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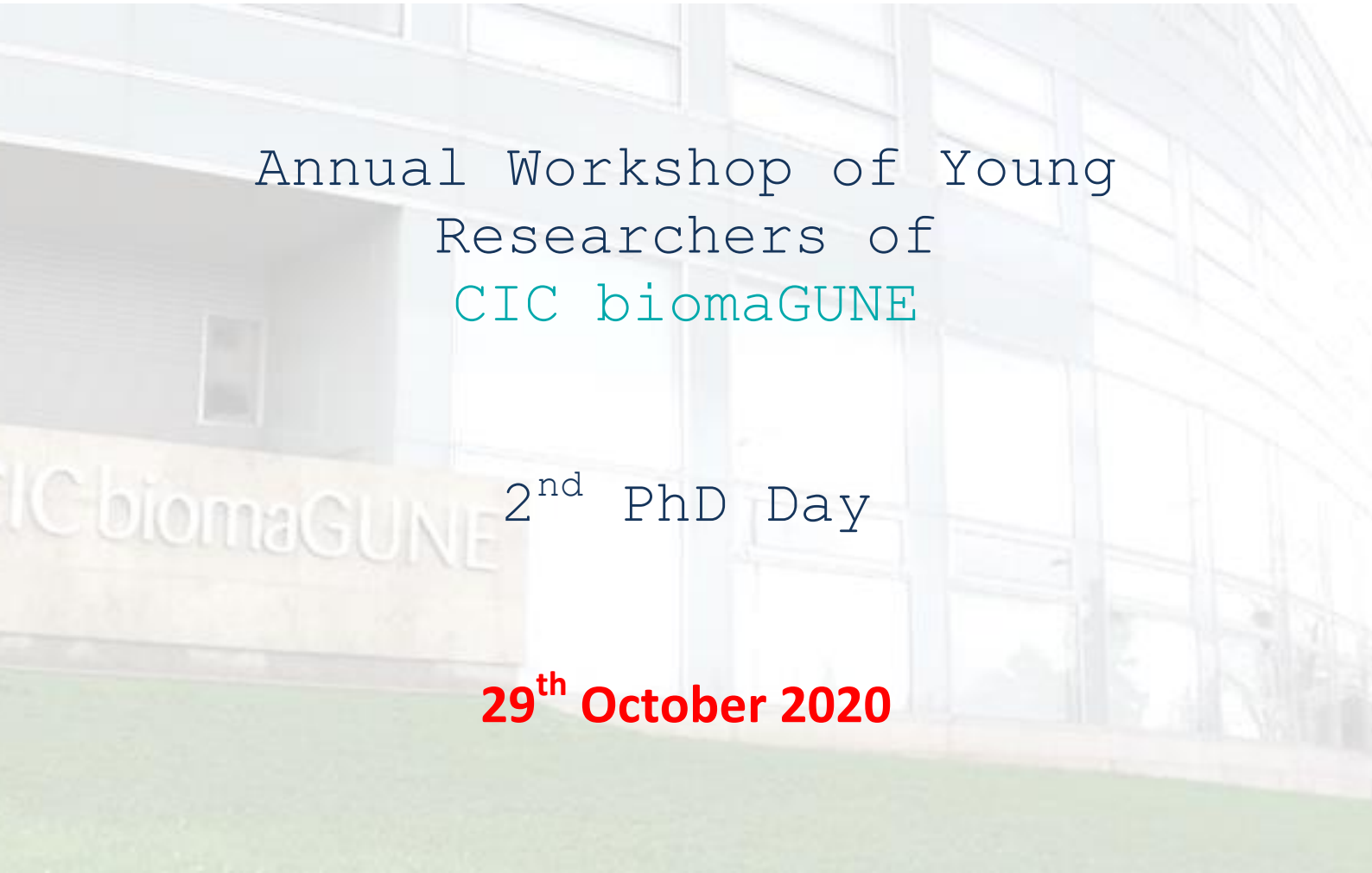
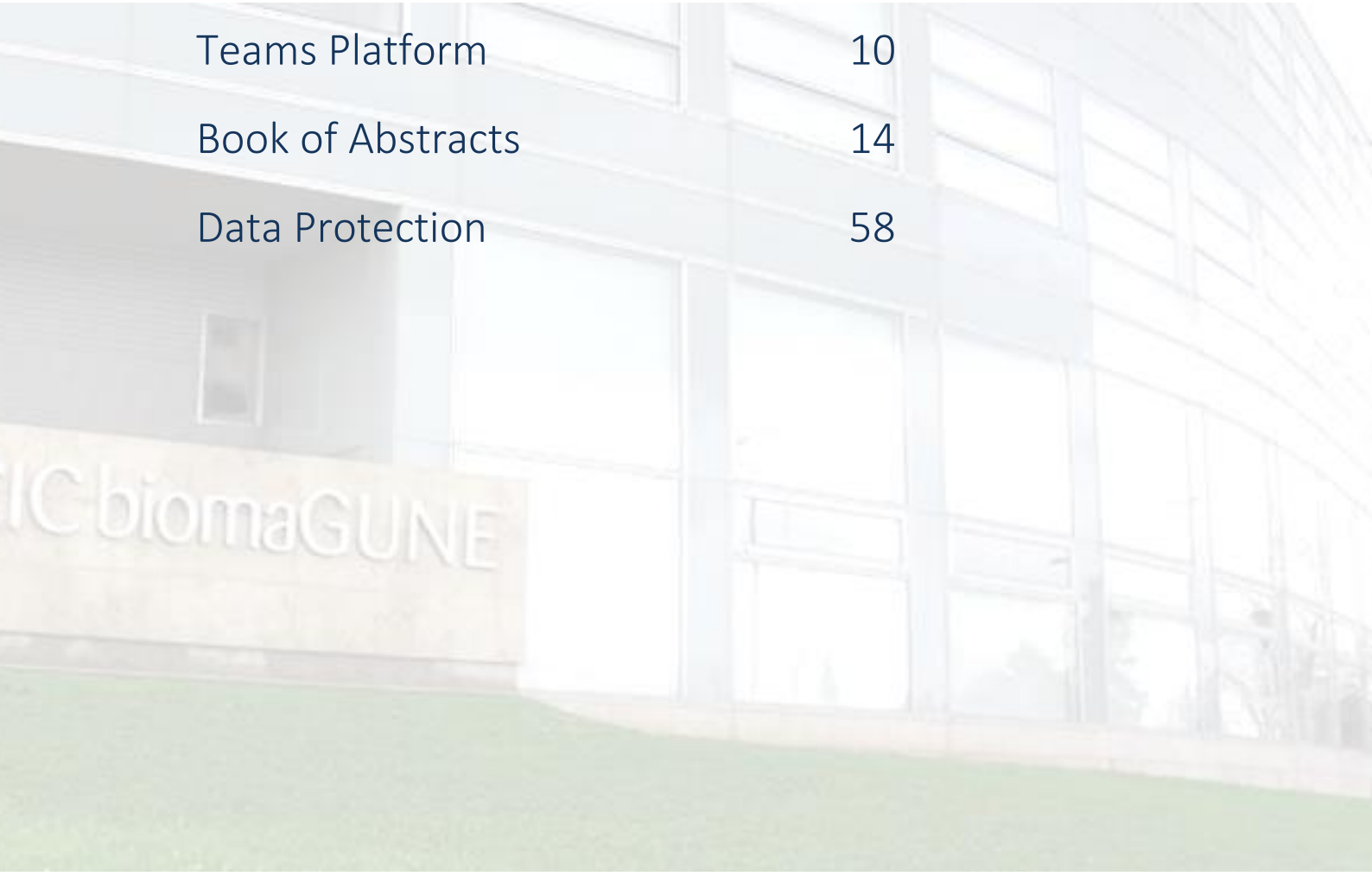


Annual Workshop of Young Researchers of CIC biomaGUNE

2nd PhD Day

29th October 2020

Index



About

The "**Annual Workshop of Young Researchers of CIC biomaGUNE**" is a yearly meeting open to all researchers, but with a special emphasis on the participation of biomaGUNE's PhD students. The event is envisaged as a 1-day symposium, where PhD students have the opportunity to present their latest results within a relaxed environment, and participate in lively scientific debates. The contributions by PhD students of second and third year will be in the form of short 5 minutes oral communications, encouraging them to focus on presenting the most important results of their research in a clear and concise way. Additionally, first year PhD students will have the opportunity to introduce their research with a poster. As such, PhD Day will tackle all research activities of CIC biomaGUNE, and will provide postdocs and PhD students with an invaluable insight into the latest achievements performed at the institute, all within a reduced timeframe.

A number of prizes will be awarded to the best oral communications and posters, but will also recognize the best contributions of the students during the discussions following the talks. These prizes will be decided by the students themselves, and the organization will provide a link for them to vote after the event.

Ultimately, PhD Day pretends to be a platform for young researchers within the institute to engage in scientific discussion, share ideas and start new collaborations. Furthermore, the event should help to improve the social and working environment at CIC biomaGUNE, which is of vital importance for the scientific development and wellbeing of researchers at the center.

Unfortunately, due to the situation with the COVID-19 this year, the 2nd CIC biomaGUNE PhD Day will take place as an online symposium using the **Teams** platform. *We are fully aware that some students might encounter with technical problems to attend, present or participate in the talks during the symposium. If that is your case we encourage you to contact the organizing committee as soon as possible, which will try to provide with alternative solutions.*

Presentations for the oral communications should be submitted by the 28th of October.

Posters (as a pdf file) and a video presentation of approximately 3 min duration should be uploaded by the 27th of October, and will be accessible to the whole institute from the 28th of October.

More information on how to upload and present your oral communications and posters can be found below in this document.

All the content of the event is strictly confidential. *Only the organizing committee and CIC biomaGUNE will be allowed to record or take pictures, which will not contain sensible information*. This graphic material might be used for promotion purposes in the future. **More information about the data protection policy for the event, and how to withdraw your consent can be found at the end of this booklet**.

Finally, remember that the PhD Day is your own space, where you as students are the center and the driving force. As such, we encourage you to participate actively, and hope that you will enjoy the experience.

See you soon

The organizing commitee

Program

Oral Communications

Thursday 29 October			
9:30-9:40	Welcome		
9:40-9:45	Rossana Passannante *Pharmacokinetics evaluation of new drugs using Positron Emission Tomography*	O-1	**Chair:** Ana Beatriz Miguel Coello
9:45-9:50	Lydia Martínez-Parra *Tailored nanoparticles for atherosclerosis diagnosis*	O-2	
9:50-9:55	Ana Joya Villanua *Magnetic Resonance Imaging evaluation of the role of hyperglycemia in experimental subarachnoid haemorrhage*	O-3	
9:55-10:00	Lucía Fadón Padilla *Characterization of Pulmonary Hypertension*	O-4	
10:00-10:05	Cristina Simó *Monitoring $^{18}F/^{124}I$ enzyme-powered nanomotors in vitro and in vivo by PET-CT*	O-5	
10:05-10:10	Riccardo Rovina *In vivo fate studies of glyco NPs and biocorona stability*	O-6	
10:10-10:15	Jose Alberto Suárez Vázquez *sncRNA charged extracelular vesicles as vehicles to the CNS*	O-7	
10:15-11:00	Global Discussion		
11:00-11:30	**Coffee Break**		
11:30-11:35	Anna Ballesteros *Isolation of N-glycans from natural sources under alkaline conditions*	O-8	**Chair:** Elisa Bindini
11:35-11:40	Elisa Lenzi *SERS bioimaging of complex 3D cellular systems*	O-9	
11:40-11:45	Cristian Salvador *Supramolecular polyamine Nanocarriers for siRNA delivery*	O-10	
11:45-11:50	Damián Pérez-Martínez *Design and Synthesis of N-glycomimetics with high affinity for DC-SIGN*	O-11	
11:50-11:55	Idoia Mikelez-Alonso *NK cell stimulation with IONP@hIL15HIS: enhanced targeting and tumor cell killing*	O-12	
11:55-12:00	Tanja Ursula Lüdtke *Biological fate of gold nanoparticles*	O-13	
12:00-12:45	Global Discussion		
12:45-13:45	**Lunch**		
13:45-13:50	Javier Santiago-Arcos *Evaluating of different carriers for the immobilization of alcohol dehydrogenases. A novel heterogeneous biocatalysts for the oxidative transformation of diols to hydroxialdehyde*	O-14	**Chair:** Maxence Fernandez

13:50-13:55	Laura Mazzei *Design of Hybrid Structures for Bioorthogonal Drug Photoactivation and Photocatalysis*	O-15	
13:55-14:00	Pilar Castellnou *One-pot multi-enzymatic synthesis for the preparation of L-glutamine as potential nitrogen-13 radiotracer*	O-16	
14:00-14:05	Cristina Risueño Fernández *Modulating the Conformational Plasticity of Tetraspanin CD81 by Ligand Binders and its Implication in Cellular and Viral Processes*	O-17	
14:05-14:10	Alba Ledesma-Fernández *Design of consensus tetratricopeptide repeat (CTPR)-based orthogonal scaffolding units for the ordered assembly of enzymes*	O-18	
14:10-14:15	Guillermo García-Marquina *Enzyme engineering and flow-reactor immobilization of the acyltransferase LovD*	O-19	
14:15-14:20	Elena López-Martínez *Tuning the Luminescence of Gold Nanoclusters Through Protein Coordination*	O-20	
14:20-15:05	Global Discussion		
15:05-15:35	**Coffee Break**		
15:35-15:40	Mathias Charconnet *Optical properties of stretchable gold nanoparticle superlattices*	O-21	**Chair:** Jhoan Toro-Mendoza
15:40-15:45	Cristina de la Encarnación Bermúdez *Magneto-plasmonic nanoparticles for multimodal bioimaging*	O-22	
15:45-15:50	Donato Mancino *Mechanical properties of CNTs based polymeric scaffolds for tissue engineering applications*	O-23	
15:50-15:55	Javier Plou *Detection of extracellular metabolites by SERS: from nanostructured 2D substrates to 3D-printed plasmonic scaffolds*	O-24	
15:55-16:00	Christian Vila-Parrondo *3D porous scaffolds for SERS sensing*	O-25	
16:00-16:05	Vished Kumar *Micelle-assisted Chiral Seeded Growth over Gold Nanocrystals*	O-26	
16:05-16:50	Global Discussion		
16:50-17:00	Closing Remarks and Photo Contest Prize announcement		

Posters

Paula Vázquez-Aristizabal *Development of customized nanoparticle-based bioinks for 3D printing of dynamic cancer models*	P-1
Cecilia Wetzl *Graphene-based functional materials for electrochemical imaging*	P-2
David Vila-Liarte *Design of colloidal plasmonic nanostructures with chiral optical activity, for bioimaging and diagnosis*	P-3
Inés Teruel *Synthesis of stable isotope-labeled O-glycan standards and their application in clinical diagnosis*	P-4
Nicolette Czarnievicz Rother *Surface engineering of transaminases to tailor protein immobilization on microreactors*	P-5
Nicoll Zeballos Lema *Immobilization of multi-enzyme systems through artificial cellulosomes in solid phase*	P-6
Unai Mendibil *Cartilage decellularized extra cellular matrix materials for preclinical products application*	P-7
Andrea Fernández-Martínez *Synthesis and Evaluation of Glycomimetics as potential Siglec-10 Immunomodulators*	P-8
Laura Pérez-Chirinos *2D self-assembled hybrid biomaterial*	P-9
Silvia Vázquez-Díaz *Biocatalytic synthesis of nano-atomic clusters for bioanalysis*	P-10
D. Andrés-Sanz *Self-sufficient Reduction of β-ketoesters Catalyzed by a Novel and Robust Thermophilic Alcohol Dehydrogenase Co-immobilized with NADH*	P-11
María Regato-Herbella *Responsive Microgels for Biomedical Applications*	P-12
Raquel Ruiz Hernández *3D models for in vitro definition of cartilage-to-bone transdifferentiation process*	P-13
Irati Aiestaran-Zelaia *Enhancing energy to achieve healthy aging and greater longevity*	P-14
María Jesús Sánchez-Guisado *Effect of dietary regimes in the development of pulmonary hypertension*	P-15

Teams Platform

Team Platform for the PhD day and Poster Session

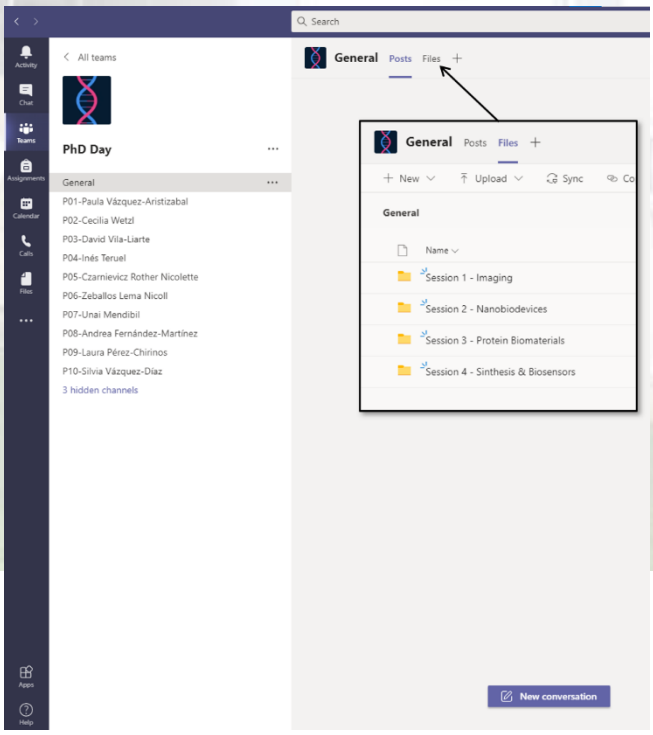
Given the current situation the event will be held online through the Teams platform to which everyone has access.

Presentations for the oral communications should be submitted by the 28th of October.

Posters (as a pdf file) and a video presentation of approximately 3 min duration should be uploaded by the 27th of October, and will be accessible to the whole institute from the 28th of October.

Prior to the submission deadline everyone will be invited to the group *PhD Day*. The event will be carried out in the tab General. Different Channels (Subgroups) have been created for the posters.

Presenters will send their oral communications to Dr. Ivan Sasselli Ramos (isasselli@cicbiomagune.es). If your presentation is too big, or you have any issues contact him to agree on an alternative submission procedure. During the event, all presentations will be loaded in one computer and the presenter will get the control of it remotely to pass the slides.



Questions for the talks will be added as *New Conversation* stating to which talk it is directed. The moderator will manage the questions in the discussion session. Additionally, the moderator and organizing committee might post questions for the audience during the talks.

Each poster presenter will be responsible of uploading their posters (as a pdf file) and video of approximately 3 min duration explaining its work to their corresponding Channel (bellow *General* with the format: *P## - Name Surname*) in the *Files* tab. In this way, each Channel will include one poster and the corresponding video in the *Files* tab.

Questions/comments to the posters will be added in each Channel as New Conversations and the presenters of the posters will be responsible to answer them.

How to record a video presentation using Microsoft Teams

---Be aware that the quality of the video depends on the internet connection. Please ask us for support if you need a computer with camera or a good wifi ---

1. Open your PowerPoint or PDF document with content you wish to share (Avoid having too many documents open)

2. Open Microsoft Teams

3. Go to the Calendar

4. Click the “Meet now” button

5. Click “Join now”

6. To start recording, select more options/ start recording from the call menu bar (Tip: you can briefly present yourself for the camera)

7. In the call menu bar now choose the presentation file to share or screen share your desktop

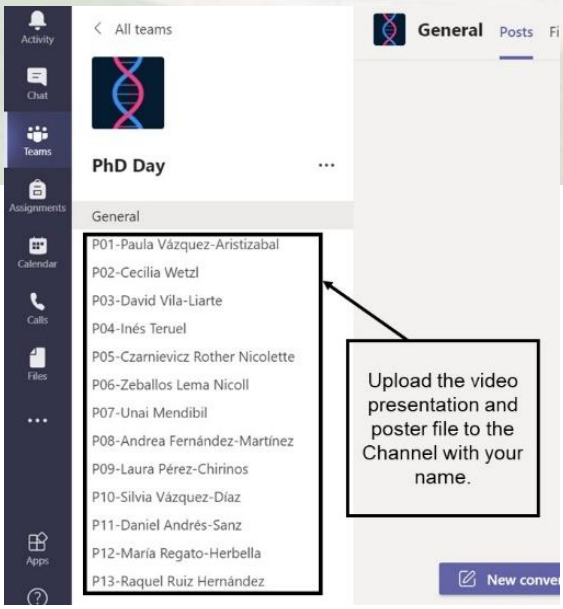
8. Present your content (You can practice and make several videos to choose the best or with a video editor you can cut/paste the best sections)

9. To stop recording, navigate back to the meeting tool bar and select “stop recording”

10. Click the “hang up” button

11. You will see your video on the Chat recent activity in Teams (Download the video to the disk by clicking on it. If you cannot directly download it, click the three dots “Open in Microsoft Streams” and download from there by clicking the three dots below the video)

12. Upload your final video to the Team “PhD day” to the designated Channel with your name (P01, P02 …… etc.)



Several options available (select the one best fit you):

13a. Click “New conversation” and on the bottom ribbons Click “Upload from my computer” and select your video

13b. Click “New conversation” and on the bottom ribbons Click “Microsoft Stream” and paste the link to the video (you can see this link on Microsoft Stream by clicking “Share”)

13c. Click “New conversation” and paste the OneDrive share link of the video

14. Confirm you video plays properly --Click send

15. Upload also your PDF poster file

---Further links with detailed instructions---

https://howto.hyms.ac.uk/knowledge-base/recording-a-narrated-presentation-via-microsoft-teams/

https://www.alma.edu/live/files/3930-create-and-share-video-ms-teamspdf

Questions and answers:

Some tutorials mention the recording is uploaded automatically to Microsoft Stream for easy management and sharing, but I am not seeing my video on Stream?

--Apparently this function is no longer supported with some Teams licenses, but you can upload manually to Stream if you wish. https://docs.microsoft.com/en-us/stream/portal-upload-teams-meeting-recording

Is there a video tutorial of the instructions above?

--Yes several tutorials are available, for example: https://www.youtube.com/watch?v=Kul6fphW5Ss

How a poster video presentation might look?

--There are different poster presentation videos available online, for example https://www.youtube.com/watch?v=7rjbB57vQh8 , but be creative to increase the delivery impact of your poster.

---------Disclaimer: The shared material and links are being provided as a convenience and for informational purposes only; they do not constitute an endorsement by the PhD day organization.--------

Book of Abstracts

Oral Presentations

Pharmacokinetics evaluation of new drugs using Positron Emission Tomography

R. Passannante[a], M. A. Morcillo[b], U. Cossío[a], V. Gómez-Vallejo[a], A. Vallejo-Illarramendi[c,d], M. Eceiza[c], M. Sagartzazu-Aizpurua[c], A. Martín[e], P. Aguiar[f], J. Bergare[g], L. Kingston[g], C. S. Elmore[g], J. M. Aizpurua[c,d], J. Llop[a].

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At early stages of drug development, there are certain questions that need to be answered, e.g.: Where is the drug accumulated? How long does it stay in the body? How is it eliminated? In this work, we investigated the drug candidate FKBP12-RyR stabilizer AHK2 (Fig. 1a), with potential application in Duchenne muscular dystrophy (DMD) using Positron Emission Tomography (PET) to answer the questions above. The target molecule was labelled either with ^{11}C (AHK2.1 and AHK2.2) or ^{3}H (Fig. 1a). Metabolism was first investigated *in vitro* using liver microsomes. PET imaging studies in rats after intravenous and oral administration at different doses (1µg/Kg and 5mg/Kg) were combined with determination of arterial blood time-activity curves (TACs) and analysis of plasma samples by high performance liquid chromatography (HPLC) to quantify radioactive metabolites. Pharmacokinetic parameters were determined by non-compartmental analysis of the metabolite-corrected arterial blood TACs. ^{11}C- and ^{3}H-labelled compounds were obtained in overall non-decay corrected radiochemical yields of 14±3% and 0.15±0.05% and, molar activities of 40-140 and 0.5-1 GBq/µmol, respectively. *In vitro* results showed that demethylation of the CH_3O-Ar residue is the main metabolic pathway, followed by oxidation of the thioether group into sulfoxyde and demethylation of $–N(CH_3)_2$. Fast metabolism was observed *in vivo*. Pharmacokinetic parameters showed short half-life and high plasma clearance (Fig. 1b). Dynamic PET imaging (Fig. 1c) showed accumulation in the gastrointestinal track when AHK2.1 was administered. Contrarily, elimination via urine was observed after administration of AHK2.2, probably reflecting the biodistribution of [^{11}C]methanol as the major metabolite.

Keywords: Pharmacokinetics, FKBP12-RyR stabilizer, radiolabeling, arterial TACs.

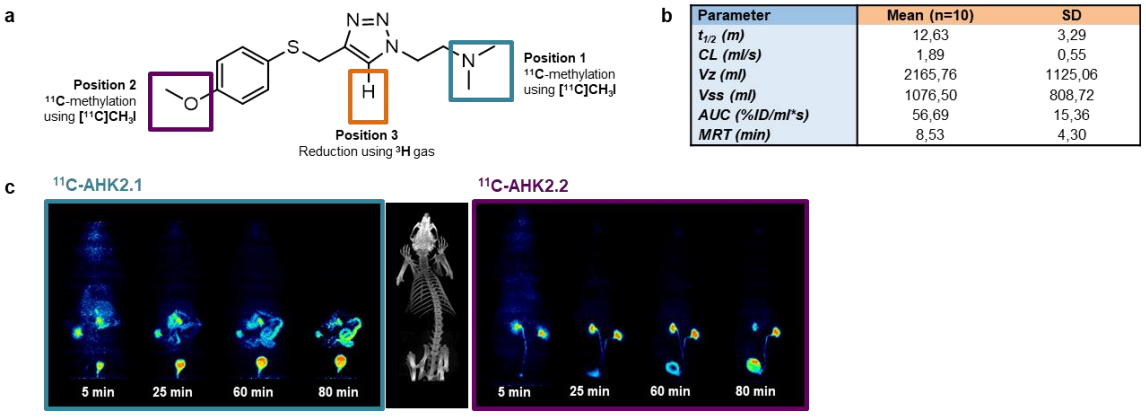

Fig. 1: a) Chemical structure of AHK2. The positions where was labelled are indicate; b) Main pharmacokinetic parameters obtained from metabolite-corrected arterial TACs; c) PET images at different time points after intravenous administration of AHK2.1 and AHK2.2.

Tailored nanoparticles for atherosclerosis diagnosis

Lydia Martínez-Parra[1], Ana Beatriz Miguel-Coello[1], Lucia Cardo[1], Natalia Herrero[1], Fernando Herranz[2], Susana Carregal-Romero[1,3], Maurizio Prato[1], Jesús Ruiz-Cabello[1,3].

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Atherosclerosis is the main cause of cardiovascular diseases and stroke and thus the leading cause of death globally. However, the atheroma plaque development is not fully understood and not adequately addressed by existing early detection assays and therapies.[1] Research in this area has focused on targeted imaging and nanomedicine. In the preclinical realm, early diagnosis has been already possible with advanced molecular imaging methods using nanoparticles (NPs) such as dextran-coated iron oxide NPs or ^{64}Cu-labeled dextran–coated iron oxide NPs for Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET) respectively.[2] First in-human trials with NPs in Phase 2 ((NCT00655473), (NCT00695305)), suggest that nanotechnology will have an impact on patient diagnosis.

In this context, our group is working in the development of ultrasmall magnetic NPs based on iron oxide and Gd-doped calcium carbonate and carbon dots to decipher which are the best nanocarriers for targeted detection of the atheroma plaque with MRI.[3,4] Recently, we have been able to demonstrate the specific accumulation of some of the aforementioned NPs by coating the NP surface with biomolecules with proved affinity towards several processed taking place in atheroma plaque such as inflammation or microcalcifications. Thus we will present some of our most promising results for the MRI-based evaluation of atherosclerotic disease with targeted imaging.

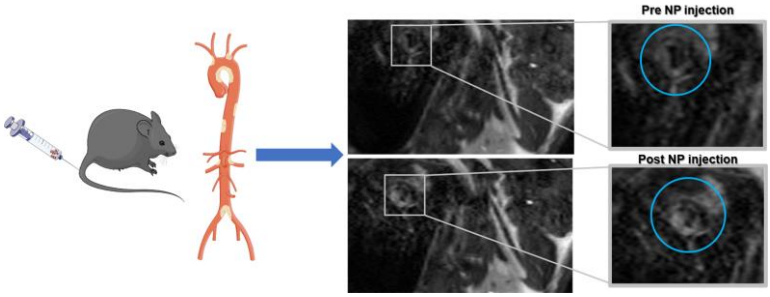

Figure 1. Administration and MRI-detection of the atheroma plaque with targeted magnetic NPs.

References

[1] Charo, I. F et al. *Nature reviews. Drug discovery* **2011**, *10*, 365.
[2] Mulder, W. J. M.et al. *Science Translational Medicine* **2014**, *6*, 239sr1.
[3] Pellico, J. et al. *Scientific Reports* **2017**, 7, 13242.
[4] Gomez, J. et al. *Journal of Material Chemistry B*, **2018**, 6, 5540.

Magnetic Resonance Imaging evaluation of the role of hyperglycemia in experimental subarachnoid haemorrhage

Ana Joya[1,2] , Sandra Plaza[1], Daniel Padró[1], Pedro Ramos-Cabrer[1,3], Jordi Llop[1,] Abraham Martín[2,3]

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Spontaneous subarachnoid haemorrhage (SAH) is a devastating cerebrovascular disorder with high mortality and morbidity[1,2]. Besides, the occurrence of hyperglycemia has been linked with the worsen outcome after SAH[3]. For this reason, the aim of this study is to evaluate the effect of hyperglycemia in a rat model of SAH using Magnetic Resonance Imaging (MRI) and neurological evaluation.

In vivo MRI imaging studies using different sequences: T2W, T2*(SWI) and Dynamic Contrast Enhanced (DCE) were carried out to evaluate the effect of hyperglycemia in different aspects at days 1 and 3 after the SAH. Hyperglycemia was induced by intraperitoneal administration of dextrose 30 minutes before SAH. Blood samples were withdrawn before and immediately after SAH to measure glucose levels. Finally, the neurological outcome was measured at days 1 and 3 after SAH.

An hyperglycemic situation during SAH worsen the neurological outcome and increases mortality rates. This mortality rate is higher if the hyperglycemia is severe, evidencing the worsening role of this condition in SAH. Moreover, hyperglycemia increases haemorrhage volumes, stroke volumes and oedema at day 1, and stroke volumes at day 3 in comparison with the normoglycemic situation (Figure 1). In addition, this condition induces a major impairment of neurofunctional evolution after SAH.

These results suggest that hyperglycemic condition during SAH worsen the pathological evolution of preclinical SAH increasing the mortality rate, volume of haemorrhage, injury around the lesion and disruption of the BBB. Likewise, these findings are in agreement with the effect of high blood glucose levels after clinical SAH on the increase of complications and the higher risk of death or functional disability.

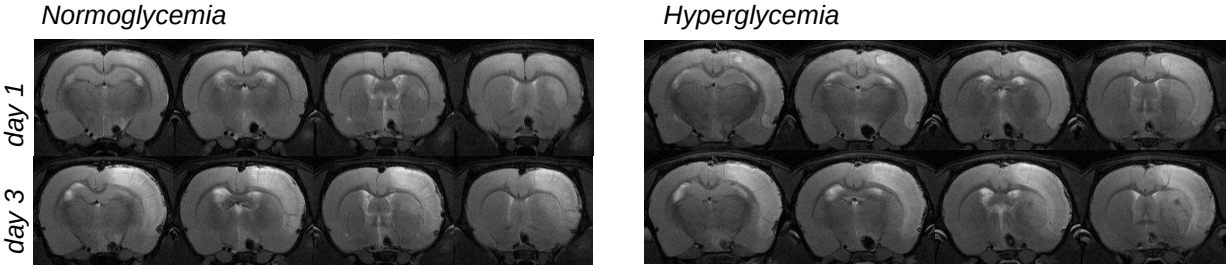

Figure 1. T2-W images of a normoglycemic and a mild hyperglycemic animal at day 1 and 3 after the SAH onset.

Keywords: Subarachnoid haemorrhage, SAH, MRI, hyperglycemia, blood brain barrier

References

1. Macdonald, et al., *Spontaneous subarachnoid haemorrhage*. Lancet, 2017. 389(10069): p. 655-666.
2. Lucke-Wold BP et al. *Aneurysmal Subarachnoid Hemorrhage and Neuroinflammation: A Comprehensive Review*. Int J Mol Sci. 2016;17(4):497.
3. Kruyt, N.D., et al., *Hyperglycemia and clinical outcome in aneurysmal subarachnoid hemorrhage: a metaanalysis*. Stroke, 2009. 40(6): p. e424-30.

Characterization of Pulmonary Hypertension

Lucía Fadón Padilla[1,2], Irati Aiestaran Zelaia[1], María Jesús Sánchez Guisado[1], Susana Carregal Romero[1], Haizpea Lasa Fernández[1], Edurne Berra[2], Jesús Ruiz-Cabello[1,3]

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Pulmonary Hypertension (PH) courses with endothelial dysfunction and remodeling of the pulmonary vessels, accompanied by metabolic reprogramming in the heart and lungs [1]. My research project aims to deep into the understanding of the physiopathological mechanisms underlying PH in order to improve diagnosis and treatment.

Two well reported and independent models of PH in rats and mice have been used. PH is induced by intraperitoneal injection of monocrotaline (MCT) in the first model and in the second one, animals are exposed to chronic hypoxia exposure together with the administration of sugen, an anti-angiogenic drug. Animals were analyzed by MRI and PET, and confirmation of the disease was performed hemodynamically through the jugular vein.

The thesis is divided in two large parts: 1. Nanotechnology for imaging/diagnosis and treatment. A) ^{68}Ga-labeled neutrophil-affinity radiotracer to monitor *in vivo* inflammation in these PH models using PET imaging. Results show increased radiotracer uptake only in the MCT model. B) Effective nanoparticle-based genetic treatment in PH. Investigation and comparison of the Jugular, intravenous and intratracheal routes of miRNA formulation administration. Preliminary results show excellent accumulation of nanoparticles in the lung vasculature with a jugular administration and mannitol osmotic challenge. 2. Contribution of environmental variables in the endophenotype of the PH. We are focused on the role of the diet (using 2-deoxyglucose, 2-DG, to mimic a hypocaloric diet), the exercise and the mitochondria (using ethidium bromide to impair mitochondrial DNA replication). RVSP, radiotracers uptake, cardiac images and histological test reveal differences between groups. In particular, a hypocaloric diet appears to protect from HP.

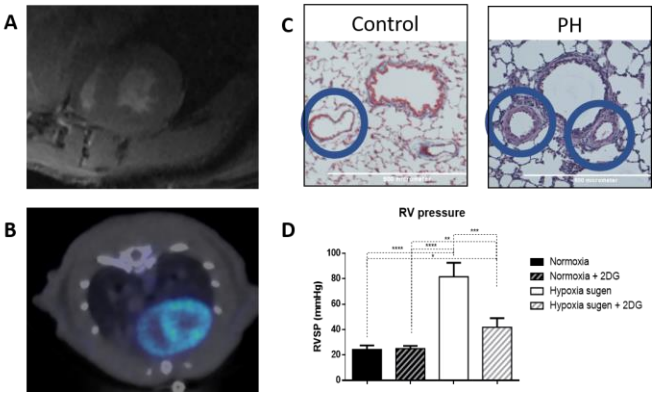

Figure 1. Characterization of the effects of 2-DG in a rat model of PH A) MRI cardiac image B) ^{18}FDG-PET heart uptake C) Histological stain of the lungs D) Right Ventricular Systolic Pressure

Keywords: Pulmonary Hypertension, mitochondria, inflammation, PET, MRI.

Monitoring $^{18}F/^{124}I$ enzyme-powered nanomotors *in vitro* and *in vivo* by PET-CT

C. Simó[1], A. C. Hortelao[2], M. Guix[2], S. Guallar-Garrido[3], E. Julián[3], D. Vilela[2], L. Rejc[1], P. Ramos-Cabrer[1], U. Cossío[1], V. Gómez-Vallejo[1], T. Patiño[2,4], S.Sánchez[2,5], J.Llop[1]

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Micro/nanomotors are micro/nanoscale devices capable to induce self-propulsion in fluid environments. Among them, enzyme powered nanomotors, which show high biocompatibility and can swim in biological fluids, have emerged as promising tools for biomedical applications[1]. Nevertheless, tracking them in vivo and at the whole body level still remains a challenge. Here, we describe the application of radiolabeling followed by Positron Emission Tomography (PET) to achieve the time-resolved *in vitro* and *in vivo* tracking of urease-powered nanomotors as potential drug delivery agents for bladder cancer. For that purpose, mesoporous silica nanoparticles (MSNP) were functionalized with urease enzymes and gold nanoparticles. Radiolabeling could be achieved using two different strategies: (i) Attachment of the ^{18}F-labeled prosthetic group 6-[^{18}F]fluoronicotinyl-2,3,5,6-tetrafluorophenyl ester ([^{18}F]FPy-TFP; 30% yield)[2], and (ii) absorption of ^{124}I at the gold surface (71% yield). PET imaging studies in 3D-printed phantoms clearly showed the self-propelling capacity of the nanomotors in the presence of urea, while no significant movement was observed in pure water (Figure 1a). Biodistribution studies in female mice after intravenous administration confirmed the suitability of PET to quantitatively track nanomotors *in vivo*, and the convenience of ^{18}F-labeling strategy. After intravesical instillation, urease-functionalized nanomotors showed motile capacity in the presence of urea (Figure 1b).

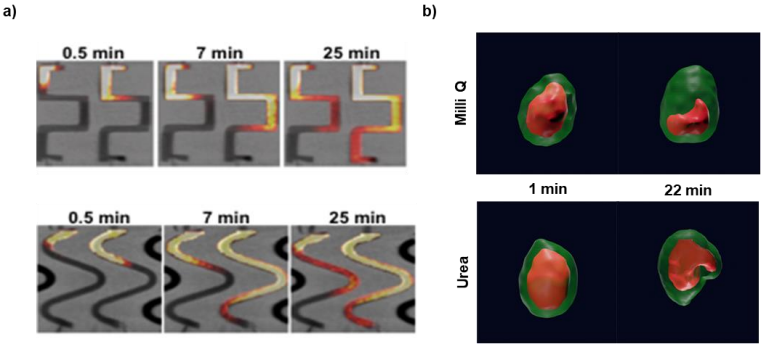

Figure 1. (a) Images obtained in a 3D printed phantoms at t= 0.5, 7 and 25 min after seeding of nanomotors in water (left) and in 300 mM aqueous urea solution (right); (b) 3D images of the *in vivo* biodistribution of nanomotors at t= 1 and 22 min after intravesical intillation in water (top) and 300 mM aqueous urea solution (bottom).

Keywords: nanomotors, PET imaging, medical imaging.

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In vivo fate studies of glyco NPs and biocorona stability.

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Glycans act as mediators in cell-cell interactions and play a pivotal role in different pathologies like ischemic processes. During an ischemia Mannose Binding Lectin (MBL) is deposited on the inner surface of injured vessels[1], where it binds to different carbohydrates and triggers the inflammatory process. Previous works suggest that compounds that bind and inhibit MBL may represent a promising therapeutic modality for stroke[1]. In this project, we propose the use of glycan-functionalized gold nanoparticles (AuGNPs) as potential theragnostic agents. First, ligands with mono- and poly-saccharides, recognizable by MBL and bearing a thiol group were synthesized by reacting (11-(Methylcarbonylthio)undecyl)-tetra(ethylene glycol) with fully protected sugars in *ca.* 35% yield (Fig. 1a); reaction of the same spacer with phthalimide followed by hydrolysis (Fig. 1a) yields amino-functionalised ligands (65% yield) to enable future radiolabelling using positron emitters. The preparation of spherical AuGNPs with a core size of *ca.* 1.6±0.7 nm was achieved via one-pot method by adding a mixture of the ligands to a solution containing $HAuCl_4$ and $NaBH_4$ in CH_2Cl_2 (Fig. 1b). Future experiments will comprise investigation of the protein corona formation using ^{19}F-DOSY-NMR[2,3], radiolabelling with positron emitters using ^{18}F-labelled prosthetic groups[4] and conducting *in vivo* biodistribution studies in both wild type and ischemic rodents.

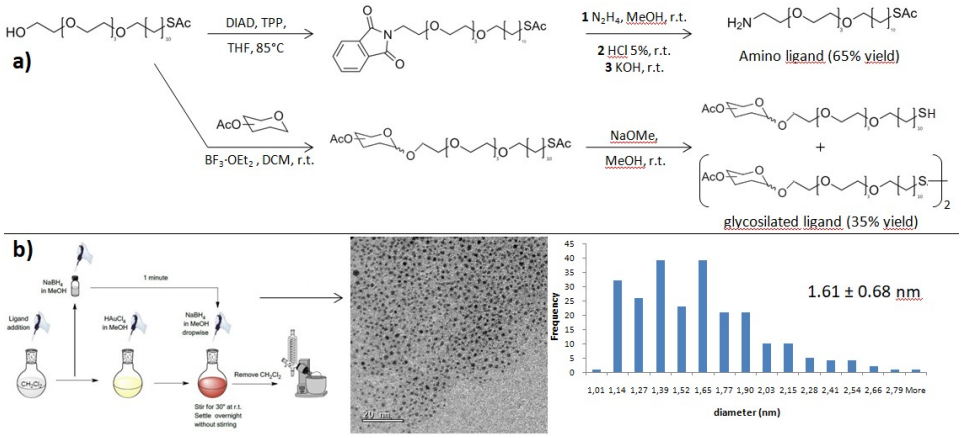

Figure 1. **(a)** ligands syntheses, **(b)** reaction scheme, TEM image and associated histogram for AuNPs.

Keywords: glycosilated gold-nanoparticles, biofunctional materials, PET imaging, ischemia.

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sncRNA charged extracelular vesicles as vehicles to the CNS

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About 98 % of human genome is represented by Non-Coding RNAs (ncRNAs), a type of RNA that doesn´t encode proteins but have other key regulatory roles in many biological processes [1]. One type of ncRNA are microRNAs, with a size of approximately 22 nucleotide long they have the capacity to modulate post-transcriptional gene expression by binding to specific sequences of target mRNA [2]. Using membrane surrounded recipients called extracellular vesicles (EVs) and liposomes, originated from different intracellular components and artificially respectively, the aim of this project is to deliver to the central nervous system (CNS) these elements charged with miRNA-219, one type of microRNA associated with different biological processes in the brain, in order to use it as a therapy to treat multiple sclerosis disease, a chronic inflammatory demyelinating disease which affects the white and gray matter of the CNS [3]–[5].

For this purpose, we fine tunned an EV developing process from different cell origins overexpressing miRNA-219 and liposomes charged with mimic-miR-219 to treat EAE induced mice, a MS disease model. It has been demonstrated in a previous study that EVs from HEK-293T cells reduce EAE severity in mice [6], so we want to test if the different cargo of EVs from different cellular origins would be able to enhance the therapeutic effect and if we can use artificial membrane compounds charged with this microRNA to emulate the same effects done by these EVs.

Keywords: miR-219, EVs, liposomes, EAE, Multiple sclerosis.

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Isolation of N-glycans from natural sources under alkaline conditions

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Complex N-glycans are sought-after biomolecules for advancing biomedical applications in glycoscience. Glycoproteins are a well-known natural source due to large variety of N-glycans present in their structure[1]. Isolation of these structures from natural sources via enzymatic or chemical methods is an alternative to their challenging synthesis. Nowadays the interest in large-scale N-glycan isolation has increased and chemical methods are being evaluated as a cost-effective alternative to enzymatic cleavage for the release of N-glycans[2].

Herein we present the optimization and scale up of an alkaline deglycosylation method[3] applied to hen's egg white, a commercially available and inexpensive glycoprotein source that shows a high structural diversity of N-glycans. The method consists in the selective hydrolysis of the N-type carbohydrate peptide bond of glycoproteins under sodium hydroxide conditions and a dedicated multistep purification process. The method was optimized on 1 g and then scaled up to 500 g of hen's egg white thus obtaining a mixture of N-glycans which was labeled with a florescent tag and purified by preparative HPLC.

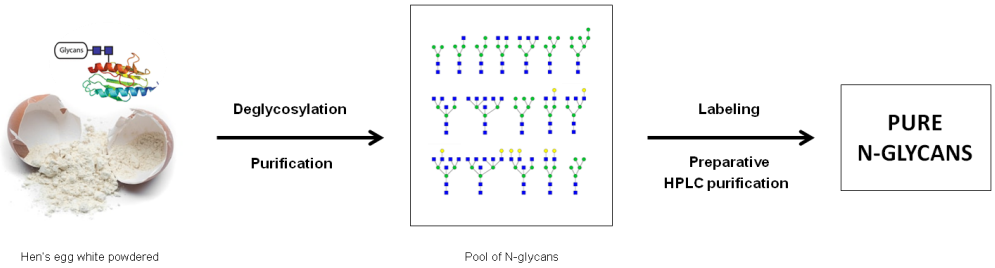

Figure 1. N-glycan isolation from Hen's egg white powdered

Keywords: glycoprotein deglycosylation, N-glycan release.

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SERS bioimaging of complex 3D cellular systems

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Developments in the fabrication of Surface Enhanced Raman Scattering (SERS) tags, which are composed of a metal nanoparticle core (mainly gold or silver), a Raman reported layer and an external hydrophilic shell, have opened up their use as tools to monitor in real-time mobility and viability of complex three dimensional (3D) cellular systems[1]. Here we have focused in the synthesis of several types of SERS tags[2] and we have studied their interaction with different cell lines. Moreover, to recreate a natural extracellular matrix environment, 3D scaffolds, which can act as cell's growth support and imaging reference, can be designed. Additionally, they can be used as internal sensors incorporating plasmonic NPs. In this line, we have focused our study on the optimization of SERS imaging conditions for 3D structures, considering penetration depth, resolution, optical heating, toxicity and cell viability. Up to now, we have created a library of SERS tags which have been used to study their interaction with MCF7 breast cancer cell line. In particular, we are controlling their durability inside the cells over time, avoiding heating toxicity under SERS measurements and quantifying the number of NPs inside the cells according to the SERS signal. At the same time, we have investigated the spatial and depth limitations of hydrogel-based scaffolds[3] (see **Figure 1**) and a doble co-culture of SERS labelled cells in a 3D printed PLGA scaffold. Once all these aspects are controlled, we aim to obtain a detailed overview of cell models in 4D (including three dimensional and temporal information).

Keywords: SERS tags, SERS imaging, 3D cell model.

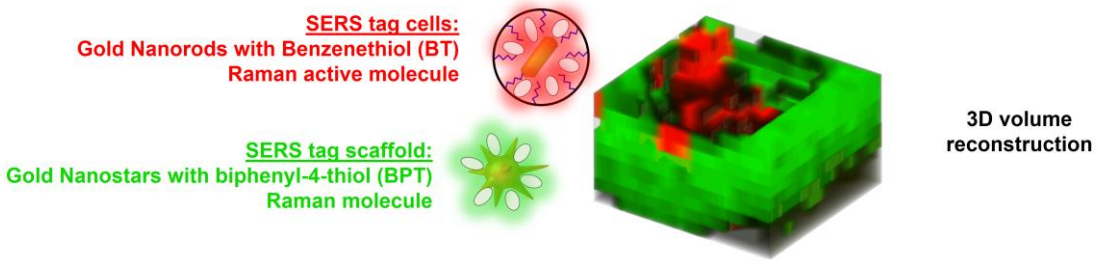

Fig.1 3D volume reconstruction of SERS labelled hydrogel-based scaffolds and cells.

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Supramolecular polyamine Nanocarriers for siRNA delivery

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In recent years, RNA interference (RNAi) technologies have emerged as a promising therapeutic tool for the treatment of different diseases[1]. For example, siRNAs can be applied to many cancer types as they can inhibit the expression of any gene of interest, being highly specific. However, a major hurdle for clinical translation is to find a suitable and safe delivery vehicle for the siRNA. Unprotected siRNAs are unstable in bloodstream due to nuclease degradation, and are not able to cross cell membranes[2]. Thus, the proposal of this Thesis is to develop and produce a novel nanocarrier for delivering siRNA systemically.

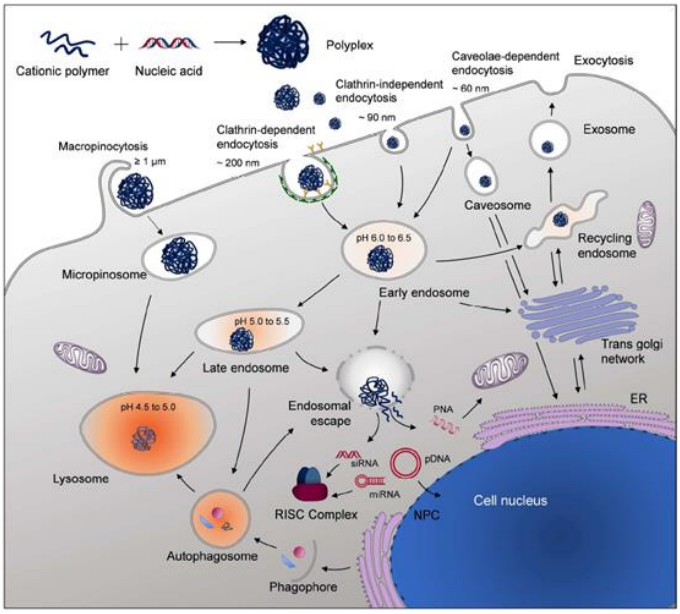

Figure 1. The gene delivery process of cationic polymers including polyplex formation,intracellular uptake via various endocytosis pathways, intracellular polyplex trafficking, endosomal escape into the cytoplasm, release of the cargo from the polymer and transport to the site of action[4]

For this porpoise supramolecular polyamine nanocarriers (PANs) have been chosen. They are based on the complexation of the phosphate groups of siRNAs with primary amines from PolyAllylamine Hydrochloride (PAH), a polyelectrolyte with MW15KDa. To improve silencing efficacy and reduce toxicity, carriers will be chemically modified with different amount of oleic acid. Size of polyplexes is characterized by Dynamic Light Scattering and Electron microscopy. The uptake of siRNA by A549 (pulmonary cells) and the delivery efficiency of these polyplexes is evaluated *in vitro* by Flow Cytometer. The decrease in fluorescence of GFP protein in A549-GFP cell line is quantified after treatment with anti-GFP siRNA. Cytotoxicity of polymers nanoparticles and complexes is evaluated as well by MTS assay. Further work will focus on the use of the polyplexes in the treatment of pulmonar cancer cells by silencing the gen CD47. Gen responsible of the growth, proliferation and aggressiveness of metastasis[3].

Keywords: siRNA, nanocarrier, polyplex, PAH, single-chain

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Design and Synthesis of N-glycomimetics with high affinity for DC-SIGN

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C-type lectin receptors (CLRs) are a super family of around 1000 carbohydrate binding proteins found on the surface of many cells, and in particular antigen presenting cell (APCs) where they are involved in self/non-self-differentiation by the immune system.[1] Certain CLRs can therefore be considered a form of glycol-immune checkpoint inhibitors. The carbohydrate Lewis antigens that commonly detected on cancer cells, bind to the C-type lectin DC-SIGN (Dendritic Cell-Specific Intercellular adhesion molecule 3-Grabbing Nonintegrin), driving to innate immune suppression. release of the immune cells from their dormant state by interfering with the pertinent carbohydrate-protein interactions could become a complementary immune therapy approach the treatment of cancer.[2] Synthetic carbohydrate chemistry has been and continues to be the most reliable, scalable and common source for glycans. Even so, the synthesis of very complex such a multiantennial N-glycans is a challenge because of its high cost in time and resources. Herein, egg yolk powder has been used as a gram-scale source of sialilglycopeptide (SGP).[3] Through organic chemistry and chemoenzymatic truncation/elongation we were able to modify the extracted SGP to prepare a scaffold for two different types of bi-antennary N-glycan mimetics, modifying the natural Lewis X domain: LDNF-type and Le^X-Type (Figure 1). In order to obtain a preliminary library of N-glycan mimetics, click reactions with a series of alkynes were performed directly on microchip array and screened against DC-SIGN. This initial screen allowed us to evaluate the potential of the resulting mimetics.

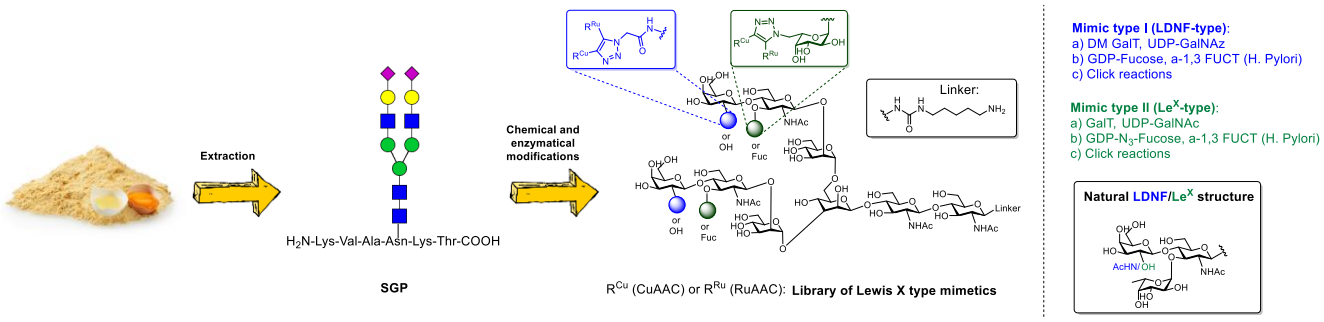

Figure 1. General overview for obtaining the glycomimetics.

Keywords: DC-SIGN, N-glycans, glycomimetics, glycan array.

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NK cell stimulation with IONP@hIL15HIS: enhanced targeting and tumor cell killing

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In the field of therapy important challenges are targeting to specific locations[1] and the induction of specific cell mediated cytotoxic activity against cancer cells. Also, not only the target localization and lysis of tumor cells is important, but also, the avoiding of said effects such as exacerbate inflammation or other cytotoxic effects must be controlled. One example with very promising results are Natural killer (NK) cells. The reason of the success is that they are antigen independent and this is translated in no graft versus host disease (GvHD) while they maintain a potent graft versus tumor effect[2].

It is known that the activation of NK cells with different cytokines such us interleukins enhances their cytolytic functions[3], which is reliable strategy for cancer cell killing. In this study we show the design and generation of a nanoformulation, based on a biocompatible, biodegradable, and traceable nanomaterial, which enhances NK cell cytotoxicity against melanoma and leukemia cell lines in vitro. Furthermore, an in vivo[4] study is currently ongoing in order to test if this nanoformulation is able to reduce melanoma tumor in the C56BL/6 mouse model.

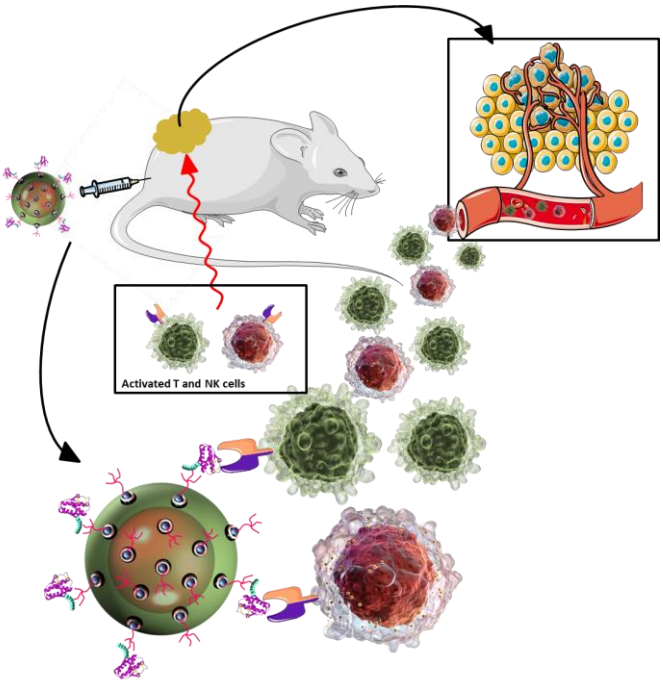

Figure1. In vivo study scheme.

Keywords: nanotherapy, immunology, cancer

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Biological fate of gold nanoparticles

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Nanoparticles offer broad application opportunities in the biomedical field. In a biological environment, nanoparticles interact with proteins and form the so-called protein corona, a coating of proteins around the nanoparticles that give them a biological identity. The protein corona is the primary nanoparticle interface in contact with cells and tissues and consequently, it has a great influence on biodistribution, translocation and cell uptake.[1,2] Here, we are interested in studying protein corona formation around glyco-functionalized nanoparticles, which may have specific interactions with proteins and lectins. Protein corona studies have been carried out by means of fluorescence correlation spectroscopy that provides information on the dynamics of protein corona and how this is affected during nanoparticle translocation in vitro. Furthermore, the biodistribution of different nanoparticles in mice after intravenous injection was investigated by their quantification in different tissues based on ICP-MS analysis.

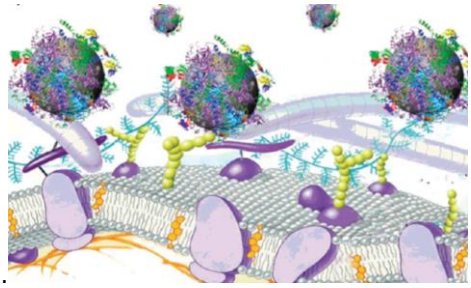

Figure 1. Nanoparticles with a protein corona interacting with a cell.[1]

Keywords: protein corona, gold nanoparticles, biodistribution.

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Evaluating of different carriers for the immobilization of alcohol dehydrogenases. A novel heterogeneous biocatalysts for the oxidative transformation of diols to hydroxialdehyde

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Chemical synthesis catalyzed by enzymes is contributing to establish a modern chemistry supported on cleaner, faster and safer chemical reactions. Immobilization of enzymes on carriers to fabricate heterogeneous biocatalyst presents an interesting approach to move enzymes into the industrial process development [1]. Furthermore, factors such as spatial organization and stability of the immobilized enzyme arise crucial to assemble robust and effective biocatalysts. Hence, we aim to develop a heterogeneous biocatalyst based on immobilized alcohol dehydrogenases to carry out the ordered double oxidation of diols to yield lactones (Scheme 1).

To this aim, we have tested different carriers with different physical-chemical properties and functionalization to site-selectively and reversibly immobilize a poly-His tagged alcohol dehydrogenise (ADH) from Bacillus stereathermophilus. Additionally, an irreversible immobilization strategy enzyme-carrier was tested by incorporating epoxide groups in the Co-functionalized agarose microbeads. We have studied the impact of the immobilization on the recovered activity, thermal stability, conformational changes, kinetic parameters for selected substrates and the enzyme spatial organization determined by confocal fluorescence microscopy (Figure 1). In this communication, we present a study for the better understanding of ADH immobilization and the application in flow reaction. Future work will involve the application of a novel cofactor-regenerative enzymatic system for ADH biocatalyst and computational studies.

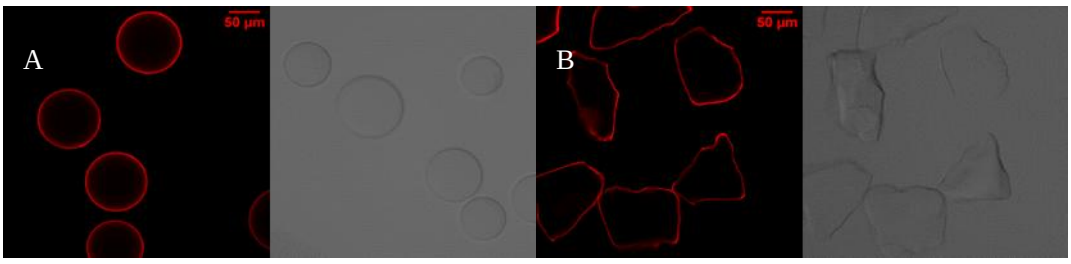

Figure 1: Spatial distribution of the immobilized ADH-Bs in Agarose (A) and SiO_2 (B) by using fluorescence microscopy

Keywords: **Biocatalyst, Sustainable, Enzymes, Immobilization**

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Design of Hybrid Structures for Bioorthogonal Drug Photoactivation and Photocatalysis

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Advances in biorthogonal catalysis are creating new opportunities to perform chemoselective transformations in complex biological environments with applications in biomedicine and bioimaging. Recently, riboflavin photocatalysis has been used to activate Pt(IV) anticancer prodrugs *in vitro* under light irradiation.[1] In this work, we propose to expand this strategy by co-immobilization of both photocatalysts and prodrugs, on engineered repeat proteins as templates[2], and on gold nanoparticles as nanozyme scaffold.[3] These systems are envisioned to have more efficient drug activation and will facilitate controlled cell internalization.

Specifically, we use tetratricopeptide repeat protein as engineered repeat protein with 4 module repetitions (CTPR4) and 1.9-nm gold nanoparticles decorated with thiol ligands bearing a TACN (1,4,7-triazacyclononane) headgroup. In this contribution, I will discuss the conjugation between CTPR4, N3-functionalized riboflavin and Pt(IV) complex by exploiting cysteine (Cys) and lysine (Lys) residues of the protein scaffold, functional group of the riboflavin and NHS ester-reactive group of the Pt(IV) complex. Currently, we have achieved a distribution in the number of functionalized riboflavins and platinum complexes linked to the CTPR4. Some preliminary light-irradiation studies at 460 nm indicated that riboflavin loaded on CTPR4 has increased photostability, compared to free riboflavin, likely due to the ability of the protein scaffold to protect the modified riboflavin from the photodegradation of its ribityl chain. Catalysis studies performed show that riboflavin preserves its photocatalytic activity upon conjugation to the protein.

On the other hand, using the nano-based system, it was observed that the nanozyme affording a catalytic system capable of activating the Pt(IV) prodrug substrate with an almost identical behavior in terms of catalytic activity of the system without AuNPs, reaching 80% conversion already within 7 min and a plateau at 90% conversion after 30 min of light irradiation with turnover frequency (TOF) of 7.4 min^{-1}.

Keywords: repeat protein, flavin, platinum prodrug, gold nanoparticles, biorthogonal photocatalysis.

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One-pot multi-enzymatic synthesis for the preparation of *L*-glutamine as potential nitrogen-13 radiotracer.

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Besides accelerated glucose metabolism, cancer cells have a notable increased demand of amino acids to support their exceptionally fast proliferation rate. Particularly, the role of glutamine in tumor metabolic pathways has gained notable attention because it is needed for nucleotide biosynthesis, a critical process for tumor cell proliferation.[1] Because of this, radiolabeled glutamine could be an excellent PET tracer for oncology imaging. Taking into account the advantages that biocatalysts offers over traditional synthetic routes,[2] here we report a one-pot multi-enzymatic reaction for the preparation of L-glutamine that will allow us to produce [^{13}N]*L*-glutamine by using ^{13}N-labeled ammonia. The reaction cascade consists of the generation of the keto-acid starting from *D*-glutamine using DAO (D-amino acid Oxidase) followed by *in situ* formation of the desired product by addition of GluDH (Glutamate Dehydrogenase) to the media (Figure 1). In this process, the proportion between DAO and GluDH has to be critically controlled to avoid the formation of the cyclic keto-acid form, much more stable than the linear one and non-reactive towards the enzyme. In addition, Catalase and Glucose Dehydrogenase were also incorporated in the reaction mixture, in order to eliminate hydrogen peroxide and regenerate the cofactor NADPH, respectively. Under optimal experimental conditions, the production of non-radioactive *L*-glutamine has been achieved with a yield of 15%. Future studies will focus on the production of [^{13}N]*L*-glutamine using [^{13}N]NH_4^+ as the radiolabelling agent. The labeled amino acid will be used in a mouse prostate cancer model by positron emission tomography (PET) imaging.

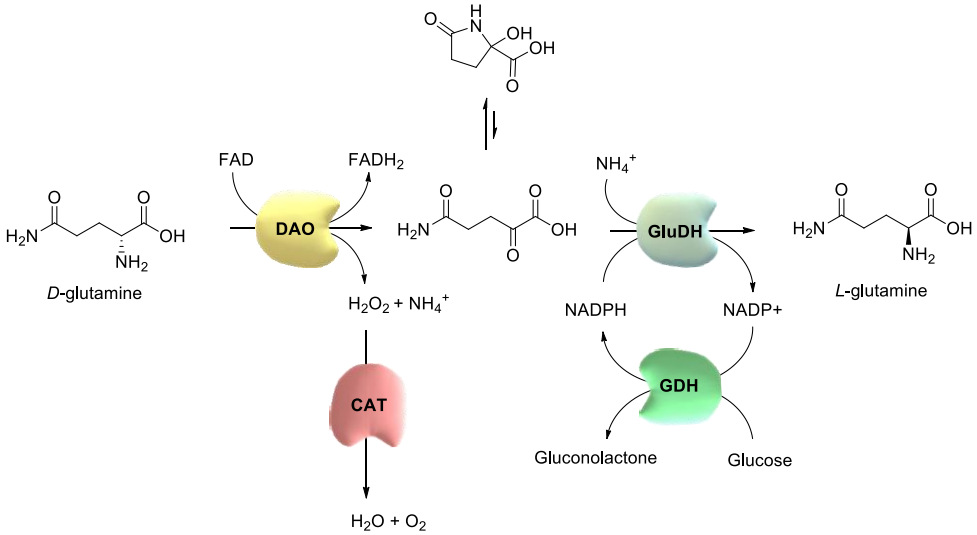

Figure 1. Enzymatic pathway for the production of L-Glutamine using D-amino acid Oxidase, Glutamate Dehydrogenase, Catalase and Glucose Dehydrogenase.

Keywords: biocatalysis, amino acids, nitrogen-13, cancer.

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Modulating the Conformational Plasticity of Tetraspanin CD81 by Ligand Binders and its Implication in Cellular and Viral Processes.

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CD81 is a tetraspanin receptor that cluster into microdomains to mediate an enormous variety of physiological processes. However, this protein is also exploited by pathogens to infect cells. In particular, CD81 is involved in the internalization of Hepatitis C Virus (HCV) via clathrin-mediated endocytosis (1,2).
Previous structural studies on the CD81 large-extracellular-loop domain ($CD81_{LEL}$) have shown that it can adopt either a closed or an open conformation depending on the environmental conditions, although the precise molecular mechanisms of this opening are not yet well-understood (3-5). We hypothesize this plasticity could be exploited by HCV to trigger conformational changes in its glycoproteins for endosomal viral membrane fusion, and genome release (6).

By performing molecular dynamics (MD) simulations on the different conformations (open-close) of $CD81_{LEL}$, we have found that the stability of the protein within the solvent is compromised at low pH ($\Delta H>0$ kJ/mol). The residues that contribute the most in this phenomenon are D139 and E188, which are located nearby the head subdomain. As consequence, the solvation profile in this region becomes loose, facilitating the dynamics of the head subdomain. *In silico* mutations E188Q and D139N stabilize the protein-solvent interaction enthalpy and restrict the conformational space that the head subdomain can explore.

Currently, we are expressing and purifying wild-type and mutant $CD81_{LEL}$ proteins in order to perform NMR experiments to verify the findings achieved with our MD simulations. With these experiments we hope to shed light about the role of residues D139 and E188 in the plasticity of $CD81_{LEL}$.

Keywords: Molecular Dynamics, NMR, CD81, Plasticity.

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Design of consensus tetratricopeptide repeat (CTPR)-based orthogonal scaffolding units for the ordered assembly of enzymes

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Rational protein design can be applied to generate scaffolds onto which different cascade enzymatic reactions can be assembled to obtain controlled multi-enzymatic systems. In this work, we explore the use of repeat proteins, in particular the consensus tetratricopeptide repeat (CTPR) module for the design of a variety of scaffolds towards the assembly of multi-enzymatic pathways. We selected two strategies based on supramolecular assembly of repeated modules[1,2] (SCAB modules) and on the biomolecular recognition of orthogonal protein-peptide pairs using the designed tetratricopeptide repeat affinity (TRAP modules)[3]. In the first approach, the enzymes are fused to the SCAB units and the assembly is driven by the SCAB modules. In the second approach, the enzymes are fused to tag peptides and assembled onto the TRAP scaffolds by specific binding to the scaffold. We aim to obtain nanometric arrangement of the multi-enzymatic systems that will result in optimized biocatalytic cascade reactions.

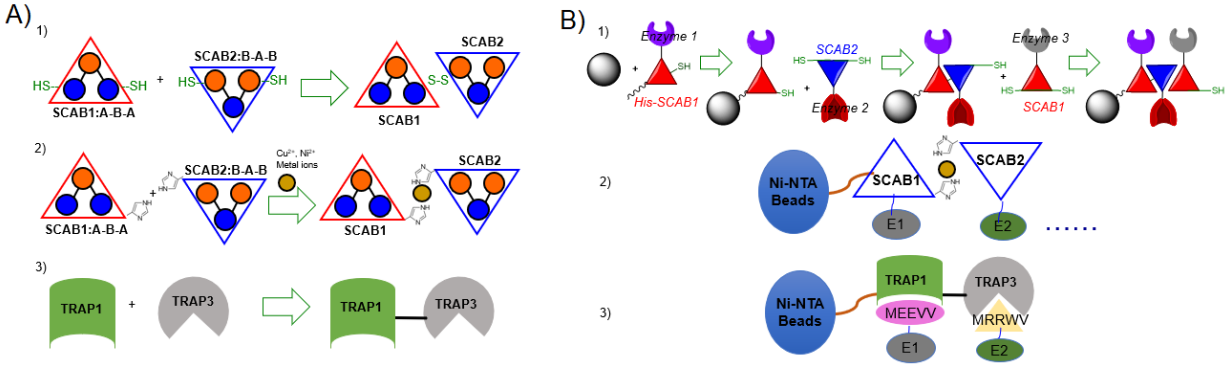

Figure 1. A) Scaffolding strategies. 1st and 2nd strategies are based on the assembly of SCAB modules and the 3rd strategy is based on TRAP proteins. B) Assembly of enzymes by different scaffolding strategies.

Keywords: protein engineering, self-assembly, enzyme-immobilization, multi-enzymatic systems

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Enzyme engineering and flow-reactor immobilization of the acyltransferase LovD

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Pharmaceutical synthesis is progressively replacing the chemical synthesis of cholesterol lowering agents by enzymatic processes. The directed evolution of acyl transferase LovD meant a breakthrough in the synthesis of simvastatin, rising to the most evolved variant LovD9 [1].

In this study, we have elucidated the contribution of different groups of mutations scattered in different locations of LovD9 for its kinetic and stability parameters. Through a rational combination of those clusters of mutations, we have generated a minimalist variant (B+C) whose catalytic productivity and thermal stability was similar to LovD9 but with 15 mutations less. This work demonstrates the synergic effect between buried and channel mutations to enhance the binding of the acyl donor and the relevance of the buried positions to speed up the acyl transfer step.

Furthermore, we identified and quantified for each variant three side reactions which could compete with the synthase activity in an enzymatic industrial process: the inhibition of the first step of the reaction by one of the substrates (monacolin J acid), the hydrolysis of the acyl-enzyme intermediate and the hydrolysis of simvastatin.

In order to design a process optimized towards simvastatin synthesis, we immobilized the B+C variant in different carriers (Ag-Co^{2+}, Ag-Co^{2+}/Epoxyde and catechol-Fe^{3+} silica). After thermal deactivation assays and batch reactions, we chose the catechol-Fe^{3+} silica as the best carrier to maintain the optimum activity and stability of the enzyme. We then performed a flow reaction with this biocatalyzer at different flow velocities, concluding that at a low flow, the hydrolysis of simvastatin avoid high conversion rates, whereas at a high flow, the hydrolysis is appeased.

Keywords: LovD, acyltransferase, simvastatin, biocatalysis, enzyme immobilization.

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Tuning the Luminescence of Gold Nanoclusters Through Protein Coordination

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Tetratricopeptide repeats (TPR) are modular proteins whose most common function in Nature is scaffolding[1]. We can control the self-assembly of consensus TPR proteins and use them as templates for metal nanomaterials[2] with valuable activities, such as catalysis, conductivity or fluorescence. Since NCs luminescence can be tuned using the coordination of small molecules[3], we hypothesize that the chemical versatility of proteins can provide a biocompatible and bioactive platform to tune NCs properties, resulting in the generation of interesting materials for biological applications, such as biolabeling. Therefore, we synthetized non-passivated ('naked') gold nanoclusters (AuNKNCs) that are not luminiscent[3], and studied the effect of the protein coordination. AuNKNCs were conjugated with two designed CTPR proteins that differ in the metal coordinating residues. The characterization of fluorescence and stability of the system revealed the role of the protein–AuNC surface interaction in the AuNC properties. These hybrids will be explored as a modular toolbox for the easy construction of biomaterials with intertwined optical and biological properties.

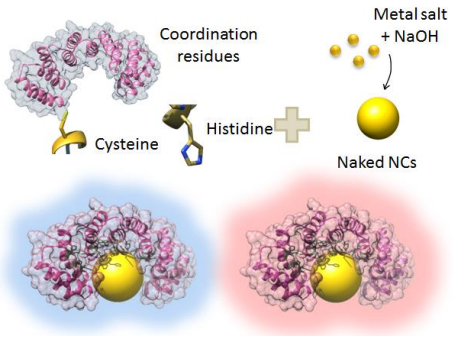

Figure 1. CTPR proteins with different coordination residues can attach non-passivated nanoclusters, producing metal NCs that hold luminescent properties

Keywords: metal nanoclusters, design protein, fluorescence tuning.

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Optical properties of stretchable gold nanoparticle superlattices

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Metallic nanoparticles (NPs) are known for their plasmonic properties allowing them to confine electric fields to the nanoscale. The size, shape and material of the NP specifically defines the wavelength, at which light is absorbed due to interaction with the metal electrons, and hence, the properties of the electric field enhancement. Periodic structures of gold nanoparticles are especially interesting as they exhibit lattice plasmons, which are diffractively coupled plasmon resonances at wavelengths depending mainly on the period of the nanostructure. Such lattice plasmons grant for ultrasensitive surface enhanced Raman spectroscopy provided they match the laser excitation [1].

Generally, periodic nanostructures featuring lattice plasmons are fabricated by electron beam lithography. Our strategy, however, consists of a bottom-up approach for creating periodic structures of gold nanoparticles. We present here a process for capillary-assisted self-assembly of nanospheres into superlattices on a flexible substrate. Structural changes of the superlattice through mechanical deformation of the flexible superlattice allows for continuous and reversible tuning of the lattice plasmon over a broad wavelength range (figure 1). Especially, we will show that matching the lattice plasmon to the laser excitation leads to an increase of the surface enhanced Raman signal.

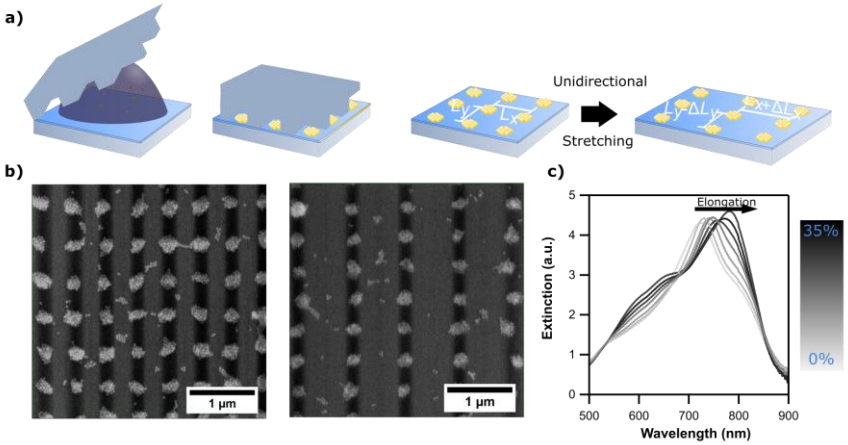

Figure 1. a) Capillary-assisted self assembly of gold nanoparticles on flexible sample. b) SEM images of a superlattice unstretched and stretched to 30%. c) Optical tuning by mechanical deformation

Keywords: Gold Nanoparticles, Self-Assembly, Lattice Plasmons, Superlattices.

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Magneto-plasmonic nanoparticles for multimodal bioimaging

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Early diagnosis is particularly relevant in diseases such as cancer increasing the chances for successful treatments. Imaging techniques constitute one of the most important advances in biomedicine representing a remarkable minimally-invasive tool for this end[1]. The development of multimodal imaging techniques based on the combination of different imaging modalities for a single objective provides advantages over the limitations of the independent techniques.

Over the last few years, nanomaterials have been used due to their unique properties for different biomedical applications including diagnosis, detection and treatment**.** Nanoparticles (NPs) based on different metals exhibit remarkable physical and chemical characteristics that make them particularly interesting for their application in this field[2].

In this study, we report the synthesis and characterization of a magneto-plasmonic system that combines iron oxide and gold. In particular, iron oxide multicore particles are synthesized and decorated with gold seeds. The amount of seeds can be tuned and is crucial for the subsequent gold overgrowth. Thus, gold shells with star shape around the iron cores are synthesized (Figure 1). The obtained system involves Surface-enhanced Raman Spectroscopy (SERS) application attributed to the gold component and the contrast agent capability of the iron for Magnetic Resonance Imaging (MRI). Thereby, it is possible to take full advantage of the promising properties of both metals for complementary multimodal bioimaging. Besides, the synthesized hybrid nanoparticles can be easily further functionalized to enhance their potential for desired applications.

Figure 1. Schematic representation of the synthesis of magneto-plasmonic NPs

Keywords: magneto-plasmonic, iron oxide-gold nanoparticles, multifunctional, multimodal bioimaging

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Mechanical properties of CNTs based polymeric scaffolds for tissue engineering applications.

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To date, the positive effect of using different kind of CNTs in cell culture is known by the scientific community in broad sense.[1] Incorporation of CNTs into hybrid polymeric scaffolds has been proven to be one of the main tactics in tissue engineering,[2] with conductive polymers leading the way in the second generation of these regenerative platforms.[3] In order to utilize CNTs based scaffold in the *in-vivo* regeneration of different biological tissue, the mechanical properties of the 3D system have to be properly tuned to match the mechanical stress response of the living matter. Variating the internal crosslink degree of the CNTs/polymer matrix can be an efficient way to deal with this non trivial challenge.

To achieve the correct mechanical response, different covalent strategies for crosslinking CNTs have been explored. Viability of the different functionalization has been assessed with different cell lines, overall highlighting promising results of these initial studies.

Keywords: CNTs, polymer matrix, tissue engineering, Young's modulus, crosslink.

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Detection of extracellular metabolites by SERS: from nanostructured 2D substrates to 3D-printed plasmonic scaffolds

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The assessment of tumor-secreted metabolites and anti-cancer drugs diffusion in the tumor environment becomes instrumental for monitoring the response of tumor upon therapeutic challenges, the stratification of cancer patients, and the identification of novel therapeutic strategies. In this regard, the *in-situ* fast detection of analytes by surface-enhanced Raman scattering (SERS) can play a critical role to accomplish these aims. SERS allows the identification of vibrational fingerprints of probe molecules in contact with plasmonic nanostructures, and its sensitivity can go as far as the single-molecule level. As a consequence, the correct integration of such nanomaterials within the biological environment represents a pivotal step to get valuable information about tumor evolution. In this direction, different nanocomposite structures are evaluated and implemented to achieve nondestructive monitoring of various cellular events.

Herein, we present different gold nanoparticles organizations with the capacity not only to enhance the signal of relevant metabolites in complex environments, as biological media, but also to provide spatiotemporal information of their diffusion in the cell environment. Additionally, the employment of printed scaffolds containing gold nanoparticles enables the evaluation of three dimensional analytes distributions, allowing a more complete understanding of the progression of the disease.

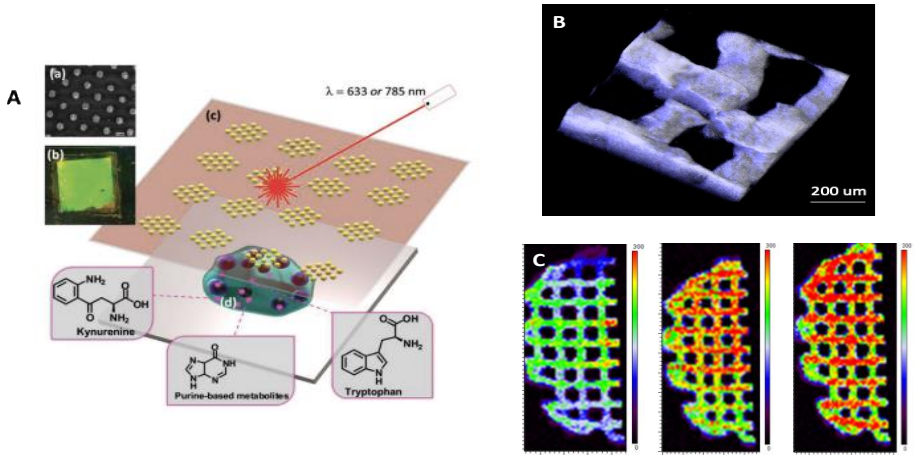

Figure 1. A) Schematic illustration of the SERS-based system to detect the accumulation of metabolites in the extracellular tumor milieu. B,C) Detection of Methylene Blue drug diffusion in 3D printed-scaffolds

Keywords: SERS, plasmonic structures, cancer metabolites, 3D cell culture, cell sensing

3D porous scaffolds for SERS sensing

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Finding a cure for cancer requires overcoming different obstacles such as the complexity of the disease itself, the variety of cancers types and the variability observed between patients. In order to fully understand the cellular mechanisms involved with this disease, *in vitro* assays are required to study the signaling pathways used for cancer cells during the evolution of the tumor. However, most of the current strategies make use of flat two-dimensional (2D) supports for cell growth, thus not being able to mimic the physicochemical properties of the *in vivo* scenario. For these reasons, 3D scaffolds represent an interesting approach for *in vitro* studies due to the wide range of modeling possibilities to enhance cell diffusion and mimic the properties of real tissues [1]. Regarding the analytical tools used to study the behavior of cancer cells, common strategies such as proteomics and fluorescence assays tend to be too invasive, thus damaging the cells and altering their behavior. In this context, we propose the use of Surface Enhanced Raman Spectroscopy (SERS) as a non-invasive sensing strategy to detect and obtain information of biomolecules present in the tumoral microenvironment [2]. The project is focused on the fabrication of 3D porous plasmonic scaffolds by means of different processes such as colloidal systems as templates for polymeric inverse opals and 3D printing of High Internal Phase Emulsion (HIPE) ceramic inks for SERS sensing. Upcoming efforts will be focused on evaluating the potential of these systems to monitor tumoral cell-secreted metabolites diffused within the 3D support.

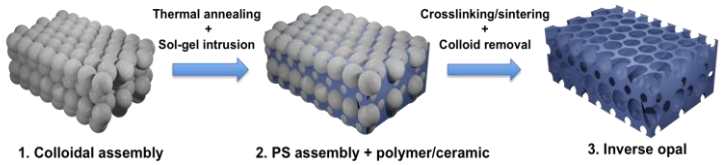

Figure 1. Stages of a colloidal synthesis process (inverse opals)

Keywords: cancer, 3D models, scaffold, SERS, sensing

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Micelle-assisted Chiral Seeded Growth over Gold Nanocrystals

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The aim of the work is to demonstrate preparation of intrinsically chiral nanorods (NRs) with helical screw like features of platinum and palladium over AuNRs. The as-synthesized chiral NRs would be important for a variety of optics, catalysis and sensing based applications due to their bimetallic composition and interesting surface morphology, exhibiting circular dichroism (CD). Here, we firstly prepare monodisperse single-crystalline AuNRs of size 130x29nm in dimension, using the modified silver-assisted growth method.[1] These AuNRs are then utilized as seeds over which either platinum or palladium growth is carried out, following our recently developed micelle-directed approach (for preparation of chiral AuNRs).[2] We exploit the use of BINOL and BINAMINE as chiral additives with cetyltrimethylammonium chloride to obtain chiroptically active, worm-like micelles.[3] These micelles tend to adsorb over the seed AuNR surface in a quasi-helical pattern, thus acting as a template to guide the seeded growth of metallic shell. The resulting NRs have screw-like features over their surface with a specific handedness. A detailed structural analysis is being carried out by employing high resolution electron microscopy in order to differentiate between the bimetallic interface, and effectively characterize their surface morphology and elemental composition. Additionally, an understanding between the NR structure and resulting optical & CD response, is also being developed. This study successfully demonstrates scalable preparation of chiral NRs with different metallic shells; and contributes to the better understanding of the chiral seeded growth, supported with detailed structural and optical analysis.

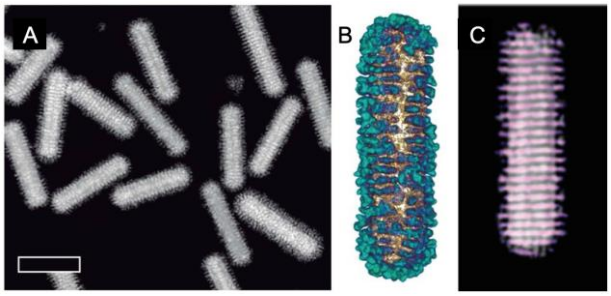

Figure 1. (A) HAADF STEM image of AuNRs with platinum grooves (scale bar:100nm); (B) Tomography reconstruction in EDX mode with platinum groove's pattern (cyan) over AuNR (golden); (C) Inverse FFT presenting the areas of NR with chiral features overlapped with projection of the tomography, indicating a right handed lower angle.

Keywords: Gold, Platinum, Palladium, Nanoparticle synthesis, and Circular Dichroism.

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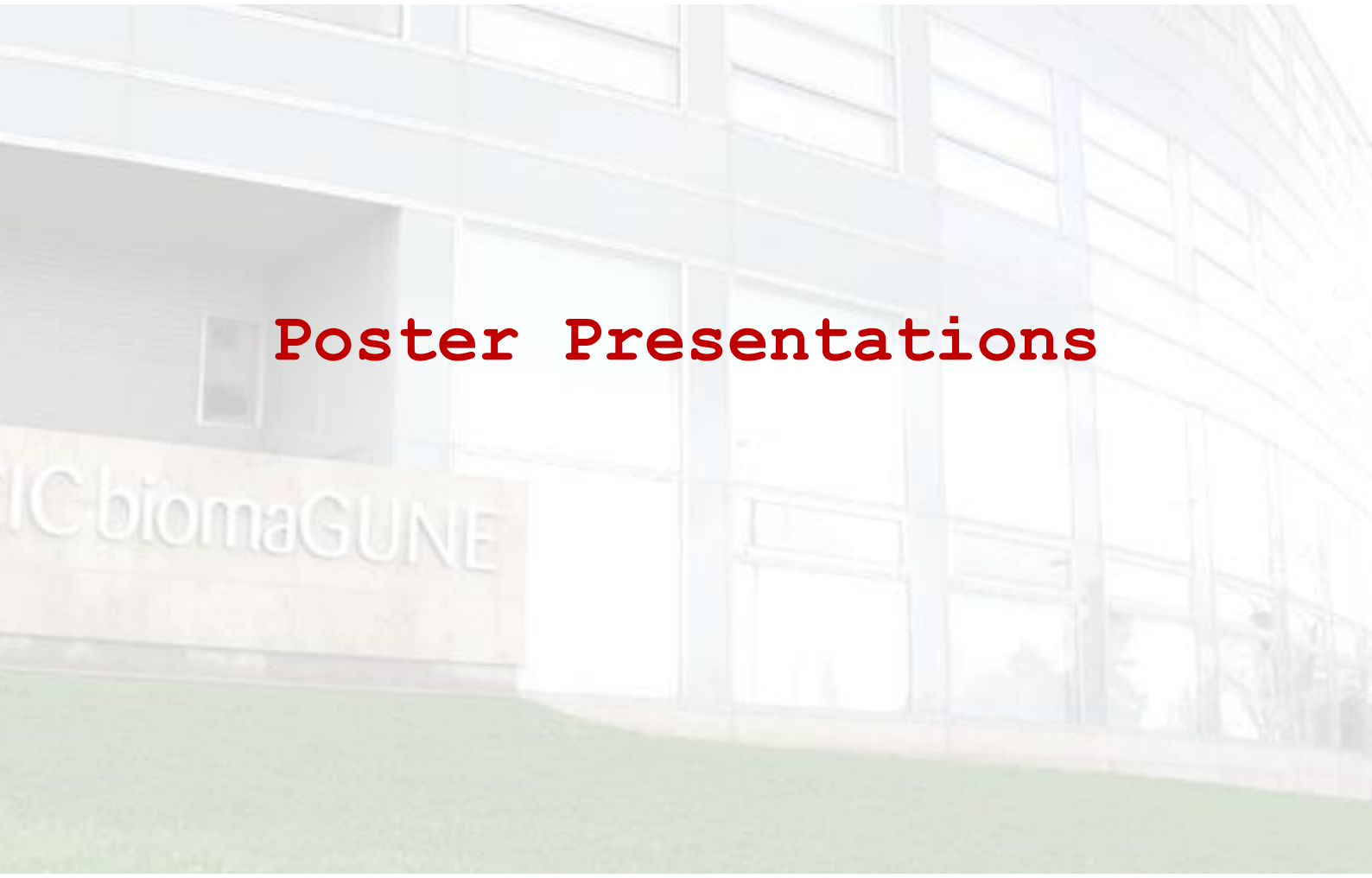

Poster Presentations

Development of customized nanoparticle-based bioinks for 3D printing of dynamic cancer models

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3D printing of natural and synthetic materials has emerged as a powerful tool in the field of tissue engineering. One such natural material is organ-derived decellularized extracellular matrix (dECM). Compared to conventional printing materials, dECM offers a customizable bioink which is richer in proteins, growth factors and other signalling molecules (1). By combining this bioink with plasmonic nanoparticles (NPs) exhibiting surface enhanced Raman scattering (SERS) abilities, a method in which cells can be grown in 3D and continuously monitored can be achieved. Compared to traditionally used fluorescent dyes, SERS-encoded NPs offer stable and high intensity signals over prolonged time periods, and can be imaged using Near-Infrared (NIR) light sources within the biological transparency window. Such a model enables the monitoring and detection of relevant metabolites and live cell behaviour in a time-dependent manner (2). Although this technique could be applied to various diseases, the prevalence and the accessibility by optical methods make breast cancer and melanoma our main targets. Porcine dermal and adipose tissue were selected as the first dECM sources based on human similarities. After thorough decellularization processes, cell-free ECMs are being tuned into printable and biocompatible inks by enzymatic digestion, which will later be customized with cancerous and stromal cells and plasmonic NPs. Developing 3D *in vitro* tumour models grown in dECM scaffolds comprising AuNPs will lead to a better understanding of cancerous and healthy cell growth and dynamics, as well as providing a model to study pharmacological effects in a relevant disease model

Keywords: bioinks, 3D printing, dECM, AuNPs, SERS.

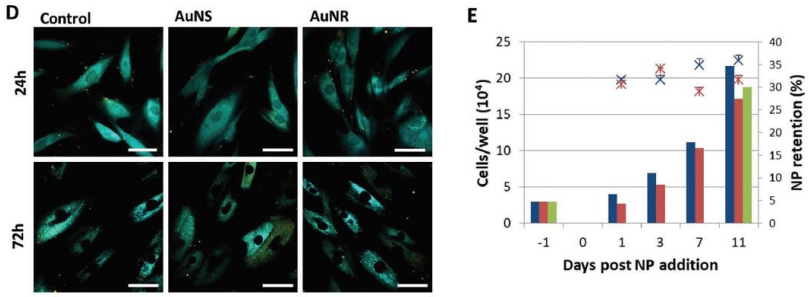

Figure 1. Cell viability and NP uptake in HDF cells reported by Jimenez de Aberasturi et al. (2020).

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Graphene-based functional materials for electrochemical imaging

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Electrochemiluminescence (ECL) is an analytical technique based on the detection of luminescence signals generated when a redox process, occurring at the surface of an electrode, induces electron transfer from high energy species (co-reactants, i.e. TPA), to a light emitting component (luminophore); thus, ECL devices measure luminescence signals produced upon application of an electrochemical stimulus, offering low backgrounds, high sensitivity (≤ 10^{-11} M), good temporal and spatial resolution, robustness and low fabrication costs. Whilst these features are attractive in different fields, including (bio)sensors and bio-imaging[1,2], we are interested in employing ECL as a tool to visualize single cells; this is an application at a very early stage but also a promising alternative approach to improve issues related to signal-to-noise and single biomolecule probing during cell imaging. For this application, one limit in ECL requires advancement: in current devices, the intensity of the detected signal depends on the distance between the luminophore and the electrode (≤ 500 nm)[3]. However, in more recent designs, the implementation with carbon-based nanomaterials, known for their peculiar conductivity properties, remarkably enhanced the efficiency of ECL devices. Here, we present a new synthetic strategy and preliminary characterization studies to optimize a hybrid system in which graphene oxide (GO) is used as a scaffold for a dual functionalization with: i) a targeting unit, specific for cell membranes and ii) $Ru(bpy)_3^{2+}$ as ECL luminophore. In our design, this system will allow anchoring the ECL probe to the cell membrane and, simultaneously, enhancing the intensity of the ECL signal.

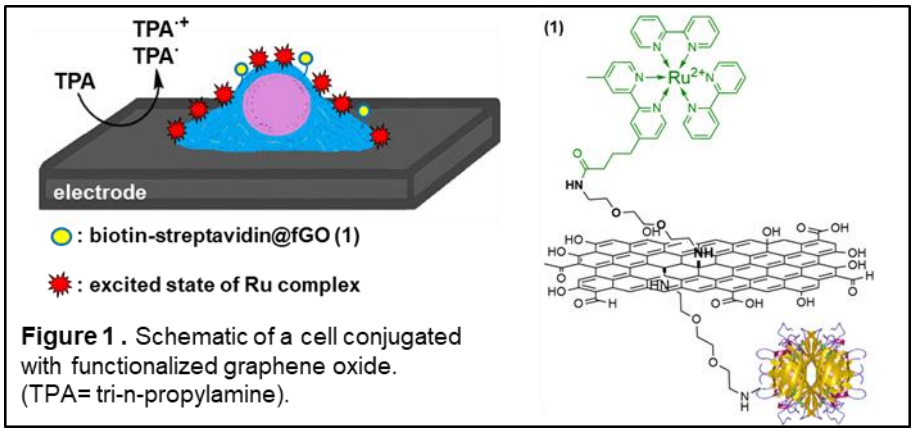

Figure 1. Schematic of a cell conjugated with functionalized graphene oxide. (TPA= tri-n-propylamine).

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Keywords: ECL, cell imaging, graphene oxide, covalent functionalisation, single cell

Design of colloidal plasmonic nanostructures with chiral optical activity, for bioimaging and diagnosis

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Conduction electrons of metallic nanoparticles react coherently to the electric field of incoming radiation, oscillating as localized surface plasmon resonances (LSPR). The characteristics of this electronic excitation depend on the size and shape of the colloids themselves, which allows tailoring their optical properties by wet-chemistry, determining their potential applications. Recently, a new concept has revolutionized the field of plasmonics: asymmetric morphologies whose mirror images cannot be superimposed display optical chirality. Hence, like enantiomeric molecules do, LSPRs can interact differently with circularly polarized light of opposite handedness, enabling novel light-managing possibilities. Our main objective is the design of chiral plasmonic systems by either the synthesis of intrinsically chiral nanoparticles or by assembly of achiral components into chiral configurations. In the latter case, chirality is obtained as a collective property when nanoparticles adopt helical morphologies through self-assembly. Thermotropic liquid crystals or amyloid fibrils can act as directors for such processes. On one hand, when mesogenic molecules are cooled down from isotropic phase, they can crystalize into helical nanofilaments [1]. Appropriate functionalization of gold nanoparticles enables their assembly (Figure 1A). Enantiomeric excess, together with optimized decoration of the helices, are necessary milestones on the way to achieve plasmonic chirality. Importantly, the same principle applies when gold nanorods assemble with amyloid fibrils (Figure 1B). In that case, diagnosis by chiral sensing becomes possible as these disease-related protein aggregates would be detected by circular dichroism signals coming from the nanorods assembly [2]. Future efforts will be devoted improve the detection limit.

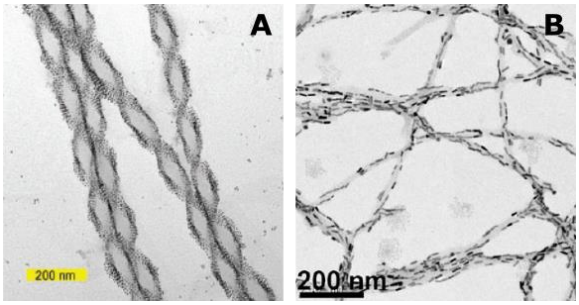

Figure 1. Au nanoparticles assembled around (A) liquid crystals [1] and (B) amyloid fibrils [2].

Keywords: plasmonic chirality, circular dichroism, helical nanofilaments, liquid crystals, amyloids

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Synthesis of stable isotope-labeled O-glycan standards and their application in clinical diagnosis

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Gastric cancer is the third most malignant disease worldwide after lung and liver cancer and its advanced stage has a poor prognosis. For this reason, this project focuses on the discovery and validation of new N- and O-glycans as serum biomarkers for early detection and for the discrimination between healthy and diseased state [1]. Innovative glycan quantification methods based on the use of isotopically labeled glycan standards will be applied. This will allow an absolute quantification of glycan levels in serum in a given clinical state, using mass spectrometry techniques. For this, the chemical synthesis of the 8 cores of O-glycans (*Figure 1*) and their enzymatic diversification will be carried out. In addition, a graphene-based biomarker detection platform is being developed and validated for the rapid and cost-effective detection of early stage disease markers. A functionalized graphene sample plate will be combined for the affinity capture of glycopeptide serum markers with ^{13}C-labeled internal standards and analyte detection by MALDI-TOF-MS to produce a quantitative analysis platform that would adapt to existing hospital instrumentation. Target compounds include sialylated Lewis X and Tn antigens (*Figure 2*).

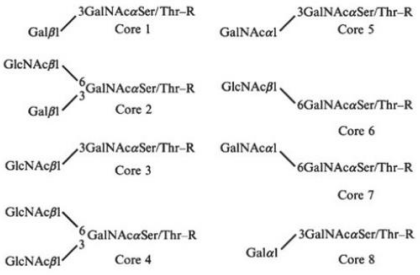

Figure 1. *O-glycans 1-8 cores.*

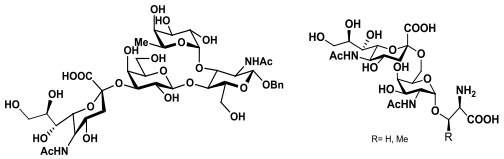

Figure 2. *Sialyl Lewis X and Sialyl T antigens.*

Keywords: O-glycans, N-glycans, gastric cancer.

References

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Surface engineering of transaminases to tailor protein immobilization on microreactors

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Nowadays, the production of chemicals involves larger amount of money, energy, time and complex downstream process for the isolation of the final products that generate the majority of the waste worldwide[1]. To overcome this problem, it is necessary to develop new production strategies that combine awareness with sustainability criteria. Remarkably, nature uses smart and efficient synthetic methods through the use of biocatalysts in multi-step pathways avoiding the intermediate isolation and purification step[2].

Biocatalysts in vitro generally demand for an immobilization step to simplify the downstream processing and to enable the re-use of the enzymes. In addition, the combination of functional materials and protein engineering in flow reactors will trigger in heterogenous biocatalysts[3]. The use of cell free systems for multistep reactions immobilized in the microreactor will facilitate the manipulation and the complexity of the process. Moreover, this system showed a high productivity for sustainable manufacture being often the preferred option to implement[3].

The aim of this project is to design strategies of protein immobilization based on protein engineering to develop microsystems for biocatalyst evaluation and develops functionalized microreactors integrating a cell free enzyme system into the microdevice. This would improve heterogenous biocatalyst's robustness, spatial orientation, and meeting the process requirements for the value-added products for diverse applications in surfactants, antioxidants and bioplastics among other. In order to reach this goal, it would be necessary to develop a toolbox for material design, surface modification techniques, protein engineering methods to fulfill the demand of preparation and implementation of heterogeneous (bio) catalysts.

Keywords: enzyme engineering, microreactor, biocatalystis, green chemistry, sustainable materials.

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Immobilization of multi-enzyme systems through artificial cellulosomes in solid phase

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Synthetic Scaffolds such Cohesin and Dockerin domains enable the spatial organization of enzymes in multi-enzymatic reactions with one of the strongest protein-protein interaction that has been reported.[1] The spatial organization facilitates the direct transfer of a substrate from one enzyme to the next one, avoids accumulating intermediates and maximize the chemical fluxes towards the target products.[2]

Using the Cohesin and Dockerin system, two types of biocatalysts in solid-phase were generated. In the first biocatalyst, Dock-wTA-Dock and Coh-ADH were separately immobilized on two different microbeads, so the intermediates must travel from one bead to the other in order to complete the chemical cascade.

The biocatalysts are able to produce acetophenone and isopentylamine from methylbenzylamine and 3-methyl-1-butanol, while the cofactor needed for the first oxidation reaction is in situ recycled by ADH using acetone as co-substrate. We demonstrate the sequential immobilization and the strength of the specific interaction. Our results showed higher performance obtained when both enzymes are co-immobilized versus when enzymes were immobilized apart on two different carriers.

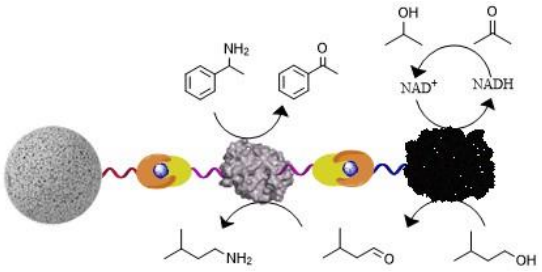

Figure 1. Scheme of enzymatic cascade

Keywords: Scaffolds, multi-enzymatic, sequential immobilization

References

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Cartilage decellularized extra cellular matrix materials for preclinical products application

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Implants are common therapeutic-surgical approach to treat cartilage injuries. However, implants act as a temporary solution, as they do not promote tissue regeneration. Extracellular matrix (ECM) is bioactive and therefore it can be used as definitive biomimetic and regenerative implant. In recent years, tissue-decellularization based ECM materials had gained interest as implantable compound. Decellularization protocols have to be tissue-specific, as each tissue reacts in a different way to the same agents due to their different composition [1]. The aim in this work is to develop a cartilage tissue-specific decellularization method which would yield a dECM material useful to be processed for in testing and regenerative studies. Starting from porcine post-mortem tissue, cartilage tissue has been isolated and processed in a variety of decellularization techniques. Resulting dECMs have been studied in terms of degree of decellularization and ECM compounds. Results suggest that the combination of trypsin-EDTA, SDS and Benzonase is the better decellularization protocol among tested ones. This method eliminates most of the DNA content, lowering it below 10 ng/mg. Overall, the matrix maintains the cartilage ECM structure and preserves GAGs and collagens. Therefore, we have been able to define a specific protocol which preserves the key molecules in dECM. The next step will be processing the dECM, both alone and combined with synthetic materials, to produce relevant 2D and 3D structures potentially useful in cartilage tissue studies. Then, generated structures will be tested for their potential application in *in vitro* and *in vivo* settings.

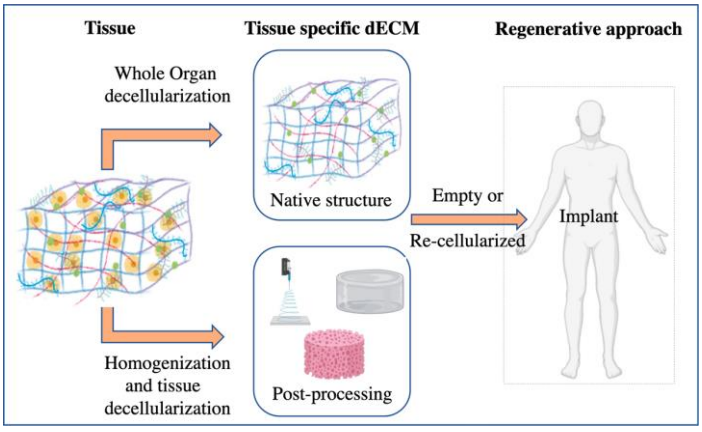

Figure 1. Schematic of organ decellularization and tissue decellularization approaches

Keywords: decelularization, regenerative medicine, cartilage,

References

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Synthesis and Evaluation of Glycomimetics as potential Siglec-10 Immunomodulators

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The sialic acid-binding immunoglobulin-like lectins (Siglecs) are important regulators of the immune system[1]. High affinity ligands derived from sialic acid that target specific Siglec can provide valuable therapeutic tools. A question of interest is how to design ligands that bind to these regulatory receptors with sufficient avidity and specificity to manipulate the immune system. In this project we focus on the design of potential ligands based on a trisaccharide scaffold that combines modifications in sialic acid and galactose; the second residue next to sialic acid known to make contacts to Siglec binding site (Figure 1) [2]. With these tools we seek to study Siglec-10 receptor binding mode to natural ligands and use this information to rationally synthesize high affinity glycomimetics with an increased inhibitory potential and a greater selectivity for Siglec-10 or other Siglecs. By this way we could use these compounds as potential leads for the development of glycan based immune therapies.

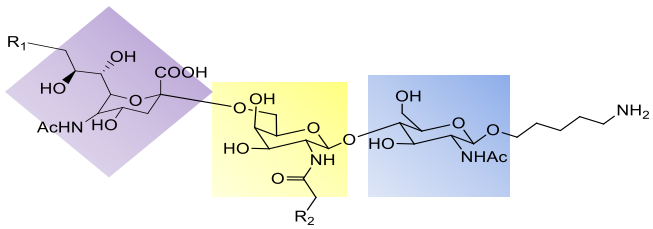

Figure 1. Structure of the trisaccharides that will be tested as potential immunomodulators

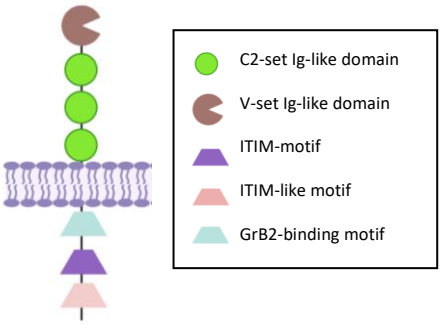

Figure 2. Siglec-10 structure

Keywords: Glycomimetics, Siglecs, Sialic acid, Galactose.

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2D self-assembled hybrid biomaterial

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The development of novel bio-inspired systems employing repeat proteins as individual biological blocks is a promising research area for applications in biotechnology and nanomedicine [1,2]. This work presents a novel approach for designing 2-dimensional (2D) biomaterials based on the self-assembly of peptide-protein hybrids (PPH) and amyloid-like peptides (ALPs). A single consensus tetratricopeptide repeat (CTPR) protein linked to an amyloid-like peptide, i.e. peptides with a structure that mimics sections of amyloid proteins, will form the peptide-protein hybrid (PPH) component, in which the peptide of this hybrid will aggregate with other amyloid-like peptides (ALPs) (Fig.1). These ALPs have been designed to co-assemble into amyloid fibers, what has been proven computationally by using Molecular Dynamics and characterized by AFM and TEM. These amyloid peptides will then drive the self-assembly all together with the peptide-protein hybrid to co-assemble into the bio-inspired material.

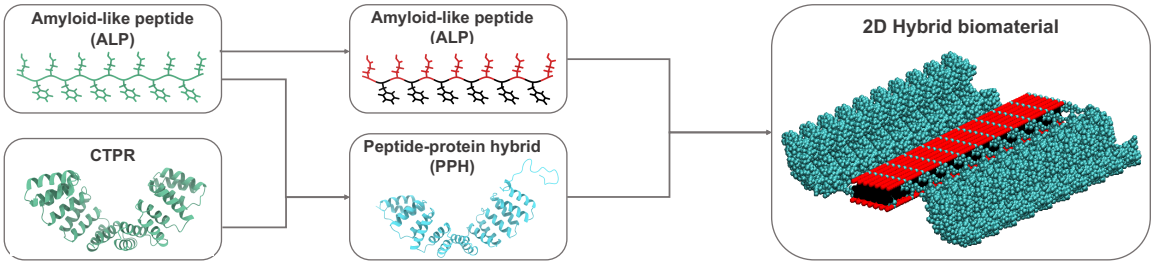

Figure 1. Outline of the project. The consensus tetratricopeptide protein (CTPR) will be linked to an amyloid-like peptide (ALP), forming the peptide-protein hybrid (PPH). Both, the PPH and free ALPs, will self-assemble into a 2-dimensional (2D) biomaterial.

Keywords: bio-inspired systems, self-assembly, peptide-protein hybrid, amyloid fibers, biomaterial.

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Biocatalytic synthesis of nano-atomic clusters for bioanalysis

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The aim of this project is the biological synthesis of atomic nanoclusters with enhanced fluorescent and catalytic properties and its implementation in biosensors and bioanalytical systems. Due to the remarkable features that metal nanoclusters exhibit such as their fluorescence and catalytic properties, they have become increasingly more relevant in sensing applications [123]. It has been proven that nanoclusters can be synthesized using antibodies as scaffolds under non-denaturing conditions [4]. CTPR designed proteins, as functional scaffolds for the stabilization of nanoclusters, allow the fusion of protein-nanocluster modules for the labeling of other recognition molecules [5]. The modification of recognition elements with metallic nanoclusters is being used for the design and development of biosensors. The electrochemical characterization of different protein-quantum dots compositions is being studied and the best configurations will be selected to achieve the development of novel detection systems improving the sensitivity of existing biosensors. As an application, the electrochemical detection of COVID-19 antibodies by means of an immunoassay is being studied. Anti-Human IgG antibodies modified with bimetallic gold and platinum nanoclusters have peroxidase like activity and high affinity for Anti-RBD antibodies. A complex will be formed between the RBD protein immobilized on a screen-printed electrode surface and Anti-RBD antibodies present in human serum of patients. The detection of the Anti-RBD antibodies will be carried out by a second incubation with the catalytic antibodies. An increase in the peroxidase like activity signal will be able to be measured by chronoamperometry if the serum contains Anti-RBD antibodies.

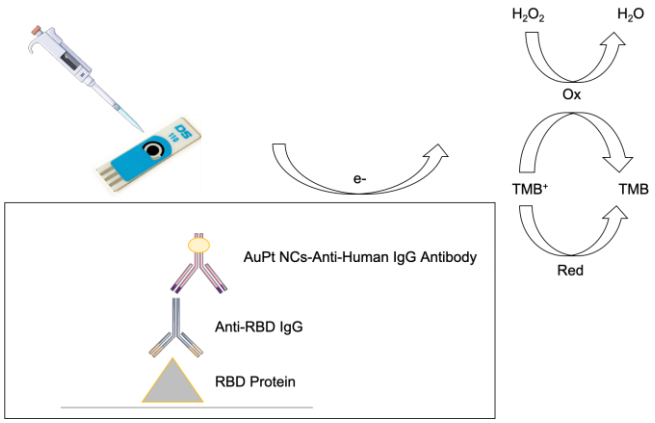

Figure 1. Scheme for the electrochemical detection of COVID-19 antibodies.

Keywords: nanoclusters, biosensors, electrochemistry, COVID-19.

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Self-sufficient Reduction of β-ketoesters Catalyzed by a Novel and Robust Thermophilic Alcohol Dehydrogenase Co-immobilized with NADH

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β-hydroxyesters are essential building blocks utilized by the pharmaceutical, agrochemical and food industries in the synthesis of functional products. Beyond the conventional production methods based on chemical synthesis or whole-cell catalysis, the asymmetric reduction of β-ketoesters with cell-free enzymes is gaining relevance. [1]

To this end, a novel thermophilic enzyme from *Thermus thermophilus* HB27 called (S)-3-hydroxybutyryl-CoA dehydrogenase (Tt27-HBDH) has been expressed, purified and biochemically characterized, determining its substrate specificity towards β-ketoesters and its dependence on NADH as a cofactor. Tt27-HBDH immobilization on agarose microporous beads activated with aldehyde groups and its subsequent functionalization with polyethyleneimine has been found the best strategy after screening multiple immobilization and post-immobilization protocols. Through this design we are able not only to increase the workability and stability of the biocatalyst but also, to co-immobilize both Tt27-HBDH and NADH on the same particle, while the cofactor is reversibly embedded in the cationic layer attached to the porous surface of the carrier. Since Tt27-HBDH catalyzes cofactor recycling by Isopropanol oxidation, we achieve a self-sufficient heterogeneous biocatalyst where NADH is available for the immobilized enzymes but its lixiviation to the reaction bulk is avoided. [2]

Taking advantage of the autofluorescence of NADH, we demonstrate the activity of the enzyme towards the immobilized cofactor through single-particle analysis which also provide information about the activity ratio between orthogonal reduction and oxidation reactions. [3] Finally, we tested this self-sufficient biocatalyst for the asymmetric reduction of β-ketoesters in batch, succeeding in the reuse of both the enzyme and the co-immobilized cofactor up to 10 reaction cycles.

Keywords: β-ketoesters, β-hidroxiesters self-sufficient heterogeneous biocatalyst, single-particle

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Responsive Microgels for Biomedical Applications

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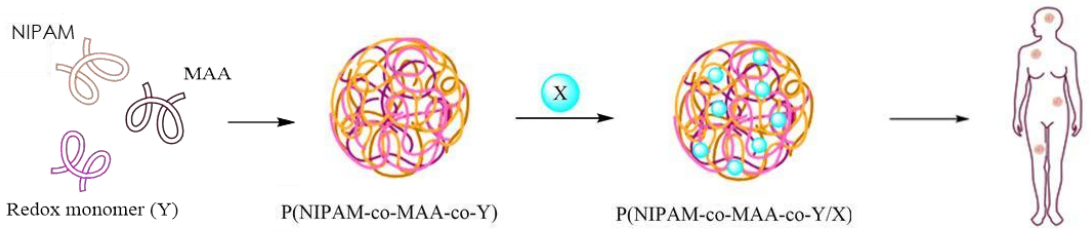
Microgels are highly versatile polymeric structures in micrometer size interesting for drug delivery of drugs with difficult introduction or action in the body. The polymers that make up these microgels have specific properties (pH-responsive, temperature-responsive and redox-responsive) that vary depending on the percentage of monomer in the microgel[1] In this way, the systems will only act on those cells that contain or produce stimulus that makes the microgel collapse or swell, releasing the drug or species inside or on the cell surface, decreasing the toxicity and increasing the selectivity of the drug. Thus, the monomers NIPAM, MAA are the base of the particles[2] and DMA[3] and MTEMA in different proportions were chosen for the formation of microgels with different sizes (from 100 to 300 nm). DLS and AFM allowed to know the size and z potential of the hydrated microgels. Size, Z potential, toxicity and biocompatibility, among others, are very important factors in the system.

Keywords: Microgels, NIPAM, redox-responsive, DMA, size.



Scheme 1: Synthesis and action of responsive microgels.

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3D models for *in vitro* definition of cartilage-to-bone transdifferentiation process

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Endochondral ossification is an essential process by which mammalian long bones are created, remodeled and repaired based on previous cartilage formation. Recent experimental evidences suggest that during endochondral ossification hypertrophic chondrocytes can become osteoblasts and osteocytes while surviving after hypertrophic phase in a process called cartilage-to-bone transdifferentiation (CtBT).[1] Up to now, molecular mechanisms related to CtBT are still largely unknown and unexplored. The objective in this work is to define the molecular mechanism triggering CtBT. For this aim, here an *in vitro* cell culture approach is implemented based on 3D gelatin scaffolds and CtBT-relevant cells. The target of the study is being able to recapitulate CtBT and recover samples at different timing to perform molecular studies. We set up the culture system with the model cell lines CH3/10T1/2 and MC3T3, and with CtBT-relevant primary cell cultures. In this sense, we isolate murine articular bone progenitors (mABPs) from newborns and differentiate through CtBT. mABPs are cartilage precursors, and therefore a good cell-model to be integrated in a scaffold culture system to recapitulate sequential differentiation processes present in CtBT. Results indicate that MC3T3 cells cultured in scaffolds for 21 days in osteogenic differentiation medium developed their own matrix, while control cells did not. Results with CH3/10T1/2 indicate serial cartilage and bone differentiation is feasible *in vitro*, while cells can be harvested during intermediate stages via digestion with collagenase. Results with mABPs indicate these primary cartilage precursor cells need a cartilage priming step to further differentiate to bone phenotype in *in vitro* culture system. In conclusion, these data show that CtBT can be recapitulated in *in vitro* culture settings. Protocols have been set up and further molecular studies are ongoing.

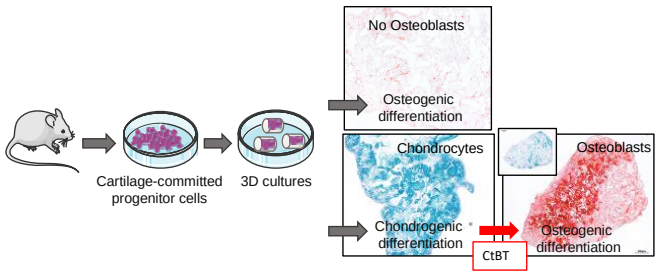

Figure 1. *schematic protocol of in vitro 3D culture of iMABPs. Preliminary data indicates that these cells do not differentiate into osteoblasts without a previous chondrogenic differentiation priming.*

Keywords: bone regeneration, transdifferentiation, chondrocytes, osteocytes

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Enhancing energy to achieve healthy aging and greater longevity

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Diet has a major impact on health and longevity. Most of the food we consume is converted to energy by the mitochondria. However, energy production by mitochondria declines with age, with a clear correlation between mitochondrial activity and aging. Moreover, mitochondrial dysfunction is increasingly recognized as an important factor in a wide range of human diseases [1]. We have recently demonstrated that normal mitochondria undergo major remodeling in response to changes in nutrient availability; and that dysfunctional mitochondria behave like intracellular parasites when sugars derived from carbohydrates are plentiful. Changing nutrient metabolism by synthethic glucose restriction (using 2-deoxy-D-glucose, 2-DG) purges cells of dysfunctional mitochondria. These findings can explain why dysfunctional mitochondria accumulate with age in animals, and they are concordant with the increased healthy lifespan of rodents fed in low calorie diets. [2].

We aim to unify the molecular and whole-body effects of intermittent caloric restriction diets on mice, in normal and disease state. This study will determine the cardiac, metabolic and cognitive function by magnetic resonance imaging (MRI), glucose and insulin tolerance test, nuclear magnetic resonance spectroscopy (NMRS), positron emission tomography (PET) and Barnes Maze test.

Our initial data show some differences in the metabolic and cardiac function between forms of administration of the hypocaloric diet through the intake of 2-DG in drinking water during 8 months. Moreover, during the second part of the study we will assess the impact of the combination of different diets on aging in order to provide metabolic readouts to offer lifespan benefits.

Keywords: aging, diet, 2-deoxy-D-glucose, caloric restriction, mitochondria.

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Effect of dietary regimes in the development of pulmonary hypertension

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Pulmonary arterial hypertension (PAH) is a rare and severe form of pulmonary hypertension. PAH is characterized by remodeling of the distal pulmonary arteries, leading to an increase in pulmonary vasculature resistance, reduced compliance, and elevated pulmonary artery pressure. It is resulting in progressive right heart failure and premature death[1]. One of the first steps of the treatment of cardiovascular disease is the change lifestyle. Nevertheless, the European Society of Cardiology (ESC) and the European Respiratory Society (ERS) Guidelines have not established specific recommendations for dietary habits or nutrient supplementation for PAH [2].

Due to the role of the oxidative phosphorylation (OXPHOS) in the PAH and the influence of the hypocaloric diet on the OXPHOS, the aim of this study is to elucidate the influence of different dietary regimes in the development of this rare disease. This study will determine the cardiac and metabolic function by magnetic resonance imaging (MRI), glucose and insulin tolerance test, positron emission tomography (PET) and nuclear magnetic resonance spectroscopy (NMRS). The flow MRI will be used as non-invasive diagnostic tool.

Our initial result showed that treatment with 2-deoxyglucose (2-DG), administered in a continuous way, improves the functional status of the heart and ameliorates the right ventricular pressure of these animals. Our findings suggest the important role of mitochondria in the evolution of this diseases and may represent a new therapeutical approach in pulmonary vascular diseases. The study will be completed to evaluate the best dietary administration and the effect this diet in aged animals.

Keywords: pulmonary arterial hypertension, diet, 2-deoxyglucose and aging.

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