We will operate on an **academic calendar** (9 months), taking off summer (June-August) with the exception of an external workshop at the CCRC in August.

We will meet twice a month on a bi-weekly basis: One meeting for **didactic instruction**, and the other for **hands-on training**. Both of these meetings will include lunch (provided by Skills Core C) followed by a journal club (didactic training) or laboratory experiments with the Shared Resources Core B.

**Didactic Instruction** (2 hrs, once a month)

- **1st hour**(lunch) will be a faculty lecture (40 min) on a topic taken from the book, "Essentials in Glycobiology" followed by a discussion (20 min).
- **2nd hour** will be a journal club. Two - three trainees will present an article by a PowerPoint presentation in 10-15 min, budgeting 10-15 min for discussion. In the first year, the trainees will be assigned a "historical" primary research article related to the lecture topic, with the purpose of demonstrating how major paradigm shifts in glycobiology were originally discovered. In the second year, the trainees will be assigned (or may suggest an article) that demonstrates more recent groundbreaking discoveries in glycobiology.

**Hands-On Training** (3+ hrs, once a month)

- **1st hour** (lunch) will involve a brief (10-20 min) introductory lecture by PEG staff, giving an overview of the methodologies about to be taught in that session. Following this introduction, PEG staff will pose an interesting scientific question to answer, ask the trainees to brainstorm how they would answer that question, and direct the trainees in the best approach to answer this question.
- **Laboratory Work** covering the topics described in the "Principles and Practice of Proteoglycan and Glycosaminoglycan Glycoscience" syllabus (listed below) will comprise the rest of the afternoon, and may require a small time commitment over the next few days. The trainees will be broken down into two training groups of 2-3 trainees per group. Each group will conduct the identical experiment and their results will be evaluated in the next 1st hour lunch session.

**Annual Workshop at the CCRC:**Please plan to have your trainee attend the 4th Warren Workshop at the CCRC (Athens, GA) in August 8 – 11, 2012 (Wed-Sat).This biennial workshop strives to bring together investigators working on the frontiers of glycan characterization in order to advance the field of glycomics, glycolipidomics, and glycoproteomics. The format of the workshop is designed to facilitate interactions and foster meaningful discussions that will encompass the nuts-and-bolts of recent technological advances, the ups-and-downs of data analysis, and the ins-and-outs of glycobioinformatics.Registration and abstract submission ends May 31st.

**Textbook:** The "Essentials in Glycobiology" textbook has been purchased by Core C and each trainee will receive their own copy at an upcoming meeting once they sign that they have received it. Relevant protocols in proteoglycan and glycosaminoglycan analysis from Methods in Enzymology reviews and Protocols in Molecular Biology have been downloaded and provided to the trainees free of charge.

**Course Title:** Principles and Practice of Proteoglycan & Glycosaminoglycan Glycoscience

**Course Instructors:** Drs. Mark Lauer, Vincent Hascall& Ron Midura

**Course Objectives:**

1) Understand the chemical & biological principles & practice regarding the removal and isolation of proteoglycans &glycosaminoglycans from tissues and cell cultures.

- A) Hydrophilicity and hydrophobicity
- B) Chaotropism and denaturation
- C) Non-ionic versus zwitterionic detergents

2) Understand the chemical principles & practice regarding the purification of proteoglycans &glycosaminoglycans from tissues and cell cultures

- A) Desalting chromatography
- B) Ion-exchange chromatography
  - i) Continuous gradient elution
  - ii) Stepwise batch elution
- C) Affinity chromatography
  - i) Hydrophobic binding
  - ii) Lectin binding
  - iii) Antibody binding

3) Understand the chemical principles & practice regarding the characterization of proteoglycans &glycosaminoglycans from tissues and cell cultures

- A) Chemical assays
  - i) Uronic acid detection
  - ii) Hexosamine detection
  - iii) Dimethyl Methylene Blue detection of sulfated GAGs
- B) Size-exclusion chromatography
- C) Removal of intact GAG chains from core protein
  - i) β-elimination reaction using alkaline borohydride
  - ii) Proteolytic digestion
    - a) Limited vs. complete
  - iii) GAG sizing analyses
- D) Removal of intact core protein from GAG chains
  - i) Enzymatic digestion of GAG chains
    - a) Chondroitin lyases to digest chondroitin or dermatan sulfate
    - b) Heparitinases to digest heparan sulfate
    - c) Keratanases to digest keratan sulfate
  - ii) Eliminase vs. hydrolase mechanisms of digestion and the resulting have degradation products.
  - iii) Core protein sizing and identification

4) Understand the chemical principles & practice regarding the characterization of proteoglycan & glycosaminoglycan fine structure from enzymatically released products

- A) Method selection
  - i) FACE, HPAE, CZE
- B) Disaccharide analysis
- C) Monosaccharide analysis
- D) Linkage oligosaccharide analysis
- E) Quantitative calculations to assess amounts of GAG per tissuemass.

5) Understand how newly synthesized proteoglycans & GAGs can be analyzed separately from the resident bulk proteoglycans & GAGs

A) Radioisotopes and their detection
B) Radioisotope-labeled precursors of GAGs (3H-glucosamine and 35S-sulfate)
C) Calculation of intracellular radiolabeled precursor specific activities
D) Agents to modulate GAG metabolism
    i) Xylosides competition
    ii) Brefeldin A
    iii) Tunicamycin

6) Understand how proteoglycans & GAGs can be detected and localized in tissue sections
A) Antibody detection of neo-epitopes after eliminase degradation of GAGs
B) Antibody detection of core protein epitopes
C) Antibody detection of core protein neo-epitopes resulting from pathology
D) Biotinylated HA-binding complex detection of hyaluronan
E) Identification of inflammatory cell binding HA matrices

7) Understand how hyaluronan binding partners and its post-translational modifications can be detected and measured
A) Western blot analysis for inter-α-inhibitor heavy chains or versican after *Streptomyces*hyaluronidase digestion

8) Understand how hyaluronidase activities can be detected in hyaluronanzymogram gels