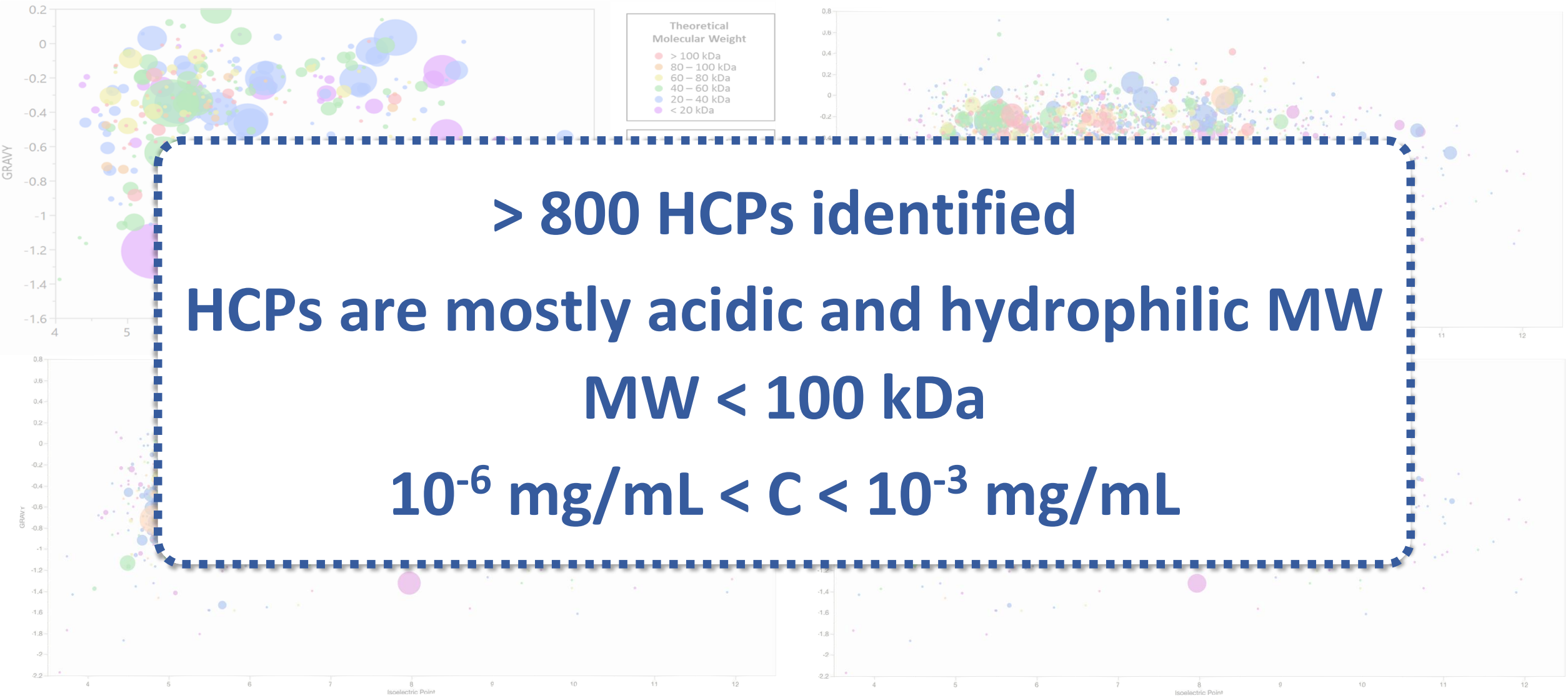
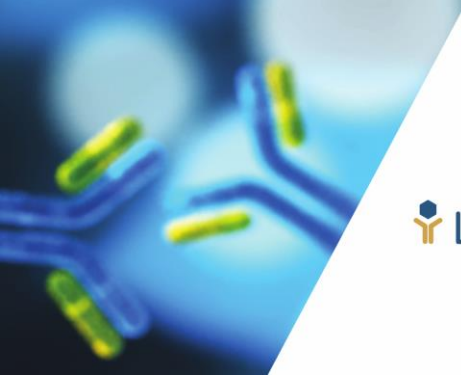




Target Impurities: HCPs

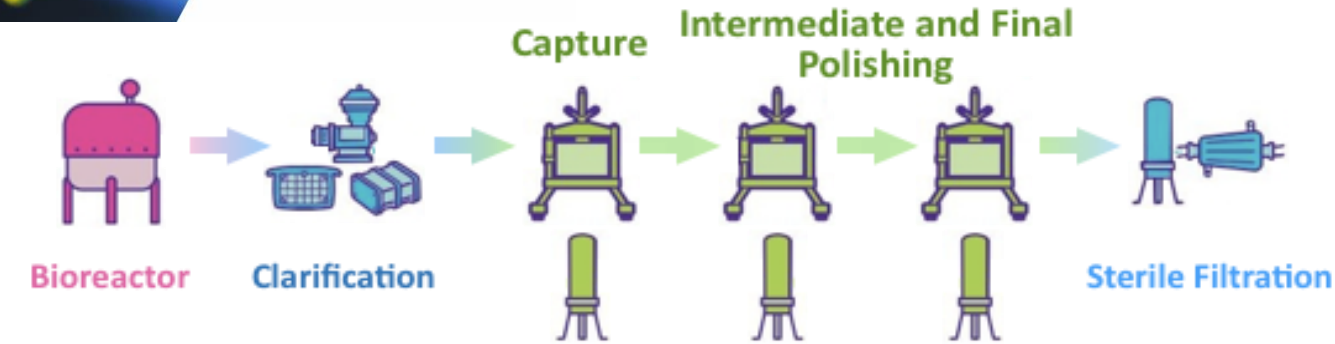
Proteomic Analysis of CHO harvests



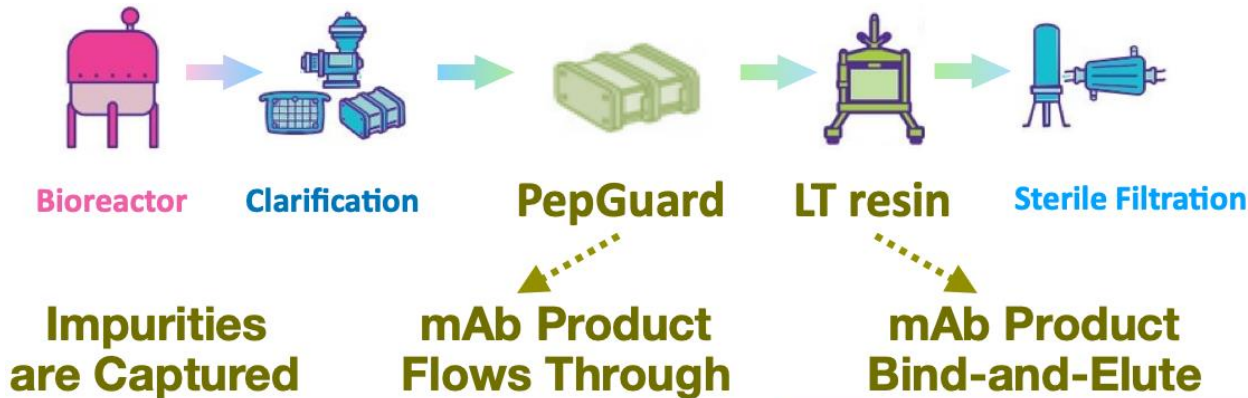


LigaTrap Technology

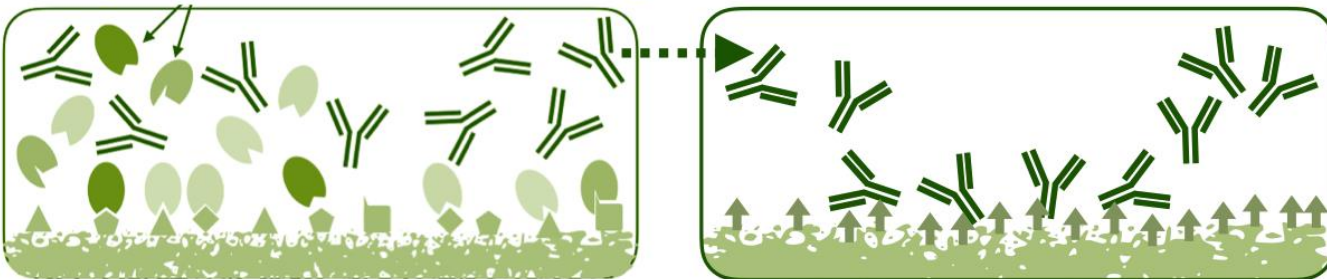
Transform mAb Manufacturing

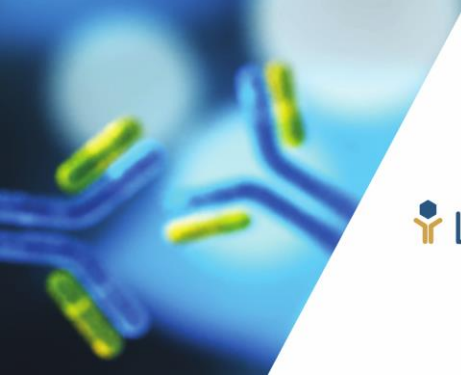


From a
3-phase batch process



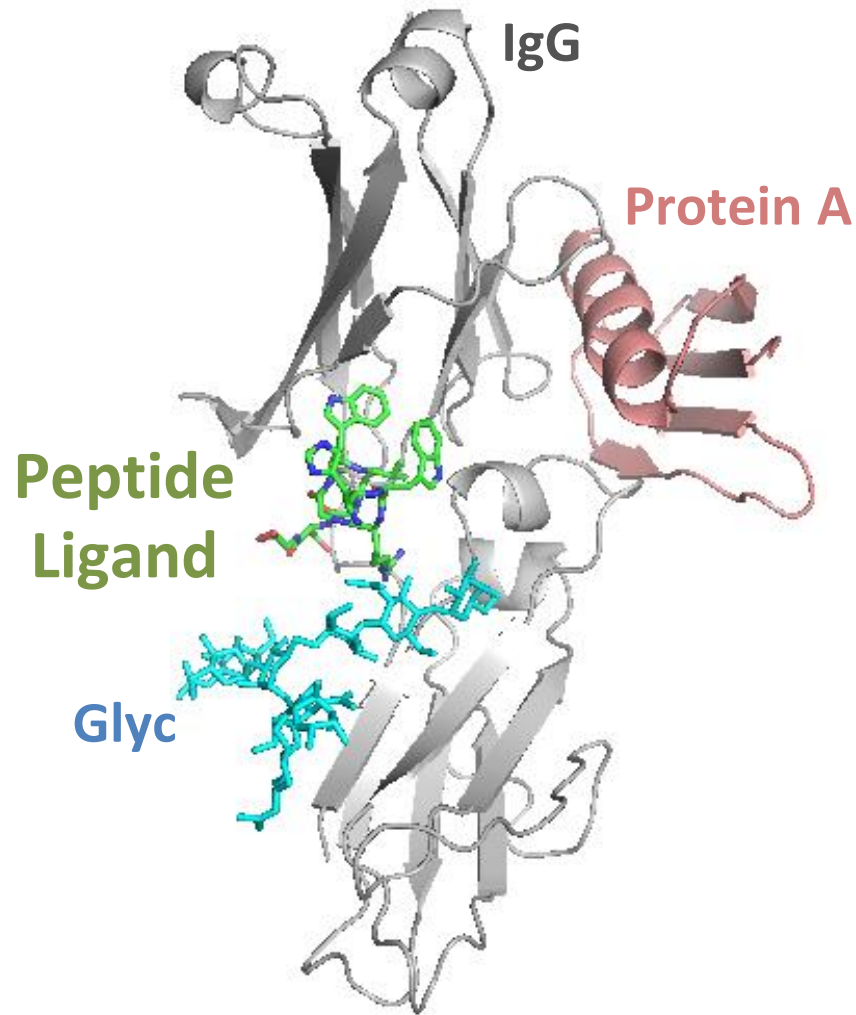
to a
Continuous Process
with
Peptide-based Media





LigaTrap Technology

mAb Capture and Concentration



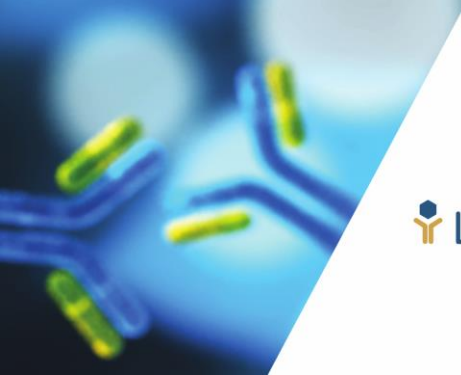
IgG-binding peptide
 $K_D \sim 10^{-6} \text{ M} \rightarrow$ Elution at pH 4
Binds all human IgG subclasses
Binds animal IgG
Safe upon re-use (> 100 cycles)



Binding capacity > 40 mg mAb / mL_R (RT: 2 min)

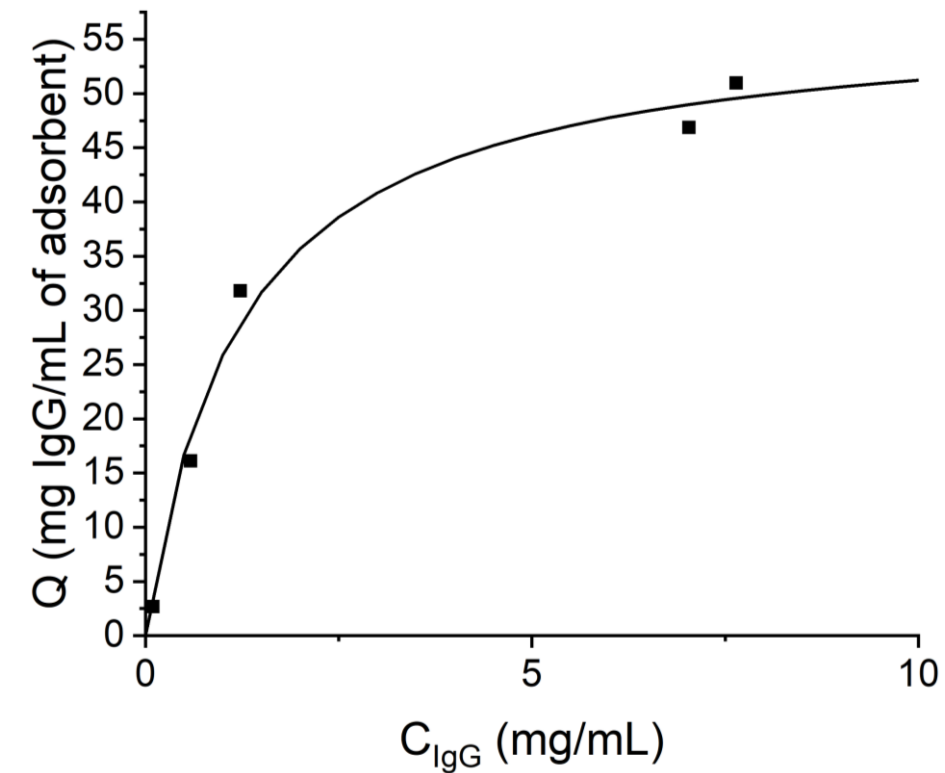
HCP LRV ~ 2.15 (direct harvest)

HCP LRV: log removal of protein impurities

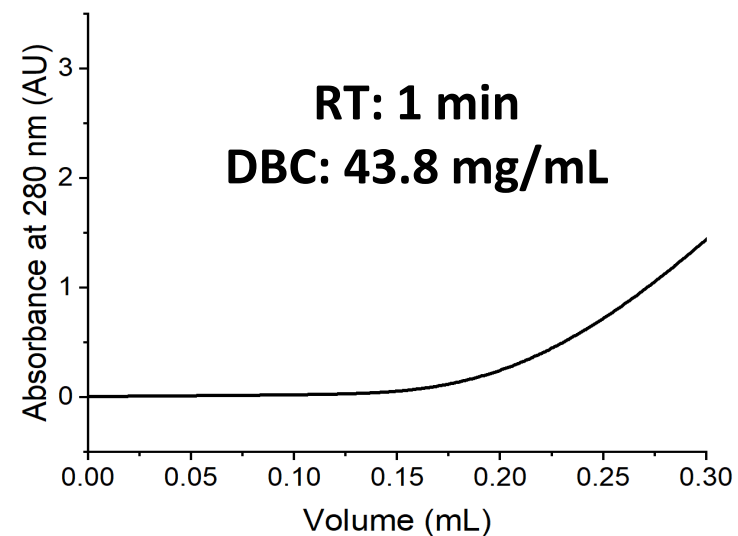
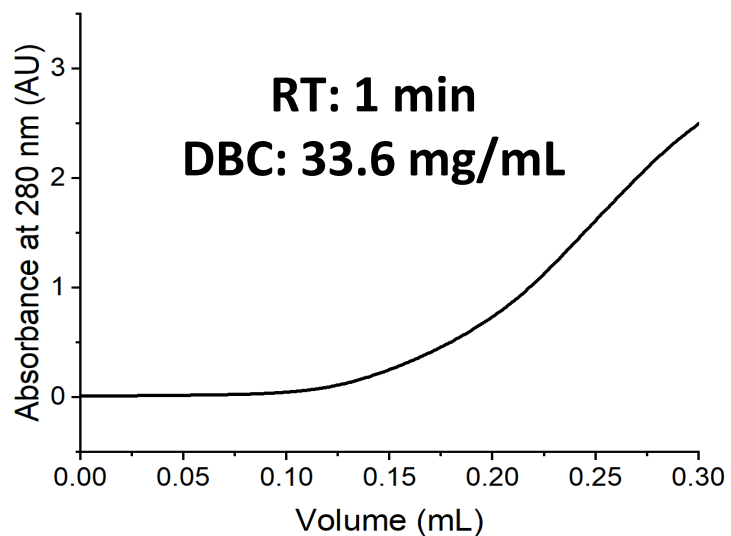
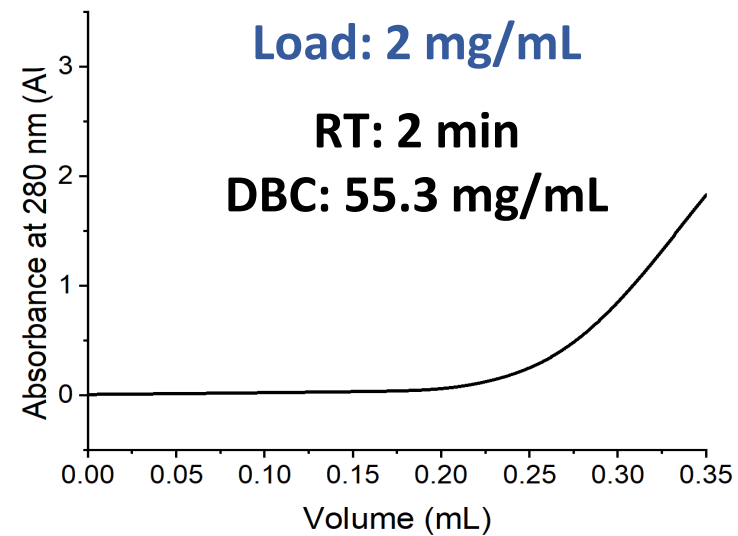
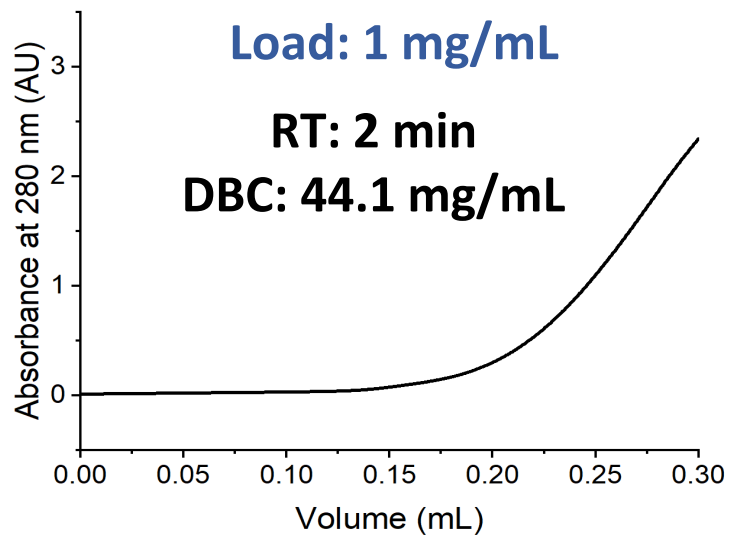


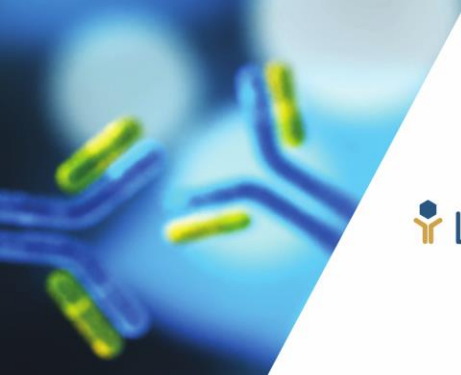
LigaTrap Technology

Binding Performance



Reusability > 100 cycles

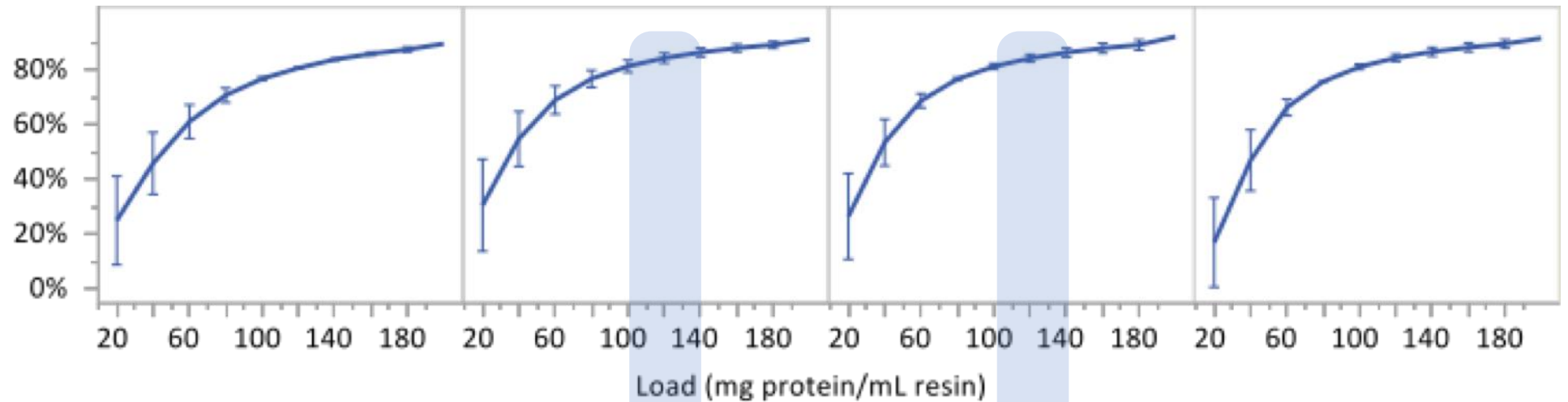




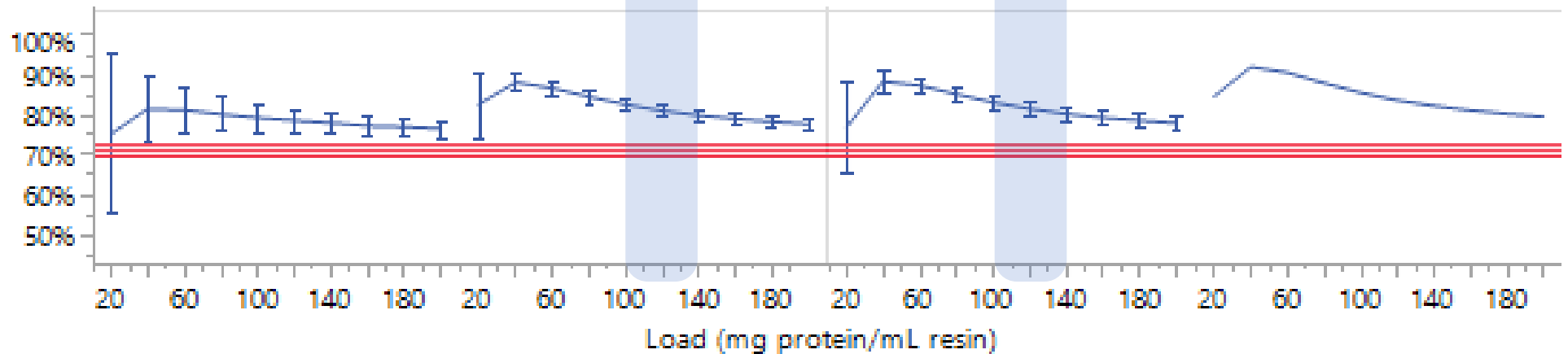
LigaGuard Technology

mAb Yield and Purity

mAb Yield



mAb Purity



“Problematic” HCPs



Protein	R1	R2	R3	Protein	R1	R2	R3
60S acidic ribosomal protein P0	■		■	Glutathione S-transferase P	■		■
60S acidic ribosomal protein P2	■			Glyceraldehyde-3-phosphate dehydrogenase	■		
Actin, alpha cardiac muscle 1	■			Heat shock cognate protein	■	■	
Actin, cytoplasmic 1	■		■	Legumain		■	
Aldose reductase-related protein 2	■			Lipoprotein lipase	■	■	
Alpha-enolase	■			Matrix metalloproteinase-19	■	■	
Cathepsin B	■	■		Metalloproteinase inhibitor 1	■		
Cathepsin D	■	■		Nidogen-1	■		
Clusterin	■			Peroxiredoxin-1			■
Cofilin-1	■			Phosphoglycerate mutase 1	■		
Elongation factor 1-alpha	■			Phospholipase-B-like protein	■		■
Elongation factor 2	■			Pyruvate kinase	■		
ER chaperone BiP		■		Sialidase 1		■	
Fructose-biphosphate aldolase	■			Vimentin	■		■

R1: Co-elute with mAbs product from Protein A

R2: Highly toxic or immunogenic

R3: Trigger mAb instability



“Problematic” HCPs

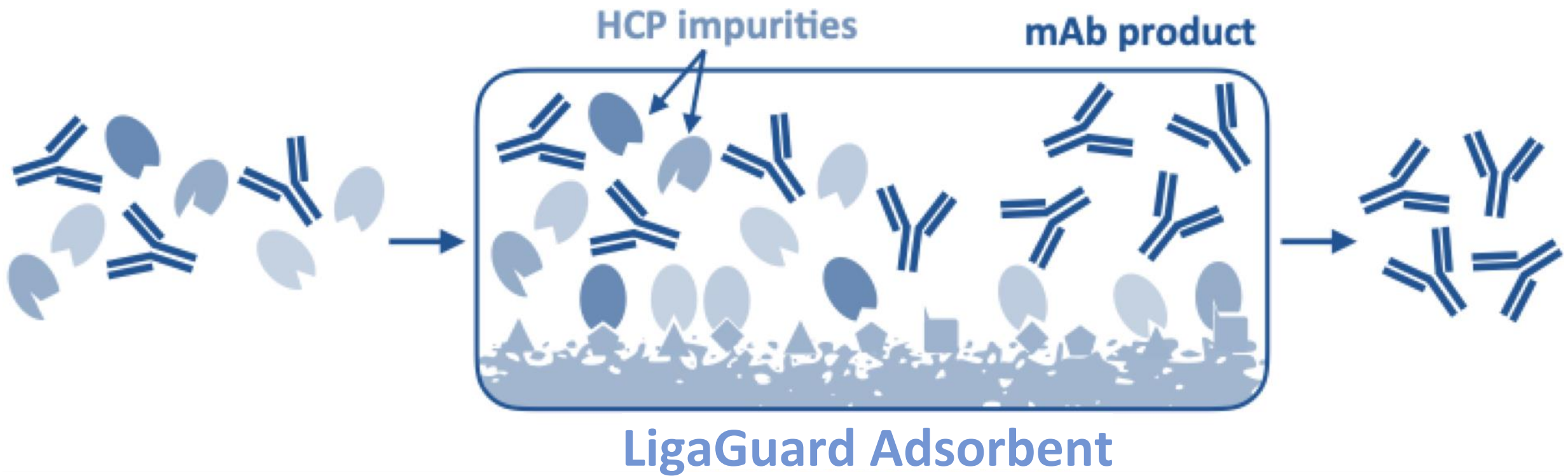
Protein	R1	R2	R3	Protein	R1	R2	R3
60S acidic ribosomal protein P0	█		█	Glutathione S-transferase P	█		█
60S acidic							
Actin, alpha							
Actin							
Aldose reductase							
Al							
Elongation factor 2	█			Pyruvate kinase	█		
ER chaperone BiP		█		Sialidase 1		█	
Fructose-biphosphate aldolase	█			Vimentin	█		█

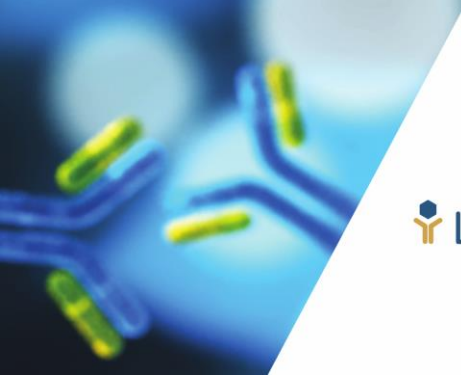
HCP LRV > 3 (down to < 100 ppm)
Difficult-to-remove HCPs
Threat to product safety

mAbs
Protein A
acidic or basic
Ab

LigaTrap Technology

LigaGuard





LigaTrap Technology

HCP Clearance and mAb Quality

Carboxypeptidase D: R > 98.5%

Cathepsin B: undetected

Cathepsin D: R > 98.5%

Cathepsin L: undetected

Cofilin 1: R > 95%

**Glutathione
undetected**

Enolase 1: R > 99.5%

Human Metalloprot Inh. 1: R > 96.5%

Legumain: undetected

Lipoprotein lipase:

Phospholipase B-like prot.: R > 98.5%

Nidogen 1: undetected

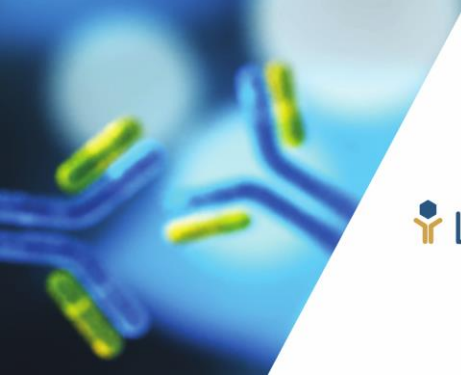
S-transferase: Peroxiredoxin 1: undetected

Peroxiredoxin 4: R > 98.5%

Global Yield ~ 70 - 74 %

Purity > 99.9%

CHO HCP LRV ~ 3.02 (ELISA)



LigaGuard Technology

mAb Yield and Purity

mAb Yield

Cut off Load at ~ 120 mg protein/mL

RT of 1 - 2 min

Yield \geq 85 %; Purity ~ 85 %

@ direct harvest application

Purity in feed ~ 70%

mAb Purity

