

Effective wash solutions to improve removal of E.coli HCP in Protein L chromatography of scFv

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Parallel chromatography

TOSOH

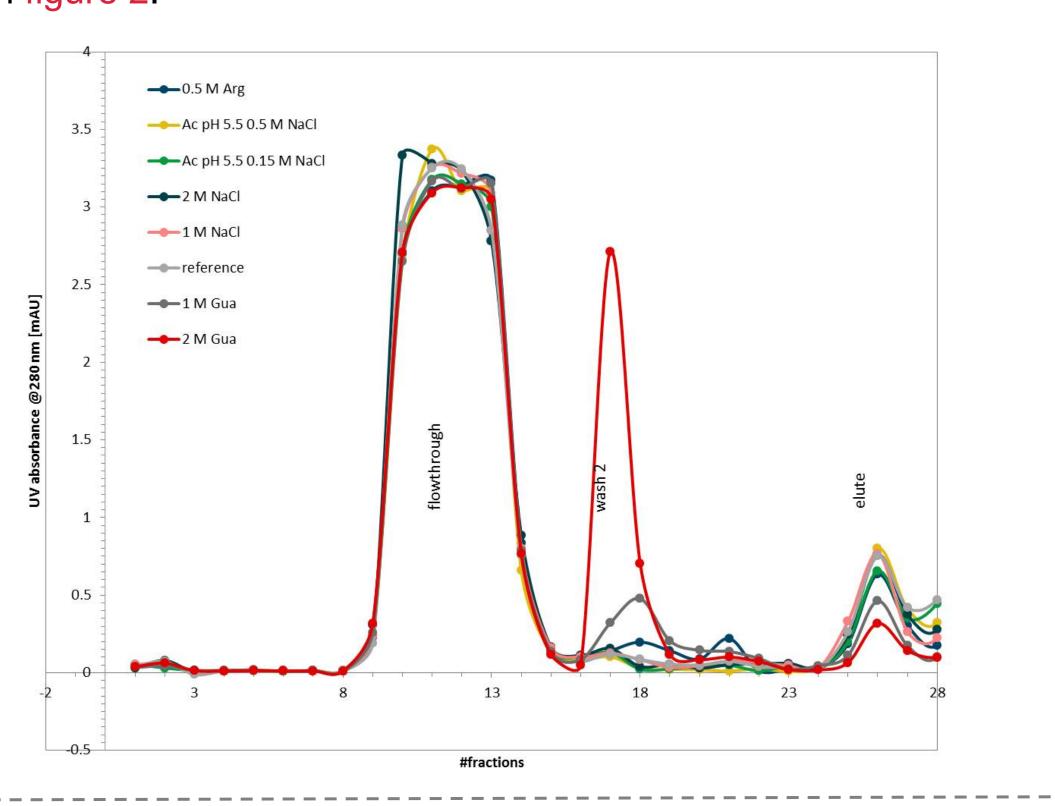
HCP of bacteria or insect cells are sticky compared to HCP of CHO cells. Thus, removal of E.coli derived HCP during affinity chromatography may need to be improved. Miscellaneous wash solutions typically used to reduce the residual amount of HCP after protein A affinity chromatography were evaluated in a parallel chromatography approach.

- 200 μl TOYOPEARL® AF-rProtein L-650F RoboColumns
- scFv from E.coli was loaded @3 min residence time
- Wash procedure: 5 CV reference buffer + 5 CV individual wash solution + 5 CV reference buffer
- Product recovery in 50 mM glycine HCl pH 2.0

The applied wash solutions are shown in table 1:

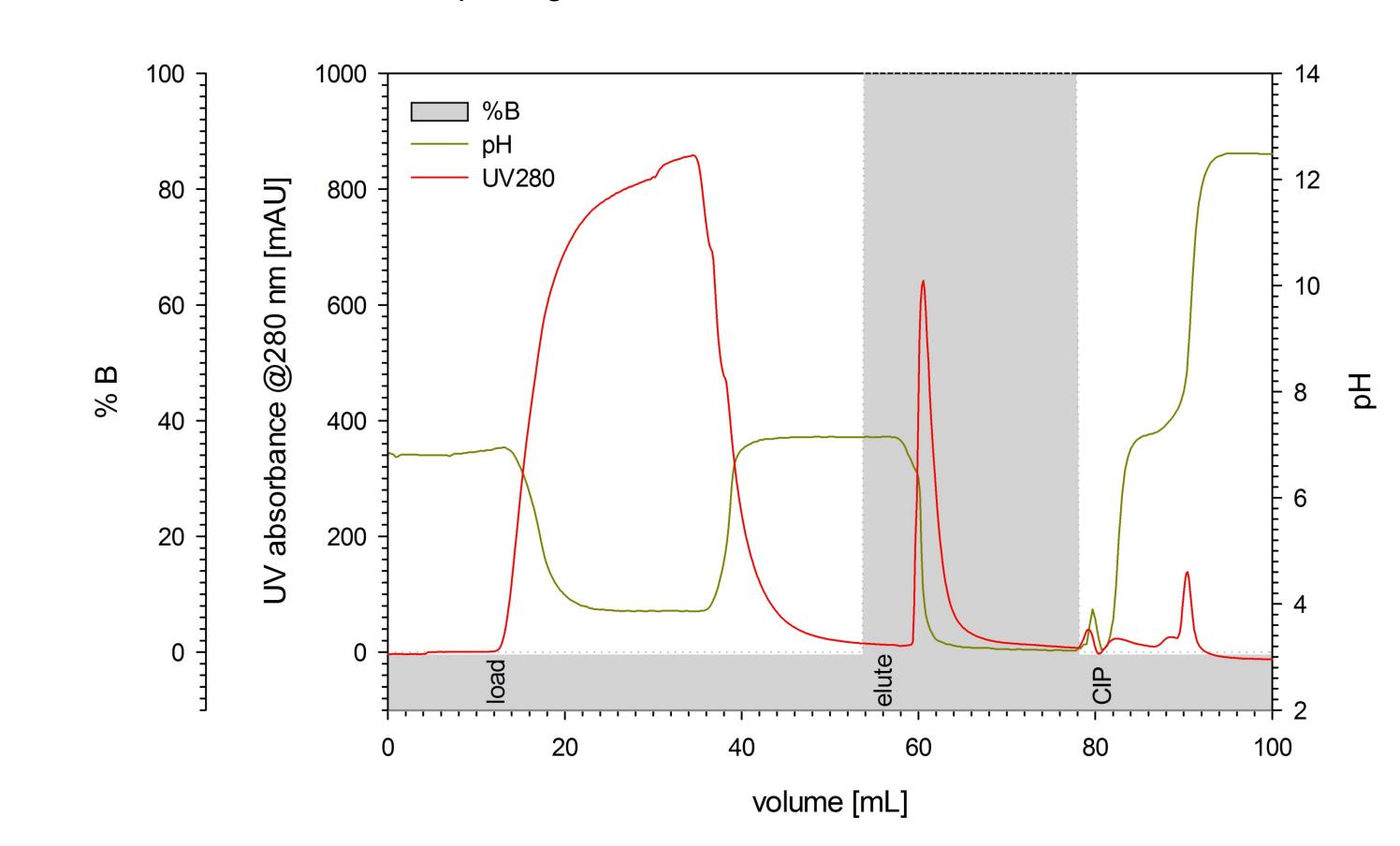
buffer	additive	additive concentration
100 mM NaP, pH 6.5 (reference)	-	_
100 mM NaP, pH 6.5	arginine	1 M, 2 M
100 mM NaP, pH 6.5	guanidinium hydrochloride	1 M, 2 M
100 mM NaP, pH 6.5	sodium chloride	0.5 M
100 mM Na acetate, pH 5.5	sodium chloride	0.15 M, 0.5 M

Pseudo-chromatograms of the parallel chromatography runs are shown in figure 2.



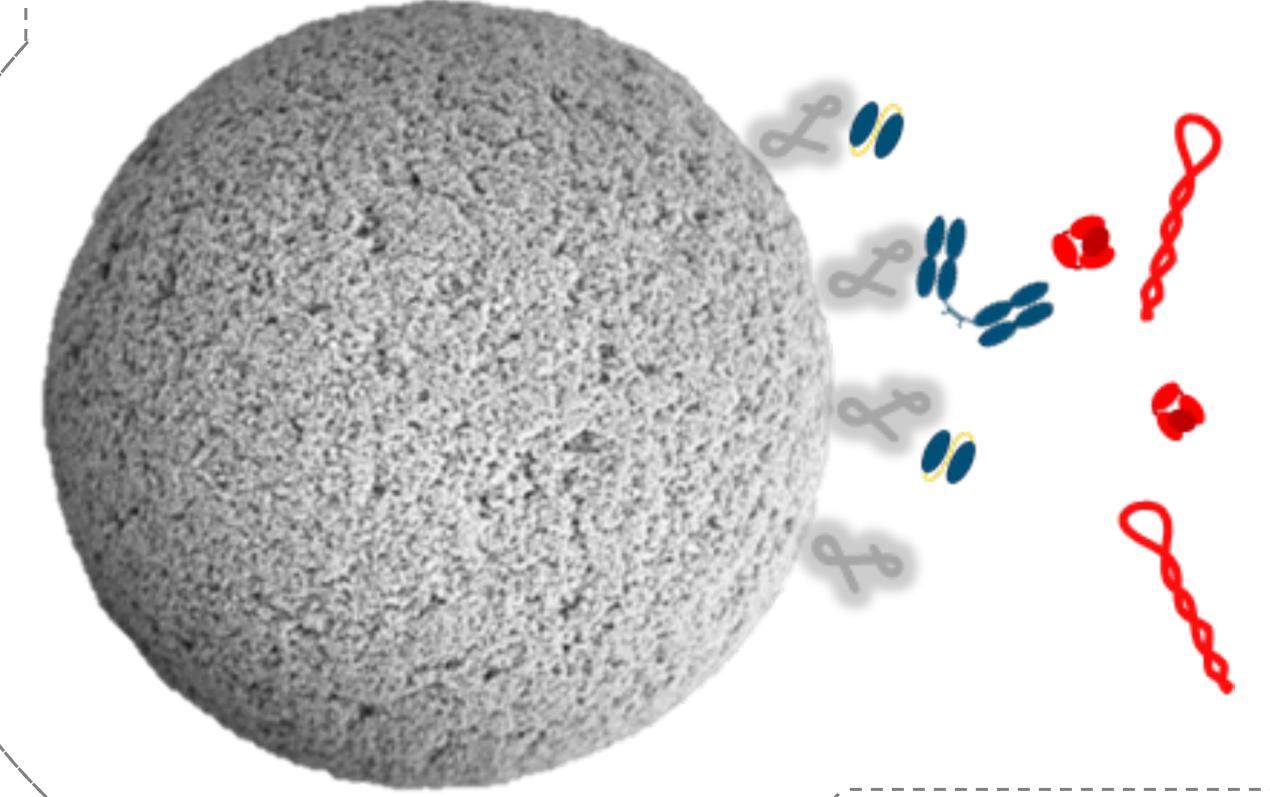
Lab-scale experiment

TOYOPEARL AF-rProtein L-650F is a Protein L affinity chromatography resin based on 45 μ m polymethacrylate particles. Typical target molecules are mAb, f(ab)₂, fab or scFv. Figure 1 shows an exemplary chromatogram of a lab-scale run for scFv capturing from *E.coli*.



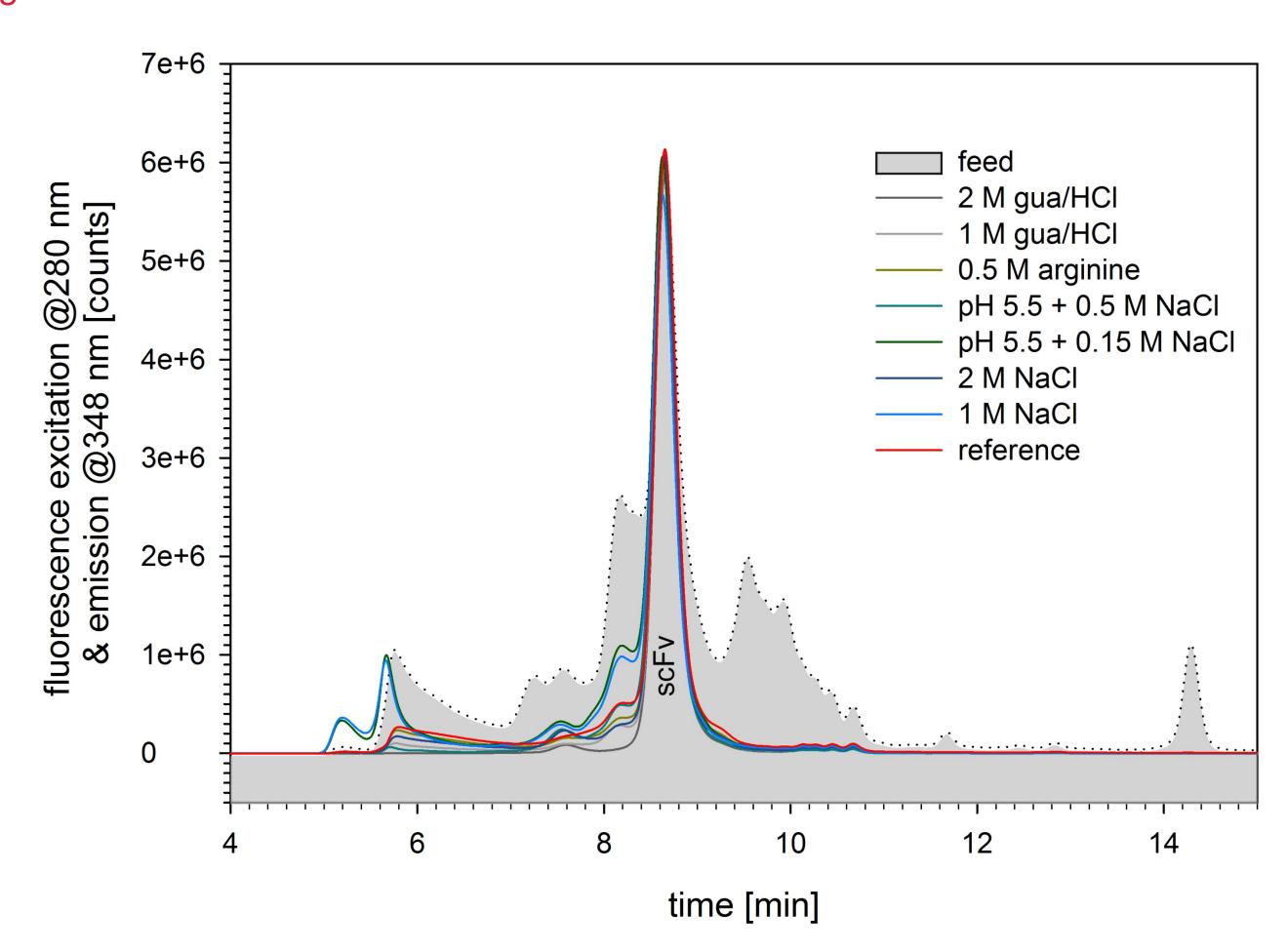
Periplasmic proteins were extracted and 15 CV of the feedstream were loaded on the column at 3 min residence time. HCP flows through the column during loading and washing with 0.1 M sodium phosphate, pH 6.5. The scFv elutes as a sharp peak in 100 mM glycine/HCl, pH 2.0

Acknowledgements: The scFv feedstream produced in *E.coli* was kindly provided by Dr. Oliver Seifert, Institute of Cell Biology and Immunology, University of Stuttgart.



SE-UHPLC

SEC analysis was conducted with TSKgel® G2000SWxl (7.8 mm ID x 30 cm L) at 1.0 mL/min. 100 mM sodium phosphate + 100 mM sodium sulfate, pH 6.7 was used for isocratic elution. 20 µl of the load and elution fractions from the parallel chromatography runs were injected. The corresponding normalized chromatograms are provided in figure 3.

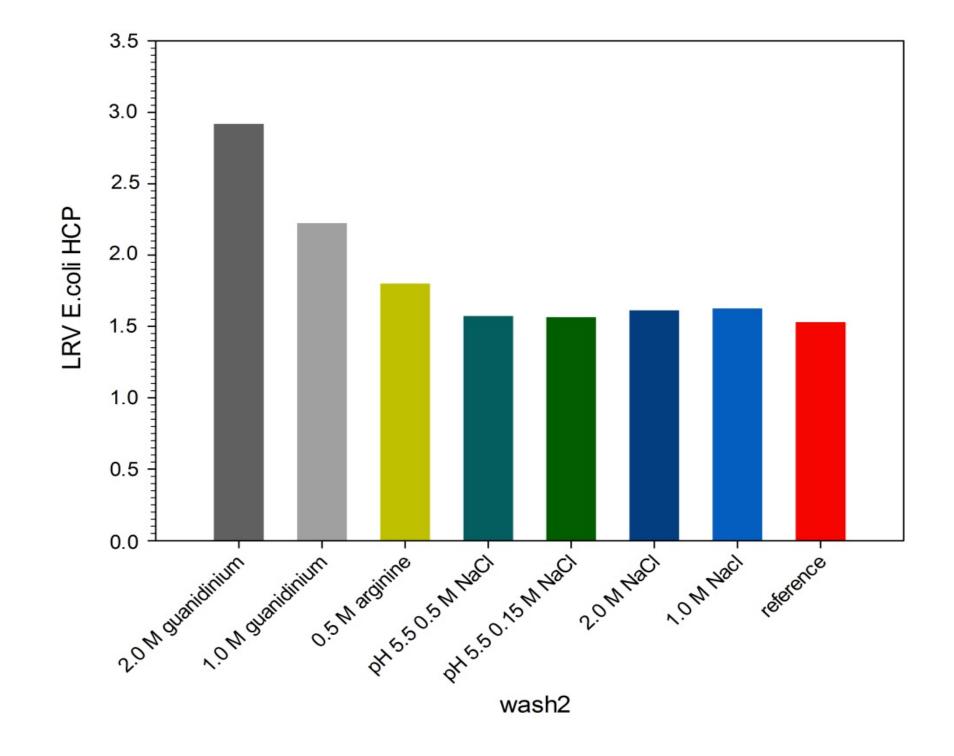


- Peaks eluting directly in front of the scFv probably represent misfolded and aggregated scFv.
- The chromatograms suggest on-column refolding of scFv
- Guanidinium hydrochloride is most efficient with regards to impurity removal and refolding.

HCP clearance

Determination of E.coli HCP contents was conducted with a dedicated commercially available ELISA kit. Log reduction values are given in figure 4.

- Guanidinium hydrochloride increases HCP clearance
- Improvement of > 1 log can be achieved compared to the reference conditions.



Protein L ligand leaching

Figure 5 shows the ligand leaching of TOYOPEARL AF-rProtein L-650F, which was tested after treatment with the different wash solutions. Protein L leaching was determined using a dedicated ELISA kit.

- Protein L leaching < 20 ng/mL.
- The applied solutions do not affect ligand leaching.

