Continuação: Imobilização e NanoBioCatalisadores (NBCs)

Vídeo #2

"… biocatalytic processes are often limited by the lack of stability of the enzymes and their short lifetime. Therefore, immobilization is key to the successful implementation of industrial processes based on enzymes."

Recent advances in nanostructured biocatalysts, Biochem. Eng. J., 2009, 44, 53–9

Imobiliza E em nanomateriais → NBCs → Assunto atual em pesquisa e indústria

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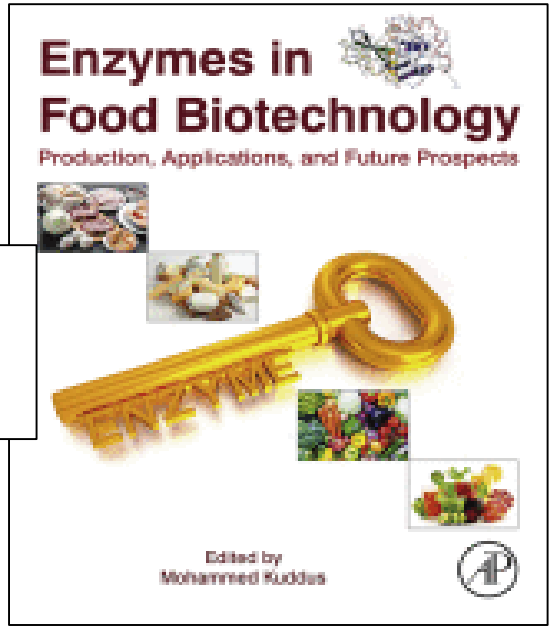
EXEMPLOS DE APLICAÇÕES:

Emerging role of nanobiocatalysts in hydrolysis of lignocellulosic biomass leading to sustainable bioethanol production, J. Catalysis Rev., 2018

Covalent immobilization of peanut β-amylase for producing industrial nanobiocatalysts: A comparative study of kinetics, stability and reusability of the immobilized enzyme, Food Chem., 2018

Chapter 45 - Application of Nanobiocatalysts on Food Waste, 2019, 785-793.



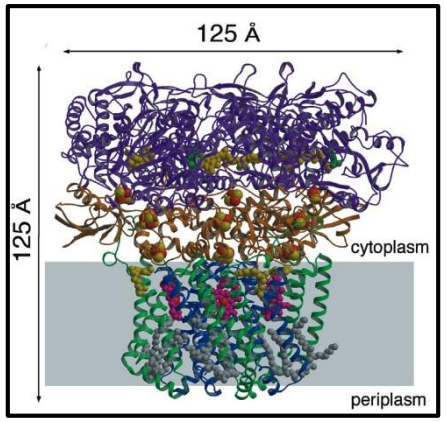
Tamanhos nanomateriais e enzimas ????

22 cm

Bola de Futebol (aproximadamente 22cm)

carbono 60 (0.7 nm)
R.Drautz

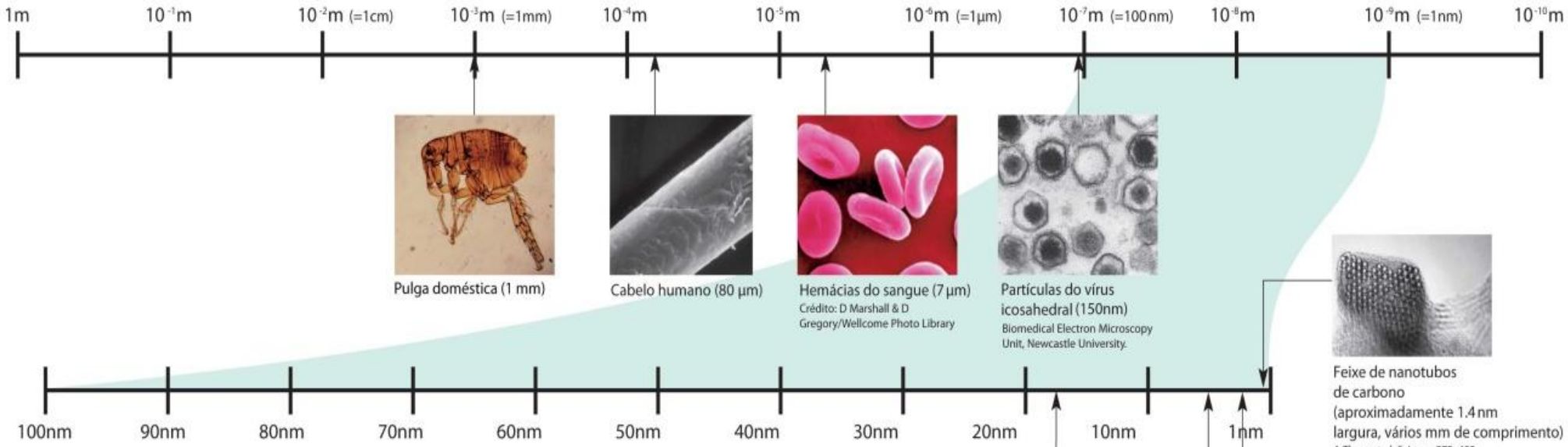
= 12,5 nm



1 nm = 10^{-9} m

1A = 1 x 10^{-10} m =

0.1 x 10^{-9} m = 0,1 nm



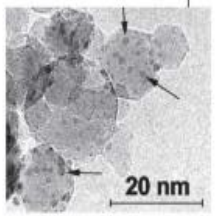
Pulga doméstica (1 mm)

Cabelo humano (80 µm)

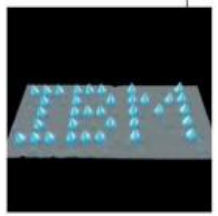
Hemácias do sangue (7 µm)
Crédito: D Marshall & D Gregory/Wellcome Photo Library

Partículas do vírus icosahedral (150nm)
Biomedical Electron Microscopy Unit, Newcastle University.

Feixe de nanotubos de carbono (aproximadamente 1.4 nm largura, vários mm de comprimento)
A Thess et al, Science 273, 483, 1996



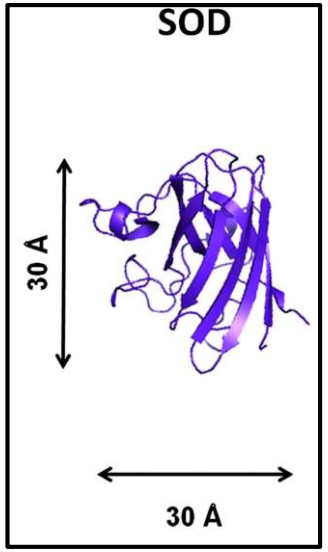
Partículas de platina e dióxido de titânio (Partículas de platina menores que 3 nm de diâmetro estão indicadas nas on titanium dioxide)
R Strobel et al, J Catal 222, 296, 2003



Cada letra do logo tem aprox. 5 nm do topo até a base.
Image reproduced by permission of IBM Research, Almaden Research Center. Unauthorized use not permitted.

Segmento de DNA (aproximadamente 2 nm de largura)

http://www.sltcaucho.org/nanociencia-e-nanotecnologia-marly-jacobi/



Nanomaterais usados para obter NBCs

Aspectos a considerar ao usá-los para empregá-los:

- ✓ **Área superficial X tamanho**
- ✓ **Porosidade**
- ✓ Capacidade de carga de enzima → nanopartículas, p. ex.
- ✓ Resistência de transferência de massa
- ✓ **Funcionalização química**
- ✓ Capacidade de penetração celular

Não Magnéticos

Organo-sílicas (silicato mesoporoso)
Materiais nanoporosos

Magnéticos

NP Magnetita (Fe_3O_4 modificadas ou não)
NP de Au
NP de Ag

Situações a considerar no preparo dos NBCs:

Funcionalizações a considerar para a imobilização:

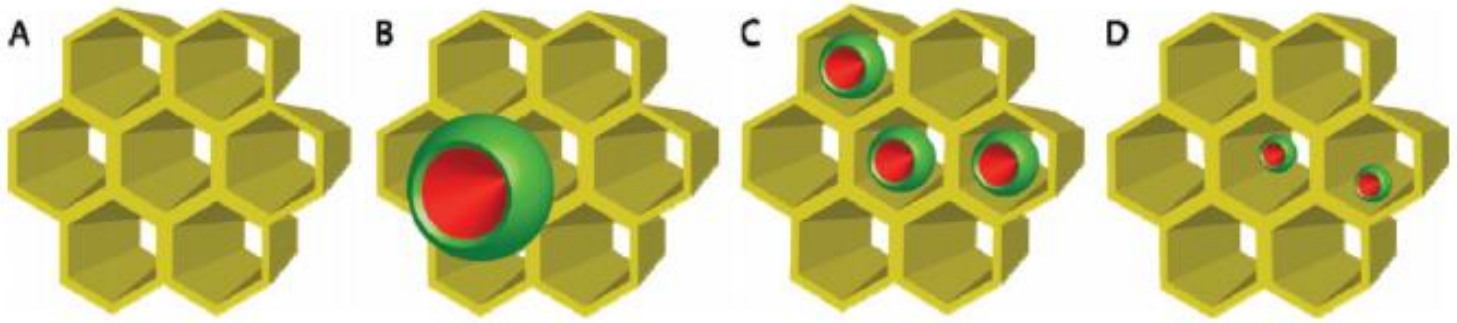


Figure 2. (A) Representative image of ordered mesoporous silicate (OMS). (B) Depicts the situation where an enzyme is too large to fit inside of the pores and is mainly adsorbed in the outer surface of the OMS. (C) Portrays enzyme adsorption in the inner pore volume when pore diameter and enzyme size matching is achieved. (D) Depicts the opposite of case B, where the pore opening is much larger than the enzyme and is susceptible to leaching.

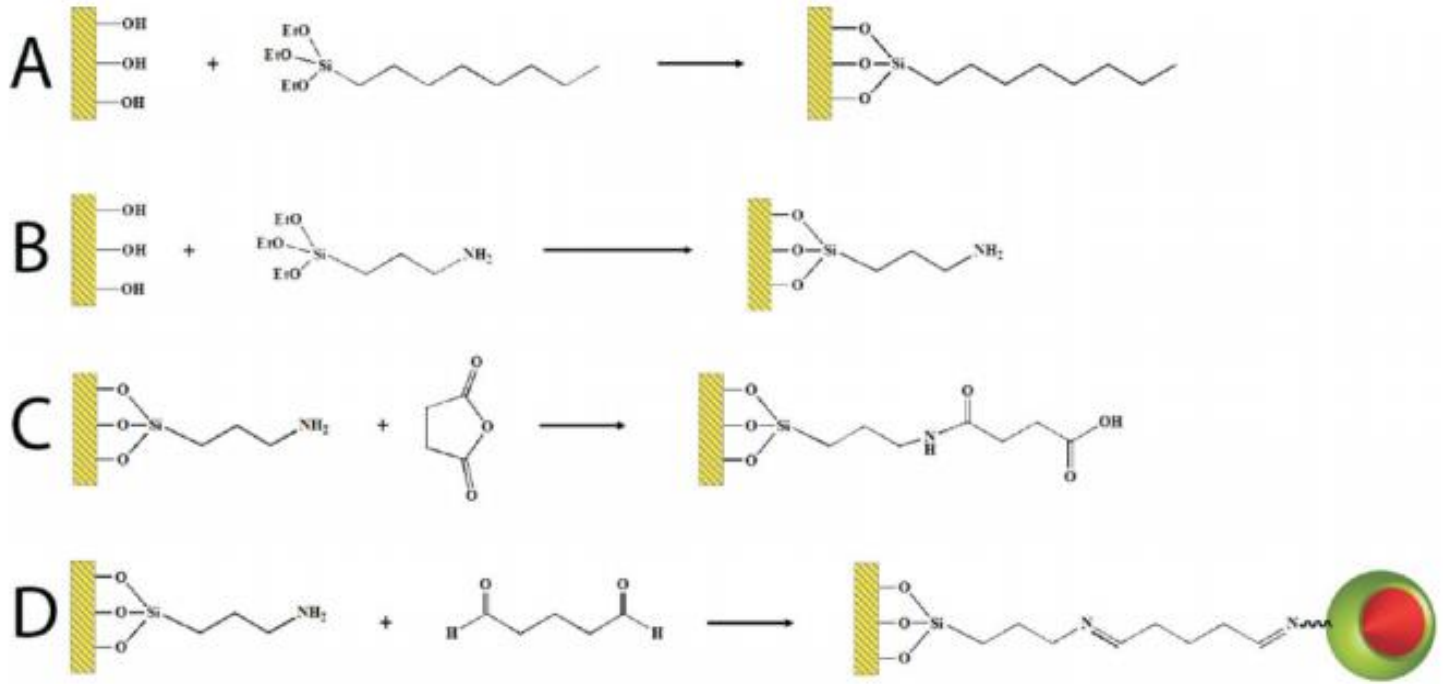


Figure 3. (A–D) Represents the functionalization of OMS material surface silanols with various functional groups. Panel A is the hydrophobic functionalization by attachment of octyltrimethoxysilane. Panel B represents the hydrophilic functionalization with the addition of 3-aminotrimethoxysilane (APTES). Panel C represents the subsequent treatment of APTES functionalized OMS with succinic anhydride for carboxylate functionalization. Panel D represents the cross-linking of APTES functionalized OMS with external anime groups found on enzymes.

Nanopartículas Magnéticas → superparamagnéticas → pouca atração entre si

Separadas dos meios reacionais → separação magnética

Imobilização de
enzimas livres ou *Cross-linked Enzyme Aggregates* (CLEAs)
em partículas de magnetita

Production of superparamagnetic nanobiocatalysts for green chemistry applications, *Appl Microbiol Biotechnol 100:7281–7296, 2016* → →

Preparação de NBC a partir de lacase*

The immobilization method consists of the production of **superparamagnetic nanoparticles by co-precipitation of FeCl2 and FeCl3.** $Fe^{2+} + 2Fe^{3+} + 8OH^- \rightarrow Fe_3O_4 + 4H_2O$
Subsequently, the particle surface is modified with an organosilane containing an amino group.
Next, the enzymes are adsorbed on the particle surface before a cross-linking agent, i.e., glutaraldehyde is added which links the amino groups on the particle surface with the amino groups of the enzymes and leads to internal cross-linking of the enzymes as well.

** reação de oxidação para despolimerizar lignina*

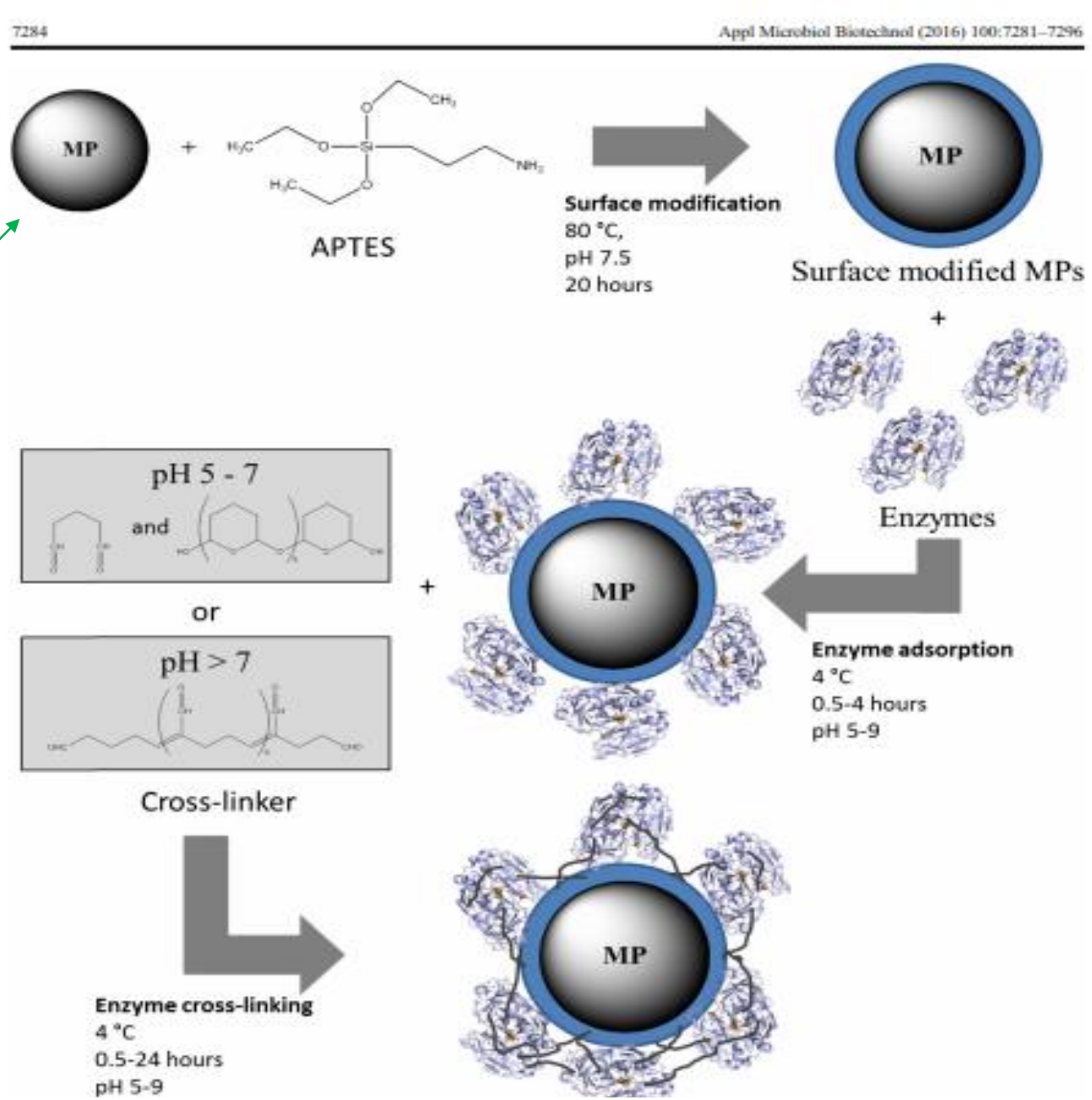
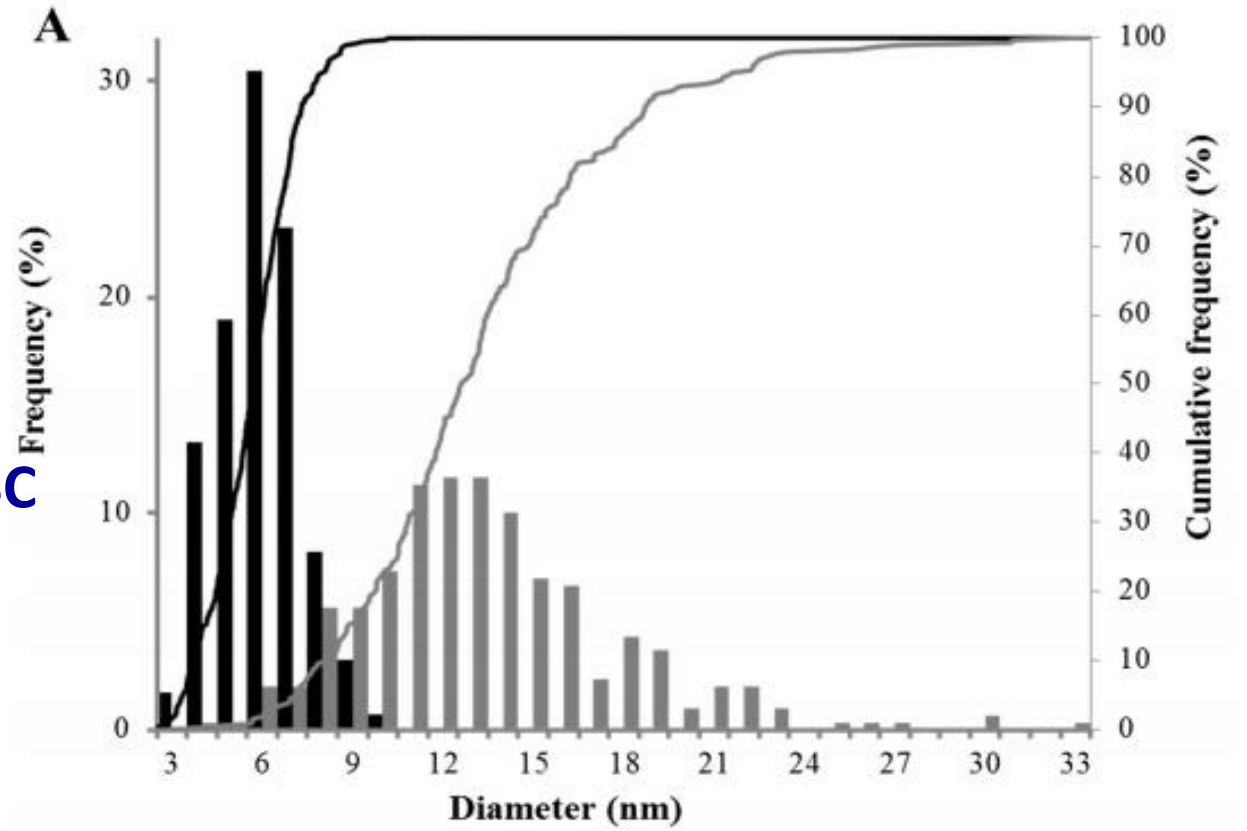
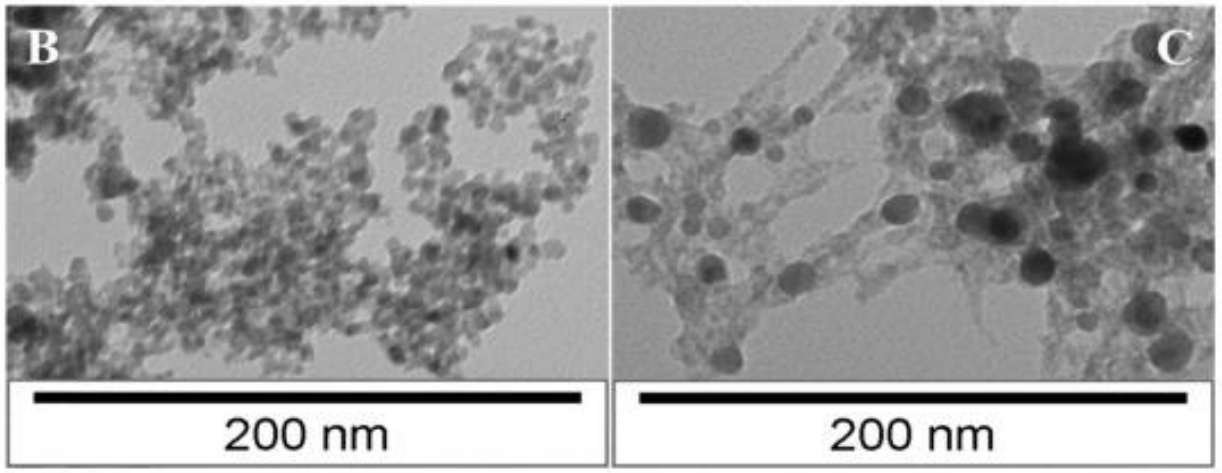


Fig 1. Descrição esquemática de "sorption-assisted surface conjugation" method desenvolvido.

Fig. 2 Distribution of particle diameters (**a**) of MP (*black*) and TVL-MP (*gray*). *Bars* show the frequency of particles with the corresponding diameter (rounded to 1 nm). *Lines* show the cumulative frequency, i.e., the percentage of particles that have at the most a diameter as displayed on the *x*-axis. TEM pictures of MP (**b**) and TVL-MP (**c**)



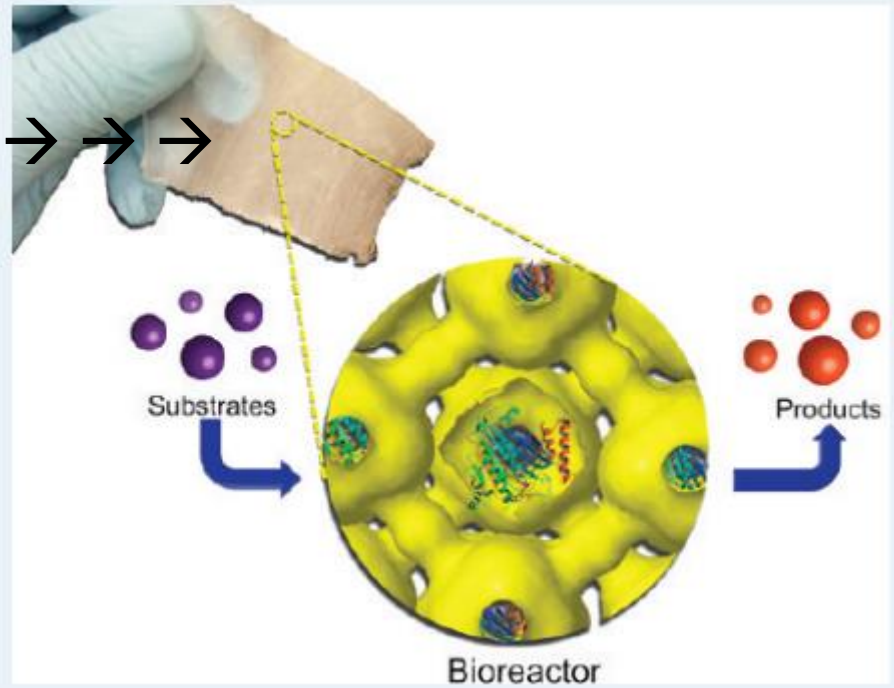


Caracterização físico-química do NBC

TEM = Microscopia de Transmissão eletrônica

- ✓ Medida do tamanho do NBC e variabilidade
- ✓ Observação de agregação ou não

Como objetivo é **USO** → → →



Caracterização do NBC tem que incluir:

1- O NBC está ativo?
2- Quanto de E/mg de NP?
3- É facilmente recuperável? Quanto?
4- No NBC a enzima teve outras propriedades melhoradas? ----------
5- como o NBC pode ser armazenado?
6- o NBC pode ser reutilizado → quantas vezes?

- ✓ Cinética & Kcat/Km
- ✓ Estabilidade química
- ✓ pH ótimo
- ✓ Termoestabilidade

EX: NBC = Fe_3O_4@sílica-αCT a partir da α-quimotripsina pancreática bovina livre

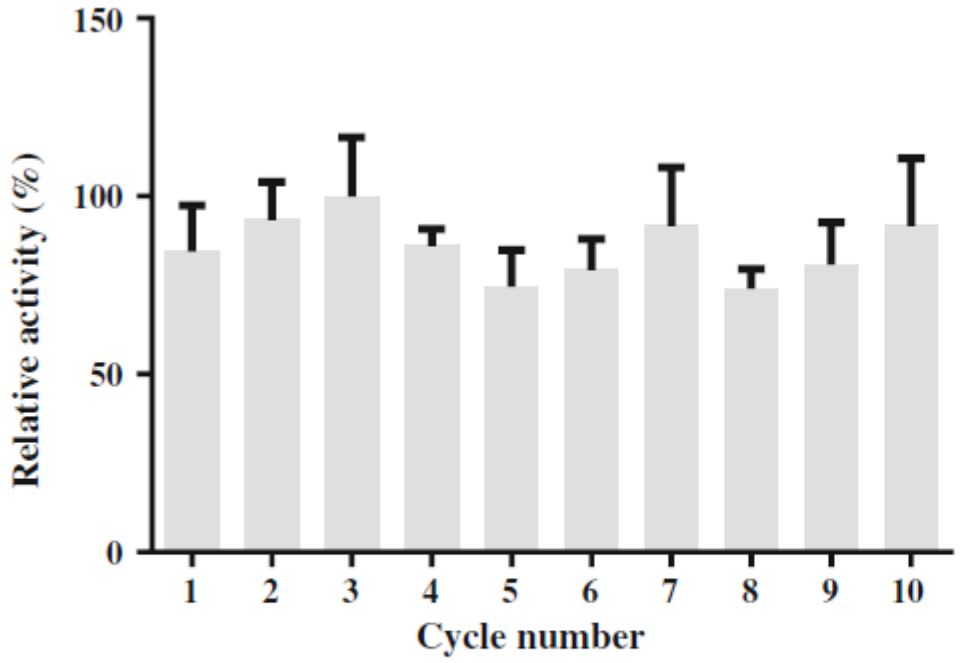


Fig. 3 Relative amidase activity measured after use of Fe_3O_4@silica-αCT stored in suspension at room temperature for ten cycles. The analysis of variance (ANOVA) gave a very high *p* value ($p = 0.2481$)

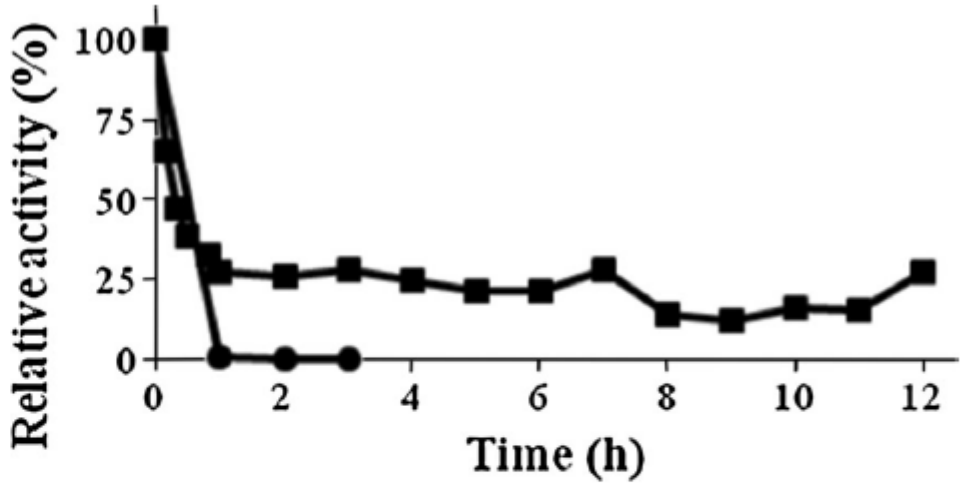


Fig. 4 Thermal stability of free αCT (*filled circle*) and Fe_3O_4@silica-αCT (*filled square*). Remaining amidase activity was determined after enzyme pre-incubation at 60 °C. The remaining activity (%) represents the ratio of residual activity after pre-incubation/initial activity

Ungaro, Vitor Augusto. Desenvolvimento, estudo e aplicações de nanobiocatalisadores proteolíticos magnéticos / São Paulo, 2020. 223 p.

Liria et al., *J Nanopart Res* (2014) 16:2612

Table 1. Recent Methods Used to Immobilize Enzymes for Catalytic Reactions

types of reactions	relevant enzymes	methods	reference
hydrolysis	β-glucosidase	adsorption	12,96
	cellulase	adsorption CLEA	12,23,48,97
	lysozyme	adsorption	18,28,29,98,99
	urease	adsorption	100
	lipase	adsorption CLEA	17,19,31,41,42,45,46,51,101−109
	alkaline serine endopeptidase	adsorption	32
	penicillin acylase	adsorption CLEA	15,30,45,47,110−112
	pepsin	adsorption	113
	invertase	adsorption	33
	papain	CLEA	83,84
	phytase	CLEA	34
	α-amylase	adsorption CLEA	29,83,115,116
	phospholipase	CLEA	49
	pectinase	CLEA	48
	xylanase	CLEA	48
	α-chymotrypsin	adsorption	101,117
	hydroxynitrile lyase	CLEA	31,32,43
	trypsin	adsorption	118
	alkaline serine protease	CLEA	119
oxidation/reduction	glucose oxidase	adsorption CLEA	11,23,34,59,60,120−123
	monooxygenase	adsorption	10
	horseradish peroxidase	adsorption CLEA	13,43,59,105,120
	galactose oxidase	CLEA	34
	laccase	adsorption CLEA	16,34,39,105
	tyrosinase	CLEA	44
	formaldehyde dehydrogenase	CLEA	34
	alcohol dehydrogenase	CLEA	34
	chloroperoxidase	adsorption CLEA	38,60,124,125
	cytochrome c	adsorption	14,126
	microperoxidase-11	adsorption	27
	hemoglobin	adsorption	58
	glutaminase	adsorption	127
	myoglobin	adsorption	18,128
	superoxide dimutase	adsorption	129
other	carbonic anhydrase	adsorption	57
	hydroxynitrile lyase	CLEA	36,37,50
	nitrile hydratase	CLEA	40,50