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Microbial extracellular production and affinity separation of industrial relevant proteins by nonfunctionalized magnetic particles

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Introduction

The polyglutamate-tag (E8) binds to nonfunctionalized magnetic nanoparticles (MNPs) with a high affinity and enables a selective capturing. The secretion of polyglutamate-tagged proteins and their direct capturing from the extracellular medium (EM) would be a highly promising approach for integrated bioprocesses. To proof the system, we fused the E8-tag to a nanobody (VHH) and expressed it with the novel enGenes-X-press-system. This system enhances the expression yield of proteins, and more importantly, enables extracellular expression. After the secretion, we incubated the extracellular medium with bare magnetic nanoparticles and could bind the E8-VHH directly.

Working Principle

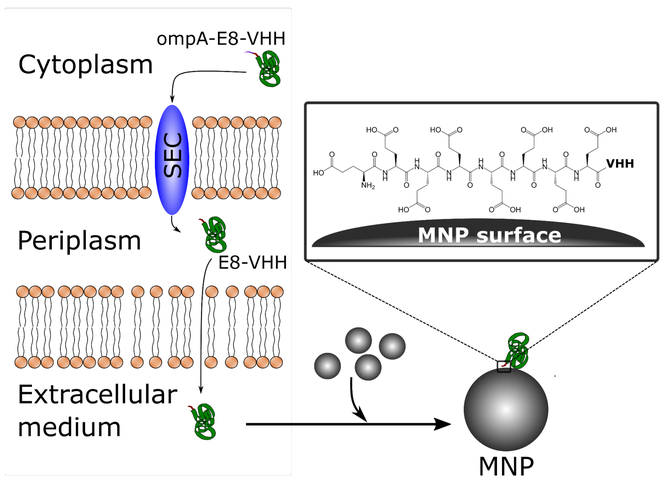


Figure 1: A polyglutamate-tagged nanobody (E8-VHH) was extracellularly expressed in an E. coli enGenes-X-press strain. By the addition of non-functionalized magnetic nanoparticles (MNP), the E8-VHH was selectively captured.

- A nanobody is tagged with the signal peptide ompA and the polyglutamate-tag (E8)
- The nanobody gets secreted via the SEC pathway and the leaky outer membrane of enGenes-X-press strain
- The E8-tagged nanobody is captured by MNPs through the E8-tag

The expression system

The enGenes-X-press strains were compared with the *E. coli* BL21(DE3) regarding growth (see Fig. 2) and secretion (see Fig. 3) of E8-VHH.

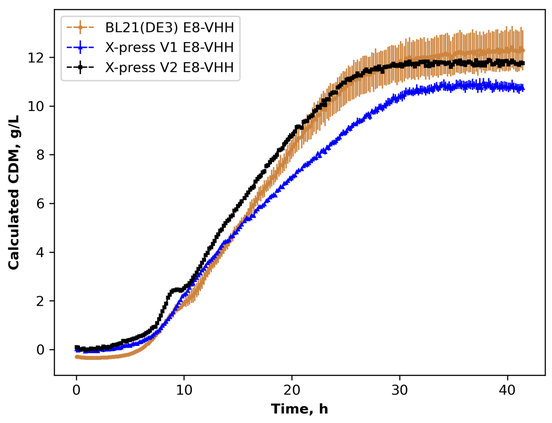
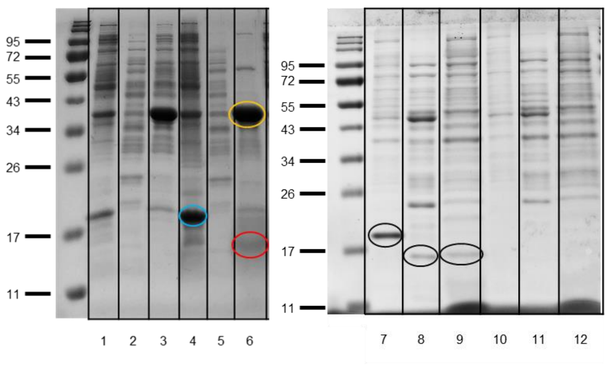


Table 1: The different genotypes of E. coli BL21(DE3) and the enGenes-X-press strains

Bacterial strain	Genotype
***E. coli* BL21(DE3)**	*E. coli* str. B F– ompT gal dcm lon hsdSB(rB–mB–) λ(DE3 [lacI lacUV5-T7p07 ind1 sam7 nin5]) [malB+]K-12(λS)
***E. coli* enGenes-X-press V1**	*E. coli* B F– ompT gal dcm lon hsdSB(rB- mB-) λ(DE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5]) attTN7::<Para-Gp2> ΔaraABCD
***E. coli* enGenes-X-press V2**	*E. coli* B F– ompT gal dcm lon hsdSB(rB–mB–) [malB+]K-12(λS) araB::T7RNAP-tetA attTN7::<Para-Gp2>

No growth limitations of X-press strains compared to *E. coli* BL21(DE3).

Figure 2: Comparison of the growth behavior of BL21(DE3) and both X-press strains (V1 and V2) bearing the E8-VHH plasmid. Strains grown as biological duplicates in a BioLector micro fermentation system in 48-well flower plates. Cultivation in a FIT fed-batch medium without arabinose and IPTG.



	BL21(DE3) E8-VHH		X-press V1 E8-VHH		X-press V2 E8-VHH		X-press V2 untransformed
1	Spheroplast	4	Spheroplast	7	Spheroplast	10	Spheroplast
2	Periplasmic fraction	5	Periplasmic fraction	8	Periplasmic fraction	11	Periplasmic fraction
3	Extracellular medium	6	Extracellular medium	9	Extracellular medium	12	Extracellular medium

The enGenes-X-press strains secreted the E8-VHH into the extracellular medium, whereas the *E. coli* BL21(DE3) did not.

The black curls show the E8-VHH expressed in X-press V2, the blue and red curl in X-press V1.

Easy and fast expression in autoinduction medium

Figure 3: SDS-PAGE of E8-VHH cultivated in E. coli BL21(DE3), X-press V1, and X-press V2 and untransformed X-press V2. BL21(DE3) and X-press V1 were cultivated in semisynthetic medium, X-press V2 samples in autoinduction medium. Different fractions (spheroplast and periplasmic fraction after chloroform treatment, extracellular medium) were loaded on a 15% polyacrylamide gel.

Capturing of E8-VHH with MNPs

The polyglutamate-tag enabled a selective binding to non-functionalized magnetic nanoparticles directly from the extracellular medium. Different factors influenced the yield and purity:

- Concentration ratio of extracellular medium to MNPs
- pH
- PEG presence

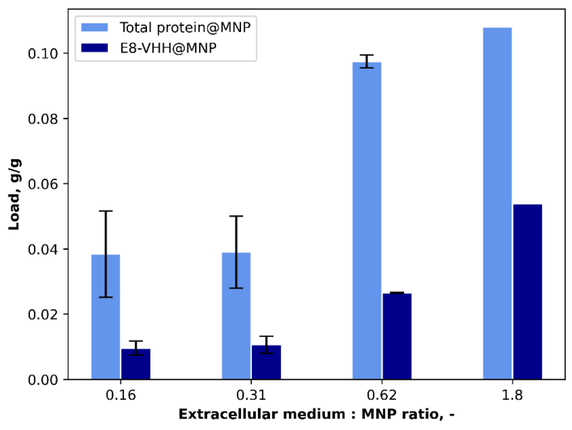


Figure 4: Influence of varied extracellular medium (EM) to MNP ratios for incubation. Load of the total proteins and E8-VHH on the MNPs after incubation (1 h, 25 °C, 1000 rpm) and two times washing with 50 mM Tris pH 7.0.

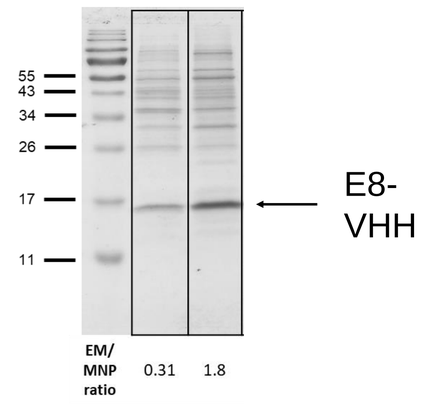


Figure 5: SDS-PAGE of E8-VHH cultivated in X-press V2 (autoinduction medium) and its extracellular medium contacted with 1 g L^{-1} MNPs for 1 h (25 °C, 1000 rpm). MNP samples were boiled and loaded onto the SDS-PAGE.

Higher extracellular medium to MNP ratios increased the purity of E8-VHH up to 50%, and thus, increasing the purification factor distinctly (6.5x).

The presence of PEG led to higher yields. The pH influenced the purity of E8-VHH in the samples w/o PEG.

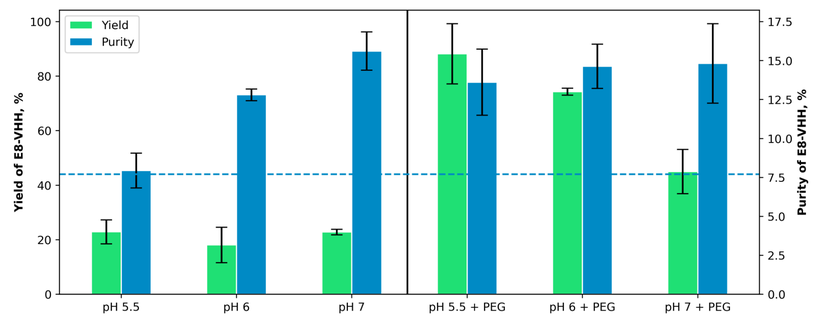


Figure 6: Yield and purity of E8-VHH bound to MNPs. The initial purity of E8-VHH in the extracellular medium is shown as a blue line (7.7%).

Summary and Outlook

The combination of the enGenes-X-press system for secretion, non-functionalized magnetic nanoparticles and the tagging of proteins with the polyglutamate-tag for selective binding promises a worthwhile integrated bioprocess for the future. We could prove the system with an E8-tagged nanobody. The yield and purity were influenced by the pH, the concentration ratio of EM to MNPs, and the addition of PEG 6000. However, more details on the transferability to other proteins are needed. Furthermore, the direct mixing of MNPs to the fermentation broth would be interesting as an *in situ* product removal (ISPR) method.

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