TECHNICAL RESOURCES & EXPERTISE WITHIN EPIEPINET

This list will help us to understand each other better, complement existing technical requirements in each partner & plan short-term missions and joint research projects

If you need further information, get in contact with us at epiepinet@gmail.com

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Index per Institution

Sapienza University of Rome

Research Groups: Eleonora Palma / Sergio Fucile / Esposito / Giollonardo / Cristina Limatola

Resources / Expertise in Molecular and Cell Biology:
• Real-time PCR • Western Blot • Cell cultures and several molecular biology techniques

Resources / Expertise in Electrophysiology and Functional Studies:
• Voltage-clamp recordings • Patch-clamp recordings • Calcium imaging • In vivo EEG recordings platform for animal models • Field and patch-clamp recordings applied to neuroimmunology • Electrophysiology in micro-transplanted Xenopus oocytes

Resources / Expertise in Human and Animal model pathology:
• Animal models of epilepsy • Clinical neurosurgery on drug-resistant epileptic patients at Neuromed IRCCS • Clinical epileptology at Policlinico Hospital

Resources / Expertise in Microscopy:
• Electron microscopy

Resources / Expertise in Genetically modified animal Models:
• **hSOD1 G93A mice**

Amsterdam UMC (AMC)

Group PIs: Eleonora Aronica, Erwin van Vliet

Resources / Expertise in Molecular and Cell Biology:
• Human astrocytes (cultures) • Inflammation • Oxidative stress • MicroRNAs • Quantitative RT-PCR • Western blot • Immunohistochemistry • ELISA

Resources / Expertise in Omics (genomics, transcriptomics, proteomics, or metabolomics):
• Genomics (somatic mutations) • Transcriptomics

Resources / Expertise in Electrophysiology and Functional Studies:
• Electrophysiology in vivo (EEG recordings mice and rats) • Electrophysiology in vitro (Multi Electrode Array) • Assays for cultured cells

Resources / Expertise in Human and Animal model pathology:
• Animal models of epilepsy (kindling and post-SE) • Stem cell-based models (TSC)

Resources / Expertise in Microscopy:
• Neuropathology • Confocal microscopy • Electron microscopy

Resources / Expertise in Genetically modified animal Models:
• Stem cell-based models (TSC)

Lund University

Group PI: Merab Kokaia

Resources / Expertise in Molecular and Cell Biology:
• Real time PCR • Western Blot • Molecular Cloning • Viral vector design and production (AAV, LV, Retroviruses) • CRISPR/Cas9 activation and interference • Cell culturing (primary neurons and cell lines) • Stem cell culturing and differentiation

Resources / Expertise in Electrophysiology and Functional Studies:
• In vitro electrophysiology: single and dual channel recordings in acute slices • Whole-cell, cell-attached, perforated patch and field potential recordings • Optogenetics in vitro and in vivo • Behavioural studies in rats and mice • In vivo EEG recordings (wired and wireless)

Resources / Expertise in Human and Animal model pathology:
• Intra-hippocampal and systemic KA model in mice and rats • Human epileptic slice cultures from surgical resections • Gene therapy with viral vectors in animal models

Resources / Expertise in Microscopy:
• Bright field • Epifluorescence • Confocal microscopy

Resources / Expertise in Genetically modified animal Models:
• Cre-dependent SPH CRISPRa mice for overexpression of endogenous genes • Cntnap2 KO mice • Cre-dependent ChR2 mice for optogenetic experiments • PV-Cre, CaMKII-Cre lines

iMM (Instituto de Medicina Molecular João Lobo Antunes)

Some of the Research Groups:

Neuronal Signalling and Synaptopathies Unit
Unit head: Ana M Sebastião;
Other PIs at the Unit: Maria José Diógenes, Sara Xapelli, Sandra Vaz; Cláudia Valente

Neural Mechanisms of Perception, Memory & Decision Unit
Unit Head: Miguel Remondes

Physical Biochemistry of Drugs & Targets Unit
Unit Head: Miguel Castanho
Other PIs of the Unit: Vera Neves

Resources / Expertise in Molecular and Cell Biology:
• RT-PCR, Cloning, Site-directed mutagenesis, RNA interference • Western blot • ELISA • Immunohistochemistry and immunocytochemistry • **Organotypic cultures** • **Primary cultures of neurons, astrocytes, microglia and neurospheres** • Cultures of cell lines • FRET

Resources / Expertise in Electrophysiology and Functional Studies:
• Recordings of **spontaneous activity in organotypic slices** • Recordings of **field excitatory postsynaptic potentials (fEPSPs)** • **Patch-clamp recordings:** EPSCs, IPSCs, tonic GABAergic currents, AP firing pattern • **In vivo electrophysiology, optogenetics and chemogenetics during complex behaviors (Miguel Remondes Group)** • **Behaviour tests** (learning, memory, anxiety, depressive-like behaviour): Morris water maze, Barnes Maze, y-maze; open-field, Novel object recognition (NOR), Novel object location, T-maze, Elevated Plus-Maze; Forced swim test, sucrose preference test, odour discrimination, social interaction; Rotarod test treadmill running; etc • **Quantification of neurotransmitters transporters activity using radio-labelled ligands** • **Quantification of neurotransmitters release using radio-labelled ligands**

Resources / Expertise in **Human and Animal model pathology**:
• Absence Seizures • Epilepsy • Amyotrophic lateral sclerosis • Alzheimer's disease • Multiple sclerosis • Rett Syndrome • Major depressive disorder

Resources / Expertise in **Microscopy**:
• Confocal microscopy • Spinning disk live-imaging microscopy • Calcium imaging (cell cultures) • Transmission electron microscopy (TEM) • Atomic Force Microscopy (Miguel Castanho Group)

Resources / Expertise in **in vivo compound/drug administration**:
• Intraperitoneal administration of drugs • Intravenous administration of drugs • Mice and rat brain stereotactic injections and mini-pump implantations

Resources / Expertise in **collection of samples**:
• Collection of cerebrospinal fluid • Dissection of brain areas (cortex, hippocampus, striatum, prefrontal cortex, cerebellum, brain stem) • Isolation of extracellular vesicle (exosomes and microvesicles) • Collection of spinal cord

Resources / Expertise in **Genetically modified animal Models**:
• IP3-R2-KO mice (lacks calcium elevation in astrocyte) • dn-SNARE mice (lacks the release of gliotransmitters) • Stargazer mice (Absence Seizures) • GAERS rats (Absence Seizures) • SOD-G93A mice (ALS) • Mecp2 knockout mice (Rett syndrome mouse model) • Nestin-CreERT2/R26R-YFP mice (to label Nestin positive cells) • 5xFAD (AD model)

Resources / Expertise in in vitro models / Biophysics: (at Miguel Castanho Lab)

- **IN VITRO BLOOD-BRAIN BARRIER (BBB) PERMEABILITY:**
  - Customised human or mouse models using confluent cell monolayers cultured in permeable tissue culture inserts.
  - Mono-culture or co-culture with brain vascular pericytes.
  - Evaluation of the drug candidates' transport across the BBB and effect on the BBB integrity.
  - Characterization of the drug transport mechanisms across the in vitro BBB models.
- **BBB PEPTIDE SHUTTLES:**
  - Peptides that when conjugated other therapeutics/drugs promote BBB transport.
- **CYTOTOXICITY SCREENING:**
  - Cells from different tissues: brain endothelial, neuronal, astrocytes, renal, liver, bronchial, derma, red blood cells.
- **BIOPHYSICS:**
  - Membrane models (LUV's)
  - Affinity constants and kinetics of binding – ligand/analyte

Other Facilities:

- **Bioimaging Unit:** Including Spinning disk confical microscopes, point scanning confocal microscopes, multi-photon microscopy, light-sheet fluorescence microscopes, widefield fluorescence microscopes

- **Biobank Unit:** The Biobanco-iMM CAML is integrated in the Academic Medical Center of Lisbon (CAML), which brings together on the same campus a research institute (Instituto de Medicina Molecular João Lobo Antunes - iMM), a medical school (Faculdade de Medicina da Universidade de Lisboa - FMUL) and an university hospital (Centro Hospitalar Lisboa Norte - CHLN, Hospital de Santa Maria - HSM).
  Biobanco-iMM CAML is available to establish agreements with researchers to provide specific services. Biobanco-iMM CAML can provide different services to the scientific community, which includes:
  - ✓ Nucleic Acid extraction (DNA and RNA) from whole blood, tissue and cells.
  - ✓ Peripheral Blood Mononuclear Cells (PBMC) isolation.
  - ✓ Primary cell culture of human skin fibroblasts.
  - ✓ Sample processing according to good laboratory practice (GLP) and Standard Operating Procedures.

- **Analytical and Structural Biochemistry Unit:** The Analytical and Structural Biochemistry (ASB) unit is devoted to the production, analysis and functional characterization of biological macromolecules including recombinant proteins, antibodies and nucleic acids. It also offers expertise in bioinformatics and structural biology for the study of structure-function relationships in biological macromolecules and in-silico drug design by computer-aided methods.

- **Zebra Fish Unit:** provides the necessary tools and technical support to initiate and develop studies using Danio rerio as animal model. These studies spam from vertebrate development, physiology to disease mechanisms, such as cancer, cardiovascular disorders, neurological diseases, inflammation, angiogenesis, muscle-associated diseases and osteoporosis. The facility

has the capacity of 400 zebra fish tanks, which accommodate 8000 adult zebra fish, quarantine, a behaviour room, to three Brightfield Stereoscopes, two Fluorescent Stereoscopes with Digital Cameras, four Microinjection Systems, a Cell Transplantation System, a DanioVision Swimming Automated Tracking System and a Swim Tunnel Respirometer for motor function studies. Besides animal care and maintenance of zebra fish lines, the facility provides services in setting up crosses, embryo microinjections and development of new protocols.

- **Rodents Unit**

- **Comparative Pathology Unit:** provides services in research histology, immunohistochemistry, transmission electron microscopy, veterinary pathology and Laser Caption Microdissection, as well as assistance in study design and training.

- **Flow Cytometry Unit:** equipped with state-of-the-art equipment including a 4-colour FACSCalibur, two 10-colour FACSFortessa, 16-colour LSRFortessa X20, and one BD Accuri C6 flow cytometers, as well as two common-use 12-colour high speed cell sorter FACSAria and a BD FACSAria Fusion that allows analysis and purification of rare populations of cells. The facility has recently acquired an imaging flow cytometer (Amnis ImageStreamX Mark II). Data analysis hardware and software are available (FlowJo, ModFit, FACSDIVA and CellQuest). The facility provides training and support to researchers in instrument operation, data analysis and interpretation.

- **Others:**

  - ✓ Surface Plasmon resonance
  - ✓ Atomic force microscopy.
  - ✓ Dynamic light scattering – nanoparticle analysers
  - ✓ Spectroscopy: Fluorescent spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR); circular dichroism (CD).