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gBIOT

Transforming Healthcare: Treatment of Inflammatory Bowel Disease with Biocatalytic Nanobots

Ana Sofia Sousa
André Mayer, Celso A. Reis, Maria Manuela Pintado & Ezequiel R. Coscueta

CRS 2025 ANNUAL MEETING & EXPOSITION
PHILADELPHIA, PA
JULY 13-18, 2025

NEXT-GENERATION DELIVERY INNOVATIONS

Gut Inflammation: *A Growing Silent Epidemic*

Inflammatory bowel disease (IBD)

IBD is a chronic **intestinal inflammation**

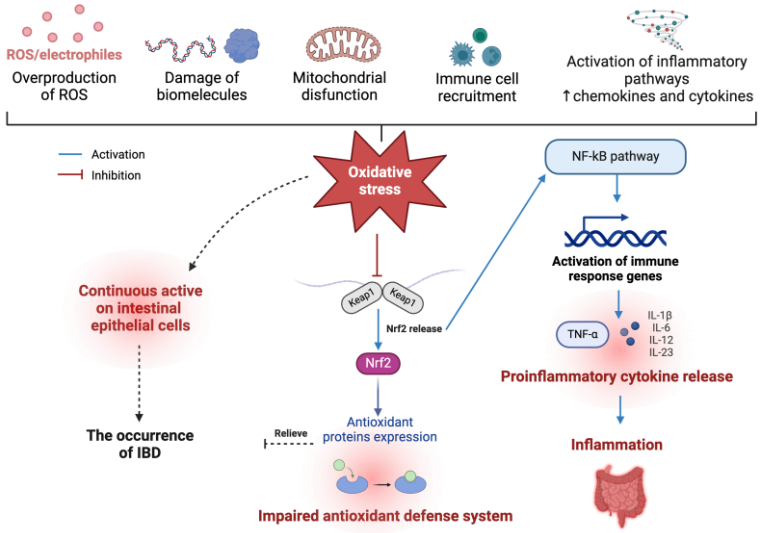
2 Subtypes: Crohn's disease and Ulcerative Colitis

Affects **6.8 million** people

Economic burden
IBD costs Europe over €4.6 billion annually in healthcare, with even higher hidden social costs.

Cancer risk
*Increased risk of **colorectal cancer** - one of the **deadliest** cancers*

How does oxidative stress drive IBD?



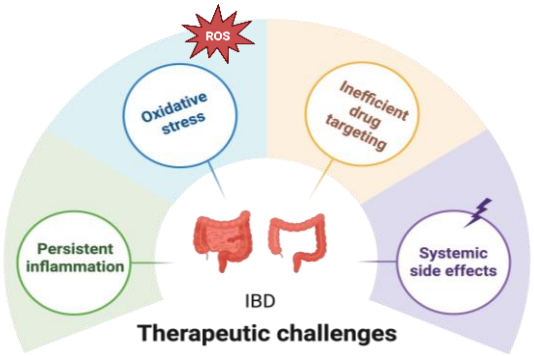
Although the exact **cause of IBD remains unclear**, **impaired gut immunoregulation** is crucial. In IBD, **oxidative stress increases**, with **excessive ROS** leading to tissue damage.

de Souza, H. *et al*. T. *Nat Rev Gastroenterol Hepatol* 2017, 14, 739–749
Muro, P. *et al*. Front Endocrinol 2024, 15.

Rethinking IBD Treatment

IBD Conventional Treatments

Corticosteroids
Aminosalicylates (ASA)
Immunosuppressants
Biologics



Treating IBD demands more than drugs — it requires **TARGETED, RESPONSIVE & SITE-SPECIFIC SOLUTIONS**.

Future

From conventional drugs to innovative nanomedicine

Nanomedice → **Nanotechnology** → **Drug delivery**

Ehrlich's "**magic bullet**" concept

Our approach

NANOBOTS (multifunctional nanoparticles):

Devices expected to do specific tasks at the atomic, molecular, and cellular levels: move, feel/manipulate their environment, and display intelligent behavior (perceiving the environment, processing perceptions, and responding to achieve an expected result).

Puccetti, M. *et al.* Pharmacol Res 2024, 201, 107086.
Andhari, S.S. *et al. Sci Rep 2020,* 10, 4703

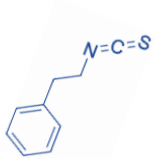
gBiOT Approach: Tiny Tools, Big Relief

AIM Developing a **biocatalytic** and **modular nanobot** design to overcome key therapeutic barriers in IBD:

- ✓ Scavenges ROS to **reduce oxidative stress**
- ✓ **Targets inflamed gut** areas with precision
- ✓ **Respond dynamically** to the complex inflamed gut environment.

A dual-action strategy combining **ROS scavenging** + **targeted delivery** to restore gut balance.

Crucial Cargo: PEITC (Phenylethyl Isothiocyanate) as an Inflammatory Modulator



Our work on PEITC related to gastrointestinal health


molecules

Review

Phenylethyl Isothiocyanate: A Bioactive Agent for Gastrointestinal Health

Ezequiel R. Coscueta [1,*], Ana Sofia Sousa [1], Celso A. Reis [2,3,4,*] and Maria Manuela Pintado [1]

Molecules **2022**, *27*(3), 794; https://doi.org/10.3390/molecules27030794


PLoS One. 2021 May 6;16(5):e0248257. doi: 10.1371/journal.pone.0248257. eCollection 2021.

Chitosan–olive oil microparticles for phenylethyl isothiocyanate delivery: Optimal formulation

Ezequiel R Coscueta [1], Ana Sofia Sousa [1], Celso A Reis [2 3 4], Manuela Pintado [1]

Affiliations + expand

PMID: 33956792 PMCID: PMC8101728 DOI: 10.1371/journal.pone.0248257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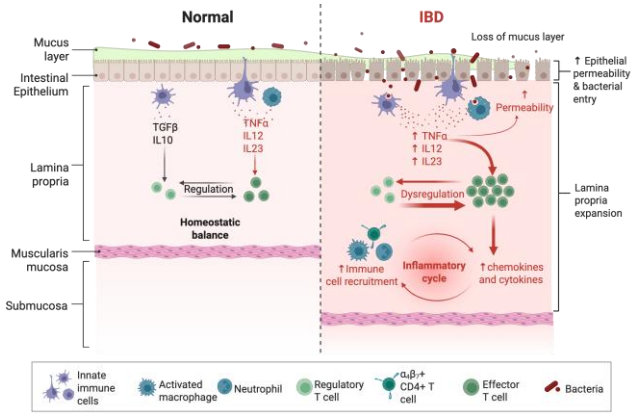
Effects of microencapsulated phenethyl isothiocyanate on gastrointestinal cancer cells and pathogenic bacteria

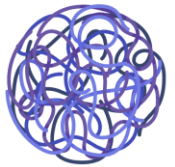
Rafaela Abrantes [a,b,c,1], Cátia C. Ramos [a,b,c,1], Ezequiel R. Coscueta [d,*], João Costa [d], Joana Gomes [a,b], Catarina Gomes [a,b], Celso A. Reis [a,b,c,e], Maria Manuela Pintado [d]

gBiOT Approach: Tiny Tools, Big Relief



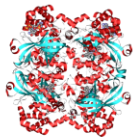
Active motion

Therapeutic bioactivity



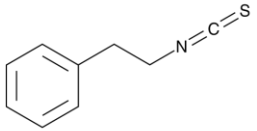
(CS/TPP)NP

+



Catalase

+



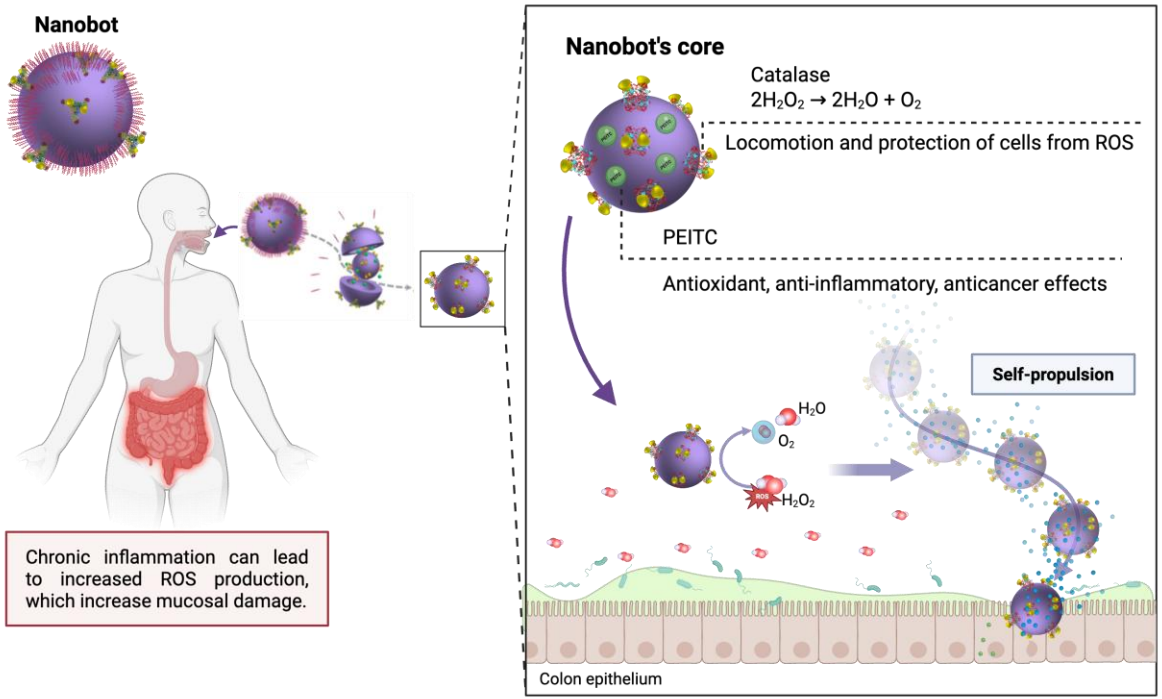
PEITC
(Phenylethyl isothiocyanate)

- *Anti-inflammatory*
- *Antioxidant*
- *Chemopreventive agent*

Ideal for IBD environments

- *Self-propulsion*
- *Improving mucosal penetration & retention*

ROS-responsive nanobots powered by catalase and driven by inflammation.



Chitosan as a strategic choice

WHY Chitosan?

The gut is not an easy place!

IBD mucus turnover is rapid → Without mucoadhesion, particles can be swept away by peristalsis or mucus clearance before reaching a lesion.

Chitosan sticks to mucus → **Local depot** on the colon wall.

The triple threat of chitosan: residence, adhesion, precision

Catalase binding using **glutaraldehyde**

IBD **ROS**

Catalase

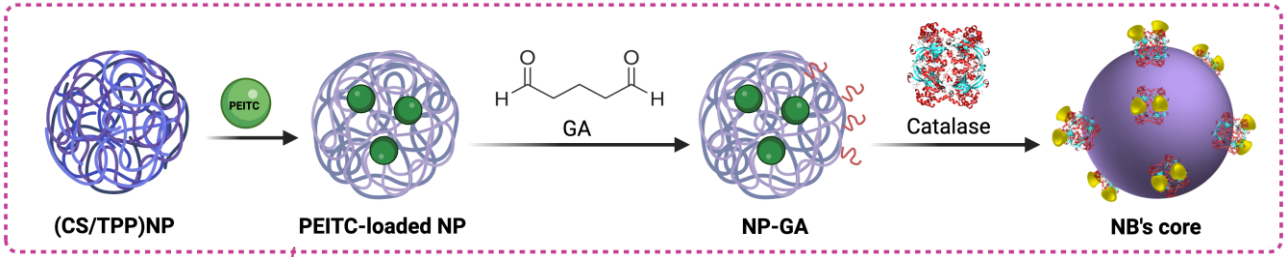
$2\ H_2O_2 \longrightarrow 2\ H_2O + O_2$

Hydrogen peroxide — Water — Oxygen

Enzyme-Driven Propulsion by O_2 microbubbles

Serra-Casablancas, M. *et al. ACS Nano* 2024 *18* (26), 16701-16714

Nanobot's core

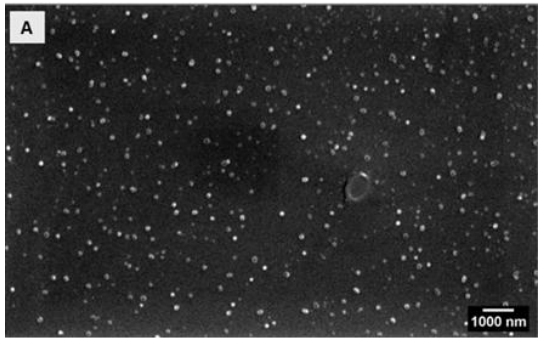


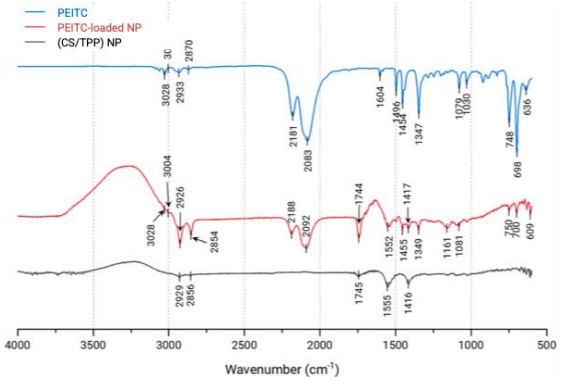
Encapsulation Efficiency (%) = 99.4 ± 0.1

Drug Loading (%) = 16.86% *(based on initial chitosan mass)*

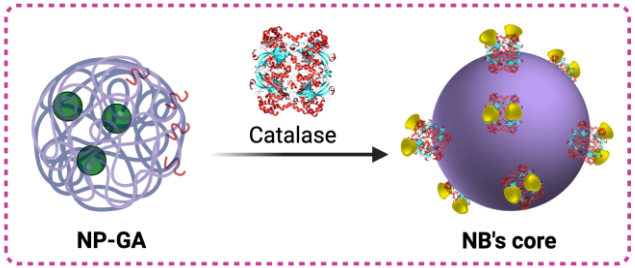
Sample	Size (nm)	PdI	ζ-potential (mV)
PEITC-loaded NP	214 ± 8	0.342 ± 0.020	43 ± 0.3
NP-GA	222 ± 26	0.247 ± 0.070	56 ± 17
NB's core	255 ± 3	0.274 ± 0.0090	23 ± 1

SEM image of NP





Nanobot's core functionality

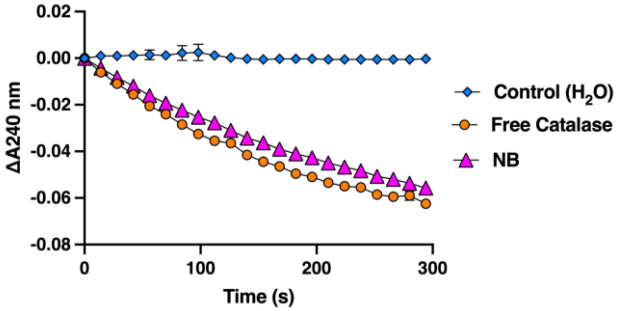


Catalase Conjugation Efficiency

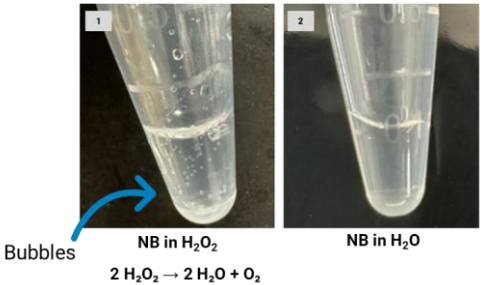
Catalase added	186 ± 9 µg/mL
Catalase conjugated	129 ± 15 µg/mL
Conjungation efficiency	**69 %**

Corrected for NP-GA background (BCA assay, n = 3).

Decomposition of H_2O_2 by catalase



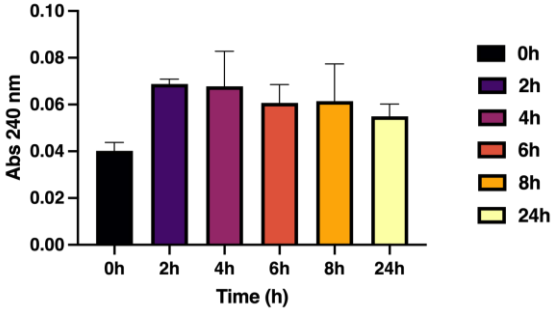
Visual evidence of oxygen being released during H_2O_2 decomposition



$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

Decomposition of H_2O_2 by catalase

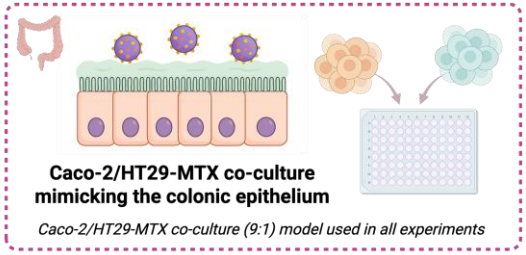
(Final Kinetic Point – H_2O_2 Degradation at pH 6.8, 37 °C)



Catalase remains enzymatically active throughout 24 h at colonic pH

Zhang, Y. et al. Appl Mater Today, 2022, 29
Saeidnia, S., & Abdollahi, M. Toxicol. Appl. Pharmacol., 2013, 273, 442–455

In vitro nanobot's core functionality



The recommended PEITC dose for prevention or therapy is 5 to 30 µM.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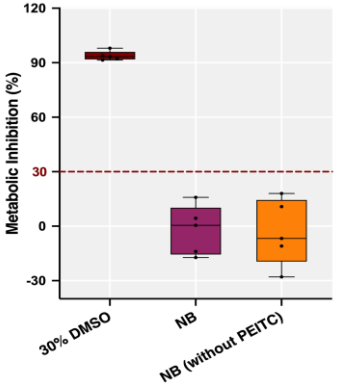
Effects of microencapsulated phenethyl isothiocyanate on gastrointestinal cancer cells and pathogenic bacteria

Rafaela Abrantes, Cátia C. Ramos, Ezequiel R. Coscueta, João Costa, Joana Gomes, Catarina Gomes, Celso A. Reis, Maria Manuela Pintado

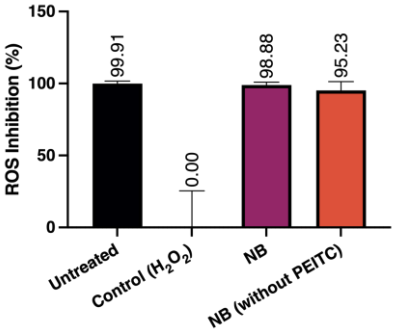
NB tested at 30 µM PEITC

Biocompatibility of NB



NB is safe at 30 µM (PEITC)

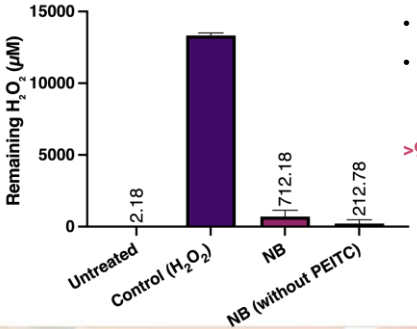
Intracellular ROS



- Control H_2O_2 10 mM
- Measured by DCFDA
- 4 h incubation

NBs protect cells from oxidative stress

Extracellular H_2O_2 Levels



- Pierce™ assay
- 4h post-treatment

>90% H_2O_2 degraded by NB

Key findings & where we're heading

- ✓ NB's core was engineered to respond to H_2O_2 and encapsulate PEITC.
- ✓ NB showed low cytotoxicity and reduced intracellular and extracellular ROS in a Caco-2/HT29-MTX co-culture.
- ✓ These findings support the potential of NB-PEITC as a targeted antioxidant platform for IBD.

Challenges

- Protect the core and ensure NB stability with controlled GI release
- Reliable and scalable NB production

Future Work

- Evaluate anti-inflammatory and antioxidant activities in IBD
- Evaluate biodistribution in GI
- Testing performance in *ex vivo* and *in vivo* models

gBiOT — *Innovation at the microscale, healing at the human scale*

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Bioactives and Bioproducts Research Group

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Thank you for your kind attention!

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"Wet or fine, the air of Portugal has a natural happiness in it, and the people of the country should be as happy and prosperous as any people in the world."

H.G. Wells, English writer.

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