Facilities, Equipment and Other Resources

A. Brief Statement of Facilities and Resources For Success of Proposed Research

Dr. Xu has built a highly interdisciplinary research laboratory and a multidisciplinary research program. The Xu lab is well equipped with the state-of-the-art instrumentation and excellent facilities and resources, including single nanoparticle optical microscopy and spectroscopy imaging stations, full ranges of cell cultures, molecular and cellular biology instrumentation and imaging equipment, and a wide variety of apparatus for nanomaterials synthesis and characterization, single nanoparticle and single cell imaging and spectroscopic characterization. The university is well equipped with a wide range of research facilities to support the proposed research activities. They include high-resolution transmission electron microscopy (HR-TEM) for characterization of sizes and shapes of single nanoparticles (NPs) and NMR for characterization of functional molecules on the surface of single NPs. Therefore, we have the state-of-the-art facilities, equipment and resources to accomplish a wide range of interdisciplinary research programs. The detailed are outlined below:

B. Facilities and Equipment in Xu's Lab at Old Dominion University

Laboratory:

The PI has about 3000 square feet of laboratory space suitable for nanomaterials synthesis and characterization, bioanalytical chemistry, biophysics, biochemistry, molecular and cellular biology research. This includes nanoparticle synthesis and characterization, single nanoparticle imaging, nanoscale analysis, characterization, and separation, single-molecule/single cell microscopy and spectroscopy, and cell culture operation. The PI has office space (250 sq. ft) for ~15 people.

Clinical:

Other modern clinical facilities including large autoclave facilities, animal research facilities (AAALAC Full Accreditation), walk-in cell culture facilities, for handling clinical samples and wastes at the university, and human tissue banks at nearby Eastern Virginia Medical School (EVMS) are accessible to our research group.

Computer:

The PI's group has 34 computers with instrumentation interfaces, and 14 computers for data analysis and modeling and simulation.

Office:

The PI has office space (250 sq. ft) for ~15 people.

Major Equipment and Resources:

- Two far-field photostable optical nanoscope (PHOTON) are currently being upgraded and further developed: each is equipped with one upright dark-field optical and fluorescence microscope (Nikon, E-400), coupled with Multispectral Imaging System (MSIS, N-MSI-VIS-FLEX, CRi, Hopkiton, MA). The MSIS is an integrated system of CCD camera (SonyICX 285) and liquid-crystal-tunable-filter (LCTF) for simultaneously acquiring dark-field optical images and LSPR spectra of massive amount of single nanoparticles (NPs) with spectral resolution of 1 nm to enable high-throughput analysis of single NPs and single cells in real time at nm resolution. The dark-field microscope is equipped with a dark-field condenser (oil, 1.43-1.20), a microscope illuminator (Halogen lamp, 100 W), a 100x objective (N.A. 0.5-1.3, iris, oil).
- One single-molecule fluorescence microscopy and spectroscopy apparatus that includes an inverted UV fluorescence microscope (Zeiss Axiovert 100/135D) with an 100x microscope objective (ultrafluar, numerical aperture = 1.2, Zeiss) coupled with an ICCD (intensified charge-coupled-device) camera system (Princeton Instruments, Intensified PentaMAX-512 EFT at 5 MHz with Gen II high resolution image intensifier), a microscope illuminator (halogen lamp, 100 W) with

an oil dark-field condenser (Oil 1.43-1.20, Nikon) for dark-field microscopy and the unfocused filtered Ar^+ laser beam at 275/305/335/350/488/514 nm (Spectra-physics, Beam Lok 2045-15/4) for fluorescence microscopy. The entire system is also interfaced with an electronic system including a PG200, a wave-generator and an oscilloscope (Tektronix, TDS 3052) for real-time kinetics measurement.

- One single molecule/single nanoparticle/single live cell microscopy and spectroscopy apparatus that includes an upright optical and fluorescence microscope (Nikon-E600) equipped with an oil dark-field condenser, a 100x objective (Nikon Plan fluor 100x oil, iris, SL. N.A. 0.5-1.3, W.D. 0.20 mm), a PID 1030x1300 pixel CCD camera (Roper Scientific, Micromax, 5-MHz-Interline) for high resolution cell imaging, a LN back-illuminated CCD camera (Roper Scientific) coupled with SpectraPro-150 (Roper Scientific) for simultaneous spectroscopic measurements, and a Coolpix 990 digital camera (Nikon) for real-color imaging. This microscopy system is capable of real-time single molecule detection (SMD), single-cell imaging and single-nanoparticle microscopy and spectroscopy measurements. The entire system is interfaced with an electronic system including a wave-generator and an oscilloscope (Tektronix, TDS 3052) for real-time kinetics measurement. The entire microscope system is located inside a CO_2 cell-culture incubator that allows us to directly observe cell growth and division while imaging and characterization of nanoprobes.
- One upright optical and fluorescence microscopes (Nikon, E-400) coupled with high-resolution CCD cameras (5-MHz, Micromax), and EMCCD camera (Roper Scientific) coupled with SpectraPro-150, and a high-speed video color camera (Sony) are suitable for imaging of single protein molecules and single ligand-receptor interactions on single live cells using single nanoparticle sensors, and for real-time monitoring of the sub-cellular mechanism in single cells.
- Two upright optical and fluorescence microscopes (Nikon, E-400): (i) One coupled with high-resolution CCD cameras (5-MHz, Micromax) and a high-speed video color camera (Sony); (ii) The other coupled with high-resolution CoolSnap-k4 CCD camera (20 MHz) that is interfaced with SpectraPro-150, for high-speed and high-resolution imaging and simultaneous spectroscopic measurements. Both systems are suitable for imaging of single ligand-receptor interactions on single live cells using nanoparticle probes and real-time imaging of subcellular response of single live cells at each cellular stage and over several generations.
- One newly installed high-resolution Confocal Fluorescence/Raman Laser Microscope and Spectrometer (Horiba, Labram HR) with the state-of-the-art detection system, several excitation laser beams, and nanoscope scanning stage for acquiring 3D images of single living cells and organisms and mapping of 3D distribution of intracellular NPs in single live cells.
- One beamLok 2045-15/4 argon ion laser with output lines at 275/305/335/350/488/514 nm (Spectra-physics, Beam Lok 2045-15/4) provides the excitation beam to all microscopic imaging systems for fluorescence microscopy and spectroscopy measurement. Each microscope is also equipped with individual high-pressure Hg lamp.
- Two fluorescence spectrometers (LS50B Perkin-elmer; Cary elipse) and three UV-vis spectrometers (Cary 5000 V-vis-NIR; Cary 3Bio; Hitachi-U3310) (two are equipped with water-bath for temperature controlled experiments) for characterization of optical properties of NPs and SMNOBS.
- One multi-mode microplate Reader (BioTek, Synergy HT) with both UV-vis and fluorescence detectors and temperature controlled and timing capabilities for study of binding affinity and binding kinetics of protein-protein interactions (SMNOBS with its analytes). The reader can also be used to measure biocompatibility and toxicity (minimum inhibitory concentration, MIC) of drug molecules and NPs.
- One customized microscope CO_2 incubator (COY laboratory products Inc.) that is able to accommodate any of all our microscope systems and allows us to real-time image subcellular response of single live cells at each cellular stage and over several generations.

- One state-of-the-art glove-box system equipped with a N_2 purging system and vacuum line, and several glassware systems for synthesis of nanoparticles.
- One well-equipped synthesis lab, including hoods, vacuum lines, digital mixers, and state-of-the-art rotary evaporator (Buchi), for synthesis, functionalization and characterization of nanoparticles
- One Nicomp 380ZLS nanoparticle size and zeta potential characterization system in our lab allows us to characterize sizes of nanoparticles in solution using dynamic light scattering (DLS) and measure zeta-potential of nanoparticles in solution.
- Fully equipped histology facilities (ThermoFisher Scientific) include a tissue processor (STP 120); a tissue embedding center (Shandon Histocentre™ 3 Embedding Center); a HM 360 Microtone (0.25-100 μm thin-layer section); a slide bath, are available in our lab to prepare ultra thin-layer section of tissue and cellular samples for characterization of intracellular nanoparticle probes.
- One Barocycler homogenizer to prepare cell samples from tissues; two high-power and digital sonifers to disrupt cells and isolate membranes and proteins from cells; One Freeze Drier (VirTis) for freezing and drying purified proteins and nanoparticles.
- One Gene pulser Xcell total system (BioRad) for gene transfection to prepare mutants, and to prepare feeder cells for culture of embryonic stem cells.
- A well-equipped microinjection system (Eppendorf: Injectman N12 Micro-Manipulator; FemtoJet Express Micro-Injector) is available in our lab to inject nanoparticles into living organisms.
- One CO_2 water-jacketed incubator for cell culture (Farma); two microbial cell incubators (Fisher); two temperature-controlled incubators for cell culture; one cell culture chamber (purifier class II safety cabinet with UV radiation, Labconco); one environ-shakers (Lab-line Orbit) for cell culture; one state-of-the-art floor shaker (Max Q 5000) with refrigerated, cell culture and incubation capabilities to culture and prepare a wide variety of the cells.
- Two compact (0.67 $feet^3$) cooled air incubators to culture zebrafish embryo stem cells and feed-layers.
- Several centrifuge systems including one L-90 and one L-70 ultra-centrifuge (Beckman) and one floor centrifuge (Beckman, model J2-21) in our lab are used to isolate and purify nanoparticles and proteins. We also have several desktop centrifuge systems in our lab.
- Three state-of-the-art cryo storage systems to store cell lines and proteins; one -80^{0}C freezer and several -20^{0}C to store proteins and cells; one 4^{0}C cool cabinet, which acts as a cool room, to isolate, purify and characterize bioactivity of proteins and single-molecule nanoparticle optical biosensors (SMNOBs).
- One small autoclave system in our lab and one large autoclave system in biology department are used to prepare sterilized solutions and containers, and to treat bio-wastes.
- One home-built high-performance capillary electrophoresis (HPCE) with laser-induced fluorescence (LIF) detection for high-speed and high-resolution separation of biomolecules.
- Two 400 MHz NMR (Varian; Bruker); One EFT-60 NMR spectrometer (Anasazi Instruments, Inc.); Two FTIR spectrometers (Nicolet); One FT-RAMAN 960 spectrometer (Thermo-Nicolet), owned by the Department are fully accessible to our research group. These spectrometers are used to characterize the functional groups attached on nanoparticle surfaces.
- Mass Spectrometers (QH-FTICR-MS 12 Tesla; TOF-MS) owned by the department and the college are fully accessible to my research group for characterization of protein molecules. We recently installed an Ultraflex TOF-TOF MALDI mass spectrometer (Bruker) in our lab, which can be used to characterize protein biomarkers of interest.
- One UHV-STM (shared between my group and colleagues in engineering department) and AFM (Veeco) in our department are used to characterize morphologies of nanoparticles.

C. Other Resources

- **The Newly-Established Electron Microscopy Laboratory** is located in our Applied Research Center (ARC) for characterization of our nanoparticles. The lab is equipped with a JEOL JEM-2100 F transmission electron microscope with TEM/STEM, high-sensitivity EDS (Oxford INCAx-sight EDS detector), and Gatan SC 1000 ORIUS CCD camera (11 megapixel) for characterization of nanoparticles. The lab is also equipped with AFM and scanning electron microscope, Raith ELPHY Quantum Electron Beam Lithography System, which is installed on scanning electron microscope (JEOL JSM-6060LV). The ELPHY Quantum features a powerful GDSII editor and viewer, precise exposure control, and multilevel lithography.

- **The other Electron Microscopy Laboratory** is located in the Department of Biological Sciences (next door to our lab building). Complete specimen preparation equipment and supplies are available for a wide variety of biological and nonbiological specimens (e.g., nanoparticles). Other tools including RMC MT2C ultramicrotome; LKB 7801A Knife maker; EMS Critical Point Dryer; Fullam Sputter Coater; several MJO-Diatome and DDK diamond knives for preparation of thin-layer sections of cells; and standard lab accessories for staining and processing of biological samples for EM imaging.

- **Clinical Related Animal Facilities**: We have a newly established state-of-the-art animal research facilities (vivarium) with ~10,000 sq. ft of animal space, housed on the 2nd floor of Innovation Research Park Building 2 on campus. The animal care and use program at ODU is a Public Health Service (PHS) assured program (Assurance Number: A3172-01) and is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC Full Accreditation Date Feb. 19. 2013). The animal facility offers full time veterinary care supervised by the attending veterinarian, Dr. Len Murray, DVM (DACLAM). The Vivarium is maintained as a specific pathogen free (SPF) facility. The staff provides technical and veterinary support for all educational and research projects utilizing laboratory animals. The Vivarium includes three suites and can handle a wide range of large and small animals. The vivarium contains a complete cage washing facility, an imaging center containing a high resolution Vevo 770 Ultrasound Imaging System, Moor LDLS laser Doppler imager, IVIS Spectrum for 3D non-invasive *in vivo* imaging with fluorescence and bioluminescence tomography, and an Olympus IV 100 Intravital Laser Scanning Microscope. The animal facility also contains a state-of-the-art catheter laboratory and separate operating rooms for large and small animals, as well as anesthesia machines for large and small animals. The vivarium is equipped with a steam autoclave and a chemical fume hood. These university facilities are fully accessible to our research group.

- **DNA Microarray Core Facility** (www.narf.vcu.edu) located at nearby Virginia Commonwealth University in Richmond, VA, is accessible to our research group. The core facility can offer all types of services including 500 Affymetrix microarry chips.

Profile of the Students: Total enrollment of ODU for the Spring 2013 is ~23,329 including 18,349 undergraduate and 4,980 graduate students. The student population is **highly diverse**. Of the undergraduates, 55% are female; 22% are African-American; 4% are Asian; and 6% are Hispanic or American Indian. The graduate population is represented similarly. Old Dominion offers 66 Bachelors degrees, 56 Masters degrees, 41 doctoral degrees, and 2 educational special degrees in six colleges including Arts and Letters, Business, Health Sciences, Sciences, Education, and Engineering. Old Dominion University is a state assisted doctoral research institution. The University's business and research activities contribute nearly $ 1 billon annually into the economy. This makes it the largest generator of new jobs in the region.

The Department of Chemistry and Biochemistry currently has 22 full-time faculty members. The department offers PhD programs in both chemistry and biochemistry, and an interdisciplinary PhD program in biomedical sciences. Our undergraduate program is accredited by the ACS. The PI is also affiliated with PhD program in Biomedical Engineering at college of engineering. She also mentors and directs PhD students in the program.