Rational design and engineering of proteins for functionalization and biocatalysis of commodity polymers

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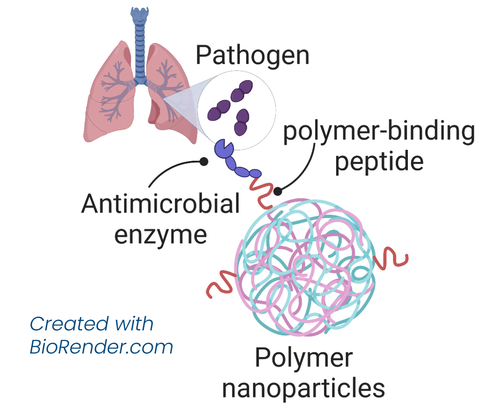
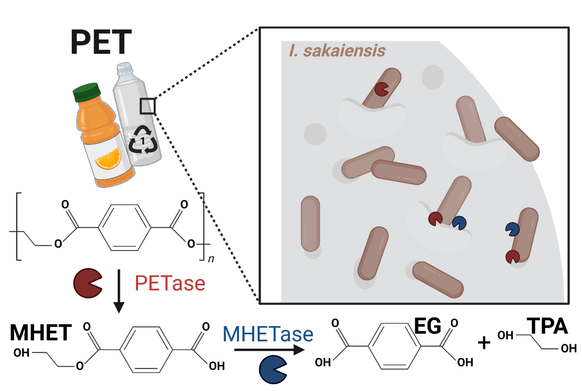
Motivation

Proteins intertwine with polymer interfaces in biological environments. These interactions are exploited to modulate the function of biopolymer films/particles or to induced their degradation at their end-of-life.



Microbial proteins and peptides are used as linkers to functionalize polymer-based nanoparticles and enhance their vehiculization through membranes.



Proteins, referred to as hydrolases (eg. PETase), catalyze the degradation of natural and synthetic polymers such as polyethylene terephthalate (PET) into recyclable building blocks.

Challenge

However, their production and deployment at the industrial scale are challenging due to i) poor loadings at polymer nanosurfaces ii) loss of activity at the temperatures needed for polymer processing, iii) short life-spam, among others.

In addition, traditional methods for characterizing protein-polymer interactions lack the precision and speed to design proteins to fulfill these applications.

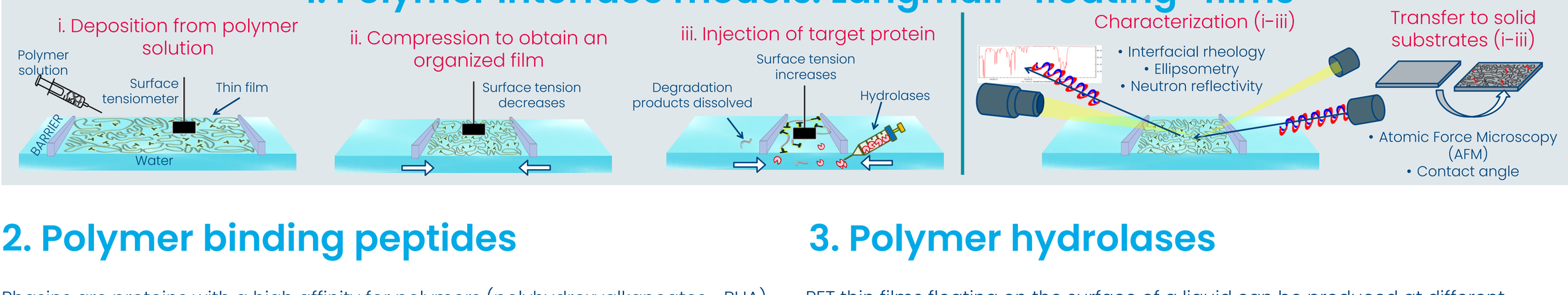
Our work focuses on

-designing and producing genetically modified polymer-binding peptides and hydrolases with enhanced binding capacity and temperature stability.

-developing model polymer interfaces, such as Langmuir thin films to evaluate protein-polymer interactions at the nanoscale.

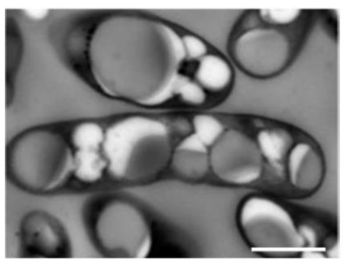
-integrating innovative interfacial techniques such as rheology, ellipsometry, and neutron reflectometry, to understand nanoscale phenomena at the polymer interfaces.

1. Polymer interface models: Langmuir "floating" films

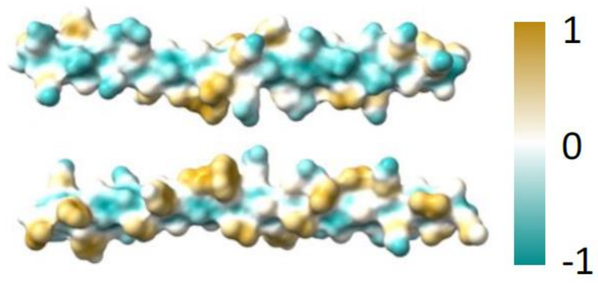


2. Polymer binding peptides

Phasins are proteins with a high affinity for polymers (polyhydroxyalkanoates –PHA) produced inside bacterial cells. Short amphiphilic peptides derived from Phasins were designed based on their amino acid sequence and hydrophobic moment.

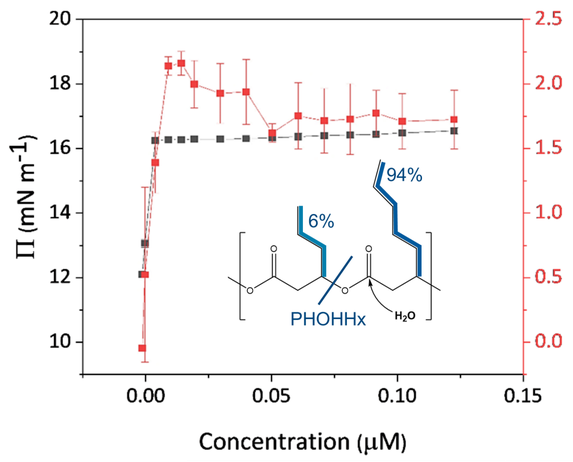


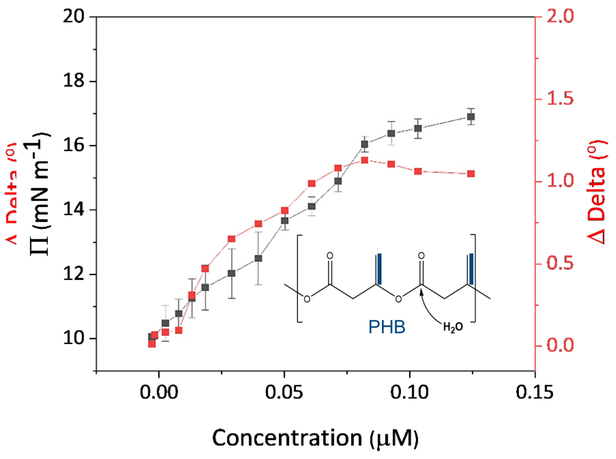
Bacterial cell accumulating PHAs, surrounded by phasins



α-helices formed by peptide MinI (minimized phasin PhaI), showing hydrophobic (brown) and hydrophilic (blue) regions

Ellipsometry measurements (angle Δ) were performed on peptides of different sizes (MinI and MinP) binding to PHAs of variable structure (PHOHHx and PHB) to provide information on kinetics, surface coverage, layer thickness, and adsorption energy.



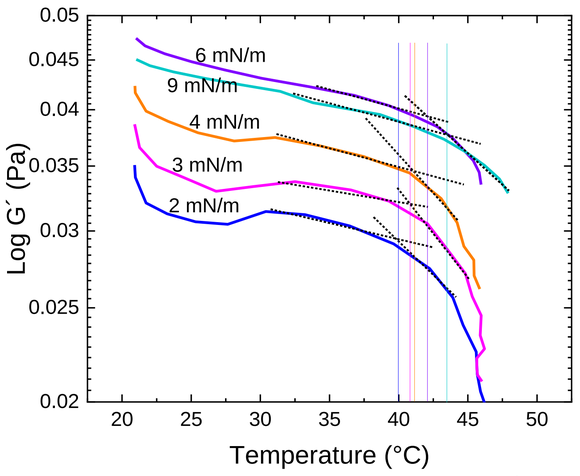


Peptide/Polymer	K_D (nM)	B_{max} (pmol/cm²)
MinP/PHOHHx	25	66
MinI/PHOHHx	< 10^{-7}	7
MinP/PHB	56	145
MinI/PHB	142	166

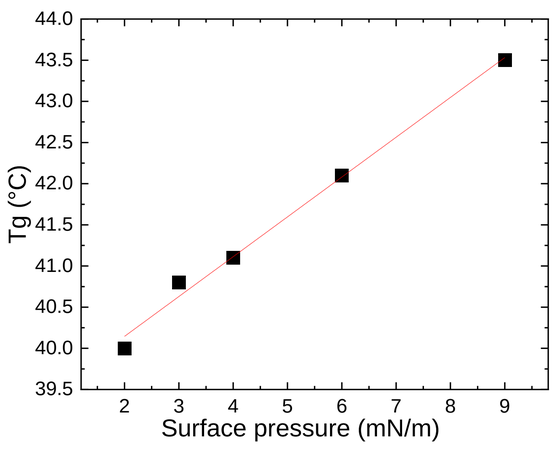
PhD student Francisco Blanco

3. Polymer hydrolases

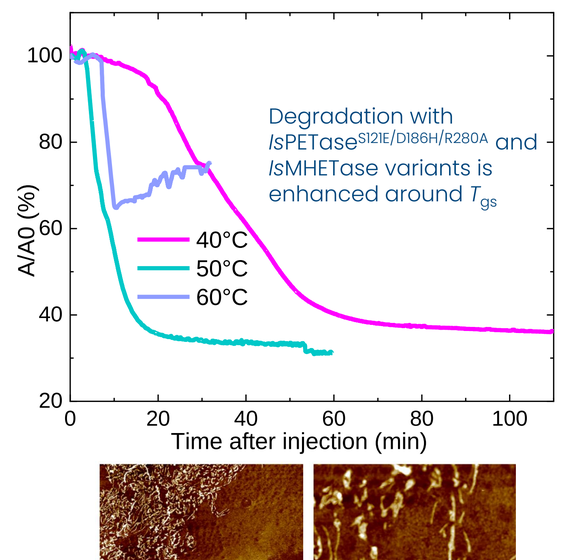
PET thin films floating on the surface of a liquid can be produced at different nanometric thicknesses varying the surface coverage (mN/m)

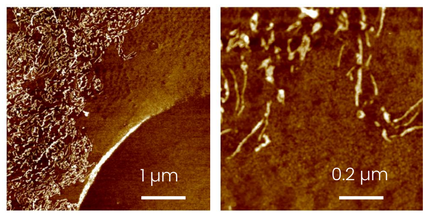


The Glass transition temperature of PET films (surface T_{gs}) is measured as storage modulus as a function of temperature



The T_{gs} of PET thin films (40-44°C) is 20°C lower than that of the bulk, enabling enzymatic hydrolysis at mild temperatures





AFM images of PET film after degradation show passivation of the surface

Outlook

We bring digital MSE forward by providing a nanoscale understanding of interfacial phenomena to enable information-guided design of proteins for key biotechnological applications.

Moving forward, we are using neutron reflectivity and Isotopic substitution, i.e. deuteration of the monolayer, to accurately quantify molecules at the surface and unveiled new mechanisms

Data of our latest excursion to the ILL Is currently in preparation in cooperation with **Gaetano Mangiapia, Jean-Francois and Moulin, Martin Müller**

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