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Biossensores Microbianos e suas Aplicações Ambientais

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Biossensores

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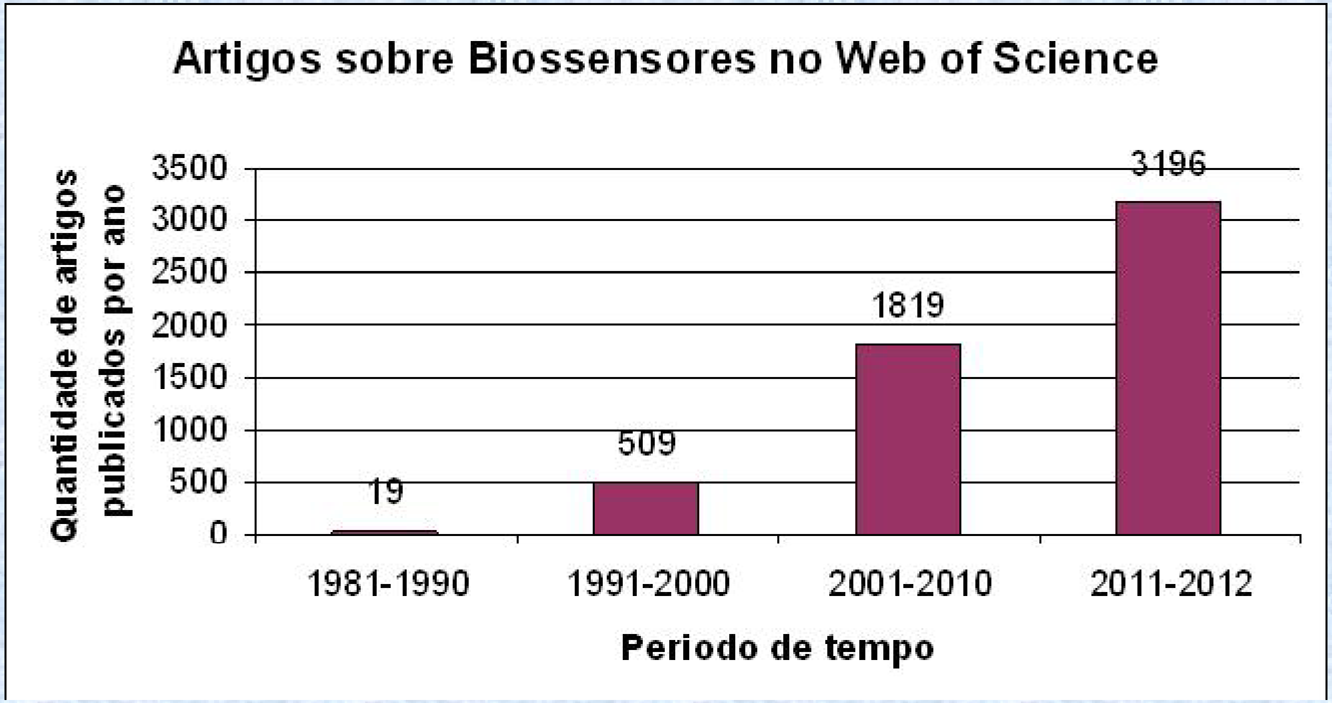
- Definição, Princípios, Características & Classificações dos Biossensores
- Biossensores Microbianos:DNA Recombinante, Genes de Resistência e Sinalizadores
- Biossensor para estudo da biodisponibilidade do Hg
- Aplicações dos Biossensores & Biossensores Comerciais disponíveis
- Perspectivas Futuras dos Biossensores

Biossensores

- Produção Acadêmica sobre Biossensores a partir da década de 1980
- Simpósio nos EUA em 1984
- Periódico: *Biosensor and Bioeletronics* em 1985



Barrocas *et al.*, 2008

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- Nova Abordagem: Biossensores
- Definição:

"Biossensor é um sistema integrado e independente, contendo um elemento biológico, responsável pela detecção do analito, em contato direto com um elemento transdutor, o qual converte a reação biológica em um sinal mensurável"

Rodriguez-Mozaz et al., 2005

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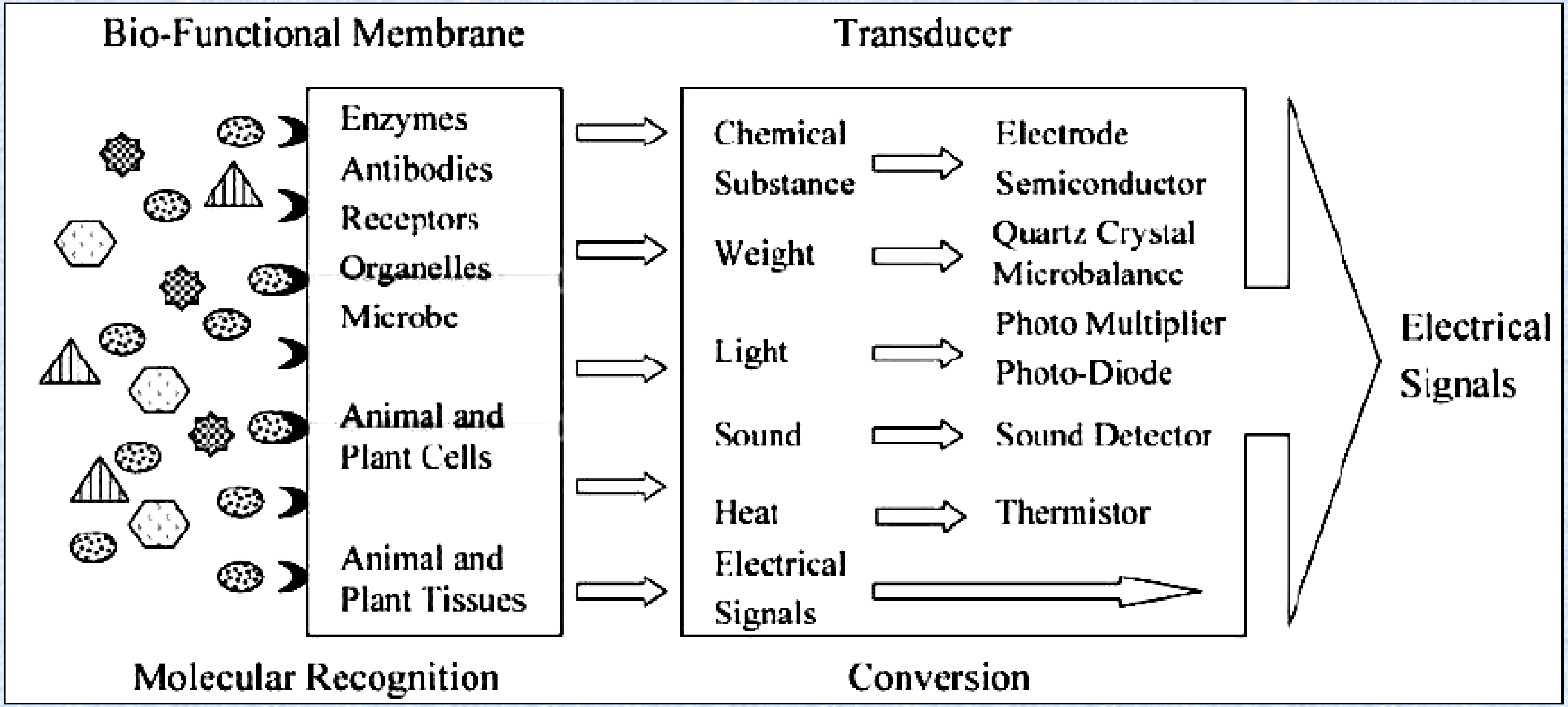
➢ Princípio dos Biossensores:



Nakamura & Karube, 2003

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➢Características e Vantagens dos Biossensores

Biosensors		Conventional methods
✓ Direct analysis/minimum sample preparation	←	× Preconcentration
✓ Simplicity; user friendly	←	× Trained personnel required
✓ Portability	←	× Laboratory methods
✓ Little sample and organic solvents consumption	←	× High sample and organic solvents consumption
✓ Cost-effective	←	× Expensive
✓ Fast results; real-time detection; feed back control	←	× Long analysis time
× Low biological material stability	→	✓ No biological stability restrictions
× Single analyte determination	→	✓ Multianalyte determination
× Mostly prototypes	→	✓ Mostly commercial
✓ Determination of biological effect	↔	✓ Chemical determination
✓ Determination of bioavailable pollutant content	↔	✓ Total pollutant content determination
✓ Sensitivity, precision, accuracy	↔	✓ Sensitivity, precision, accuracy

Rodriguez-Mozaz *et al.*, 2004

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- Classificação dos Biossensores:
  - Método de Conversão do Sinal
  - Elemento Biológico de Detecção

Rodriguez-Mozaz *et al*., 2004

Signal transduction	
Electrochemical	amperometric conductimetric impedimetric potentiometric
Optical	absorption fluorescence/phosphorescence bio/chemiluminescence reflectance raman scattering refractive index
Mass sensitive	surface acoustic wave biosensors cantilever biosensors
Thermometric	
Recognising Biomolecule	
Antibodies (Immunosensors)	monoclonal policlonal
Protein Receptors	metabotropic receptors ionotropic receptors
Whole Cells	microbial sensors mammalian cells tissue
Nucleic Acids	hybridisation low weight compound interaction
Enzymes	

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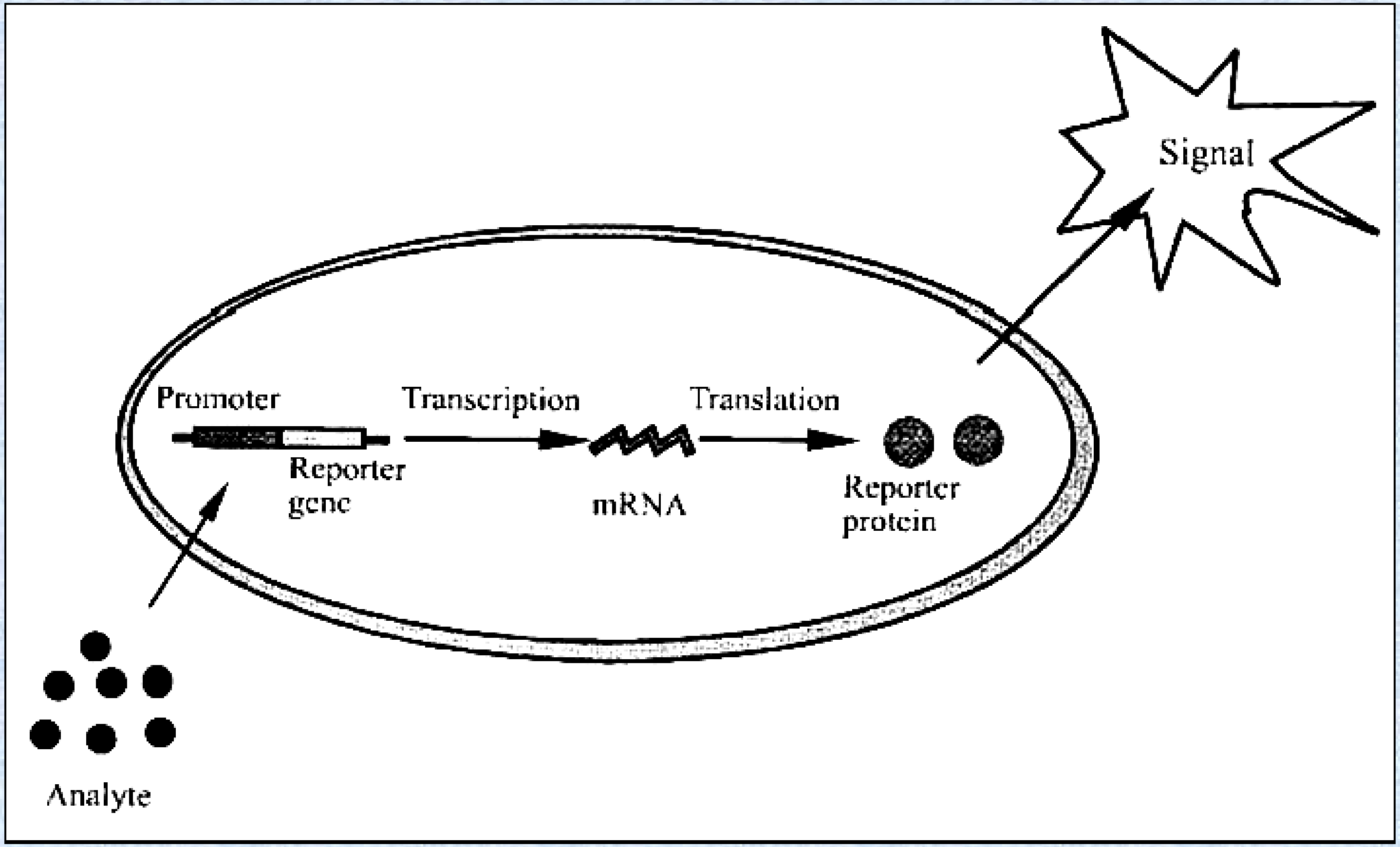
- Princípio dos Biossensores Microbianos:



Köhler *et al.*, 2000

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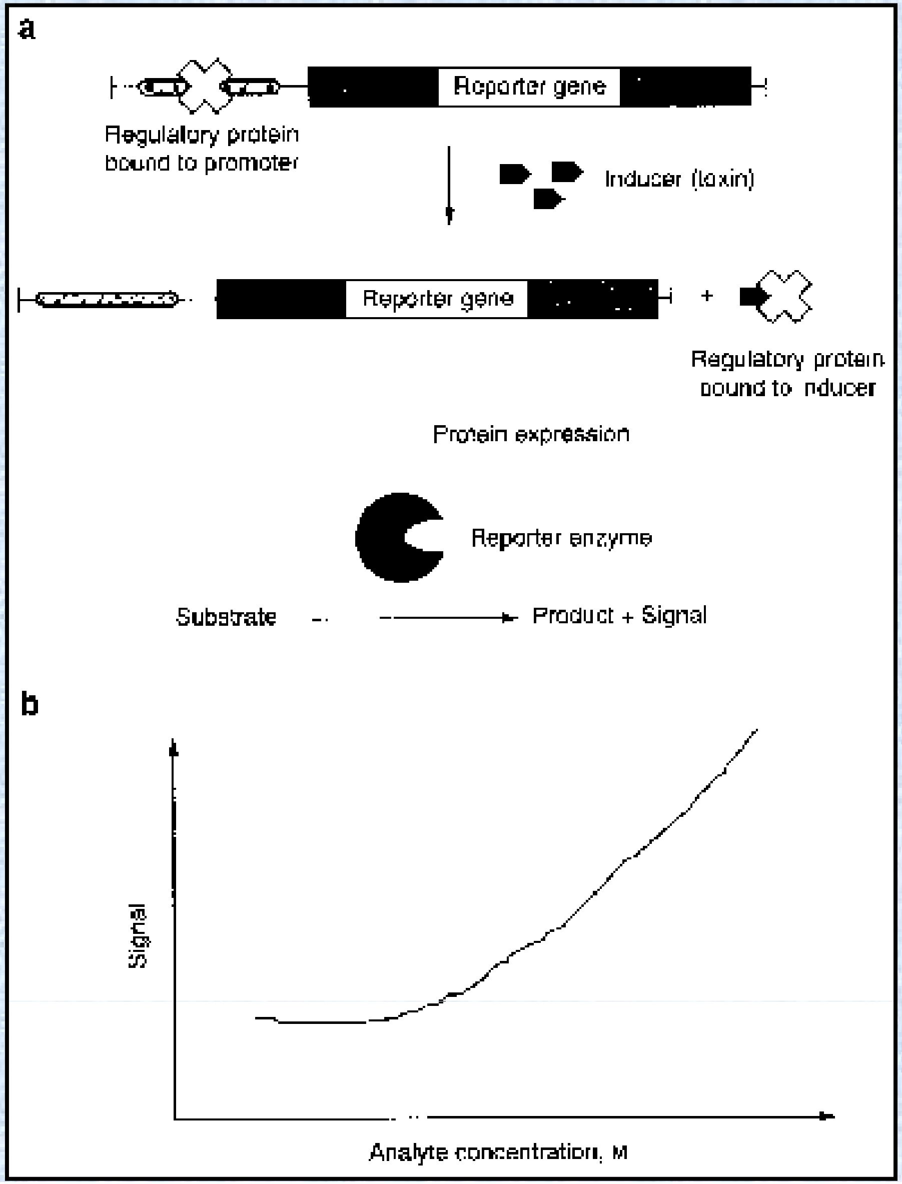
- Construção do Biossensor Microbiano: Tecnologia do DNA Recombinante
  - Gene Sinalizador
  - Promotor do Gene de Resistência



Ramanathan *et al*., 1994

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- Mecanismos Genéticos de Resistência Bacteriana a Metais:

☹ 1. Hg^{2+}. *mer*. Hg^{2+} and organomercurials are enzymatically detoxified.

☹ 2. AsO_4^{3-}, AsO_2^{-}, SbO^{+}. *ars*. Arsenate is enzymatically reduced to arsenite by ArsC Arsenite and antimonite are "pumped" out by the membrane protein ArsB that functions chemiosmotically alone or with the additional ArsA protein as an ATPase.

☹ 3. Cd^{2+}. *cadA*. Cd^{2+} (and Zn^{2+}) are pumped from Gram positive bacteria by a P-type ATPase with a phospho-aspartate intermediate.

☹ 4. Cd^{2+}, Zn^{2+}, Co^{2+}, and Ni^{2+}. *czc*. Cd^{2+}, Zn^{2+}, Co^{2+}, and Ni^{2+} are pumped from Gram negative bacteria by a three polypeptide membrane complex that functions as a divalent cation/2 H^{+} antiporter. The complex consists of an inner membrane protein (CzcA), an outer membrane protein (CzcC) and a protein associated with both membranes (CzcB).

☹ 5. Ag^{+}. *sil*. Ag^{+} resistance results from pumping from bacteria by three polypeptide chemiosmotic exchanger plus a P-type ATPase.

☹ 6. Cu^{2+}. *cop*. Plasmid Cu^{2+} resistance results from a four polypeptide complex, consisting of an inner membrane protein, an outer membrane protein, and two periplasmic copper-binding proteins. In *Pseudomonas*, Cop results in periplasmic sequestration of Cu^{2+}. In addition, chromosomally-encoded P-type ATPases provide partial resistance by effluxing Cu^{2+} or Cu^{+}.

☹ 7. CrO_4^{2-}. *chr*. Chromate resistance results from a single membrane polypeptide that causes reduced net cellular uptake, but efflux has not been demonstrated.

☹ 8. TeO_3^{2-}. *tel*. Tellurite resistance results from any of several genetically-unrelated plasmid systems. Although reduction to metallic Te^{0} frequently occurs, this does not seem to be the primary resistance mechanism.

☹ 9. Pb^{2+}. *pum*. Lead resistance appears to be due to an efflux ATPase in Gram negative and accumulation of intracellular $Pb_3(PO_4)_2$ in Gram positive bacteria.

Resistance systems await understanding for bismuth (Bi), boron (B), thallium (Tl) and tin (Sn).

Silver, 1988

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➢Genes de Sinalização de Biossensores Microbianos:

- ❑ Bioluminescência é conveniente: análises *in vivo* e reutilizáveis
- ❑ Relação direta como o Metabolismo Celular: Biodisponibilidade

Reporter protein	Reporter gene	Origin	Potential substrate	Detection method
Bacterial luciferase	*lux*	One of several luminescent bacteria	Long chain aldehydes (C9–C14)	Luminescence
Insect luciferase	*luc*	Fireflies, click beetles	Luciferin	Luminescence
β-Galactosidase	*lacZ*	*E. coli*	Galactopyranosides	Colorimetric, electrochemical, fluorescence, chemiluminescence
Green fluorescent protein	*gfp*	*Aequorea victoria*	–	Fluorescence
Alkaline phosphatase	*phoA*	Various	Phosporylated organics	Colorimetric, chemiluminescence
β-Glucuronidase	*uidA (gusA, gurA)*	*E. coli*	β-Glucuronides	Colorimetric, fluorescence, luminescence
β-Lactamase	*bla*	*E.coli*	Lactamides	Colorimetric

Köhler *et al*., 2000

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➢Exemplos: Biossensores Microbianos

- ❑ Específicos
- ❑ Genéricos (ex: toxidade)

Analyte	Promoter (origin)	Reporter	Micro-organism	Time for induction	Concentration
Inorganic compounds					
Aluminium	*fliC (E. coli)*	*luxAB (V. harveyi)*	*E. coli*	20 min	40–400 µM
Antimonite, arsenite	*arsRD'*	*lacZ*	*E. coli*	17 h	100 µM
				30 min	10^{-11} M
Arsenate, arsenite	*arsRDABC, arsRBC (E. coli, S. aureus)*	*luxAB (V. harveyi)*			
	arsR	*luc (firefly)*	*S. aureus*	1 h	ca. 0.01–10 µM
Arsenic	*ars (S. aureus)*	*blaZ*	*S. aureus, E. coli*		
	arsB (S. aureus)	*luxAB (V. harveyi)*	*S. aureus, E. coli*	1–2 h	
Cadmium	*cadA (S. aureus)*	*luxAB (V. harveyi)*	*S. aureus, E. coli*	1–2 h	1–100 µM
	cadA, cadC (S. aureus)	*blaZ*	*S. aureus*	1.5 h	0.5–100 µM
	cadCo/p	*luc (firefly)*	*B. subtilis*	3 h	10 nM
			S. aureus	2 h	3.3 nM
	lac (E. coli)	*lux (V. fischeri)*		1 h	>0.42 µM
Chromate	*chr (A. eutrophus)*	*lux*	*A. eutrophus*	1–2 h	1–50 nM
Copper		*luxABCDE (V. fisheri)*	*E. coli*		1 µM–1 mM
Heavy metals		*luxAB (V. harveyi)*			
Iron	*pupA (P. putida)*	*luxCDABE (V. fischeri)*	*P. putida*	Hours	10^{-2}–1 µM
Inorganic mercury	*mer (Tn21)*	*luc (firefly)*	*E. coli*		≤0.1 fM
	merTPAP (TN501)	*lacZ, lux (V. fischeri)*	*E. coli*		0.02–0.2 µM
	mer (S. aureus)	*blaZ (S. aureus)*			5 µM
	mer (Tn21)	*luxAB (V. harveyi)*	*E. coli*	2–3 min	10^{-9} M
	mer (Tn21)	*luxCDABE (V. fischeri)*	*E. coli*	40 min	0.5–5 µM
	mer (Tn21)	*luxCDABE (V.a fischeri)*	*E. coli*		10 pM
	mer	*lux (V. fischeri)*	*E. coli*	30 min	10 nM–4 µM
	mer	*luxABCDE (V. fisheri)*	*E. coli*		0.1 nM–1 µM
	merR-T'	*luxCDABE (V. fischeri)*	*E. coli*	70 min	0.025 nM
	mer (S. marcescens)	*lux (V. fischeri)*	*E. coli*	90 min	

Köhler *et al.*, 2000

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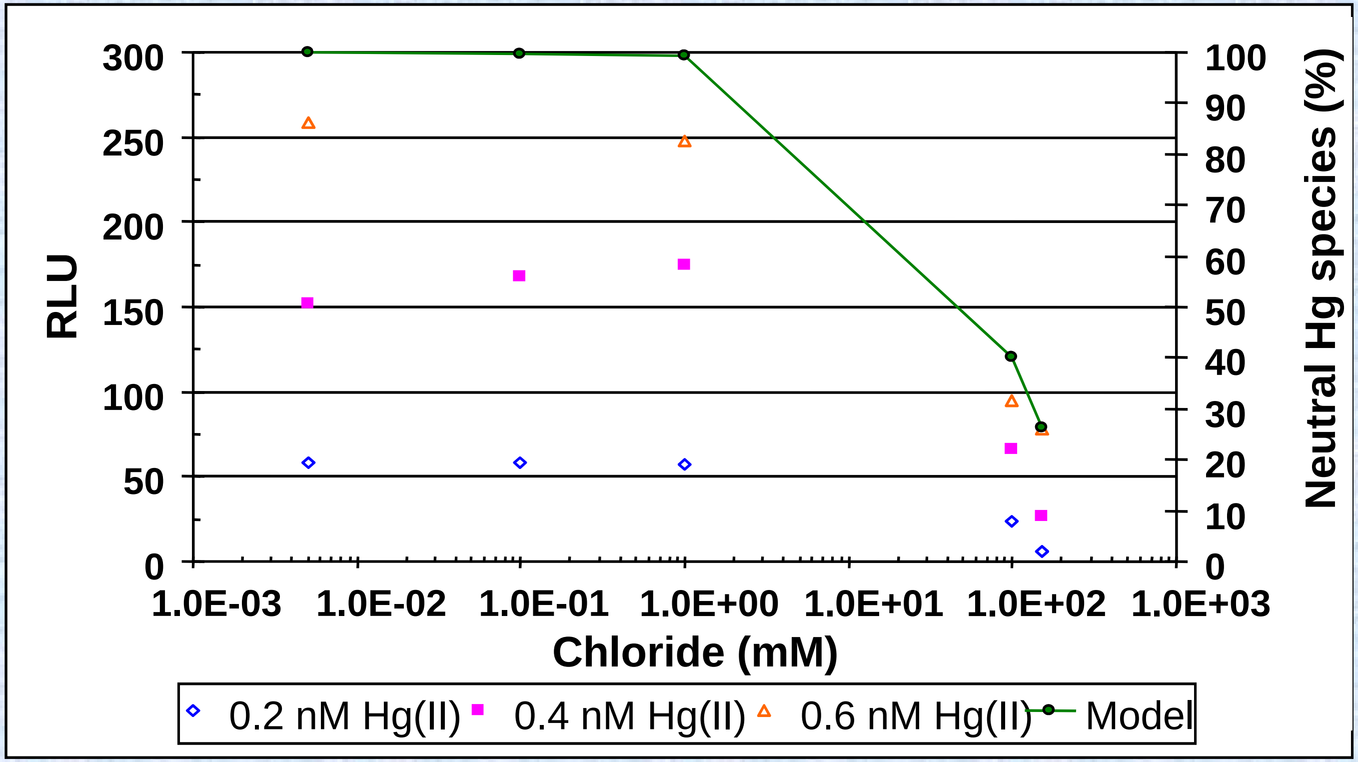
➢Exemplo: Biossensor de Hg

- ❑ Complexos neutros de Hg são mais Biodisponíveis que complexos aniônicos

Cl total (mM)	Hg species (%)			
	$Hg(OH)_2^o$	$Hg(Cl)_2^o$	$Hg(Cl)_3^-$	$Hg(Cl)_4^{2-}$
0.005	99.8	0.2		
0.1	61.1	38.7		
1	1.6	97.6	0.8	
100		40	31.8	28.3
150		26.4	31.5	42



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➢ Áreas de Aplicações dos Biossensores:

- ❑ Clínica
- ❑ Alimentos
- ❑ Ambiental
  - ✓ Monitoramento/Screening
  - ✓ Avaliação da Biodisponibilidade
  - ✓ Avaliação do Efeito/Toxicidade
  - ✓ Exemplos: DBO, Fenol, Fosfato, Metais e Pesticidas

} Avaliação de Risco Ambiental

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- Exemplos da Aplicação de Biossensores a Amostras Ambientais:

Analyte	Matrix	Transducing and recognition element
Pesticides and estrone	River water	Optical immunochemical
Phenols	Wastewater	Electrochemical enzymatic
Linear alkyl benzene sulphonate (LAS)	River water	Electrochemical bacteria
Toxicity	Wastewater	Electrochemical bacteria
Toxicity	Wastewater	Optical bacteria
Alkanes	Groundwater	Optical bacteria
Estrogens and xenoestrogens	Real water samples (lake and a sewage plant)	Optical human estrogen receptor (EC)
BOD	River water	Optical pseudomona
Zinc dichromate chromate	Soil (extract)	Optical bacteria
Mercury arsenite	Soil (extract)	Optical pseudomonas
Daunomicyn PCBs, aflatoxin	River water (preconcentrated)	Electrochemical DNA
Chlamydia trachomatis (DNA)	River water (preconcentrated)	Electrochemical DNA

Rodriguez-Mozaz *et al*., 2005

➢ Biossensores Comerciais existentes no mercado:

Instrument	Company	Transducing and recognition element
BIACORE	Biacore AB (Uppsala, Sweden)	Optical BI
IBIS	Windsor Scientific Ltd. (Berks, UK)	Optical BI
SPR-CELLIA	Nippon Laser and Electronics Lab (Japan)	Optical whole cells or macromolecules
Spreeta	Texas Instruments Inc. (Dallas, USA)	Optical BI
BIOS-1	Artificial Sensing Instruments (Zurich, Switzerland)	Optical BI
	Amersham International	Optical immunoreagent
	XanTec Bioanalytics GmbH (Münster, Germany)	Optical BI
Kinomics Plasmoon™	BioTul AG (Munich, Germany)	Optical BI
IASys plus ™–	Affinity Sensors, (UK)	Optical Antibody
REMEDIOS	Remedios (Aberdeen, Scotland)	Optical whole cell
Cellsense	Euroclon Ltd. (Yorkshire, UK)	Electrochemical *Escherichia coli*
PZ 106 Immunobiosensor System	Universal Sensors, (Kinsale, IR)	Piezoelectric antibody
ARAS BOD	Dr. Bruno Lange GmbH (Duesseldorf, Germany)	Electrochemical whole cell
ToxSenTM	Abtech Scientific Inc., (Yardley, USA)	Electrochemical BI
	Universal Sensors Inc., (New Orleans, USA)	Electrochemical enzymes
NECi's Nitrate Biosensor	Nitrate Elimination Co. Inc., (Michigan, USA)	Amperometric enzyme
eTag Assay System	ACLARA Bioscience (Mountain View, USA)	Optical eTag reporters

Rodriguez-Mozaz *et al*., 2005

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- Biossensores Comerciais existentes no mercado:
  - RIANA

Rodriguez-Mozaz et al., 2005


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➢ Perspectivas Futuras dos Biossensores:

- ❑ Aperfeiçoamento das Tecnologias dos Transdutores
  - ✓ Nanotecnologia & Miniaturização
  - ✓ Matrizes Multi-sensores
- ❑ Desenvolvimento de Novos Bioelementos
  - ✓ Biomateriais desenvolvidos por Engenharia Genética
  - ✓ Materiais Sintéticos
- ❑ Aperfeiçoamento das Técnicas de Construção
  - ✓ Imobilização


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- Perspectivas Futuras dos Biossensores:
  - Desenvolvimento de novos Biossensores para a Análise de Contaminantes Emergentes;
  - Ferramenta para avaliação rápida de amostras ambientais *in loco*;
  - Possível uso na biorremediação de áreas contaminadas;
  - Aumento do número de Patentes e de Sistemas Comerciais no mercado;
  - Desenvolvimento de Estações Analíticas Autônomas Integradas Remotamente.


FIM
Obrigado pela sua atenção
Perguntas???