**Nano-Bio Materials for Therapeutic Applications: Synthesis of Encapsulated Sulfanilamide Antibiotics**

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**Summary- Broader Impacts Statement**

Summary of Proposed Work –

Funds are being requested to fund summer internships at Albany State University for two (2) undergraduate students who will be trained in three different aspects of nanotechnology research. This research project encompasses the use of nanotechnology in i) detection/sensing, ii) engineering and iii) applications. These students will be supervised by the faculty at Albany State University, Department of Chemistry & Forensic Sciences which is adequately equipped with modern instrumentation, and the space needed to accommodate a Research Experience for Undergraduate summer program in nanotechnology.

One transformative aspect of this project is to introduce underserved economically disadvantaged and minority students, including women, of southwest Georgia to nanotechnology and its practical applications. Preparing these students to integrate the workforce with up to date knowledge in cutting edge science and technology is a national security priority and will reinvigorate the local and regional economy. Research Experience for Undergraduate (REU) tops the list of best practices for successful students training and their seamless transition into the workforce. Our innovative approach to the REU proposes training tracks associated to emerging industries and biotech firms. Proposed research projects, herein, are interdisciplinary and designed to expose students to research encompassing more than one STEM discipline, from fundamental principles of chemical synthesis of nanoparticles to their physio-chemical characterization and/or bio-chemical evaluation in living organisms as well as their use in modern, technologically advanced devices.

Specific objectives include

1. Implementation of interdisciplinary research in the STEM fields of chemistry, biology, physics, and forensic sciences.
2. ii. Increase students’ content knowledge of the field of nanoscience and nanotechnology: 100% of students that engage in activities will demonstrate growth in nanotechnology content knowledge.
3. iii. Improve student research and critical thinking skills: 90% of students will demonstrate growth in research and problem solving skills.

**Background and Hypothesis**

Infectious diseases, and drug resistance to common antibiotic drugs continue to represent a major threat to human health [1]. In developing countries, millions of children die each year due to infections that are otherwise treatable. Sulfa drugs or sulfanilamides are inexpensive, yet effective antibacterial drugs that have been known for more than a century [2]. Several types of sulfa drugs are marketed for treatment of common bacterial infections (skin, bladder, respiratory or urinary track etc.) in addition to being effective against malaria. Sulfanilamides control the growth of bacteria (bacteriostatic) by inhibiting folic acid synthesis needed for bacterial growth. Sulfanilamide is structurally similar to *p*-aminobenzoic acid (PABA) which is part of dihydrofolate. Bacterial growth is hindered by the progressive replacement of PABA by sulfanilamide during dihydrofolate biosynthesis in bacteria. Therefore sulfanilamides are considered inhibitors of dihydropteroate synthetase. Several analogs of sulfanilamide have been synthesized, and described in textbooks. Metal complexes of sulfanilamides are water soluble but have also been linked to higher toxicity and increasingly linked to clinical resistance to sulfanilamides [3].Over the past few decades, dendrimers have emerged as versatile drug delivery and targeting vehicles with increasing market share owing to markedly improved outcomes and efficiency when administered with Doxorubicin (Doxil®) and paclitaxel (Abraxane®) as first-line treatments in various cancer types. Dendrimers are macromolecules which behave as monodispersed nano-reactors with ligand sites on both their surface and inside suitable for conjugation/entrapment with drugs, and are therefore useful in nano-medicine. Dendrimers host their guest-molecules with a molecular level of dispersion to the latter that increases the bio-availability of the nanocomposites thus formed. Highly branched, tree-like dendrimers such as polyamidoamine (PAMAM) are structurally well-defined. For instance, PAMAM-paclitaxel conjugates have demonstrated good stability under physiological conditions and greater permeability across Caco-2 cell and porcine brain endothelial cells monolayers than paclitaxel alone, making it a choice nanocarrier for poorly water-soluble drugs. ***We hypothesize that improved pharmacokinetics, biodistribution and controlled release of a PAMAM-Sulfa drug can markedly improve the antibacterial properties of sulfanilamides and overcome resistance****.* We propose to test the toxicity of a small library of synthetic PAMAM-Sulfanilamides towards *E.Coli, Luteus Myccocus,* and other bacteria. Our preliminary results indicated that the dendrimer PAMAM is nontoxic and unable to interfere with the results of the proposed study [4].

**First year: Students will synthesize multigram amount of novel *poly(amido)amine-sulfanilamide* complexesor simplyPAMAM-Sulfa, from commercially available materials.**

During the first year, students will synthesize multigram quantities of a small library of novel *poly(amido)amine-sulfanilamide* complexesor simplyPAMAM-Sulfa, as outlined in the *scheme 1* below. N-acetylsulfanilyl chloride **1** will be refluxed with an amine (R) to yield compound **2** which, upon treatment with diluted hydrochloride acid will afford sulfanilamide **3**. The latter will be stirred with PAMAM at room temperature to afford the PAMAM.Sulfa drug **4**. In order to build a small library of these dendrimer-sulfanilamide conjugates, amines such as imidazole, benzimidazole, indole and others known to possess and/or enhance biological properties of therapeutics will be included in the design and synthesis of PAMAM-sulfanilamides. Characterization of each complex using spectroscopic methods such as nuclear magnetic resonance (NMR), infrared (IR), scanning electron microscopy (SEM), and high resolution Mass Spectrometry will then be done by students who will learn the use of modern analytical instruments in our department.

*Scheme 1: Synthesis of a library of PAMAM.Sulfa conjugates.*



**Second year: biological testing using the Kirby-Bauer test for gram positive and/or gram negative bacteria.**

In the second year, students will proceed with biological testing of the synthetic PAMAN.Sulfa conjugates **4** on gram positive and/or gram negative bacteria using the Kirby-Bauer test. Students will use different concentrations of a solution of the PAMAM-Sulfa complexes to determine their respective zone of inhibition in *E.Coli*, and *Luteus Myccocus* primarily. Promising candidates will be selected for further evaluation in subsequent studies on *staphylococcus* and other bacteria. The agar diffusion method will be used to analyze bacterial sensitivity to these potential antibiotics. A sterile disc of filter paper (called a waffer) will be impregnated with various concentration of the PAMAM-Sulfa conjugate and placed on a plate of nutrient medium seeded with *E.Coli* or another bacterium. Students will then incubate the plate at physiological temperature. After 24 hours, students will observe the plates for clear zones of inhibition (zones of no growth) surrounding the disc. The size of the zone of inhibition will then be used to infer the effectiveness of the PAMAM-Sulfa conjugate against those organisms as well as to compare the effectiveness of these Sulfa drugs quantitatively. (See Appendix for Table 1: Zones of Inhibition (in mm) of PAMAM-Sulfa Conjugates in Different Bacteria)

**Third year: Result Optimization:**

Our preliminary results [4] have clearly demonstrated that PAMAM-Sulfanilamide SP [c] works better than sulfanilamide SP [a] alone in inhibiting the spread of *M.Luteus* (orange marker) and/or *E.Coli* (green marker) as shown in the image below.

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The current study aims at generalizing and improving those findings amongst analogous conjugates. After analyzing the results of the biological testing (Project 2), promising candidates amongst the PAMAM-Sulfa conjugates will be selected for further evaluation. Compounds displaying large zones if inhibition against some of the bacteria will be, further, evaluated by students in subsequent *in vitro* studies. Students will study the particular timing of the onset on inhibition in bacteria as well as the spread thereof in order to further characterize the potency of the PAMAM-Sulfa conjugates as potential therapeutics.

**References:**

[1] The US Centers for Disease Control and Prevention (CDC) **Annual Report 2013** updated

April 14, 2017; www.cdc.gov/narms.

[2] Paul Gelmo (May 14, 1908) "Über Sulfamide der p-Amidobenzolsulfonsäure," **Journal für**

**Praktische Chemie**, 77 : 369-382.

[3] Skӧld, O. **Drug Resist Updat**. 2000 Jun;3(3):155-160.

[4] Whitfield Z, Mandouma, G. 2017 Georgia Academy of Science Annual Meeting, Young Harris

GA, March 24-25, 2017.

**Appendix**

Table 1. Zones of Inhibition (in mm) of PAMAM-Sulfa Conjugates in Different Bacteria

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| R (in Amines) | E.Coli | Luteus Mycoccus | Staphilococcus | Observation |
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R groups

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**Project time line**

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Weeks | | | | | | | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Literature search and current progress in the research area | X |  |  |  |  |  |  |  |
| Synthesis of PAMAM-Sulfanilamide Conjugates |  | X | X | X |  |  |  |  |
| Characterization of the PAMAM-Sulfanilamide Conjugates by NMR, IR, GCMS, and SEM |  |  | X | X | X | X |  |  |
| Biological Testing by the Kirby-Bauer Test |  |  |  | X | X | X | X |  |
| Calculation of Zone of Inhibition |  |  |  | X | X | X | X |  |
| Result analysis |  |  |  |  |  |  | X | X |
| Summarization of whole project and manuscript preparation |  |  |  |  |  |  | X | X |