Title: Biocatalytic Nanobots: Modulating Oxidative Stress in Cellular Models of Inflammatory Bowel Disease

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Introduction: Inflammatory bowel disease (IBD) is a chronic disorder marked by elevated levels of reactive oxygen species (ROS), which exacerbate local inflammation and lead to intestinal mucosal damage (1). Intelligent nanotechnologies, such as biocatalytic nanobots (NBs), offer targeted strategies to reduce oxidative stress (2). This study investigates a self-propelled NB designed to neutralize extracellular ROS and maintain biocompatibility with colonic cells, paving the way for future integrations of bioactive compounds to address intracellular stress further.

Methods: The NB core was fabricated using chitosan via ionic gelation with sodium tripolyphosphate (TPP) as the crosslinking agent. A biotin-avidin coupling system was used to anchor catalase onto the NB surface, promoting the decomposition of hydrogen peroxide (H_2O_2) into oxygen (O_2) and enabling propulsion. Mobility was assessed via fluorescence microscopy in ultrapure water (control) and H_2O_2 solutions (30 µM and 300 µM), with the analysis of the mean square displacement (MSD), mean squared speed (MSS), diffusion coefficient, average distance, trajectory bending, and displacement efficiency. Biocompatibility was evaluated on co-culture of colon cell lines (Caco-2 and HT29-MTX), while extracellular and intracellular ROS levels were quantified via the Pierce Quantitative Peroxide and DCFDA/H2DCFDA assays, respectively.

Results: The NB exhibited increased propulsion in H_2O_2 solutions, with the MSD as a function of time, highlighting a significant difference in mobility favoring H_2O_2. The comparison of the MSD's intersection and slope reinforced the improved mobility under H_2O_2 conditions. The MSS did not indicate differences between H_2O and H_2O_2. There was a noteworthy increase in the diffusion coefficient in H_2O_2. The particles traveled a markedly greater distance in H_2O_2, confirming better directional movement in this medium. The trajectory curvature was not different, suggesting similar randomness in movement patterns across both media. The displacement efficiency was significantly greater in H_2O_2, indicating more direct movement from the origin. These findings confirmed robust movement in ROS-rich environments. The NB displayed no detectable cytotoxicity in co-culture of Caco-2 and HT29-MTX cells, and the reduction of extracellular H_2O_2 correlated with a significant decrease in intracellular ROS. This underscores the indirect impact of the neutralization of extracellular ROS on intracellular oxidative stress.

Conclusions and Impact: This self-propelled, biocatalytic NB platform offers a powerful approach to addressing oxidative stress in IBD, operating at the extracellular level while maintaining cell viability. Enhanced propulsion in ROS-rich conditions positions these NBs for site-specific applications in inflamed tissues. Future developments will include bioactive payloads to target intracellular ROS, thereby expanding this technology's therapeutic potential for advanced IBD management. Ongoing work will incorporate additional modules to enhance stability, guide navigation, and broaden therapeutic functionalities.

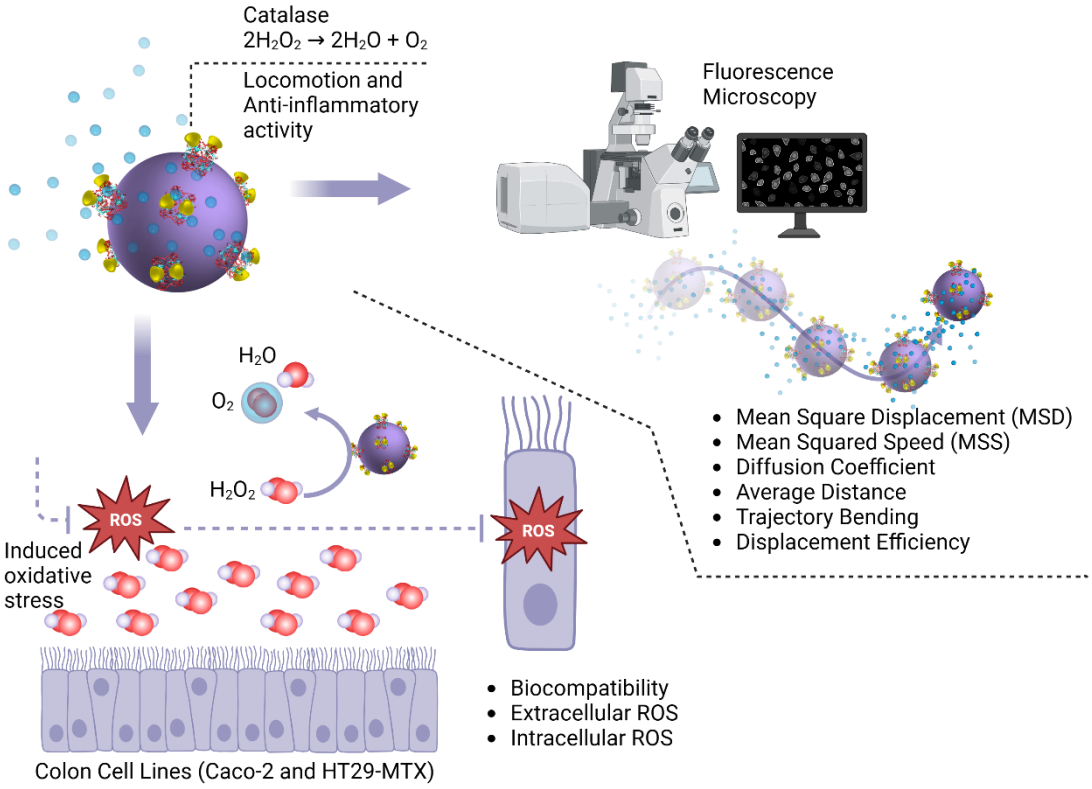

Figure 1. Mechanism of Polymeric and Biocatalytic Nanobot Action and ROS Responsiveness in a Cellular Model. The schematic illustrates the functioning of a nanobot designed to mitigate reactive oxygen species (ROS), thereby reducing oxidative damage at the cellular level. It includes the monitoring of the nanobot's locomotion and ROS-responsive activity using advanced imaging techniques such as fluorescence microscopy.

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Presenter biography: Dr. Ezequiel Coscueta, a Senior Researcher at the CBQF and an Invited Assistant Professor at the Faculty of Biotechnology, Universidade Católica Portuguesa, holds a Ph.D. in Biological Sciences and a B.Sc. in Biotechnology. His expertise spans green chemistry, peptidomics, and nanotechnology, with special emphasis on the nutraceutical sector. As the PI of the gBiOT project, he leads groundbreaking research in nanotechnological solutions for intestinal health.

Learning Objectives:

Describe the mechanism of biocatalytic nanobots that neutralize extracellular ROS.
Analyze the correlation between nanobot propulsion and its functionality in ROS-enriched environments.
Assess the biocompatibility and broader therapeutic potential of nanobots in colon cell models.