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Tagging 3-Hydroxy-4-Pyridinone Iron Chelators with Rhodamine B Derivatives is Essential to Target Mycobacterium Avium Infection
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The increasing development of bacterial resistance to traditional antibiotics has reached alarming levels and a strong effort to develop new antimicrobial agents is imperative. The process of iron acquisition represents a pathway, which can be successfully targeted by novel therapeutic tools and iron restriction has been shown to improve the outcome of a number of infectious diseases.
Our group designed a new type of tripodal iron chelators which incorporate fluorescent labels that allow following its pathways within the cell. The results demonstrate that these chelators can limit the access of iron to bacteria and have a significant inhibitory effect on the intramacrophagic growth of M. avium. Furthermore, it was found that chelation of iron is a determinant but not sufficient property for antimicrobial activity. 1,2.
In order to identify key molecular features essential for biological activity we designed parent hexadentate and bidentate chelators, in which different structural groups are introduced in the molecular framework. The results show that rhodamine B isothiocyanate labeled chelators exhibit the highest biological activity and suggest that the high affinity of rhodamine B towards lipid phases may be determinant to situate the chelator in a favourable position to compete with mycobacterial siderophores.
The work developed in model membranes using fluorescence and electron paramagnetic resonance spectroscopies will be rationalized from the chemical, biophysical and biological points of view.
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1. Rangel, M. et al, J. Biol. Inorg. Chem., 2010, 15, 861.
2. Gomes, M.S. et al Microbes and Infection, 2010, 12, 287.

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Langmuir Monolayers of Bacterial Outer Membranes
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The increase of Gram-negative bacteria super-resistant to antibiotics is a major problem in medicine. These bacteria possess a double membrane system, with the external leaflet of the outer membrane composed of lipopolyssaccharides (LPS) that act as the first barrier to drugs. Thus, bacteria's susceptibility to lipophilic antibiotics is highly correlated with the permeability of this LPS barrier. In this study, we extracted LPS from two isogenic Salmonella sp strains, using the method of Galanos. These strains contain alterations in the lipid A portion of their LPS regulated by the PhoPQ-system. These modifications include palmitoylation of the 3-hydroxyl group in the 3-OH-myristyl residue, addition of 4-aminoarabinose to the 4' phosphate group and addition of a 2-hydroxy group to the myristate residue at the position 3'. Langmuir monolayers were formed of both LPS and compression isotherms indicated that PhoP- (LPS from the strain with no modifications) occupies a limiting area of 150Å2, while for PhoPc (LPS from the strain with the modifications cited above) this value is 175Å2. These are in agreement with previous report for other Salmonella sp LPS. Epifluorescence microscopy reveals PhoP- has a very homogeneous, fluid-like, morphology throughout the compression without discrete domain formation. In contrast, PhoPc displays circular-shaped domains that increase in size and number with the compression. The addition of novobiocin (0.4 μg mL-1) to the subphase promoted slight changes in PhoP- morphology, suggesting that this monolayer was already fluid enough to incorporate this antibiotic. For PhoPc monolayer, changes in morphology were observed only for high concentrations (2.5x) of novobiocin at which the domains started to aggregate generating structures like a ''pearl necklace''.

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Structure of Glycocalyx
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Glycocalyx is a highly charged layer of membrane-bound biological macromolecules attached to a cell membrane. This layer functions as a barrier between a cell and its surrounding. Glycocalyx also serves as a mediator for cell-cell interactions and protects a cell membrane from the direct action of physical forces and stresses allowing the membrane to maintain its integrity. Glycocalyx is also involved in development and progression of many diseases. Glycocalyx is composed of glycosaminoglycans, proteoglycans and other glycoproteins bearing acidic oligosaccharides and terminal sialic acids. Most glycocalyx associated proteins are transmembrane that can be linked to the cytoskeleton. This linkage not only restricts their position and constitutes the foundation of the glycocalyx structure, but it also allows signal transduction from the external to the internal parts of a cell. Here we focus on the endothelial cells' glycocalyx.
The aim of this project is to employ Molecular Dynamics simulations to gain insight into the molecular structure and functions of the glycocalyx. This choice is justified by the fact that experimental studies of the glycocalyx are to a large extent restricted by its dynamic and soft nature driven by thermal fluctuations, implying that the glycocalyx has no unique state or structure but it instead is expected to fluctuate significantly in time. Only recent progress in the development of molecular simulation techniques and the availability of larger computer resources have fostered studies of complex biological systems such the glycolalyx itself.

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Bacterial Signal Indole Modifies the Physicochemical Properties of Lipid Membranes
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Indole is a biological molecule produced by more than 85 bacterial species. It acts as an inter-cellular signal, influencing multiple aspects of bacterial physiology and has proved to be an important factor in the transition to stationary phase. It also promotes resistance to a range of drugs and toxins and is involved in preventing plasmid instability. Aspects of bacterial ecology and host-pathogen interactions which respond to indole include biofilm formation and the expression of virulence factors.
As indole is widely affecting cellular organisms here we study the effect of indole on the physicochemical properties of the lipid membrane. We use the intrinsic florescence of the indole molecule to show that indole is freely diffusing through the lipid membrane barrier. Using electrophysiology we show that the indole is able to collapse membrane potential. We also show that the rate of mitochondrial oxygen consumption increases when the indole concentration is raised from 0 to 1mM. Therefore our results demonstrate that indole is capable of lowering the energetic barrier for proton permeation across lipid membranes. Concluding from these results to obtain an enhanced protonophoric activity we proposed a point mutation on the indole molecule 4F-indole. Confirming our interpretation on the mechanistic details of indole induced proton transport, 4F-indole is two time more efficient in uncoupling the oxidative phosphorylation in isolate mitochondria. Further using light spectroscopy we find the partition coefficient for indole into lipids in aqueous environment.Then using differential scanning calorimetry we show that indole modifies the phase transition of *Escherichia coli* cell membranes. These findings offer an explanation for the multiple roles that indole has in membrane biology, bacterial cell cycle control and potentially for the design of antibiotics that target the cell membrane.

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Critical Temperatures in Plasma Membrane Vesicles are Dependent on the Cell Cycle
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Giant plasma membrane vesicles (GPMVs) isolated from RBL-2H3 cells appear uniform at physiological temperatures, contain coexisting liquid-ordered and liquid-disordered phases at low temperatures, and experience micron-sized critical fluctuations close to their critical temperature. We observe a broad distribution of critical temperatures in GPMVs isolated from a dish of seemingly identical cells even though each individual vesicle has a well defined critical temperature. In addition, we observe that the average transition temperature of GPMVs isolated from a population of cells is inversely proportional to the surface density of cells growing in the culture dish. Since it is known that cellular doubling times are reduced in more densely plated cells due to contact inhibition, we hypothesized that critical temperatures are linked