

# Conversion of Feed, Side & Waste Streams to High Value Protein Ingredient Products

*Searching for the Protein Biominer's Philosopher's Stone*

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# Talking Points

- Protein biomining for unique and/or added value
- Intro to proteins, chromatography and Expanded Bed Adsorption (EBA)
- Considerations for the use of EBA in DSP in different business sectors
- An illustrative business case
- PROMINENT

# The Philosopher's Stone

## *Parallels to Protein Biomining*



The philosopher's stone, or stone of the philosophers (*lapis philosophorum*) is a legendary alchemical substance capable of turning base metals such as mercury into gold....

The philosopher's stone was the central symbol of the mystical terminology of alchemy, symbolizing perfection at its finest, enlightenment, and heavenly bliss. Efforts to discover the philosopher's stone were known as the *Magnum Opus*.

### **Central Idea:**

- Transforming low(er) value starting materials to high value products
- A process that is achievable and cost-effective

# Extracting (mining) proteins for (added) value... ...serendipity



EUR million

## INCOME STATEMENT

Revenue

EUR growth

Organic growth

EBITDA

EBITDA margin

Depreciation, amortization and impairment losses

EBIT before special items

EBIT margin before special items

Special items and net financial expenses

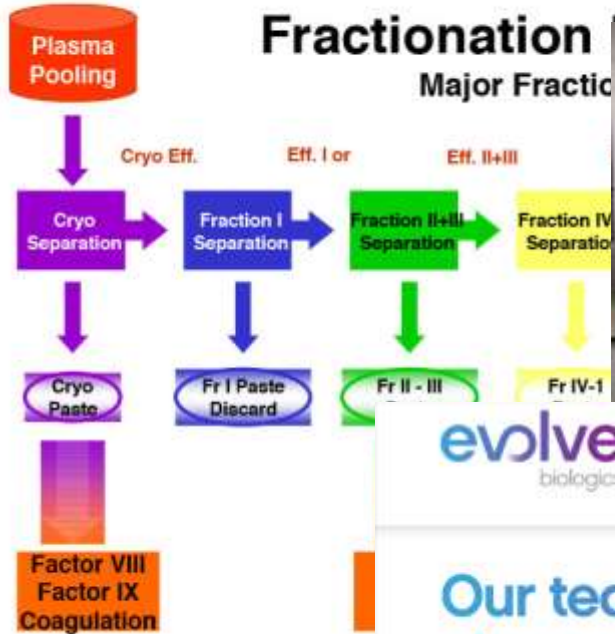
**Profit before tax**

	Food Cultures & Enzymes	Health & Nutrition	Natural Colors	<b>2016/17</b> Group
Revenue	617.5	224.7	220.3	1,062.5
EUR growth	9%	22%	10%	12%
Organic growth	9%	14%	10%	10%
EBITDA	249.5	83.5	35.1	368.1
EBITDA margin	40.4%	37.2%	16.0%	34.6%
Depreciation, amortization and impairment losses	(36.4)	(18.0)	(6.6)	(61.0)
EBIT before special items	213.1	65.5	28.5	307.1
EBIT margin before special items	34.5%	29.2%	12.9%	28.9%
Special items and net financial expenses				(16.2)
<b>Profit before tax</b>				<b>290.9</b>

# Extracting (mining) proteins for (added) value... ...targeted



FIGURE 73.—Edwin J. Cohn, Ph.D.



The screenshot shows the Evolve Biologics website. The header includes the company logo and navigation links: [About Us](#), [Our Technology](#), [Our Pipeline](#), and [Our Leadership](#). The main content area features a large blue headline: **Our technology could be a game-changer for our industry.** Below this, there is a paragraph of text: "Our proprietary technique, known as PlasmaCap EBA™, has the potential to extract more value from each liter of donor plasma as compared to the legacy technology in the plasma products industry." Another paragraph follows: "Our unique process involves passing plasma through a series of sequential chromatography columns. Each column is designed with proprietary adsorbent beads that selectively extract plasma proteins with optimal protein binding." A final paragraph states: "This results in both enhanced product yield and purity for proteins used in proven therapies. We believe our process also has the potential to treat other rare disorders in the coming years." To the right of the text is a circular graphic with a blue-to-purple gradient, containing a stylized image of a chromatography column and the text: "Learn more about PlasmaCap EBA."



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# Protein Characteristics and Implications for DSP

## Bovine Lactoferrin (bLf)

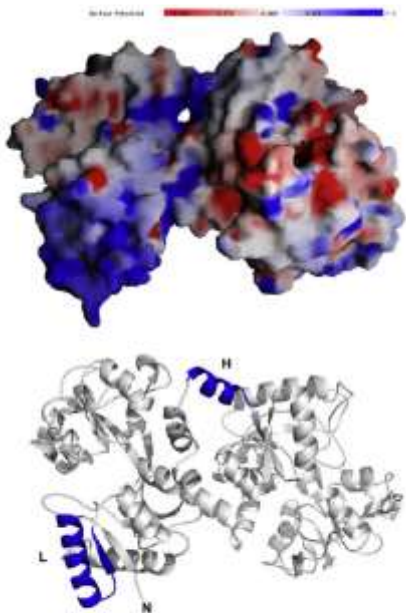
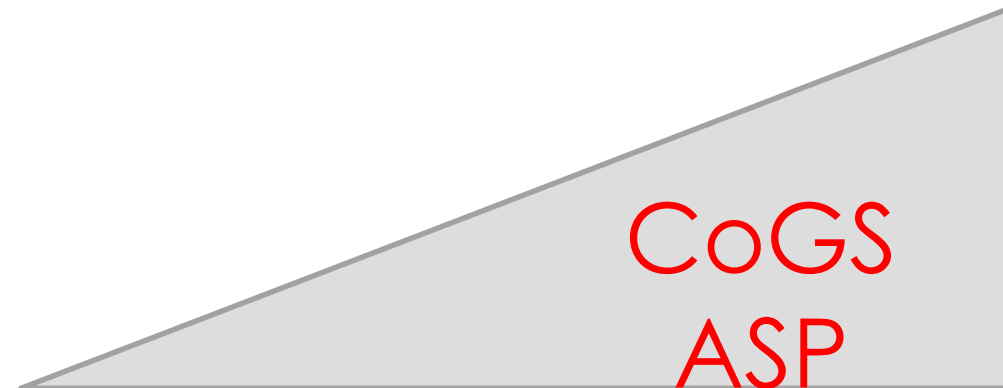


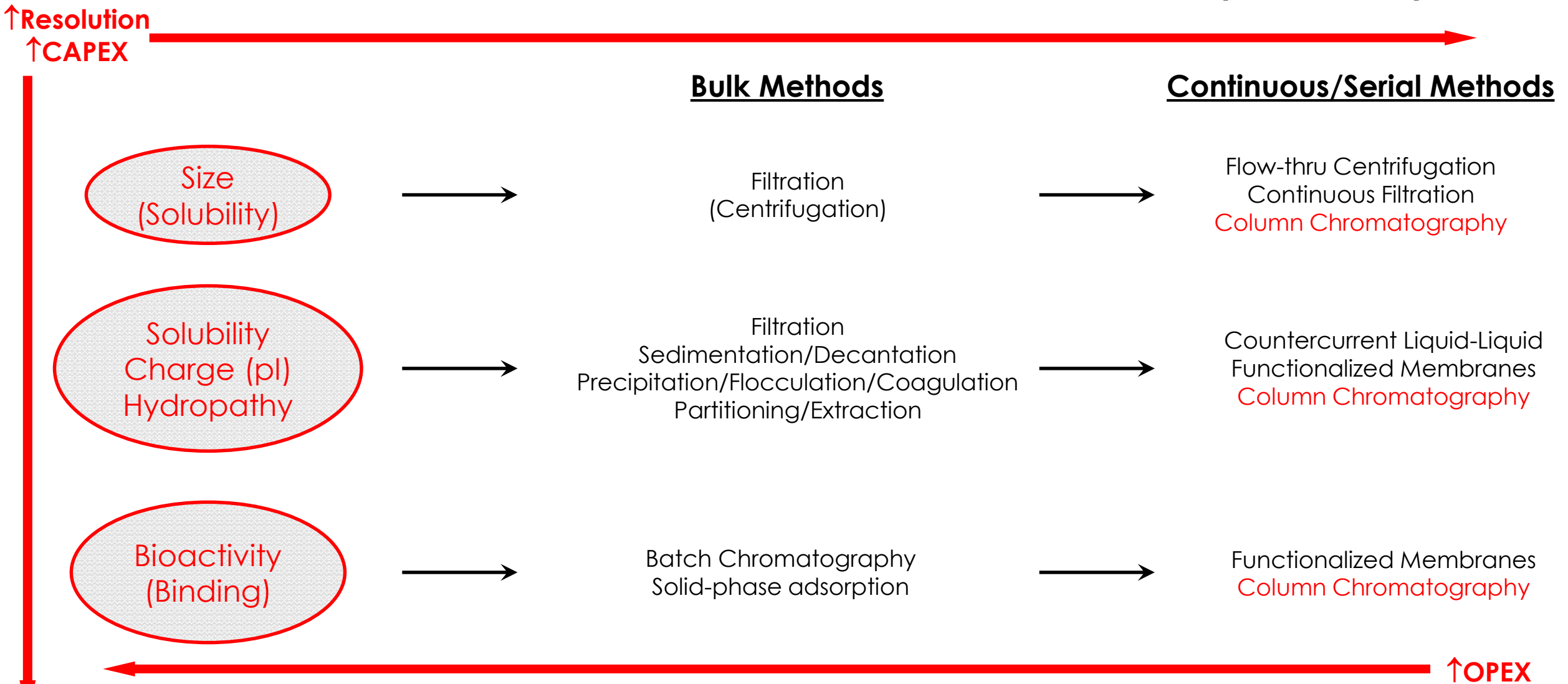
Fig. 3. Charge distribution on the Lf surface. In the upper diagram, shown in space-filling form, positively charged regions are shown in blue, and negatively charged in red. The lower diagram shows how the upper one corresponds with the polypeptide fold. The regions of greatest positive charge are at the N-terminus (N) and around the antibacterial lactoferrin domain (L) and the connecting helix (H), both shown in blue in the lower figure.

- Proteins are specialized, highly heterogenous and differentiable by diverse physical and chemical properties
  - Size, solubility, charge, hydropathy, bioactivity (binding)
- Protein physical and chemical properties can be exploited for their isolation & purification
  - Filtration, precipitation, partitioning, chromatography
- Proteins are generally fragile biomolecules that require gentler processing in order to maintain functionality and bioactivity
  - **Target Applications: Nutrition/Bulk vs. Functionality/Bioactivity**

Baker & Baker (2009) *Biochimie* **91**, 3-10



# Protein Separation Modalities in DSP (liquids)



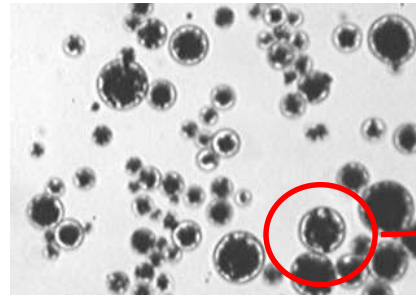
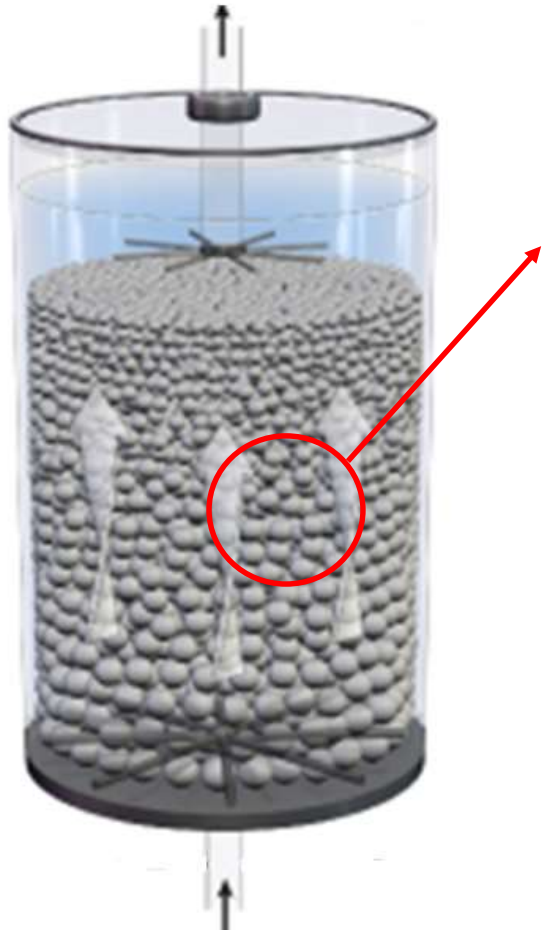
# Packed Bed Chromatography



- Monodisperse particles
  - 40-180  $\mu\text{m}$
- Uniform Beds, low dispersion - high resolution (higher maintenance)
- Lower throughput – lower flow rates (1.5 - 5 cm/min; 80 cm column  $\approx$  25 L/h), bed height dependent
- Requires clarified feedstreams; prone to fouling
- CIP – challenging, mesh supports prone to biofilm formation



# Upfront EBA Chromatography



Functionalized Agarose

Tungsten carbide

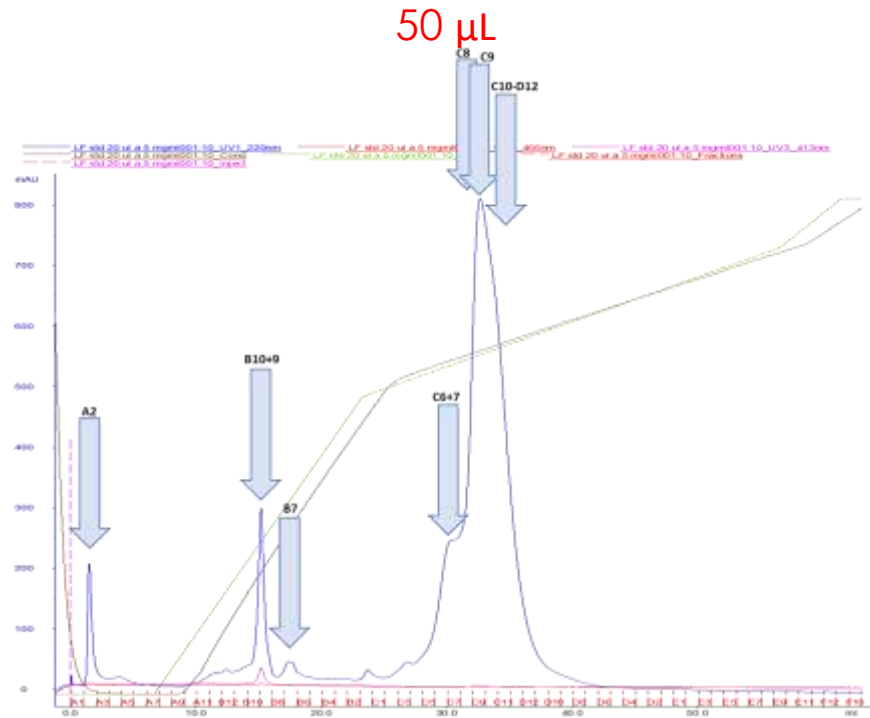
- Polydisperse beads
  - Crosslinked, functionalized agarose WC conglomerate
  - Density 2.5 – 3.5 g/mL
  - Size 60 – 300  $\mu\text{m}$
- Moderate resolution
  - “Stabilized Bed”
  - Adsorption-desorption, step gradient process
- High flow rates (5-30 cm/min; 80 cm diameter  $\approx$  150 L/min)
- Can tolerate particulates in feedstream
  - Clarification, capture, purification in one step
- Inlet and column design improve fluid distribution & minimize support screens that can promote biofilm development

# Analytical Packed Bed vs. Process EBA

## Bovine Lactoferrin & Lactoperoxidase

### Packed Bed Analytical

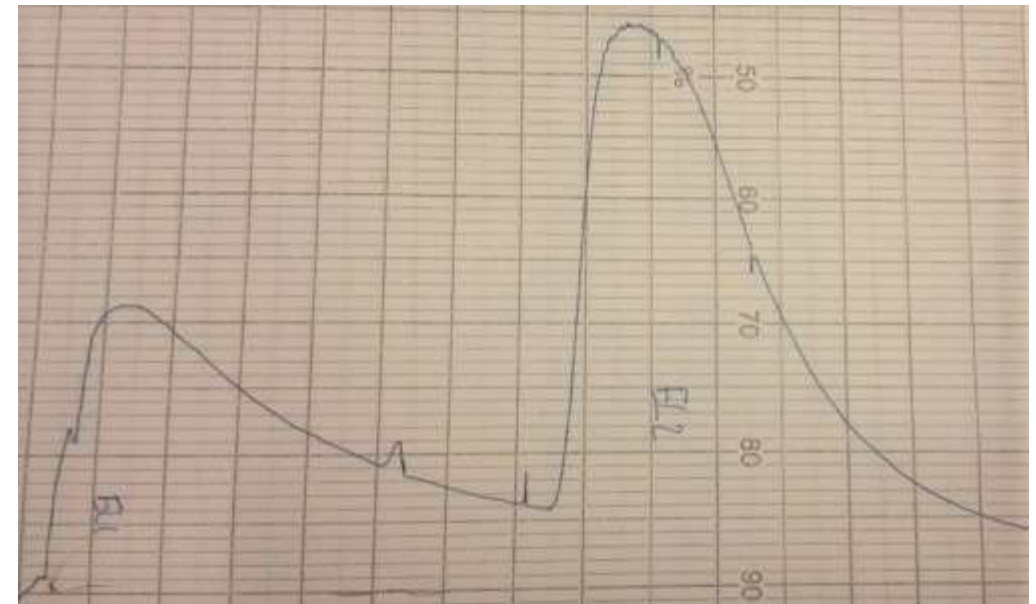
Mono S, 4.6 x 100 mm analytical column



### EBA Preparative

15 cm i.d., 50 cm Bed height, LactoPro SP

≈ 700 L



# Selling Prices vs. Application for Protein Products

## Implications for DSP

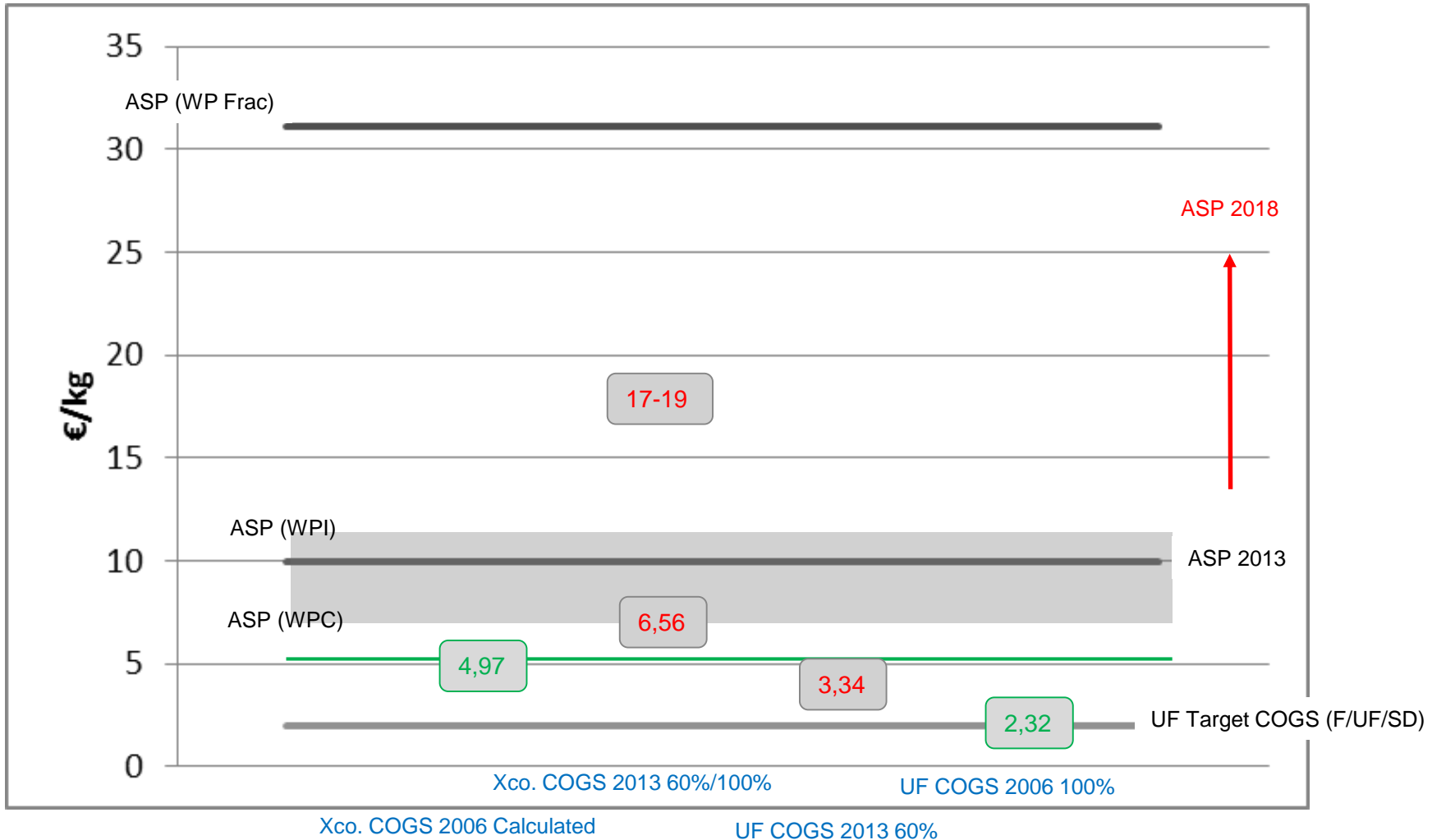
Application	Prices (€/kg)	Protein Ingredients	Products
Pharmaceuticals	> 1000	mAbs, insulin	Herceptin, Tresiba
Nutraceuticals	100-1000	Lactoferrin	Infant formulas
Functional Food Ingredients	5-100	WPI, $\beta$ -lactoglobulin, Solanic 300	Stabilizers, emulsifiers, foaming agents, gelling agents
Nutritional Ingredients	1-100	Solanic 100, WPC	Protein Bars, Drinks
Feed Ingredients	1-20	Crude protein mixtures, enzymes	Bonemeal, fishmeal, plasma, phytase



Purity

# Protein prices

## Concentrate, Isolates, Fraction and Plant Protein Case



# Other Considerations...

- The feedstream often has value that must not be compromised and should be maximized...
- “Transformation” of the feedstream should be considered

## Commercial, Large-Scale Production of Bovine Lactoferrin (bLf): Sources of Product Heterogeneity and Implications for Applications



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### Introduction

Upfront Chromatography is commissioning a commercial pilot plant based on Expanded Bed Adsorption (EBA) chromatography for the production of bLf and bovine lactoperoxidase (bLp). The plant is designed as a turnkey solution for seamless integration into dairy production lines and at the current pilot scale is capable of accepting a feed stream of unpasteurized skim milk at 9000 L/h. When commissioned, the plant will yield on the order of 4 kg per 8 hour production cycle of bLf. Process design requirements are 95% purity, >90% protein content and 100% native following down stream processing (DSP). In addition, the treated skim milk is returned to the dairy stream for further processing.



# The PROMiNENT Framework

Valorizing side and waste streams from  
wheat and rice industry

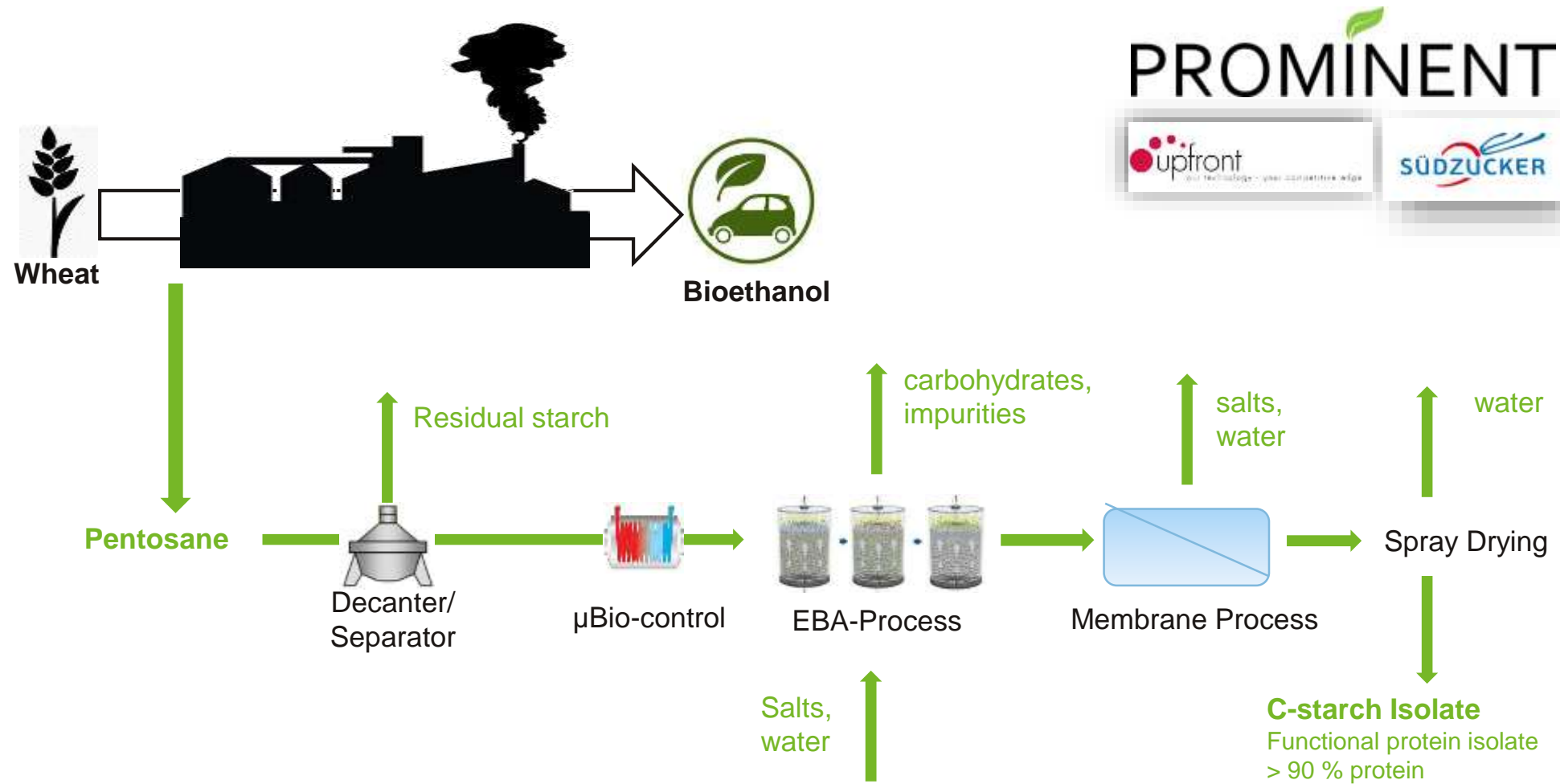
This project has received funding from the Bio-Based Industries Joint Undertaking under the European Union's Horizon 2020 research and innovation programme under grant agreement No 668953.





# Valorization of Sidestream by Adsorption Technology

## Concept B: Isolate (> 90 % Protein)



# Valorization of Wheat Sidestream in PROMINENT

- EBA protocols developed to extract highly functional, high protein content isolates from low value sidestream
- Analysis and testing of isolates indicate very favorable protein ingredient properties meeting or surpassing properties of most current market protein-based food ingredients
- Pilot scale production for partner application development in May at 10s of kg scale

# Scale



# Conclusions

- EBA is an attractive chromatographic method and has advantages for high volume, streamlined DSP
- The use of EBA (and other sophisticated) DSP methods requires a VERY complete understanding of the business case for most protein products of non-pharmaceutical nature
- EBA is currently in use and/or being developed for a wide range of protein products
- EBA has allowed the development of very promising protein isolates from wheat sidestreams within the PROMINENT project