University of Burgos (UBU) is the TAT-CF project coordinator. The principal investigator at UBU is Roberto Quesada, professor in the department of Chemistry. The main role of UBU is to synthesize the compounds to be tested in TAT-CF project. Key staff at UBU are pioneers in the field of development and structure-activity relationships analysis of transmembrane anion transporters.

Steinbeis-Innovationszentrum Zellkulturtechnik

SIZ Zellkulturtechnik (Mannheim, Germany) belongs to the Steinbeis gGmbH (Stuttgart, Germany) and is a private research institute. In the frame of the TAT-CF project we develop genetically encoded fluorescent indicators for chloride and bicarbonate using our proprietary IGAMI technology.

The Biophysics Institute at Genova is part of the Italian National Research Council (CNR). The group, led by Oscar Moran, is responsible for the biophysical characterization of the mechanism of the new anionophores, and for the characterization of anion transport in living cells.

Istituto Giannina Gaslini (IGG) is one of the partners in the TAT-CF project, with long lasting experience in studying cystic fibrosis pathophysiology and pharmacology. The group, belonging to the Medical Genetics Unit, is responsible for performing the primary screening and participates in the investigation of the biophysical and physiological properties of the new compounds.

bioneer:FARMA

Bioneer Pharma (BIONEER A/S), owned by the Technical University of Denmark, is a non-for-profit, R&D based company that delivers services to pharmaceutical and biotechnological companies.
BIONEER A/S will characterize the anionophores developed in TAT-CF project and will assess their transport across cell culture. Furthermore, BIONEER A/S will evaluate efficacy and will carry out studies of ADME-tox, bioavailability, biodistribution and PK profiles of lead compounds as well as develop suitable drug delivery systems.

Praxis Biopraxis Research

Biopraxis Research AIE (BIOPRAXIS) was constituted to drive and support all the research and innovation activities for Praxis Pharmaceutical Group.
BIOPRAXIS will participate in compound efficacy studies and in the assessment of their antimicrobial activity. BIOPRAXIS will also be in charge of Structure-Activity-Relationship (SAR) studies and of the development of the optimal formulation and drug delivery system for the active compounds. Additionally, BIOPRAXIS is the leader of Dissemination and Exploitation activities.

AVIDIN BIOTECHNOLOGY

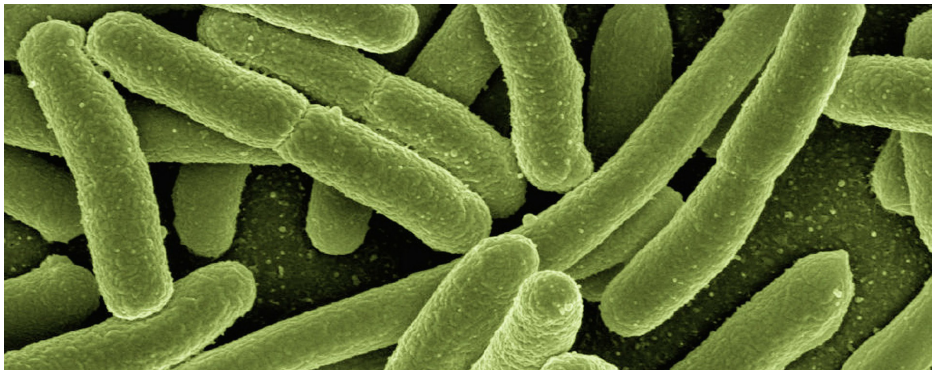
Avidin is the leader of WP4 (Medicinal Chemistry), in which the company coordinates the structure-activity relationship studies and evaluation of the drugability of potential anionophore compounds along with in silico optimization of selected hits, pharmacophore identification and novel synthetic modifications supporting WP1 (Chemistry) to improve the drugability of lead compounds. Our company will also participate in WP5 (Preclinical studies), where Avidin will determine ADME-tox, bioavailability, biodistribution and PK/PD in animal non-GLP studies. In WP6 (Drug delivery and formulation) Avidin will participate in determining the optimal formulation of the lead transporter compounds.

CSIC

The Consejo Superior de Investigaciones Científicas (CSIC) is the largest public research organization in Spain, which is distinguished by its multidisciplinary nature, approaching all knowledge areas, from basic research to topmost advanced technological developments.
The CSIC group will be responsible for the ex-vivo and in-vivo preclinical experiments. The group will also be in charge of producing the iPS cell line collection from patients with different mutations and genetic backgrounds.

tat-cf

Toward a new cystic fibrosis therapy



The threat of antimicrobial resistance

During the last decades an increasing ability of microorganisms to resist to the major antimicrobials used has been recognized *(EC COM (2011) 748)*. This phenomenon, neither surprising nor new, is becoming a global public health problem, as antimicrobial resistance **(AMR)** accumulates and accelerates in time. AMR is referred to microbes that have acquired the ability to survive exposures to clinically active concentrations of drugs that would kill otherwise sensitive organisms of the same species. Although resistance could be regarded as a natural biological phenomenon, it has been enhanced by a wide variety of human factors (misuse of human and veterinary antimicrobials, and their illegal use as growth promoters in livestock). These are the driving forces of the selective process, only the "best" drug resistant pathogens survive, multiply and spread. The effectiveness of current antibiotic treatments has been seriously jeopardized, entailing morbidity and mortality increases, as well as higher health care expenditures.

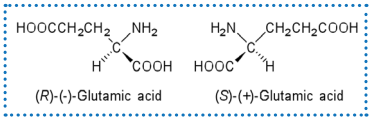
In addition, recent studies carried out by the WHO on the antibiotic development pipeline have proven that **there is a deep lack of development of novel antimicrobial treatments** (ECDC/EMEA Joint Tech. Report, 2009; Bulletin of the World Health Organization 2016). This situation is partly caused by low investment in R&D activities related to the discovery of novel antimicrobials, as these have been considered as a less attractive market niches by major pharmaceutical companies, which have prioritized the development of drugs with better economic return. The European Union has boosted an in R&D in the EU and worldwide, including H2020 and IMI EU research programs (e.g. New Drugs for Bad Bugs), as antimicrobial resistance represents a major threat to the success of almost all branches of medical practice, jeopardizing the efficacy and future sustainability of public health systems. Furthermore, in addition to the dreadful human losses (25,000 each year), this results in disturbing overall societal costs (extra healthcare costs and productivity losses) of at least 1.5 billion Euros in the EU.

This situation also affects Cystic Fibrosis patients in a severe way. CF patients develop respiratory tract infections with several degrees of resistance. Once infected, patients cannot easily clear the microorganisms, which critically injuries lungs, resulting in the most significant cause of death for CF patients.

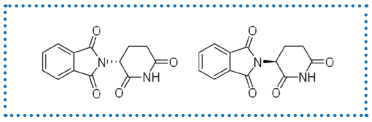
We, as society, need innovative approaches to tackle this challenge. These new solutions may be based on the use of nature-inspired substances (i.e. bacteriophagues), new presentation of existing drugs (i.e. nanoformulations) or small molecules with a radically new mode of action, which differs from those which are common to other treatments, making the rapid development of resistance mechanisms more difficult. This could be the case of the compounds developed by TAT-CF project which will be presented in the next issue.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 667079.

Chirality in Drug Research

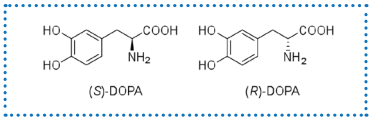


Two molecules are considered enantiomers if they share the same chemical composition, have four different substituents attached to a central carbon atom (called a chiral carbon atom or a centre of asymmetry), but they cannot be superimposed on their mirror images. The presence of one or more centres of asymmetry results in optical activity of enantiomers. If the enantiomer changes the rotation of the plane of polarized light clockwise, it is marked as (+) and if an anticlockwise change is observed, the symbol (-) is used.[1-3] Glutamic acid, a simple mono acid and neurotransmitter, naturally occurs as the (*S*)-(+)-enantiomer, but the (*R*)-(-)-enantiomer can be achieved by means of asymmetric synthesis.[3] The symbols (*R*) and (*S*) are used alternatively with the symbols D and L. An equimolar mixture of two enantiomers is called a racemic mixture (racemate), is optically inactive and is denoted by the symbols (*RS*), DL or (±).[1, 2].

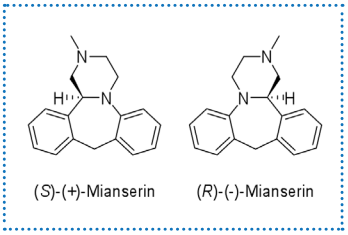


Although enantiomers of a chiral molecule are indistinguishable in an achiral environment due to displaying identical physical and chemical properties, they might display very different biological activities in living organisms that are themselves chiral systems. Taking into consideration the above mentioned and the fact that approximately 50% of drugs available on the market are chiral, and about half of these are mixtures of the (*R*)- and (*S*)-enantiomer, from a pharmacological point of view it is crucial to determine biological activities of both isomers at the early stage of the drug development program.[1]

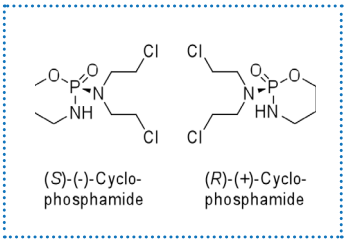
The terrible thalidomide tragedy observed at the turn of the 1950s and 1960s highlights the importance of research on biological activity of each of enantiomers. Both (*R*)-(+)-thalidomide and (*S*)-(-)-thalidomide exhibit similar sedative activity in animal models, but only the (*S*)-(-)-enantiomer is strongly teratogenic. This was unknown when thalidomide (THA) was introduced as a racemic mixture to the market in 1956.[3] Numerous cases of neuropathies in females and their newborns lead to the withdrawal of the drug from the market in 1961. Unfortunately, its fatal effects are observed, especially in the countries of the Western Europe, until today.



Stereospecific toxicity of DOPA, the precursor of dopamine (DA), the neurotransmitter deficient in the brains of the patients suffering from Parkinson´s disease, has been attributed to the (*R*)-enantiomer. In the early 1960s (*RS*)-DOPA was administered to parkinsonian patients, the therapy was found beneficial, but severe side effects like granulocytopenia were observed. Now (*S*)-DOPA, more commonly known as L-DOPA, is used exclusively for the treatment of parkinsonism.[3]



Mianserin (MIAN) is an atypical tetracyclic antidepressant available as a racemic mixture, but its activity lies mainly in the (*S*)-(+)-enantiomer that is 200-300 times more active as an norepinephrine (NE) reuptake inhibitor (NRI) than the (*R*)-(-)-enantiomer. Moreover MIAN inhibits reuptake of dopamine (DA) and serotonine (5-HT) weakly, is an antagonist of presynaptic α_2-adrenergic receptors, and a potent antagonist of histamine and serotonine receptors. Antidepressant activity of mianserin is clearly enantioselective as the (*S*)-(+)-enantiomer is the one that blocks presynaptic α_2-adrenergic receptors predominantly. Interestingly, sedative effects of the drug attributed to its antihistaminic activity are non-enantioselective. MIAN is marketed as a racemic mixture in order to balance antidepressant activity that resides mainly in the (*S*)-(+)-enantiomer and sedative effects caused by both enantiomers.[3]



From a structural point of view cyclophosphamide (CP), a well-established anticancer drug, is a very interesting molecule as the reason for its chirality is not a carbon atom, but a phosphorus atom. Although both (*R*)-(+)-cyclophosphamide and (*S*)-(-)- cyclophosphamide have the same LD_{50}s, the (*S*)-(-)-enantiomer was found to be twice as effective as the (*R*)-(+)-enantiomer in killing tumour cells. As a result, the therapeutic index for (*S*)-(-)-CP is about two times greater than that for (*R*)-(+)-CP and it is also 1.3 times higher than that for the racemic mixture. These observations indicate that it would be better to use the (*S*)-(-)-cyclophosphamide as a less toxic antitumour drug.[2]

Drug enantiomers often not only exhibit different pharmacodynamic activities, but also different pharmacokinetics (absorption, elimination, distribution, metabolic and renal clearance). Moreover, enantiomer-enantiomer and enantiomer-other drug interactions might be observed.[2]

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2. Williams, K.; Lee, E. Drugs 1985, 30, 333.

3. Coutts, R. T.; Baker, G. B. Chirality 1989, 1, 99.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 667079.

Biosensor cell line for measuring bicarbonate transport activity of anionophore compounds

Cystic fibrosis (CF) is a genetic disease that results in the accumulation of thick mucus that does not clear properly from the lungs, intestine, and most exocrine glands. The disease is caused by a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. In normal CFTR expressing epithelial cells chloride and bicarbonate are transported at high rates across the plasma membrane. In mild forms of cystic fibrosis bicarbonate transport is significantly diminished. CFTR mutations resulting in more severe forms of CF do not support bicarbonate transport but, surprisingly, may support chloride transport. Therefore, the severity of CF disease correlates better with defects in bicarbonate transport than with defects in chloride transport[1]. The effects of a defective bicarbonate secretion on the maturation of the mucus were overseen until Quinton[1] and others[2] provided evidence that bicarbonate was essential for proper mucus release and mucin expansion. The maturation of mucus is a complex process. Before being secreted highly negatively charged mucin polymer molecules are tightly packed at low pH and high calcium ion concentration within intracellular granules. The negative charges are shielded by protons and calcium ions. During mucin exocytosis secreted mucin undergoes a maturation process in which the volume changes as much as 100-fold within seconds[3]. The poor solubility of calcium bicarbonate and the proton binding activity of bicarbonate support the removal of calcium ions and protons from the mucus matrix. It was demonstrated that bicarbonate plays a critical role in determining the rheology of mucus by controlling swelling most likely by removal of calcium ions, which aggregates mucus polymers by divalent cross-linking[4].

TAT-CF develops small anionophore compounds that are able to transport bicarbonate and chloride anions across the epithelial membrane complementing for the defective CFTR ion transport activity. For the measurement of intracellular bicarbonate concentration changes a biosensor cell line was developed. This cell line expresses a genetically encoded sensor and reporter. The sensor senses bicarbonate resulting in a generation of cAMP which is reported by a cAMP-dependent fluorescent signal. The reporter CEPAC consists of a cAMP binding domain fused to a blue and a yellow fluorescent protein domain at the amino- and carboxy-terminal end respectively. After binding of cAMP to the reporter a conformation change results in an anti-FRET effect (Figure 1). The change of concomitant fluorescent properties is measured by the fluorescence emission ratio (E480/E530). Based on this sensor cell line bicarbonate influx and efflux assays were developed for the characterization of the bicarbonate transport activity of several compounds developed during the TAT-CF project. Bicarbonate transport activities of several compound classes will be completed in the following months together with toxicity data. These activities will allow us to select final compounds with bicarbonate transport activities and tolerable toxicity.

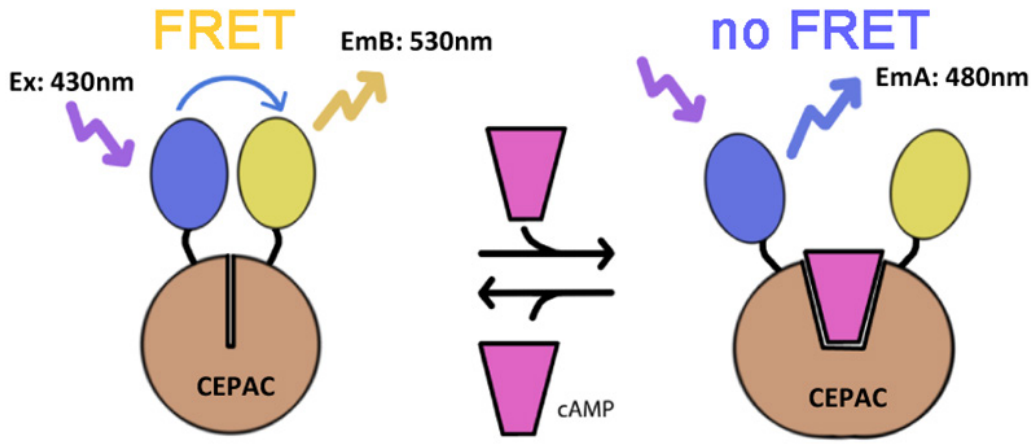

Figure 1 cAMP Reporter CEPAC

Without cAMP the distance between the N- and C-terminal located blue and yellow dye proteins allows a Förster resonance energy transfer (FRET) from the donor (blue protein) to the acceptor (yellow protein) resulting in an emission of yellow light after excitation of the blue dye. After cAMP binding the distance increases and FRET is no more possible. In that case no yellow emission follows excitation of the blue dye. The fluorescence ratio (Em480/Em530) increase follows directly an increase of the cAMP concentration.

[1] *Quinton PM. The neglected ion: HCO^3 Nat Med 2001;7:292–3.*

[2] *Choi JY, Muallem D, Kiselyov K, Lee MG, Thomas PJ, Muallem S. Aberrant CFTR-dependent HCO^3 – transport in mutations associated with cystic fibrosis. Nature 2001;410:94–7.*

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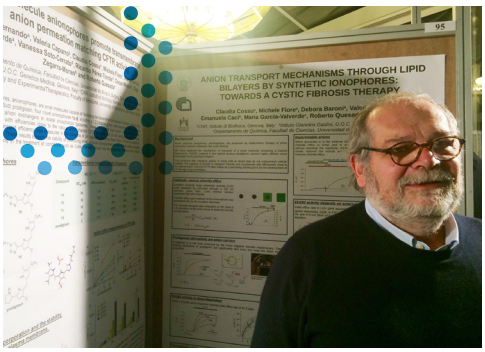
[4] *Chen et al. A new role for bicarbonate in mucus formation. Am J Physiol Lung Mol Physiol 2010; 299:L542-L549.*

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 667079.

Next project meeting and Workshop in Budapest

TAT-CF will hold its next periodic meeting in June 2018. The meeting of the Steering Committee will include the review of the project progress, milestones and planning of actions for the last six months together with the fourth TAT-CF Workshop. During this workshop there will be scientific presentations by TAT-CF partners to enhance the collaboration among partners, contribute to the project progress and reinforce the unity of the consortium and serve as learning experience for TAT-CF recruited researchers, at an operational level, highlighting the work of young researchers. The project will enter into its last six months of development, so crucial decisions will be taken in order to speed up the project and to get all the planned aims. The meeting will be hosted by AVIDIN at Danubius Hotel Gellert in Budapest, Hungary, to facilitate logistics to the project partners.

TAT-CF at the European Cystic Fibrosis Society (ECFS) Basic Science Conference



The 15th ECFS Basic Science Conference organized by the European Cystic Fibrosis Foundation was held in Loutraki, Greece, from 21st to 24th of March, 2018. The TAT-CF team was represented by members of Universidad de Burgos, Istituto Gianina Gaslini and Istituto Biofisica Italiano. Some of our latest results were disseminated and two posters ("Small molecule anionophores promote transmembrane anion permeation matching CFTR activity" and "Anion transport mechanisms through lipid bilayers by synthetic ionophores: towards a cystic fibrosis therapy") successfully presented, attracting a good deal of attention. The conference clustered more than 200 scientists from around the world to deliver lectures and discuss their work. Symposiums on CFTR Expression, Folding, Trafficking and Activity, Cell Physiology and Ion Transport, Model systems, Mucus and Mucins, Inflammation, Host-pathogen interactions, Therapeutic Approaches and Translational CF Research provided the latest developments in the field. Encouraging news about new advances of combo therapies addressing the underlying cause of cystic fibrosis (CF), which is the functional defect of the cystic fibrosis transmembrane conductance regulator (CFTR) protein owing to mutations in the CFTR gene, were presented. TAT-CF strategy aiming at a mutant independent therapy was highlighted because of its potential to treat mutations resulting in no CFTR being produced.

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