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Understanding K. phaffii (Pichia pastoris) Host Cell Proteins: Proteomic Analysis and Flow-through Affinity Clearance

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Abstract

K. phaffii is a versatile expression system that is increasingly utilized to produce biological therapeutics – including enzymes, engineered antibodies, and gene-editing tools – that feature multiple subunits and complex post-translational modifications. Two major roadblocks limit the adoption of *K. phaffii* in industrial biomanufacturing: its proteome, while known, has not been linked to downstream process operations and detailed knowledge is missing on problematic host cell proteins (HCPs) that endanger patient safety or product stability; furthermore, the purification toolbox has not evolved beyond the capture of monospecific antibodies, and few solutions are available for engineered antibody fragments and other protein therapeutics. To unlock the potential of yeast-based biopharmaceutical manufacturing, this study presents *(i)* a secretome survey of *K. phaffii* cell culture harvests that highlights HCPs with predicted immunogenicity, ability to cause product instability by proteolysis or degradation of excipients, and potential to interfere with purification operations via product association or co-elution; and *(ii)* a novel affinity adsorbent functionalized with peptide ligands that target the whole spectrum of *K. phaffii* HCPs – PichiaGuard – designed for the enrichment of therapeutic proteins in flow-through mode. The PichiaGuard adsorbent features high HCP binding capacity (˜25 g per liter of resin) and successfully purified a monoclonal antibody and an ScFv fragment from clarified *K. phaffii* harvests, affording up to 80% product yield, and a >300-fold removal of HCPs. Notably, PichiaGuard outperformed commercial ion exchange and mixed-mode resins in removing high-risk HCPs – including aspartic proteases, ribosomal subunits, and other peptidases – thus demonstrating its value in modern biopharmaceutical processing.

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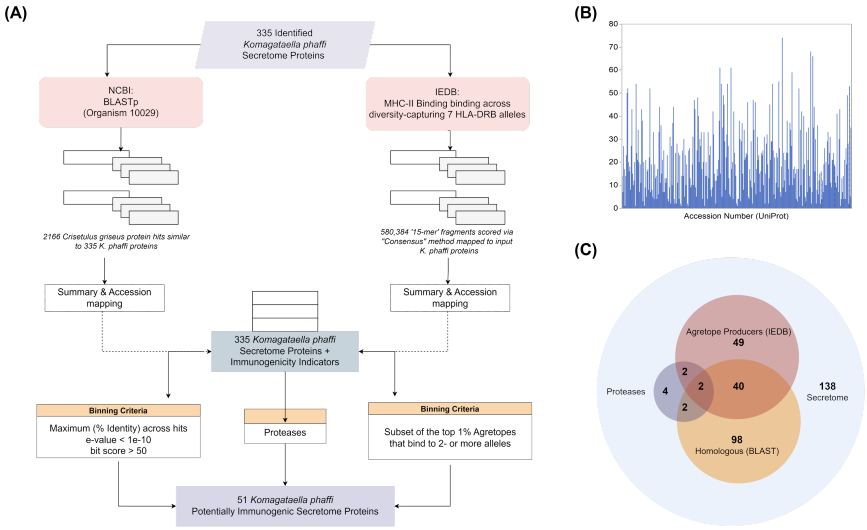

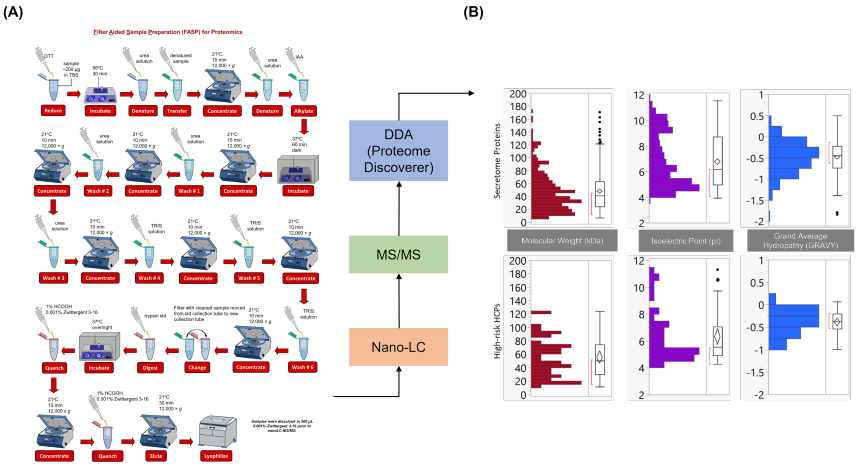

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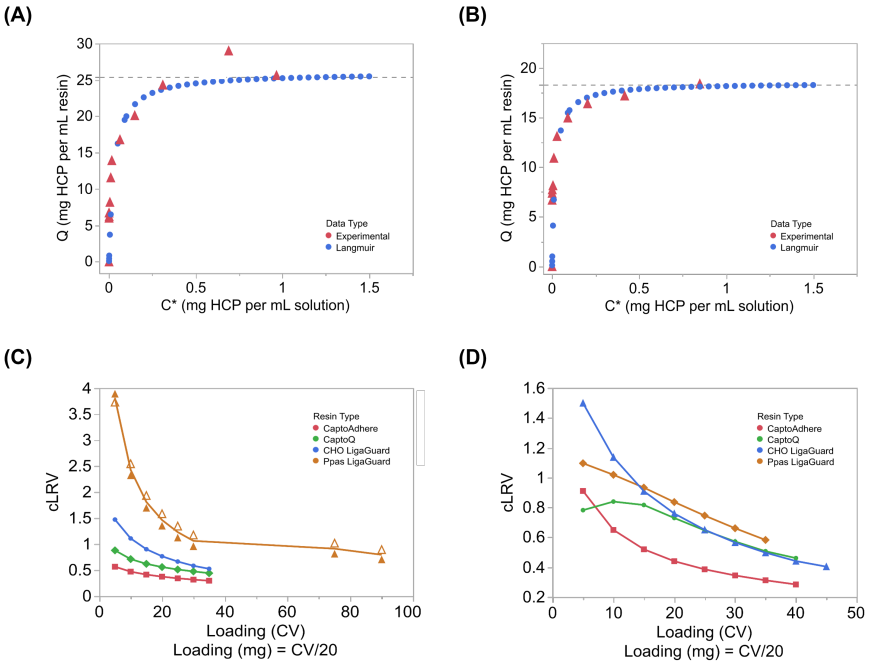

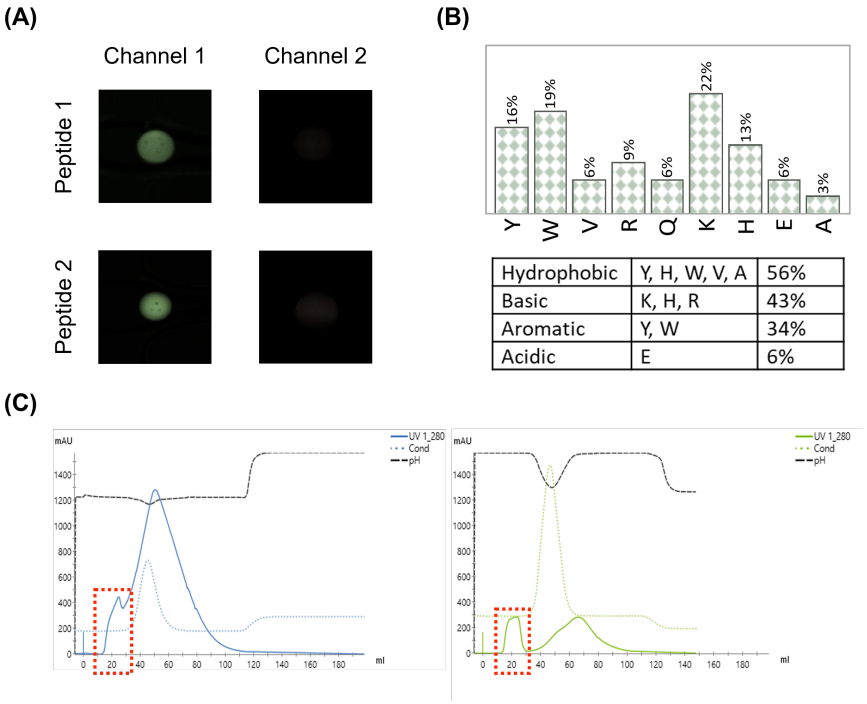

Hydrophobic	Y, H, W, V, A	56%
Basic	K, H, R	43%
Aromatic	Y, W	34%
Acidic	E	6%

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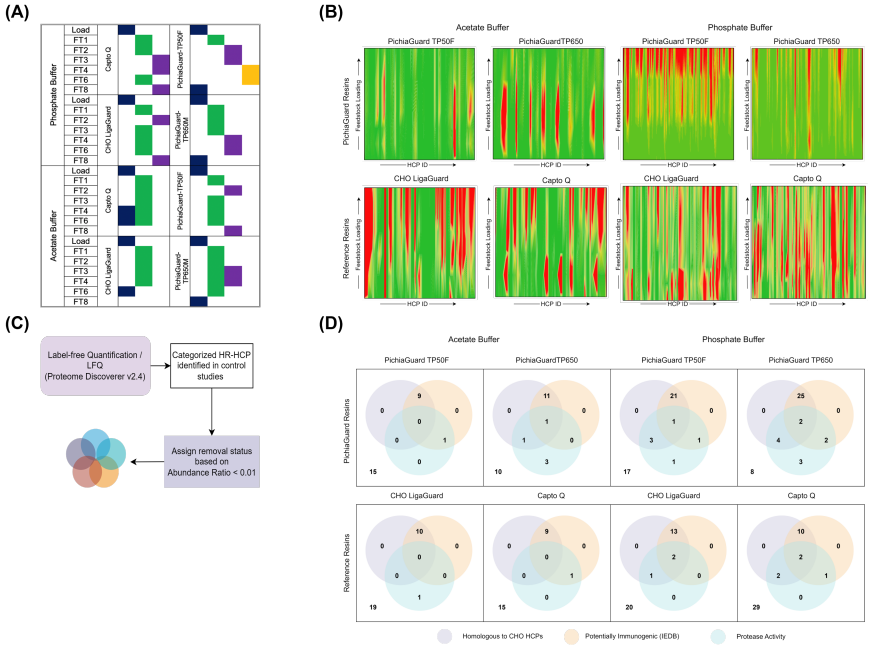

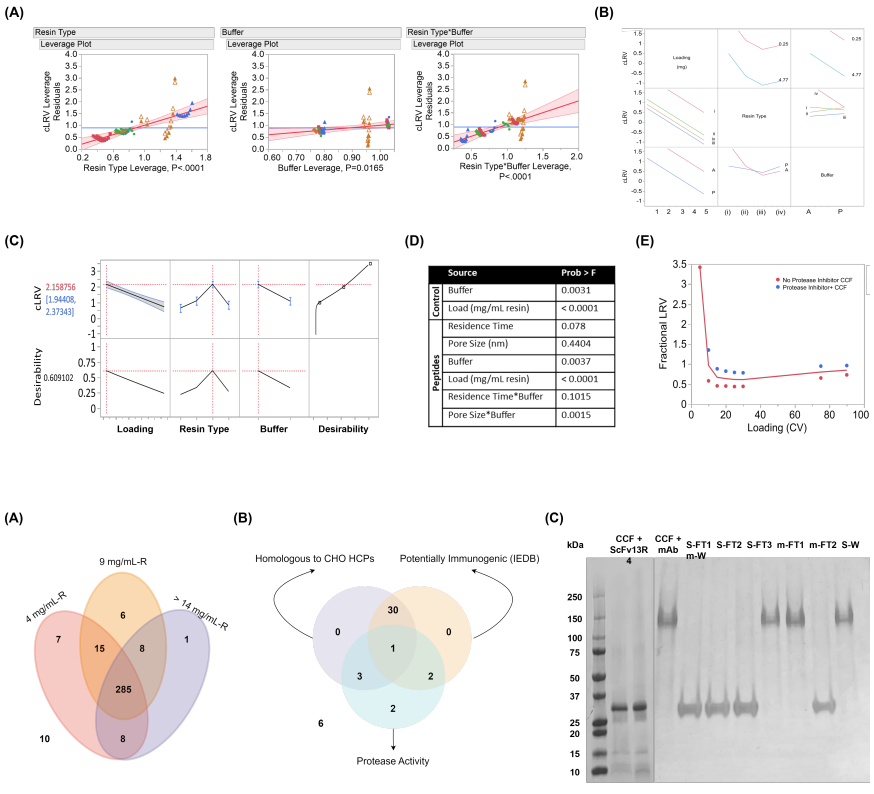

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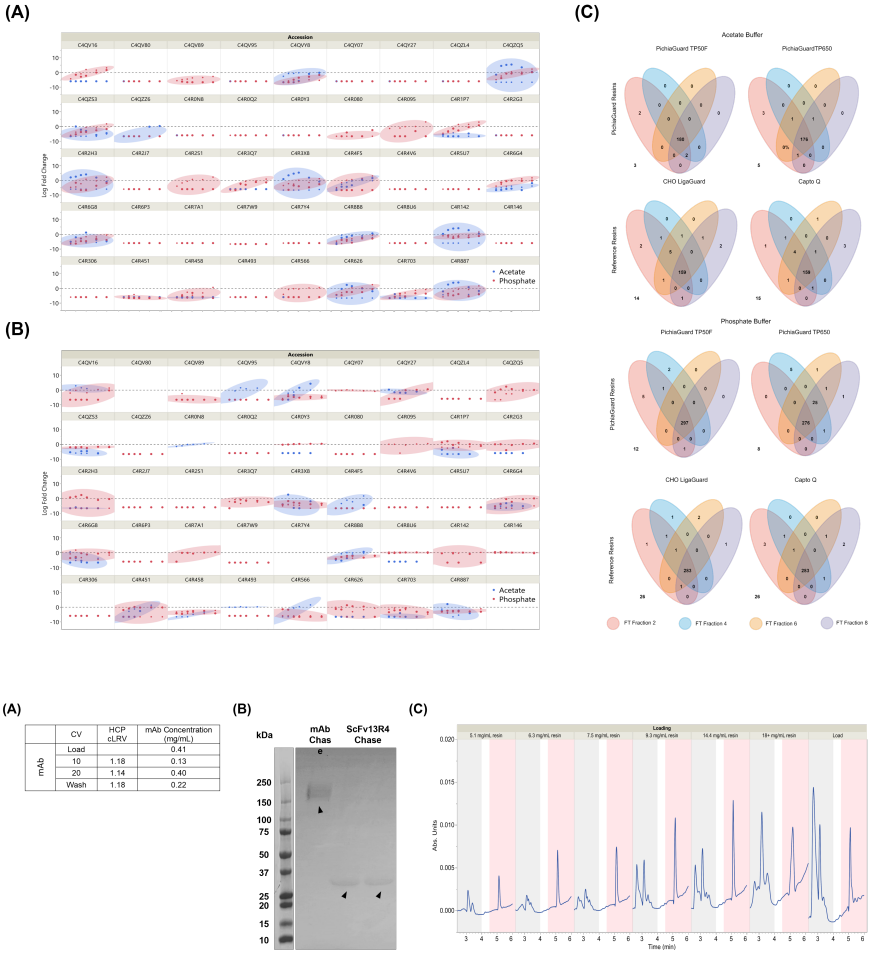

(A)

	CV	HCP cLRV	mAb Concentration (mg/mL)
mAb	Load		0.41
	10	1.18	0.13
	20	1.14	0.40
	Wash	1.18	0.22

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