



### **Technology Profiles for Commercial Exploitation**

1. Mouse models of cancer for in vivo testing of novel therapeutics
2. A unique human alpha-synuclein inducible neuroblastoma cell line
3. Metabolism of peptide hormones/ drugs. Stability ranking and SAR information
4. Quantification of bioactive peptides/proteins by mass spectrometry:  
Applicability to pharmacokinetics or biomarker validation
5. Directed differentiation of embryonic stem cells
6. Drug screening assays using zebrafish
7. Biomarker Discovery in whole tissue biopsies and  
pharmacoproteomic assessment of novel pharmacologic agents in cell  
based systems using quantitative proteomic methods.

## Technology Profiles for Commercial Exploitation

Technology 1	Title: Use of mouse models of cancer for in vivo testing of novel therapeutics
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For the past 20 years, mouse models of cancer have been used primarily to study the basic biology of cancer. Validation of new drugs, on the other hand, relies on cell culture and xenograft models. Though rapid, these approaches have the disadvantage of studying the effect of drugs on tumor cells that are growing outside their natural niche.

We have developed a method that allows us to conditionally express any protein of interest in the tissue of choice in the mouse. The approach is based on the Cre/lox system and utilizes a highly and ubiquitously expressed locus, the translation elongation factor 1a (Efl) locus. As a proof of principle we developed a mouse line that conditionally expresses a Kras cDNA with a G12D substitution which is very common in human cancer. Using different Cre lines we were able to express the oncogenic Kras (Kras\*) in various tissues including the breast, the pancreas, the prostate, the hemopoetic system, the intestine, the skin and other tissues. Almost invariably, the mice developed highly invasive carcinomas which often resemble the respective human tumors. In one example, we used a mammary gland specific Cre to activate the oncogene in the mammary epithelium. The mice quickly developed invasive carcinomas which upon characterization were characterized as basal-like. Because these tumors overexpress Igf1r, we were able to successfully treat them with an inhibitor which is specific to the receptor (Klinakis et al 2009). This could be the prototype for studies that could potentially include oncogenes and tumor suppressors of interest as well as new drugs and combinations of drugs.

### Main applications

- Validation of new drugs
- Identification of biomarkers

Type of partners/collaboration sought	We are seeking partners from the pharmaceutical industry that would have an interest in the development of novel antitumor drugs.
Research group:	Laboratory of genetics and gene therapy
Group Leader:	Apostolos Klinakis, PhD
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## Technology Profiles for Commercial Exploitation

Technology 2

Title: Generation of a unique human alpha-synuclein inducible neuroblastoma cell line (patented)

The need for appropriate cell systems as models for Parkinson's disease is high. We have therefore generated inducible a-synuclein human neuroblastoma SHSY5Y cell lines under the control of the TET OFF promoter. Cell lines express the known alpha-synuclein mutations (A53T, A30P,) as well as the WT form.

Our results show that the gene is tightly regulated and, upon induction of expression, **soluble cytosolic alpha-synuclein oligomeric species form** in all cell lines. We have used this system to investigate the mechanisms through which such soluble oligomers form, develop and are degraded, as well as the impact that they may have on the neuronal degradation machinery. We have found that WT alpha-synuclein oligomer formation tightly correlates with death in this cell system. (Vekrellis et al., 2009, J. Neurochem.). Additionally, we found that WT alpha-synuclein **is physiologically secreted** from expressing cells and can be toxic to neighboring neurons. Such studies may have implications not only for Parkinson's disease, but also for other neurodegenerative diseases in which cytosolic soluble oligomers may play a pathogenic role.

### Main applications

- Cell death model for Parkinson's Disease
- Synuclein oligomer characterization/manipulation
- Discovery/development of novel drugs that inhibit or delay protein oligomerization and therefore death.

Type of partners/collaboration sought

We are seeking partners from the pharmaceutical industry that would have an interest in the development of "toxic oligomer" modifying drugs or pharmaceutical and biotechnology companies with an interest in protein misfolding and neurodegenerative diseases.

Research group:

Division of Basic Neurosciences, Lab. For Neurodegenerative Diseases

Group Leader:

Leonidas Stefanis, Kostas Vekrellis

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Group size:

12

## Technology Profiles for Commercial Exploitation

Technology 3

Title: Metabolism of peptide hormones/ drugs. Stability ranking and SAR information.

Peptide hormones are primarily metabolized by the kidney, mediated by enzymes found in the kidney basolateral membrane. We have developed a robust and versatile approach with the aim of ***in vitro* metabolic studies** for **peptide hormones and drugs, allowing peptide stability evaluation and metabolite characterization.**

Peptides of interest (hormones, drugs) are incubated with mouse kidney membrane preparations prior to analysis by Liquid Chromatography - tandem Mass Spectrometry (LC-MS/MS). LC-MS/MS is ideal for quantitative measurements (monitoring of metabolite formation/peptide degradation as a function of with time) and metabolite structure elucidation. We have applied and validated our methodology carrying out an extensive evaluation of novel gonadotropin releasing hormone (GnRH) peptide analogues. **Excellent correlation** has been demonstrated regarding ***in vitro/ in vivo* peptide stability** data.

The described protocol/tool is applicable for screening peptide analogues and in depth metabolic studies. It can also be applied to several peptide hormones (e.g. incretins, gonadotropins) and peptide drugs (or mimetics).

### Main applications

- Peptide stability of peptide hormones/drugs
- Metabolite characterization and monitoring
- Discovery/development of
  - **novel or generic peptide drugs,**
  - **protein drugs**

Type of partners/collaboration sought

We are seeking partners from the pharmaceutical industry that would have an interest in the development of novel peptide drugs or pharmaceutical and biotechnology companies with an interest in protein biosimilar drugs. Research groups working in the biomedical field (proteomics, peptide drug pharmacology etc).

Research group:

Division of Pharmacology - Pharmacotechnology

Group Leader:

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Group size:

7

## Technology Profiles for Commercial Exploitation

Technology 4	Title: Quantification of bioactive peptides/proteins by mass spectrometry: Applicability to pharmacokinetics or biomarker validation.
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We have developed approaches for the **absolute quantification of peptides and proteins** (drugs or biomarkers) in biological fluids or tissues. The analytical methodology employed is state-of-the-art Liquid Chromatography - tandem Mass Spectrometry (LC/ESI-MS/MS) ideal for quantitative measurements in biological fluids. We have applied these methodologies for peptides of ~1 kDa (MT-II, leuprolide). We have developed more complex methodologies for the quantification of larger polypeptides such as incretin hormones (GIP ~ 5kDa). The Division of Pharmacology-Pharmacotechnology of BRFAA, has been accredited according to the standard «**EN ISO/IEC 17025:2005**» for the analysis of the peptide drug leuprolide in plasma. In addition, it operates under the principles of Good Laboratory Practice (GLP), a necessary requirement for the clinical study of drugs.

Other methodologies for peptide/protein quantification include immunoassays which often lack the necessary selectivity for distinction between the peptide/protein, recombinant forms or transformation products. In many cases suitable antibodies for each protein analyte are very expensive or not available at all. LC/ESI-MS/MS method development is rapid, versatile and applicable to various matrices, thus providing assays that are suitable for both preclinical and clinical studies. LC/ESI-MS/MS offers near to absolute structural specificity, analyte detection in low volumes and ease of multiplexing. However, there is currently a lack of robust MS based methods for peptide/protein absolute quantification.

### Main applications

- Pharmacokinetics and biodistribution/bioavailability of bioactive peptides **following administration** (pre-clinical or clinical)
- Discovery/development of
  - **novel or generic peptide drugs,**
  - **protein drugs**
- Monitoring of peptide/protein **biomarkers - verification/validation**

Type of partners/collaboration sought	We are seeking partners from the pharmaceutical industry that would have an interest in the development of novel peptide drugs or pharmaceutical and biotechnology companies with an interest in protein biosimilar drugs.  Developers of diagnostic kits would have an interest in these technologies in order to validate putative biomarkers prior to commercial development and exploitation.
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Group size:	7

## Technology Profiles for Commercial Exploitation

Technology 5

Title: Directed differentiation of embryonic stem cells

Using a combination of extracellular signals and inducible expression of key transcription factors we have directed the differentiation of mouse embryonic stem cells to glucose responsive insulin producing cells and neural progenitors corresponding to distinct levels of the developing neural tube. We have used this approach to identify novel genes and processes that are targets of these key transcription factors and are currently validating our findings in vivo using mouse, chick and zebrafish embryos. We are extending this approach to human embryonic stem cells and improving our methodologies incorporating findings from our functional genomics approach. Specifically regarding pancreas development we have identified novel signalling pathways involved in endocrine cell specification and migration to form islets. These findings may also help in devising ways to stimulate endogenous pancreatic stem cells towards restoring b cell mass and function.

### Main applications

- Cell Therapy
- Tissue regeneration
- Drug screening

Type of partners/collaboration sought

We are seeking partners from the pharmaceutical industry that would have an interest in the development of directed differentiation of human embryonic stem cells into pancreas endocrine cells and specific subtypes of neural cells for cell therapy and drug screening

Research groups working in the developmental biology of the endocrine pancreas and the neural tube

Research group:

Division of Developmental Biology

Group Leader:

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Group size:

6

## Technology Profiles for Commercial Exploitation

Technology 6

Title: Drug screening assays using zebrafish.

The zebrafish embryo has become an important vertebrate model for assessing drug effects. Zebrafish has a plethora of advantages including small size, external fertilization, plethora of progeny and rapid development. Most of the organs develop within the first five days after fertilization and can be easily and non-invasively observed. It is well suited for studies in genetics, embryology, development, and cell biology. Zebrafish embryos exhibit unique characteristics, including ease of maintenance and drug administration, short reproductive cycle, and transparency that permits visual assessment of developing cells and organs. Because of these advantages, zebrafish bioassays are cheaper and faster than mouse assays, and are suitable for large-scale drug screening particularly for assessing toxicity, angiogenesis, and apoptosis. In addition, the effects of a compound can be studied *in vivo* and can provide more accurate data than cell base assays.

Increasing pharmacological data in a plethora of chemical compounds, demonstrates that toxic response, teratogenic effects, and LC50 in zebrafish are comparable to results in mice. The effects of compounds on various organs can be observed in the transparent animals without complicated processing, demonstrating the efficiency of toxicity assays using zebrafish embryos. However, we have developed a number of transgenic lines that can facilitate the analyses and observation of specific organs (liver, pancreas, heart, vasculature, brain). Furthermore, we are focusing on mutant lines with cardiovascular phenotypes and we are currently characterizing the genetic lesions in more than a dozen of them. These can be used to assay cardiovascular therapeutic potentials of compounds.

Type of partners/collaboration sought

We are seeking partners from the pharmaceutical industry that would have an interest in rapid, *in vivo* assays of toxicological studies during embryonic development or in specific organs.

We have also developed models of cardiovascular disease where novel therapeutic compounds can be tested.

Research group:

Division of Developmental Biology

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Group size:

5

## Technology Profiles for Commercial Exploitation

Technology 7

**Title: Biomarker Discovery in whole tissue biopsies and pharmacoproteomic assessment of novel pharmacologic agents in cell based systems using quantitative proteomic methods.**

Clinical tissue specimens and their proximal fluids pose many challenges in their analysis and characterization due to their heterogeneity and complexity (the occurrence of multiple cell types). We have developed sensitive and reproducible quantitative proteomic approaches that will allow the comprehensive analysis of protein expression (including their *in vivo* modifications) with the aim in the **discovery of candidate biomarkers with potentially predictive, diagnostic and prognostic significance and in the discovery of novel and potentially viable pharmacologic drug targets** (2008 JPR, 2009 JPR).

Key features of our quantitative proteomic methods include the use of robust tissue procurement and protein processing protocols, multi-dimensional liquid chromatographic techniques (MDLC), stable isotope labeling (with iTRAQ reagents), two-dimensional gel electrophoresis (2DGE) based and high-resolution tandem mass spectrometry (MS-MS) techniques. **These LC-MS methods exhibit great versatility and reproducibility and can be tailored to address the intrinsic biological constraints of the various clinical tissue specimens** (i.e. breast and prostate whole tissue biopsies).

As an extension to our protein expression studies of whole tissues specimens, we have developed LC-MS methods that **allow for quantitative and temporal protein expression studies of cell culture models** (i.e. DU-145, PC3, LnCAP, MCF7) administered with biologically active drug agents. These methods allow for direct protein-ligand interaction studies through the use of **non-covalent activity based proteomic profiling techniques** and indirect, enzyme-induction studies at cell cycle relevant time points (2 manuscripts in preparation). Collectively, **these pharmacoproteomic methods allow for the elucidation of the combinatorial signal modulation effects of novel pharmacologic agents.**

### Main application areas

- Discovery/development of
  - **Candidate biomarkers with potentially predictive, diagnostic and prognostic significance,**
  - **Novel pharmacologic drug targets**
  - **Characterization of novel mechanisms of drug action (in cell culture models)**
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Type of partners/collaboration sought

We are seeking partners from the pharmaceutical industry interested in:

- Participating in a multi-center biomarker verification and validation study of interest to either breast and prostate cancer based on whole tissue specimens and their proximal fluids traceable to well defined clinical designs,
- Screening pharmacologic agents with pre-clinical data for their multi-target and multi-factorial signal modulation effects in cell culture systems

Research group:

Biotechnology

Group Leaders:

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Group size:

6 (LC-MS), 10 (2DGE)