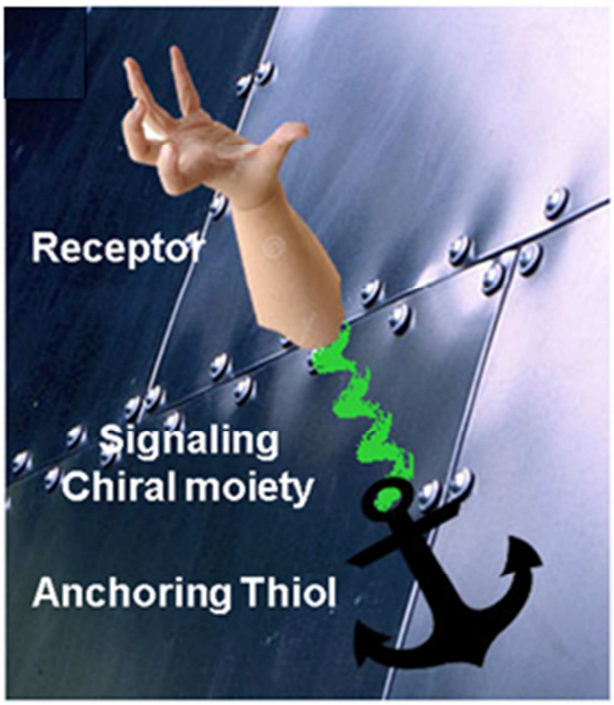
INTERNATIONAL IBERIAN NANOTECHNOLOGY LABORATORY

COLLABORATIVE PROJECT IDEA CONTEST

Nano *Upstanding* ***Chiral*** *Architectures for* ***BioSens****ing*



NanoChiralBioSens

(UVigo–CHUS–INL–TUM)

OCTOBER 2014

INFORMATION

1. Concept Notes must be emailed to vicinv@uvigo.es by **13.11.2014**.
2. All participants will be contacted and informed of the outcome of deliberations of the Technical Evaluation Committee: **a ranked list of projects.** The highest ranked projects in the list shall be invited to complete a detailed Project Proposal.
3. Evaluation criteria and key Elements to be assessed by the Evaluation Committee

- Impact of the project
- Alignment with Galician S3 Strategy and INL research areas or INL interest areas
- Project implementation, Technical Quality of the proposed project idea and economic-financial adequacy.

SUMMARY SHEET

Contact Information

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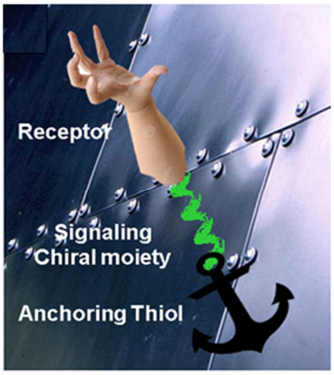
Project Details

Project Title	***Nano*** *Upstanding* ***Chiral*** *Architectures for* ***BioSens****ing* ***NanoChiralBioSens***
Project Team Members (Titles & Role)	1. José Lorenzo Alonso Gómez, coordinator (University of Vigo): • **Development of functionalized chiral systems for the construction of Upstanding Chiral Architectures.** • **Evaluation of the chiroptical amplification and sensing of the chiral surfaces.** 2. Florian Klappenberger (Technical University of Munich): • **Low-temperature scanning probe investigations for high-resolution characterization of the Upstanding Chiral Architectures.** 3. Stefano Chiussi (Universidade de Vigo): • **LabOnChip-compatible production of cm^2-sized transparent surfaces with Upstanding Chiral Architectures, stable under ambient conditions.** 4. Dmitri Petrovykh (International Iberian Nanotechnology Laboratory): • **Characterization of structure and composition of Upstanding Chiral Architectures on cm^2-sized transparent surfaces using complementary spectroscopy and microscopy methods** 5. Ramiro Couceiro (IDIS Complexo Hospitalario Universitario de Santiago): • **Evaluation of sensitivity of the chiral surfaces to targets relevant for cell-free circulating DNA detection and recognition**
Thematic Area(s)	**Main Sector(s)**
Health	**Biosensing, Lab-On-a-Chip, Medical Devices, Circulating DNA**
Total Budget	**228.000 €**
Execution Time	**24 Months**

PROJECT OUTLINE

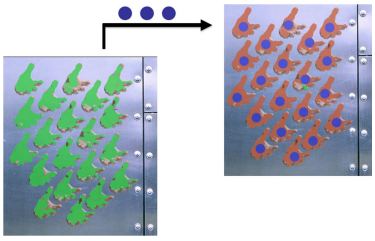
A. Research problem addressed



Establishing the proof-of-principle for chiroptical sensing with surfaces functionalized for biosensing

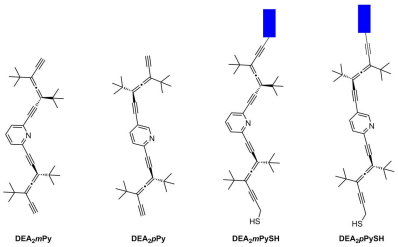
Population aging is one of the major problems facing Europe in the coming years – according to the UN in 2050 more than 38% of the population in Spain will be over 60 years old– and this inevitably leads to an increase in chronic diseases and in the need for personalized medical monitoring and care. Medical care controlled by individual patients is, therefore, essential to sustainably face this demographic development. The advance of telemedicine through biosensors paves way for remote monitoring, prevention, and diagnosis, avoiding unnecessary treatments and travel as well as reducing waiting times. To reduce costs and expand the variety of monitored diseases as well as detection limits and accuracy, new sensing methodologies for biomarkers are required. Chiral molecular systems that exhibit strong optical responses (chiroptical systems) may open possibilities for unique biosensing modalities because they are highly sensitive to conformational changes and supramolecular interactions, both properties are important for biomolecular recognition. Chiroptical systems are typically characterized by circular dichroism (CD) and optical rotation dispersion (ORD) measurements.[1,2] The weak intrinsic chiroptical activity of commonly used chiroptical systems as well as the missing control over their structure, however, have limited their use in sensing applications. Conversely, access to nanostructures with very strong chiroptical responses would open novel possibilities for using them as transducers. We have already prepared cyclic[3] and open[4] allene oligomers showing outstanding chiroptical properties and we have been able to induce strong chiral amplification on self-assembled nanoparticles.[5] Very recently, we successfully transferred the chirality from enantiopure allenes to surfaces via the construction of Upstanding Chiral Architectures (UCAs).[6] The use of such enantiopure allenes with extraordinarily strong chiroptical responses as highly sensitive and specific optical transducers opens a broad range of possibilities for the construction of unique chiroptical sensors.

B. Objectives



The aim of this project is to build stable Upstanding Chiral Architectures (UCAs) with strong chiroptical responses and to explore their use for the construction of sensors in the context of detecting biomarkers (biosensing). This project is complemented by the recently funded Explora Ciencia project (*Smart Chiral Frameworks to Control and Inhibit Corrosion*, CTQ2013-50575-EXP, PI J.L. Alonso-Gómez).

Three key factors provide the basis and motivation for developing the proposed chiroptical sensing modality: 1) circular dichroism changes induced by complexation of chiral allenes permit solutes detection in ppm quantities,[7] 2) synthesis of chiral allenes that are able to form monolayers via self-assembly[6] and 3) chiroptical transduction reports for protein monolayers,[8] which exhibit considerably weaker chiroptical responses than do chiral allenes. Consequently, it should be feasible to use self-assembled monolayers of UCAs with extraordinary chiroptical responses as transducers. In order to target the goal of producing stable UCAs, the following specific objectives are proposed:

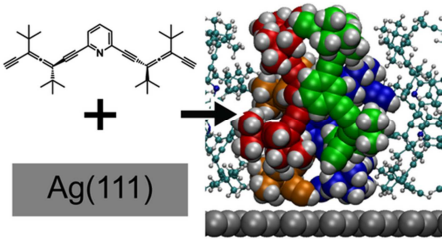


Development of functionalized chiral systems for the construction of Upstanding Chiral Architectures (UVigo)

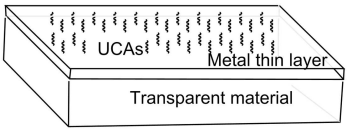
Enantiopure **DEA$_2$*m*Py** shows chiral transfer from the molecular level to the overall chiral surface.[6] We expect that this morphological self-assembly will be improved by using *para*-substituted **DEA$_2$*p*Py**. In addition, the incorporation of thiol groups for surface attachment in one terminal position

of the molecule is expected to considerably enhance the UCA stability with **DEA$_2$*m*PySH** and **DEA$_2$*p*PySH**. In order to prove the transduction capabilities of UCAs, functional groups will be incorporated in the structure that forms the most stable UCAs at the terminal position exposed to solution (blue rectangles). As a proof-of-principle, imidazole group will be incorporated for pH sensing.

Low-temperature studies for high-resolution characterization of the Upstanding Chiral Architectures (TUM)



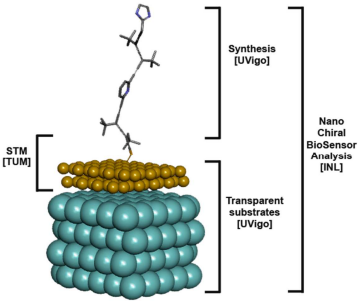
Synthesized chiral frameworks will be studied by state-of-the-art scanning probe techniques for the understanding of model systems at the single molecule level. Employing scanning tunneling microscopy, we unraveled the morphological self-assembly principles behind the formation of the UCAs.[6] Now, we want to address the anchoring of the UCAs to the metal surfaces via strongly chemisorbing functional groups aiming at controlled and stable adsorption and extend the investigation techniques to atomic force microscopy to obtain deeper insight into intermolecular forces and adsorption strengths. Our first set of test molecules described above follows the thiolate approach, which is extremely successful in the field of self-assembled monolayers (SAMs). As model systems, the (111) surfaces of coinage metals (Au, Ag, and Cu) will be used. In the very unlikely case that thiol anchoring and allene integrity are incompatible, stable adsorption via other functional groups will be evaluated. For example, bidentate coordination of the carboxylate group appears to be a possible alternative approach. The results from the STM studies will help in the design of new systems for the construction of stable UCAs. It is expected that investigating this set of building blocks will clarify how room-temperature stable monolayer films with allene moieties can be achieved. This result will allow the fabrication of UCA monolayers under normal lab conditions.



LabOnChip-compatible production of cm^2-sized transparent surfaces with Upstanding Chiral Architectures, stable under ambient conditions. (UVigo)

Transparent surfaces of cm^2-size (Au and Ag on SiO_2) will be produced by thermal evaporation. These surfaces will be functionalized with the UCAs that were previously tested at TUM. Functionalization will be performed via thermal evaporation, sublimation and/or spin coating, to obtain chiral surfaces that are stable at room temperature. An evaluation of this thermal stability as well as the impact of 193 nm laser pulses for cleaning and micro/nano-structuring UCAs will also be performed. Particular attention will be devoted to guarantee the production of UCAs monolayers, either via self-assembly, or if SAMs of self-limiting processes are not reliable, via thinning through thermal, laser-assisted, or wet-chemistry treatments. Characterization of thickness, growth rates, composition, and structure will be achieved through Quartz microbalance, AFM-Raman/TERS measurements. Thermal stability will be evaluated via Mass Spectrometry and Time Resolved Reflectivity (TRR). Further detailed high-resolution surface characterization, performed at INL, will be essential in order to compare the cm^2-sized transparent surfaces with the results from the STM at TUM.

Characterization of structure and composition of Upstanding Chiral Architectures on cm^2-sized transparent surfaces using complementary spectroscopy and microscopy methods (INL)

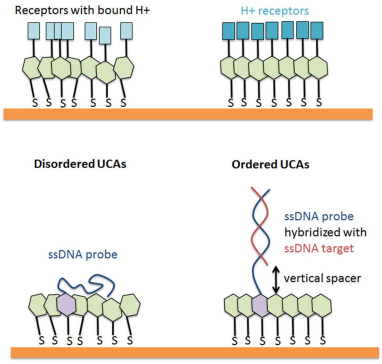


Comprehensive structural, compositional, and optical characterization of the cm^2-sized transparent surfaces using high-resolution spectroscopy and microcopy techniques (XPS, HR-SEM, FTIR, SE, HR-AFM, Raman) will be performed at INL. Possible benefits of using chiroptical transducers alone or in combination with advanced INL sensors (GFETs, spintronic devices), to enable the enhancement of their performance, will be evaluated. The possibilities for future sensing and biosensing device technologies based on UCAs will be studied and future joint projects on

larger international scale will be considered. Particularly, once chiroptical sensing with UCAs has been proved, miniaturization of chiroptical detectors for various wavelengths will be a key point to enable the transfer of this technology for commercialization.

Evaluation of chiral amplification and chiroptical sensing of cm^2-sized transparent surfaces functionalized with Upstanding chiral architectures (UVigo)

The sensitivity of chiroptical responses for the produced cm^2-sized transparent surfaces will be tested by circular dichroism. Comparison of the chiroptical responses in solution and as monolayers will determine the degree of chiral amplification using UCAs. The sensitivity of these chiroptical responses will be tested first by detecting pH changes as a proof-of-principle. Once chiral amplification and pH sensing are achieved, chiroptical biosensing will be studied with chiral surfaces intercalating DNA probes for free circulating DNA (ctDNA). Complexation of the ssDNA probe to the ssDNA target may change the conformation of several UCAs in the surface, inducing consequently a chiroptical signal. Although in this project detection will be used based primarily on electrostatic interactions in proof-of-principle experiments, the chiral nature of the UCAs may offer in the long term unique possibilities for chiral/enantiomer-specific detection of proteins and other biomolecules via their interactions with appropriately designed UCAs. Identification of specific and nonspecific CD changes will be evaluated using chromophores having strong absorbance at distinct characteristic wavelengths, ranging from 180 to 500 nm. Our expertise in theoretical simulation of chiroptical properties will improve the understanding of the mechanisms that underlie the sensing responses, thus enabling the design of chiral molecules for the construction of UCAs with optimized properties for biosensing.



Evaluation of sensitivity of the chiral surfaces to targets relevant for cell-free circulating DNA detection and recognition

Developing non-invasive methodologies for detecting and tracking tumor progression is a major challenge in Oncology. Recent studies[9] have shown the importance of using free circulating DNA (ctDNA) as potential biomarkers for early detection in patients' liquid biopsies. It is known that clinically relevant mutation of biomarkers, such as KRAS, are present in >99.2% of cholorectal cancer patients biopsies.[10] Furthermore, there is an actual presence of ctDNA even when circulating tumor cells are not present in the analyzed biopsies. These observations imply that ctDNA is a useful, sensitive, and specific biomarker, which can serve as a stepping stone for the further development of point-of-care medical devices, in particular for patients affected by multiple kinds of cancer.

In this project, we will evaluate the potential of chiroptical detection of low-concentration KRAS markers in terms of both specificity and sensitivity. Based on our findings, future improvements of sensing methods for ctDNA detection will enable production of valuable point-of-care tools for physicians and cancer researchers in order to provide them with a quick and early prognosis.

C. **Alignment with Galician S3 Strategy:** This project addresses **Priority 3.1**, *Active ageing*, of **Challenge 3,** *New healthy lifestyle model based on active ageing of population*. Only in 2008, about 12.7 million cancer cases and 7.6 million cancer deaths were estimated worldwide. The world population growth and ageing is increasing these numbers every year. The Galicia–North Portugal Euroregion has a particular demographic ageing problem within Europe, due to the loss of population and decrease of new births. Therefore, more efficient and less expensive cancer detection methodologies can provide a significant benefit for population of Galicia.

This proposal combines three key proofs-of-concept from previous work and a team with complementary expertise to achieve its goals. The understanding of chiroptical properties and synthesis or powerful chiroptical systems at UVigo (J.L. Alonso-Gómez) combined with the expertise of F. Klappenberger (TUM) in Scanning

Tunneling Microscopy already enabled the construction of Upstanding Chiral Architectures with tremendous potential for sensing applications. This team is now complemented by a SERGAS-CHUS expert (R. Couceiro) on cell-free circulating tumor DNA detection, an UVigo expert (S. Chiussi) on the production of thin film semiconductors, biomaterials and reprivatized substrates , and INL (D.Y. Petrovykh) with extensive competence in the design and characterization of functionalized surfaces. The combined complementary expertise of this new team, therefore, opens possibilities for the development of unique and novel biosensors.
Validation of the chiroptical transduction mechanism for biosensing in this project will enable a medium-term impact in personalized medicine, e.g. via integration (after appropriate miniaturization) into multi-modal biosensing platforms. We stress, however, that the DNA detection, as proposed in this project, is often used as proof-of-principle validation of novel biosensing approaches in complex environments. Accordingly, the long-term impact of this project will be in establishing the basis for unique and novel biosensing modalities that exploit chirality of UCAs for biorecognition of hard-to-detect targets that are particularly relevant for ageing population, such as protein structures related to Alzheimer's disease or viral particles.

D. **Alignment with INL Research Areas:** Development of biosensors is a critical component of INL strategy in two of the laboratory's main research areas: *nanomedicine and food and environmental security*. Including complementary sensing/transduction modalities is a promising strategy for addressing complex biosensing challenges via integrated multi-modal platforms. INL's expertise and infrastructure will be important not only for characterizing the materials developed in this project, but also for guiding the design and development of these materials in the context of integration with existing and future device platforms.

E. **Expected Output:** The proof-of-principle for chiroptical sensing with biofunctionalized surfaces will be established, which will open opportunities for using chiral monolayers for unique biosensing modalities. Additionally, opening a new field of this relevance will be of ultimate importance for the application of an ERC consolidator grant by the PI in 2017, and certainly will open opportunities for international funding.

F. **Impact:** *Impacts in the regional economy:* As indicated above, the primary expected impacts of this project in the medium and long term will be via establishing the basis for unique personalized point-of-care diagnostics that will be particularly suitable for diseases that are strongly affecting the ageing population of the Galicia-North Portugal Euroregion.
Creating and/or enhancing international collaboration: The team of this project incorporates a fruitful existing collaboration between J.L. Alonso-Gómez (UVigo, Spain) and F. Klappenberger (TUM, Germany); the results from this collaboration provide the basis for the proposed project. Incorporation of R. Couceiro (SERGAS, Spain), S. Chiussi (UVigo, Spain), and D.Y. Petrovykh (INL) will not only re-enforce the existing collaboration, but also will ensure the success of this project thanks to the synergy and complementarity among the partners.
Improving Innovation Capacity and the integration of new knowledge in the industry: This proposal has a clear target: development of unique and novel biosensors. Significant research and development at both fundamental scientific and technological levels will be required to build the S&T capacity in this area to the level appropriate for transferring to the biomedical device industry. While project is based on previously demonstrated principles, their combination and the proposed area of applications are highly novel therefore, technological development will be complemented by producing new fundamental knowledge and rational design principles in chirality, surface imaging, surface preparation, and sensing devices.

G. **Strategy for Valuation and Transfer:** The main deliverables of this project will be publications in scientific journals and presentations at international conferences. Appropriate IP protection and contacts with industry will be a priority throughout the project to ensure that the design and technology development resulting from the project will have a high potential for being transferrable to biomedical device industry. A workshop will be held to bring together interested partners from academia and industry.

PROJECT BUDGET

	BUDGET CATEGORY	COST		
		2015	2016	TOTAL
1.0	**Personnel costs**			
	Ph.D two years student for design and synthesis of chiral compounds UVigo	18.000	18.000	36.000
	Post-doc one year surfaces preparation at UVigo	15.000	15.000	30.000
2.0	**Contracts/Technical assistance**			
	— *INL Contracts*	35.000	35.000	70.000
	— *Training*			
	— *Consultancies*			
	— *International collaborations*			
	— *Technology Transfer activities*			
3.0	**Materials & Supplies**			
	Chemicals and solvents for synthesis UVigo	15.000	15.000	30.000
	Molecular Biology CHUS	6.000	10.000	16.000
	Cell Culture CHUS	5.000	9.000	14.000
	Surface preparation at UVigo	7.000	7.000	14.000
	Surface characterization at INL	2.000	2.000	4.000
4.0	**Equipment**			
5.0	**Travel and subsistence costs** *(Research stays, International collaborations, joint activities, meetings…)*			
	Travels between Spain-Portugal-Germany and conferences	6.000	8.000	14.000
6.0	**Other costs** *(Indicate)*			
	TOTAL	109.000	119.000	228.000

PROJECT IMPLEMENTATION / WORK PLAN

Activity 1	Timeframe
UVigo - J.L. Alonso-Gómez; Design and synthesis of the chiral systems.	Months 1-8
Chiroptical characterization in solution.	Months 6-8
TUM - F. Klappenberger; Low temperature STM measurements, comparison with the already known UCA	Months 3-8
MD simulations to characterize in detail the architecture of the new UCAs. Thermal stability studies.	Months 6-8
Results from the first results at TUM will be essential for the design of more stable UCAs. Therefore new synthesis at UVigo will be conducted and studied at TUM in a feedback loop along the project.	Months 6-24
This milestone will provide the basis for the following milestones, since the most promising chiral molecules will be used for the next parts.	
Milestone: Stable UCAs constructed in Ultra-High Vacuum	**Projected Date: 8th Month**
Activity 2	**Timeframe**
UVigo - S.Chiussi; workshop will be held to bring together interested partners from academia and industry.	Months 7-8
Transparent surfaces functionalized with the most stable UCAs from Milestone 1.	Months 8-12
Thermal stability studies for conventional thermal and ultrafast laser assisted thermal treatment. Optimization of monolayer formation.	Months 12-16
INL - D.Y. Petrovykh; Comprehensive structural, compositional, and optical characterization of the cm^2-sized transparent metal films with UCAs using high-resolution spectroscopy and microscopy. Comparison to results from TUM.	Months 8-16
UVigo - J.L. Alonso-Gómez; Synthesis of chiral molecules bearing an imidazole group.	Months 8-12
Chiroptical characterization of the UCAs, study of the chiral amplification.	Months 10-16
Comparison with the responses in solution and the pH sensitivity of UCAs functionalized with imidazole.	Months 12-16

TUM - F. Klappenberger; Low temperature STM measurements, comparison with the stable UCAs from Milestone 1 and the imidazole derivatives. MD simulations to characterize in detail the architecture of the new UCAs. Establishment of AFM characterization and investigation of molecular forces on the single-molecule level.	Months 12-16
Milestone: RT-Stable UCAs constructed in normal conditions presenting chiral amplification and pH sensing	**Projected Date: 16th Month**
Activity 3	**Timeframe**
CHUS - R. Couceiro; Design of the most appropriate DNA probes for the combination with the most stable UCAs.	Months 10-20
Comparison of chiroptical biosensing with state-of-the-art methodologies.	Months 20-24
UVigo - S.Chiussi; construction of transparent metal films functionalized with the most stable UCAs intercalating DNA probes.	Months 16-20
INL - D. Petrovykh; Comprehensive structural, compositional, and optical characterization of the functionalized chiroptical transparent surfaces using high-resolution spectroscopy and microscopy. (Month 16-20)	Months 16-22
UVigo - J.L. Alonso-Gómez; Chirotical characterization of the UCAs intercalating DNA probes and biosensing evaluation in collaboration with **CHUS** and **INL**.	Months 18-24
Dissemination of results by preparation of manuscripts for relevant scientific articles and assistance to international conferences.	Months 4-24
Organization of a congress related with the chirality in surfaces and chiroptical sensing	Months 20-24
Milestone: UCAs functionalized with DNA probes; DNA hybridization detection using biofunctionalized UCAs	**Projected Date: 24th Month**

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