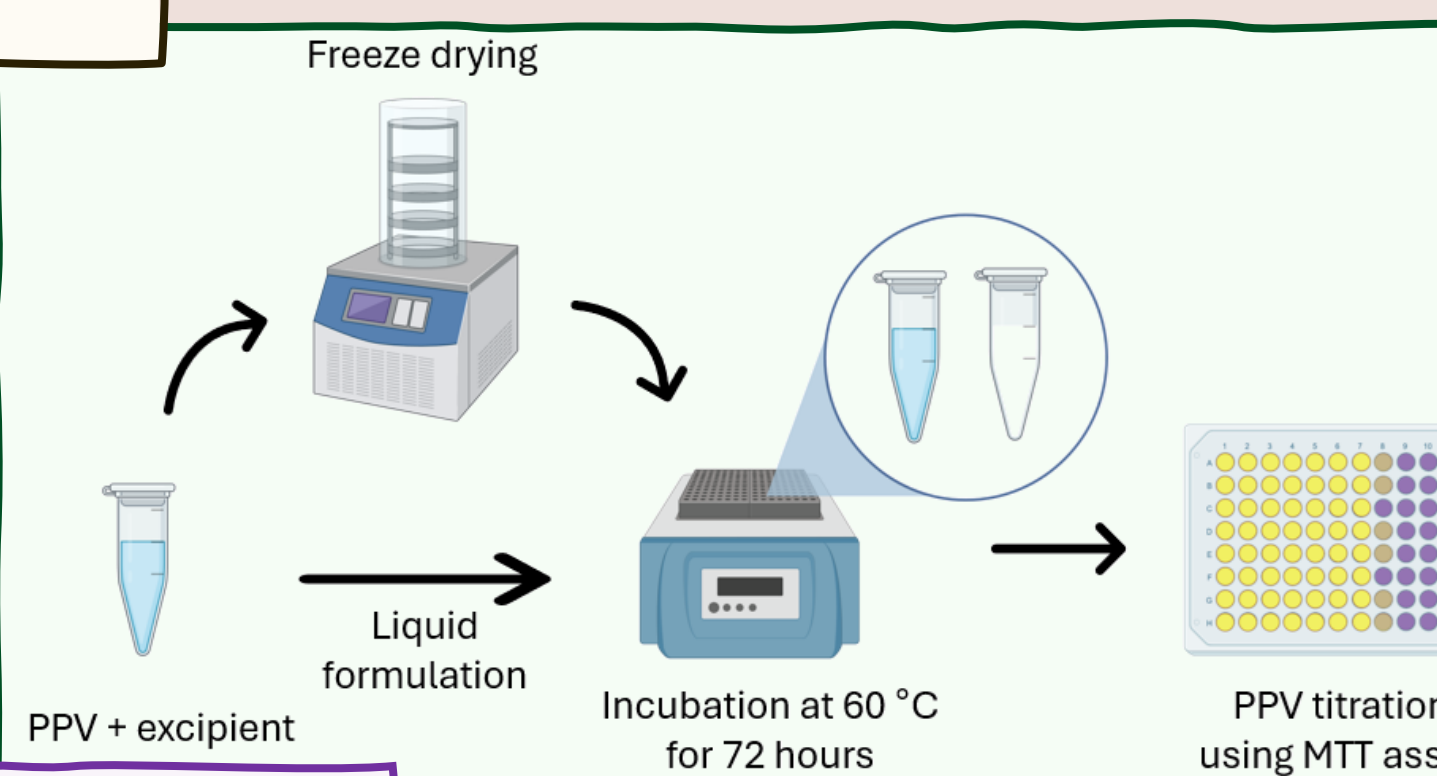


Protein Thermal Shift Assays

How can we evaluate excipient effects on protein folding?

Virus Infectivity Assays

How do excipients influence the shelf life of vaccines?



Molecular Dynamics

What molecular-level features underlie excipient mechanisms?

Excipient Case Study: Arginine

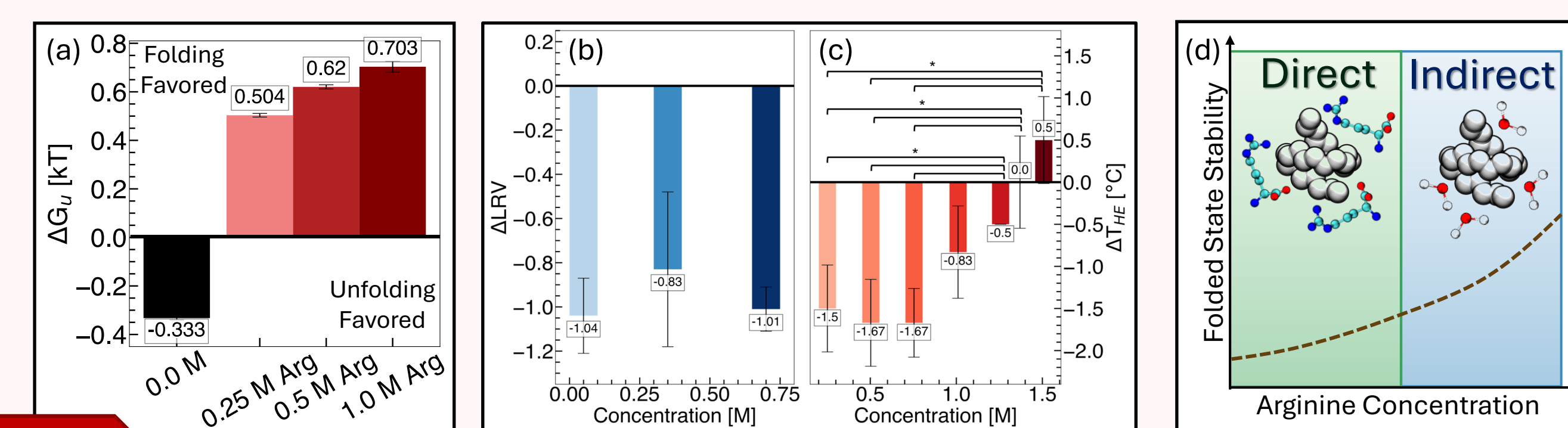
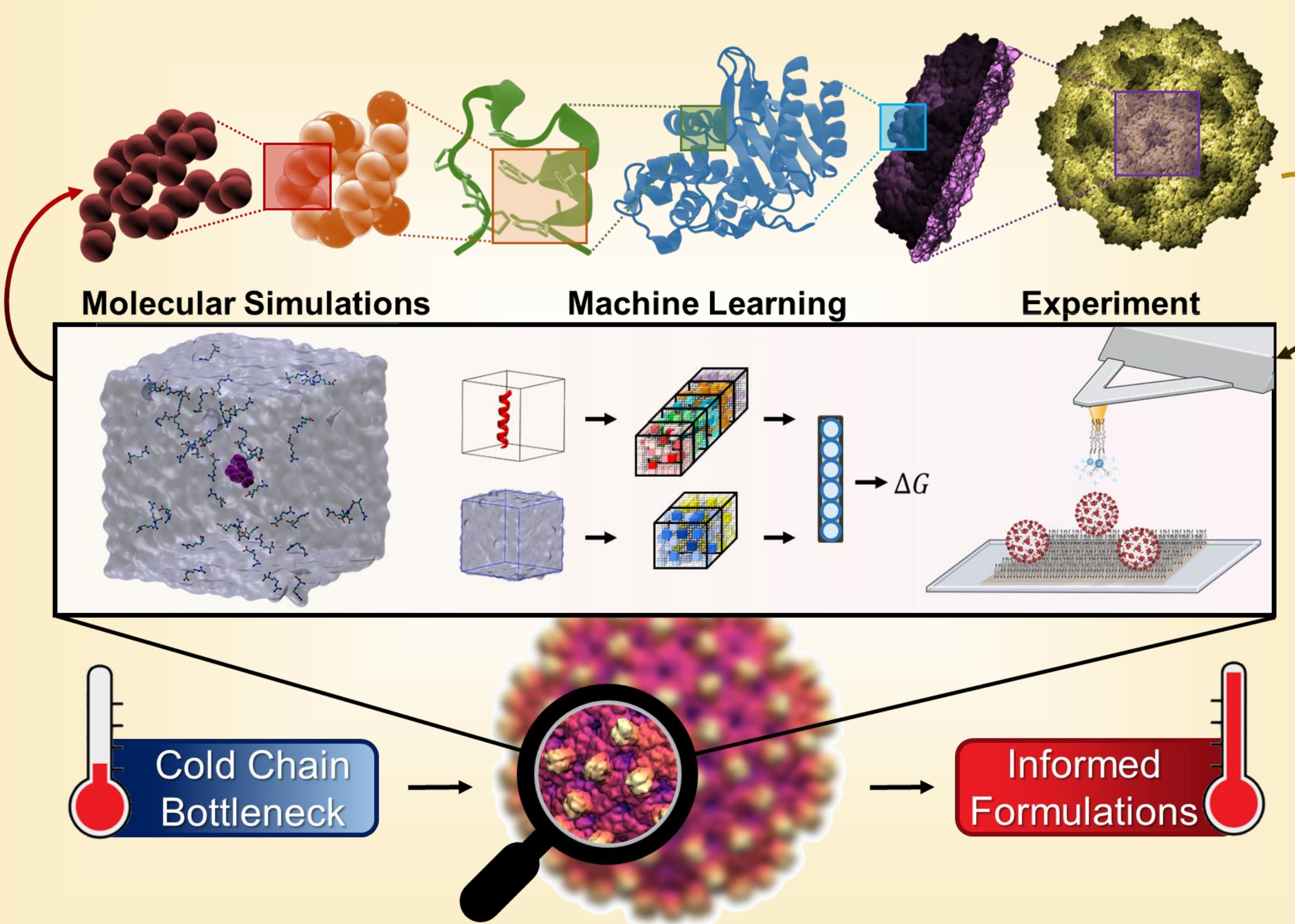


Fig. 4

- Arginine is a widely used excipient with a wide range of reported effects
- Arginine stabilizes a hydrophobic polymer (Fig. 4a), but not hen egg white lysozyme (HEWL) (Fig. 4b) or porcine parvovirus (PPV) (Fig. 4c)
- Hydrophobic interactions not the dominant part of stabilization for HEWL and PPV!

The multi-faceted, complex effects of the excipient arginine can be rationalized due to its positioning at the **edge of a mechanistic flip**^[1]

MULTISCALE INVESTIGATION OF VIRUS STABILITY



Insights Across Experimental Paradigms

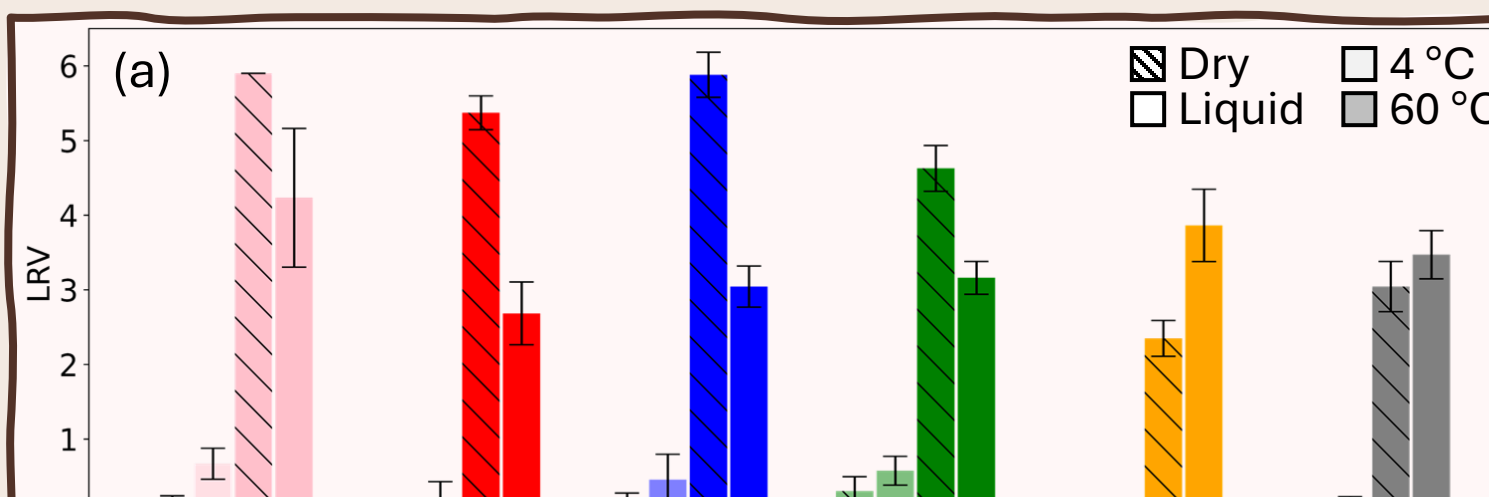


Fig. 5

- PPV stabilization by amino acids, sugars and sugar alcohols at 0.7 M (Fig. 5a)
- Dried samples had increased titer loss relative to liquid samples

Sugar alcohols best stabilized PPV, followed by glutamate

- Hydrophobic exposure temperature (HET) of HEWL with excipient concentration (Fig. 5b)
 - Combinations of excipients provided additive behavior unlike observations in hydrophobic polymer models^[2]
- Glutamate best stabilized HEWL, followed by sugars and positively charged amino acids**

Predicting Excipient Effects

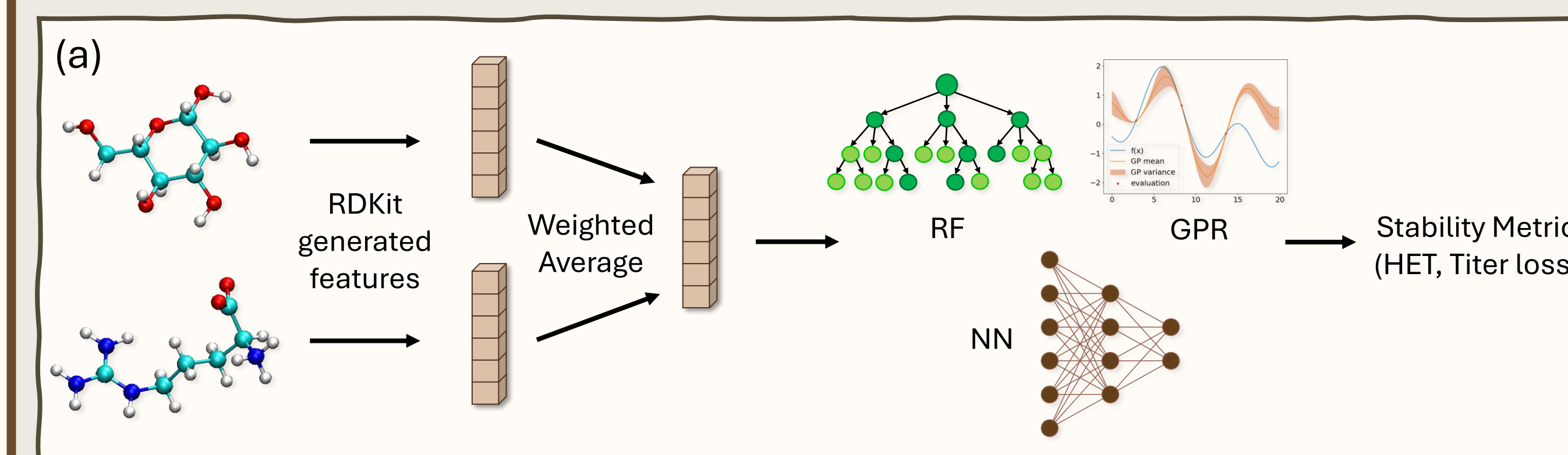
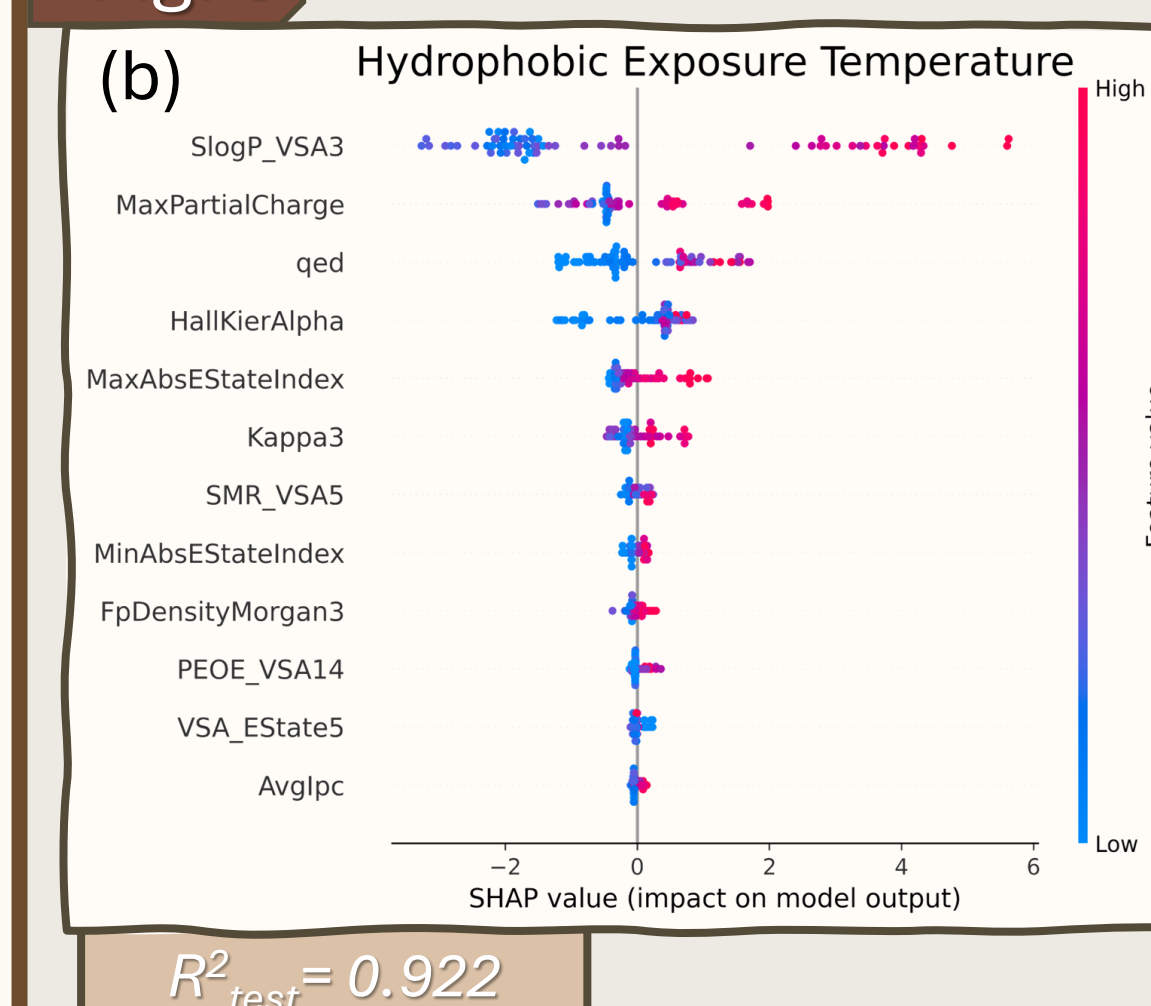


Fig. 6



- Predict the thermal stability of HEWL and PPV in different excipient solutions (Fig. 6a)
- Screened through combinations of 20 excipients and predicted Tryptophan and Histidine as HEWL stabilizers
- Model for PPV data had higher errors primarily due to data heterogeneity

Specific level of **excipient hydrophobic surface area** stabilizes HEWL better

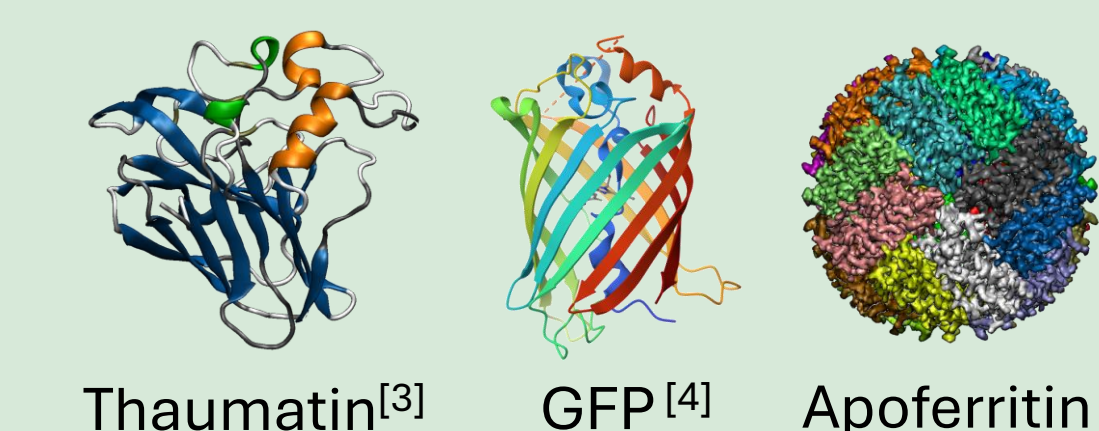
$R^2_{test} = 0.922$

Conclusions

- A combination of experiments and simulations can provide a detailed understanding behind the stabilizing role of excipients in biological formulations
- An underlying common mechanism is present that varies between proteins/viruses as showcased by focusing on arginine
- ML models aid in identifying key excipient features imparting stability and can be used for excipient selection

Ongoing and Future Work

- Perform experiments on additional small model cationic thaumatin, anionic green fluorescent protein (GFP) and apoferritin
- Study additional minute virus of mice (MVM) to probe effect of viral surface features on their stability
- Develop **multi-fidelity GPR model** to predict excipient effects on a new protein/virus



1. Modified kernel:

$$k_{\theta}(x, x') = \theta_0 \exp\left(-\frac{\|x - x'\|^2}{2\sigma^2}\right) \exp\left(-\frac{|f_{\theta}(x) - f_{\theta}(x')|^2}{2\sigma_f^2}\right)$$

$$+ \theta_1 \exp\left(-\frac{\|x - x'\|^2}{2\sigma^2}\right)$$
 2. One-hot encoded representation:
 In vitro: 0, In vivo: 1

References

- J.W.P. Zajac, P. Muralikrishnan, I. Tohidian, X. Zeng, C.L. Heldt, S.L. Perry, S. Sarupria, *Flipping Out: Role of Arginine in Hydrophobic Interactions and Biological Formulation Design*, arXiv:2403.11305v1 (2024)
- J.W.P. Zajac, P. Muralikrishnan, C.L. Heldt, S.L. Perry, S. Sarupria, *Impact of Co-Excipient Selection on Hydrophobic Polymer Folding: Insights for Optimal Formulation Design*, arXiv:2407.00885 (2024)
- Q. Ma, G. M. Sheldrick, *Thaumatococcus Structure at 1.05 Å resolution* <https://doi.org/10.2210/pdb1trqw/pdb>
- Lin, C. Y., & Boxer, S. G. (2020). Unusual spectroscopic and electric field sensitivity of chromophores with short hydrogen bonds: GFP and PYP as model systems. *The Journal of Physical Chemistry B*, 124(43), 9513-9525. <https://doi.org/10.2210/pdb60G9/pdb>
- Yip, K. M., Fischer, N., Paknia, E., Chari, A., & Stark, H. (2020). Atomic-resolution protein structure determination by cryo-EM. *Nature*, 587(7832), 157-161. <https://doi.org/10.2210/pdb7A6A/pdb>

