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Identifying Key Drivers of Nanobody Crystallization using Machine Learning

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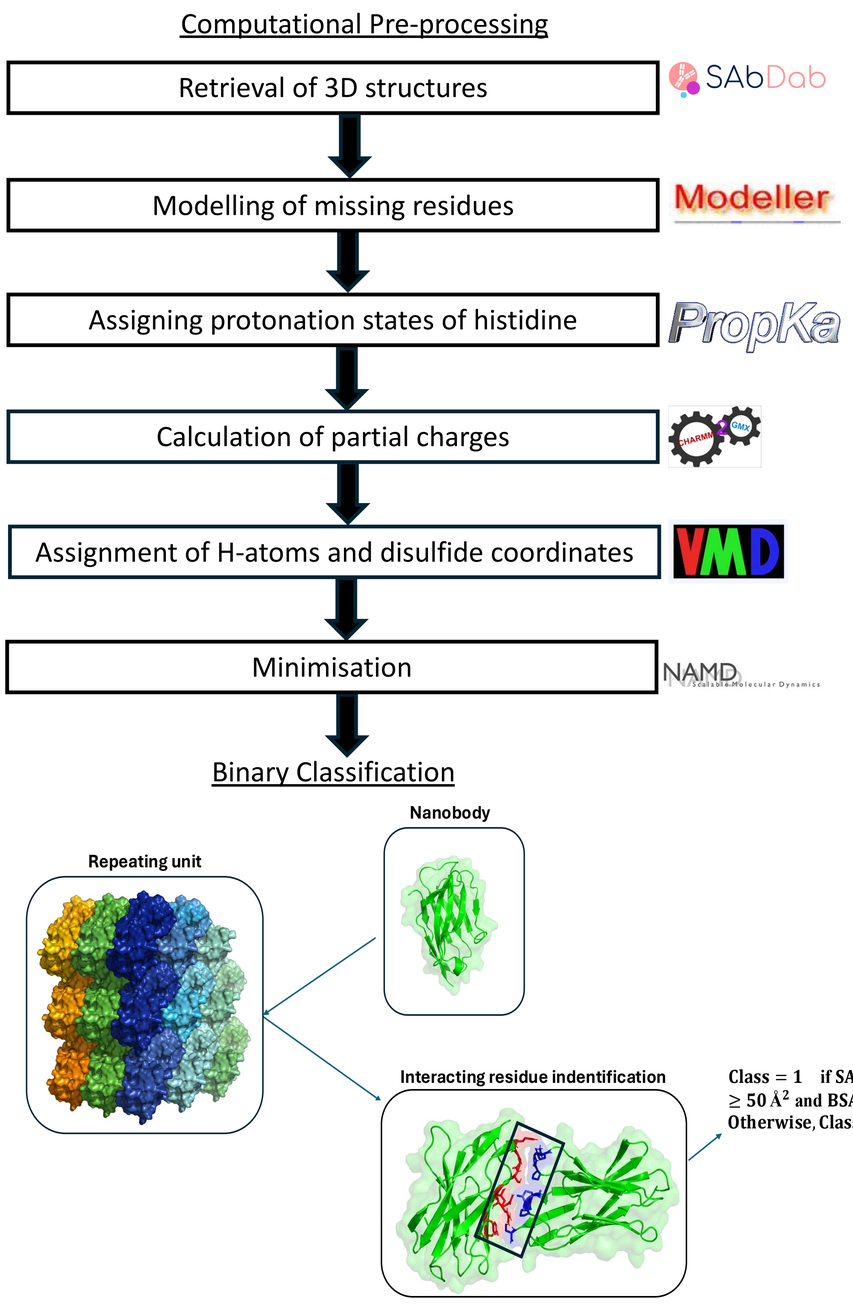
INTRODUCTION

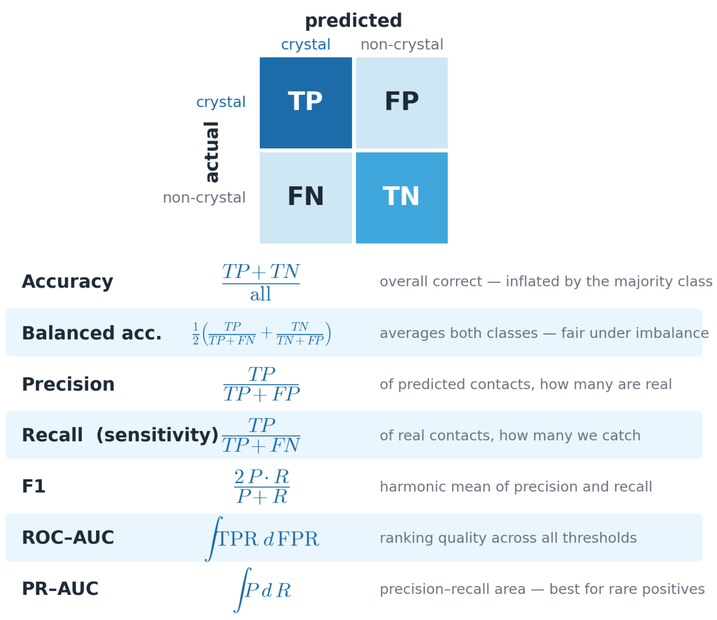
Antibody-based therapeutics are essential biopharmaceuticals, yet their crystallization—crucial for structural characterization, downstream processing, and advanced formulation—remains severely hindered by their large size, conformational flexibility, and a scarcity of structural data. To overcome this bottleneck, this study aims to decode the residue-level determinants of crystal formation and establish a machine learning framework to rationally predict crystallization-enhancing mutations. Using nanobodies as a highly tractable proof-of-concept system, we curated monomeric structures to classify interface residues as crystal-site or non-crystal-site based on different residue level decsriptors.

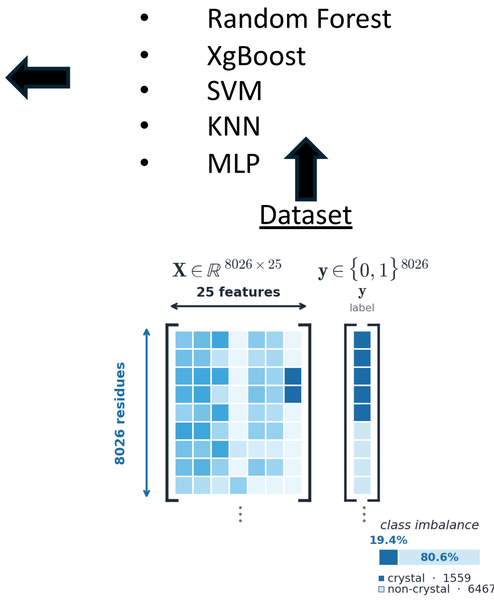
AIM

To identify the specific molecular rules governing **crystallization propensity** by evaluating key structural features through optimized machine learning algorithms, ultimately providing a predictive tool to guide the **mutation design in nanobodies**.

METHOD



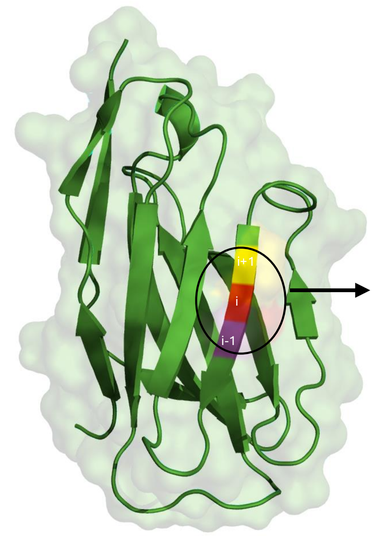




Feature engineering

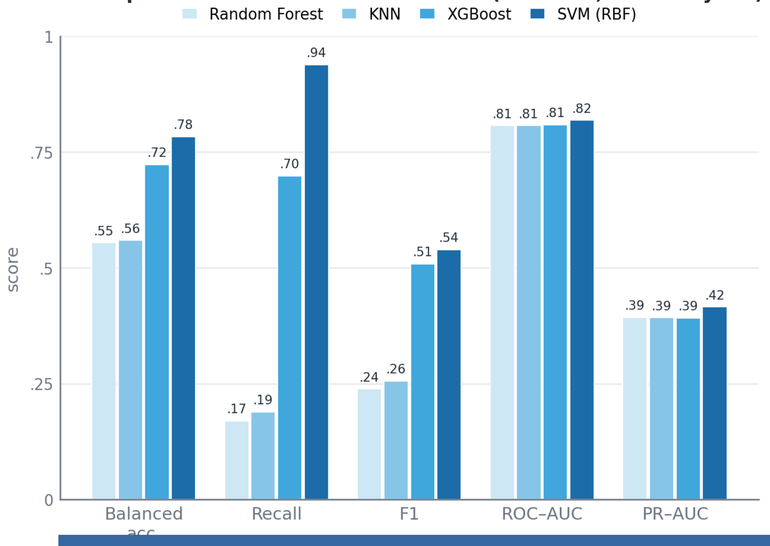
Feature extraction per residue

- Solvent accessible surface area
- Protrusion index
- Fractional exposure
- Spatial aggregation propensity
- Charge(sum of partial charges)
- Hydrophobicity
- Depth index



i_{nb} [≤ 5 Å and ≤ 7 Å
h
Type of residue any type, hydrophobicpolar-charged, hydrophobic-charged, & charged-polar

RESULTS



Model performance

held-out residues · n = 1606 · 19.4% crystal

Model	Precision	Recall	F1	Balanced acc.	ROC-AUC	PR-AUC
Random Forest	**.40**	.17	.24	.55	.81	.39
KNN	.40	.19	.26	.56	.81	.39
XGBoost	.40	.70	.51	.72	.81	.39
SVM (RBF)	.38	**.94**	**.54**	**.78**	**.82**	**.42**

shaded = best model for that metric

DISCUSSION

These preliminary results show comparable ranking power across all four classifiers (ROC-AUC ≈ 0.81). The imbalance-aware SVM and XGBoost recover crystal-contact residues well (recall 0.94 and 0.70), suggesting the SAP-adjacent-FE and surface-exposure descriptors carry real signal, though precision stays low at 19.4% positives. Full 10-fold cross-validation and SHAP analysis are still pending.

FUTURE WORK

1. 10-fold cross-validation, structure-grouped to prevent leakage
2. SMOTE oversampling to counter class imbalance
3. Compare SMOTE against current class-weighting approach
4. In silico mutations
5. Finally,validate these mutations *in vitro*

REFERENCES

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