UNIVERSITY OF LOUISIANA AT LAFAYETTE

STEP Committee

Technology Fee Application

Purchase of a Centrifuge for Chemistry Laboratory Courses

Drs. Wu Xu and August Gallo

Name of Submitter
(Faculty or Staff Only)

Department of Chemistry

Organization

Title: Purchase of a Centrifuge for Chemistry Laboratory Courses
Date: 07/13/2021
Contact Person: Wu Xu
Participating Faculty: Drs. Wu Xu and August Gallo
Address: Department of Chemistry, P.O. Box 44270, University of Louisiana at Lafayette, 70504
Phone Number: 337-482-5684 (Dr. Xu)
Email: wxx6941@louisiana.edu (Dr. Xu)
Department/College/Organization: Chemistry/Sciences

ABSTRACT:

Centrifugation, a fundamental technique in all chemistry and biology laboratories, could separate and characterize inorganic or organic compounds or macromolecules. The technique has a broad spectrum of applications in academia and industry due to its extremely high purity of molecular separation according to their size, shape, density, viscosity of the medium and rotor speed. In addition, centrifugation can be integrated with a variety of spectroscopic techniques to have extremely high power for characterizing molecules ranging from small to large molecules. Chemistry teaching and research labs need a refrigerated floor centrifuge in order for our faculty to efficiently prepare solutions and samples for the labs, and for students in chemistry labs to perform certain chemical reactions and culture microorganisms and mammalian cells. Following the strategic plan of our College, we recently obtained approval for a shared Ph.D. program in Earth and Energy Sciences, and for a new Master program in Industrial Chemistry. This will lay the foundation for a research-oriented program at the graduate level. Our department is undergoing a dramatic transition from a program with primary teaching focus to a unit with a strong research presence. The Department of Chemistry had a >20 year-old floor centrifuge and it did not work. We have submitted the proposals to BoR enhancement program for requisition of a floor centrifuge to support teaching twice in the past. The reviewers commented that the centrifuge is a basic instrument. We feel that a STEP technology fee application is the most sensible way to attempt to address our instrumental deficiencies. This situation compels us to submit this proposal for university support.

Purchase of a Centrifuge for Chemistry Laboratory Courses

A. Purpose of grant and impact to student body as a whole

In line with the University's mission, our department strives to achieve regional and national recognition for excellence in research and teaching. Following the strategic plan of our College, we recently obtained approval for a shared Ph.D. program in Earth and Energy Sciences. This will lay the foundation for a graduate level, research oriented program, which is found in all other units within our College. Though the recent hires of several new faculty members with strong research background, our department is undergoing a dramatic transition from a program with primary teaching focus to a unit with a strong research presence. A second new program, a M.S. degree in Industrial Chemistry, is approved as well. Existing instrumentation, while very much in line with the needs of a strong undergraduate program, does not fully support our developing research needs. Equipment will provide the foundation for the successful solicitation of federal funding, future collaborations, to attract additional talent when hiring, and for the continued growth of the new graduate program to achieve national competitiveness.

The department's mission has been focused on undergraduate teaching and training. Currently, the Department of Chemistry offers ten lab courses: CHEM 112 (General Chemistry for Education Majors), CHEM 115 (General Chemistry), CHEM 222 (Analytical), CHEM 252 and 452 (Inorganic), CHEM 233 (Organic I), CHEM 234 (Organic II), CHEM 311 (Physical I), CHEM 312 (Physical II), CHEM 319 (Biochemistry) and CHEM 362/462 (Undergraduate Research I/II) for the students of the Colleges of Science, Engineering, and Education, as well as courses designed for those interested in non-technical fields.

Dr. Xu along with many faculty members in the Department of Chemistry teach CHEM 115. CHEM 115 (General Chemistry Lab) is designed to reinforce concepts learned in lectures of general chemistry (CHEM 107 and CHEM 108) and provides an introduction into basic laboratory techniques, the experimental methods, and the presentation of scientific data, as well as direct experiences with chemical principles and the properties and reactions of substances and molecules. This lab is a required course for undergraduates majoring in biology, chemistry, physics, chemical engineering, civil engineering, petroleum engineering, and kinesiology. The department typically offers eight sections of CHEM 115 in fall semesters and nine sections in spring semesters. There are also four to five summer sections of CHEM 115, although the number of sections is somewhat variable. Each section has ~25 students (American Chemical Society (ACS) limits number of students in any chemistry lab to 25), and every section is typically full, and has a substantial wait list. It is estimated that approximately 500 students take this lab every year.

Table 1. List of undergraduate majors which require CHEM 115 for graduation

	CHEM 115 General Chemistry
Biology	X
Microbiology	X
Biodiversity	X
Chemistry	X
Chem Eng	X
Civil Eng	X
Petroleum Eng	X
Physics	X
Kinesiology	X

Chemistry is one of traditional and fundamental fields. Its applications are to address the challenges facing the world by combating diseases, providing clean water and safe food, developing new sources of energy, developing new materials, and confronting climate changes through interdisciplinary nature of chemistry. To obtain skills in solving world challenges, it requires an appropriate suite of modern chemical instrumentation and specialized lab apparatus to analyze chemical properties of small and large molecules to support undergraduate instructional and research missions. All chemistry labs need a certain type of equipment for students to learn lab skills and collect data. Centrifugation has its uniqueness of providing first-principle hydrodynamic theory to define size, shape, and interactions of macromolecules and thermodynamic laws to define molecule mass, assembly stoichiometry, association constants, and solution nonideality. Because sedimentation relies on principal property of mass and fundamental laws of gravitation, it is a primary method for which the results are absolute and do not depend on a comparison to standards. Consequently, sedimentation can be used to analyze the solution behavior of nearly any type of molecules over a wide range of concentrations and in a wide variety of solvents. Furthermore, a broad range of particle sizes might be analyzed by using different rotor speeds. Sedimentation has the merits of being rapid, non-destructive, and simple to use. Because it is nondestructive, samples such as cells, proteins and DNA/RNA may be recovered for further tests following centrifuge. For many questions, there is no satisfactory substitute method of analysis. Centrifugation is a unique investigational tool employed in many areas of analytical science and industry.

Figure 1. The old floor refrigerated centrifuge in biochemistry lab. It is not functioning.

There is a floor refrigerated centrifuge that was purchased in > twenty years ago (**Figure 1**). It is no longer functioning. *Currently, there is no floor refrigerated centrifuge in any of the chemistry labs.* One particularly advantageous property of centrifugation is that, because recent developments in analytical procedures allow the high resolving power of sedimentation velocity methods to be coupled to sedimentation equilibrium approaches and applied to both static and dynamic associations, students can extract macromolecules with extremely high purity under physiological conditions. The main purpose of the proposal is to purchase a centrifuge with its accessories for CHEM 317, CHEM 417, CHEM 319 lab, CHEM 115 lab, CHEM 362/462 lab and other chemistry labs. The centrifuge will be also used in all other chemistry labs. This situation requires us to initiate this STEP proposal, seeking support from our university. Students will benefit in the following ways:

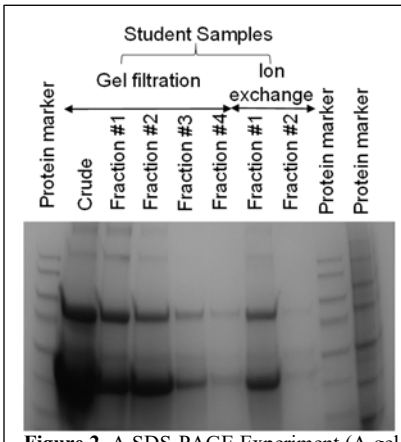

Figure 2. A SDS-PAGE Experiment (A gel picture from a student who took Biochemistry Lab, CHEM 319, in the past.)

(i) The centrifuge has been used on daily basis in biochemistry teaching and research labs in the past twelve years. The objective of Experiments 3-6 is to purify lysozyme from egg white and to characterize the enzyme by enzymatic reaction. Experiments 3-6 involve (1) isolation of proteins from egg white by size exclusion and ion exchange chromatography; (2) protein content analysis by Bradford, Folin-Lowry and UV-Vis methods; (3) separation of the purified proteins by SDS-PAGE. A refrigerated centrifuge is required to prepare samples for the experiments (**Figure 2**). In addition to protein

purification from egg white, students will isolate cytoplasmic proteins, nuclear proteins and whole cell proteins from mammalian cell cultures, and resolve proteins in SDS-PAGE based on our published methods to characterize proteins. Experiments 9-11 were designed based on Dr. Xu's EMBO J paper. Four endogenous genes were dependent on the physical interactions between CREB and CBP in response to cAMP signaling. The endogenous cAMP responsible genes were verified by luciferase reporter assay. Two of the constructed reporter genes: *cfos* and *Areg*, were used in experiments 9-11. The objective of these three labs is to identify plasmids using two independent methods: enzyme digestion and polymerase chain reactions. These three labs include (1) isolation of plasmid DNAs: *cfos* and *Areg* luciferase reporter constructs; (2) enzyme digestion of the plasmids and gel electrophoresis; (3) PCR and gel electrophoresis. Preparation of bacterial cells and isolation of plasmid DNAs require a refrigerated centrifuge (**Figures 3** and **4)**. The objectives of Experiments 13-14 are to learn mammalian cell culture, to isolate and to characterize signaling-dependent mRNAs. Experiments 13-14 include (1) mammalian cell culture and cell treatment by anti-cancer drugs [1] and cAMP activator; (2) RNA isolation and reverse transcriptase reaction; (3) semi-quantification of RNA by RT-PCR. A refrigerated centrifuge is needed to prepare RNA samples (**Figure 5**).

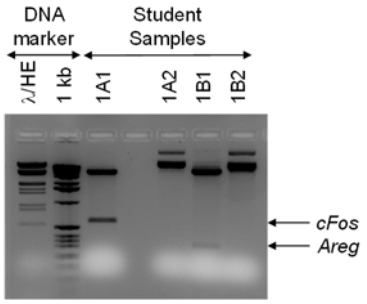

Figure 3. A plasmid DNA synthesis and characterization by enzyme digestion and electrophoresis (A gel picture from a student who took Biochemistry Lab, CHEM 319, in the past.)

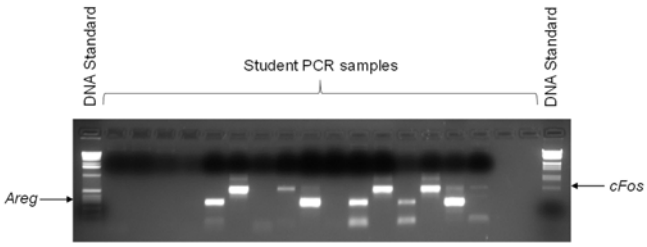

Figure 4. DNA fragments were synthesized by PCR and analyzed by electrophoresis (A gel picture from a student who took Biochemistry Lab, CHEM 319, in the past.)

(ii) CHEM 320 Introduction to Polymer Science: In Chapter 7, Characterization of Polymers, determination of molecular weight and polymer microstructures will be discussed. Matyjaszewski and co-workers have recently demonstrated that modern polymer synthesis methodologies can be implemented within the classical chemistry undergraduate curriculum with ease by providing two examples for undergraduate lab experiments at the nexus of polymer synthesis. A centrifuge will be introduced to integrate synthetic polymers with biopolymers;

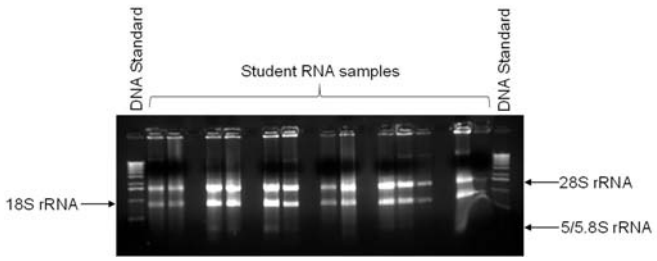

Figure 5. Total RNAs were isolated from NIH 3T3 cells and characterized by electrophoresis (A gel picture from a student who took Biochemistry Lab, CHEM 319, in the past.)

(iii) CHEM 115 is designed to reinforce concepts learned in lectures of general chemistry and provides an introduction into basic lab techniques, the experimental methods, and the presentation of scientific data, as well as direct experiences with chemical and/or biochemical principles and the properties and reactions of substances and molecules. Molecular separation and characterization by centrifugation and spectroscopy will become the fundamental technique in general chemistry lab. A centrifuge is important equipment for faculty members to prepare certain solutions for students;

(iv) CHEM 233 and 234-Organic Chemistry I and II Labs: In the organic laboratories, students are introduced to synthesis, purification and characterization techniques of small to large organic compounds. Drs. Gallo and Junk are in the process of upgrading our organic teaching labs and plan to significantly upgrade training in the area of separation techniques. Currently, only recrystallization and liquid-liquid extraction are covered by two separate experiments in our Organic Chemistry Laboratory. A more inclusive lab, which introduces a range of important separation techniques, would be more appropriate. This lab familiarizes students with the common separation techniques of distillation, vacuum filtration, recrystallization, extraction, and centrifugation. This lab will consist of two lab sessions, in which students will work in groups to master all of the above methods and draw conclusions about the efficacy of each one. With respect to centrifugation, students will prepare and attempt to separate a silica colloid by filtration (failure), then by centrifugation. It is important to establish student proficiency in this technique, notably in preparation for biochemistry.

(v) CHEM 362 and CHEM 462: Research quality is an important indicator of success of an academic program since high quality research will better prepare students to pursue intellectual inquiry. The faculty members have been able to obtain funding to enhance their research activities. Dr. Xu has obtained support from ITRS program, an NSF EPSCoR award, and a RCS award. A number of independent and collaborative projects have been developed by our faculty members. We are very proud of our undergraduates. The proposed instruments are essential for undergraduate research. One important objective for requesting funding is to encourage students to be exposed to research and advanced instrumentation early in their education. Many undergraduates are bright, highly motivated, and determined to succeed, but lack academic preparation and essential lab skills to work at a higher cognitive level and/or research environment. Students with hands-on experience obtained from lab courses or research experience tend to perform better in their course study. Dr. Xu has been conducting surveys on undergraduate research with the goal of integration of research with teaching. Students are interested in learning lab techniques. Twenty-seven undergraduates have been the coauthors in thirteen peer-reviewed publications in the last fourteen years: Sophia Zhou [2], Ali Faust, Ashlin Naquin, Avery Walton, Peter Kishbaugh [3], Oliver Y. Achi, Justin G. Hanks [4], André Delavault [5], Anne Odoux, Darren Jindal, Tamara C. Tamas, Benjamin W.H. Lim, Drake Pollard [6], Anne Le Roux, Quentin Dutercq [7], Benjamin Amire-Brahimi [8], Aline Henry, Jennifer M. Battle, Mathieu Micault [9], Sean P. Comiskey, Kara H. Otero, Corey L. Michel, Wesley M. Juneau [10], Amelie Laujac [11], Julie A. Craft, Patricia R. Fontenot, Marion Barens [12], Andrew T. Nelson, Leah N. Segura [13], Pierre E. Doulain, and Caroline A. Landry [14]. Ten undergraduates: Maria D. Abascal, Lauren E. Cole [15], Emma E. Domangue, Nina A. Landry, Rachel A. Needham, Aimee A. Rodwig, Luke J. Pryor, Artavion L. Cook, and Madeline E. Gautreaux[2], and Lillian D. Lestelle [16] were acknowledged in three papers. As a result, the students are more likely to pursue an occupation in science or other professional fields. Research will provide students with a group-based work environment. The interaction with other students (from biology and engineering backgrounds at undergraduate and graduate levels) allows the growth of interpersonal, organizational, and teamwork skills that will better prepare all involved students for their future careers. The centrifuge is an essential and basic instrument for undergraduate teaching and research.

(vi) The instrument will be used in new graduate courses. Many courses are being developed. In addition, this centrifuge is essential for graduate research.

B. Projected lifetime of enhancement

The refrigerated floor centrifuge should be in a good working condition in the laboratory for ten years, only requiring regular basic maintenance.

C. Person(s) responsible for
Implementation: Wu Xu and August Gallo
Installation: Wu Xu and August Gallo
Maintenance: Departmental committee made of up faculty members
Operation: Faculty of Chemistry Department
Training: Wu Xu will conduct training for faculty members, following their training by the manufacturer

Budget Proposal

1. Equipment $19,125

Name	Qty	Description	Price
Centrifuge	1	Sorvall LYNX 6000 Superspeed Centrifuge	$11,000
BIOFlex HC	1	4x1000mL40x50mLconical24 x microplates Rotor	$2,500
Fiberlite	1	F20-12x50 LEX 12 x 50 mL Rotor	$3,000
Fiberlite	1	F23-48x1.5 48 x 1.5 mL Rotor	$2,500
FREIGHT CHARG E			$125.00
		Total:	$1,9125

2. Software $ 0.00
No software is required.

3. Supplies $0.00

4. Maintenance $ 0.00
Routine maintenance will be covered by department.

5. Personnel $ 0.00
No personnel required

6. Other $ 0.00
None

TOTAL: $19,125

D. Other relevant information

None

E. Previous STEP projects

Dr. Xu successfully authored the STEP proposals “Smart Classrooms in Chemistry”, funded in 2014 or 2015.

F. The papers published with undergraduate students as the coauthors or participants acknowledged.

1. Srivastava RS, Fronczek FR, Perkins RS, Fukuyama T, Xu W: **Anticancer activities of the ruthenium carboxylato, amido and pyridine complexes**. *International Journal of Oncology* 2010, **36**(6):1591-1598.
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3. W X, X-J X, AK F, M L, X L, F C, AA N, AC W, PW K, J-Y J: **All-Atomic Molecular Dynamic Studies of Human and Drosophila CDK8: Insights into Their Kinase Domains, the LXXLL Motifs, and Drug Binding Site**. *International Journal of Molecular Sciences* 2020, **21**(20).
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5. Delavault A, Fronczek FR, Xu W, Srivastava RS: **Ionic η5-Cp-Ruthenium (II) complexes as potential anticancer agents**. *Journal of Organometallic Chemistry* 2018, **875**:29-34.
6. Odoux A, Jindal D, Tamas TC, Lim BWH, Pollard D, Xu W: **Experimental and molecular dynamics studies showed that CBP KIX mutation affects the stability of CBP:c-Myb complex**. *Computational Biology and Chemistry* 2016, **62**:47-59.
7. Massoud SS, Perkins RS, Louka FR, Xu W, Le Roux A, Dutercq Q, Fischer RC, Mautner FA, Handa M, Hiraoka Y *et al*: **Efficient hydrolytic cleavage of plasmid DNA by chloro-cobalt(ii) complexes based on sterically hindered pyridyl tripod tetraamine ligands: synthesis, crystal structure and DNA cleavage**. *Dalton Transactions* 2014, **43**(26):10086-10103.
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9. Xu W, Xue L, Sun Y, Henry A, Battle JM, Micault M, Morris SW: **Bcl10 is an essential regulator for A20 gene expression**. *Journal of Physiology and Biochemistry* 2013, **69**(4):821-834.
10. Massoud SS, Perkins RS, Knierim KD, Comiskey SP, Otero KH, Michel CL, Juneau WM, Albering JH, Mautner FA, Xu W: **Effect of the chelate ring size on the cleavage activity of DNA by copper(II) complexes containing pyridyl groups**. *Inorganica Chimica Acta* 2013, **399**:177-184.
11. Xu W, Wang Y, Taylor E, Laujac A, Gao L, Savikhin S, Chitnis PR: **Mutational Analysis of Photosystem I of Synechocystis sp. PCC 6803: The Role of Four Conserved Aromatic Residues in the j-helix of PsaB**. *PLoS ONE* 2011, **6**(9):e24625.
12. Xu W, Craft JA, Fontenot PR, Barens M, Knierim KD, Albering JrH, Mautner FA, Massoud SS: **Effect of the central metal ion on the cleavage of DNA by [M(TPA)Cl]ClO4 complexes (M = CoII, CuII and ZnII, TPA = tris(2-pyridylmethyl)amine): An efficient artificial nuclease for DNA cleavage**. *Inorganica Chimica Acta* 2011, **373**(1):159-166.
13. Liu Y, Gallo AA, Bajpai RK, Chistoserdov A, Nelson AT, Segura LN, Xu W: **The diversity and molecular modelling analysis of B12-dependent and B12-independent glycerol dehydratases**. *Int J of Bioinformatics Research and Applications* 2010, **6**(5):484-507.
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complexes: The syntheses, structural characterization and thermal analysis of six new species. *Polyhedron* 2009, **28**(17):3849-3857.

15. Srivastava RS, Fronczek FR, Perkins RS, Fukuyama T, Xu W: **Anticancer activities of the ruthenium carboxylato, amido and pyridine complexes**. *International journal of oncology* 2010, **36**(6):1591-1598.
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