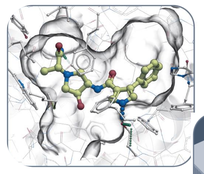

Binding Sites Identification of ClfA-Annexin A2 Complex using Protein-Protein Docking Meta-Approach

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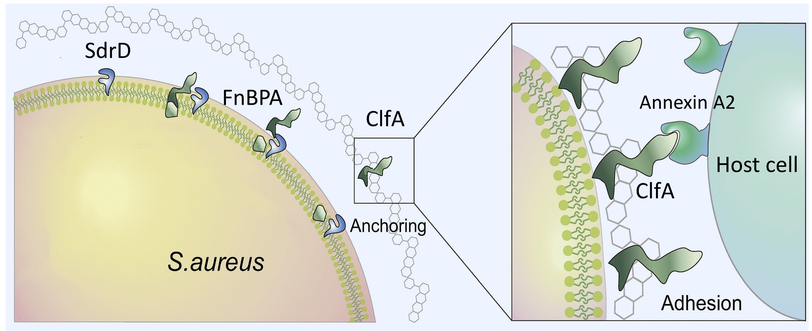
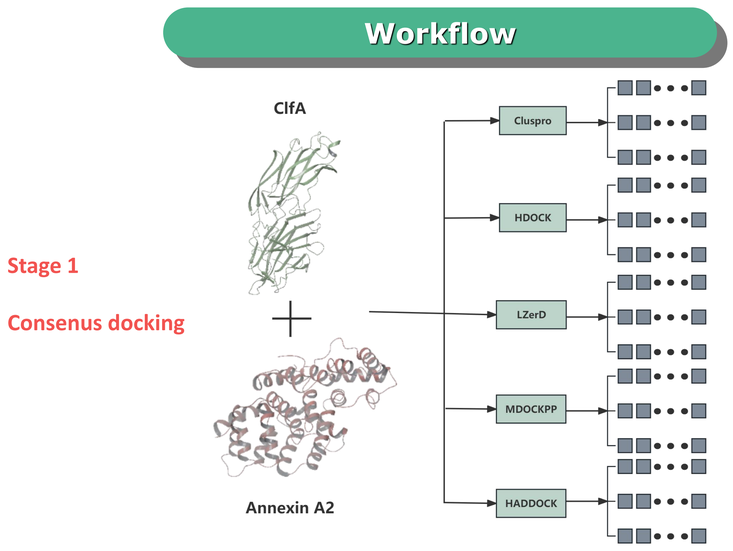
Introduction

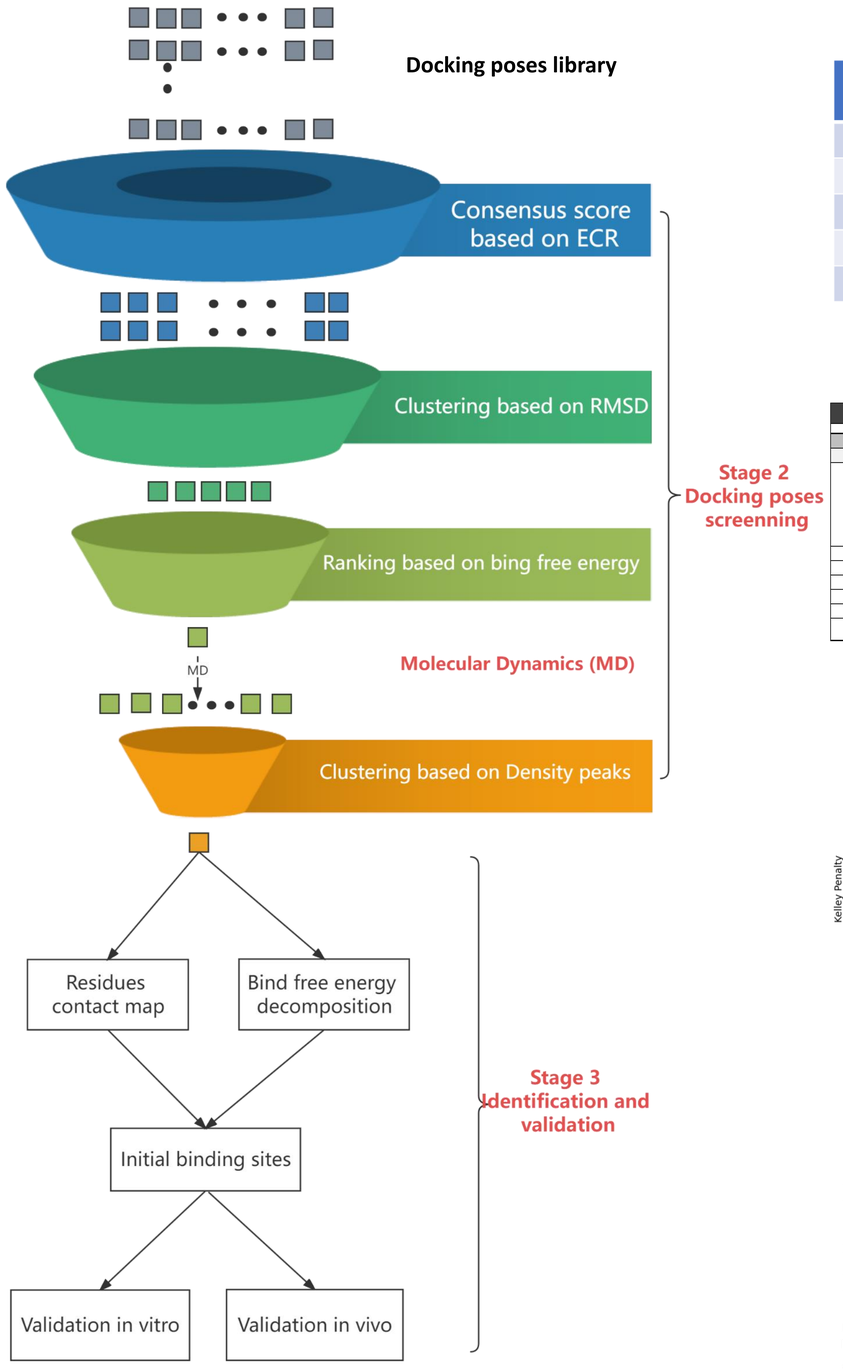
S. aureus **is a leading cause of contagious mastitis in cattle and has long challenged effective control measures in the dairy industry (Côté-Gravel et al., 2019). As global trends shift towards antibiotic-free animal production and given the suboptimal efficacy of vaccines against bovine mastitis (Rainard et al., 2017), alternative strategies are being explored to enhance bovine resistance to *S. aureus*-induced infections. Among the arsenal of virulence factors employed by *S. aureus*, adhesins stand out. Adhesins play a crucial role in facilitating *S. aureus* adherence to host cell surfaces, thereby laying the foundation for infection initiation. Notably, twenty-four adhesins have been identified, each bearing a signature LPXTG binding motif at the A domain and a flexible repeat domain (Foster, 2019). Clumping factor A (ClfA) takes center stage as the primary *S. aureus* adhesin responsible for anchoring the bacterium to the host cell (Claes et al., 2017). Our previous research has unveiled a direct interaction between ClfA and Annexin A2 on the surface of bovine mammary epithelial cells (Ashraf et al., 2017). Furthermore, it has been demonstrated that the ClfA-Annexin A2 complex exhibits remarkable binding strength, withstanding substantial external mechanical forces, in the range of 1500-1700 pN (Wang et al., 2023). Despite the pivotal role of this binding partnership, the precise binding sites responsible for conferring this exceptional covalent binding capability remain shrouded in obscurity. In this study, we employed a protein ensemble docking in conjunction with a Meta-approach to discern the pivotal residues involved in the robust binding process within the ClfA-Annexin A2 complex. To heighten predictive accuracy, we harnessed the capabilities of five distinct protein docking tools. Through the exponential consensus ranking (ECR)-based consensus docking strategy, our aim is to dwarf the limitation derived the single docking tool and heighten predictive accuracy. Furthermore, we complemented this approach with molecular dynamics simulations, followed by and Root Mean Squared Deviation (RMSD) based clustering to ensure the selection of the most favorable docking poses from the docking pose library. To substantiate these predictions, we conducted an alanine replacement to induce mutations at the predicted binding sites, thus allowing for the evaluation of the *in silico* changes in binding free energy. To further validate our findings, we plan to perform GST-pull down assays and Coimmunoprecipitation assays, both *in vitro* and *in vivo*, to confirm the roles of the predicted key residues. Our anticipated results will provide more profound understanding of the pathogenic mechanisms underpinning *S. aureus* infections in the mammary gland. Ultimately, this knowledge will provide a pathway for the development of more effective prevention and therapeutic strategies, delivering valuable benefits to the dairy industry.**



Workflow



Workflow



Results

Annexin A2 ensembles generation by MD

The best ensembles (Top 5)	1000ns*2 (5000 frames)	RMSD with docking	PCA vaule
Top1	1807	1.71	-10.0,3.4
Top2	1250	1.73	-11.2,4.9
Top3	679	1.61	-12.5,3.4
Top4	4776	1.82	17.1,-2.5
Top5	4773	1.84	17.0,5.3

Consensus score based ECR

Consensus docking

STEP 1

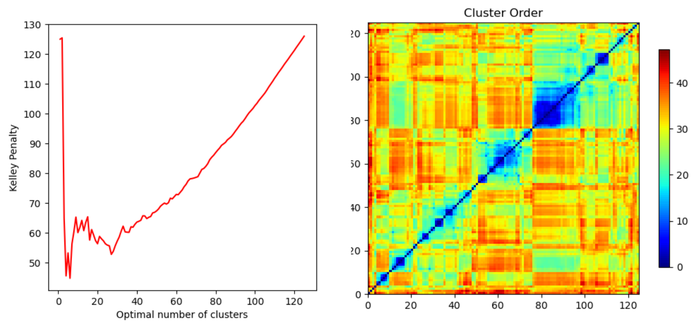
Consensus ranking for binding poses for each ClfA, using the exponential consensus ranking (ECR) formula:

$$P(i) = \frac{1}{\sigma}\sum_{j=1}^{p} \exp\left(-\frac{R^{j}_{i,\lambda}}{\sigma}\right)$$

	1N67		2VR3		5JQ6	
	ECR	Ranking $R_{i,\lambda}$	ECR	Ranking	ECR	Ranking
Annexin A2 TOP 1	0.397	2	0.427	1	0.391	3
Annexin A2 TOP 2	0.404	1	0.402	2	0.395	2
Annexin A2 TOP 3	0.388	3	0.331	5	0.337	5
Annexin A2 TOP 4	0.323	5	0.359	3	0.396	1
Annexin A2 TOP 5	0.359	4	0.351	4	0.352	4

Legend: the first column is the name of the ligands and the first line the name of the macromolecule. Which macromolecule has the ECR and ranking.

Clustering of docking poses



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