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DNA to assay-ready proteins in 48 hours. Rapid protein expression and purification on the eProtein Discovery™ system and binding confirmation on Biacore™ SPR System

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Introduction

Conventional methods to optimize and obtain proteins can take weeks to months especially if the expressed proteins are not soluble, not correctly folded or not active, resulting in multiple repeats in protein optimization workflow with new constructs. This leads to frustration, high cost and time loss. Here we have combined two cutting edge life science tools that enable rapid protein obtainability on the eProtein Discovery™ system, and functional activity via protein-protein interaction studies on Biacore™ SPR system. This workflow is demonstrated using a difficult-to-express protein VEGF165, known to contain disulfide bonds.

Objective: To obtain soluble VEGF165 protein and confirm functional activity via protein-protein interaction studies.

Methods

Expression and purification screen and scale-up using the eProtein Discovery™ system

The eProtein Discovery system is an integrated platform that enables rapid protein access in 3 simple steps (Figure 1).

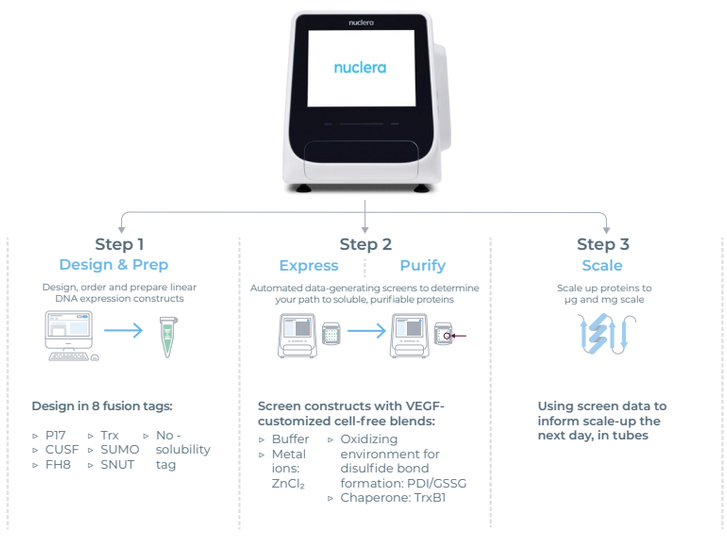


Figure 1. The eProtein Discovery™ workflow.

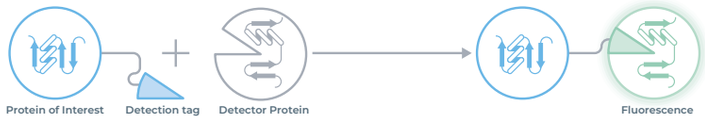


Figure 2. Fluorescence complementation technology used within the eProtein Discovery Cartridge to detect soluble protein expression.

Biacore assay

Biacore SPR systems allow you to monitor the interaction between biomolecules in real time using the phenomenon of surface plasmon resonance (SPR). Biacore SPR systems are used as powerful tools in research, drug discovery, quality control and manufacturing to provide data on, for example, kinetics, affinity, concentration and specificity. In this study, Biacore 1S+ SPR system (Figure 3) is used to study the interaction between VEGF165 and Bevacizumab via Protein-Protein interaction.

Figure 3: Biacore 1S+ SPR system equipped with six flow cells that can be used individually or in combinations to enhance the analytical flexibility.

Biacore Single-Cycle Kinetics (SCK)™ is used to study the binding kinetics of VEGF165 over Bevacizumab. Serial concentrations of 0.78, 1.56, 3.125, 6.25, 12.5, and 25 nM VEGF165 are injected over Bevacizumab captured on Sensor Chip Protein A.

Biacore SCK method details: Bevacizumab capture level 75 RU, Association time 120 s, Dissociation time 3600 s, Flow rate 30 µL/min, Temperature 25 °C, Running buffer PBS-P+. The run data is fitted with 1:1 binding model to derive the binding constants.

Results

Expression and purification screen on the instrument identifies construct and cell-free blend combinations that generates the highest purified yield

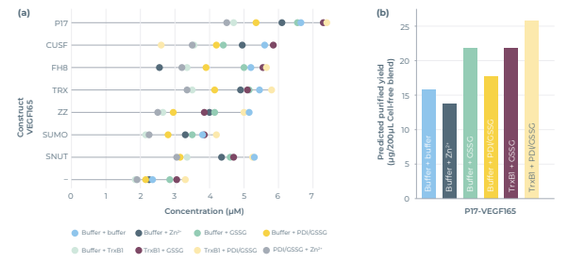


Figure 4. Expression and purification results. (a) Lollipop diagram shows expression yield of VEGF165 variants expressed in different cell-free blends. (b) Cartridge predicted scale-up yield to aid in scale-up planning.

The highest VEGF165 expression was achieved with construct P17 tag in the presence of chaperone (TrxB1) and oxidizing condition (PDI/GSSG mix) (Figure 4). This combination promotes solubility through the presence of P17 solubility fusion tag and additives that support disulfide bond formation. The result is consistent with published research showing that VEGF expresses well with solubility fusion tag in an E. coli strain that helps disulfide bond formation[1].

Scale-up expression produces VEGF165 of high quantity and purity

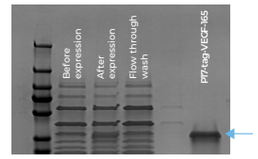
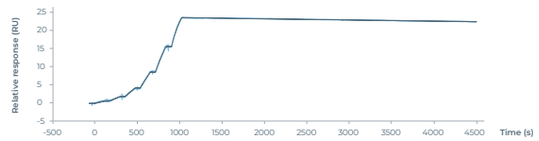


Figure 5. Scale-up expression and purification of P17-VEGF165 yielded 106 µg soluble protein from 1 mL cell-free blend at high purity

Biacore assay confirms functional activity of VEGF165 to Bevacizumab



Kinetics model	k_a (1/Ms)	k_d (1/s)	K_D (M)	R_{max} (RU)	Kinetics Chi² (RU²)	U-value
1:1 binding	4,43e+05	1,60e-05	3,62e-11	27,6	4,48e-03	1

Figure 6: Sensorgrams (blue curve) along with 1:1 fitted curve (black curve) for Biacore Single-Cycle Kinetics (SCK)™ of VEGF165 over Bevacizumab captured on Sensor Chip Protein A. Table represents the kinetic parameters for these interactions. Biacore assay confirms the functional activity of VEGF165 with an affinity constant (KD) of 36 pm.

Conclusions

- Less than 48h turnaround time from DNA to 106 µg of pure protein
- Biacore assay confirms the functional activity of VEGF165 over Bevacizumab with an affinity of 36 pM.

The combination of rapid cell-free protein expression and purification screen on the eProtein Discovery System, followed by scale-up protein production the next day had significantly shortened the time to obtain protein for functional validation on Biacore™ system.

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Reference 1: Nguyen, M. *et al*. Prokaryotic Soluble Overexpression and Purification of Human VEGF165 by Fusion to a Maltose Binding Protein Tag. PLOS ONE 11(5): e0156296. doi:10.1371/journal.