Thursday June 27 2024

Theme: INVESTIGATE THE LIVING WORLD, MEASURE, IMAGE AND INTERACT

Invited speaker Taha Benyattou

Piégeage optique à l’aide de cavité de cavité à mode de Bloch lent : Depuis la manipulation de nano-objets vers la mesure le mesure de déformabilité de cellules uniques

Ali KHEIR ALDINE, Lotfi BERGUIGA, Cécile JAMOIS, Marie-Charlotte AUDRY-DESCAMPS, Magalie FAIVRE et Taha BENYATTOU

INL, UMR5270, 69622 Villeurbanne, France.

L’utilisation de nanostructure photonique a ouvert depuis plus d’une décennie de nouveaux horizons pour le piégeage optique. Nous montrerons dans un premier temps comment l’utilisation du champ proche a permis le piégeage et la manipulation de nanoparticules. La seconde partie de cet exposé sera dédiée aux applications dans le domaine du vivant. En particulier, nous détaillerons l’approche que nous avons développée pour la mesure tout optique de la déformabilité de cellules uniques.

GROSJEAN Marc – LiPhy

Shaping evanescent waves for optogenetic activation of living cells

Total internal reflection fluorescence (TIRF) microscopy is a well-known technique allowing to confine the light close to the surface of a glass substrate. This axial confinement is based on the generation of evanescent waves. In TIRF microscopy however, there is no control of the light intensity in the transverse plane. Here, we propose a method to create evanescent patterns, which uses a fast-switching digital micro-mirror device to shape the wavefront of the beam sent into the back focal plane of the objective. The patterns are confined in the three dimensions of space and would allow better spatial control in photo-activation or photo-conversion experiments in living cells, e.g. to target processes located at cell membranes.

ARFAQUI Khouloud – LTM

Trapping of biological objects on SOI optical photonic crystal micro-resonators

Multidrug-resistant bacteria are spreading rapidly that the World Health Organisation (WHO) predicts 10 million deaths a year by 2050. This resistance results in part from the intensive use of non-specific antibiotics commonly prescribed to manage bacterial infections.

In this context, we are developing a new interdisciplinary methodology for antimicrobial susceptibility testing (AST), based on photonic crystal (PhC) micro-cavities fabricated on silicon-on-insulator (SOI) substrates. Using highly localised resonances, nano-objects can be trapped by the electric field gradient in the near-field of the resonator. The very high sensitivity of the resonance frequency to the environment allows also for the real time monitoring of the trapped object in the transmitted optical signal.

To ensure the feasibility of this measurement in a clinical context, it is essential to control all forces and interactions existing between SOI and the trapped biological object. We report here on some of the important parameters influencing these interactions, such as surface charges or the ionic strength...
of the suspension solution. The aim is to find a parameters combination that is as universal as possible to effectively trap any bacterium.

We will present our state of the art of this project and the methods currently used to study the interactions between SOI and biological objects.

**CHATELAIN Lucas – Liphy**

*Graph modeling of cellular porosity in dentin*

The mechanosensing function of teeth is thought to be determined by the odontoblasts cells located in the central cavity of the tooth (the pulp). They possess a single long process extending up to the interface between enamel and dentin, which regularly branches in secondary exploratory processes. The precise role of these branches is still unclear but since they often connect neighboring processes we believe dentin cellular porosity forms a vast network, the topology of which can be modeled and analyzed using graph theory, as already done for bones. Fluorescent confocal laser scanning microscopy has been shown to provide an accurate 3D visualization over larger regions allowing to better observe the smaller branches. However, this method presents limitations such as the restricted imaging depth and resolution and relies on image and graph processing algorithms to extract the porosity network. Thus, an assessment of the errors that might be generated during this complex process is required. Using a manually corrected ground truth, we show how the physics of imaging and data processing impacts various graph metrics, thus setting an overall framework for future analysis of dentin mechanosensing.

**FICK Jochen - Institut Néel**

*3D optical trapping and motion dynamics of pseudomonas.aeruginosa bacteria*

We will present optical trapping and manipulation of pseudomonas.aeruginosa bacteria using our optical fiber tweezers setup. Stable trapping at low light intensity was realized using fiber tips and optical fibers with printed diffractive optical elements. Special considerations will be given to the motion of un-trapped bacteria in order to determine the bacteria vitality after optical trapping.

**Theme** BIO-INSPIRED PHYSICS AND ENGINEERING: BIOMOLECULES, BIOMATERIALS, STRUCTURE-FUNCTION COUPLINGS

Invited speaker: Jérôme Sohier (LTBI Lyon)

Title: *Biomimetic hydrogels: structural versatility as a tool for modulating cellular interaction and reproducing complex tissues.*

Hydrogels are valuable tools for mimicking the extracellular matrix structure and qualities due to their biocompatibility, mechanical properties, permeability to oxygen and nutrients, and high water content. They can be designed, tuned, and formulated to either favor and reproduce interactions with cells or to mimic the structure and function of complex tissues. To explore these potentials, we have developed synthetic hydrogels with versatile and standardized properties that can replicate a wide range of tissue viscoelastic properties.

In an initial investigation, we evaluated their potential for cellular interaction and determined their ability to modulate human skin and muscle cell behavior based on the hydrogels' inherent structural
and mechanical properties. Through innovative formulations, we then further elucidated structure-function relationships in 3D culture models of muscle tissue. We then explored the possibility of reproducing the structural complexity and unique mechanical behavior of human vocal folds. From precise characterization of native human tissues, we mimicked both the collagen network and the elastin/ground substance of the extracellular matrix with polymer fibers and hydrogel composition. This approach allowed us to successfully replicate the non-linear, anisotropic, and viscoelastic mechanical behavior of the vocal folds. These examples will be presented, with an emphasis on the importance of structure-function relationships in biomimetic designs.

ZAHRAA Khalil – LMGP

Adhesive proteins inspired by nature

In the field of adhesives, current synthetic options are problematic in wet environments and raise toxicity questions. In addition to toxicity concerns, medical glue can exhibit a low mechanical strength, particularly for applications involving biomaterials. Our research seeks to develop adhesive products inspired by organic adhesion mechanisms found in particular arthropods, such as barnacles. Barnacles have unique surface self-assembly capabilities via amyloid fiber creation, allowing them to adhere to various surfaces. Our research aims to understand better how surface variables such as material, chemical, and physical characteristics impact adhesive protein auto-assembly and fibrillation. We specifically investigate synthetic recombinant protein models, such as the M19 inspired by Megabalanus Rosa cement protein previously discovered and researched for its function in the adhesion process. We study the adhesive properties, the adsorption, and the auto-assembly process of the recombinant protein in function to different surfaces using advanced methods like atomic force microscopy and modern biochemical approaches such as fluorescence spectroscopy and enzyme-linked immunosorbent test (ELISA). Our findings show that the auto-assembly process is tightly connected to surface substrate types, resulting in variances in the fibrillation and adsorption of the adhesive protein. Our research focuses on this complex relationship between surface adhesion and protein self-assembly process.

THIBIEROZ Nathan – BRM

Development of a biomimetic high throughput essay to study neuroblastoma cell proliferation and differentiation

Neuroblastoma (NB) is one of the most common cancer for children, from 7 to 10% of all pediatric cancers. Because of its heterogeneity, no specific treatments are available. Extracellular matrix composition and stiffness was found to have an impact on the proliferation and differentiation of NB. What could be the combined effect of those two parameters? We aim to develop a model closer to in vivo conditions to study neuroblastoma, through the engineering of a biomimetic environment for cell culture, in a high throughput manner. We combine two biomaterials. The Streptavidin (SAv) biomimetic platform to control the chemistry, and Layer by Layer (LbL) film to control stiffness.

Neurite length of cells is commonly used to analyze neural cells differentiation. To measure this parameter, we developed a new image analysis macro. It can also be used for proliferation and cell size analysis, among other. Our objective is to create an open, accessible tool for neurite and neural cell analysis in high content, with minimal operator input and means of verification.
With these tools in hands, we will be able to study NB using the SY-SY5Y and N2A cell lines, in an automated, standardized, and parallelized manner.

BECHARD-DRIMARACCI Emilie - Institut Néel

Enhancing functional maturation of neural networks by co-culturing neurons with astrocytes

Astrocytes are ubiquitous non-neuronal cells in the brain. Beyond their supportive role for neurons, astrocytic influence on neuronal activity and their bidirectional communication has emerged as a crucial modulator of the tripartite synapse function. Astrocytic calcium signalling is one of the main mechanisms involved in these reciprocal interactions. Synaptic activity influences calcium oscillations in astrocytes, and calcium elevation stimulates the release of gliotransmitters to regulate synaptic transmission. Furthermore, it has been proposed that astrocytic calcium disruption contributes to brain disease.

To understand the impact of astrocytes on the functional maturation of neurons and their spontaneous firing, we seeded primary neurons and astrocytes in 3 different culture conditions: neuron monoculture with Neurobasal medium, neuron monoculture with Neurobasal medium supplemented with astrocytes and neuron-astrocyte coculture.

Extracted from hippocampi of mouse embryos (E15), cells are seeded on glass coverslips pre-coated with 5 µg/cm² of Poly-L-Lysine, at a density of 100K cell/cm². Using the whole-cell configuration of patch-clamp we assess the evolution of spontaneous firing of neurons from DIV5 to DIV15 depending on the culture conditions. In parallel, we use GCaMP6 transfection or Fluo-4 probe to monitor calcium transient in astrocytes.

NICOLAS Alice – LTM

Quantitative analysis of the mechanical properties of healthy and cancer lung tissue for the design of mechano-mimetic cultures

Mechanical properties of tissues are increasingly recognized as crucial in disease progression. Here we investigate the mechanical properties of normal and adenocarcinoma lung tissues from 18 patients using indentation-type atomic force microscopy. We show that these tissues exhibit a predominant linear elastic behavior. Microscale tissue stiffness and shape descriptors of stiffness texture are extracted from maps of the Young’s modulus. Furthermore, a correlation between tissue composition and stiffness is performed. Combining these parameters with photolithography, stiffness-textured polyacrylamide hydrogels are engineered, resulting in culture substrates that mimic the tumor tissue's stiffness distribution. By culturing A549 cells on these hydrogels, the influence of substrate stiffness texture on cellular behavior is evaluated. The development of this versatile mechanomimetic platform reveals its potential applicability to other human tissues and is envisioned as an in vitro model to improve the predictability of drug screening.

Friday June 28 2024

Theme DYNAMICS OF LIVING SYSTEMS: OUT-OF-EQUILIBRIUM SYSTEMS, SPATIO-TEMPORAL EVOLUTION

Invited speaker: Cécile Leduc (Institut Jacques Monod, Paris, dynamique du cytosquelette)

Title: Mechanics and dynamics of single intermediate filaments

Intermediate filaments (IF) are involved in key cellular functions including polarization, migration, and protection against large deformations. These functions are related to their remarkable ability to extend
without breaking, a capacity that should be determined by the molecular organization of subunits within filaments. However, this structure-mechanics relationship remains poorly understood at the molecular level. I will present how, using super-resolution microscopy (SRM), we show that vimentin filaments exhibit a \( \sim 49 \) nm axial repeat both in cells and in vitro. Using an SRM compatible stretching device, we also provide evidence that the extensibility of vimentin is due to the unfolding of its subunits and not to their sliding, thus establishing a direct link between the structural organization and its mechanical properties [1]. In a second part, I will show recent results on the in vitro reconstitution of vimentin assembly dynamics. We show that vimentin filaments can spontaneously break without cofactors or post-translational modifications, but this fragmentation limits assembly only at very long-time scale (>18h) [2]. We also uncovered the mechanism responsible for fragmentation which involves subunit exchange. Our results show how vimentin self-repair to protect their integrity and provide new insights into the physical understanding of the intermediate filament length regulation.

REFERENCES


ORSI Guillermo – IAB

Biophysical transitions that organize the genome as a liquid crystal in cricket sperm.

Spermiogenesis is a radical process of differentiation whereby sperm cells acquire a compact and specialized morphology to cope with the constraints of sexual reproduction while preserving their main cargo, an intact copy of the paternal genome. In animals, this often involves the replacement of most histones by sperm-specific nuclear basic proteins (SNBPs). Here, I will discuss how a multidisciplinary approach combining confocal, electron and super-resolution microscopy with polymer physics revealed a unique architecture of sperm chromatin in the needle-shaped nucleus of the cricket Gryllus bimaculatus. Accompanying spermatid differentiation and shaping, the SNBP-based genome was strikingly reorganized as \( \sim 25 \)nm-thick fibers orderly spooled along the elongated nucleus axis, favoring its ultracompaction. These elegant transition may be recapitulated by a surprisingly simple biophysical principle based on a nucleated rigidification of chromatin linked to the histone-to-SNBP transition within a confined nuclear space. Our work highlights a liquid crystal-like mode of higher-order genome organization in cricket sperm completely distinct from nucleosomal chromatin, illustrating the diversity of non-canonical modes of DNA organization.

ZABLOTSKY Amir – LIPhy

Emergence and impact of lattice defects on microtubule stability and dynamics

Microtubules are a central structure in living cells, involved in cell division, migration, and intracellular transport. Therefore, they are a primary drug target against severe pathologies, among them neurodegenerative diseases and cancer. A complete understanding of the mechanisms regulating their dynamics and stability is a central issue in cell biology and a key challenge for human health.

Common textbook knowledge states that microtubules are hollow cylindrical structures formed by \( \alpha\beta \)-tubulin heterodimers arranged in a quasi-crystalline lattice, whose dynamics is restricted to elongation and shortening at their tips.
Despite extensive research on the MT tip dynamics, in particular on the dynamic instability, less attention has been paid to the dynamics of the bulk MT lattice due to the prevailing notion of its frozen and static state. Recent experiments have now unveiled the presence of monomer-sized vacancies within the MT lattice, which challenges the current idea of an inert shaft lattice. Moreover, this discovery has renewed the debate on the MT tip dynamics, which excludes the presence of vacancies. Employing kinetic Monte Carlo simulations and analytical approaches, we investigate the formation and dynamics of monomer vacancies, depending on the lattice binding energy and anisotropy, two crucial parameters, which are not directly accessible experimentally. Furthermore we study how the lattice properties and the presence of defects influence the dynamics of fracture propagation in end-stabilized MTs in the absence of free tubulin, a process which has been studied recently experimentally.

Our investigation reveals that only specific ranges of lattice binding energies and anisotropies allow for the formation of monomer vacancies. Furthermore, we observe a strong correlation between defect frequency and a transition from helical to uncorrelated MT polymerization modes. Simulating the dynamics of end-stabilized MTs in the absence of free tubulin, we also demonstrate how the presence of defects and the lattice parameters (binding energy, anisotropy) influence the dynamics of fracture propagation along the lattice.

We show that only weak lattice anisotropies are consistent with the experimental data on defect formation and MT shaft breakage. Our result thus indicates a much weaker lattice anisotropy than previously assumed, which calls into question our current picture of MT tip growth.

As a perspective our modelling framework lays the foundation to study the role of MT lattice defects in globally regulating the stability and architecture of the MT lattice in vivo."

**NDIAYE, Anne-Betty - LPCV**

*What makes a cell one? On the interplay between cytoskeletal self-organization and cell size regulation*

Cell size varies over several orders of magnitude, from micrometers for stem cells to centimeters for oocytes. Cytoskeletal networks play a prominent role in the maintenance of structural integrity in relationship to cell size and shape determination. Such maintenance of structural integrity upon changes in metabolic or environmental context requires a strong coupling between cytoskeletal organization and cell size. In this study, we aim at investigating the interplay between cytoskeletal self-organization and the maintenance of mechanical integrity for cells to produce an integrated response to environmental cues. Particularly, we focus on the relationship between cell size and cytoskeletal self-organization.

We adapted a method based on membrane fusion mediated by the viral fusion protein VSV G in order to induce rapid changes in cell size, and monitor the following reorganization of actin and microtubule networks architectures and dynamics.

Our preliminary results suggest the existence of a size-dependent reorganization of the microtubule network from a centrosome-driven organization towards the formation of parallel microtubule bundles that correlates with an increased microtubule stability in larger systems. The impact of such structural and dynamical changes in the ability of fused systems to establish pole-to-pole coordination and undergo polarization and migration is currently studied.

**MIHALCESCU Irina – LIPhy**
When lowering temperature, the in vivo circadian clock in cyanobacteria abide by the in vitro protein clock trough a Hopf bifurcation

Circadian clock in cyanobacteria is one of the most studied self-sustained oscillators in living matter. It had been shown that the in vitro clock protein at the heart of this oscillator, undergoes a Hopf bifurcation when temperature is lowered. Here, we studied the in vivo clock in single cyanobacteria when temperature is lowered. We first untangle the circadian clock behavior, from the bacterial cold shock response. Next, we show that the kinetic response of the oscillatory tracks can be described by a unique simple model of Stuart Landau oscillator in vivo, as well in vitro. The implications in the overall complete framework of an in vitro biochemical oscillator embedded into in vivo circadian clock are then discussed.

Theme: PHYSICAL INTERACTIONS: CELL MECHANICS, MICROENVIRONMENT, RADIATION

Invited speaker; Virginie Chamard (Institut Fresnel Marseille)

Title: Physico-chemistry insights in calcareous biomineralisation gained from advanced x-ray and optical microscopy approaches

Biomineralization integrates complex physical and chemical processes bio-controlled by living organisms through ionic concentration regulation and organic molecules production. The capability to tune, from ambient conditions crystallisation, the structural, optical and mechanical properties of hard tissues motivates extensive research to transfer biomimetic approaches into material science studies. This urges a detailed understanding of the underlying processes at play in Nature.

Our work focuses specifically on the mechanisms underlying the production of calcareous hard tissues in marine species (oysters, corals, coccoliths, etc...). While it is clear that non-classical crystallisation processes govern the formation of most of these biominerals, major questions are pending regarding the involved physico-chemical pathways. These include the identification of - possibly - several metastable precursors and the nature of their successive transitions. Our strategy is based on the investigation of early-mineralized units, located at the growth front of the biomineralizing tissue, which we characterize with highly sensitive and highly spatially resolved optical or x-ray microscopy approaches [1]. In particular:
- To monitor the different chemical states (e.g., amorphous or crystallized carbonates, organics molecules), a highly sensitive coherent Raman microscopy approach is developed. It allows mapping molecular bond concentrations and demonstrates suitability for the in vivo imaging of a growing biomineral (e.g., Pinctada margaritifera oyster shell) [2].
- To address the crystallization process, we make use of x-ray scanning nano-diffraction at novel 4th generation synchrotron source. It provides nanoscale spatially resolved and crystalline-sensitive study of a biomineralizing animal (Crassostrea gigas shell and Stylophora pistillata coral) [3, 4].

In this presentation, I will show recent results, which bring new insights in calcareous biomineralization pathways.

References
Mechanobiological effects on living cells induced by magneto-mechanical stimulation

The influence of mechanical forces and stresses on living molecules, cells and tissues, has attracted considerable interest over the last two decades, giving rise to the new discipline of mechanobiology. Magnetic nanoparticles are particularly well suited for generating local mechanical forces on cells, remotely controlled by the application of a static or low frequency (2-20 Hz) alternating magnetic field. Over the past fifteen years, studies demonstrated that such magnetomechanical stimulations can induce cancer cell death (apoptosis or necrosis), through purely mechanical effects of nanoparticles without heat generation.

Using our magnetic vortex microdisks (diameter ~1µm, 60-100nm thick, gold coated for biocompatibility), we demonstrated renal and glioma cancer cells destruction, triggered by the low-frequency vibration of such anisotropic particles. Cell viability, motility, proliferation and cytoskeleton disorganization after magnetomechanical treatments are currently under investigation, considering the influence of the microenvironment (stiffness).

Furthermore, we showed that this alternating field-mediated nanoparticles actuation technique is capable of stimulating insulin secretion in pancreatic cells. The use of magnetic vortices is also being considered for neuroregeneration to favor neuronal growth after spinal cord lesion.

These studies demonstrate the interest and effectiveness of anisotropic magnetic nanoparticles in the field of mechanobiology, to be explored for potential future medical treatments.

MARQUEZ-VIVAS Genesis – LIPhy

Self-sustained velocity waves and pattern emergence in tissues

Multicellular arrangements govern a diverse range of morphogenetic events, relying on complex interplay between chemical and mechanical signals that contribute to supra-cellular organization. These mechanical cues can initiate various biological processes, including embryonic development, tissue repair, and regeneration. Recent studies have underscored the presence of long-range mechanical excitations at the supra-cellular level. Remarkably, our recent observations indicate that they exhibit a distinct wavelength significantly larger than the cell size and a period several times shorter than the typical duration of a cell cycle. By confining monolayers using micropatterns, we determined critical dimensions affecting monolayer behavior, elucidating standing wave formation and cell movements at nodes and antinodes. We propose that periodic stretching may impact the cell cytoskeleton, influencing cell division and differentiation. Our study investigates if mechanical stimulation induces transcriptomic differences between cells at wave nodes and antinodes. Utilizing photoactivatable MDCK cells, we illuminated specific monolayer locations, enabling cell sorting for transcriptomic analysis. Overall, our findings may shed light on how mechanical signals propagate and translate into effective tissue formation.

DELORME Rachel – LPSC

Biophysical modeling for innovative radiotherapies using low-energy ions: BNCT and TAT applications

There is a great interest of using targeted radiotherapies such as Boron Neutron Capture Therapy (BNCT) and Targeted Alpha Therapy (TAT) for aggressive, diffused and metastatic cancers, because they combine advantages of a molecular targeting of cancerous cells with the local emission of very efficient low-energy ions. Such ions have high linear energy transfers (LET), which characteristic is known to be associated with an increased biological efficiency to kill cells compared to photons. The ions deposit all their kinetic energy (< 10 MeV) within tens of micrometers (few cells), limiting the damage to the targeted cancerous cells, mostly sparing surrounding healthy tissues. As it depends both
on the molecular vector uptake and the very short range of radiation, the dose deposition may be very heterogeneous at tumor and micrometer levels. In order to predict biological efficacy of BNCT or TAT treatments, nano/micro-dosimetry are essential approaches to include heterogeneous dose-deposition effects in calculations. Our team work in collaboration with IP2I (Lyon) in order to develop a calculation chain able to improve biological dose prediction for such therapies. We combine the use of very detailed Geant4-based Monte Carlo particle transportation simulations in realistic multicellular geometries and the biophysical model NanOx (Nanodosimetry and Oxidative stress) to calculate both 3D physical dose distributions and biological indexes (such as mean cell survival and Tumor Control Probability) for a given treatment condition. This calculation chain is flexible and allows to investigate the influence of a great number of irradiation, distribution or geometrical parameters. This talk will especially focus on a theoretical study performed to evaluate the importance of radionuclide cell internalization and tumoral uptake heterogeneity in different conditions of microtumors treated with TAT on physical and biological endpoints.

GOURRIER Aurélien – LIPHY

Minimal ingredients from physics to properly study cellular networks in mineralized tissues: a guarantee for a serious headache

"Understanding mechanosensing in bones and teeth represents a daunting challenge. First, owing to the small length scales (> 100 nm), imaging is non trivial and requires advanced methods, e.g.: FIB-SEM, synchrotron X-ray holotomography or optical super-resolution. Each method requires in-depth understanding of the fundamental mechanisms of wave propagation and scattering in mineralized tissues to achieve a satisfying result.

Secondly, because of the multiscale topological complexity, it is very difficult to extract the minimum information needed to obtain a reliable abstract representation of the cellular network. In addition to standard tools from physics, advanced applied mathematics tools need to be developed from graph theory and mathematical morphology.

Altogether, this requires combining expertise in instrumental development and analytical methods rooted in a diversity of traditional physics fields, including large instruments, microscopy and complex systems.

Over the last 12 years, we have explored all these fields at the LIPHY and developed a very specific expertise on mineralized tissues. This leads to a relatively distinct form of biophysics than this traditionally developed for cellular biology or soft tissues. A very brief outline of this work will be given while highlighting currently missing information and possible future developments that could lead to new local collaborations."
List of Posters:

- **LETROU Mathieu**, MC2 – LIPhy, “Influence of micron-size roughness on biofilm formation”
- **DAVID Dylan**, CEA –Pheliqs- SiNaPS, “Caractérisation du phénotype optique bactérien par piégeage sur microcavité »
- **ARFAOUI Khouloud**, LTM, « Trapping of biological objects on SOI optical photonic crystal micro-resonators”
- **NDIAYE Anne-Betty**, LPCV, “What makes a cell one? On the interplay between cytoskeletal self-organization and cell size regulation”
- **BRAM Thibault**, MicroTiss-LIPhy, “Roles of cell forces and ECM remodeling on fibrous tissue self-assembly.”
- **PISHKARI Niloofar**, MicroTiss-LIPhy, “How to make cells dance to your tune”
- **BECHARD-DRIMARACCI Emilie**, Institut Néel, “Enhancing functional maturation of neural networks by co-culturing neurons with astrocytes”