**Baylor-Rice SRP: Polycyclic aromatic hydrocarbons: Maternal Exposure, Toxicity, and Remediation**

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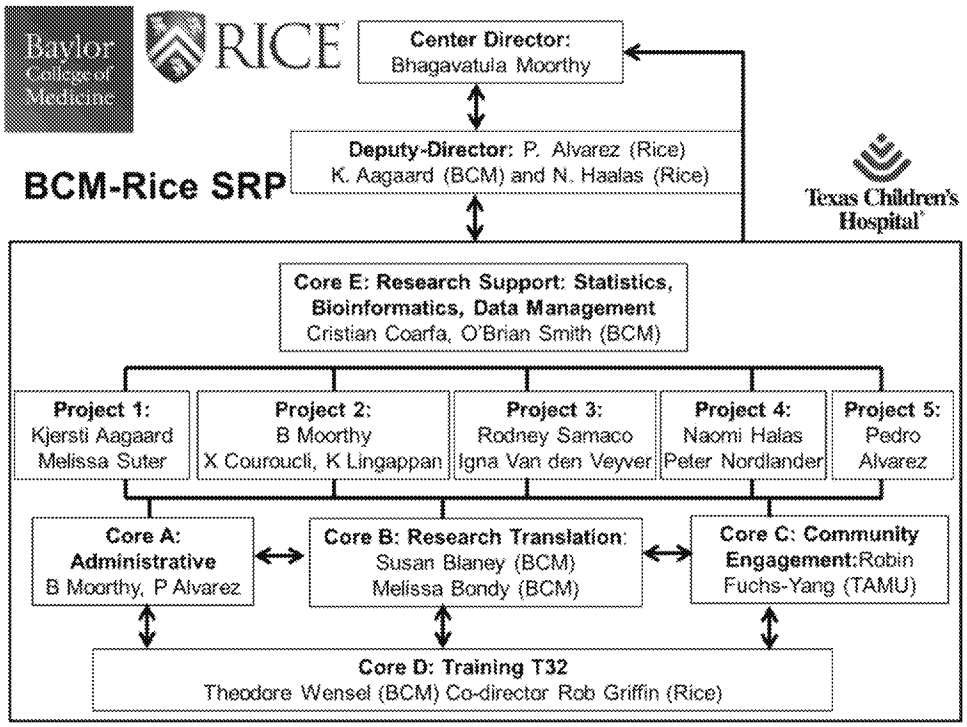
**Specific Aims of the Overall Center**




The 5 projects and 5 cores of the Baylor-Rice Superfund Research Program (SRP) involve biomedical research on the molecular mechanisms by which polycyclic aromatic hydrocarbons (PAHs) elicit health risks [chronic lung disease (CLD), also termed bronchpulmonary dysplasia (BPD), and neurological deficits (e.g., autism spectrum diseases (ASD) to human populations via maternal exposures. This will be combined with environmental science research on (i) tool development [surface-enhanced Raman spectroscopy (SERS)] that can detect and measure PAHs at 1 ppb in the environment (air, water, soil) and (ii) remediation of PAHs in the environment (soil and sediments) via use of photocatalytic nanoparticles and novel thermolysis approaches.

There is significant human exposure to PAHs through cigarette smoke, diet (e.g., charcoal-broiled steak), diesel exhausts. PAH exposure also occurs through contaminated soil and sediments, and water at various superfund sites across the US, including multiple sites in the greater Houston area. The **overarching hypothesis** of this SRP is that maternal exposure to PAHs increases the risk for preterm deliveries and the morbidities associated with preterm birth in the newborn that perisist through adulthood. The overall scientific aims of the SRP are:

**Specific Aim 1.** To measure PAH levels [benzo(a)pyrene (BP) and benzo(k)fluoranthrene (BkF) in serum and placentae from term and preterm births, and perform epigenomic and transcriptomic analyses in cytotrophoblasts isolated from term and preterm birth placenta (project 1 in conjunction with metabolomics studies (bioinformatics and statistics core (E) and project 4 that will develop ultrasensitive tools (e.g., SERS). We also seek the assistance of training core (B), research translation core (RTC) (core C), community engagement core (D) for these studies, with overall direction from administrative core A.

**Specific Aim** 2. Define the molecular mechanisms by which maternal PAHs potentiate chronic lung disease [bronchopulmonary dysplasia) in newborn mice (project 2 in conjunction with project 1, cores A-E) and test the hypothesis that maternal PAH exposure in humans enhances the risk of preterm birth, which in turn could lead to BPD in premature infants and ASD in toddlers that persists through adulthood (project 2 in conjunction with projects 1, 3, 4, and core A-E).

**Specific Aim** 3. To understand the molecular mechanisms by which PAHs, alone or in combination with hyperoxia, contributes to neurobehavioral outcomes and test whether these environmental risk factors modify phenotypic severity in ASD rodent models (project 3 in conjunction with projects 2, cores A-E, and project 4). Specifically, we will test the central hypothesis that prenatal PAH exposure, alone or in combination with postnatal hyperoxia, acts synergistically with a susceptible genotype to cause an individual to develop neurobehavioral deficits reminiscent of autism and intellectual and developmental disability (IDD).

**Specific Aim** 4.. To develop high-sensitivity (SERS) methods to detect PAHs at ppb levels in (Project 4) biological samples (projects 1-3) and in air, water, and soil (projects 4 and 5, in conjunction with cores A-E). In addition, we will design and develop high-efficiency photocatalytic nanoparticle substrates for PAH decomposition (projects 4 and 5).

**Specific Aim** 5. To develop a novel remediation technology to treat sediments contaminated with PAHs in a manner that completely removes the associated health risks while adding value to the impacted media. Our **hypothesis** is that pyrolysis of contaminated sediments will convert all PAHs into a biochar-like material, thus completely eliminating toxicity while enhancing soil fertility. We will test for proof of principle of PAH remediation by determining the toxicity of parent PAHs and their derivatives in human pulmonary cell culture systems (Project 2 in conjunction with projects 4 and 5).

Our work addresses the four SRP mandates, in an integrated manner, in the following ways: 1. **Detection, assessment, and evaluation of the effect of hazardous substances on human health**: Projects 1-5 and cores A-E). 2. **Methods to assess the risks to human health presented by hazardous substances**: Projects 1-3 in conjunction with projects 4 and 5 and cores A-E. 3. **Methods and Technologies to Detect Hazardous Substances in the environment**: Project 4 in conjunction with projects 1 and 2, and cores C, D, and E. 4. **Basic biological, chemical, and physical methods to reduce the amount and toxicity of hazardous substances**: Project 5 in conjunction with projects 2 and 4 and cores A-E.

Thus, using an integrated approach comprising multiple institutions, multiple investigators entailing scientists, physicians, engineers, community workers, trainees (students and postdoctoral fellows), and stakeholders (NIEHS, EPA, ASTDR), and local government bodies we will accomplish the goals of this SRP that will benefit the communities living near the superfund sites in the Houston area, and this will also be applicable to superfund sites across the nation.

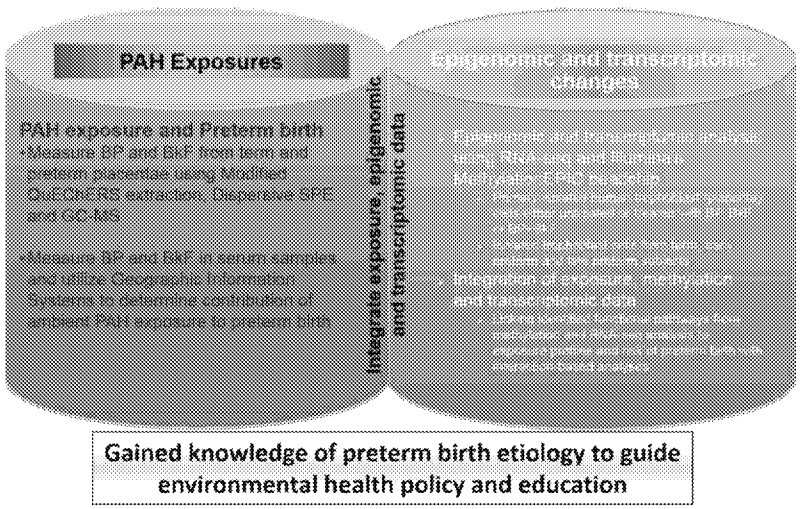
**Project 1: Maternal PAH exposure and Preterm Birth**

Project leader: Kjersti Aagaard, M.D., Ph.D. Co-leader: Melissa Suter, Ph.D. (BCM)

Baylor College of Medicine, Houston, TX

**Specific Aims**:

In this project proposal, we will delve into the molecular underpinnings of preterm births (PTB) occurring in association with environmental exposure to two prevalent PAHs: BP and BkF. Based on ours and prior studies, our **central hypothesis** underlying our proposal is that *these ambient exposures will be associated with an increase in PTB, which is mediated through epigenomic-driven modulations in placental gene expression.* We hypothesize that state-of-the-science genome-wide placental transcriptomics and epigenomics will reveal readily translatable insights on the underlying molecular mechanisms. We further hypothesize that use of Geographic Information Systems (GIS) with our existing robust, universal perinatal database and biorepository (PeriBank) will provide further population-based




***Overview of Project*** We will measure PAH levels (BP and BkF) in serum and placentae from term and preterm births. We will also perform epigenomic and transcriptomic analyses in cytotrophoblasts isolated from term and preterm birth placenta. Utilization of Geographic Information Systems will allow us to determine the specific contribution of these PAHs to preterm birth and to gain information on the mechanism behind

insight into how and where women are exposed to these PAHs.

**Specific Aim 1. Measure PAH derivatives from existing term and preterm birth population-based and enriched cohorts to determine the contribution of PAH exposure to preterm birth.** Exposure to environmental PAHs has long been known to be associated with an increased risk of cancer, but their contribution to PTB is poorly understood. We will measure the serum (maternal and cord blood) and cumulative (placental) levels of BP and BkF in *readily available, banked* samples from subjects stratified by weeks gestation (term [37-40 weeks], early preterm [>24-32 weeks] and late preterm [>32 to 37 weeks]) using dispersive solid phase extraction (SPE) and gas chromatography mass spectrometry (GC-MS). Specifically, we will measure PAHs in banked maternal and fetal (cord blood) serum which had been drawn 4 separate times throughout gestation, as well as from the placental parenchymal tissue. We hypothesize that relative increases in PAH exposures will be associated with PTB. We further hypothesize that PAH levels will be dose dependent, with a tight inverse correlation between log variation and severity of disease (early preterm birth, measured as weeks gestation).

**Specific Aim 2. Use our established whole genome transcriptome and epigenome pipelines to measure variation in DNA methylation and associative gene expression in the placenta, and integrate the data with PAH levels.** We have previously shown site specific genome-wide DNA methylation changes which significantly correlated with changes in placental gene expression following maternal tobacco smoke exposure. Based on these observations, we hypothesize that there will be measureable and significant alterations in placental DNA methylation and gene expression in association with duration and dose of PAH exposure.

**Aim 2a.** We will isolate and culture primary trophoblasts from term placentae and treat with either BP, BkF or BP+BkF. RNA-seq and 850K DNA methylation array analyses will be performed on treated and untreated cells. We hypothesize that these experiments will enable us to determine the direct contribution of these two PAHs to epigenomic and transcriptomic changes *in vitro*.

**Aim 2b.** Placentae will be collected from subjects and stratified by weeks gestation (term, early preterm and late preterm). From these placentae, primary cytotrophoblasts will be isolated and utilized for RNA-seq and 850K DNA methylation analyses. From the same placenta samples, BP and BkF levels will be measured via GC-MS. We will integrate PAH exposure level with genome-wide data to identify networks driving the association of PAH exposure and PTB, and employ Bayesian and supervised learning approaches in causal analysis.

**Specific Aim 3. Employ Geographic Information Systems (GIS) to determine the contribution of PAH exposure to preterm birth.** We have recently demonstrated that exposure to vehicular traffic emissions is associated with PTB. We hypothesize that preterm birth risk varies by vehicle miles traveled (which serves as a surrogate for duration and dose of PAH exposure). For this aim we will utilize *readily available, banked* specimens of maternal blood from a large, population-based cohort to measure PAH levels using GC-MS. Specimens will be chosen based on zip code of residence by trimester of pregnancy, and PAH levels will be stratified by zip code of residence, zip code of employment and vehicle miles traveled in order to determine the direct contribution of PAH exposure to PTB due to vehicular traffic emissions.

**Project 2.**

Role of CYP1 enzymes in PAH-mediated exacerbation of chronic lung disease (bronchopulmonary dysplasia (BPD).

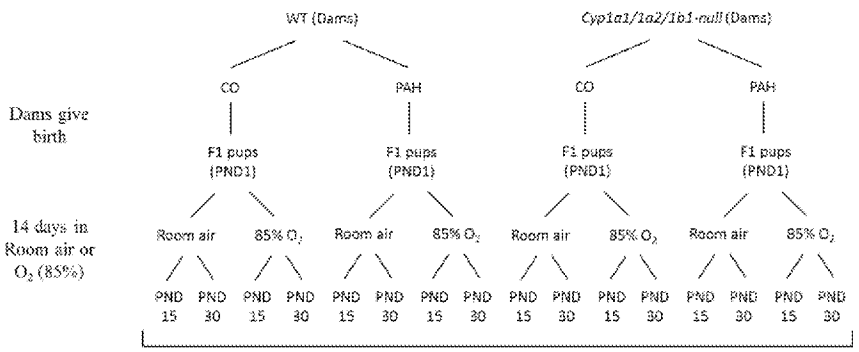
Project Leader: Bhagavatula Moorthy, Ph.D. (BCM)

Co-Project Leaders: Xanthi Couroucli, M.D. (BCM) and Krithika Lingappan, M.D. (BCM)

## Specific Aims

Pregnant women, who are exposed to polyclic aromatic hydrocarbons (PAHs) through cigarette smoke, diet, or other sources (diesel exhausts, air pollution and drinking water contamination in women living near superfund sites), are at a higher risk for preterm delivery. Preterm delivery requires the neonate to be subjected to supplemental oxygen (hyperoxia), and this in turn could lead to chronic lung disease/ bronchopulmonary dysplasia (BPD). BPD is associated with long-term long-term morbidities, and many of the complications are life-long. Maternal PAH exposure could also lead to neurological deficits (e.g., autism spectrum diseases (ASD)] in children born to these mothers, and it is known that premature birth is a risk factor for the development of ASD and other neurologcial diseases. Because term as well as preterm infants suffering from pulmonary insufficiency are frequently administered supplemental oxygen, we hypothesize that hypothesize that prenatal PAH exposure will exacerbate the effects of postnatal supplemental oxygen. The mechanisms by which PAHs potentiate BPD in infants are not well understood. Because 10% of all deliveries in the US are premature, and because the infants born to these mothers are at high risk for developing BPD, which leads to arrested lung development, and morbidity, there is an urgent need to develop strategies to prevent and/or treat BPD in these infants. The central hypothesis of this project is that prenatal administration of PAHs [i.e. benzo[a]pyrene (BP), benzo(k)fluoranthene (BkF), or BP + BkF], which are which are defined as class B2 carcinogens by USEPA, will differentially exacerbate lung injury and alveolar simplification in neonatal mice following postnatal hyperoxia, and that this effect will be altered in mice lacking the gene for cytochrome P450 *(Cyp)1a1, 1a2, or 1b1 genes* by mechanisms entailing a combination of genotoxic and epigenetic mechanisms. In order to test this hypothesis, we propose the following **Specific Aims**:

**Specific Aim 1.** To test the hypothesis that prenatal exposure of wild type (WT) (C57BL/6J) mice to BP or BP + BkF will result in exacerbation of lung injury and alveolar simplification following postnatal hyperoxia, and this effect will be altered in mice lacking the gene for *Cyp1a1, 1a2*, or *Cyp1b1* genes Timed pregnant WT mice, or mice lacking the gene for *Cyp1a1, 1a2 or Cyp1b1-null* will be treated orally with corn oil (vehicle control) BP or BP + BkF on gestational days 14-19, and newborns will be delivered at full term (day 22). The newborns will be maintained in room air or exposed to hyperoxia (85% $O_2$) for 14 days, and animals will be sacrificed on postnatal day (PND) 15. Some of the animals will be weaned of hyperoxia on day 15, and will be allowed to grow in room air until PND 30 or 45. While a sub-set of these animals will be sacrificed to determine persistence of abnormal lung alveolarization on PND 30 and 45, another sub-set of animals will be tested for neurobehavioral outcomes in collaboration with Dr. Rodney Samaco of the (Project leader of project 3). Extent of pulmonary and hepatic CYP1A1, 1A2 and 1B1 expression will be studied. Lung injury and inflammation alveolarization and vascular development will be studied by histology, immunohistochemistry, and morphometry, respectively. We will also save placentas of the dams during delivery for analysis of PAHs, PAH-DNA adducts, and CYP profiling. The placental studies will be conducted in collaboration with Dr. Kjersti Aagaard and Melissa Suter (Project leaders of project 1).




**Specific Aim 2.** To determine the mechanisms by which prenatal PAHs will alter the susceptibility of neonatal mice to hyperoxia. We hypothesize that utilization of transcriptomics and epigenomics will yield mechanistic insight into the causative etiology of BPD in these mice. PAH-DNA adducts and oxidative DNA lesions will be determined in lungs of mice (used in Specific Aim 1) exposed prenatally to PAHs the $^{32}$P-postlabeling assay. Levels of PAHs and their metabolites will be determined by GC-MS analyses at the metabolomics part of the Bioinformatics and Statistical core (Core E). RNA-Seq analyses will be conducted to determine the molecular pathways by which PAHs modulate lung injury. Concomitantly, we will perform a thorough epigenetic analysis to determine the pulmonary reprogramming which occurs by virtue of prenatal exposure to PAHs. Specifically, we will study global DNA methylation using methylated DNA immunoprecipitation (MeDIP) followed by hybridization to a promoter array chip (MeDIP-chip). We will perform ChIP-Seq using a modification enriched

in active chromatin (H3K4me3) and repressed chromatin (H3K9me3). Lastly, we will perform microRNA profiling to determine the role of microRNAs in the potentiation of oxygen toxicity by PAHs. These studies will be conducted in conjunction with Dr. Kjersti Aaagard of the Department of OBGYN at Baylor (Project 1).

**Specific Aim 3.** To test the hypothesis that mothers exposed to PAHs (that are present in superfund sites) are at a greater risk for preterm delivery, and that these infants will show increased susceptibility to develop BPD than those with lesser or no exposure. We also hypothesize that premature neonates have a greater risk for developing ASD as toddlers, and that PAH exposure augments this risk. In this prospective study, we will enroll mothers (term and preterm) (n=1000) who are admitted to the Ben Taub General Hospital (BTGH) or Texas Children's Hospital's (TCH) Pavilion for Women (PFW), and collect cord blood at the time of delivery for determination of PAH levels. We will also test if the term or preterm infants who had significant maternal PAH exposure will have a greater tendency to develop ASD when they are about 18 months old, and if this phenomenon is exacerbated in infants who had BPD. The patients will be screened for ASD and other behavioral disorders at the follow-up clinic at the TCH.

**Specific Aim 4.** To determine the mechanisms of toxicity of PAHs on human pulmonary cells (e.g., primary cells, BEAS-2B or H441 cells). We will test if the proposed remediation experiments in projects 4 and 5 using photocatalytic nanoparticles will results in attenuation of toxicity in human pulmonary cells.

The proposed studies should provide critical and conceptual foundation(s) for achieving our long-term goals, which are the elucidation of the molecular mechanisms of oxygen-induced lung injury, and the development of rational strategies for the prevention and treatment of BPD and ASD in infants.

## Project 3.

## Effect of prenatal PAH and postnatal hyperoxia on neurobehavioral outcome in rodents

Project Leader: Rodney Samaco, Ph.D. (Baylor College of Medicine)

Co-Project leader: Ignatia Van den Veyver, M.D. (Baylor College of Medicine)

### Specific Aims:

Social behavior and cognition are governed by both genetic and environmental factors, yet the environmental factors that may influence normal neurobehavioral outcome remains poorly explored despite the need to better understand it for human health. This is underscored by numerous recent findings implicating dozens of exogenous substances such as environmental pollutants and toxins in the pathophysiology of autism spectrum disorders (ASDs). ASDs, whose core features include marked deficits in social interaction and communication, and restricted repetitive behaviors, are a prominent public health concern with a current prevalence 1:68, with up to 1:42 in boys. Co-morbidities, such as intellectual disability, seizures and impaired fine motor coordination are common.

Although greater than one hundred genes and genetic susceptibility loci have been associated with syndromic ASDs or non-syndromic autism, the penetrance of autism and autism-related phenotypes for many cases is incomplete. Several hypotheses have been put forth to explain the phenomenon of incomplete penetrance; however, it is conceivable that a combination of both genetic and environmental risk factors that affect early development must be considered to reconcile the relatively high heritability with the rising incidence of autism. Among the common environmental risk factors present in air and diet, evidence suggests that prenatal exposure to polycyclic aromatic hydrocarbons (PAHs) such as benzo[a]pyrene (BP) may have an adverse effect on neurobehavioral outcome. Moreover, early postnatal supra-physiological changes in oxygen leading to hyperoxic conditions is another common environmental risk factor that is strongly linked to preterm birth associated with impaired lung development such as bronchopulmonary dysplasia (BPD, see Project 2) and neurodevelopmental impairment (NDI). In this regard, it remains unclear whether these effects on normal brain function/development are due to PAH exposure alone or is exacerbated due to 'sensitized' genetic perturbations and/or postnatal hyperoxia exposure. Therefore, the overarching hypothesis for this proposal is

that prenatal PAH exposure, alone or in combination with postnatal hyperoxia, acts synergistically with a susceptible genotype to cause an individual to develop neurobehavioral deficits reminiscent of autism and intellectual and developmental disability (IDD). If true, we anticipate that prenatal PAH exposure alone or in combination with postnatal hyperoxia in human populations may be an important, previously unaccounted for risk factor contributing to the clinical heterogeneity of ASDs. In particular PAHs could act in combination with genetic ASD susceptibility factors including male sex to enhance disease severity. To address this hypothesis, we propose to study the consequences of prenatal PAH exposure on neurobehavioral outcome in two rodent models of syndromic autism. **The goal of this proposal is to understand the mechanism of how PAHs, either alone or in combination with hyperoxia, contribute to neurobehavioral outcome and test whether these risk environmental risk factors modify phenotypic severity in ASD rodent models.**

**Specific Aim 1. Determine the relationship between prenatal PAH exposure, postnatal hyperoxia, genetic susceptibility and alterations in behavior.** To test whether prenatal PAH exposure alone, or in combination with postnatal hyperoxia exacerbates neurobehavioral outcome in a genetically sensitized background, we will expose pregnant mice harboring mutations in i) genes of the cytochrome P450 family (from Project 2, as a model of prematurity associated with BPD and NDI) or ii) ASD genes, *Fmr1* or *Mecp2,* to PAH, and expose male and female progeny during early postnatal life to normoxia or hyperoxia, and test for alterations in sociability and learning and memory during both juvenile and adult stages of life.

**Specific Aim 2. Examine the molecular consequences of prenatal PAH exposure and postnatal hyperoxia in mouse models of ASD.** To investigate whether PAH and/or hyperoxia exposure may increase phenotypic severity in ASD mouse models due to changes in molecular and biochemical substrates, we will analyze RNA by RNAseq and protein expression changes by metabolomics studies using the brains of ASD mice exposed to these environmental risk factors (prenatal PAH, early postnatal hyperoxia) either alone or in paired exposures.

**Specific Aim 3. Confirm the phenotypic and molecular consequences of prenatal PAH exposure and postnatal hyperoxia in ASD rat models.** To complement this work and as an independent approach to confirm whether these risk factors indeed have an effect on genetically sensitized animal models of ASD, we will conduct identical behavioral and molecular evaluations on genetic rat models of *Fmr1* and *Mecp2.* Studies of genetic ASD rat models provide a unique opportunity to test our hypothesis in a second mammalian species.

The features of the proposed work should maximize our understanding of how either prenatal PAH exposure and/or postnatal hyperoxia, in combination with genetic susceptibility causes poor neurological outcome. Taken together, this work is an important first step in forging a definitive link specifically between these specific environmental risk factors and ASD-like phenotypes. Our findings will have broad implications, given the importance of gene x environment interactions in the pathogenesis of ASDs and possibly other IDD.

## Project 4:

## Ultrasensitive environmental detection and enhanced remediation strategies for PAHs based on optically active engineered nanomaterials

Project Leader: Naomi Halas, NAE, NAS , Rice University, Houston, TX
Co-Project Leader: Peter Nordlander, Ph.D. Rice University, Houston, TX

### Specific Aims

**PAH Detection**: The presence of PAHs in air, drinking water and soil results in possible human exposure and ingestion with numerous deleterious human health and environmental consequences, only a subset of which have been investigated. While initial attempts have been made over the past decade to develop analysis tools

for detection and quantitation of PAHs in environmental samples, large laboratory-sized analysis instrumentation (typically GC-MS) still remains the gold standard for PAH detection and quantitative analysis. The most promising new approach for this problem is based on surface-enhanced Raman spectroscopy (SERS), where the weak Raman signature of PAH molecules is enhanced by its proximity to metallic nanostructures with strong local fields $|E|$ that enhance the Raman signal by $|E|^4$. When the local fields are strongly enhanced by the optically excited surface plasmon of the metal nanostructure, SERS detection levels can be lowered drastically, into the single-molecule regime. However, in environmental applications to date, such sensitivities have not yet been demonstrated, with detection concentrations remaining in the tens of ppm range, entirely insufficient for environmental PAH monitoring applications.

Based on our decades-long expertise in the accurate electromagnetic design, nanofabrication, and characterization of metallic nanostructures with well-controlled surface plasmons and local electromagnetic properties for chemical sensing applications, we propose to develop high-sensitivity (ppb) methods for PAH detection and quantification. Our goal is to achieve detection sensitivities equivalent to GC-MS-based methods but in ultracompact geometries that can ultimately be field-able testing and monitoring tools. Our efforts will be focused specifically on the development of Raman-based and Surface-enhanced Raman-based methods to achieve this goal. In a tightly integrated theoretical-experimental, predictive design-based approach, we intend to execute the following **Specific Aims**:

1. demonstrate hydrophobic Au-based SERS substrates and quantify detection limits of test PAHs anthracene and pyrene from aqueous PAH solutions and water-based samples, based on proven test designs based on our prior research;
2. characterize airborne PAH sample detection in the form of condensed aerosol samples on hydrophobic Au-based SERS substrates and quantify detection limits;
3. develop sustainable, low-cost hydrophobic Aluminum-based SERS substrates for short-wavelength PAH SERS detection, to exploit both electromagnetic enhancement due to the plasmonic properties of the substrate and resonant electronic enhancement of the analyte molecules themselves. The design, fabrication and testing will be performed both on aqueous PAH solutions of test PAHs, and on environmental air, water-based and soil samples;
4. develop SERS-based substrates and sampling strategies for the testing and analysis of physiological samples, to provide analytical information for assessment of patient exposure levels (Projects 1 and 2);
5. design and develop sustainable, Aluminum nanocrystal-based standalone SERS enhancers specifically for soil analysis. For testing, the nanoparticles will be placed on prepared solid-phase samples for SERS PAH quantification;
6. utilize both Au-based and Al-based SERS sensors to analyze the pyrolysis-based PAH remediation (Alvarez project) outcomes;
7. develop and demonstrate an ultracompact, all-in-one Raman detector/analyzer for PAHs in air, water-borne, and soil sediment samples. We have recently demonstrated narrowband photon detection in the near-IR based on nanoantenna-diodes invented in our research group. Here the SERS substrate and the detector are combined in the same device. These devices, which can detect optical frequencies within a narrow spectral band, can be combined in an array to serve as an ultracompact spectroscopic detector, entirely eliminating the need for the bulky and sensitive monochromators currently used for spectroscopic detection. We will design detectors for specific PAH molecules and test detection limits of this approach.

The ultimate outcomes of this research will be robust, fieldable detection methods for PAH contaminant molecules that can be used to analyze physiological, airborne, water-based and soil sediment samples, that can be widely used for environmental PAH monitoring in the full range of settings that directly address project goals.

## Project 5.

## Pyrolytic Conversion of PAHs in Contaminated Sediments into Char to Eliminate Toxicity and Enhance Soil Fertility

Project Leader: Pedro J.J. Alvarez, Ph.D. Rice University, Houston, TX

## Specific Aims

Cost-effective remediation of sites impacted by PAHs and other persistent hazardous pollutants is of critical importance to mitigate exposure and protect public health. Whereas numerous remediation approaches have been developed, many are marginally cost-effective or impractical to treat contaminated sediments. Thus there is a pressing need for technological innovation that leads to faster, more economical and more sustainable remediation of contaminated sediments at Superfund sites, particularly those located near large population centers such as sites along the Houston Ship Channel and Galveston Bay sites.

We seek to develop a novel remediation technology to treat sediments contaminated with PAHs in a manner that completely removes the associated health risks while adding value to the impacted media. Our **hypothesis** is that pyrolysis of contaminated sediments will convert all PAHs into a biochar-like material, thus completely eliminating toxicity while enhancing soil fertility. Biochar, a form of charcoal usually produced by pyrolysis of carbon-rich biomass, draws tremendous interest worldwide due to its demonstrated ability to enhance soil fertility, facilitate soil water management, sequester $CO_2$, and manage organic waste. Thus, pyrolytic conversion of PAHs and possibly other recalcitrant organic hazardous pollutants into char would facilitate broad regulatory and public acceptance of this novel technology, as well as subsequent site re-development or ecosystem restoration and re-greening efforts. **Specific Aims** for this project include to:

**Specific Aim 1. Demonstrate the potential to convert PAHs present in Superfund site sediments into biochar-like material through pyrolysis.**

**Specific Aim 2. Characterize the reaction mechanisms and end products to guide safe and cost-efficient application**

**Specific Aim 2a.** Use thermal analysis and online mass spectrometry to discern the physical and chemical processes occurring during pyrolysis, and characterize the chemical composition and dispersion of the produced char. We will determine if the produced char is present in discrete carbonaceous particles or coats the surface of soil particles that may have acted as catalysts for its formation.
**Specific Aim 2b.** Characterize the treated sediments and the produced char (surface chemistry, chemical stability, porosity, density, water-holding capacity, and ability to hold plant-available water) as a function of reaction conditions (e.g., contact time, temperature, $\%O_2$, moisture, etc.) to inform reaction mechanisms and guide reactor optimization efforts.

**Specific Aim 3. Maximize the benefits of soil pyrolysis (PAH removal, improved soil fertility) while minimizing associated costs.**
Optimization entails lowering both pyrolysis times (and thus enhancing processing capacity) and temperatures (and thus energy requirements and carbon footprint) required to reach the desired level of PAH reduction, and serves to reduce costs associated with energy and processing time. Important tasks include:

**Specific Aim 3a.** Assess the fertility of the pyrolyzed sediments in greenhouse studies relative to untreated contaminated soil and clean background soil as controls. Measurements of % seed germination and biomass yield will be used for this assessment. We will use various plant species, including *Arabidopsis thaliana* whose genome is well understood allowing assessment of stress and transcriptomic response.
**Specific Aim 3b.** Analyze the composition of volatiles released during pyrolysis, determine their heat content, and decide whether they should be combusted for energy recovery or treated and disposed.
**Specific Aim 3c.** Develop a process simulator to forecast energy requirements and implementation costs.
**Specific Aim 3d.** Investigate the stability and potential toxicity of pyrolyzed sediments to gain regulatory confidence, maximize benefits such as improved ecosystem restoration and re-greening efforts, and ensure no future site liability. Proposed experiments include leaching tests and earthworm assays.

Pyrolytic treatment would proceed at much lower temperatures than soil incineration and other thermal treatment technologies commonly used at Superfund sites, thus overcoming two major drawbacks of thermal treatment: high energy demand and loss of soil fertility due to the destruction of soil organic carbon and other nutrients. We envision that pyrolysis will be especially advantageous for fast on-site remediation of excavated (or dredged) PAH source zones and related hot spots, eliminating disposal liability while turning a problem (contaminated sediments) into a commodity (fertile soil).

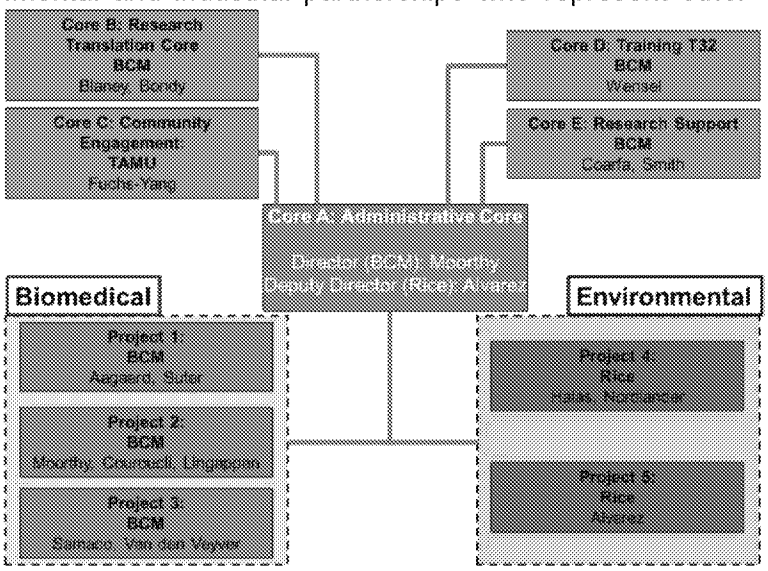
**Cores A:**

**Administrative Core**

Core Leader: Bhagavatula Moorthy, PhD, Baylor College of Medicine, Houston, TX
Co-leader: Pedro Alvarez, PhD, Rice University, Houston, TX

**Specific Aims**

The Administrative Core is primarily responsible for the overall financial management, planning of the enrichments activities, and the coordination of the research activities. The Administrative Core is the focal point of communication among the four research projects and the other three cores, to our Internal Advisory Committee (IAC) and External Advisory Board (EAB) members as well as, via the closely aligned Research Translation Core, to our sister SRP Centers, governmental and industrial partnerships who represent other professional stakeholders, and to the general public community. In order to foster interdisciplinary interactions, the Administrative Core will coordinate monthly seminars, Baylor-Rice SRP Newsletter every other month, weekly projects and cores meetings, monthly IAC meetings, and the annual EAB meetings. In addition, the Administrative Core provides assistance with coordinating and scheduling of the training and research activities of the SRP Center. These goals will be led by the Director with the help of the Deputy Director, two Associate Directors, along with guidance and support from the Program Manager of the center.




**Specific Aim 1**: Foster Continuous Interactions and communications within center investigators.

Professors Bhagavatula Moorthy, and Pedro Alverez are the Administrative Core Director and Deputy Directors, respectively. In addition, Dr. Kjersti Aagaard of BCM and Dr. Naomi Halas of Rice will be Associate Directors of the core. They will work closely with the Project Leaders and Core Leaders to promote the SRP research activities through the Research Translation Core. This effort will be accomplished through regular face-to-face meetings with the Internal and External Advisory Board members and the Program researchers and trainees, and an annual retreat.

**Specific Aim 2**: Oversee Fiscal and Data Management.

The Administrative Core will manage the fiscal resources for the entire Program ensuring that adequate and appropriate allocation of funds is maintained throughout the funding period. Project Leaders and Core Leaders will be informed of expenditures and available balances on a monthly basis. The Core will provide data management support for the Projects and Cores by tracking scientific publications, maintaining trainee records, updating Institutional Review Board (IRB) and Institutional Animal Care and Use Committee (IACUC) protocol approvals, and helping interact with the Baylor/Rice licensing Office on any patent applications.

**Specific Aim 3**: Communication strategies

The Administrative Core will be involved in all aspects of internal and external communication, and for this purpose, it is linked closely with the Research Translation Core, as well as the CEC. We will create a new website that will facilitate interactions among various investigators at Baylor, Rice, and the CEC leader at Texas A&M University. The website will highlight SRP research news, any publications, webinars, seminars,

workshops, visits by guest speakers, and other activities. This mechanism will allow us to reach a wide range of audiences. The Administrative Core will be instrumental in bringing the Projects and Cores together for cross-disciplinary collaborations and interactions.

**Specific Aim 4**: Coordination and Planning of Meetings, Research and Enrichment Activities
The Administrative Core will plan all of the meetings for the Baylor-Rice SRP Center such as monthly meetings of the IAC), weekly projects and cores meetings, and the annual meetings for the EAB, an annual retreat, etc to promote the research activities within the SRP Center to foster trans-disciplinary collaborations. In addition, the Administrative Core will develop monthly seminars and share research information. These translational enrichment activities will also be an excellent training experience for students and postdoctoral researchers within the SRP Center.

**Specific Aim 5**: Facilitation of Disseminating Research Outcomes with the Research Translation Core

The Administrative Core will work closely with the RTC Leaders to ensure productive and effective communication with other peer SRP Centers and with governmental and industrial groups who represent other professional stakeholders, such as Environmental Protection Agency (EPA), the Agency for Toxic Substances & Disease Registry (ATSDR) the Houston Department of Public Health as well as our community advocacy groups and the general public. This facilitation is critically important for the technology exchange through the Research Translation Core (RTC) to advance the research objectives to potential end-users.

## Core B:
## Research Translation Core

Core Leader: Susan Blaney, M.D. (BCM)
Co-leader: Melissa Bondy, Ph.D. (BCM)

**Specific Aims**

The objectives of the Research Translation Core (RTC) include: (a) communication within this SRP Center at the individual Project and Core levels, with NIEHS staff, and among other SRP Centers; (b) communication with our partners at governmental agencies (e.g., Department of Public Health); (c) effective technology transfer; and (d) information dissemination to other end-users. While the biomedical and Environmental Science research projects in this SRP address the needs to develop and apply next-generation tools to determine the amount, transport, and chemical and biological mechanisms of action of PAHs typically found in Superfund environments, it is critical to our mission to make certain that this research is actively translated to public health. In order to achieve these goals, we have the following Aims:

**Specific Aim 1:** Coordinate communication of research activities - within the BCM-Rice SRP, and across the SRP Centers of the US.

a. A communications network structure has been designed to enable facile communication of research ideas and results, novel training tools and engagement/translation opportunities within the Baylor-Rice SRP Center. The RTC will work with each project and core leader and identify and coordinate research translation activities. We will design a website that will help achieve this goal.

b. An effective dissemination strategy for research results is presented, and we will make the data available to the National SRP effort, the NIEHS management and other stakeholders at monthly intervals. We will publish a quarterly Baylor-Rice newsletter to capture the findings of the SRP center. The newsletter will be posted on the Baylor-Rice SRP website and will also be communicated to other SRP centers in the nation and to the NIEHS staff.

**Specific Aim 2:** Develop partnerships with government agencies and provide them with data and expertise to enhance risk assessment-based decisions.

a. We will establish communications with the NIEHS, EPA, ASTDR, and also with the state and local health departments, so they have firsthand information of the SRP activities. The SRP will also receive feedback from their counterparts in the public sector.
b. We will establish bi-directional interactions by inviting the various local, state, and federal agencies to seminars and/or webinars that will be used to share important findings of the various projects and cores, and question-answer sessions at these meetings will facilitate constructive feedback from the stakeholders.

**Specific Aim 3**: Promote technology transfer by identifying potential end-users of technologies, assays and resources developed by the Baylor-Rice SRP projects.

a. We will seek the help of our existing technology transfer offices of Baylor-Rice to help with filing patents and also to disseminate information to end users. We will establish technology transfer through patents, small business technology transfer grants, and by creating web-accessible data sharing systems that may help towards improvement in risk assessment and also help in the moving biomarker research towards epidemiological, clinical, and population based applications.
b. Newly filed intellectual property will be actively shared with regulators and with community groups.
c. An Intellectual Property training will be offered to our trainees and investigators on how to protect intellectual property in such a way that its value is maximized for both the end user and the patenting entity.

**Specific Aim 4:** We will disseminate superfund relevant information to other end users using the following approaches.

a. We will target end users such as formal/informal educational groups, hazardous waste practitioners, the lay public and other academic researches by organizing yearly workshops that will be organized and these end users will be invited to attend. In addition, we will work with CEC leaders who will disseminate the information to various communities. The RTC leader will also travel to national meetings and present the major findings at these forums.
b. We will develop web-broadcasting tools to accommodate stakeholders in remote locations or with limited travel options.

## Core C:
## Community Engagement Core

Core Leader: Robin Fuchs-Young, Ph.D. Texas A&M University, College Station, TX

### Specific Aims

The Community Engagement Core (CEC) of the Baylor/Rice Superfund Research Program will develop, implement and evaluate projects aimed at identifying and responding to community concerns through effective, bidirectional communication about risks, health effects, prevention and remediation of environmental exposures affecting human health. The research focus of the BR Superfund program is on polycyclic aromatic hydrocarbons (PAHs) and their effects on preterm birth and susceptibility to obstructive respiratory diseases, which presents a unique opportunity for effective communication of critical and cutting edge research results. The CEC has and will continue to develop partnerships with Community stakeholders to enable the development of effective response and prevention strategies to minimize the underappreciated health effects due to PAH exposure through ambient air, diet and other activities.

In keeping with the overall mission to translate scientific discoveries and accomplishments from the Superfund Research Programs (SRPs) to stakeholders and to incorporate community concerns and priorities to inform Center science, the CEC will utilize a variety of approaches to engage and share information with residents and opinion leaders about detection and effects of PAHs and other Superfund chemicals. A primary goal of is to respond to articulated needs of the communities and their opinion leaders and to translate

research findings into accurate, usable information about potential impacts on vulnerable individuals and prevention.

Engagement of target communities has already been initiated (see letters of collaboration), and discussions with community-based organizations, key informants and opinion leaders have been incorporated into the development of CEC. Planned activities include Community Science Nights, Town Halls, educational programs for teachers and collaborations with the Research Translation and Training Cores that will enhance understanding of environmental exposures, foster community involvement and build community capacity.

The Core will establish and hold regular meetings of a Stakeholder Advisory Board (SAB) to facilitate assimilation of community concerns and to communicate research findings of this and other Superfund Research Centers. Design of community events will ensure that needs and goals of these communities are a continuing focus of SRP Center research. The Specific Aims for the Community Engagement Core are:

**Specific Aim 1. To communicate with the communities, their opinion leaders and grass roots organizations about impacts and prevention of hazardous environmental exposures.**

CEC will utilize new and existing communication networks and approaches to communicate with the community residents about the health effects of environmental exposures and to translate Superfund Program research discoveries, with emphasis on PAHs and their effects on preterm birth.

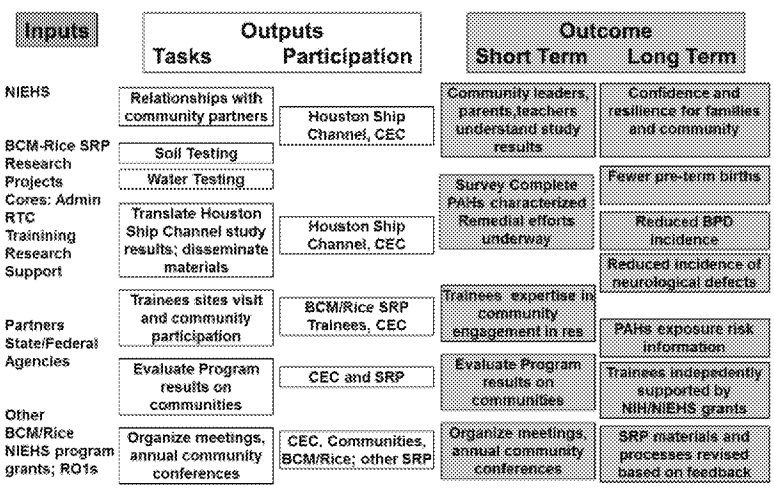
**Specific Aim 2. To provide training in the principles of Community Based Participatory Research (CBPR) for Center scientists and facilitate participation of community residents in Center research.**

With increased emphasis on investigator-initiated research translation, the CEC will collaborate with the *Training Core*, to provide education for students, post-doctoral fellows and faculty to foster understanding and incorporation of CBRP principles in all aspects of Center research and community engagement.

**Specific Aim 3. To foster communication with and involvement of local and state policy makers in response to community needs for environmental risk assessment, education and prevention.**

CEC will collaborate with the RTC and Projects 1 and 2 to respond to community needs for analysis of environmental toxicants and to provide accurate, usable and understandable information to guide prevention and remediation strategies.

**Specific Aim 4. To engage K-12 educators *in target communities* in translating superfund research discoveries and environmental health science into school programs, and classroom lessons and activities.**




CEC will engage teachers and school administrators to design and implement educational activities that translate SRP findings, and knowledge about environmental exposures and prevention strategies, into classroom lessons that enhance scientific literacy and recruitment of the next generation of EHS scientists.

**Specific Aim 5. To evaluate all activities and programs for effectiveness and impact on at-risk communities and individuals.**

An ongoing and continuous evaluation process will utilize a mixed methods approach, involving the preparation of logic maps for each type/category of activity and the design of evaluative instruments that capture the most important formative and summative information. These instruments will be designed in collaboration with our external evaluator, the SAB, opinion leaders and residents, and will include collection of quantifiable subjective and objective measurements (# participants, downloads, views, responses, etc.) to guide programmatic revisions

(formative), as needed, and to assess and document the benefits, impacts and outcomes of community engagement activities (summative).

**Core D: Training Core**

Leader: Theodore Wensel, Ph.D. (BCM)
Co-leader: Robert Griffin, Ph.D. (Rice University)

**Background**: In order to promote trainees' understanding of this unique context of Superfund research, the Training Core (TC) will enhance opportunities for cross-disciplinary training as well as research translation and community engagement experience for all Baylor-Rice SRP trainees. The TC will thereby promote trainee intellectual and professional growth and also ensure the development of a cohort of scientists who are prepared to lead future environmental science research that is responsive to public health needs. The central objective of our Training Core is to provide education and training to our SRP Scientists and engineers – those who will work on developing and applying next-generation science-based engineering approaches to research on hazardous chemicals in the areas of the Houston Ship Channel, Galveston Bay, etc. The SRP offers an unprecedented opportunity to apply basic scientific and engineering tools and hypothesis driven research to problems that are practical and pressing needs of society. The training received by students and postdoctoral researchers on this project will be enormously valuable as they progress in their careers. Programs such as this one are significant because they create a framework in which researchers working on the small details of a problem are also given the opportunity to see how that problem fits into a much larger and more complex picture. The chemicals to be studied in the field of PAHs are relevant as they are present in soil, sediments, air, and water around almost every superfund site across the nation. The training program will, however, be more generic and include a broader array of compounds typical of those found at other Superfund sites in the U.S. These are very significant human health priorities, as maternal PAH exposure can lead to many diseases in the newborn and determining the mechanisms of PAH-induced toxicities and approaches to remediate and prevent toxicity is of the highest priority.

**Program Plan**:
Program Administration: Dr. Theodore Wensel, Ph.D., Professor and Chairman of Biochemistry at BCM will lead the training core. He has vast experience in training of graduate and under-graduate students. He is already directing a T32 on The Houston Area Molecular Biophysics Program at BCM for many years, and this program has guided and directed research training in molecular biophysics for Ph.D. students in the Houston-Galveston area since 1989. This program 's major goal is allow continued excellence and innovation in training outstanding students working at the cutting edges of this increasingly important field. This program directs students from the five graduate schools of the Gulf Coast Consortium (Baylor College of Medicine, Rice University, University of Texas Health Sciences Center, Houston, University of Texas Medical Branch, and University of Houston) the program provides for common didactic and seminar courses, two local annual research conferences, monthly trainee meetings, attendance at national meetings, and annual presentation and review of trainee research progress. In addition to Dr. Wensel's experience in graduate training, he is also a leader in the field of photoreceptor membranes of the eye. He is extremely well published and well-funded. His administrative skills are evidenced by he being a successful chairman of a highly successful department of Biochemistry at BCM. The Training core will be co-directed by Dr. Robert Griffin of the Rice University.

Program Faculty: The center faculty will be drawn from BCM and Rice University. We plan to recruit 8-12 from BCM and 6-8 faculty from Rice, who have extensive experience in training students and postdocs. For those not having adequate experience we will plan to ensure successful trainee guidance by these individuals. The PIs on each project will all be mentors on the training core, and will include in their budget funds to cover their stipends.
Proposed Training: Our Training Program will employ an educational paradigm that cross-trains scientists and engineers in the needed areas of toxicology, Biochemistry, Pharmacology, Molecular and Cell Biology, Genetics, Neuroscience, and inter-disciplinary programs such as Translational Biology and Molecular Medicine (TBMM), Clinical Scientist Training Program at BCM, and several graduate disciplines in fundamental science (Biology, Physics, Chemistry) and engineering programs at Rice University. The breadth of the training opportunities on this project is vast, with many opportunities for trans-disciplinary collaborations. We plan to develop a course entitled, "**Fundamentals of Molecular Toxicology and Environmental Health"** This course

will be taught by faculty from BCM, Rice, and University of Texas SPH, University of Texas-Houston Medical School, University of Houston, etc. We expect to have at least 12 students and 4 postdoctoral fellows to participate in the training core. In addition, the training core trainees will be encouraged to various graduate courses at BCM in Molecular Cell Biology, TBMM and other programs, and they will also take courses at Rice, UTSPH, etc. Graduate students at Rice will also take course in Environmental Sciences, so they will be able to solve problems pertaining to these issues of the SRP. The students will have the opportunities to participate in the training activities of the RTC and CEC cores.

Program Evaluation: The program will be evaluated to review and determine the quality and effectiveness of the Training core. This will include the utilization of NIH Career Trac metrics to be evaluated, including Training core activities completed, degree completion, publications, fellowship honors, as well as plans to obtain feedback to help identify weaknesses and to provide suggestions to improve the program.

Trainee Candidates: Candidates for the Ph.D program of the SRP will need to have a minimum of Bachelor's degree preferably in any of the disciplines (e.g., Toxicology, Biology, Environmental Sciences, Chemistry, Physics etc.) that will be helpful in the SRP activities. Students will be selected from a pool of students accepted to the existing graduate programs at BCM and Rice. We also plan to recruit students from under-represented minorities, and we will leverage the resources already available at BCM and Rice to support these activities.

Institutional Environment and Commitment: Both BCM and Rice have very strong existing graduate programs, and we will synergize the SRP training core with these programs, which will strengthen the training core of the SRP. We have included letters of support from BCM and Rice that show funds and resources that will be available to support the core. The training core of the SRP differs from the existing training programs of BCM and Rice in that this core will have a highly structured program consisting students and postdoctoral fellows from diverse backgrounds to come together under one umbrella and focus on the projects and cores of this SRP.

**Recruitment and Retention Plan to Enhance Diversity**

Graduate students supported on this grant will enter the program from their respective academic departments. The standards at BCM and Rice are very high; acceptance rates are typically in the 10% range, ensuring a high quality workforce. Postdoctoral researchers are recruited based upon the quality of their undergraduate experience and their record of publication. Recruitment from underrepresented groups involves a structured program of visits to historically minority schools, a summer college research program that recruits from predominantly minority and financially challenged groups (MSRP), and an aggressive Internet and letter writing recruitment strategy involving peers at other institutions.

**Plans for Instruction in the Responsible Conduct Research (RCR)**

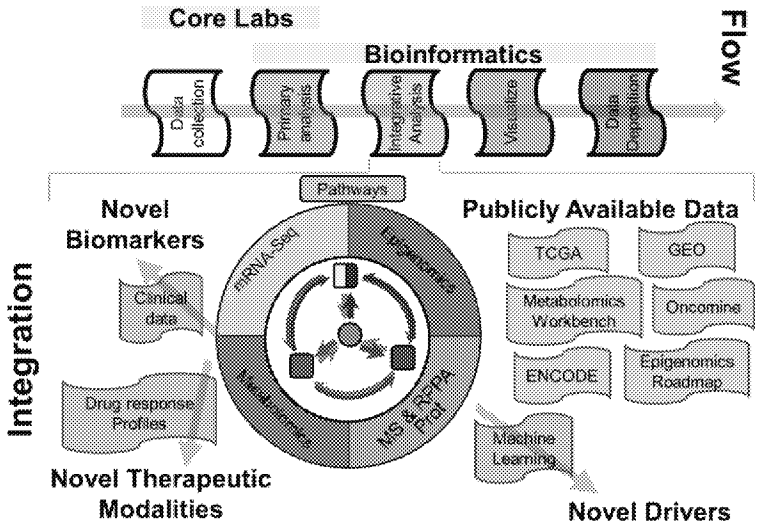
We have a structured plan at BCM and Rice for the Responsible conduct of research. All students are required to participate in training in the responsible conduct of research. BCM and Rice expect all researchers to carry out their work according to the highest ethical and professional standards -a requirement critical for excellence in science and engineering, as well as for maintaining public trust. The RCR is a framework for transferring these standards to researchers and is considered a critical component of scholarly and career development at both institutions. Our program is highly interactive and includes both trainees and faculty supervisors.

**Cores E:**

**Research Support: Statistics, Bioinformatics, and Data Management Core**

Core Leader: Cristian Coarfa, PhD, Baylor College of Medicine, Hosuton, TX
Co-Leader: O'Brian Smith, PhD, Baylor College of Medicine, Houston, TX

## Specific Aims




**Specific Aim 1: Provide expert advice for statistics/bioinformatics experimental design**
Dr. Coarfa and Dr. Smith will conduct regular meetings with the investigators from P1-P5 research projects, and ensure that experimental designs provide sufficient power for the specific questions answered or hypotheses tested. When needed, they will engage also core labs leaders to ensure feasibility of proposed experimental designs with respect to the profiling technology proposed.

**Specific Aim 2: Provide expert statistics/bioinformatics analysis**
The core directors will ensure a robust and consistent workflow to manage, analyze, integrate, interpret, disseminate and store data produced by the SRP projects. Core E will assist with upfront design, primary analysis and integration of proteomics, metabolomics, genomics (Cistrome/ChIP-Seq, whole-genome or exome sequencing), epigenomics (DNA methylation), and transcriptomics (RNA-Seq) data. The Core has developed an effective two tier system for analyzing data. Tier 1 is primary data analysis performed by laboratory research staff working closely with the Statistics/Bioinformatics Core and includes normalization, QA/QC, positive & negative controls, simple statistics and data grouping. Tier 2 is performed solely by the Statistics/Bioinformatics group and includes more complex data groupings and statistics, graphical representations, data integration, data base relationships, pathway analysis, visualization tools and interpretation. The Statistics/Bioinformatics team will utilize the Dan L Duncan NCI Cancer Center's high performance 600 core compute cluster with direct attached, extensible 200 TB NetApp storage located behind the BCM firewall at the Tier III Energy Transfer DataCenter (ETDC). All cluster nodes and appliances are accessible via a 10g Ethernet (10gE) switched local area network (LAN) to perform Tier 1 and 2 analysis using standard pipelines and algorithms and a GalaxyPlatform server to expose novel algorithms as analysis pipelines. GSEA and visualization: Integrative Genome Viewer (IGV) or the UCSC Genome Browser (http://genome.ucsc.edu/). We use principal components analysis (PCA) and hierarchical clustering for data visualization. Given the relative novelty of metabolomics analysis, in compared to the more established transcriptomics, genomics, and epigenomics, we will engage with Dr. Naggireddy Putluri, Director of the BCM Metabolomics core in associated experimental design and analysis.

**Specific Aim 3: Conduct mission-related statistical and bioinformatics methods research**
We will disseminate methodological developments in integration of multi-omics assays using peer-reviewed publications, web-available software, and also make specific tools, pipelines, and workflows available via a Galaxy platform.

**Specific Aim 4: Provide education/training for the SRP trainees**
The Statistics and Bioinformatics core will regularly provide workshops and seminar in collaboration with the Core B Training to educate pre- and post- doctoral trainees in statistics and bioinformatics techniques for analysis of the SRP projects generated profiling data, and make tools, pipelines, and workflows available via a Galaxy platform server.

**Specific Aim 5: Provide state-of-the-art data management** We will carry out data deposition to public repositories such as Gene Expression Omnibus (GEO), Short Reads Archive (SRA), Metabolome Exchange, and others, in close collaboration with BCM Research IT. We enlisted the assistance of Dr. Neil McKenna, leader of the Nuclear Receptor Atlas (NURSA) and Transcriptomine projects, with a 10+ years of experience handling large data repositories and data deposition. We will also educate the SRP projects in the importance of collecting accurate and extensive metadata at the time of omics profiling, with the long term goal of enabling efficient community search and access to the SRP projects generated omics data, and further development of scientific and technological advances based on the SRP findings.