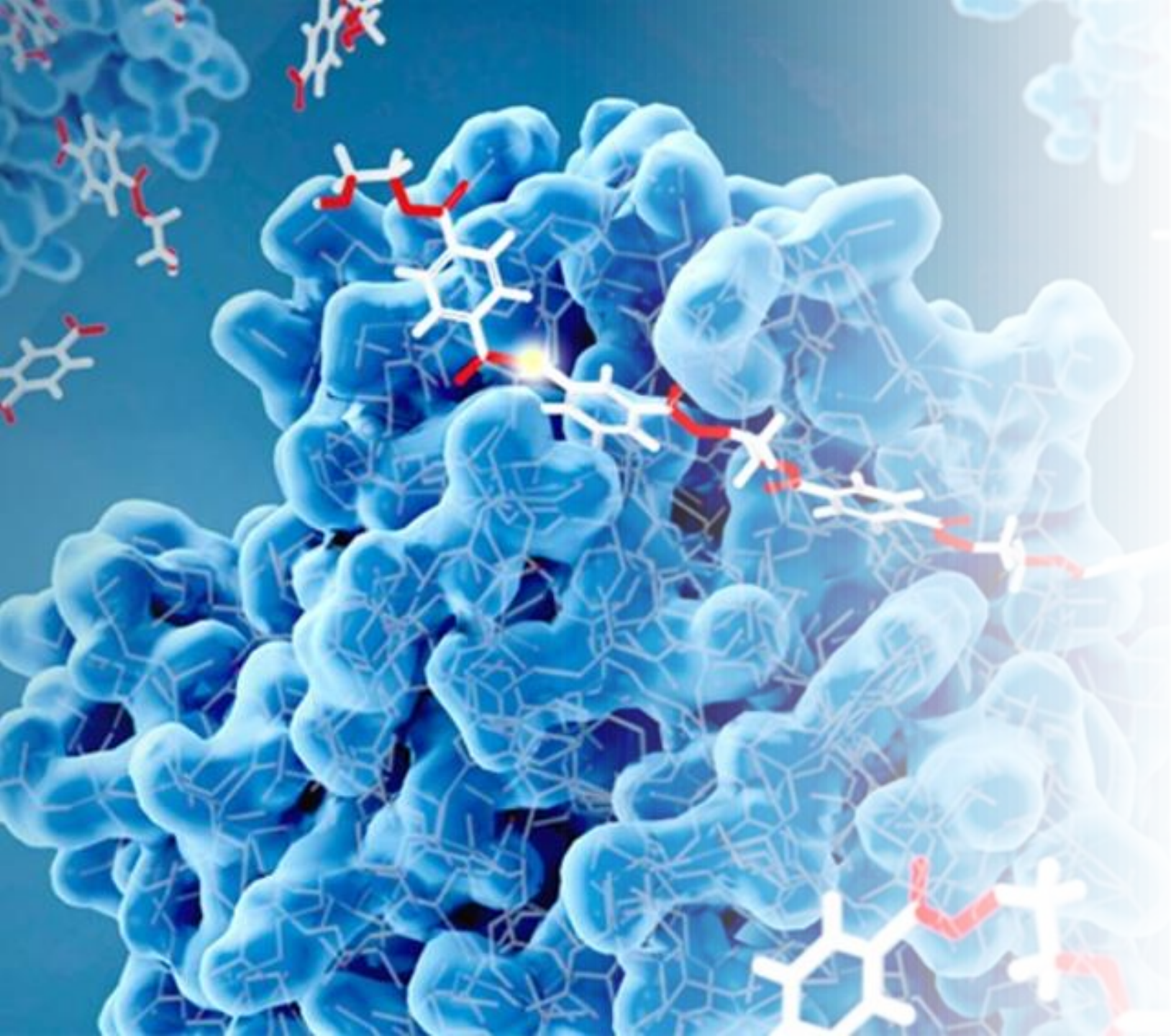

Enzymatic PET degradation

A biophysical route to optimize the process

PhD in Physics-XXXVII Cycle

A.Y. 2021/2022

Francesco Millucci

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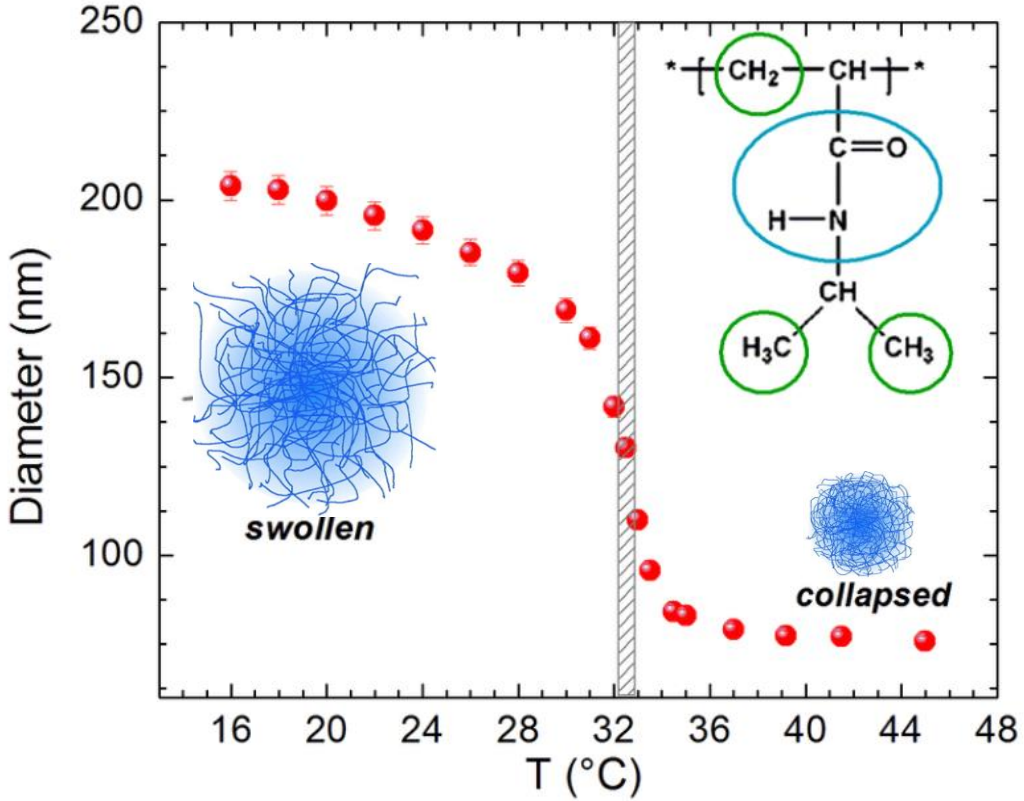
21/02/2019
BSc Physics

30/09/2021
MSc Physics

1/01/2022
PhD-PON scholarship

MSc thesis:

Volume Phase Transition in PNIPAM microgels



Key takeaways:

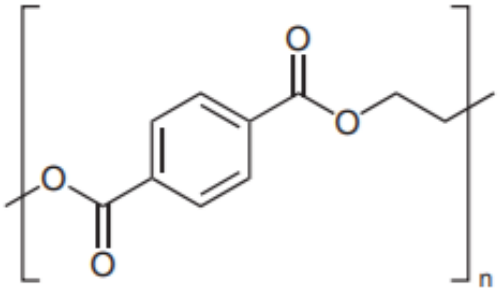
01 Polymer/protein **interactions with solvent** drive conformational changes

02 **Physics** (i.e. spectroscopy) provides fundamental information at both **colloidal** and **molecular** level

Article | Published: 08 April 2020

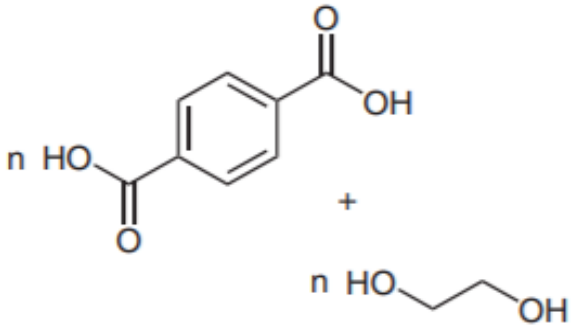
An engineered PET depolymerase to break down and recycle plastic bottles

V. Tournier et al.



PET (polyethylene terephtalate)

leaf branch compost cutinase *variant (e-LCC)*



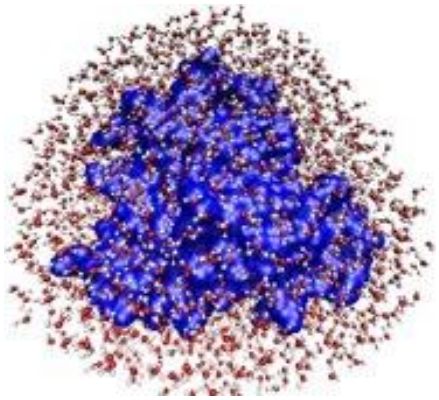
Terephtalic acid + Ethylene glycol

As PET depolymerization process requires **high temperature** (T_g~70°C) for **prolonged times,** enzymes need remarkable **thermal stability**

Main Idea: Process optimization through **modification of solvent environment**

Changing cosolvents, pH, ionic strength etc... it is possible to act on:

Enzyme



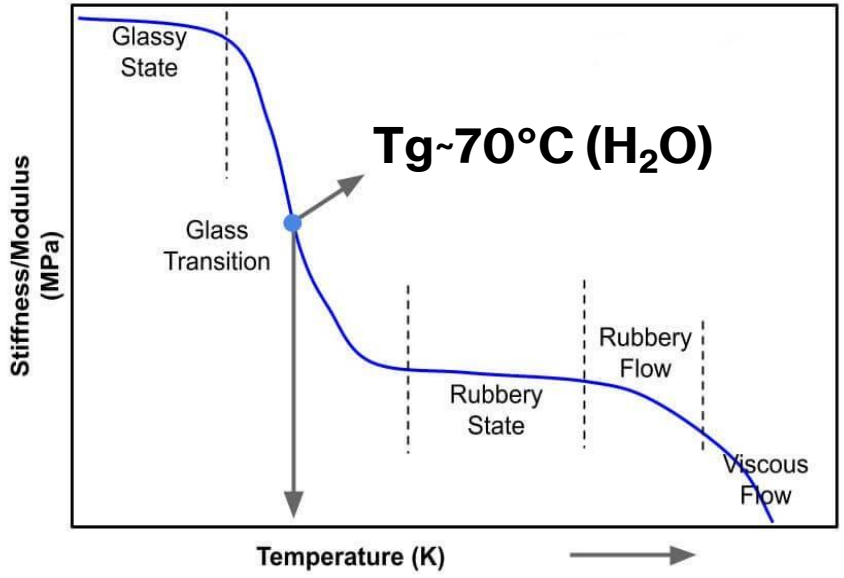
- Improve **thermal stability**
- Increase **catalytic efficiency**

Reach **100% depolymerization** in the least amount of time

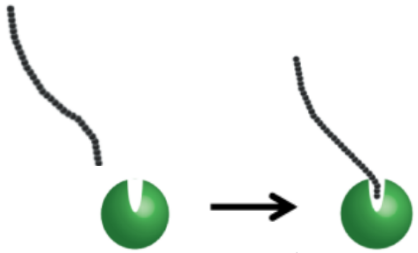
Main Idea: Process optimization through **modification of solvent environment**

Changing cosolvents, pH, ionic strength etc... it is possible to act on:

PET substrate



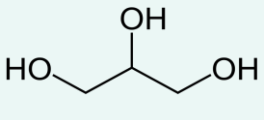
Increase **chain mobility** (depress T_g)



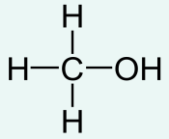
Reduce **operative temperature**

Solvent candidates

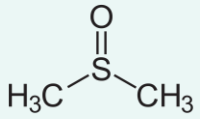
Organic Solvents



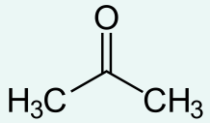
Glycerol



Methanol



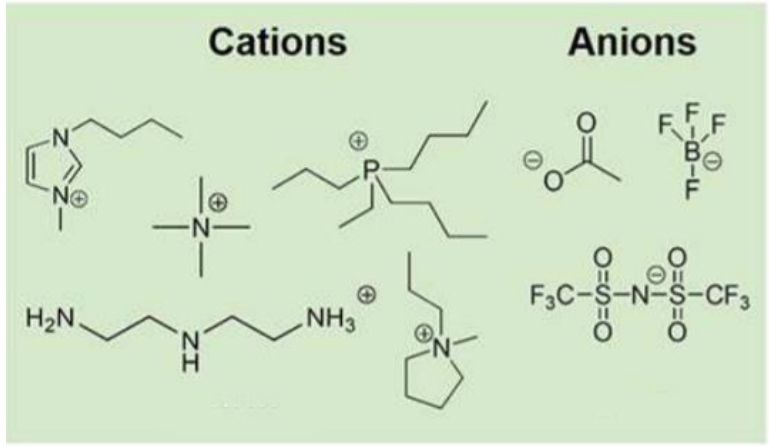
DMSO



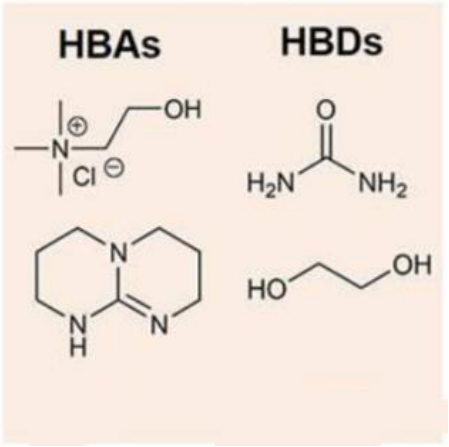
Acetone

- Enzyme **stabilization**
- PET **Plasticization**

Ionic liquids



Deep eutectic solvents



Green Solvents

- ➢ High Thermal stability
- ➢ Low-volatility
- ➢ Non flammable
- ➢ High Reciclability
- ➢ Low toxicity
- ➢ Low cost (DES only)

Combined expertise

- Drug design
- Molecular modelling
- Protein-solvent interactions
- Spectroscopy of condensed matter

Computational methods

Both **Molecular docking** and **Molecular Dynamics** simulations will be employed to study enzyme/PET interactions with solvent

Main Targets:

01 Enzyme's **conformational changes** (unbound and complexed with PET)

02 **Catalytic distance** between active site and substrate

03 PET **chain mobility**

Experimental techniques

TECHNIQUE	Properties probed
Circular Dichroism (CD)	**Secondary and tertiary** enzyme's structures
FTIR & Raman (UV-resonant and Vis)	
Photocorrelation spectroscopy	**Hydrodynamic radius**
Calorimetry	**Degree of crystallinity** and **glass transition temperature** of PET substrate

Experimental techniques

TECHNIQUE	Properties probed
Quasi-elastic neutron scattering (QENS)	**Dynamics** of both PET and enzyme (complementarity with **MD**!)

Isotopic substitution →

σ_{inc} **(barn)**

Chemistry, Biology and Biotechnology Department (Unipg)

Raman, FTIR, analytical chemistry

IUVS-Beamline@Elettra (Trieste)

Raman (UV-resonant)

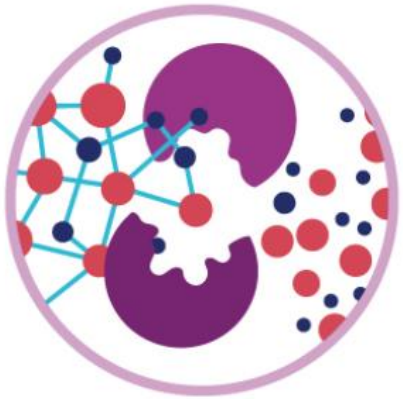
Institute Laue-Langevin (Grenoble)

Neutron scattering

Partnership for Structural Biology (Grenoble)

Enzymes Expression and purification

Project's impact



1 Develop a **new reaction protocol** exportable to recycling company

2 Provide the **physical basis** to realize a microplastic biosensor

3 Improve our understanding of **protein-solvent interaction** physics

Thank you for your attention!