



ProGenosis

HYBRID PROTEINS PLATFORM

Welcome to the world of Bifunctional Hybrid Proteins ...

New opportunities in

Protein Domain Studies

Our fundamental mission is helping our clients to maximize the return on their R&D investments.

www.progenosis.com

TABLE OF CONTENTS

1. Corporate profile
2. Introduction
3. Presentation of the technology
4. BHP for expression of difficult protein
5. Platform organization
6. Contact us

Mission = ProGenosis provides customized services in R&D for Life Sciences companies; with a primary focus on receptor-ligand interaction.

Core Business = Simplify the complexity of the interaction environment by creating BioTools for studying protein domains.

Technology platform = Construction of *Bifunctional Hybrid Protein (BHP)* for expression of difficult protein.

OVERVIEW OF THE BHP PLATFORM

The Bifunctional Hybrid Proteins (BHP) technology platform is a highly convenient and functional research enabling tool for displaying protein(s) polypeptide(s) or protein domain(s) of interest (yellow in slide 7) within the native structure of an enzyme scaffold, β -lactamase (blue and green in slide 7). The exogenous displayed polypeptide is reconstructed as a biologically active molecule. The fact that the exogenously displayed polypeptide is actually inserted into the enzyme scaffold avoids numerous pitfalls inherent in the techniques relying on fusion or chemical conjugation (slide 8), and offers stability, reproducibility, uniformity and greater control of the final product.

As a result, BHP technology must be considered as a useful BioTool for protein domain studies.

Understanding the interaction sites of proteins and reconstructing these as biologically active molecules remains a critical challenge in drug discovery. Recreating the protein interaction sites in a spatial, constrained orientation that closely mimics the native protein structure is a real challenge and a difficult task to undertake. Protein interaction sites, once identified, are some time hard to manage at the laboratory level. The BHP technology simplifies the complexity of the receptor-ligand interaction environment and brings it into a easily-manageable unit (BHP) for protein domain studies and development activities.

The main benefits of the BHP technology platform:

1. The exogenous polypeptide is displayed within a **protein environment**, which mimics wild-type protein constraints. It is often more difficult to achieve the same feature with fused peptides or chemical conjugations.
2. Due to the fact that the technology enables an actual **gene insertion** and not a fusion, the folding of both the exogenous polypeptide and the enzyme scaffold are strongly inter-dependent. Consequently, the correct folding of the enzyme scaffold, and hence its biological activity, is conditioned by the correct folding of the exogenous polypeptide of interest.
3. BHP technology uses a very robust and efficient **positive drug-based screening system**, which enables the easy and straightforward selection of fully soluble and well-folded BHPs. The β -lactamase enzyme enables bacteria to become resistant to antibiotics. Thus, when a bacterium fails to produce a soluble or active BHP, it dies in the presence of an antibiotic (slide 16).
4. The proprietary scaffold (29 kDa) exhibits a **highly permissive ability**. The molecular weight of successful insertions ranges from 2 kDa up to 40 kDa. This occurs without suffering the loss of either the enzymatic activity of the scaffold or the biological activity of the exogenous polypeptide (slide 13). We strongly believe that the β -lactamase scaffold could accommodate even larger inserts.
5. BHP technology is especially relevant for the cost-efficient **expression of proteins that are difficult to produce**, thanks to the use of the positive selection system, as only soluble BHPs will be produced efficiently (slide 14).

IMPROVEMENT OVER OTHER STANDARD APPROACHES

Difficult to express proteins and protein domain studies

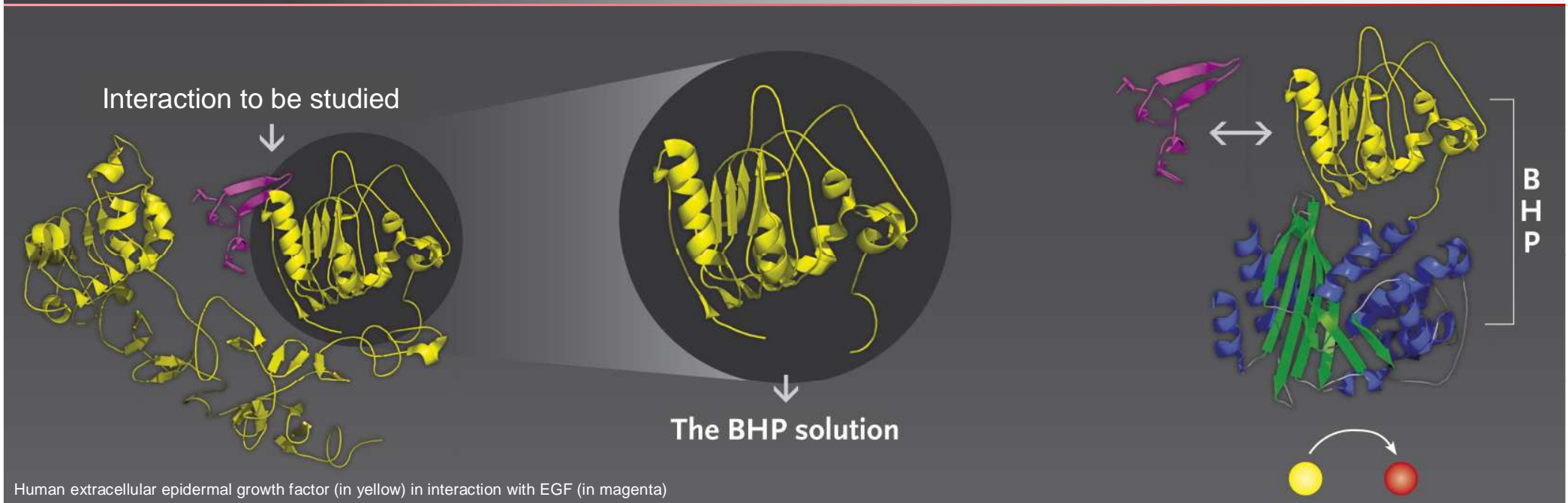
Since we have a **positive drug-based screening system** for controlling expression based on the β -lactamase activity, one guarantees the production of soluble recombinant proteins in the E. coli. (slide 16).

Rather than a polypeptide fusion, insertion makes the folding of both the exogenous polypeptide and the enzyme scaffold depend strongly on each other. Consequently, the correct folding of the BHP scaffold, and hence its enzymatic activity, are conditioned by the correct folding of the entire brand new macromolecule. This connection dramatically enhances the likelihood of obtaining soluble production, since the exogenous polypeptide is expressed in a **protein environment** comparable to that which occurs with the wild-type protein.

Should post-translational modification(s) of the recombinant proteins be required, the right construct can be transferred to the suitable heterologous expression system such as yeast, and mammalian cells.

We have also engineered some vectors enabling the cleaving off of the exogenous protein domain from the carrier protein by means of specific proteolysis cleavage.

One can use BHP technology, with the insert of interest, for crystallography X-ray 3-D protein determination work, as the β -lactamase phases are well-characterized. Furthermore, the β -lactamase enzymatic activity can be helpful in discriminating functional protein crystals from crystal aggregates during the crystallization screening step.



Complexity

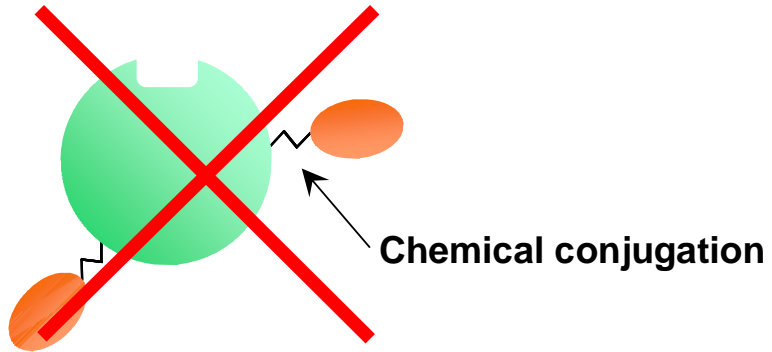
Simplicity

FUNCTIONALITY

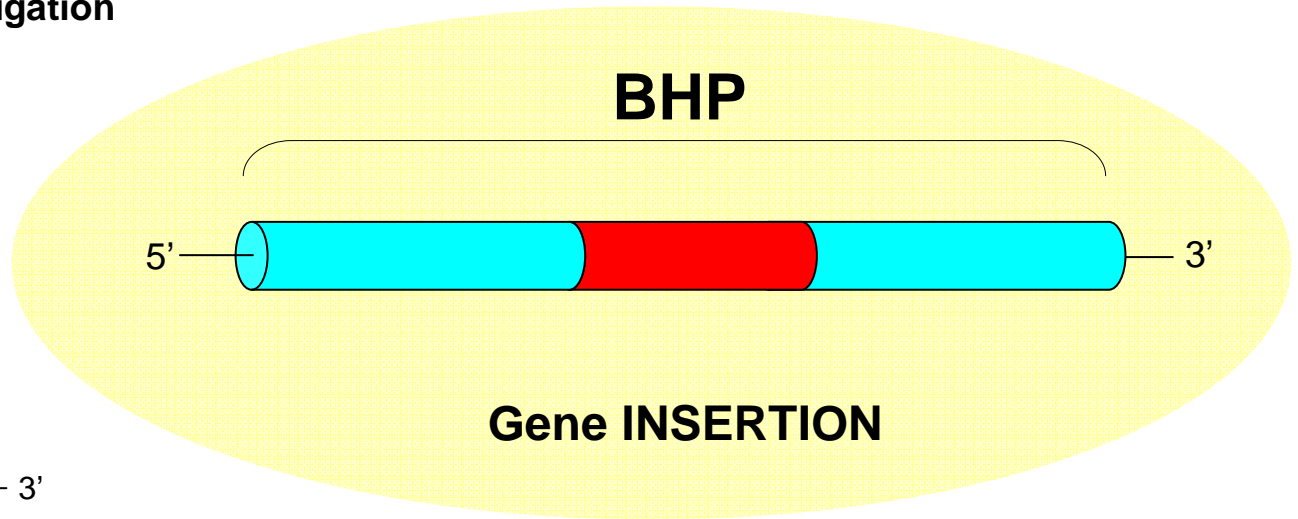
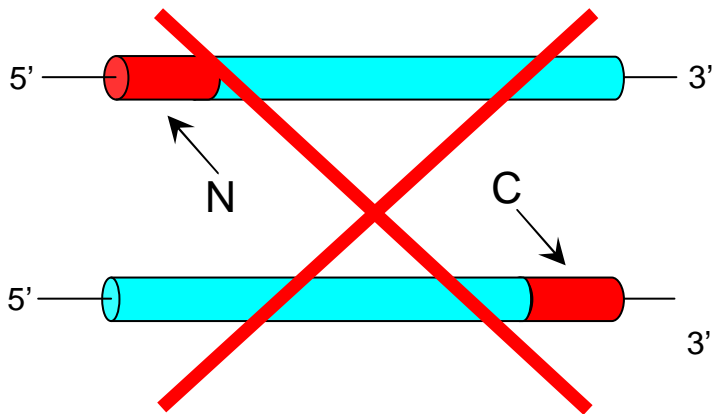
Scientists are always being dealing with quite complex proteins involved in intricate environment of interactions. Those proteins are sometimes unavailable as a functional and suitable material. Rather than use the whole protein of interest, the BHP technology allows the dissociation of the different structural elements of a protein.

In this way, one simplifies the complexity of the protein-protein interaction environment and brings it into an easily-manageable unit, the BHP, for use in protein domain studies.

Our technologies have been validated by multiple research projects related to industrial final products.

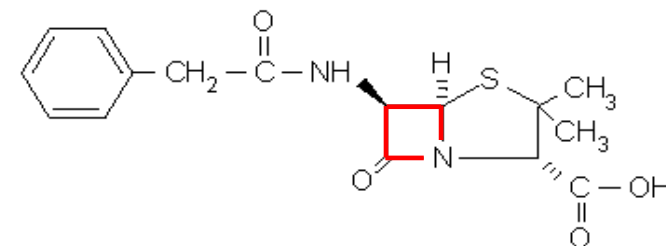


Gene FUSION



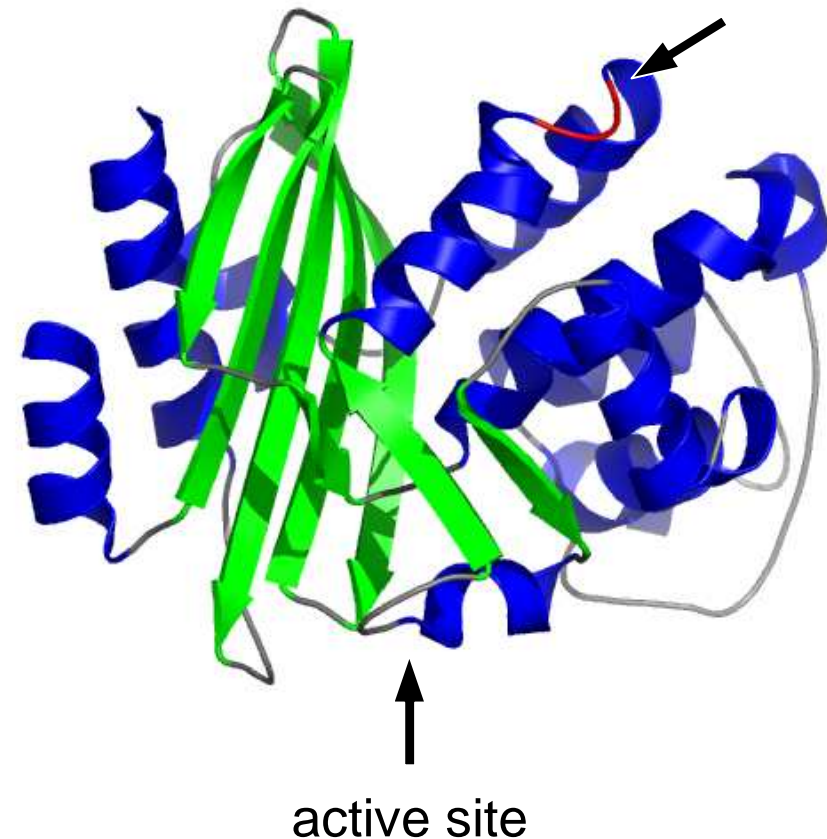
 Exogenous polypeptide

- The core of the technology is the β -lactamase enzyme
- β -lactamase = active-site serine hydrolytic enzyme that catalyses the hydrolysis of the β -lactam ring of penicillins related antibiotics
- β -lactam ring = high reactional amide bond



Chemical structure of Penicillin antibiotic

- Insertion of exogenous peptide in a high permissive loop
- The loop position is diametrically opposed to the enzyme active site
- Accessibility of the loop to the solvent
- The enzymatic activity is maintained
→ correct fold
- The β -lactamase is used as a carrier protein and reporter enzyme



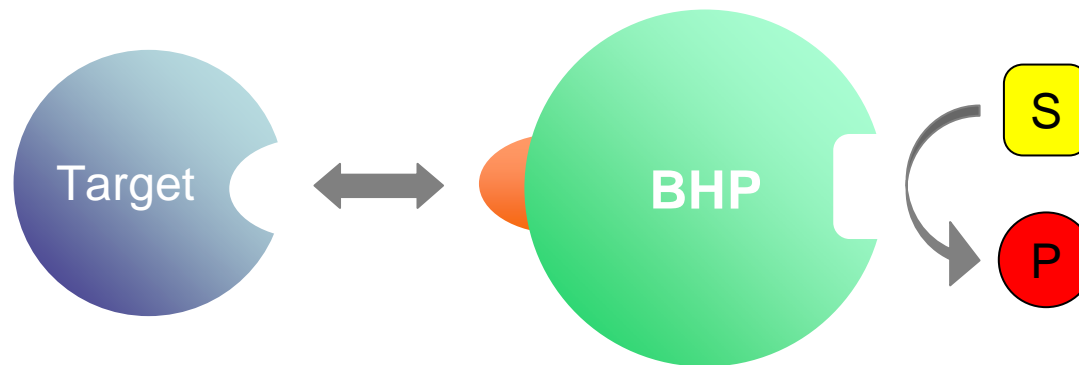
Insertion of polypeptide with specific affinity

RECEPTOR [to recognize]

LIGAND [to be recognized]



Bifunctional Hybrid Protein (BHP)



Benefits of ProGenosis technology

- Biological function displayed in a protein scaffold.
- Correctly folded, soluble and fully functional protein.
- Positive screening procedure.
- Enzymatic reporting system.
- Bypass of wild-type protein production.
- Avoidance of the problem of toxic, insoluble, difficult to produce or unfunctional proteins.
- No chemical conjugation → stability and uniformity of BHP.
- Recombinant protein → greater control of the final product.
- Reduction in procurement costs and in the loss of biological information.

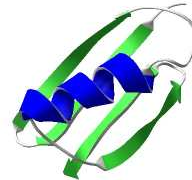
Heat stable enterotoxin
STa I from *E. coli* (2 kDa)



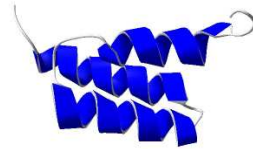
Hemagglutinin Epitope HA from
the Influenza virus (2 kDa)



One Fc-binding domain of the
Streptococcus pyogenes
protein G (6 kDa)



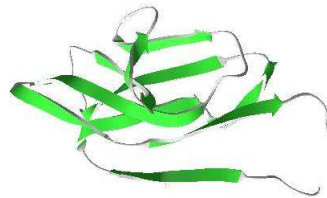
One Fc-binding domain of the
Staphylococcus aureus
protein A (6 kDa)



One Fab-binding domain of the
Peptostreptococcus magnus
protein L (9 kDa)



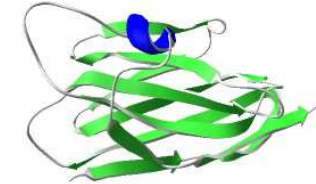
Human FcεRIα (12 kDa)



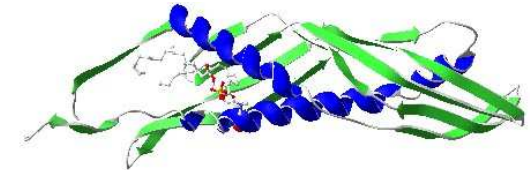
Fibronectin binding domain
from *Staphylococcus aureus*
(13 kDa)



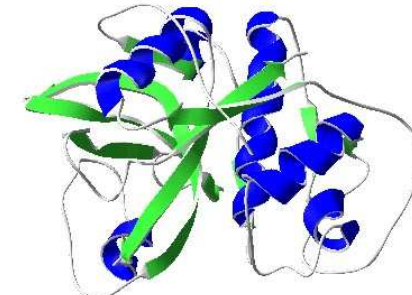
V_HH of camel antibody
(15 kDa)



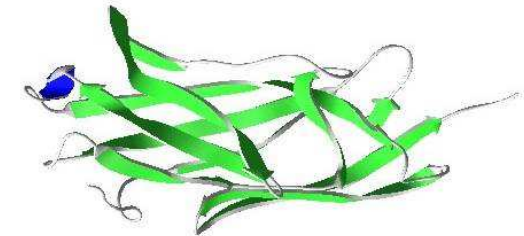
Human LPS binding-domain
BPI (25kDa)



Human allergen (35 kDa)



Clumping Factor A from
Staphylococcus aureus
(39 kDa)



Polypeptides	Source	Size	Special features	BHP benefits
Heat stable enterotoxin STa I	<i>E. Coli</i>	13 aa	<ul style="list-style-type: none"> • 3 disulphide brigdes • hapten • no antibody available • difficult to produce 	<ul style="list-style-type: none"> • well produced once internalised • well folded with disulphide links completed • raised of neutralizing antibodies
FcεRIα	Human	12 kDa	<ul style="list-style-type: none"> • no production in <i>E. coli</i> • unstable • IgE specific 	<ul style="list-style-type: none"> • well produced once internalised • stability is increased • direct reporting system
Single heavy-chain variable domain (V _H H) fragment	Camel	15 kDa	<ul style="list-style-type: none"> • 5 disulphide brigdes 	<ul style="list-style-type: none"> • direct reporting system • tool for drug discovery
Clumping Factor A	<i>Staphylococcus aureus</i>	39 kDa	<ul style="list-style-type: none"> • require to be well folded for specificity 	<ul style="list-style-type: none"> • well produced once internalised • well folded • high affinity against human fibrinogen
Human allergen	Insect	35 kDa	<ul style="list-style-type: none"> • 3 disulphide brigdes • glycosylated protein • difficult to produce 	<ul style="list-style-type: none"> • well produced once internalised • well folded with disulphide links completed • enzymatically active • direct reporting system
Chitin binding domain	Human	8,4 kDa	<ul style="list-style-type: none"> • 3 disulphide brigdes • difficult to produce • no structure available 	<ul style="list-style-type: none"> • well produced once internalised • well folded • direct reporting system • tool for drug discovery

BHP for the production of fully soluble and active recombinant protein

- Positive screening due to β -lactamase resistance phenotype
- Soluble protein can be guaranty
- β -lactamase is used as a « Tagging system » for cell-to-gel analysis

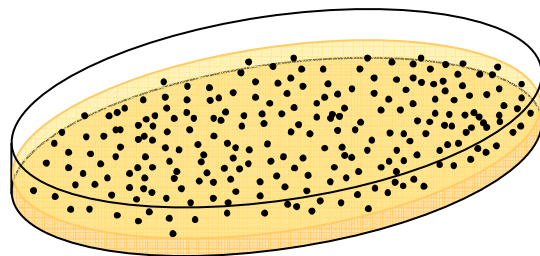
Positive screening of producing bacteria

If the BHP expression failed → no β -lactamase protection

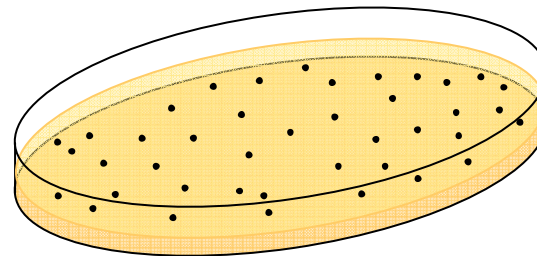
If the BHP expression is ok → the *E. coli* strain can resist to antibiotics



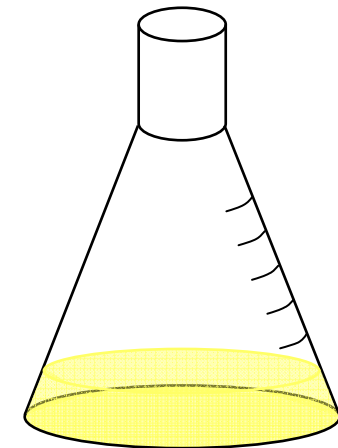
Selection of producing bacteria only



Without antibiotic

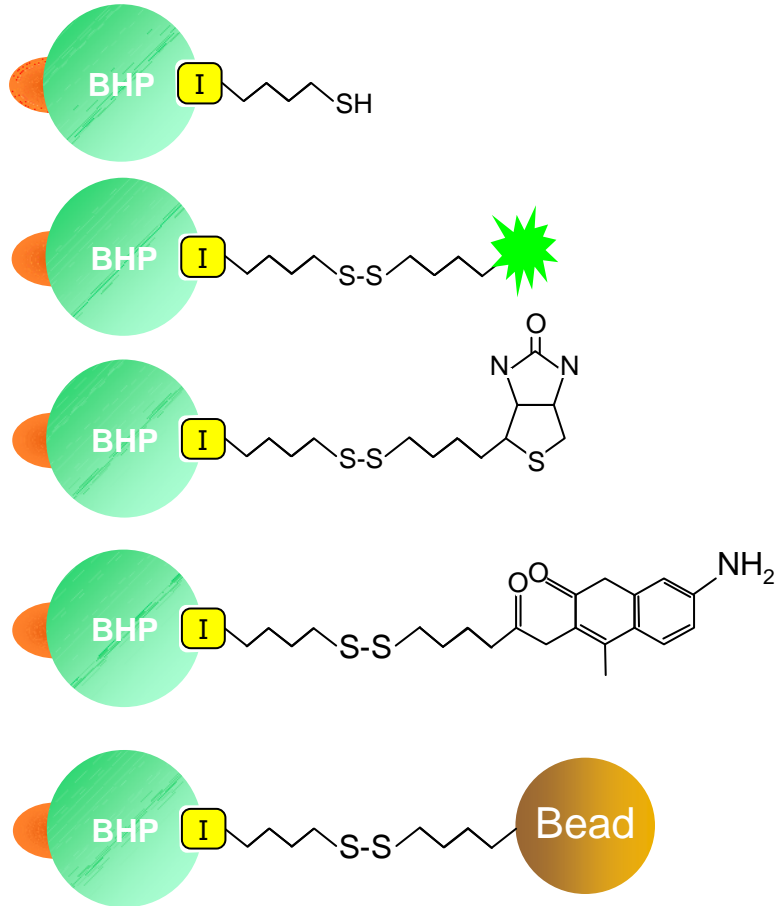


With antibiotic



Go ahead in production

Expressing, Capturing and Detecting BHP



Various β -lactam ligands which carry a variety of functionalities

- Covalent and specific bond formation with inhibitor
- Cell imaging and protein analysis
- Cell-To-Gel Analysis
- Specific labelling of protein in living or fixed cells or in vitro
- Oriented immobilization available (micro array)
- Control of sample completeness by enzymatic assay
- Enzymatic activity to monitor purification
- Easier purification by using biotin ligand or magnetic bead



All-In-One Laboratory Tool

ProGenosis' working procedure does consist in consecutive milestones, the Task. Each Task corresponding to a very well defined set of jobs. At the completion of any of these tasks, the customer is free to stop the project or re-direct it.

Task	Article	Description
1	Gene analysis and synthesize of the coding gene	<p>ProGenosis advise to chemically synthesize the structural gene coding for the protein of interest in order to:</p> <ol style="list-style-type: none"> 1. Avoid PCR mutations and truncated fragments or variants coming from a human cDNA library. 2. Optimize the codon usage for <i>E.coli</i> expression. 3. Avoid regions of very high (>80%) or very low (<30%) GC content. 4. Avoid internal TATA-boxes and ribosomal entry sites. 5. Avoid repeat sequences and RNA secondary structures. <p>If the customer can provide ProGenosis with the coding gene using the appropriate codon usage, the task 1 can be skipped.</p>
2	PCR amplification, sub-cloning & sequencing	<p>Exogenous gene is introduced into ProGenosis standard expression vector in order to express BHP.</p> <p>β-lactamase (BHP carrier protein) positive clones are selected in one step on Petri dishes with increasing amount of antibiotic (selective pressure).</p> <p>Sequencing of the inserted genes is carried out.</p> <p>ProGenosis delivers a report about the success of the cloning permissiveness and the correctness of the nucleotide sequence.</p> <p>At this stage, the customer can decide to go on or to terminate the project.</p>
3	Production of BHP	<p>The hybrid protein producing clone with the best production yield is used for production purpose in <i>E. coli</i>, in order to carry out a medium scale purification.</p> <p>The first purification step is carried out with IMAC chromatography. Generally, we obtained 70% of purity. If necessary, a second purification step is performed on MonoQ anion-exchange or any appropriate chromatography support.</p>
4	Binding assay	<p>ProGenosis proposes to carry out a binding assay with purified BHP with or without competitor (+ negative control, + positive control).</p> <p>This assay is performed with the specific customer' mAbs immobilized on a microtiter plate. In parallel, BHP is also immobilized on the bottom of the well for the same experiment.</p> <p>The same binding assay is performed with an unspecific mAb.</p> <p>Any other biological out-sourced assay can be considered, as well as customer in house assay.</p> <p>Results are delivered to client into a comprehensive report.</p>
5	Mutagenesis	<p>A directed or random mutagenesis work can be carried out on the coding gene in order to identified key amino-acid involved in the binding with the antibody, with the target or the ligand.</p>

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