

PURITY. ACTIVITY. PRECISION.

Making Proteins Work for You

CONTRACT RESEARCH SERVICES

GENETIC ENGINEERING
& PROTEIN EXPRESSION



RAW MATERIALS PRODUCTION



PROTEIN PURIFICATION



PROTEIN CHARACTERIZATION



ASSAYS & ASSAY DEVELOPMENT

GENETIC ENGINEERING & PROTEIN EXPRESSION



High level of protein expression is critical for efficient and economical purification of active recombinant proteins. We offer a number of expression systems designed to meet specific goals, ranging from expression of tagged proteins as antigens for antibody generation to production of biopharmaceuticals for clinical use. Our experts can optimize expression by engineering an expression vector with a strong promoter, an efficient ribosome binding site, a high copy number and combining it with an appropriate host. Expression also depends on the gene sequence, stability, solubility, and folding pathway of the protein product. Accordingly, we will optimize these protein expression parameters when such optimization is necessary.

GENETIC ENGINEERING

- generation of a gene by PCR
- engineering into an appropriate vector
- restriction mapping to confirm identity and integrity of a construct
- DNA sequencing of an insert and consensus sequence derivation
- mini-plasmid preparation and DNA storage

EXPRESSION SYSTEMS

- *E. coli* and mammalian expression systems
- commonly used vectors and hosts for expression or systems specified by the customer
- proprietary high efficiency *E. coli* expression system offered under separate license for large scale production destined for the clinic

PROTEIN EXPRESSION OPTIONS

- stable and transient expression in mammalian expression systems
- secreted or intracellular proteins in mammalian expression systems
- cytosolic, periplasmic or secreted proteins in bacterial expression systems
- tagged or untagged proteins

EXPRESSION ASSESSMENT

- soluble vs. inclusion bodies analysis for bacterial expression systems
- SDS-PAGE, Western, ELISA or functional analysis of cell lysates or conditioned media

EXPRESSION OPTIMIZATION

- efficient promoter
- ribosome binding site
- vector copy number
- appropriate host
- growth conditions including media and additives selection, temperature, induction

RAW MATERIALS PRODUCTION



Raw materials determine the purification strategy or even the success of purification. Protein extraction/recovery could be a very important purification step in itself. Time and effort invested at this stage often result in substantially shorter purification protocols and higher yields of active protein. Our experience with various methods of protein extraction, fractionation and enrichment for both recombinant and natural sources enables us to design the most optimal production process for your starting materials.

PRODUCTION OF RECOMBINANT PROTEINS

- conditioned media up to 200L
- mammalian cell pellet
- bacterial paste from up to 50L of culture

LYSIS OF TISSUES OR CELL PELLETS by one or a combination of the following methods:

- sonication
- homogenization
- detergent solubilization
- hypotonic solution treatment

INCLUSION BODIES REFOLDING SCREEN

RAW MATERIALS ENRICHMENT

- ammonium sulfate precipitation
- sucrose gradient centrifugation
- differential detergent extraction
- partition into Triton X-114
- treatment for membrane preparations
- inclusion body isolation, wash, solubilization and refolding

CONCENTRATION OF RAW MATERIALS BY

- centrifugation
- tangential flow filtration (TFF)

QUANTITATIVE ANALYSIS OF THE TARGET PROTEIN EXPRESSION

PROTEIN PURIFICATION



Our skills in protein purification were gained over years of purification of protein pharmaceuticals, drug targets, biological system components and biochemical reagents. Each protein has its own physico-chemical and biological properties that dictate a tailored extraction, purification and characterization.

Our purification strategy aims at a homogeneous active protein preparation achieved in two, or less often, three purification steps by thorough selection and optimization of the capture step, incorporation of a gel filtration step to remove aggregates, degradation products and other contaminants, selection of buffer conditions that stabilize biological activity and prevent product degradation. Although most proteins require individual approach, we are confident that we can handle your protein. We will purify it cost-efficiently, characterize it according to your specifications and deliver it to you in an active and application-compatible form.

PURIFICATION FEATURES

- common purification scales range from 0.001g to 5g
- proteins are purified according to customer-specified purity
- purification methods from customer-supplied, published protocols or improved protocols
- *de novo* protocols tailored to customer's requirements
- any purification mode can be used
- purification method development for transfer to a GMP facility
- dedicated columns are used in each project
- efficiency is provided by the automation and precision of AKTA systems from Amersham BioSciences (currently GE)

ANALYSIS OF PURIFICATION

- SDS-PAGE and/or dot/Western blotting are routinely used for fraction analysis, additional assays can be requested
- final protein purity is determined by densitometry from Coomassie-stained gels
- final products are supplied with a certificate of analysis, purification report and, if applicable, a batch record

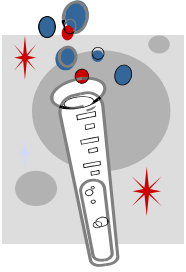
FINAL PRODUCT

- certificate of analysis is tailored to customer specification
- final products are dispensed into customer-specified aliquot sizes

AUXILIARY SERVICES

- development of affinity media
- preformulation screening
- endotoxin removal
- tag removal
- conjugation to biotin or fluorescent dye

PROTEIN CHARACTERIZATION



Analytical characterization ensures the identity, purity, structural and conformational integrity, and function of the protein. We perform a number of routine protein analyses throughout all project stages. If needed, we can submit your samples for additional methods of characterization. We also offer specialized protein characterization services for purified proteins.

ANALYSIS OF RAW, INTERMEDIATE AND FINAL PROTEIN PRODUCTS

- electrophoresis (SDS-PAGE, native-PAGE, IEF-PAGE, urea-PAGE)
- Western/dot blot
- enzyme activity assays by light absorbance or fluorescence
- ELISA (direct or sandwich)
- protein assay (A_{280} , BCA or equivalent)
- antibody isotyping
- endotoxin measurement (LAL assay)
- contaminating DNA assay
- UV-Vis absorption spectrum

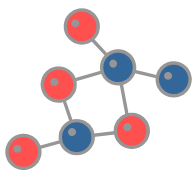
PREPARATION AND SUBMISSION OF PROTEIN SAMPLES FOR

- amino acid analysis
- N-terminus analysis
- mass-spectrometry identification and analysis
- extinction coefficient determination

PROTEIN CHARACTERIZATION

- analytical size exclusion chromatography (native MW estimate, aggregation analysis)
- analytical ion-exchange chromatography (oxidation)
- purity analysis by densitometry
- UV-Vis light absorption spectroscopy
- analysis of oxidation, degradation and aggregation products
- protein deglycosylation analysis
- protein-protein interactions by co-immunoprecipitation, spectroscopy, chromatography or BIACORE
- binding site characterization (n , B_{max} , K_d , Hill coefficient)
- enzyme steady state kinetics (K_m and V_{max})
- inhibitor characterization
- type of inhibition, IC_{50} and K_i determination

ASSAYS & ASSAY DEVELOPMENT



We offer our experience in assay development for detailed mechanistic studies of a target protein, bioanalytical measurements and high-throughput screening. Since we thoroughly characterize the materials for which an assay is developed or used our assays have maximized signal windows, low variability and are economical. These assays can have diverse applications such as analysis of enzyme activities, small molecule binding to proteins, protein/protein interactions, nucleic acids/protein interactions. In addition we offer our services for outsourcing your assays or mechanistic studies.

FUNCTIONAL MODES

- protein-protein, protein/nucleic acid, protein/small molecule or peptide binding interactions
- receptor functional assays
- enzymatic activity
- inhibition

ASSAY TARGETS

- antibodies
- enzymes, receptors and inhibitors
- peptides and small molecules

PREFERRED DETECTION METHODS

- fluorescence intensity
- fluorescence polarization
- fluorescence energy resonance transfer (FRET)
- luminescence
- light absorbance

ASSAY FORMATS

- 96-well plate
- single sample (for UV absorbance)
- HTS-compatible
- ELISA

ASSAY DELIVERABLES

- binding site characterization (B_{max} and K_d)
- enzyme steady state kinetic parameters determination (K_m and V_{max})
- inhibitor characterization and analysis of tight binding inhibitor kinetics
- single point inhibition for multiple inhibitors at a specified concentration
- IC_{50} and K_i determination
- concentration determination for proteins, peptides and small molecule

WHY ARVYS?

GET ARVYS EXPERTISE

- ✓ We integrate our expertise in genetic engineering, protein expression, protein purification, protein characterization and assay development for each project to ensure project's success.
- ✓ We monitor activity and stability of proteins through every step of purification procedures thus minimizing presence of aggregated or denatured target protein in your preparations.
- ✓ Our instrumentation and experience allow us to develop a variety of biochemical and biophysical assays and use them for protein, peptide, and small molecule characterization.
- ✓ We reduce your cost by maximizing the activity and stability of your protein product.
- ✓ We deliver endotoxin-free active proteins for your *in vivo* studies.

WHY DO YOU NEED EXPERT HELP TO PURIFY AND CHARACTERIZE PROTEINS?

Your protein which appears as a single protein band on SDS-PAGE could be heterogeneous due to:

- Aggregated, modified or partially degraded forms of the target protein
- Other proteins with the same rate of migration on SDS-PAGE
- Products from faster growing cells which may come from incomplete clonal selection or clone modification
- Presence of highly active impurities which are not easily visualized, such as endotoxin

Heterogeneity in preparations results in the following problems:

- Presence of even small amounts of denatured or misfolded proteins may trigger further protein denaturation leading to instability during manipulations and storage
- Small impurities may contribute disproportionately to the product concentration measurements resulting in erroneous data interpretation
- In *in vivo* studies, denatured protein, contaminants and endotoxin may induce unwanted biological responses
- In screening experiments, denatured protein may bind inhibitors and significantly affect K_i and IC_{50} values

Your protein preparation may be fully or partially inactive due to:

- Failure to form a native conformation during production or renaturation procedures
- Denaturation, degradation or modification
- Alteration by protein tagging or labeling
- Shielding of an active site by contaminating ligands

Loss or alteration of functional activity results in the following problems:

- Ligand binding parameters are changed
- Larger amounts of protein have to be used
- Result consistency may be affected
- Increased contribution from background activity reduces assay window
- Biological responses are too low to be measured
- Your protein preparation may generate inconsistent and unreliable data.

ABOUT US

ALEXANDER VINITSKY, Ph.D.

Principal and Co-founder

Alexander Vinitzky's experience in protein biochemistry and enzymology spans over 15 years in academia and pharmaceutical industry. Prior to co-founding ARVYS Proteins, he held a faculty position at the Mount Sinai Medical School and senior staff positions at Bristol-Myers Squibb and Palatin Technologies. Among his achievements are contributions to the improved understanding of the mechanism and functions of regulated protein degradation and to the characterization of proteins involved in Alzheimer's disease. As a part of Dr. Vinitzky's effort in the pharmaceutical industry, he directed purification and characterization of protein targets for potential therapeutics and developed numerous assays for screening of compounds against those drug targets. Dr. Vinitzky is an expert in biophysical/biochemical characterization of proteins and peptides and in assay development. He holds a B.A. in Biology and a Ph.D. in Biochemistry from New York University. His post-doctoral work was carried out at the Mount Sinai School of Medicine.

YELENA SHEPTOVITSKY, Ph.D., M.B.A.

Principal and Co-founder

Yelena Sheptovitsky has over 13 years of research and development experience. She held senior staff positions at International Bioimmune Systems, Inc. and Acorda Therapeutics, Inc. As a protein biochemistry team leader, she contributed to the development of protein-based therapeutics including enzymes, growth factors and antibodies of various isotypes. Throughout her career Dr. Sheptovitsky worked with various integral membrane proteins that function as tumor antigens, cell adhesion molecules and electron-transfer systems. Her areas of expertise are purification, characterization, stabilization and formulation of proteins, antigen identification. Dr. Sheptovitsky has a M.Sc. degree in Chemistry from Moscow State University, a Ph.D. in Biochemistry from Yale University and conducted her post-doctoral work at Memorial Sloan-Kettering Cancer Center. In addition to her scientific background Dr. Sheptovitsky holds a M.B.A. degree from the University of Connecticut.

NIKOS PANAYOTATOS, Ph.D.

Consulting Director

Nikos Panayotatos has over 25 years of academic, industrial and consulting experience in R&D of biologics. He has held senior positions at Biogen, Michigan Biotechnology Institute and Regeneron Pharmaceuticals. He has planned and directed the development of many recombinant proteins as therapeutics, such as interferon, cytokines and neurotrophic factors. He has invented gene expression systems, protein production technology, rationally designed proteins and novel methods for delivering small molecules for therapeutic use. His work is described in over 40 major publications, 17 US patents and over 50 patents worldwide. Dr. Panayotatos has received degrees from the University of Athens (B.Sc.), Ohio University (M.Sc.) and Loyola University of Chicago (Ph.D.) and conducted post-doctoral work at the University of Wisconsin.

WORKING WITH US

PROJECT SCOPE

Each project is tailored to the customer's specifications of scale, purity, analytical characterization and activity. We help our customers plan their projects in the most cost-efficient manner.

QUALITY ASSURANCE

We set high standards for our services and stay current with the latest developments in science and technology. To ensure project coordination, a project director from our senior scientific staff is assigned to every project. A project director manages all project stages – initial evaluation, quotation, planning and execution, preparation of final reports, shipments and technical support – and makes sure that a project is performed according to the customer's specifications. A project director is the customer's main contact at ARVYS.

PRICING POLICY

Our goal is to make outsourcing to ARVYS an affordable option for life scientists from both industry and academia, and yet to provide our services at the highest professional level. We are committed to being open with customers about how we price projects. Our web site has a **List of Services** where we break up our services to a single assay or a single experimental step. In our quotation we will break up our charges according to this list.

TURNAROUND TIMES

Turnaround times are provided with our official quotes. For some projects, we might break up a full project timeline into milestone timelines and provide customers with turnaround times for each step. We recognize that speed and timeliness are crucial for our clients, and we set aggressive turnaround times. At the same time, we thoroughly evaluate our human and technical resources to make sure the timelines are realistic. Once the timelines are set, we make every effort to meet them.

TECHNICAL SUPPORT

After completion of a project, we continue working with our customers for the period of 6 months to help them troubleshoot any project-related problems.

CONFIDENTIALITY

We recognize that confidentiality is vital for many of our customers. In such cases, Confidential Disclosure Agreement is executed prior to any project discussions and the following work is performed under strict confidentiality.