Slovenská technická univerzita v Bratislave
Fakulta chemickej a potravinárskej technológie
Ústav analytickej chémie

Využitie nanomateriálov pre chemické senzory

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Obsah

1. Nanotechnológia všeobecne

2. Nanomateriály ako stavebné súčasti senzorov

3. Senzory s nanorozmermi

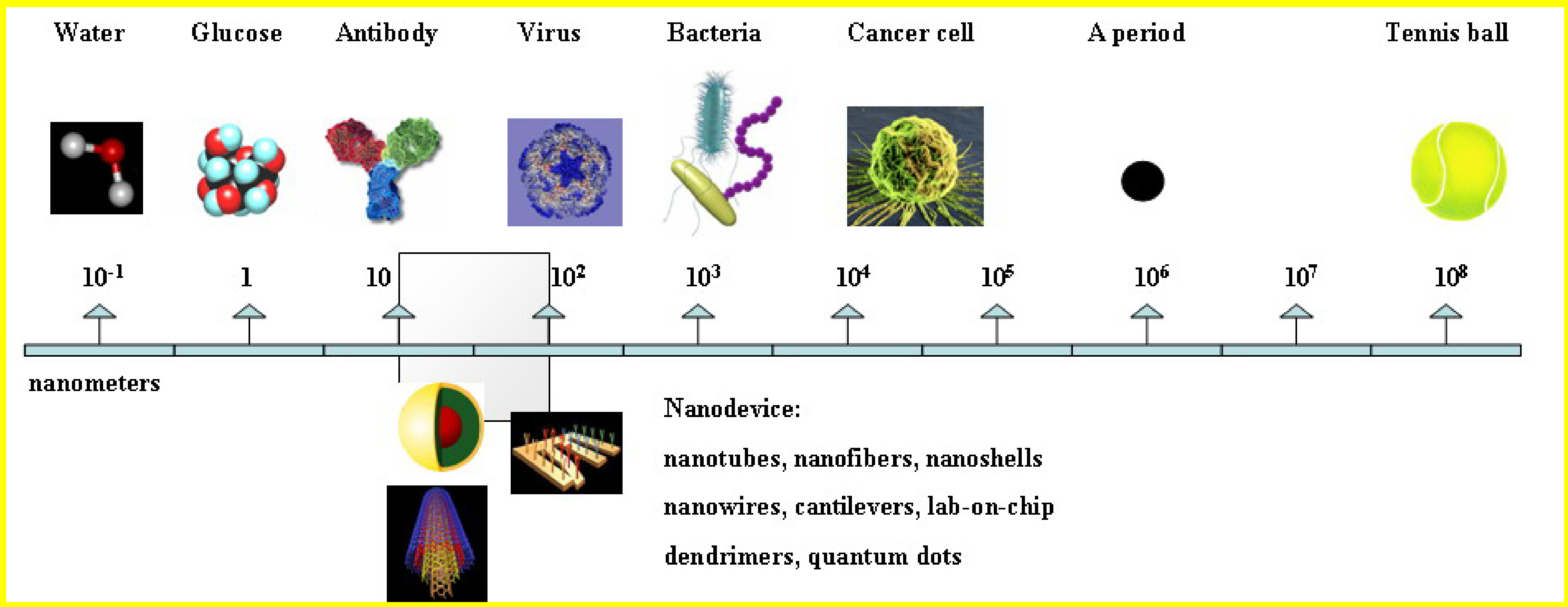
4. Riziká nanotechnológií

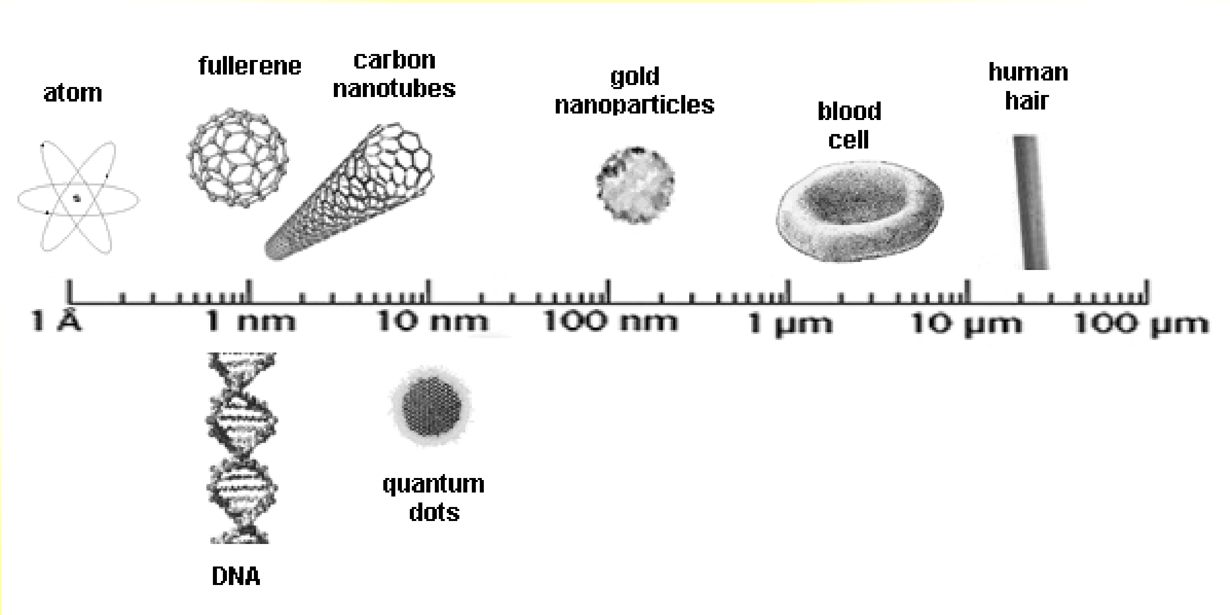
5. Záver

Čo je nanotechnológia?

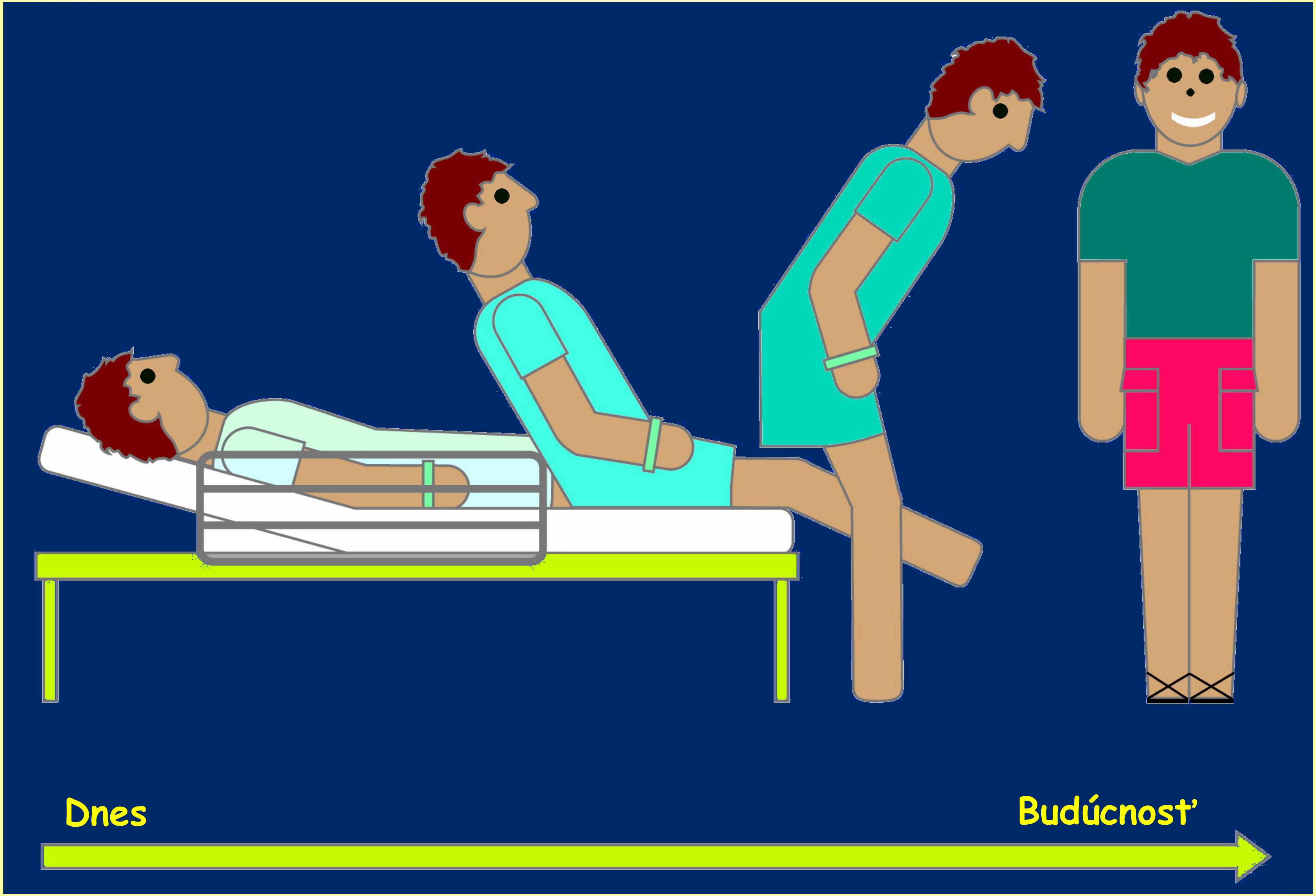
Nanotechnológia zahrňuje štúdium, manipuláciu, úpravu a použitie materiálov, zariadení a systémov s rozmerom / rozmermi typicky menšími než 100 nm.

Usporiadania v nanometrovej škále





Prečo nanotechnológia?



Unikátne fyzikálne a chemické črty nanomateriálov spôsobuje:

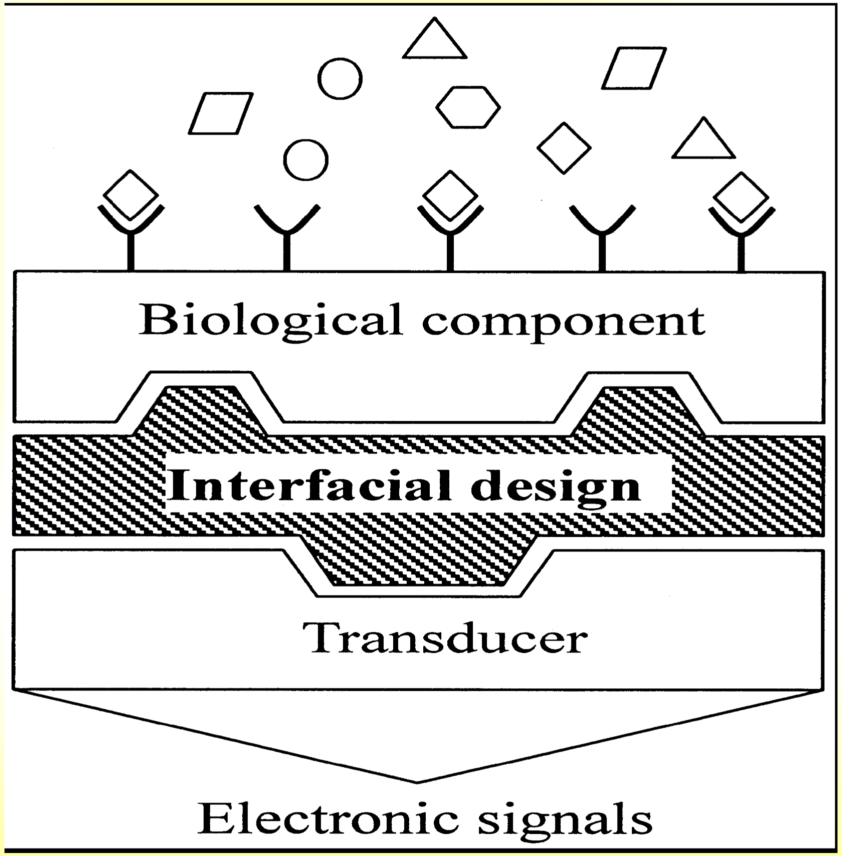
- efekt veľkosti kvanta (the quantum size effect)
- efekt mini veľkosti (mini size effect)
- povrchový efekt (surface effect)
- tunelový makrokvantový efekt (macro-quantum tunnel effect).

V prípade senzorov sú výsledkom použitia nanomateriálov:

- nové techniky prenosu signálu,
- zlepšenie imobilizácie biologickej zložky,
- zjednoduchšenie a zrýchlenie analýzy s vysokou citlivosťou a selektivitou,
- *in vivo* analýzy.

Ref.: C. Jianrong et al., Biotechnol. Advances 22, 505 (2004)

Ako využiť nanomateriály a zlepšiť (špecifický) signál chemického senzora?



Markery

Interface

Informácie - literárne prehľady

T. Vo-Dinh et al., Nanosensors and biochips, frontiers in biomolecular diagnostics, Sens. Actuators B, 74, 2 (2001)

Q. Zhao et al., Electrochemical sensors based on carbon nanotubes, Electroanalysis 14, 1609 (2002)

D. Hernandez-Santos et al., Metal nanoparticles based electroanalysis, Electroanalysis 14, 1225 (2002)

J. Wang, Nanoparticle-based electrochemical DNA detection, Anal. Chim. Acta 500, 247 (2003)

C. Jianrong et al., Nanotechnology and biosensors, Biotechnol. Advances 22, 505 (2004)

C.N.R. Rao et al., Nanotubes and nanowires, Chem. Eng. Sci. 59, 4665 (2004)

J. Wang, Carbon-nanotube based electrochemical biosensors, Electroanalysis 17, 7 (2005)

M. Valcarcel et al., Present and future applications of carbon nanotubes to analytical science, Anal. Bioanal. Chem., 382, 1783 (2005)

A. Merkoci et al., Nanoparticles for DNA labeling, TRAC 24, 341 (2005)

A. Merkoci, Carbon nanotubes in analytical sciences, Microchim. Acta 152, 157 (2006)

P. He et al., Applications of carbon nanotubes in electrochemical DNA biosensors, Microchim. Acta 152, 175 (2006)

K. Balasubramanian, M. Burghard, Biosensors based on carbon nanotubes, Anal. Bioanal. Chem. in press.

N. Sinha et al., Carbon nanotube-based sensors, J. Nanosci. Nanotechnol. 6, 573 (2006)

M. Trojanowicz, Analytical applications of carbon nanotubes: a review, TRAC 25, 480 (2006)

Eftekhari (Ed.): Nanostructured Materials in Electrochemistry, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, 2007.

Electrochemical sensors based on unidimensional nanostructures

A. C. Pereira, A. Kisner and L. T. Kubota

Instituto de Química, Universidade Estadual de Campinas, Campinas,SP, Brazil

Sensors based on low-dimensional materials are not a novelty in the current days. In fact, the miniaturization of chemical and biochemical sensors has a long history, since the works of Wise et al. [*IEEE Trans. Bio-Medical Eng.* ***1970***, *17*, *238*] in the end of sixties and Bergveld [ibid. ***1970***, *17*, *70*, ***1972***, *19*, *342*] in the beginning of seventies that could be denominated as pioneers.

Ref.: Eftekhari (Ed.): Nanostructured Materials in Electrochemistry, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, 2007.

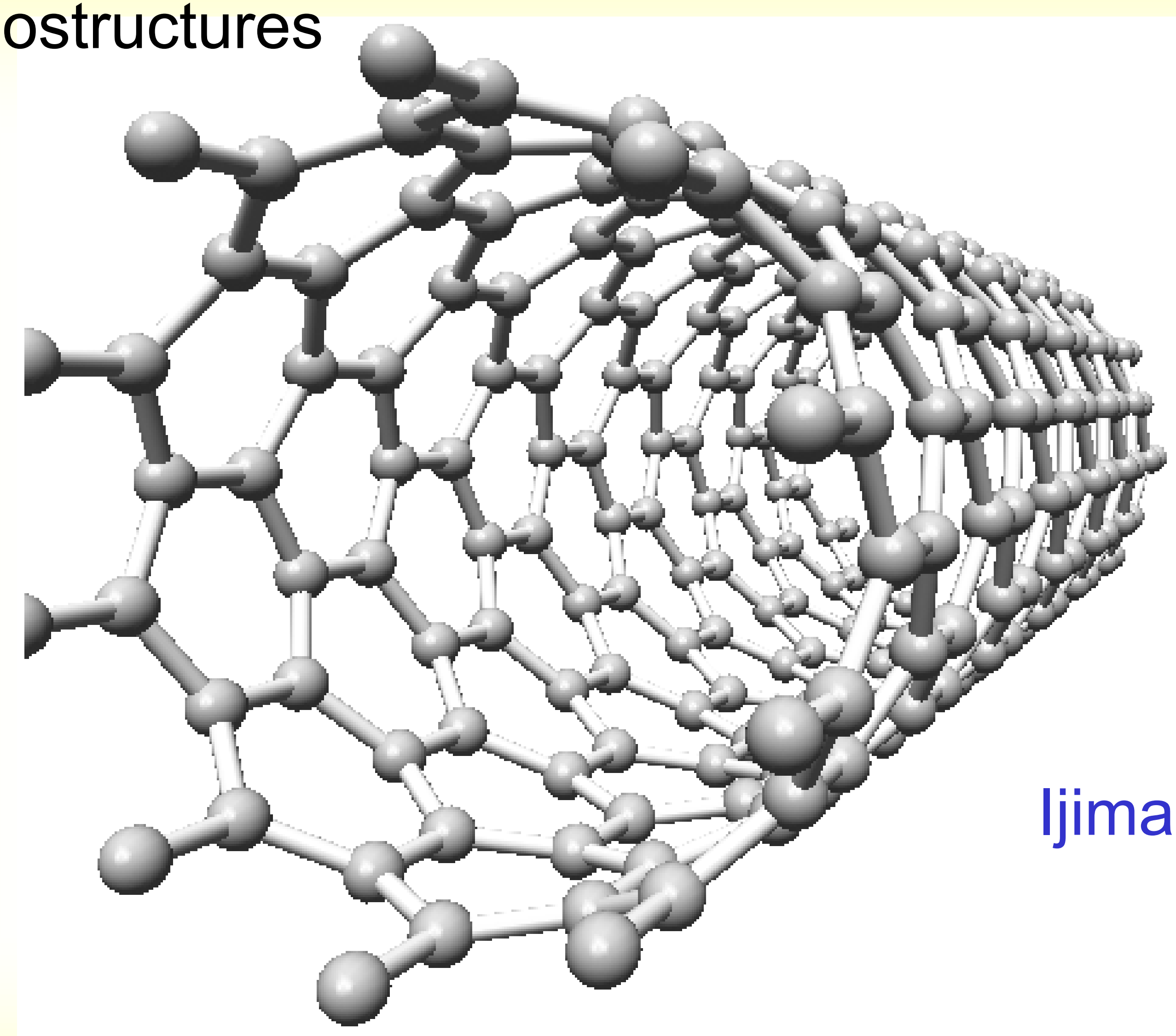
However, over the last few years a new wave of highly sensitive materials has reached scientific and industrial interests. These nanostructured materials, as they are now widely recognized, are interesting due to their promising new applications, added to the novel dimension-dependent properties exhibited by them.

In this way, unidimensional (1D) structures such as nanowires and nanotubes are expected to be excellent primary transducers in systems like biosensors.

Moreover, the relative facility to integrate these 1D materials with microelectronic techniques, these wires or tubes in nanoscale can act as active devices providing a set of new nanobiotechnology, such as the biochips.

„Carbon nanotubes"

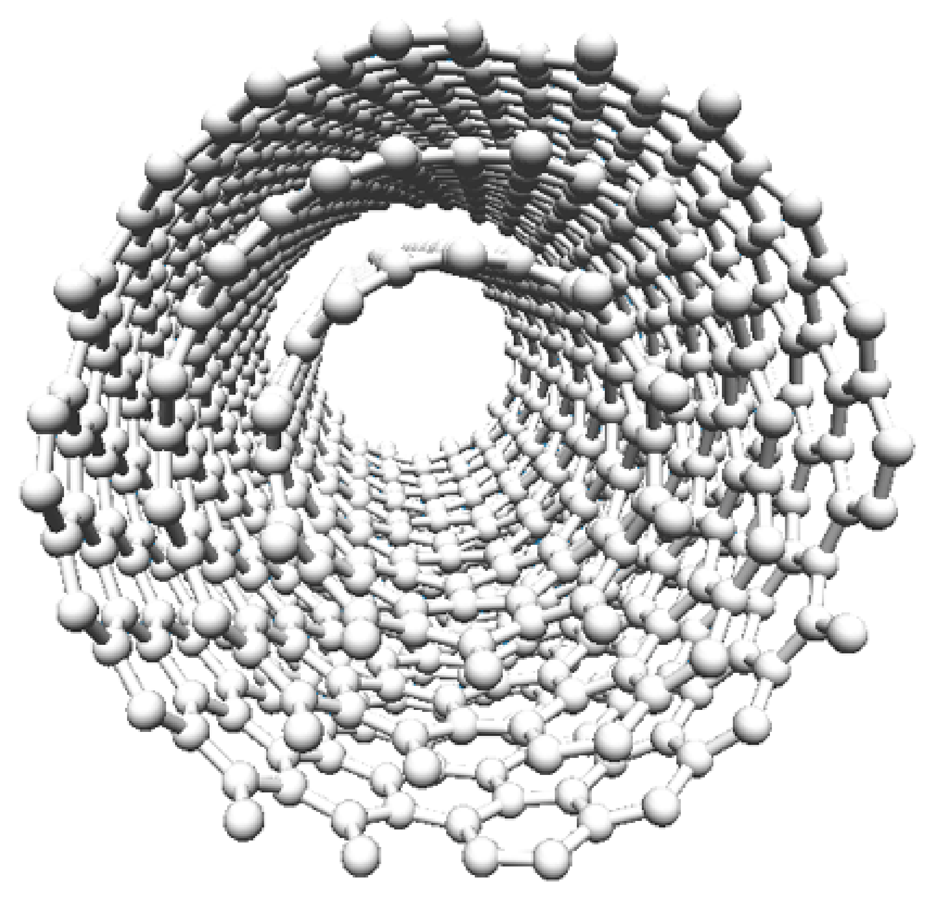
Wire-like nanostructures

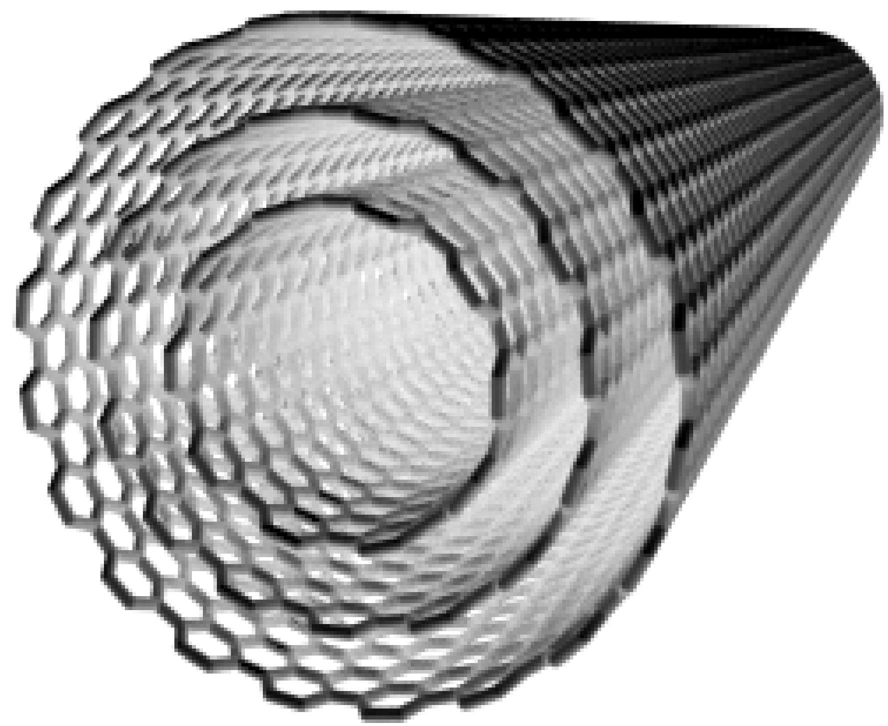


Ijima, r. 1993

Jednovrstvové (single walled nanotubes, SWNT)

priemer 0.2-2 nm, dĺžka 0.6-4 nm



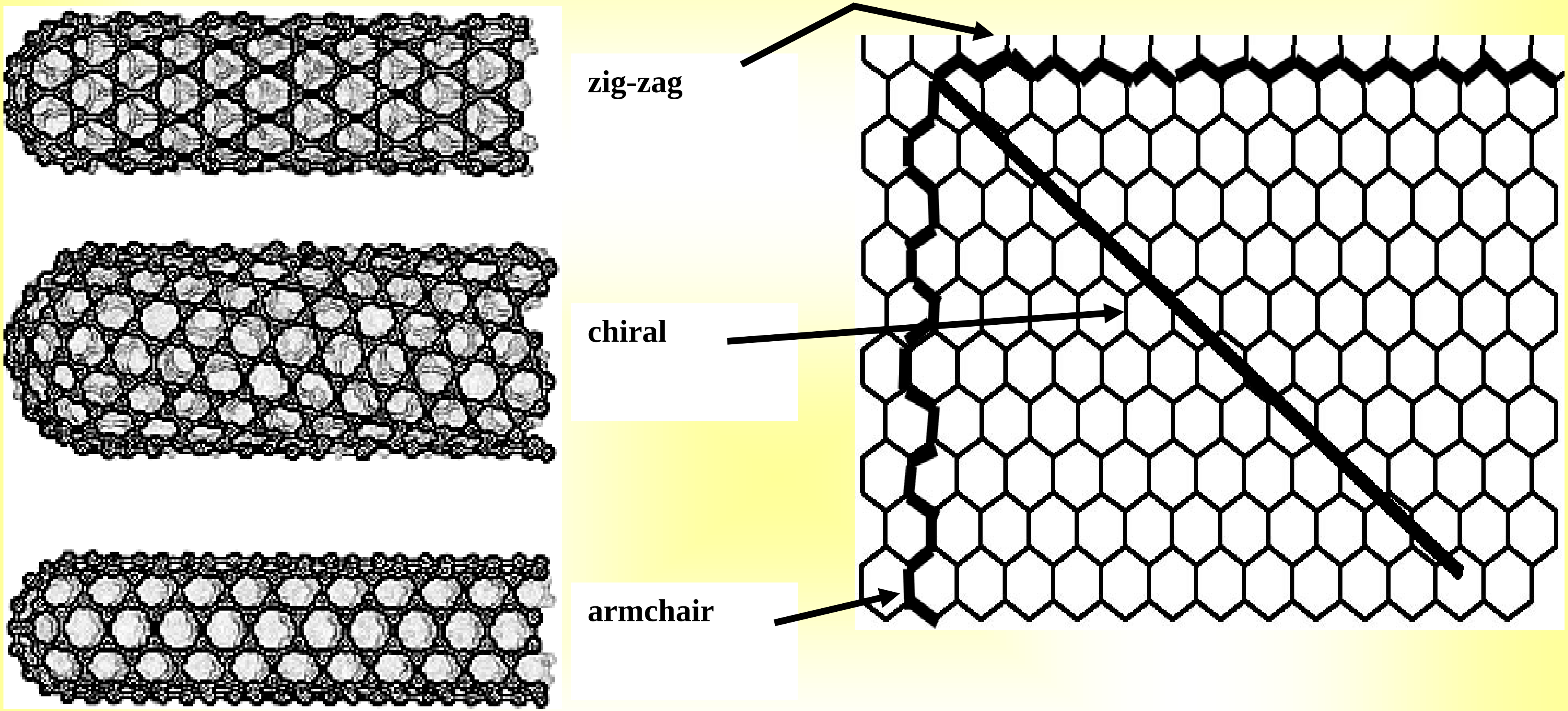


Ijima, r. 1991

Viacvrstvové (multiwalled nanotubes, MWNT)

vonkajší priemer 2-100 nm, vnútorný priemer 1-10 nm,

dĺžka 5-500 nm



Chirality of carbon nanotubes

Zig-zag and chiral are semiconductors, armchair have metallic properties

Vlastnosti CNT:

- jedinečné elektrochemické, chemické, optické, mechanické a štruktúrne vlastnosti

- zlepšenie elektrochemickej reaktivity biomolekúl (GOD, NADH)

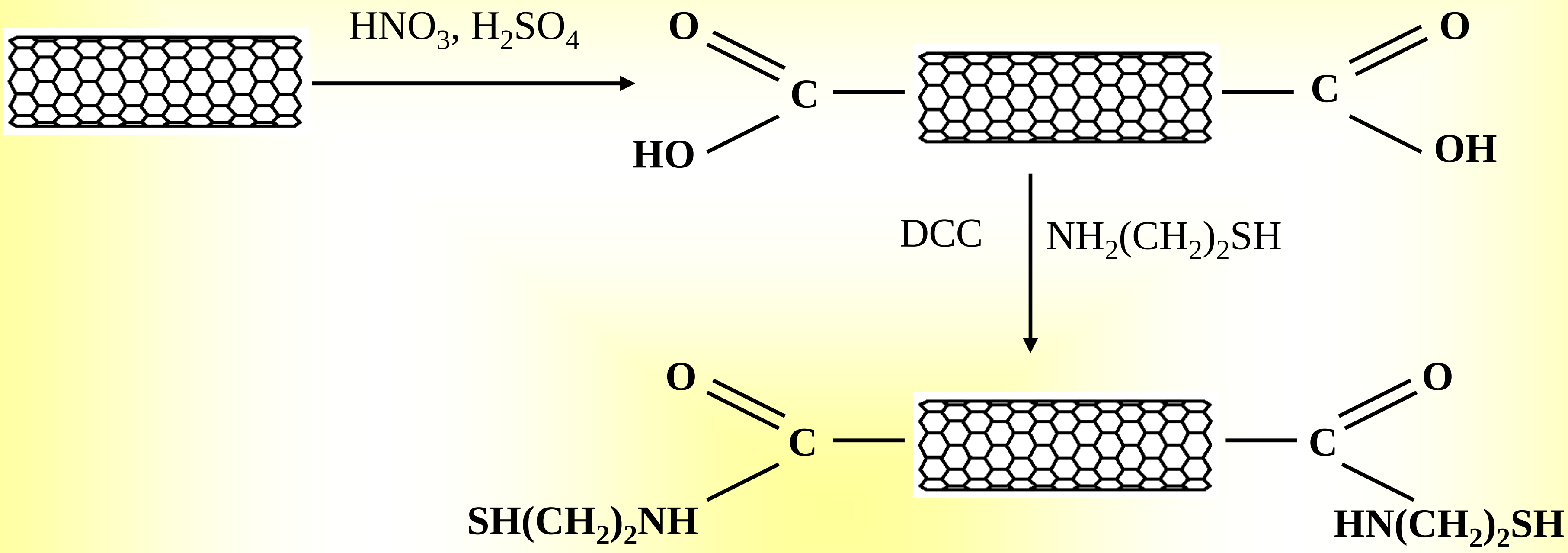
- zlepšenie akumulácie biomolekúl

- sú hydrofóbne, možno ich dispergovať:
    - v nepolárnych rozpúšťadlách
    - v polyméroch (Nafion), sol-gel
    - vo vode

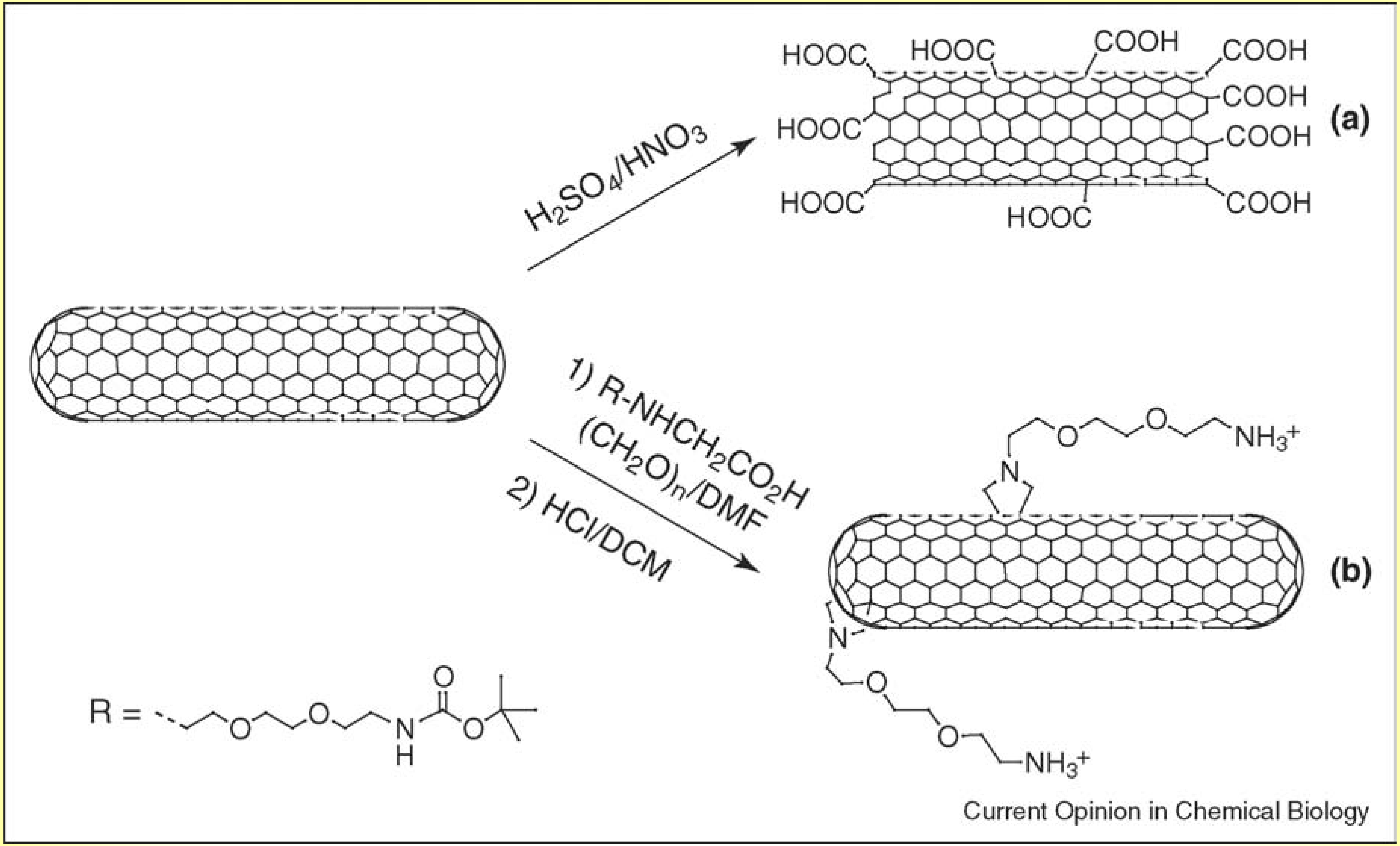
Solubilizácia SWNT vo vode

Functional group	SWNT equivalent solubility in water (mg/ml)	References
Oxidative treatments		
98% H_2SO_4/70% HNO_3 (3:1)	1.77 (pH 3)	[6]
98% H_2SO_4/30% H_2O_2 (9:1)	>0.15 ($3<pH<12$)	[7]
98% H_2SO_4/$(NH_4)_2S_2O_8$/P_2O_5-$KMnO_4$-H_2SO_4	$>$ 0.66 (pH 3)	[8]
Non-covalent stabilization		
Sodium dodecylbenzene sulfonate (SDBS)	20	[9]
Sodium dodecyl sulfate (SDS)	≤ 0.1	[9]
Triton X-100	≤ 0.5	[9]
γ-Cyclodextrin	<0.2	[10]
Covalent stabilization		
Glucose	0.1	[11]
DNA	–	[12, 13]
Enzymes	–	[14]

Ref.: M. Valcárcel et al.: Anal. Bioanal. Chem. 382, 1783 (2005)



Functionalization of carbon nanotubes
(DCC – dicyclohexylcarbodiimide)



Ref.: A.Bianco et al., Current Topics in Chem. Biol. 9, 674, 2005

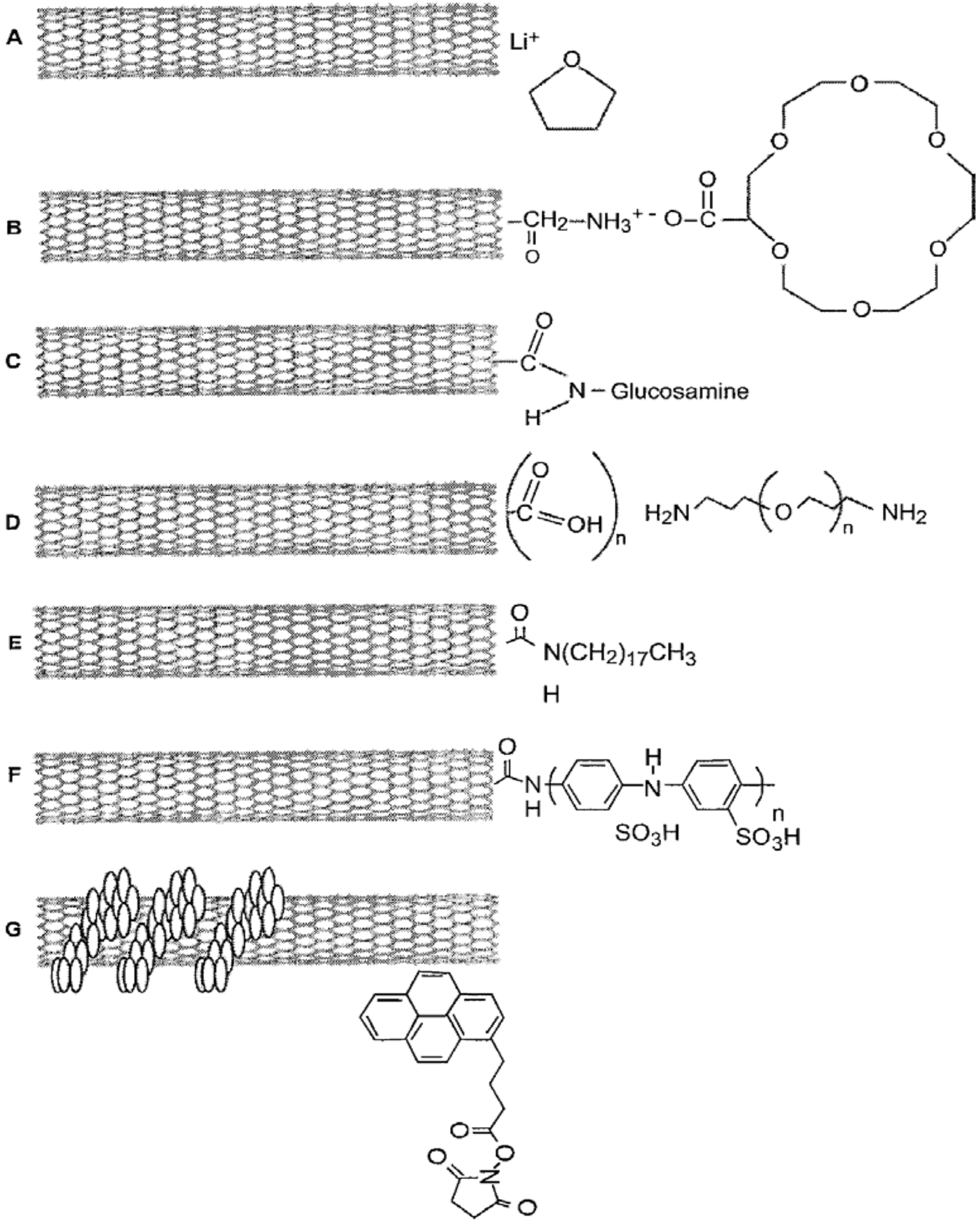
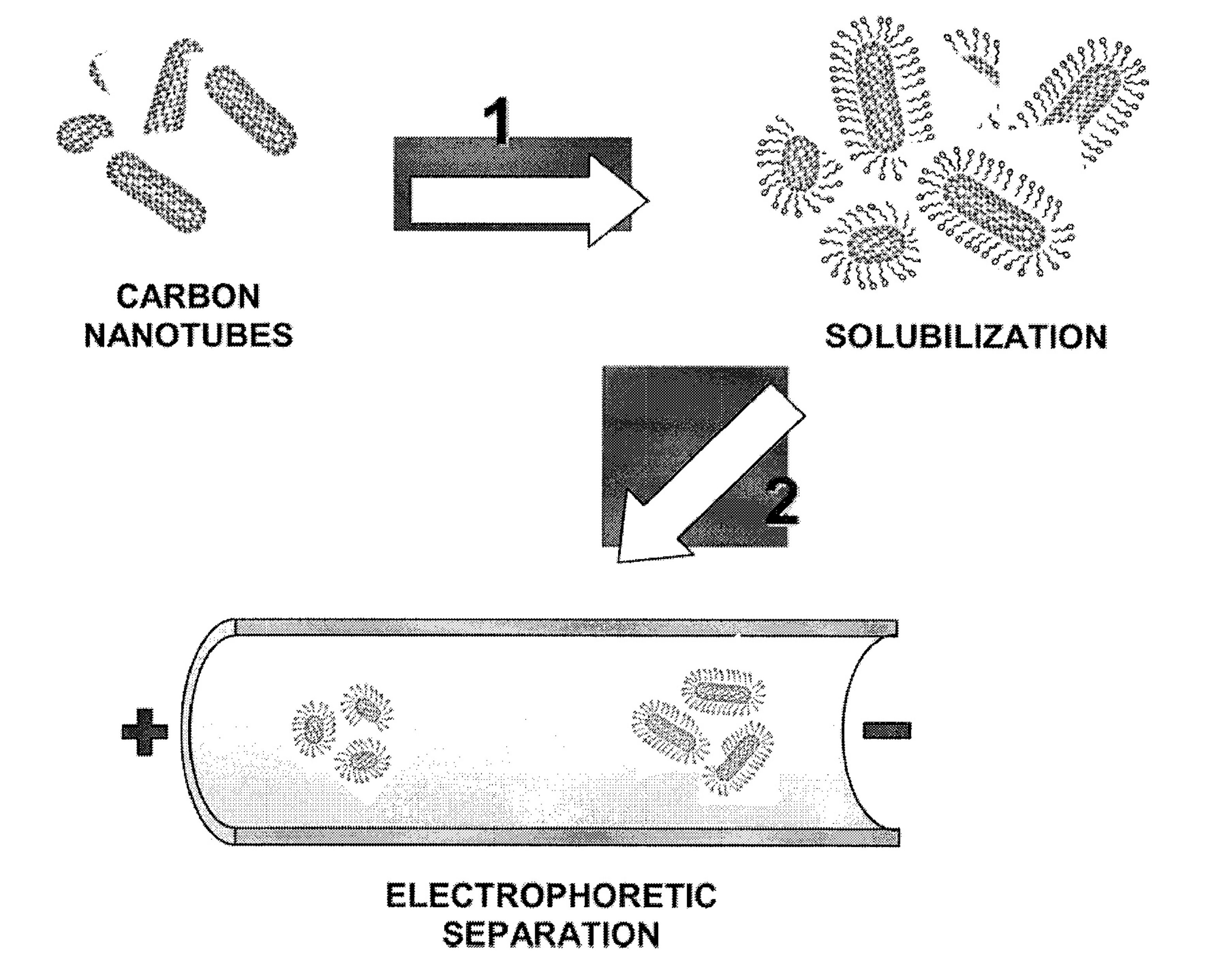


Fig. 2. Schematic of typical CNT solubilization alternatives:; (A) CNT-Li^+ conducting polyelectrolyte; (B) with amino group of 2-aminomethyl-18-crown-6 ether, (C) by amide bonds with glucosamine; (D) by diamine-terminated oligomeric poly(ethylene glycol); (E) octadecylamine (ODA) functionalized CNTs and (F) poly(m-aminobenzene sulfonic acid) (PABS) functionalized SWNTs. (G), left: supramolecular wrapping with polymer; right: noncovalent scheme that involves π-stacking of 1-pyrenebutanoic acid succinimidyl ester onto the sidewalls of SWCNTs. For details, see text

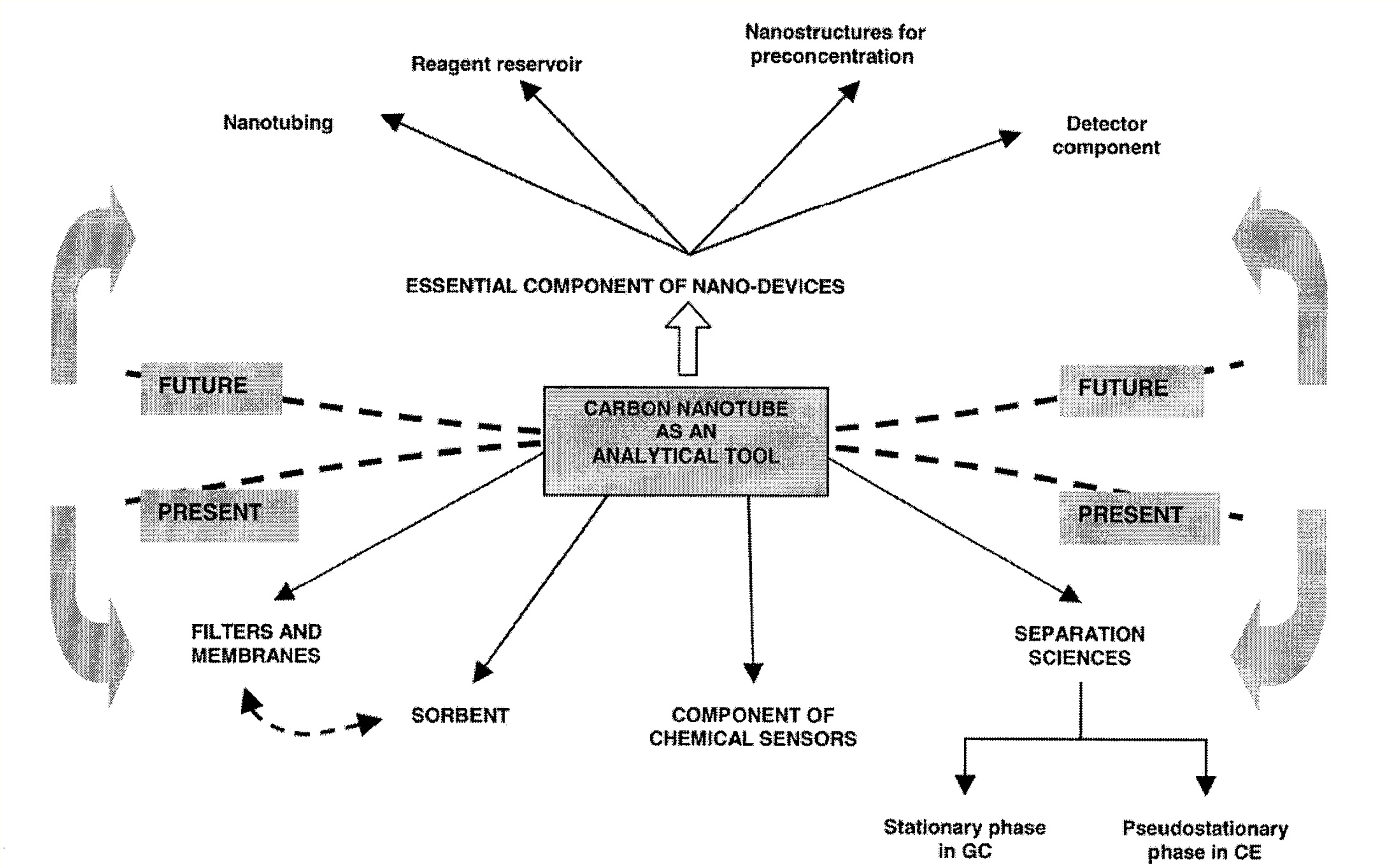
Ref.: A. Merkoci: Microchim. Acta 152, 157 (2006)

Charakterizácia a analýza CNT pomocou kapilárnej elektroforézy



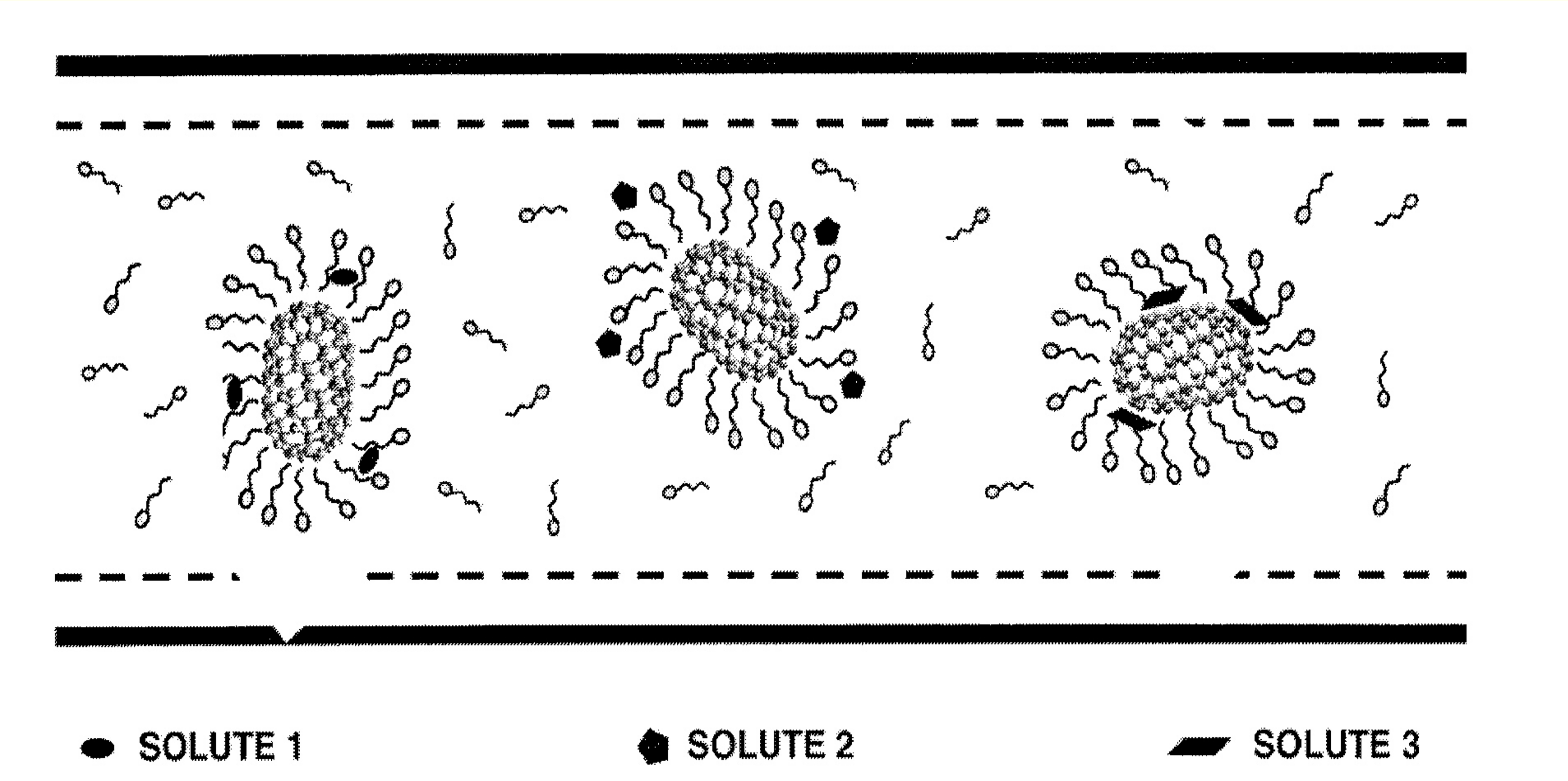
Ref.: M. Valcárcel et al.: Anal. Bioanal. Chem. 382, 1783 (2005)

Súčasné a potenciálne analytické využitie CNT



Ref.: M. Valcárcel et al.: Anal. Bioanal. Chem. 382, 1783 (2005)

Micelárna elektrokinetická chromatografia s CNT



Látka 1 interaguje ako ko-surfaktant, látka 2 interaguje na povrchu, látka 3 interaguje na povrchu CNT

Ref.: M. Valcárcel et al.: Anal. Bioanal. Chem. 382, 1783 (2005)

Elektrochemické senzory na báze CNT

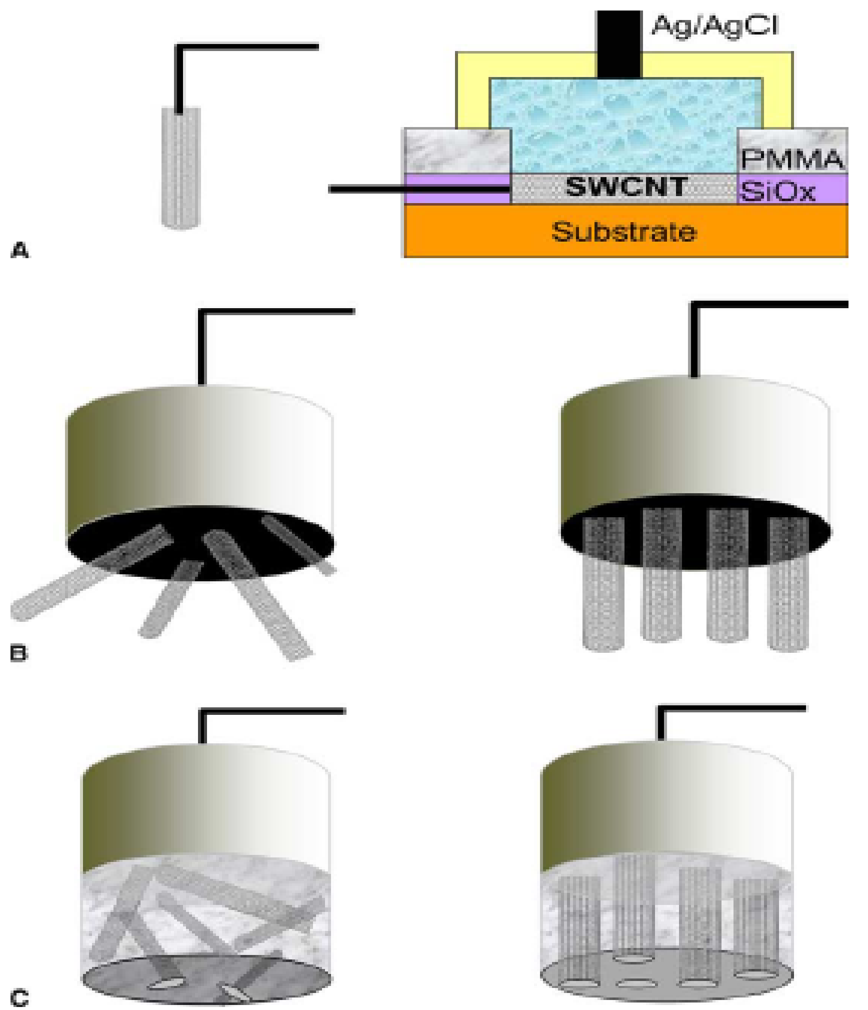
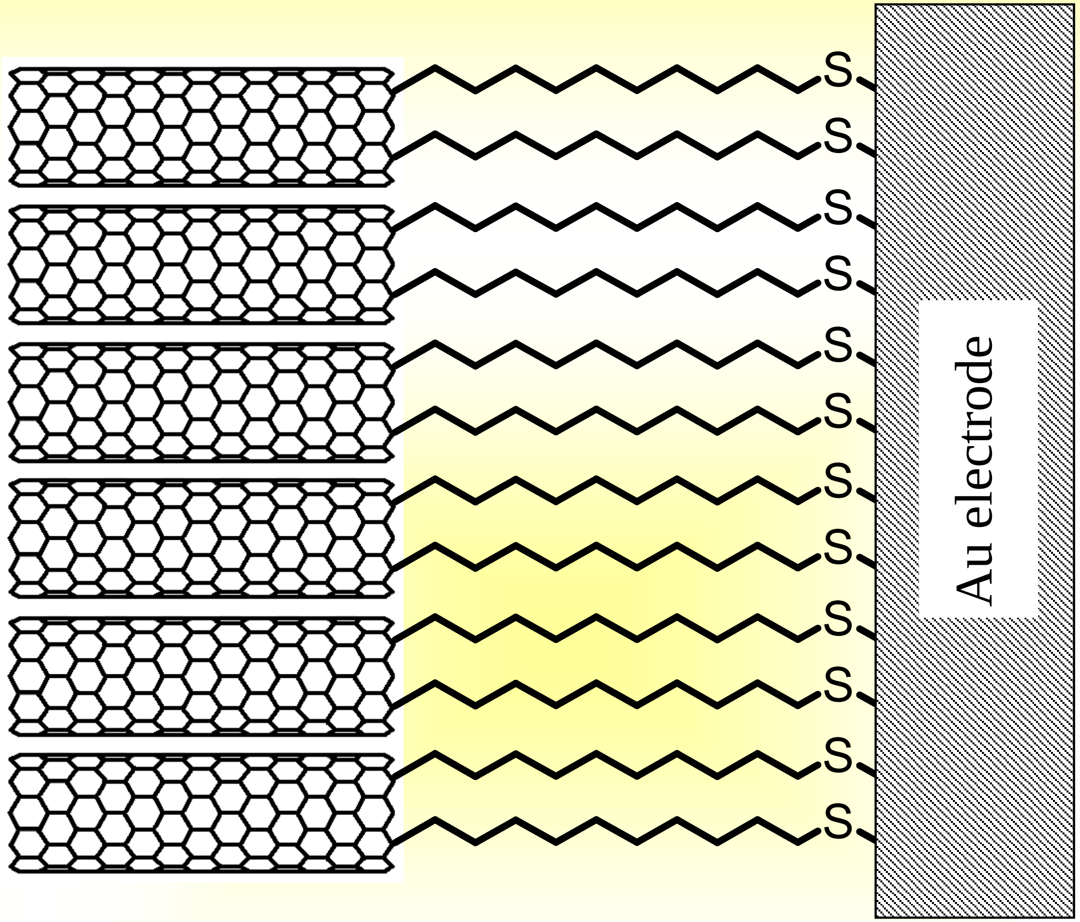
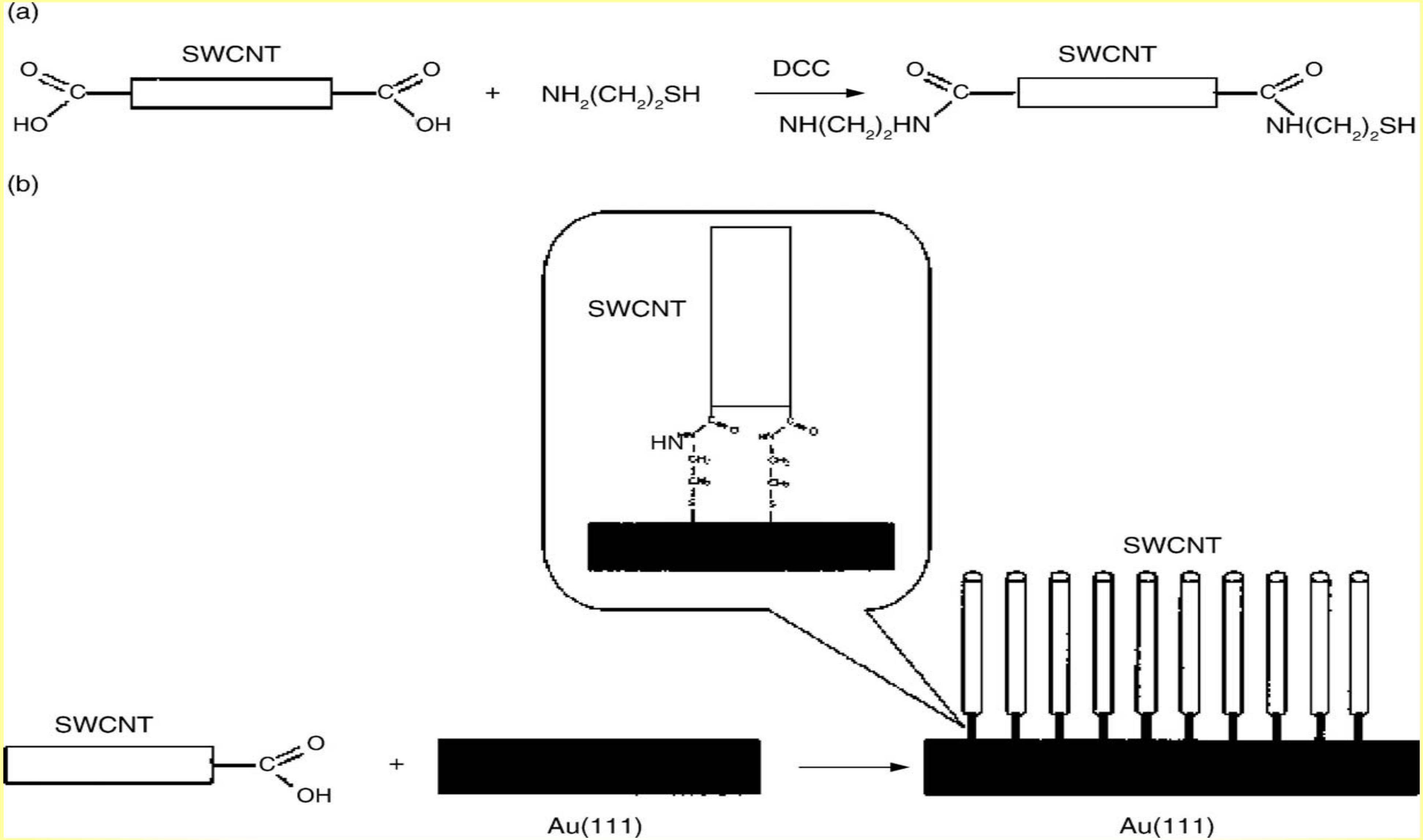


Figure 3. Three configurations of CNT integration in electrochemical sensors. (A) Use of individual CNT: for AFM tips (left) or individual SWCNT onto a Si wafer substrate (right). SiOx and PMMA are used as insulating layers in which windows are opened to expose the SWCNT; (B) electrode surface modifiers: non-oriented (left) and oriented (right) CNT configuration; and, (C) composites with non-oriented (left) and oriented (right) CNT configuration.

Ref.: A. Merkoci et al.: TRAC 24, 826 (2006)

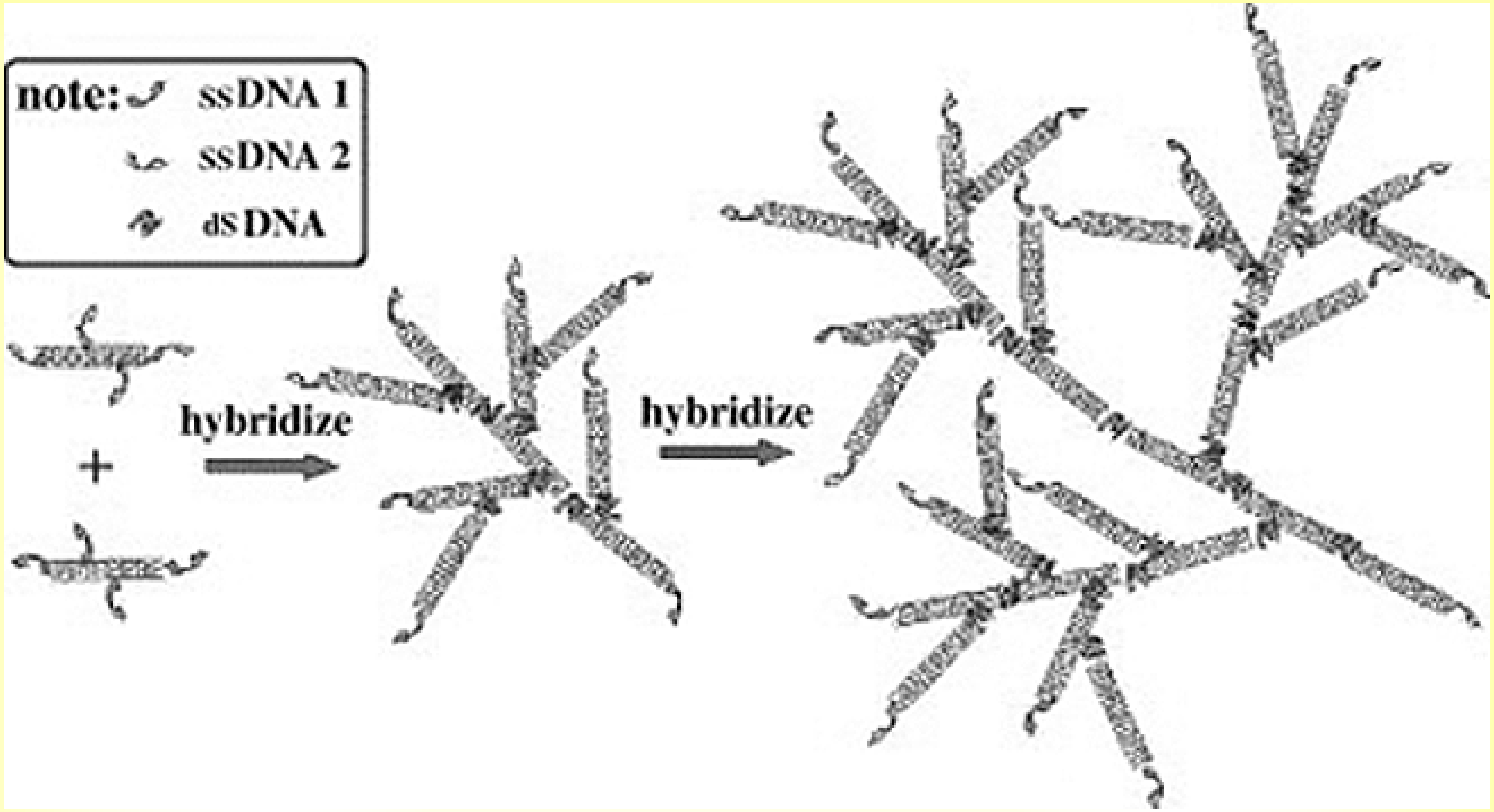


Highly ordered self-assembled carbon nanotubes

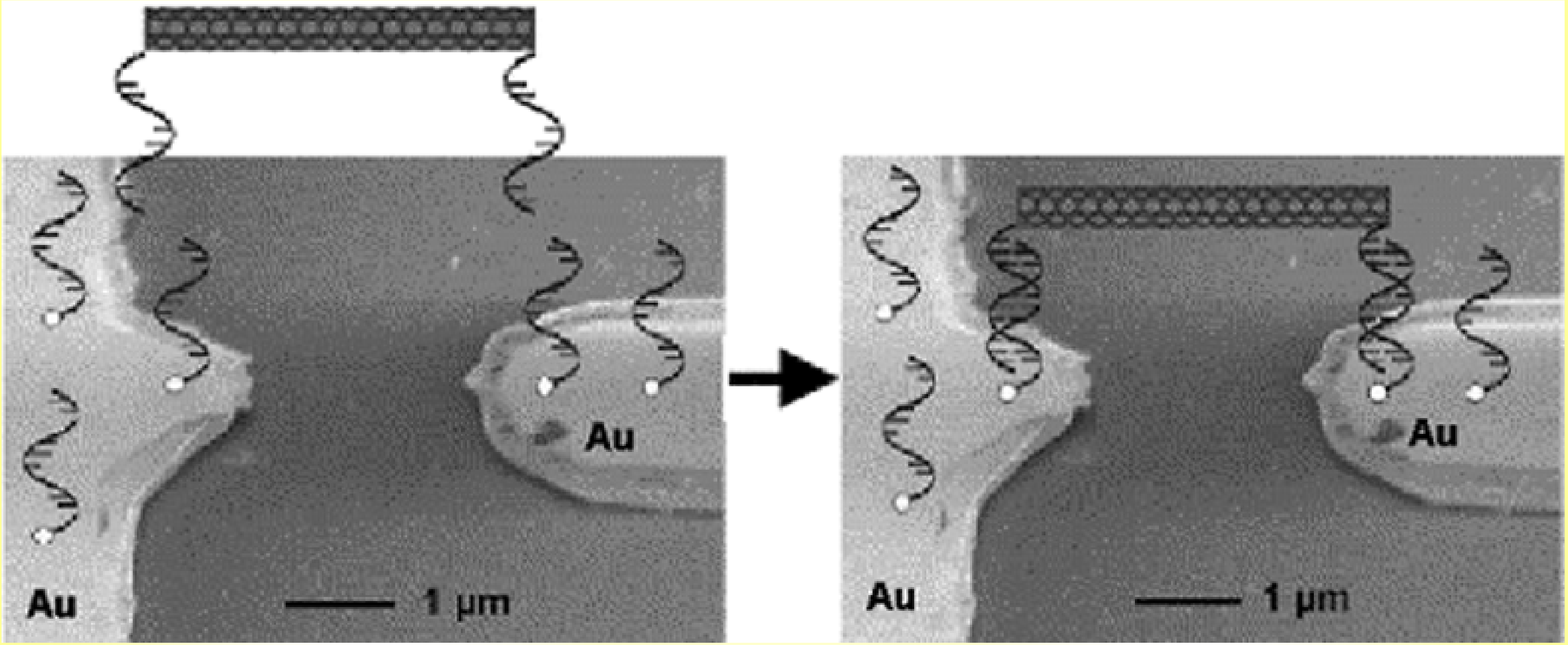


Fabrication of aligned nanotube electrode arrays by self-assembly

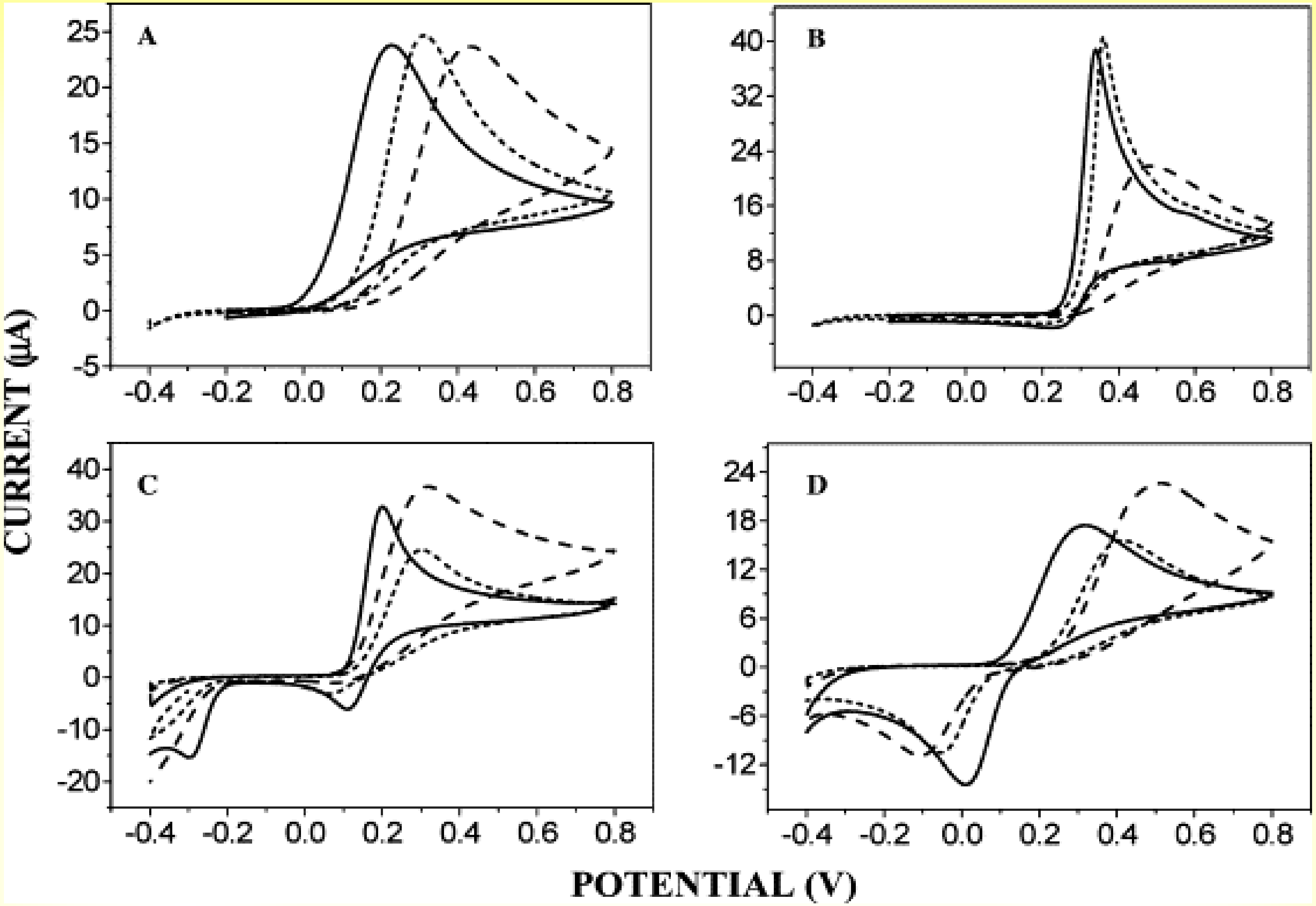
Ref.: Z. Liu et al: Langmuir16 (2000) 3569.



Schematic representation of the formation process of self-assembling of dsDNA-SWNTs



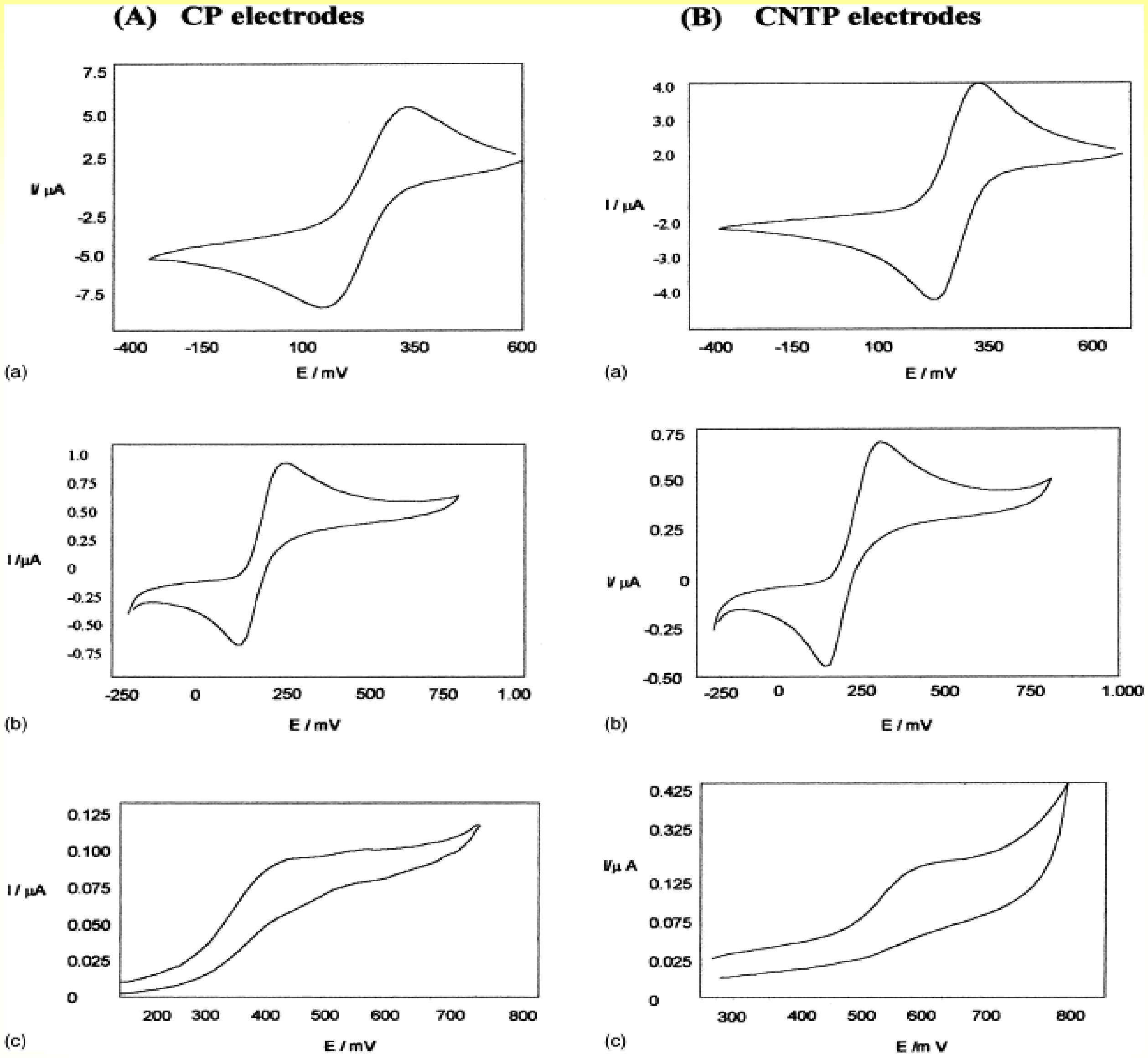
Schematic representation of DNA-mediated deposition of SWNT between two gold electrodes



CVs for 1.0 × 10^{-3} M ascorbic acid (A), uric acid (B), dopamine (C) and dopac (D) at different electrodes: (- - -) CPE; (…) CPE with 10% CNT; (—) CNTPE.

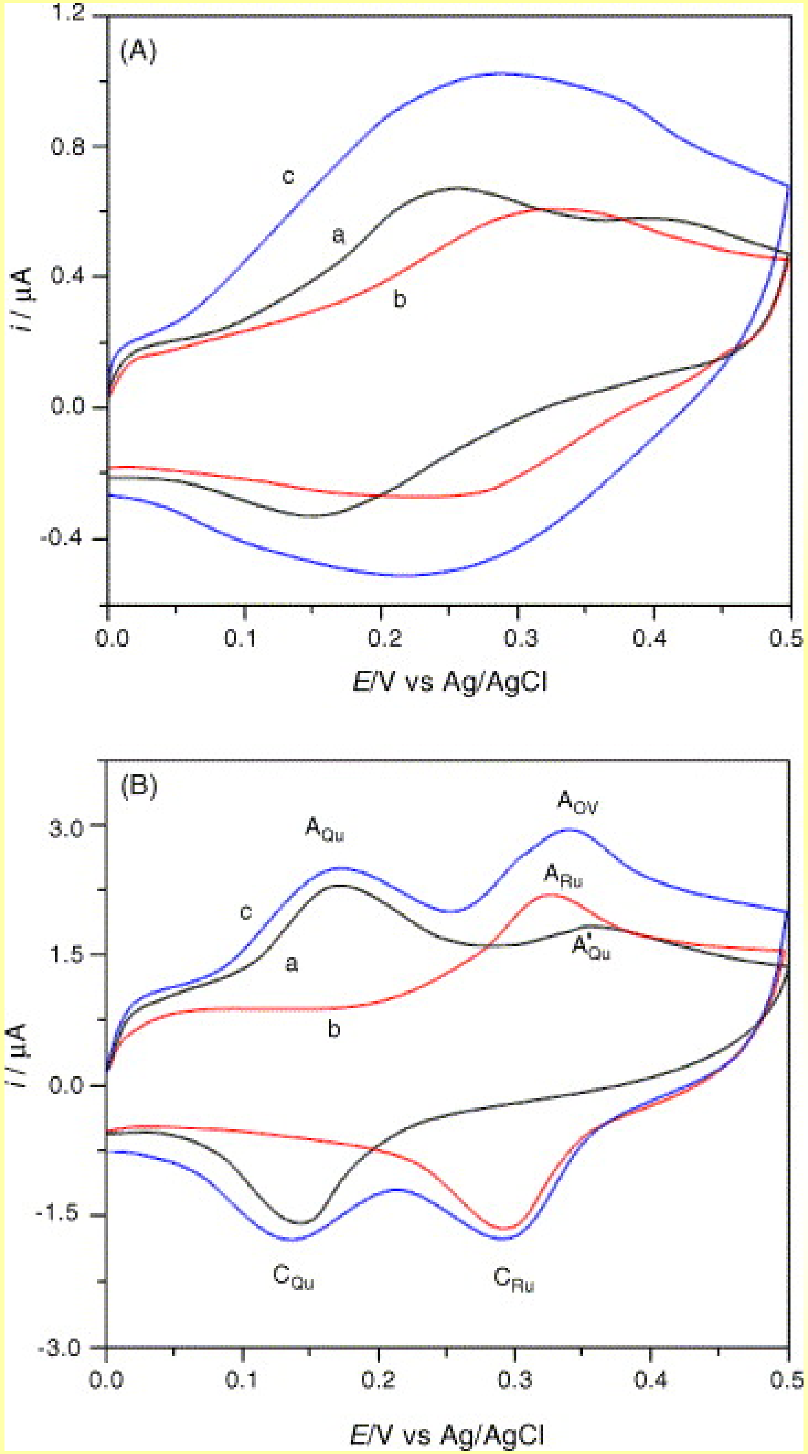
Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40. Scan rate: 0.100 V s^{-1}.

Ref.: M. D. Rubianes, G. A. Rivas, Electrochem. Commun. 5, 689 (2003).



CVs for 1×10^{-3} M potassium ferricyanide (a), 0.1×10^{-3} M dopamine (b), 0.1×10^{-3} M NADH (c) at CPE (A) and CNTPE (B), the CNT/oil ratio 60/40 (w/w). 0.2 M phopshate buffer (pH 7.0); scan rate, 100 mV/s.

Ref.: F. Valentini et al., Sensors Actuators B: Chem. 100, 2004, 117 (2004).



CVs of quercetin (a), rutin (b) and quercetin plus rutin (c)

at GCE (A)

and CNTPE (B)

B–R buffer (pH 7.0), concentration of each analyte: 1 μM; E_{ads} = 0.0 V; t_{ads} = 300 s; scan rate = 50 mV/s.

Ref.: Xiang-Qin Lin, Sensors Actuators B: Chem., in press.

Modifikátory elektrochemických biosenzorov na báze CNT

Nanotube	Combined/immobilized	Determination	References
MWNT-COOH	DNA	Specific hybridization	[42]
MWNT	β-cyclodextrin	Uric acid	[43]
MWNt	3-(mercaptopropyl)trimethoxilane	Fluphenazine	[44]
CNT	Mineral oil	Sulfide	[45]
CNT	Nafion/glucose oxidase	Glucose	[46]
CNT	Teflon/alcohol dehydrogenase/NAD^+	Ethanol	[47]

Ref.: M. Valcárcel et al.: Anal. Bioanal. Chem. 382, 1783 (2005)

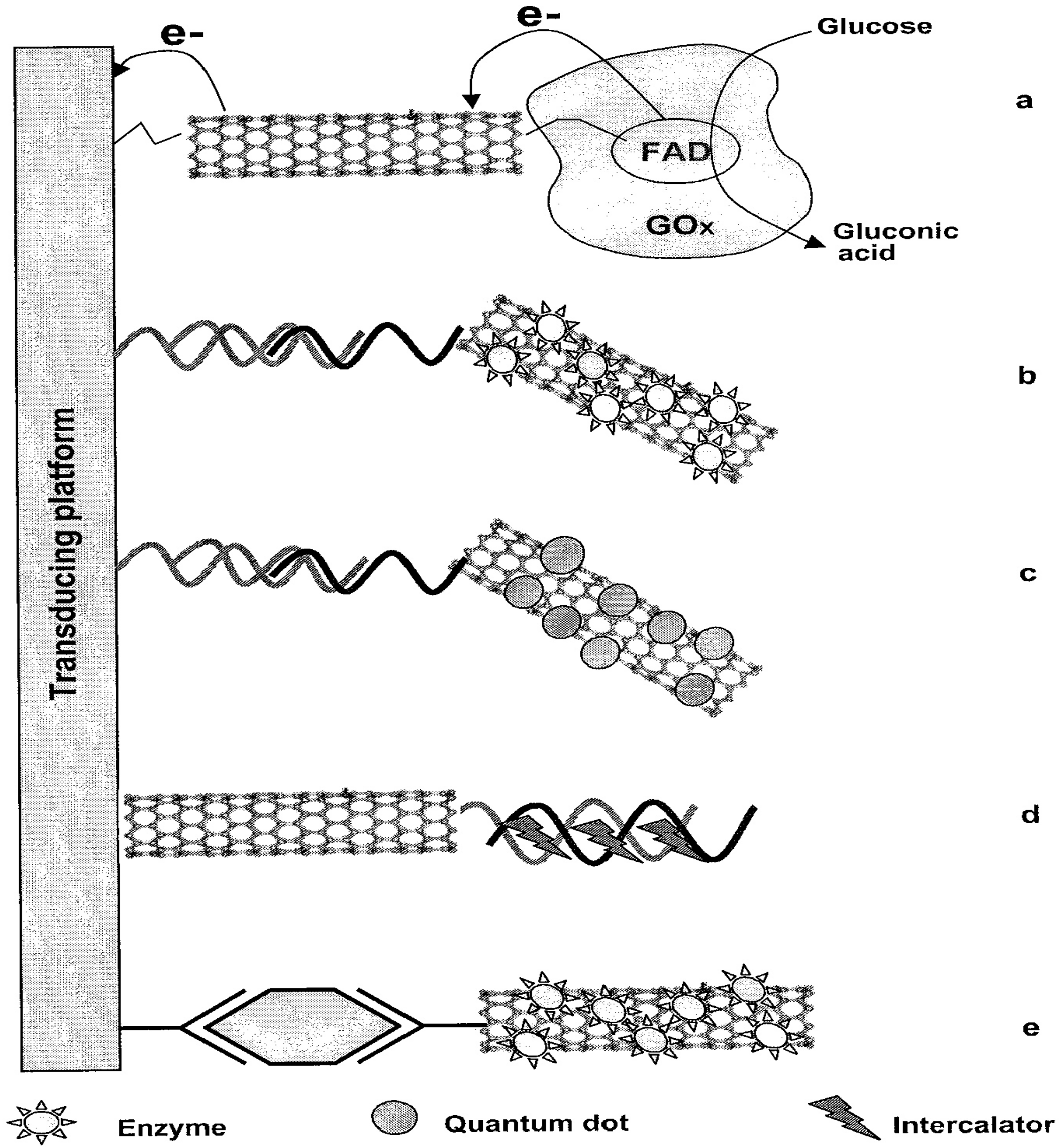
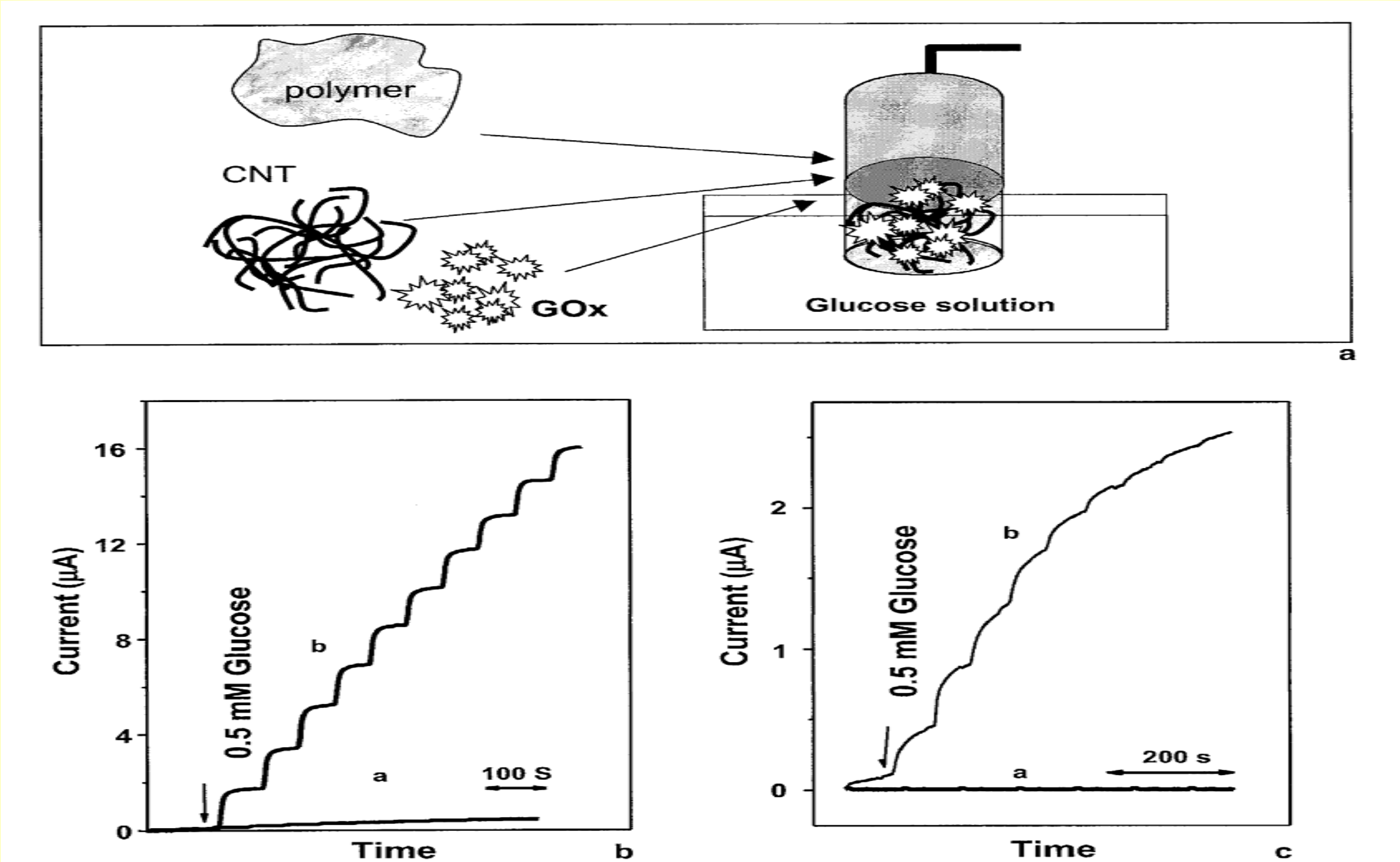


Fig. 6. Schematics of various strategies used to integrate CNTs in electrochemical sensors. (a) Direct electron transfer through the transducer and the redox centre (FAD) of GOx via CNT. (Adapted from references [90, 91]); (b) DNA detection via labelling with CNT loaded with enzymes. (c) DNA detection via labelling with CNT loaded with quantum dots. (d) DNA detection via labelling with CNT loaded with enzymes. (e) Immunosensing via antibody labelling with CNT loaded with enzymes

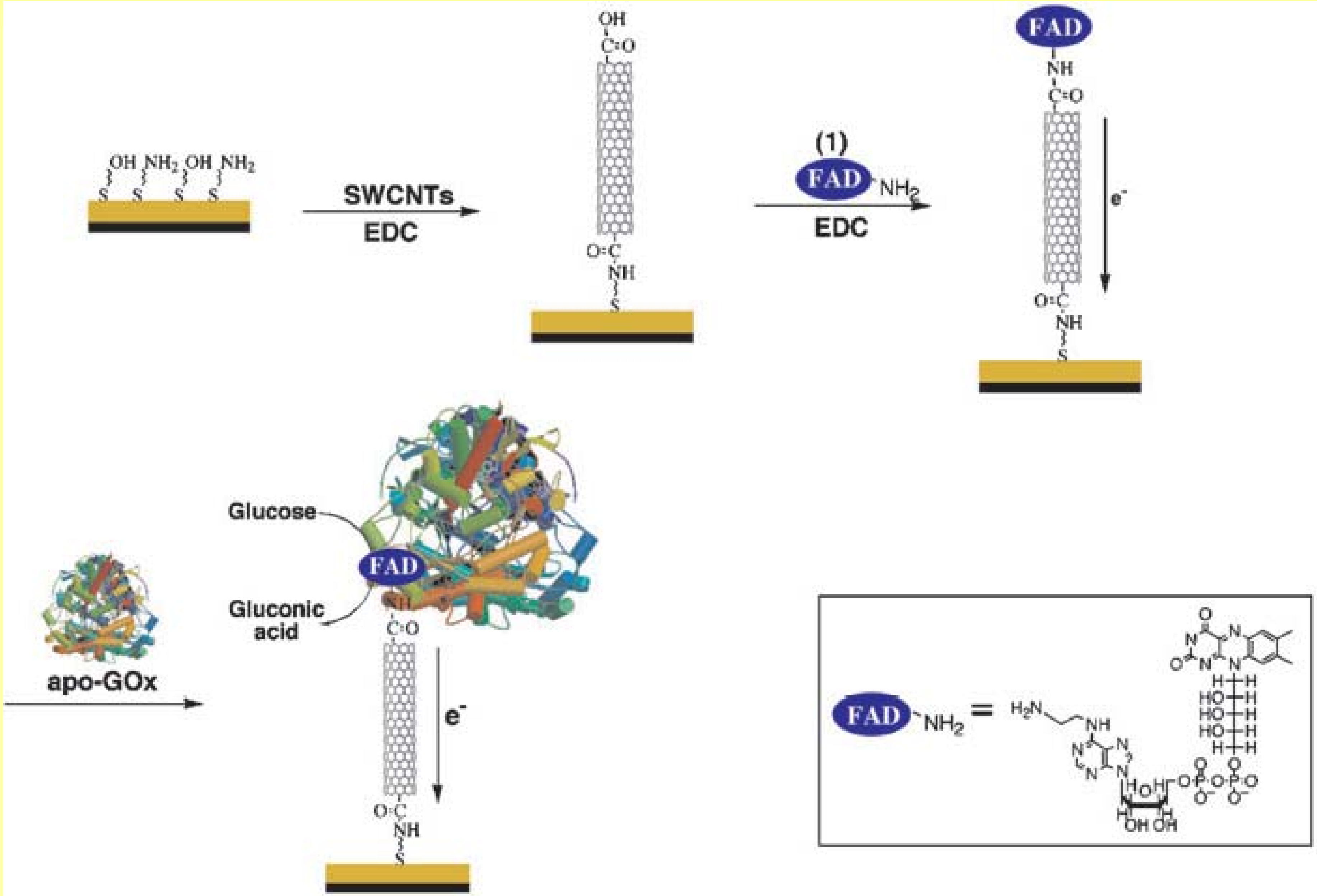
Ref.: A. Merkoci: Microchim. Acta 152, 157 (2006)

Glukózový biosenzor na báze CNT



(b) grafitový kompozit, E = +0,90 V,
(c) CNT kompozit: 18 % MWNT, 2 % GOX, 80 % epoxyd, E = + 0,55 V.

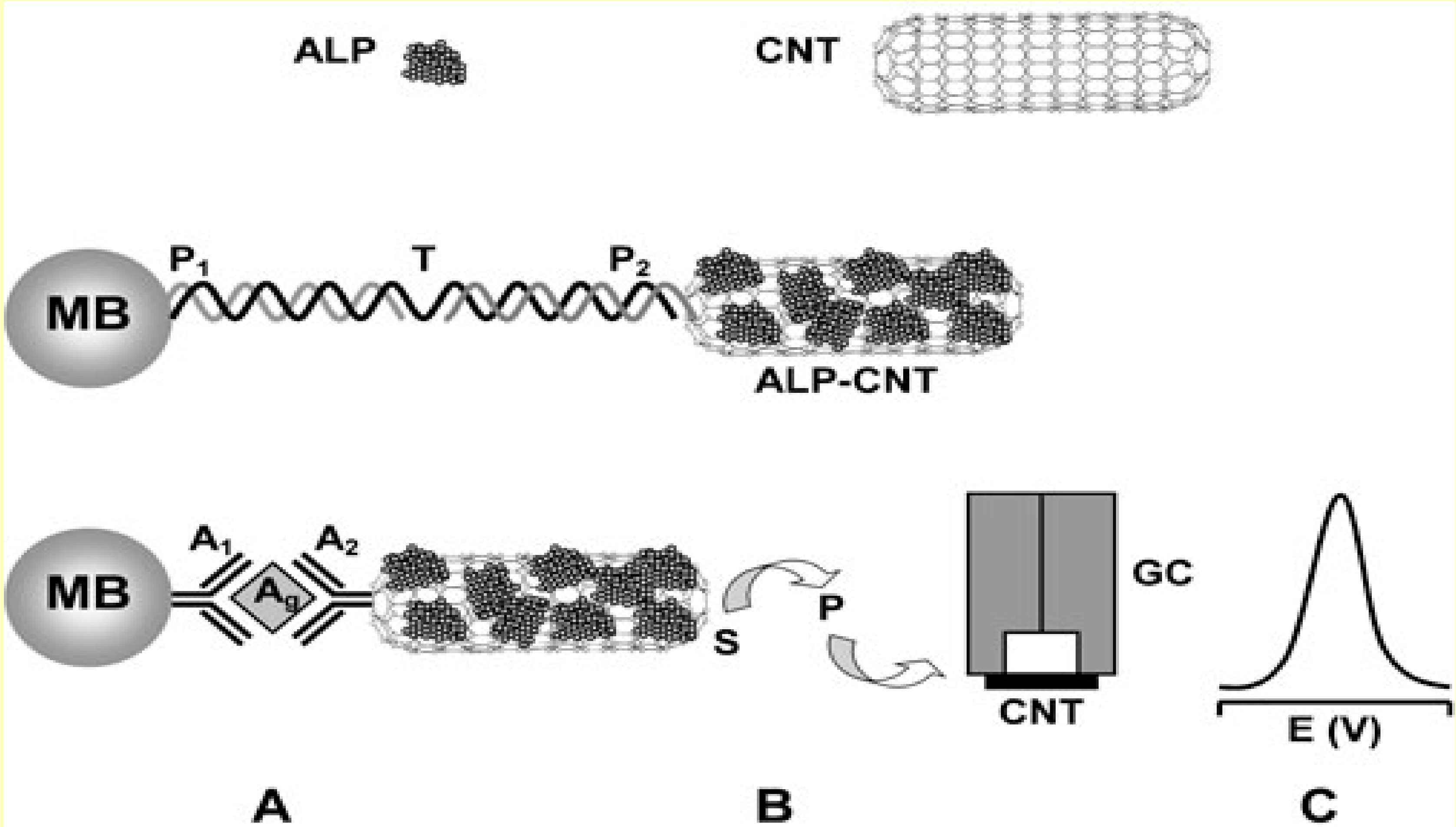
Ref.: A. Merkoci: Microchim. Acta 152, 157 (2006)



Assembly of the SWCNT electrically-contacted glucose oxidase electrode

Ref.: F. Patolsky et al., Angew. Chem. Int. Edit. 2004, 43, 2113.

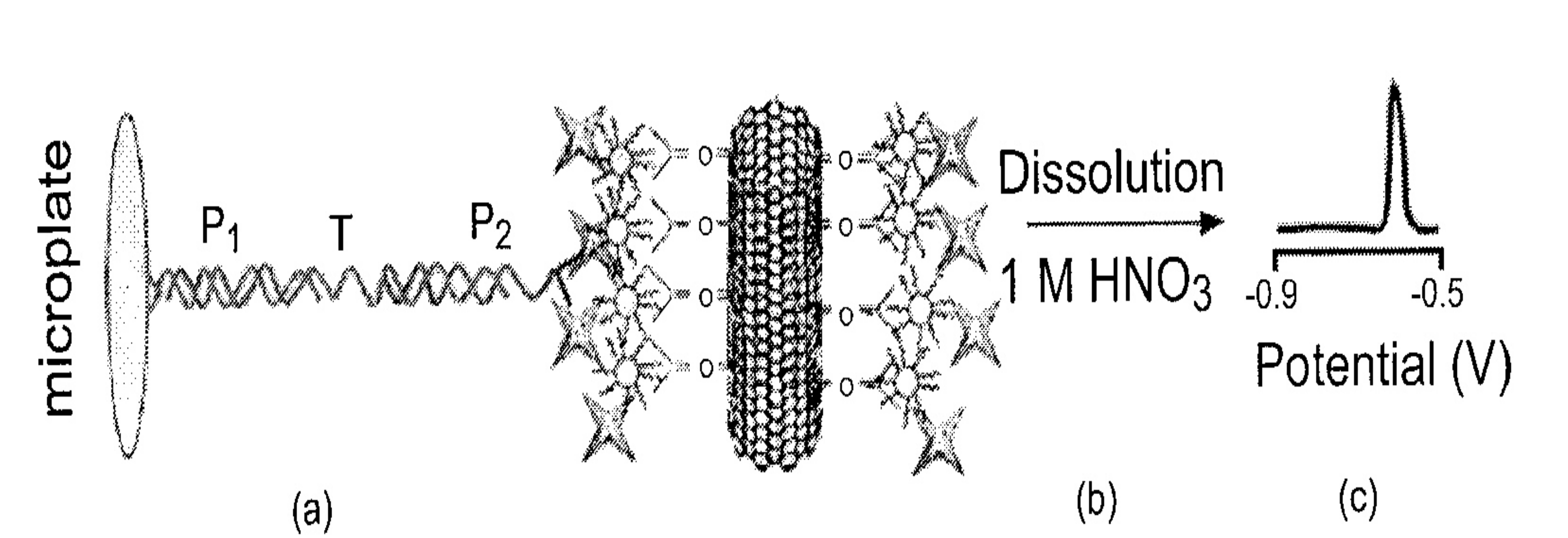
CNT-derived amplification of the recognition and transduction events



A) Capture of the alkaline-phosphatase (ALP)-loaded CNT tags to the streptavidin-modified magnetic beads by the DNA or antibody recognition events; B) addition of the substrate and enzymatic reaction;
C) electrochemical detection of the product of the enzymatic reaction at CNT-modified glassy carbon electrode.

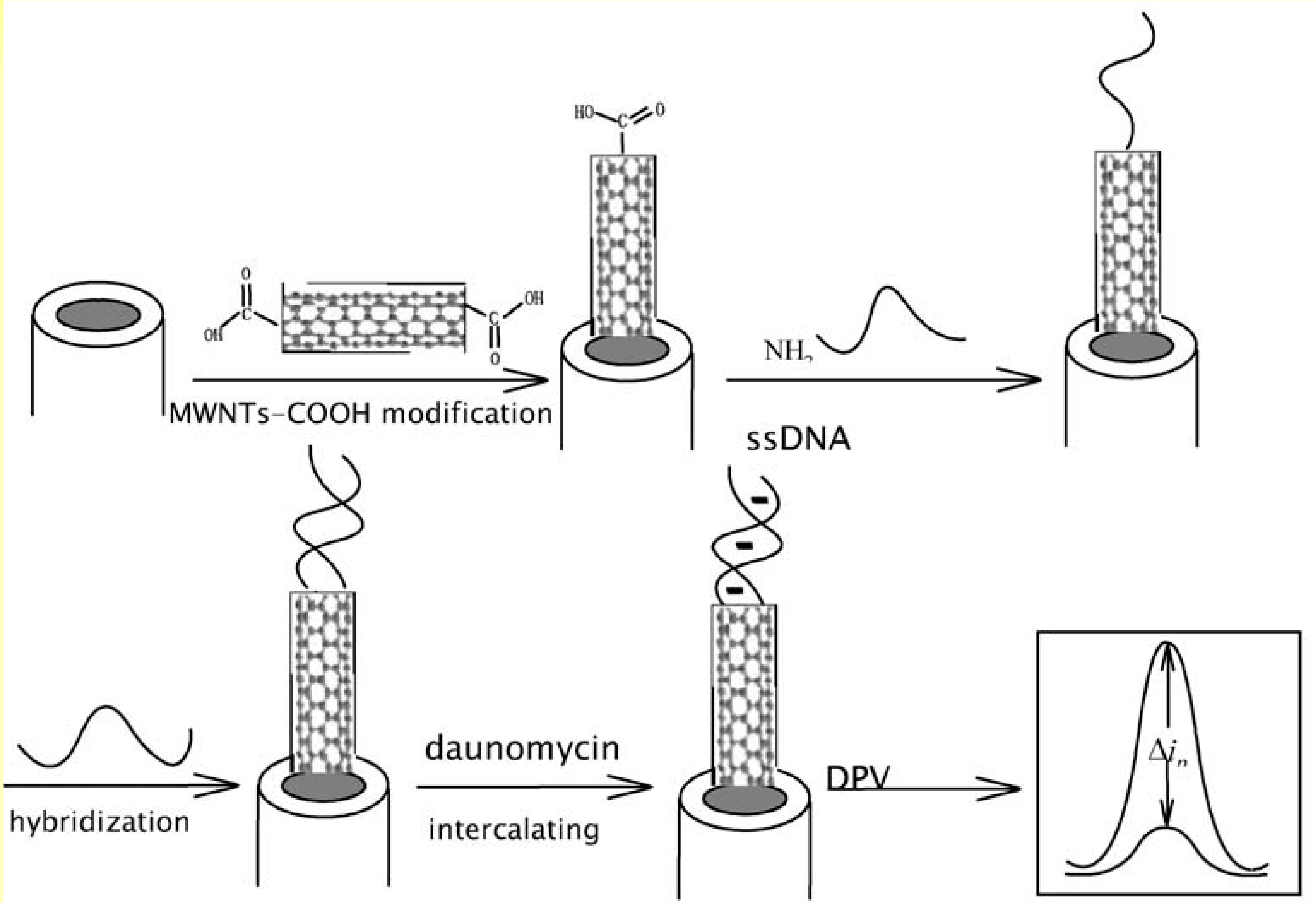
Ref.: J. Wang, J. Am. Chem. Soc. 2004, 126, 3010.

Detekcia hybridizácie DNA



(a) duálna hybridizácia s využitím CNT nabitými s 500 časticami CdS, (b) rozpustenie CdS, (c) detekcia Cd na MFE/GCE.
P_1 – sonda 1, T – cieľová DNA, P_2 – sonda 2.

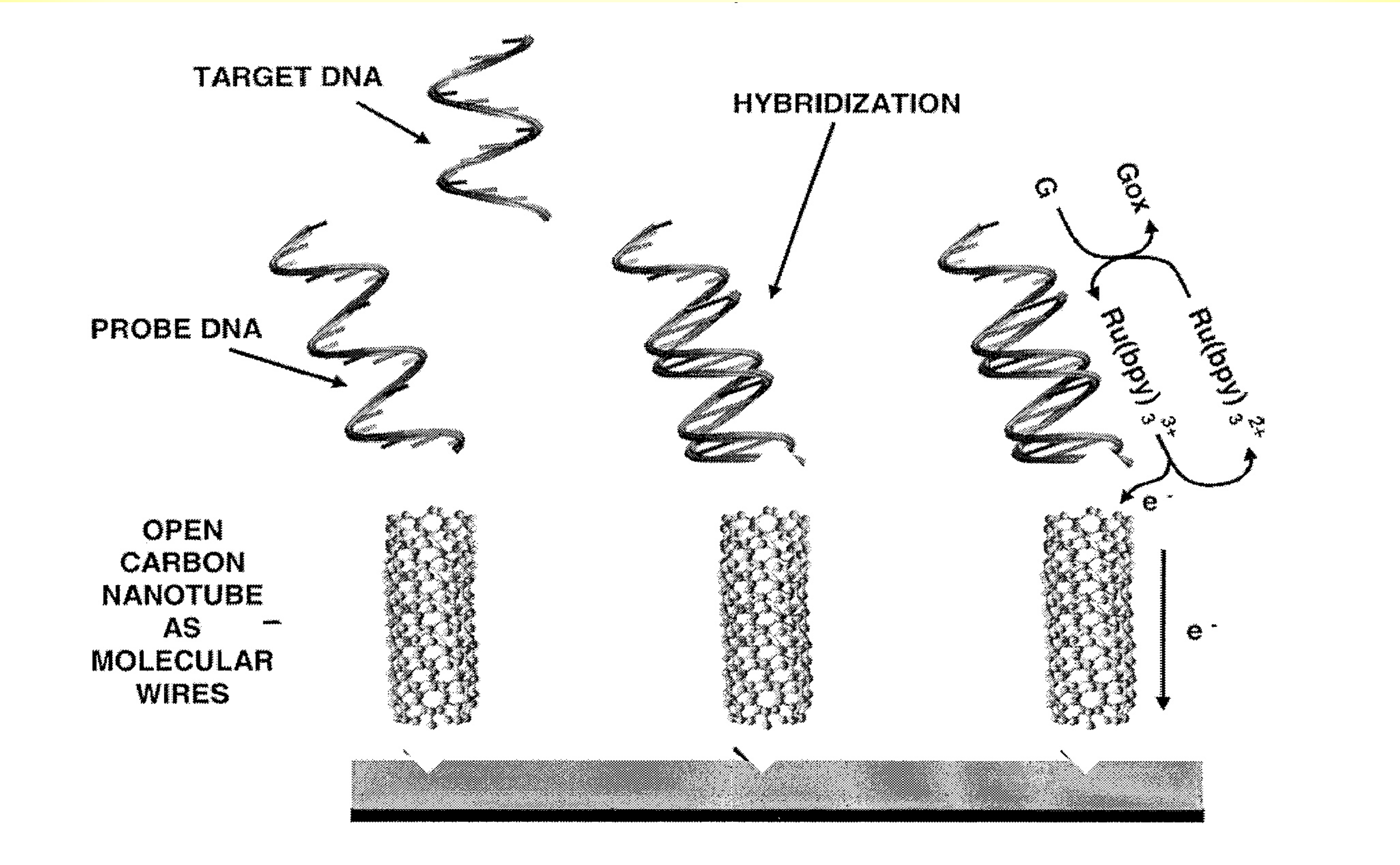
Ref.: P. He et al., Microchim. Acta 152, 175 (2006)



Enhanced electrochemical detection of DNA hybridization based on the MWNTs–COOH constructed DNA biosensor

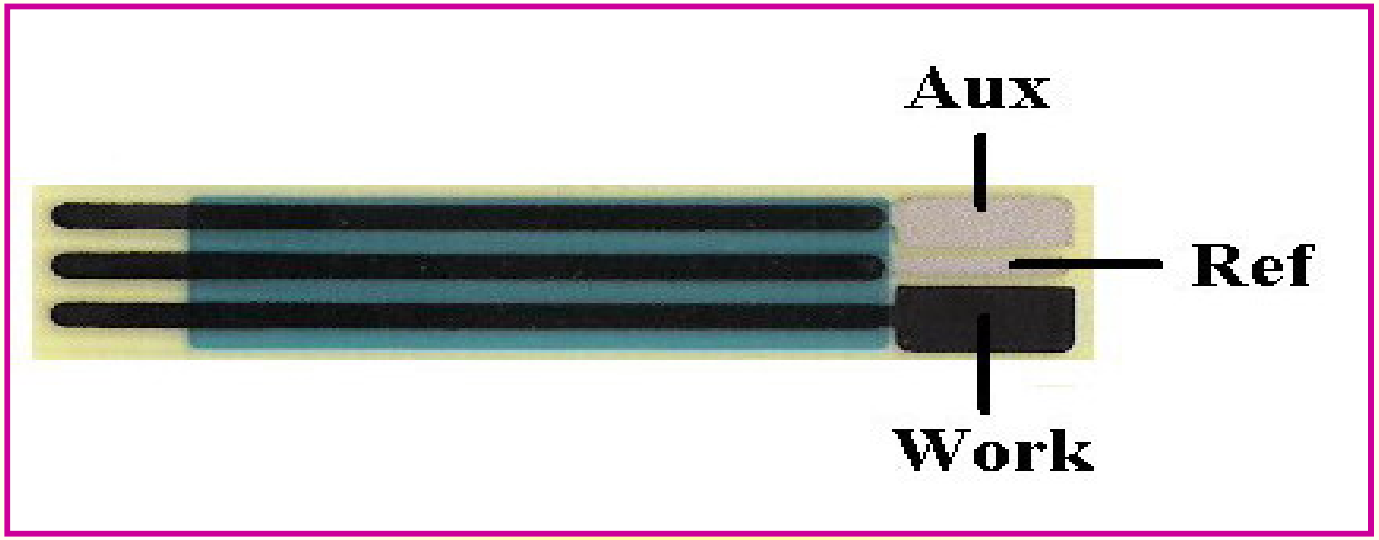
Ref.: H. Cai et al., Anal. Bioanal. Chem. (2003) 375, 287–293

Detekcia hybridizácie DNA s redoxným mediátorom



Ref.: J. Li et al.: Nano Lett 3, 597 (2003)

Elektrochemický „screen-printed“ senzor (SPE) a detekcia degradácie DNA



Work - pracovná uhlíková elektróda (25 mm^2) modifikovaná s 5 µl 0,1 $mg.cm^{-3}$ DNA

Ref - referenčná elektróda (Ag/AgCl/SPE s potenciálom 0,284 V vs konvenčná Ag/AgCl/3 $mol.dm^{-3}$ KCl)

Aux - pomocná elektróda

Opakovateľnostť signálu $[Co(phen)_3]^{3+}$: RSD 12 % (n = 15).

Chemická modifikácia SPE povrchu

Carbon nanotubes (MWNT) o.d. 40-60 nm, l. 0.5-500 μm

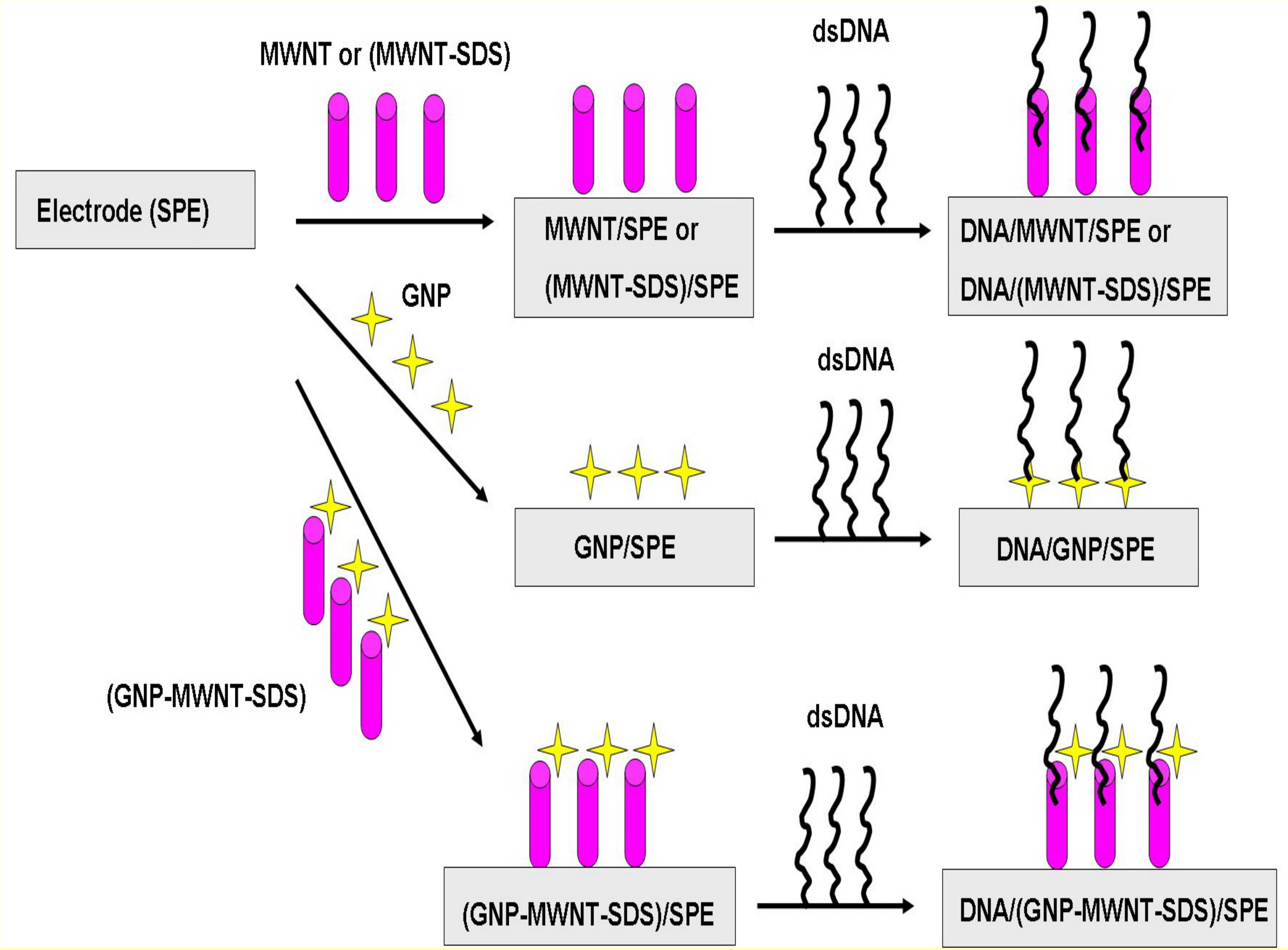
1 mg MWNT / 2 ml DMF – slabá disperzia!

1 mg MWNT / 2 ml 1% SDS

1 mg MWNT / 2 ml 0.5 % CHIT in acetic acid

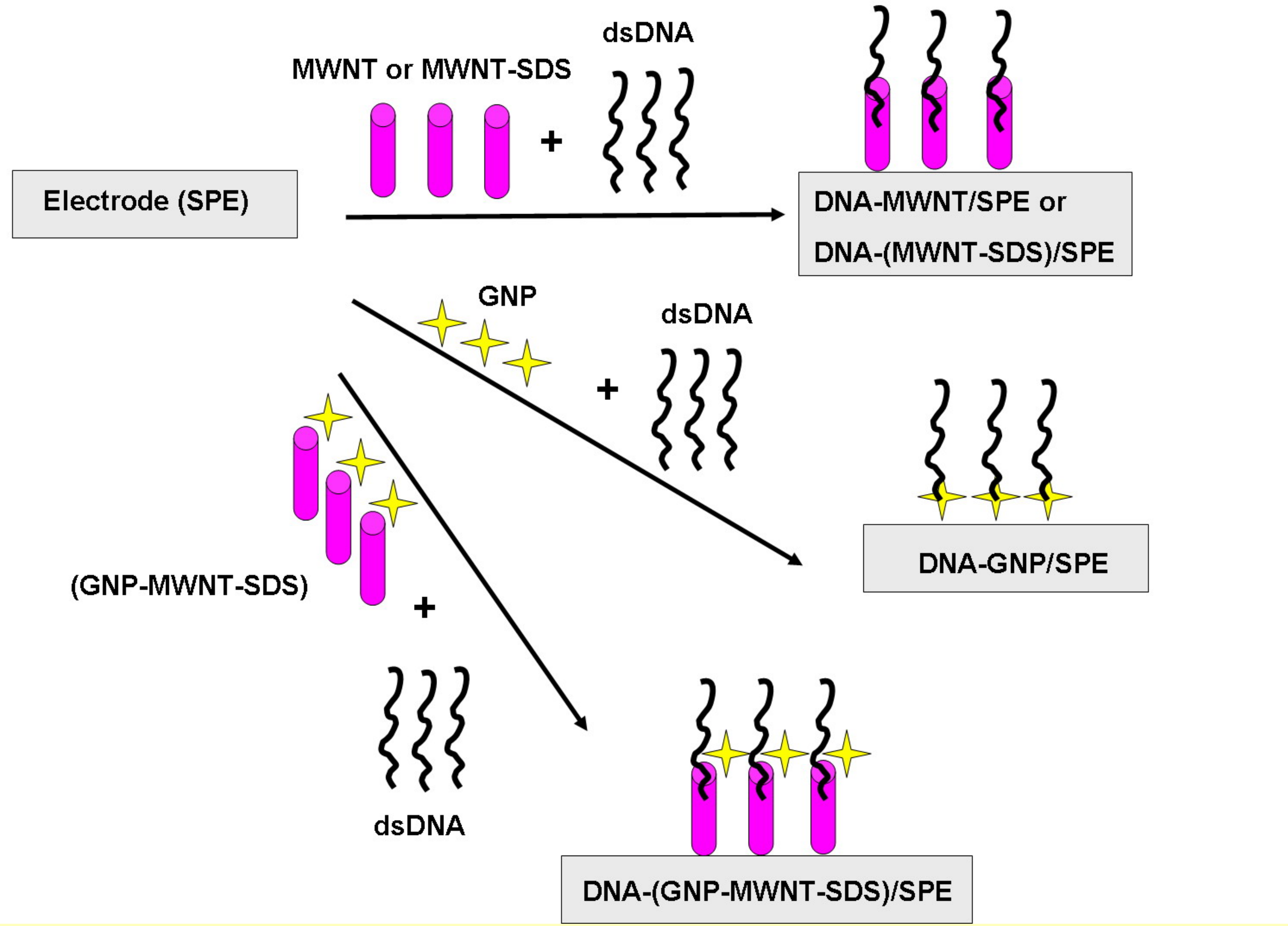
1 mg MWNT / 2 ml 1 mM PPY in DMF

Layer-to-layer coverage (DNA / CNT / SPE)

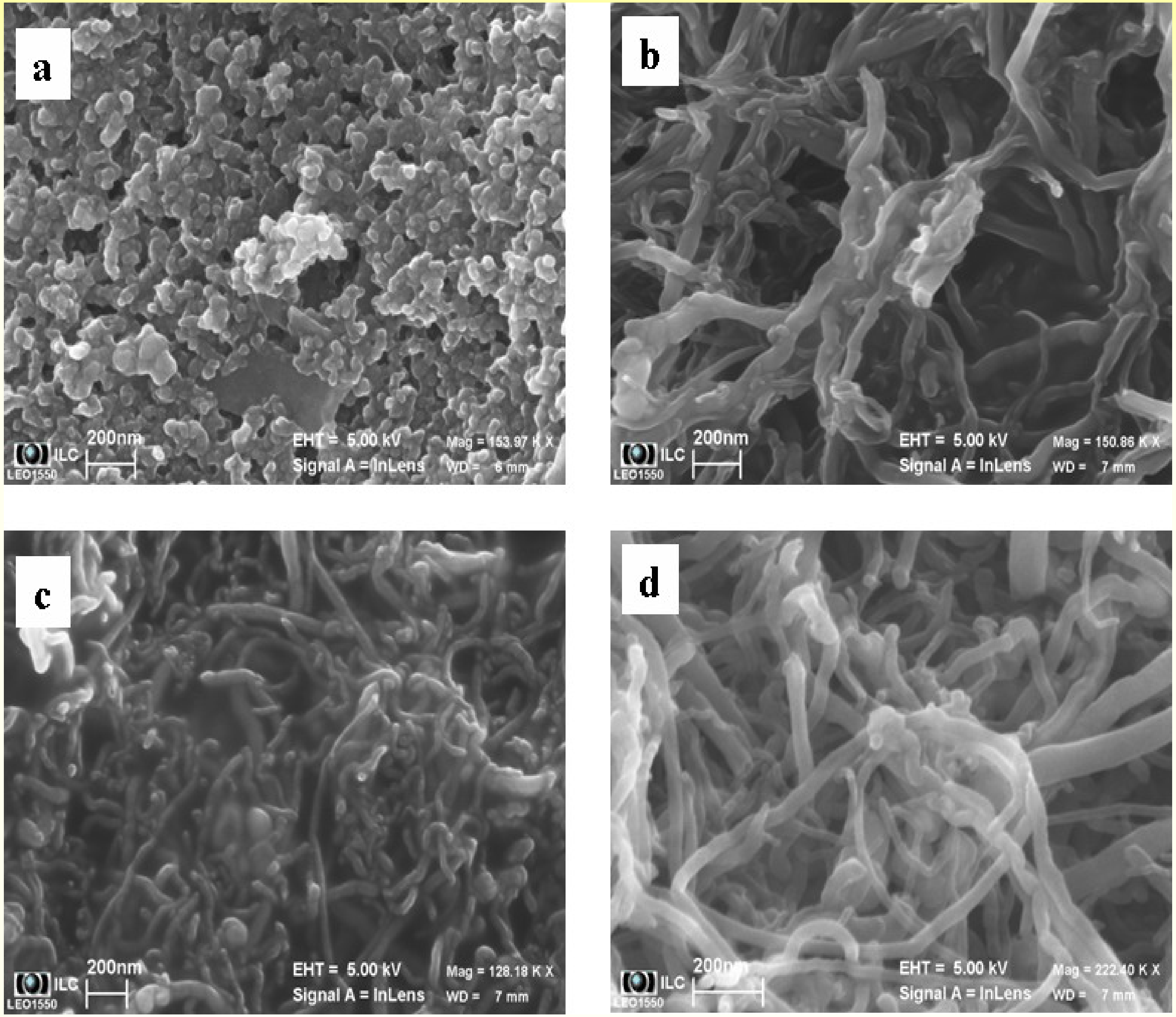


Ref.: R. Ovádeková et al., Anal. Bioanal. Chem., 2006.

Mixed coverage (DNA - CNT / SPE)



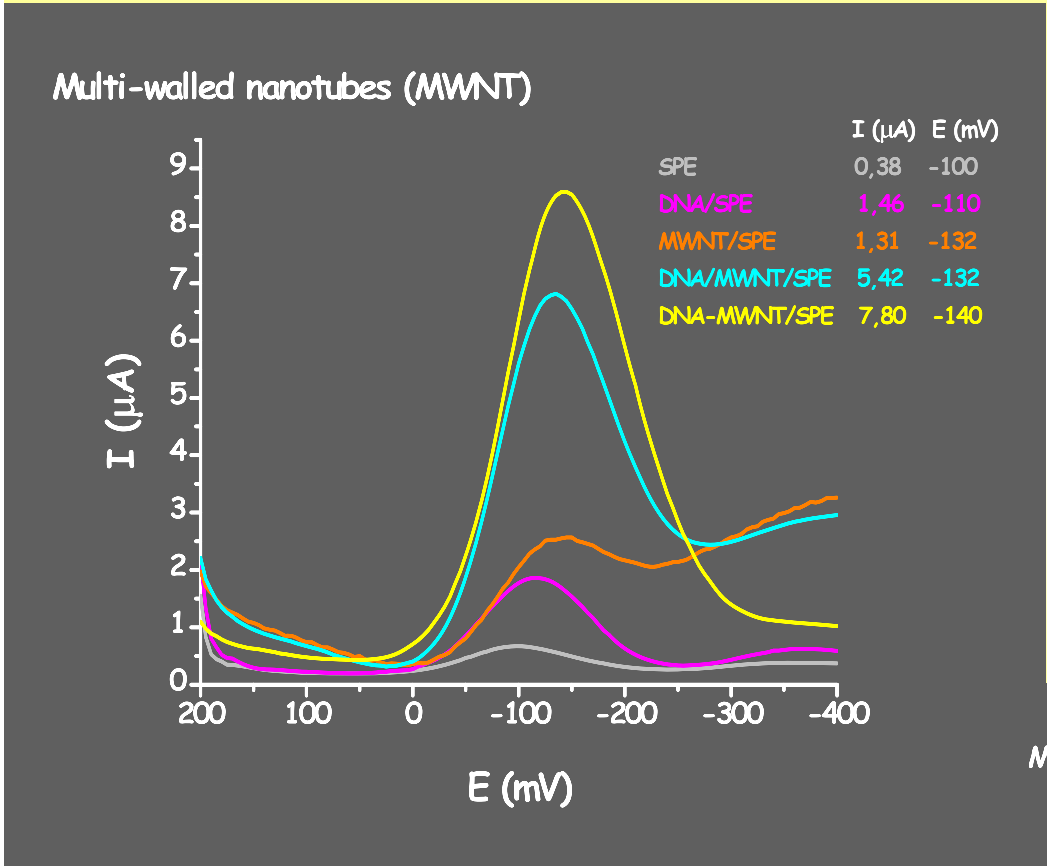
Ref.: R. Ovádeková et al., Anal. Bioanal. Chem., 2006.

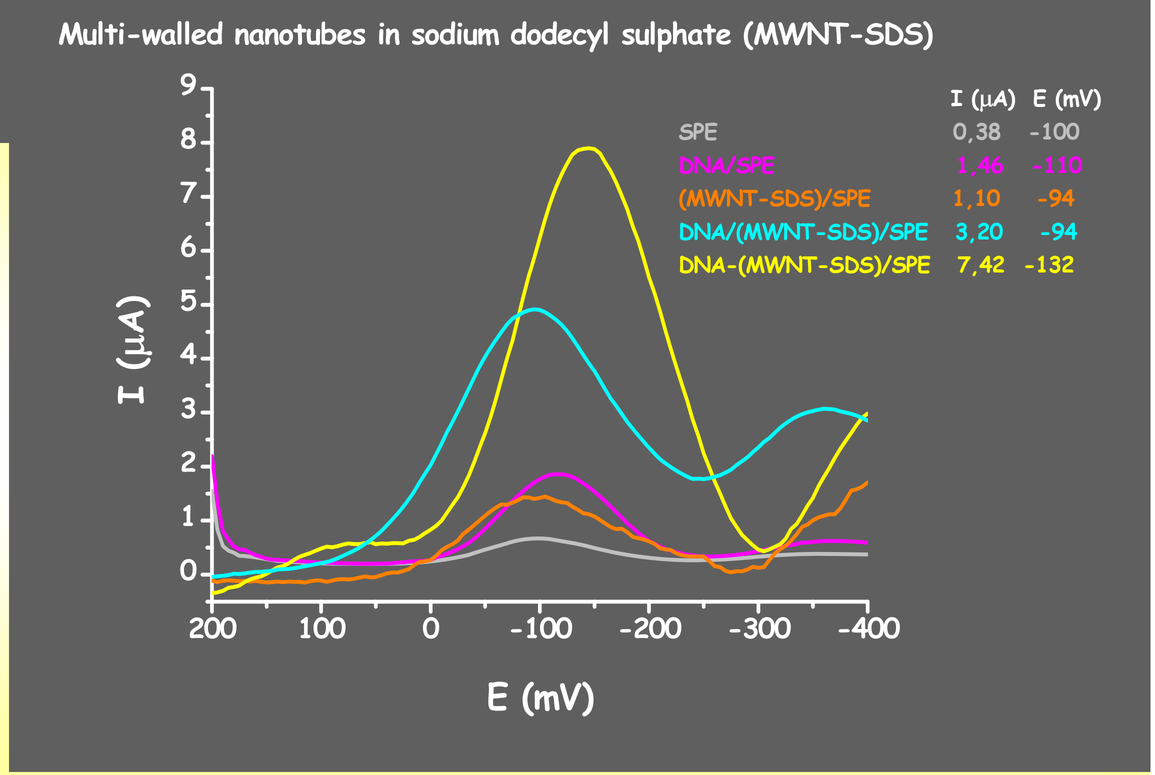


SEM images of DNA/SPE (a), MWNT/SPE (b), DNA/MWNT/SPE (c) and DNA-MWNT/SPE (d).

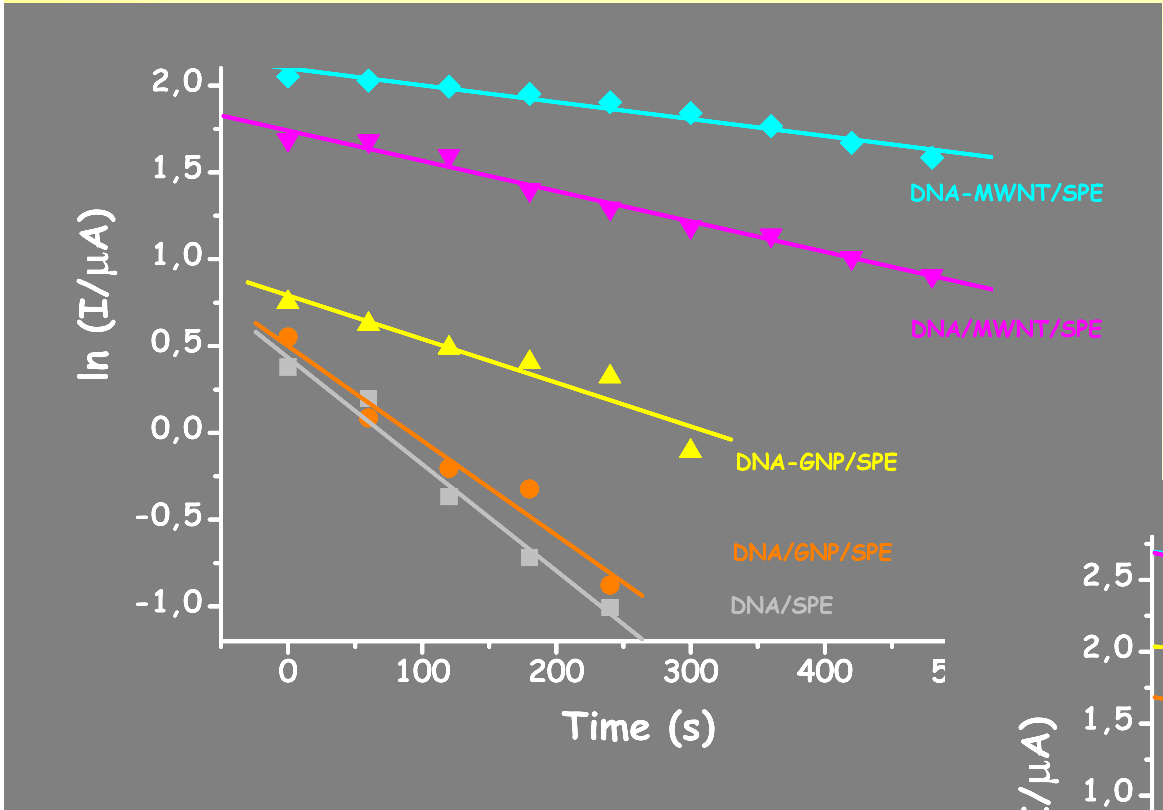
Signál DNA markera
$[Co(phen)_3]^{3+}$

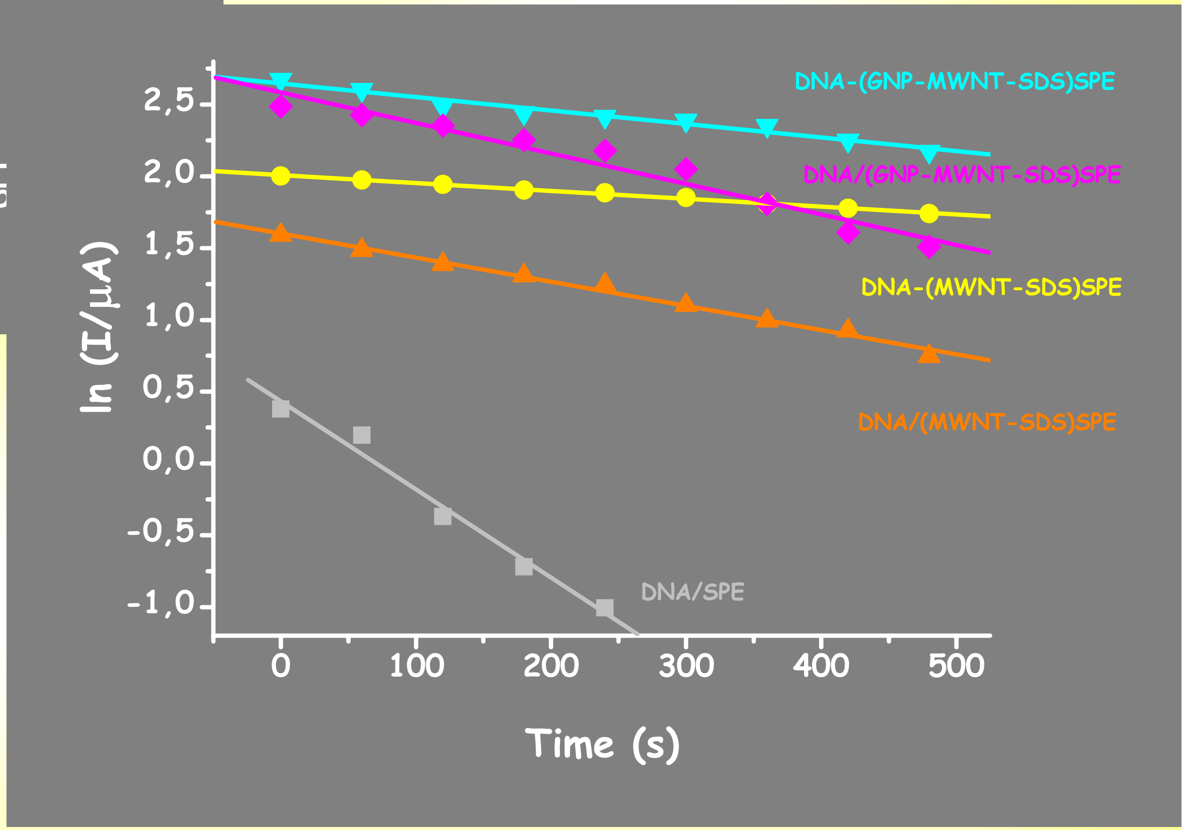
Conditions: DPV,
$5x10-7$ mol/l $[Co(phen)_3]^{3+}$
in 5 mmol/l phosphate buffer pH 7.0,
120 s accumulation





Rýchlostná konštanta disociácie dsDNA-$[Co(phen)_3]^{3+}$

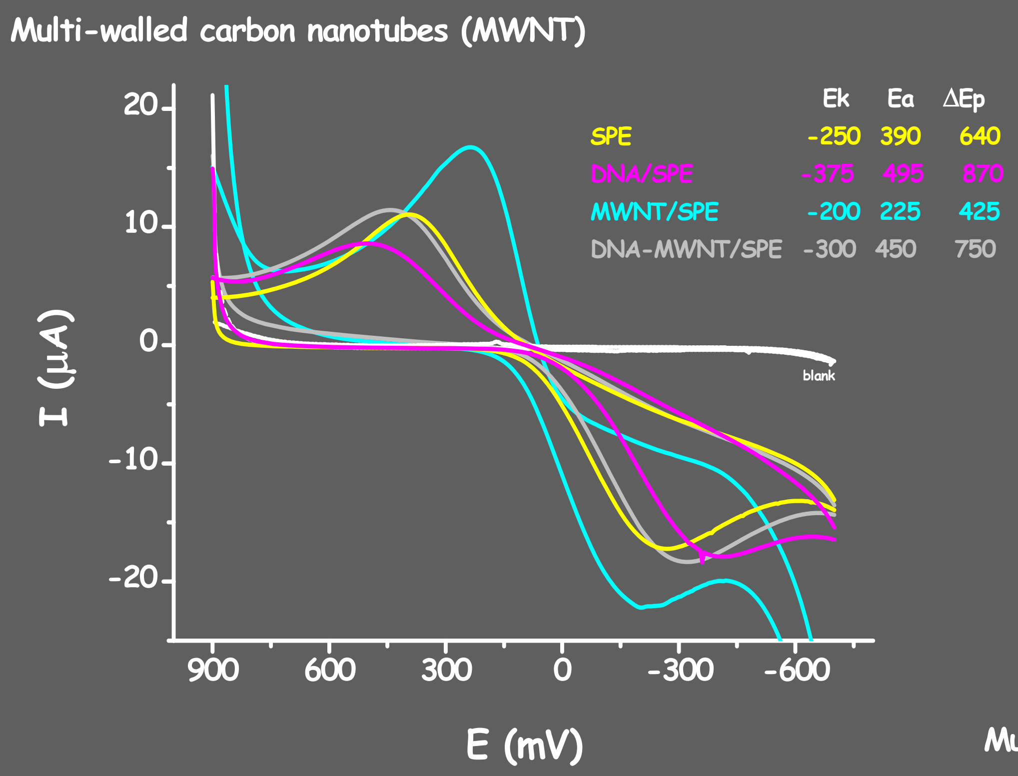


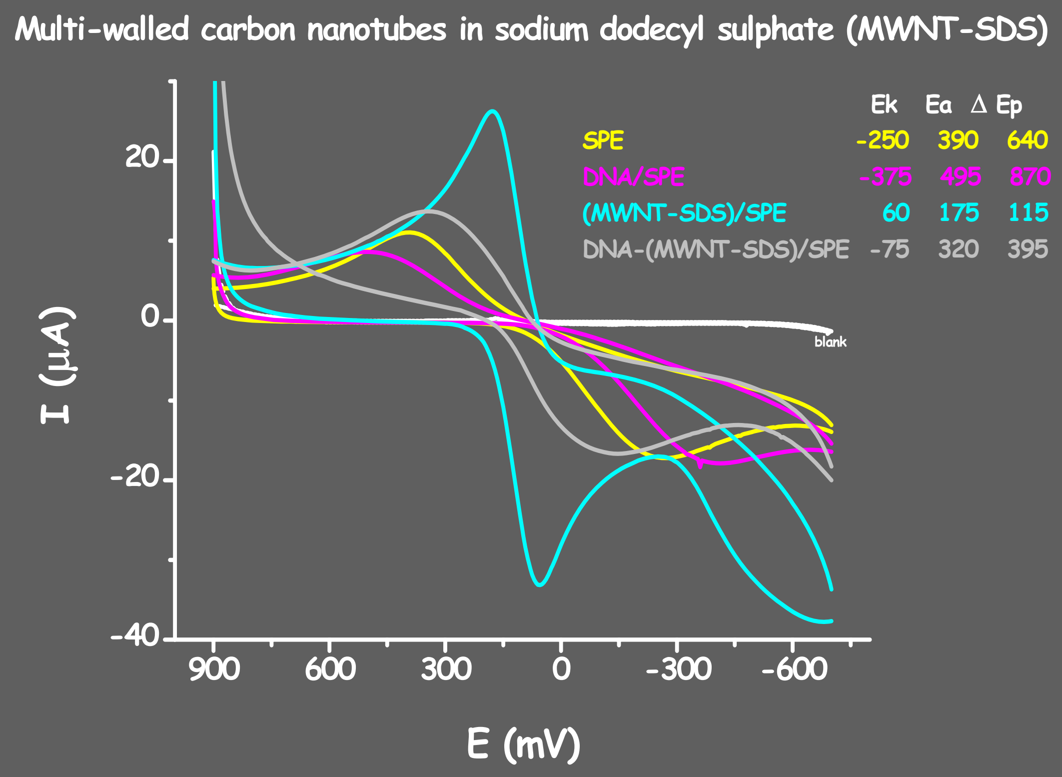


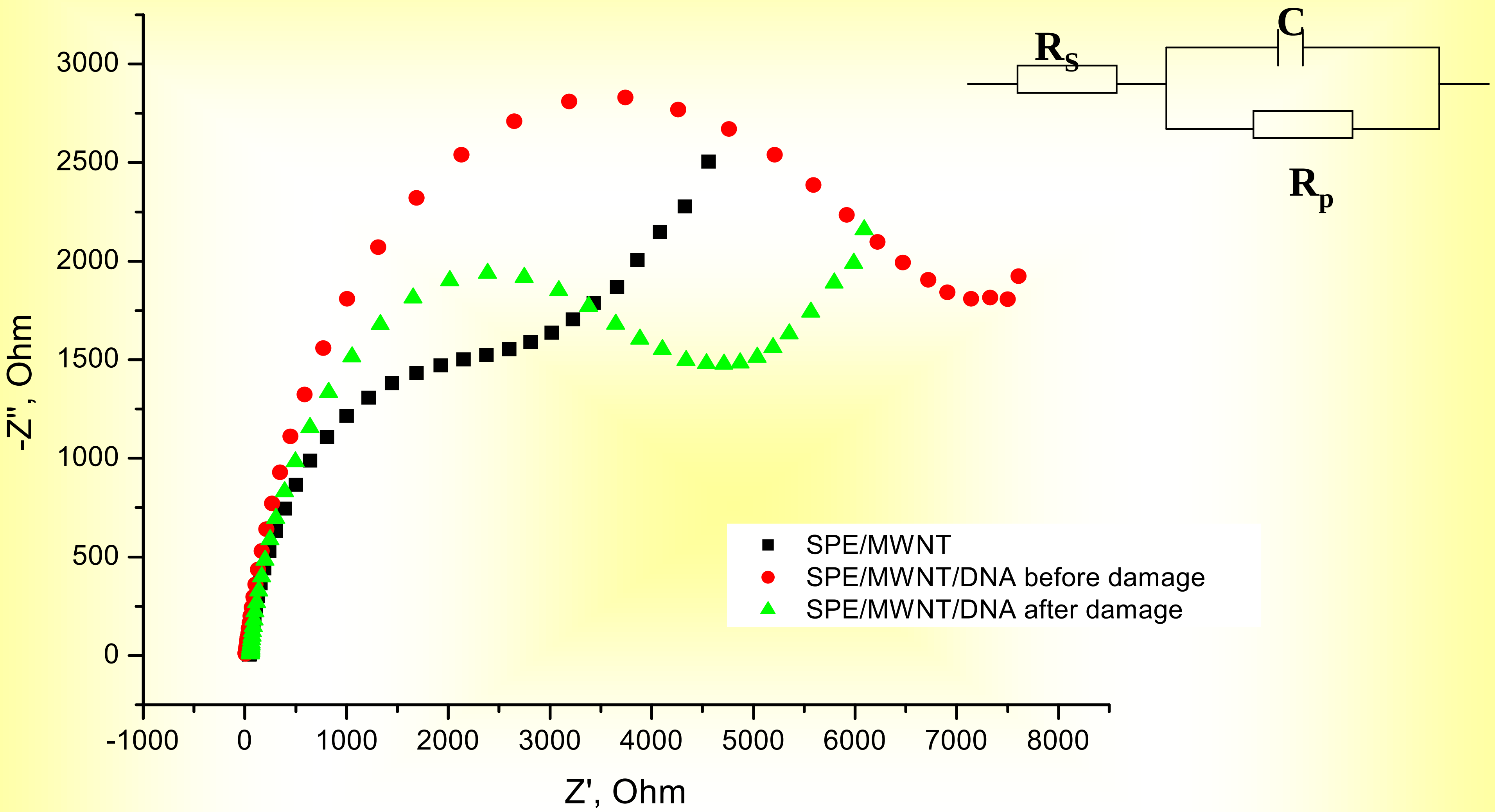
Disociácia meraná v 5 mmol/l fosforečnanovom tlmivom roztoku pH 7.0 po 60 s

Signály indikátora v roztoku: 1 mmol/l $K_3[Fe(CN)_6]$

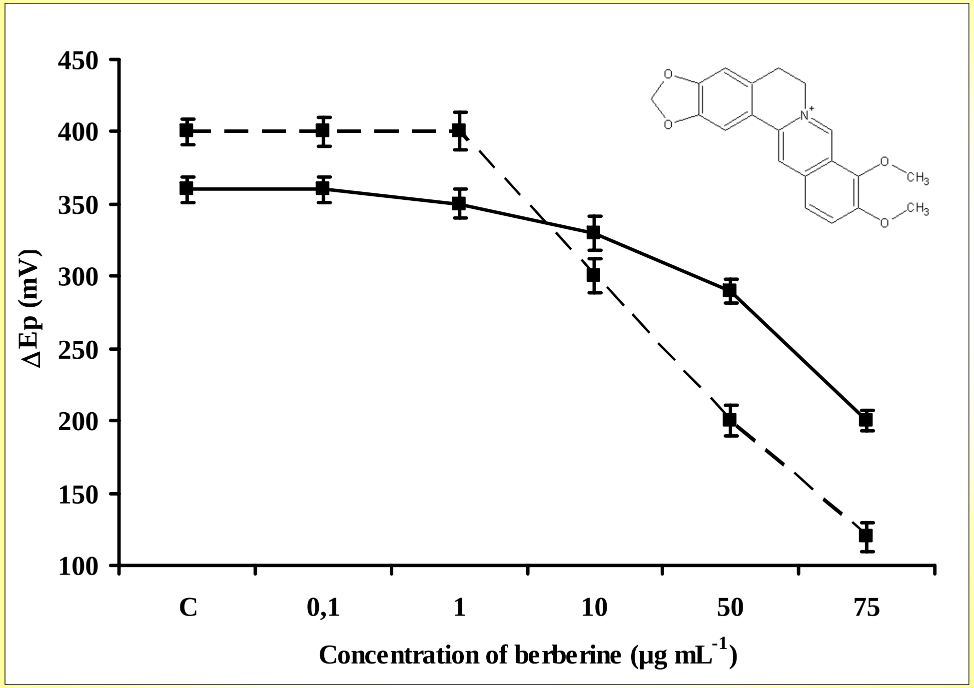
Conditions: CV,
0.1 mol/l PBS pH 7.0,
scan rate 50 mV/s.

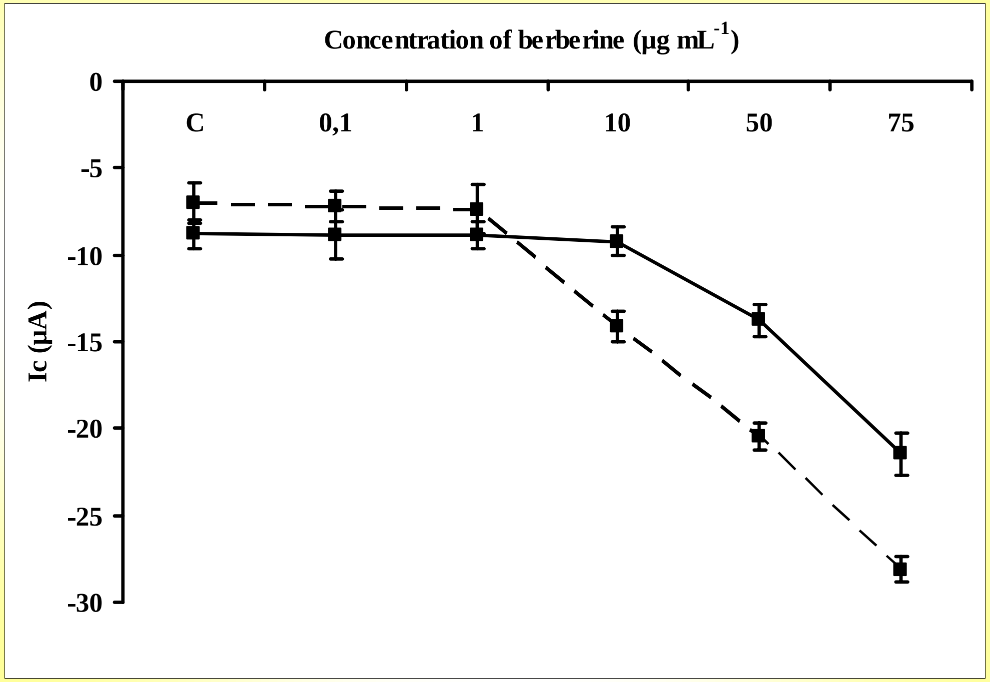






Typical complex impedance plots for $1x10^{-3}$ M $K_3[Fe(CN)_6]$ indicating DNA damage on DNA/MWNT/SPE.

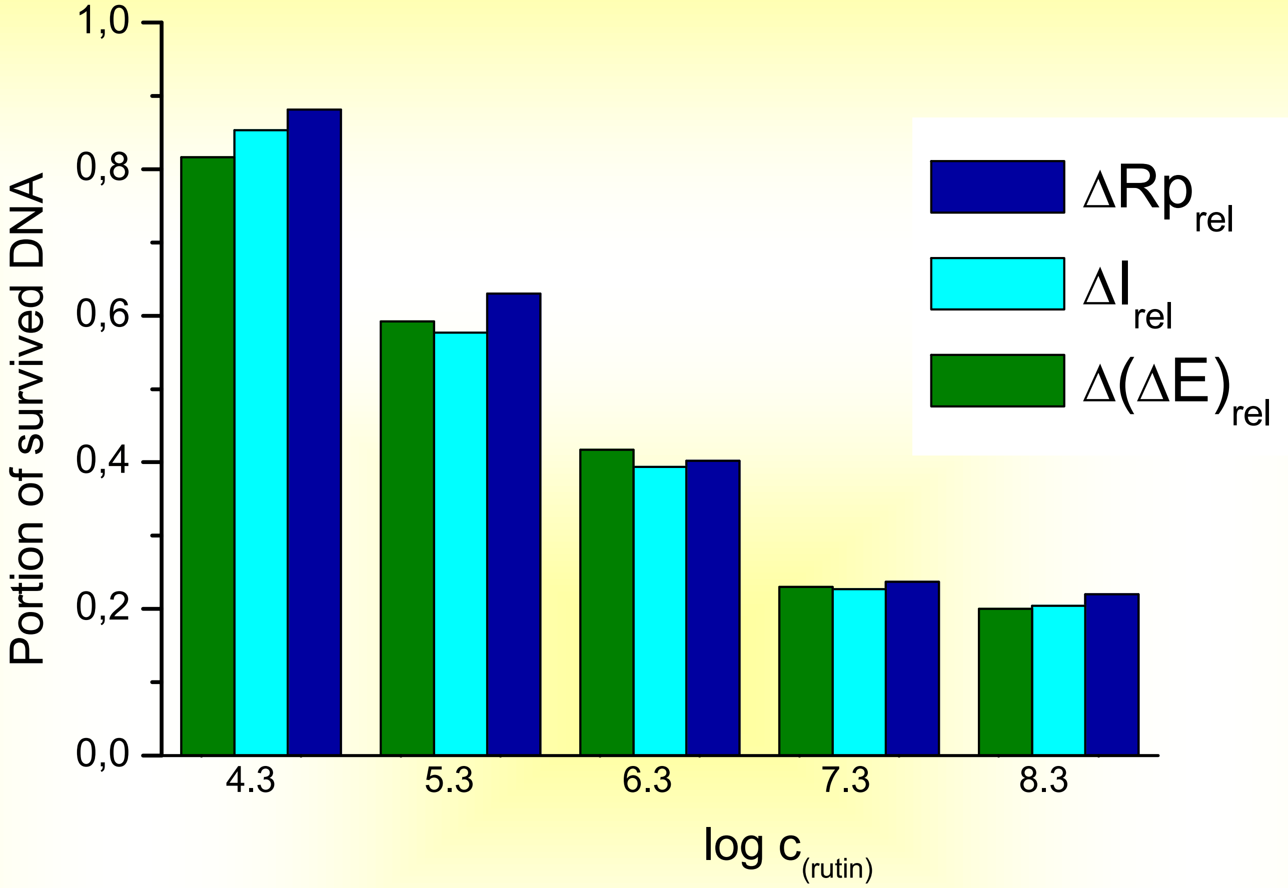




The berberine concentration dependence of the $K_3[Fe(CN)]_6$ CV peak potential separation (a) and cathodic peak current measured at 57 mV (b) obtained at DNA-(MWNT-SDS)/SPE.

Sensors with DNA from U937 cells (---) and keratinocytes (___) were firstly incubated in berberine solution in 5 mM PBS for 10 min. under stirring. C is control without berberine. Condition: 1 M $K_3[Fe(CN)]_6$ in 0.1 M PBS, pH 7.0, scan rate 50 mV/s.

Ref.: R. Ovádeková et al., Anal. Bioanal. Chem., 2006.

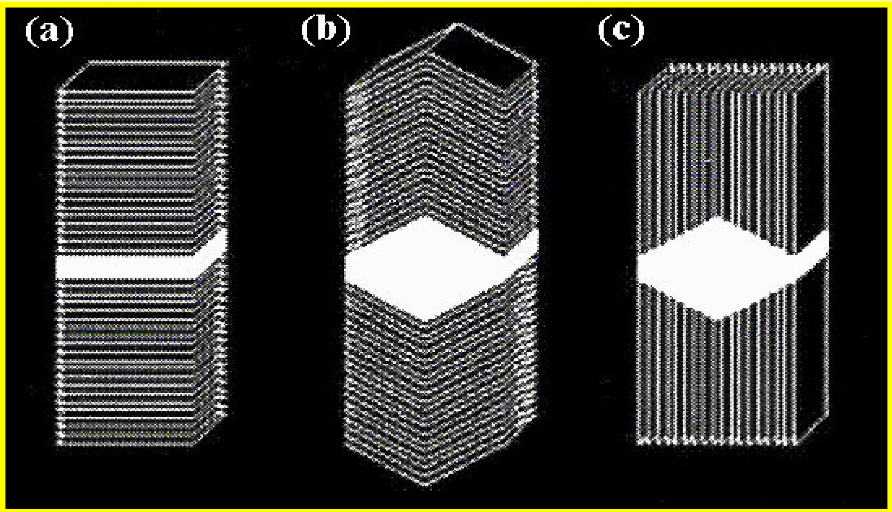


Antioxidant effect of rutin on DNA damage evaluated by using CV and EIS.

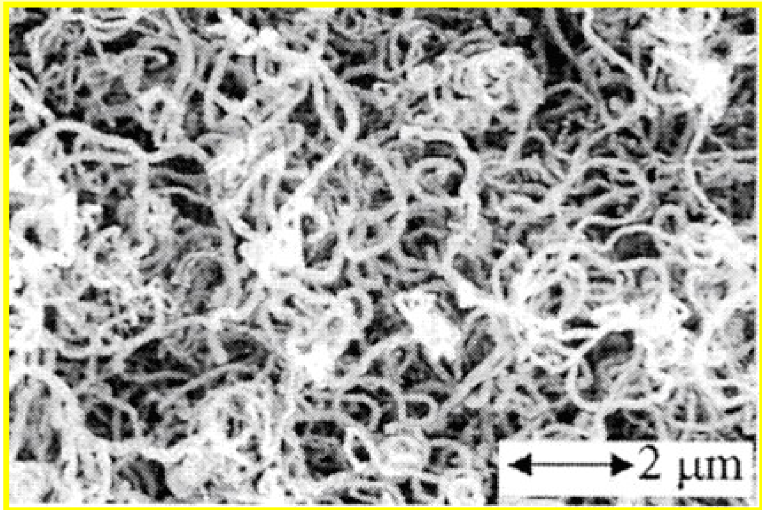
5 min incubation of calf thymus DNA/MWNT/SPE in a mixture of $1.0x10^{-6}$ M Fe^{2+}, $2.5x10^{-4}$ M H_2O_2, $1.0x10^{-6}$ M ascorbic acid and rutin under with stirring

“Carbon nanofibers"

Prvýkrát uvedené v r. 1889 (Hughes a Chambers).
Priemer 2 až 100 nm, dĺžka 5 až 100 μm.
Vysoká mechanická pevnosť, voliteľné povrchové vlastnosti, veľký povrch a dostupnosť, neprítomnosť mikropórov, vynikajúca elektrická vodivosť a absorbčná schopnosť.



Typy CNF: platničkový (a), rybej kosti (b) a pásikový (c).



„Carbon nanofibers“ s priemerom 100 nm

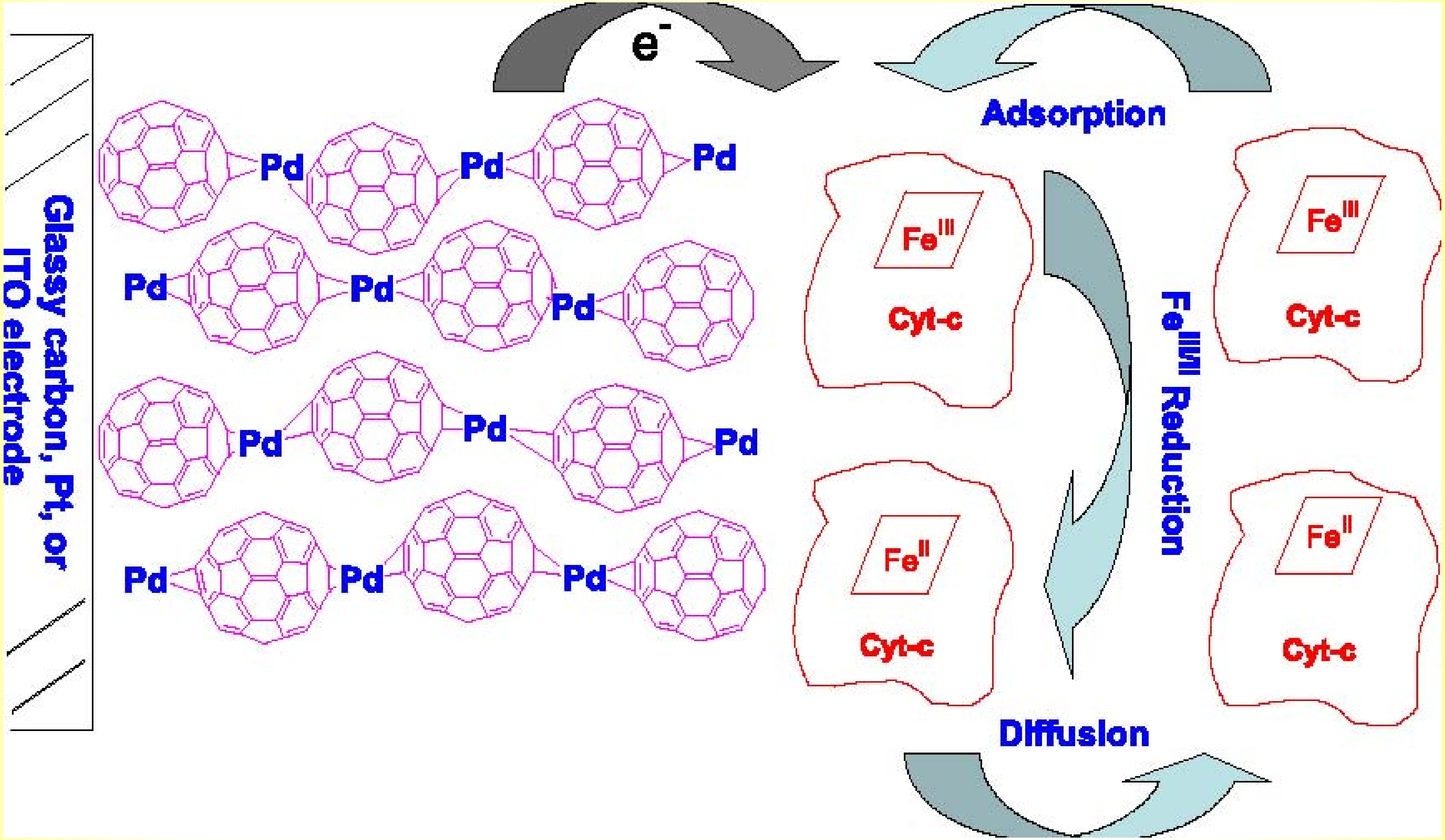
Využitie:

Aromatické molekuly ako hydrochinón, benzochinón a fenol sa silne adsorbujú na materiál z uhlíkových vlákien s dramatickým účinkom na ich elektrochemické chovanie.

Kompozitné elektródy z uhlíkových nanovlákien majú potenciálny význam v elektroanalýze.

Ref: M.L. Pedano and G.A. Rivas, Electrochem. Commun. 6, 10 (2004)..

„Fullerenes“



Proposed mechanism of cytochrome c immobilization and electrochemical reduction by C60-Pd polymer film modified electrode

Ref.: F. D'Souza et al.: Bioelectrochem. 66, 2005, 35-40.

„Nanoparticles"

Koloidné zlaté nanočastice: 3-20 nm
pripravené redukciou Au(III)

Quantum dots – kryštalické klastre
niekoľko sto až tisíc atómov,
polovodivé materiály s 3 rozmermi v škále cca 10 nm
pripravené:
„pattern formation“ (miceláciou surfaktantov),
termolýzou organokovových zlúčenín v horúcom rozpúšťadle,
cestou reverzných miciel (zmesi vody v oleji),
elektrochemickým vylúčením.

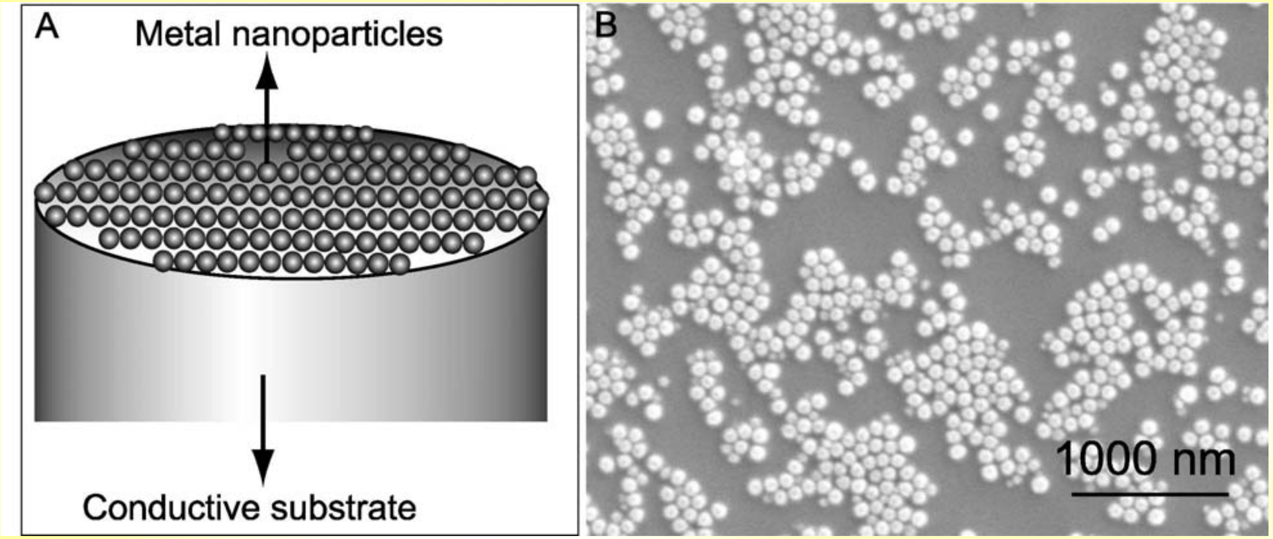
Koloidná stabilita a bioanalytické skúšanie vo vodnom prostredí vyžadujú:
rozpustnosť vo vode
zachytenie do micely

Metallic Nanoparticles: Applications in Electroanalysis

N. S. Lawrence, H.-P. Liang

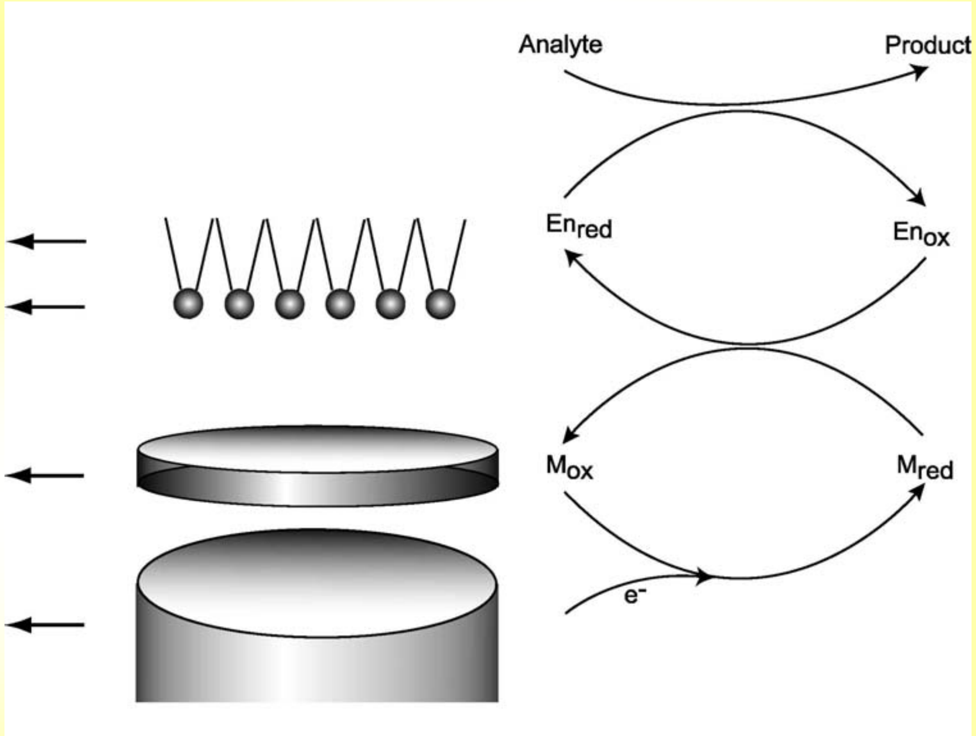
The field of nanoparticles has recently come to the forefront of analytical research. The combination of nanoscale structures coupled to the high electrocatalytic activity observed at a host of metals makes them desirable sensing materials, to counter problems of poor sensitivity and selectivity.

Ref.: Eftekhari (Ed.): Nanostructured Materials in Electrochemistry, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, 2007.

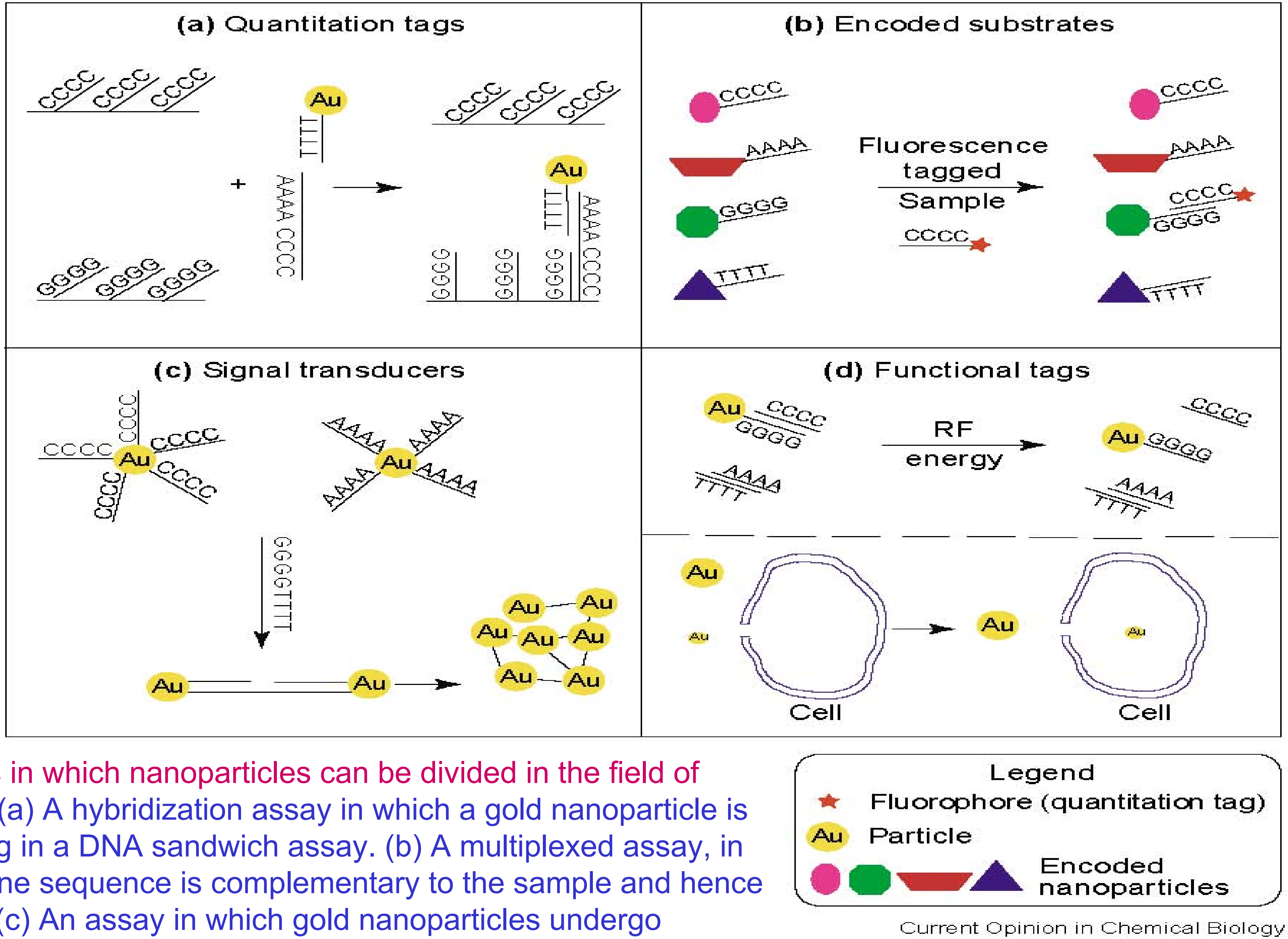


Nanoparticle modified electrode and SEM image

Nanoparticle based mediator biosensor along with the corresponding detection mechanism

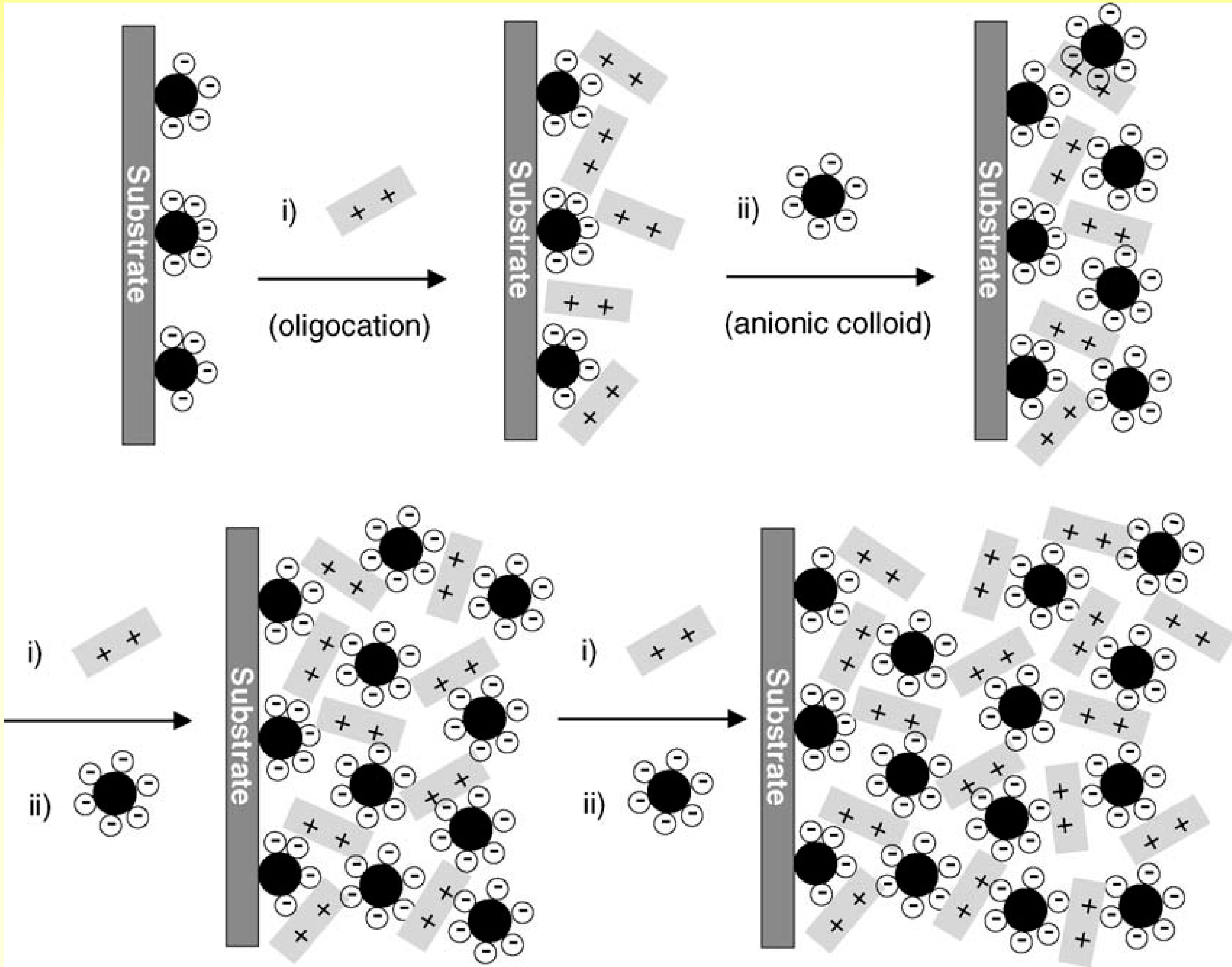


Ref.: N. S. Lawrence, H.-P. Liang, in press.



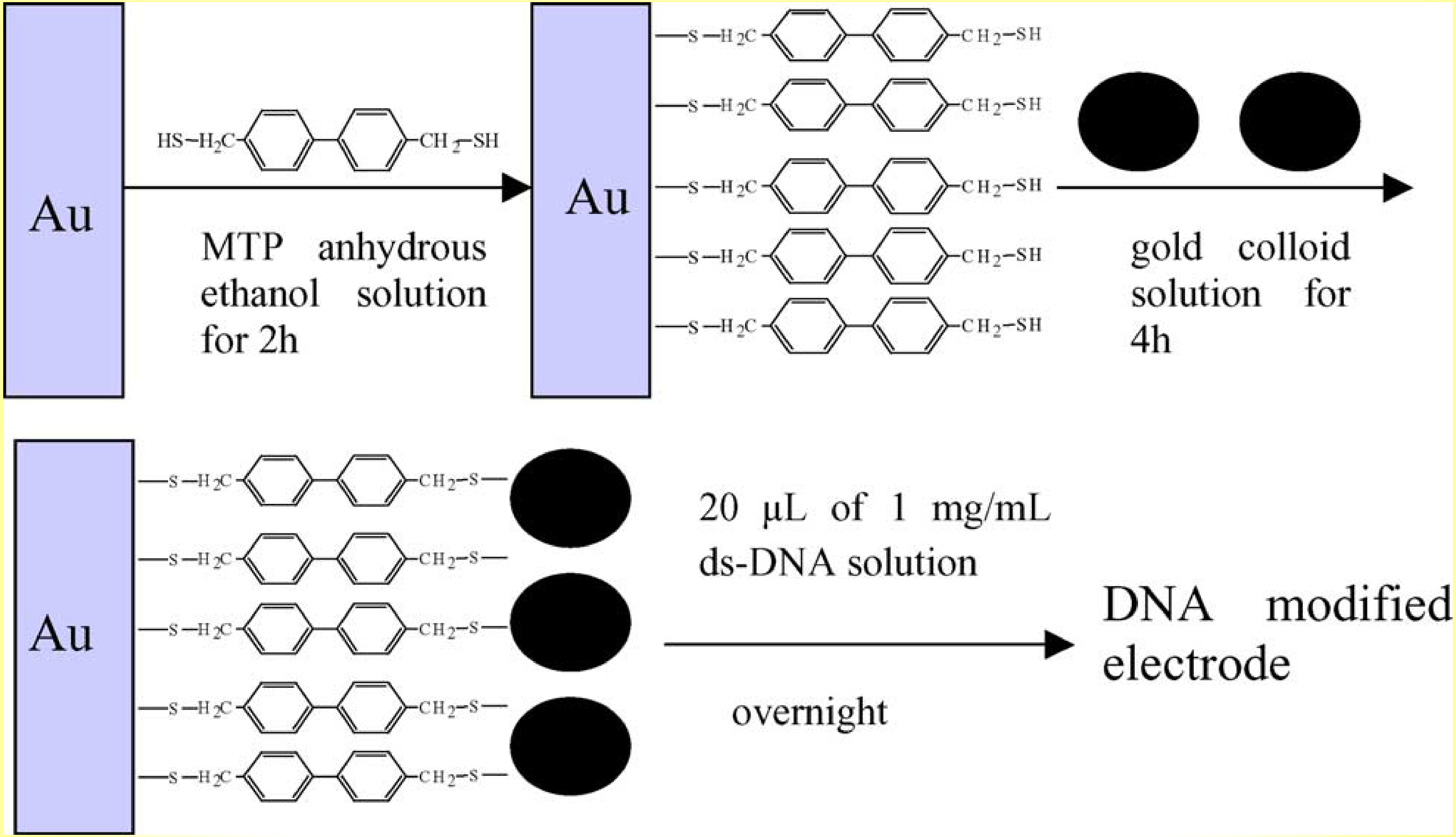
Four formats in which nanoparticles can be divided in the field of bioanalysis. (a) A hybridization assay in which a gold nanoparticle is used as a tag in a DNA sandwich assay. (b) A multiplexed assay, in which only one sequence is complementary to the sample and hence fluorescent. (c) An assay in which gold nanoparticles undergo aggregation due to cDNA sequences bringing the nanoparticles into close proximity. (d) The upper cartoon depicts localized heating of a single DNA sequence tagged with a gold nanoparticle, leading to selective dehybridization. The lower cartoon depicts a cellular assay in which only gold nanoparticles of a particular size can enter a cell.

Ref.: S. G. Penn, L. Hey, M. J. Natan, Current Opinion in Chemical Biology 2003, 7, 609–615



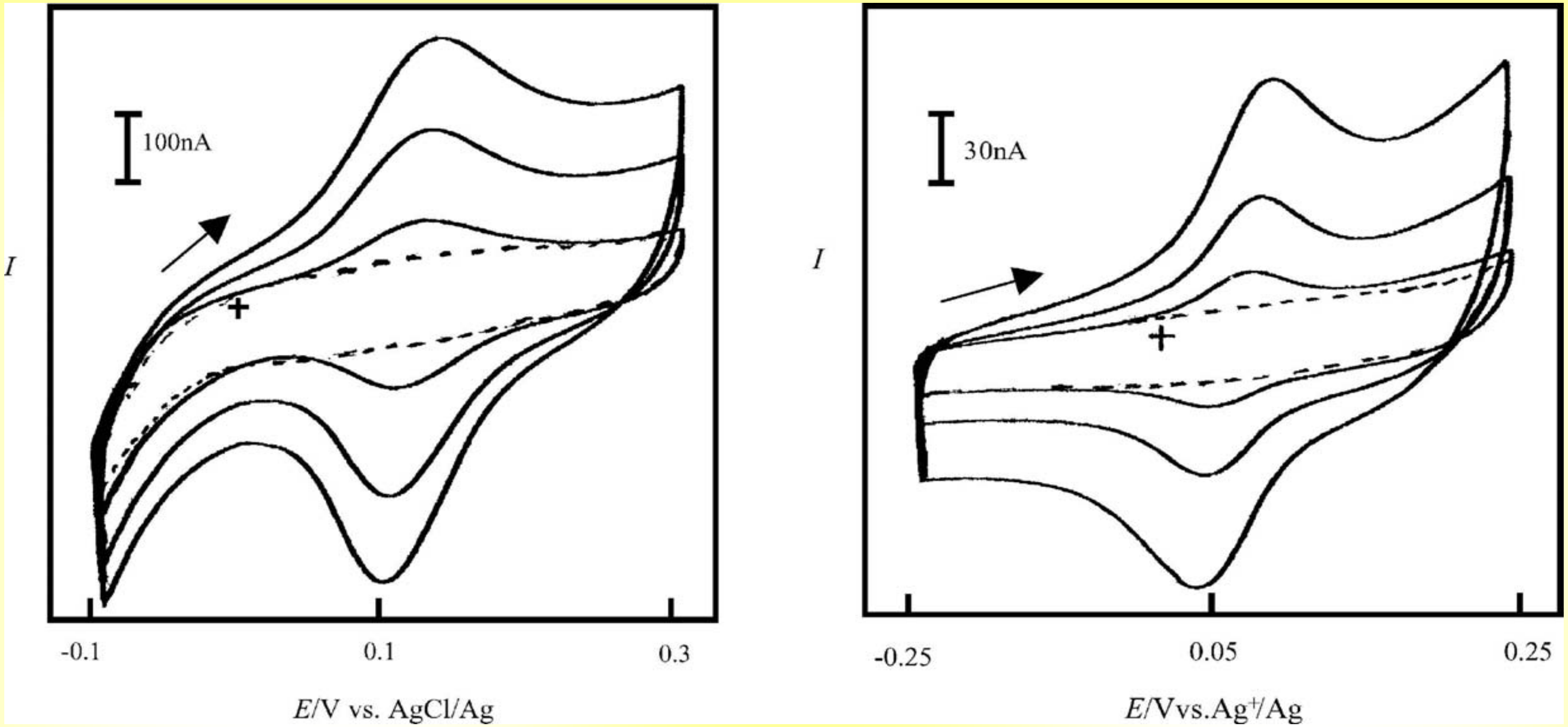
Construction of multilayer Au-nanoparticle structures based on electrostatic interactions. The first layer of Au-nanoparticles is attached to the glass-siloxane surface. The various layers are then constructed using a positively charged cross-linker (step (i) in the upper figure). Cross-linkers may be anything from a small molecule (e.g. C60) to other nanoparticles, but they must bear multiple charges.

Ref.: A.N. Shipway, E. Katz, I. Willner, Chem. Phys. Chem. 1, 2000, 18–52



Schematic diagram of the preparation of DNA modified electrode

Ref.: B. Jin, X. Ji, T. Nakamura, Electrochim. Acta 50 (2004) 1049–1055

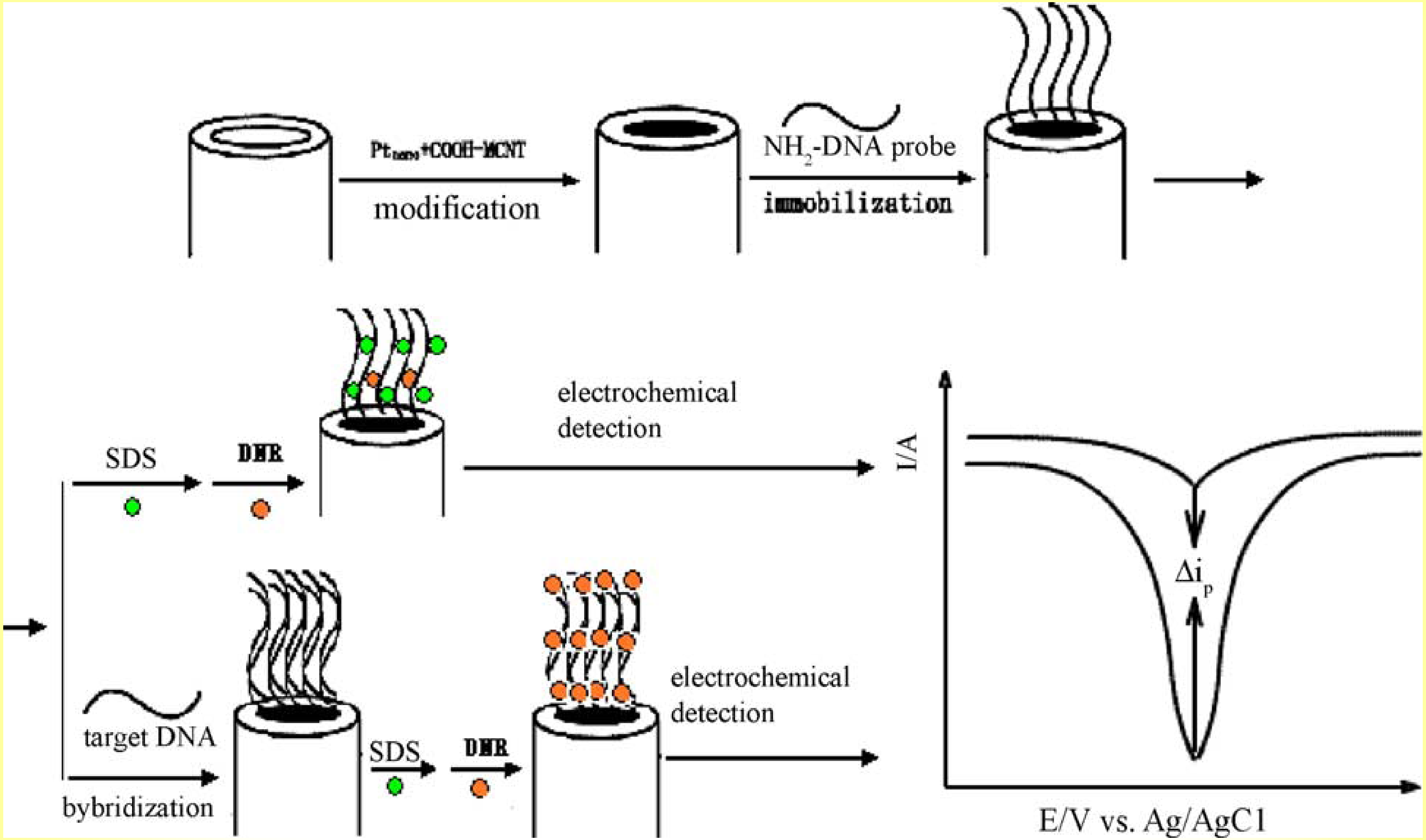


Cyclic voltammograms of $Co(phen)_3^{3+}$ adsorbed on DNA modified (6 nm nanogold) electrode in aqueous and AN solutions.

Left: aqueous system with 50 mM NaCl + 20 mM Tris–HCl (pH 7.0), potential scan rates *v* = 10, 20, 40 mV/s, reference electrode; Ag/AgCl.

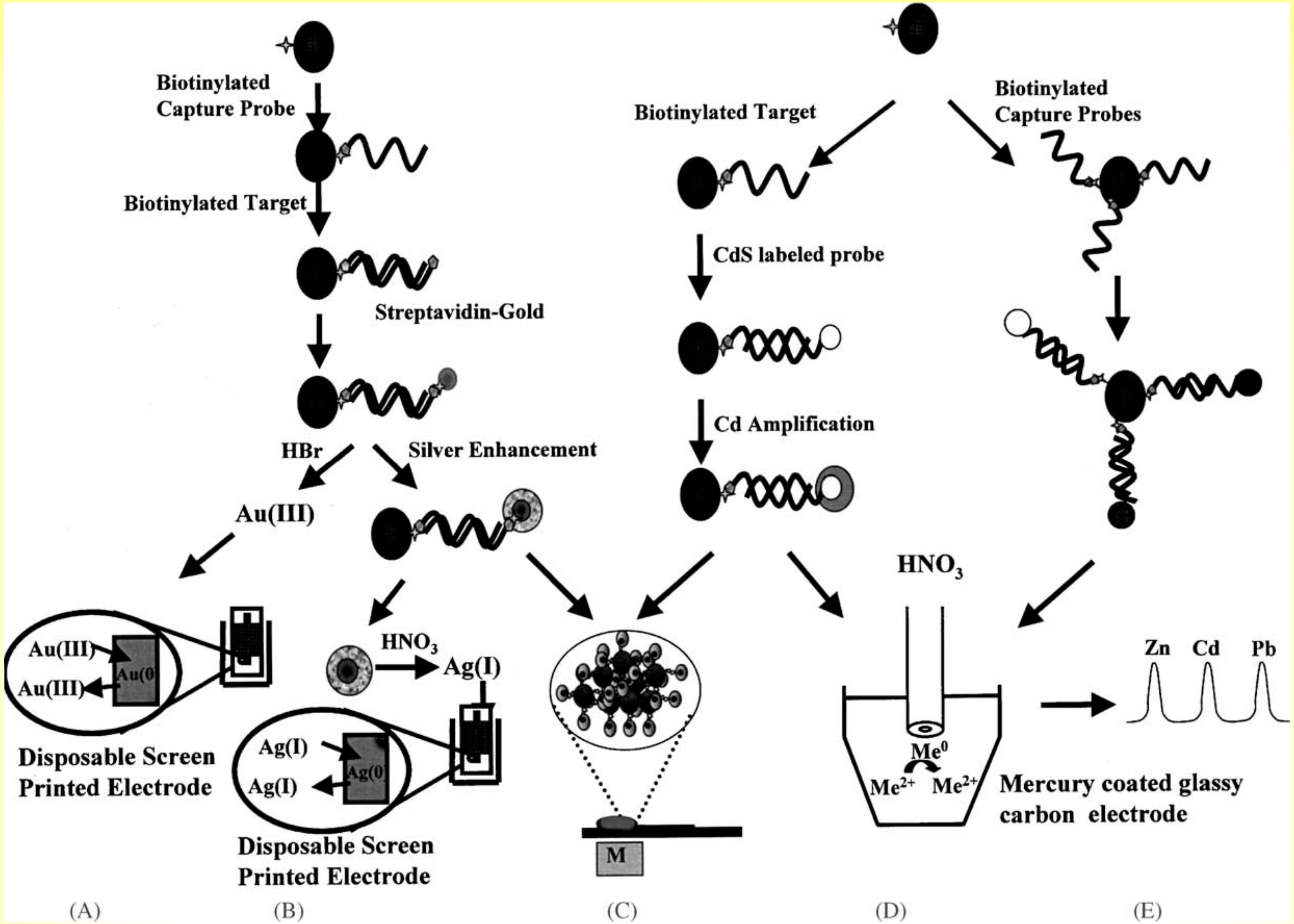
Right: AN system with 50 mM TEAP, potential scan rates *v* = 10, 20, 40 mV/s, ref. electrode; 0.01 M Ag+/Ag. Dashed lines were obtained by using gold nanoparticle electrode (without ds-DNA) instead of DNA modified electrode under the same experimental conditions (*v* = 10 mV/s).

Ref.: B. Jin,X. Ji, T. Nakamura, Electrochimica Acta 50 (2004) 1049–1055



Schematic representation of the electrochemical detection of DNA hybridization based on platinum nanoparticles combined with MWCNT

Ref.: N. Zhu, Z. Chang, P. He,Y. Fang, Analytica Chimica Acta 545 (2005) 21–26

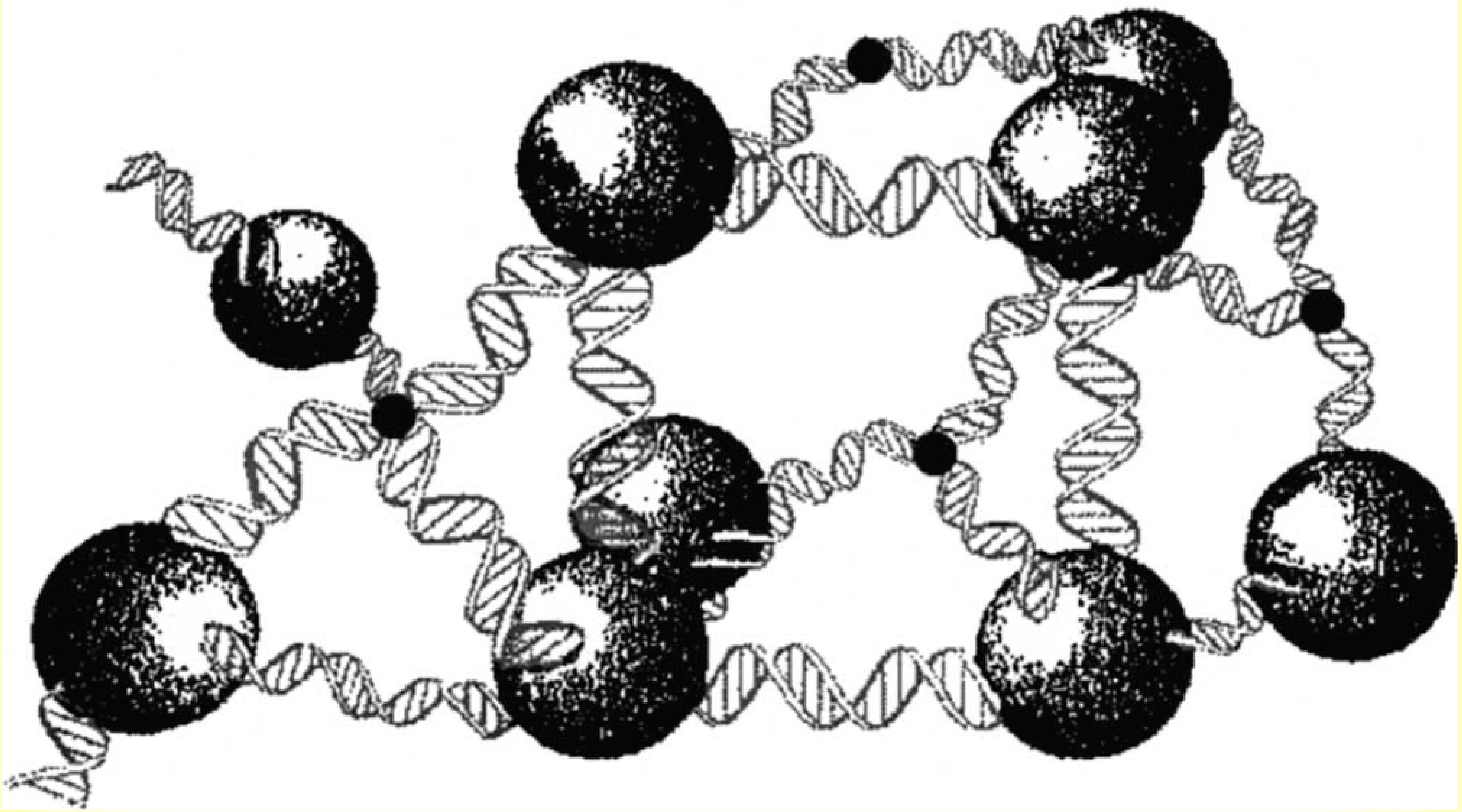


Particle-based protocols for electrochemical detection of DNA. These assays involve the introduction of the probe-coated magnetic beads, addition of the target/hybridization event, magnetic removal of unwanted materials, binding of the metal and amplified electrochemical detection of the dissolved gold (A), silver (B) and cadmium sulfide (D) nanoparticles. Me: metal tag. Also shown are solid-state stripping (C) and multi-target (E) detection protocols

Ref.: J. Wang, Anal. Chim. Acta 500 (2003) 247–257

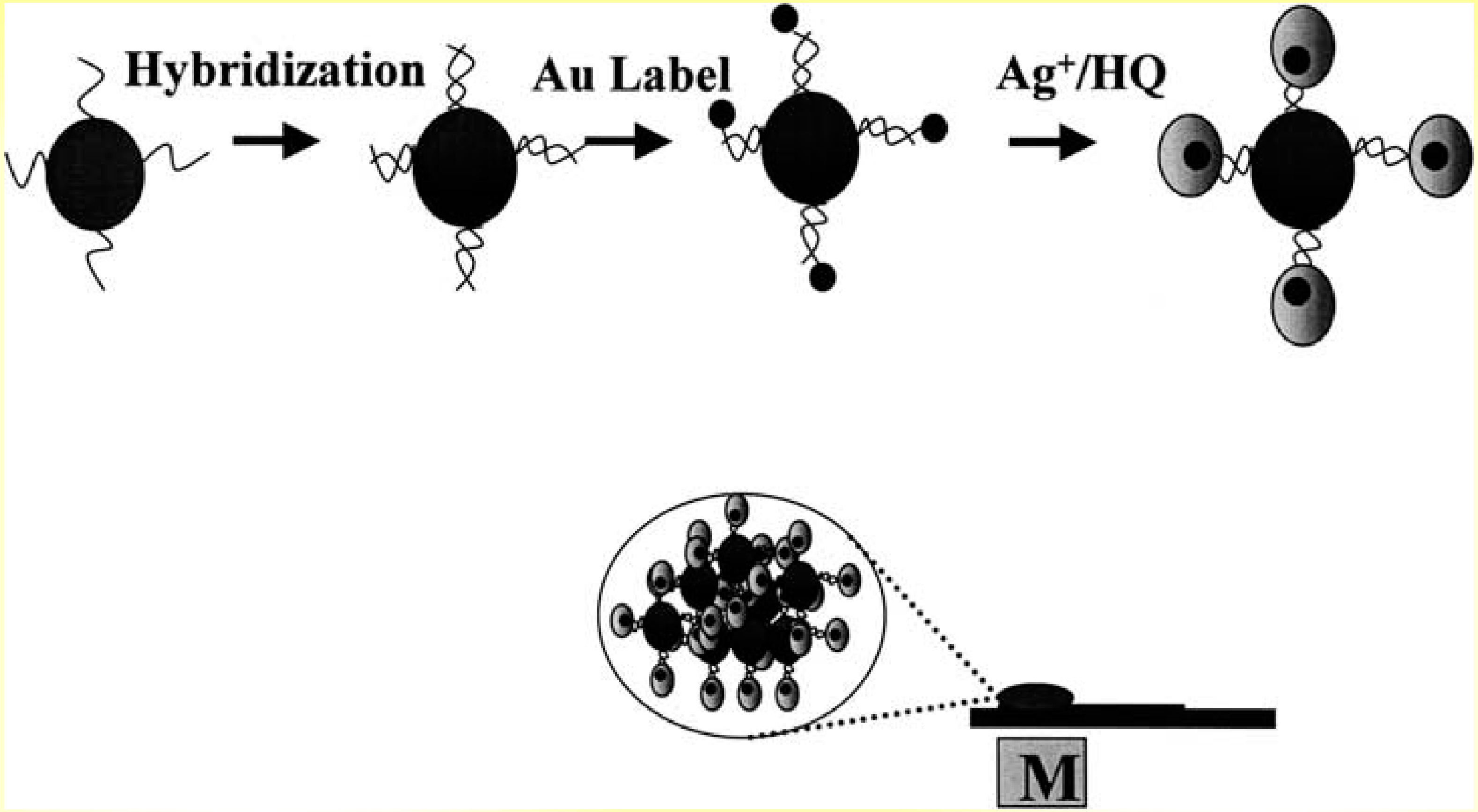
DNA-LINKED PARTICLE NETWORK

(Assembly of magnetic spheres / DNA hybridization gold nanoparticles)



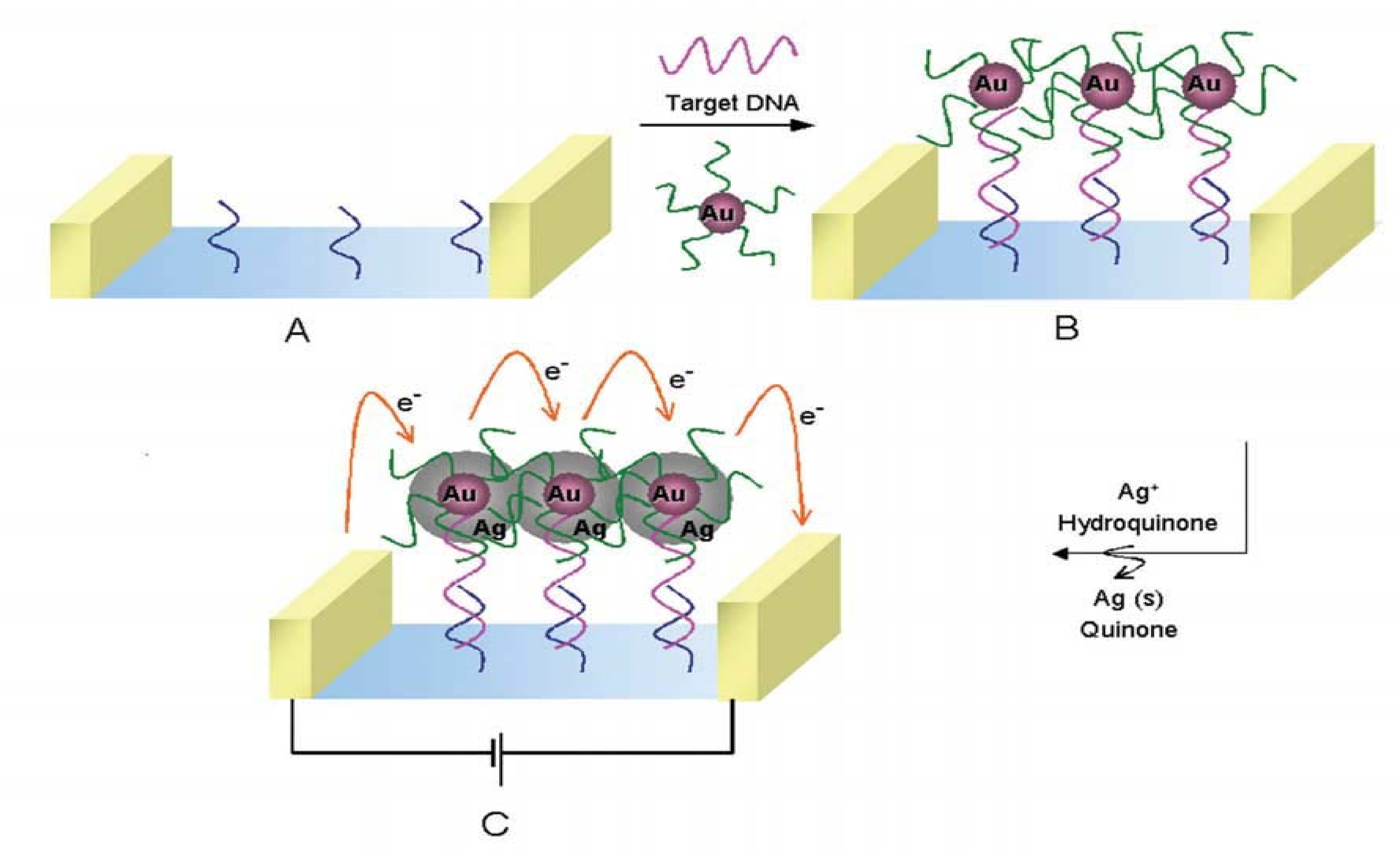
DNA-linked particle nanostructure assembly associated with the hybridization of probe-coated magnetic bead and gold nanoparticle-conjugated target

Ref.: J. Wang / Analytica Chimica Acta 500 (2003) 247–257



Schematic of the magnetically-induced solid-state electrochemical detection of DNA hybridization. The assay involves introduction of the probe-coated magnetic beads, the hybridization event (with the biotinylated target), capture of the streptavidin-coated gold particles, catalytic silver deposition on the gold nanoparticle tags, and positioning of an external magnet (M) under the electrode to attract the particle–DNA assembly and solid-state chronopotentiometric detection

Ref.: J. Wang, D. Xu, R. Polsky, J. Am. Chem. Soc. 124 (2002) 4208.



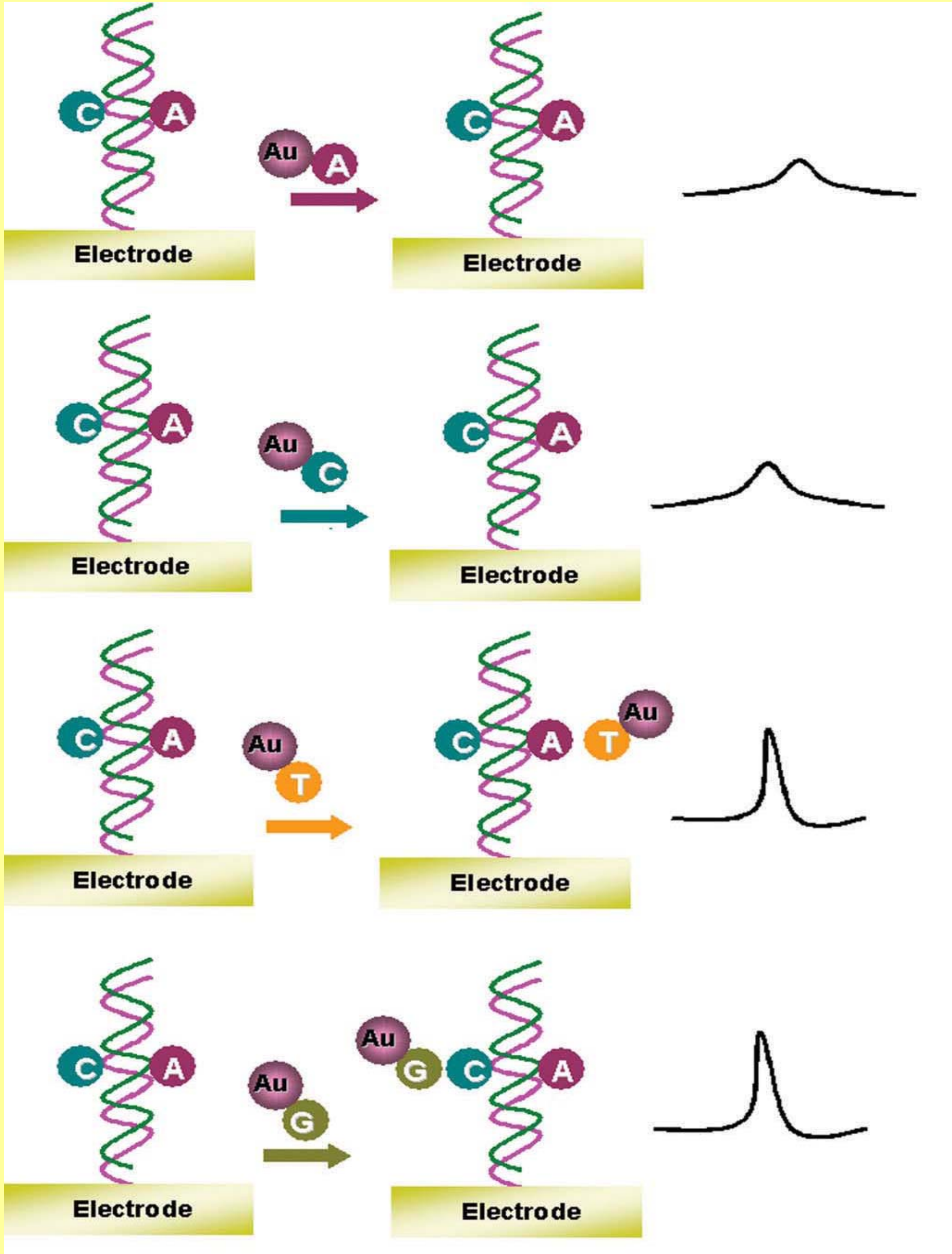
Electrical detection of DNA hybridization using Au nanoparticle labels.

(a) Immobilization of capture probes in the gap between two electrodes.

(b) Hybridization with target DNA and Au nanoparticle-labeled detection probe.

(c) Reductive deposition of Ag, creating a bridge that decreases resistance.

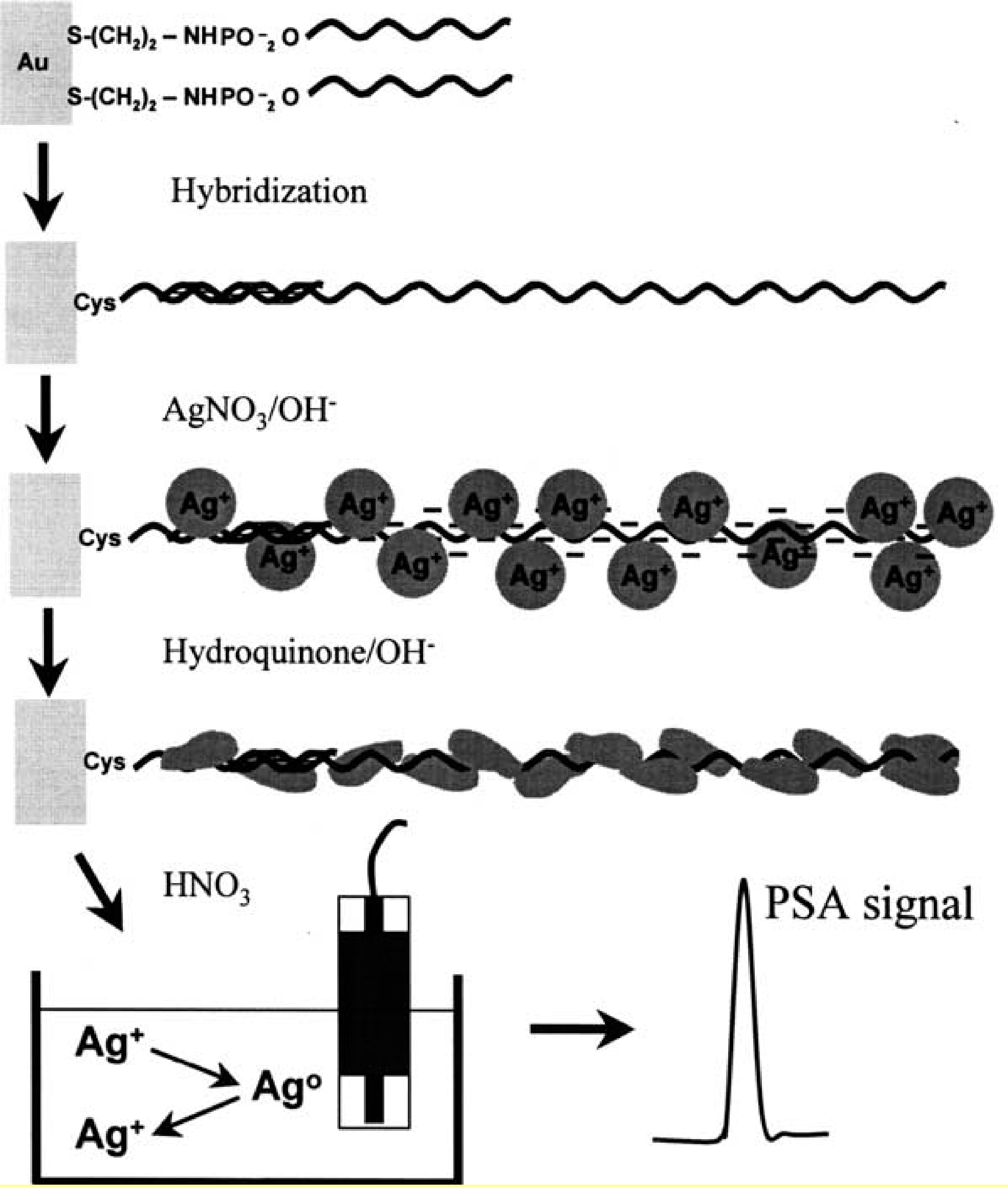
Ref.: Park, S. J., et al., Science(2002) 295, 1503



Electrochemical identification of a single-nucleotide polymorphism.

Four Au nanoparticle-labeled monobases are added in separate assays.
The comparison of the Au redox signal generated from each assay indicates the identity of the mismatch.

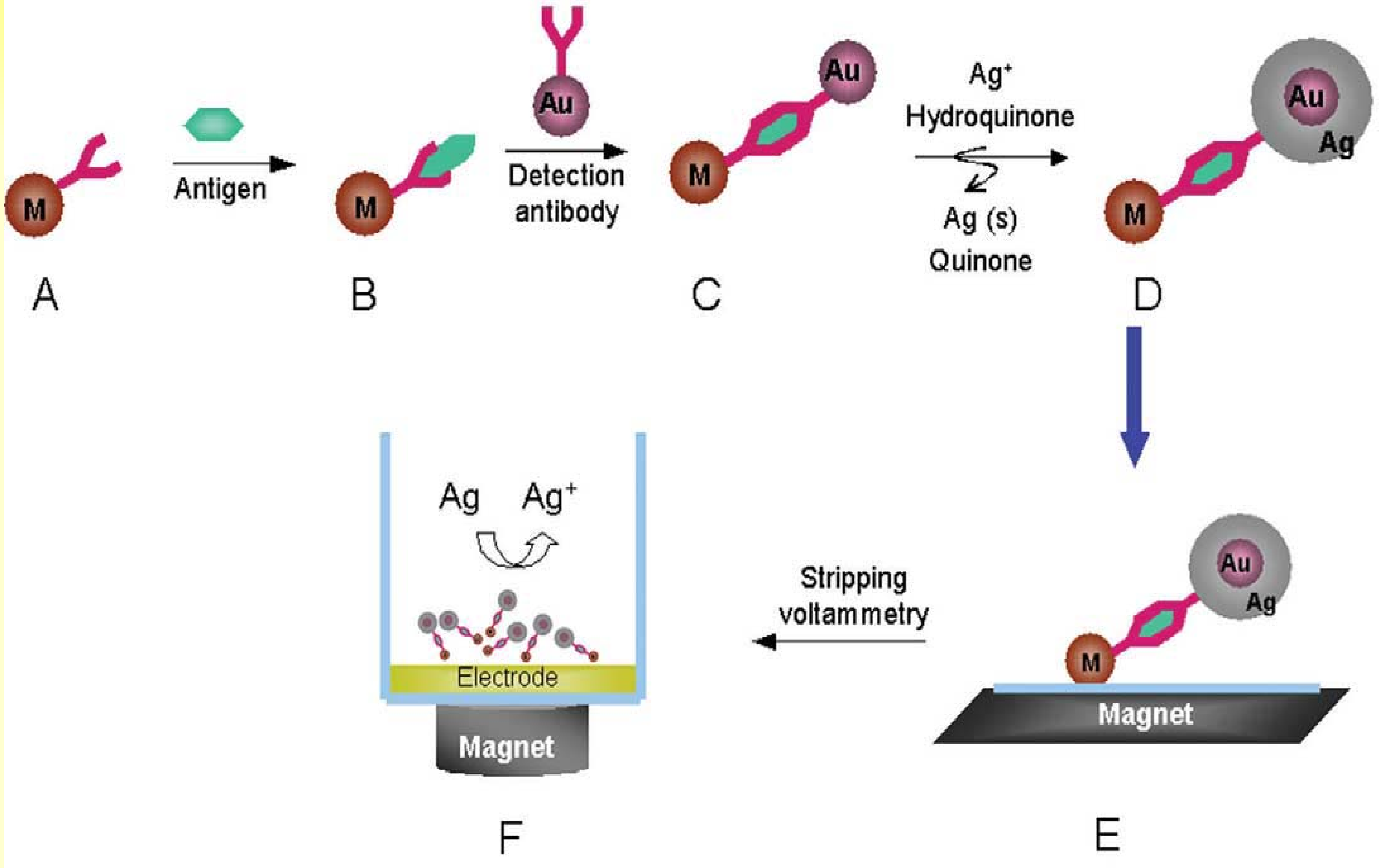
Ref.: Kerman, K. et al., Anal. Chem.(2004) 76, 1877



Schematic representation of the protocol used for electrical detection of silver clusters produced along the DNA backbone.

From top: formation of a self-assembled cystamine monolayer; immobilization of ssDNA ‘probe’ through the 5’-phosphate groups of ssDNA by the formation of phosphoramidate bond with the amino groups of the electrode surface; hybridization of the complementary target; ‘loading’ of the silver ion onto the DNA; hydroquinone-catalyzed reduction of silver ions to form silver aggregates on the DNA backbone; dissolution of the silver aggregates in a acid solution and transfer to detection cell; stripping potentiometric detection

Ref.: J. Wang et al., Electrochem. Commun. 5 (2003) 83



Electrochemical stripping detection of antigen using magnetic beads and Au nanoparticle labels. (a) Immobilization of capture antibody on a magnetic bead. (b) Binding with target antigen. (c) Recognition by detection antibody. (d) Nanoparticle-catalyzed enlargement by Ag deposition. (e) Collection of magnetic beads and the antibody-Ag complex using an external magnet. (f) Stripping voltammetric detection of the collected Au/Ag nanoparticle label.

Ref.: Liu, G.-D., and Lin, Y.-H., J. Nanosci. Nanotech. (2005) 5, 1060

Quantum dots

Quantum dots (QD) are also nanostructured materials known as zero-dimensional material.

A quantum dot is a location that can contain a single electrical charge, single electron. The presence or absence of electron changes the properties of QD and they can be then used for several purposes, such as information storage or transducers in sensors.

They are semiconductor nanocrystals roughly spherical in shape with the diameter of 1-12 nm.

Rast CdS QD nanočastíc

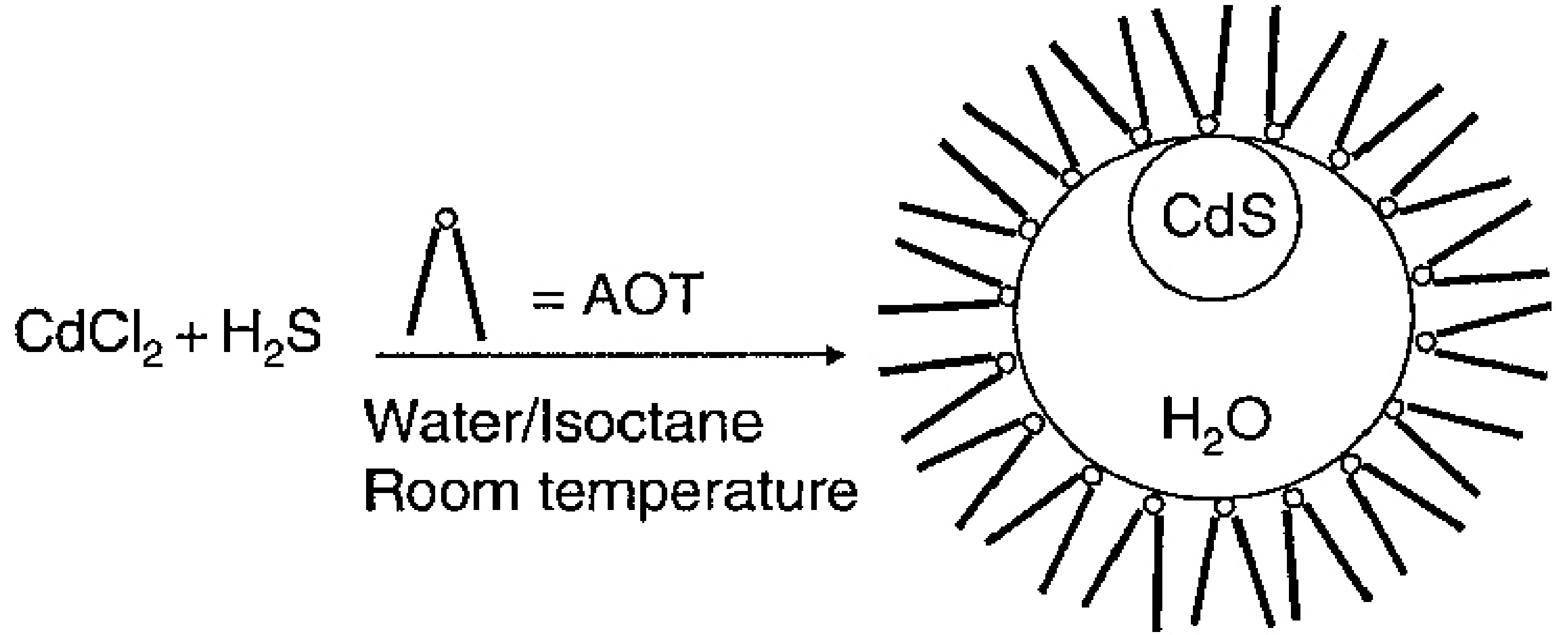


Figure 2. Growth of CdS QD nanoparticles in reverse micelles. This technique exploits natural geometrical structures created by water-in-oil mixtures upon adding an amphilic surfactant, such as sodium dioctyl sulfosuccinate (AOT). By varying the water content of the mixture, it was shown that the size of the water droplets suspended in the oil phase could be varied systematically.

Ref.: A. Merkoci et al., TRAC 24, 341 (2005)

CdS QD v micelách

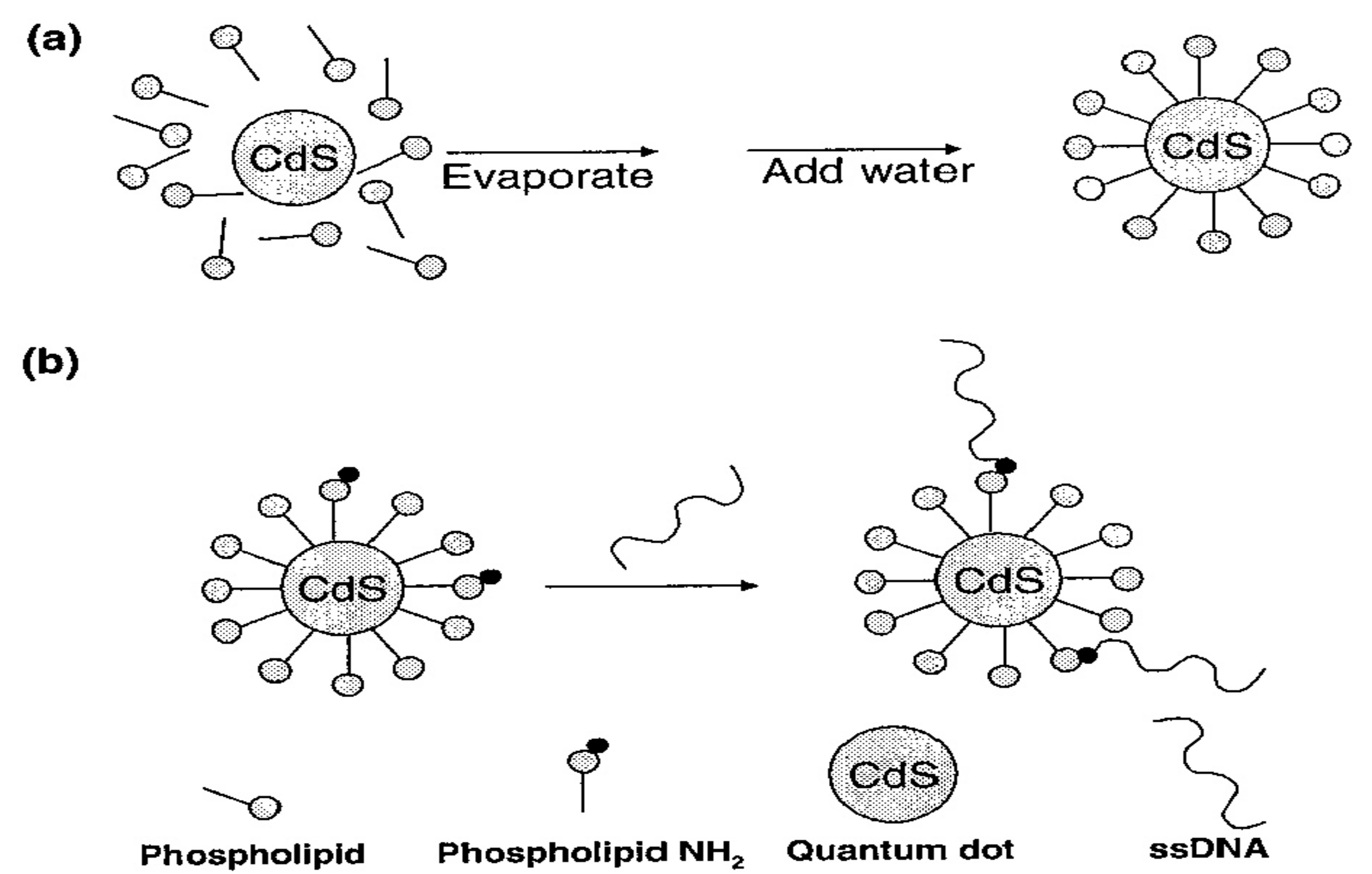


Figure 3. (a) Encapsulation of QDs in micelles; and, (b) QD–micelle conjugation with single-stranded DNA (ssDNA). CdS QDs can be encapsulated in the hydrophobic core of a micelle composed of a mixture of *n*-poly(ethyleneglycol) phosphatidylethanolamine (PEG–PE) and phosphatidylcholine (PC).

Ref.: A. Merkoci et al., TRAC 24, 341 (2005)

Modifikácia CdS QD

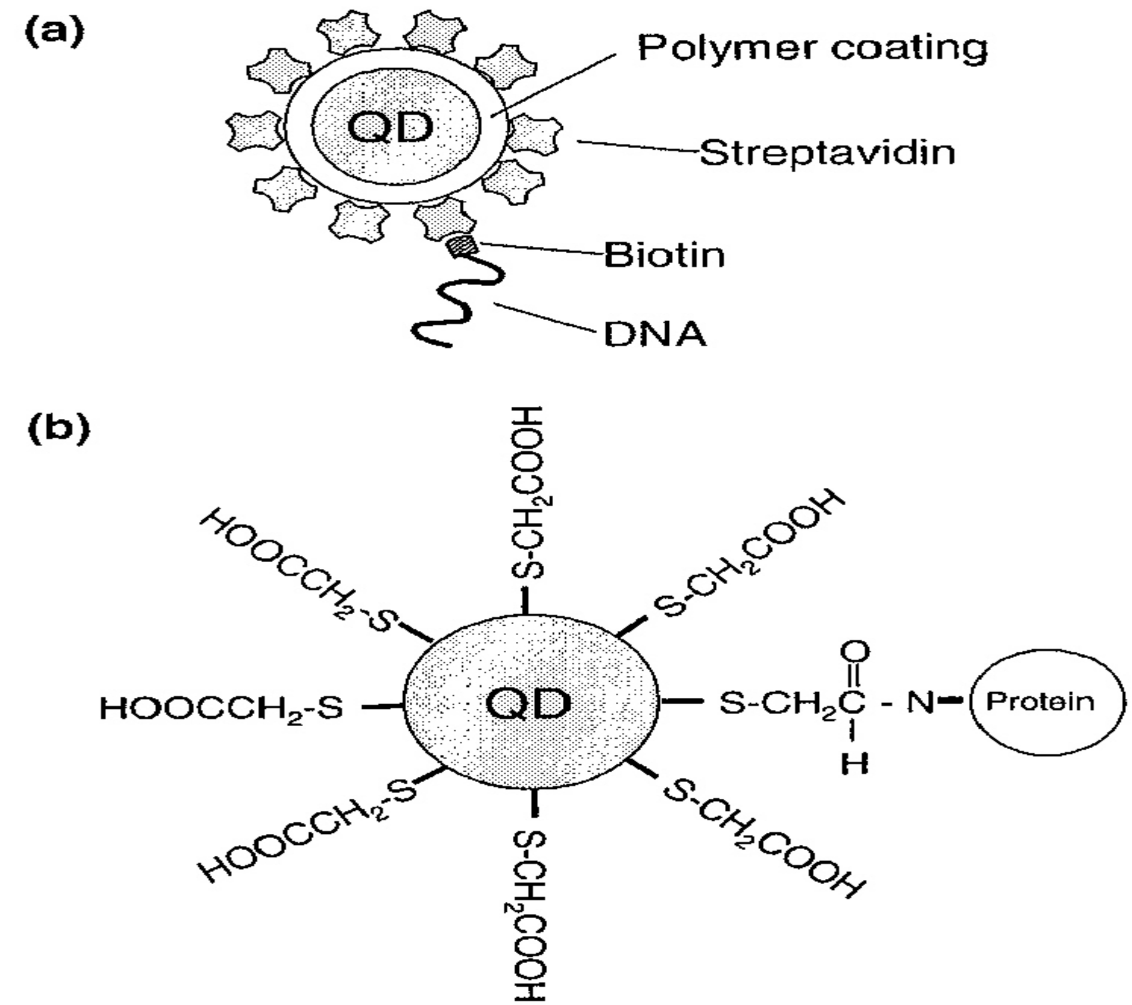


Figure 4. (a) A QD coated with a polymer modified with streptavidin. A biotin-functionalized DNA is attached onto the surface. (b) Schematic of a CdS QD that is covalently coupled to a protein through mercaptoacetic acid by using ethyl-3-(dimethyl-aminopropyl)carbodiimide) as a bifunctional reagent.

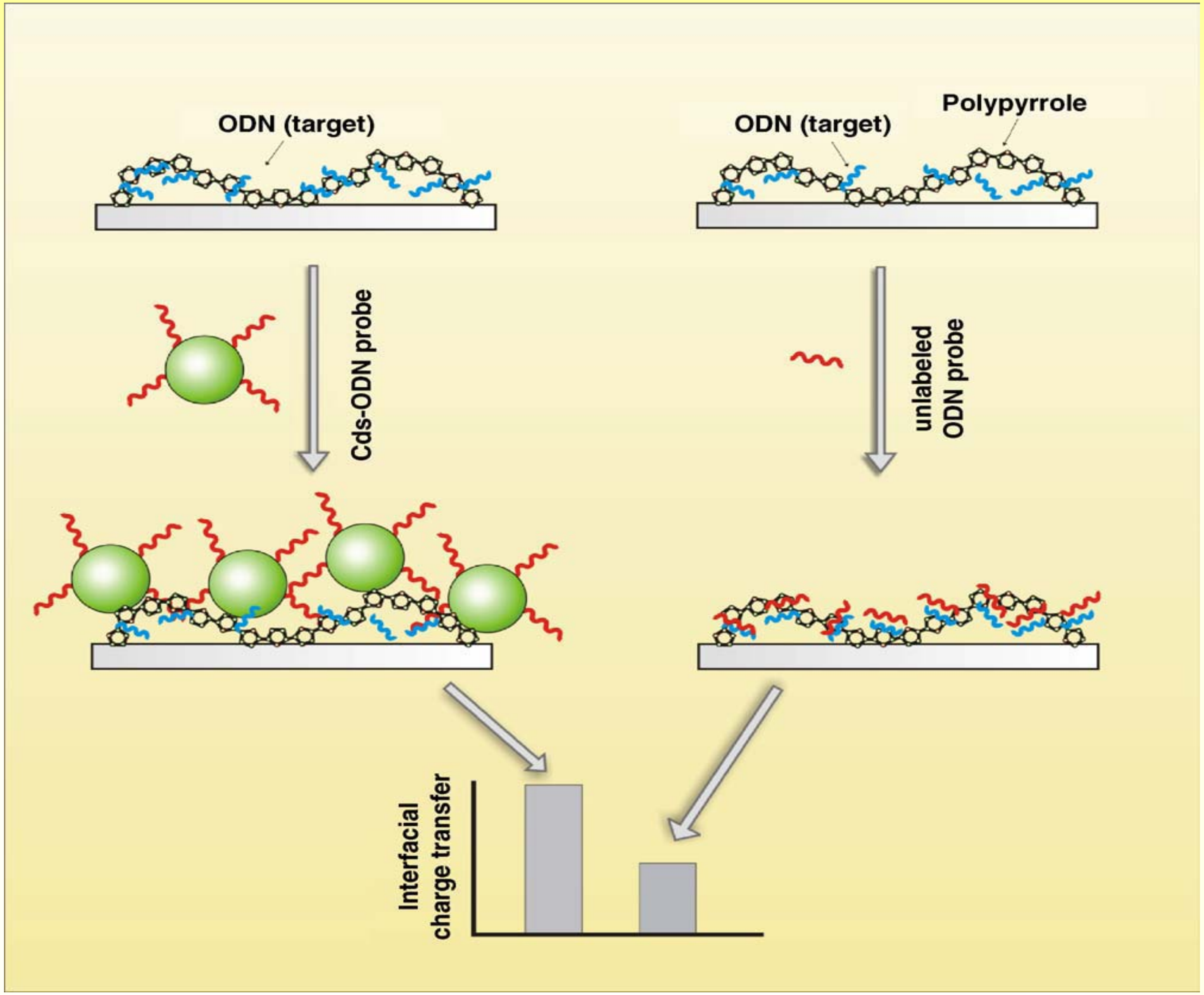
Ref.: A. Merkoci et al., TRAC 24, 341 (2005)

Table 1. Reported nanoparticle labels and analytical parameters of the assays developed

Nanoparticle label	Label connection with DNA	Detection technique	Hybridization separate from detection	DNA detection limits	RSD	Reference
Au	Au–SH–DNA	DPV at pencil-graphite electrode	No	0.78 fmol/mL	~8%	[37]
Au	Au–SH–DNA	PSA and silver catalytic enhancement at screen-printed electrodes	Yes	150 pg/mL	7%	[39,40]
Au	Au–SH–DNA	Conductivity at micro-electrodes	No	500 fM	–	[41]
Au carried into PVC beads	PVC (Au) streptavidin–biotin–DNA	PSA and silver catalytic enhancement at screen-printed electrodes	Yes	40 pg/mL	13%	[45]
CdS QDs	CdS NH–DNA	EIS with gold electrode	No	1.43×10^{-10} M	–	[44]
CNTs loaded with CdS QDs	CNT–CdS–streptavidin–biotin–DNA	DPV at Hg-film electrode	Yes	40 pg/mL	6.4%	[25]
Au–Fe (core/shell)	Fe–Au–SH–DNA	DPV at Hg-film electrode	Yes	50 ng/mL	6.3%	[43]
CdS QDs	CdS–SH–DNA	PSA and catalytic enhancement with Cd at screen-printed electrodes	Yes	20 ng/mL	6%	[42]
CdS QDs PbS QDs ZnS QDs	CdS–SH–DNA PbS–SH–DNA ZnS–SH–DNA	Simultaneous detection with SWV at Hg-film electrode	Yes	5 ng/mL	9.4%	[46]

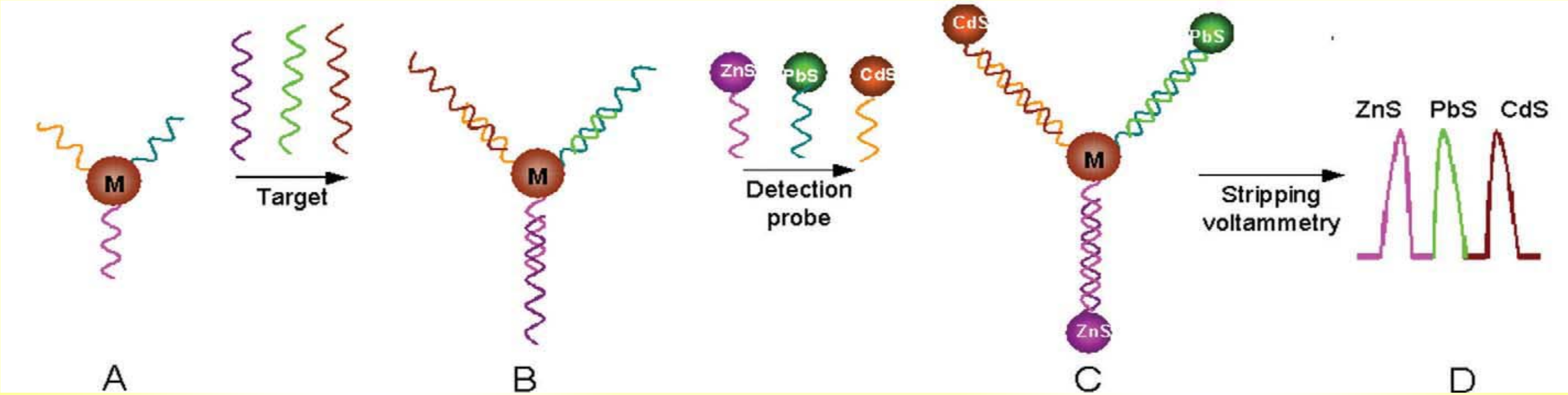
PSA, Potentiometric stripping analysis; DPV, Differential pulse voltammetry; SWV, Square wave voltammetry; EIS, Electrochemical impedance spectroscopy.

Ref.: A. Merkoci et al., TRAC 24, 341 (2005)



Mechanism underlying the sensor response.
(Top) Formation of a layer of CdS–ODN nanoparticles on the PPY–ODN film after hybridization.
(Bottom) Binding of unlabeled ODN probes to the PPY–ODN film.

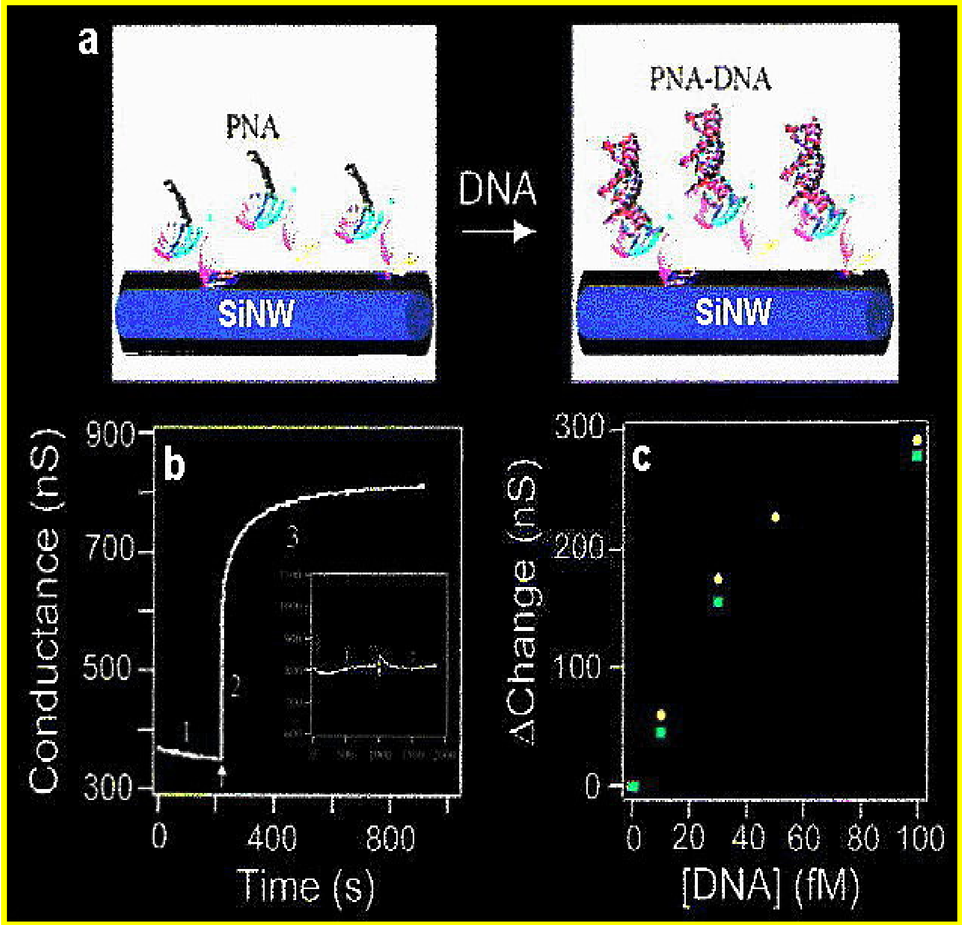
Ref.: H. Peng et al., Biosens. Bioelectron 21 (2006) 1727–1736



Multitarget electrochemical detection of DNA using QD labels.
(a) Introduction of probe-modified magnetic beads.
(b) Hybridization with DNA targets.
(c) Second hybridization with QD-labeled probes.
(d) Dissolution of QDs and electrochemical detection.

Ref.: J. Wang et al., J. Am. Chem. Soc.(2003) 125, 3214

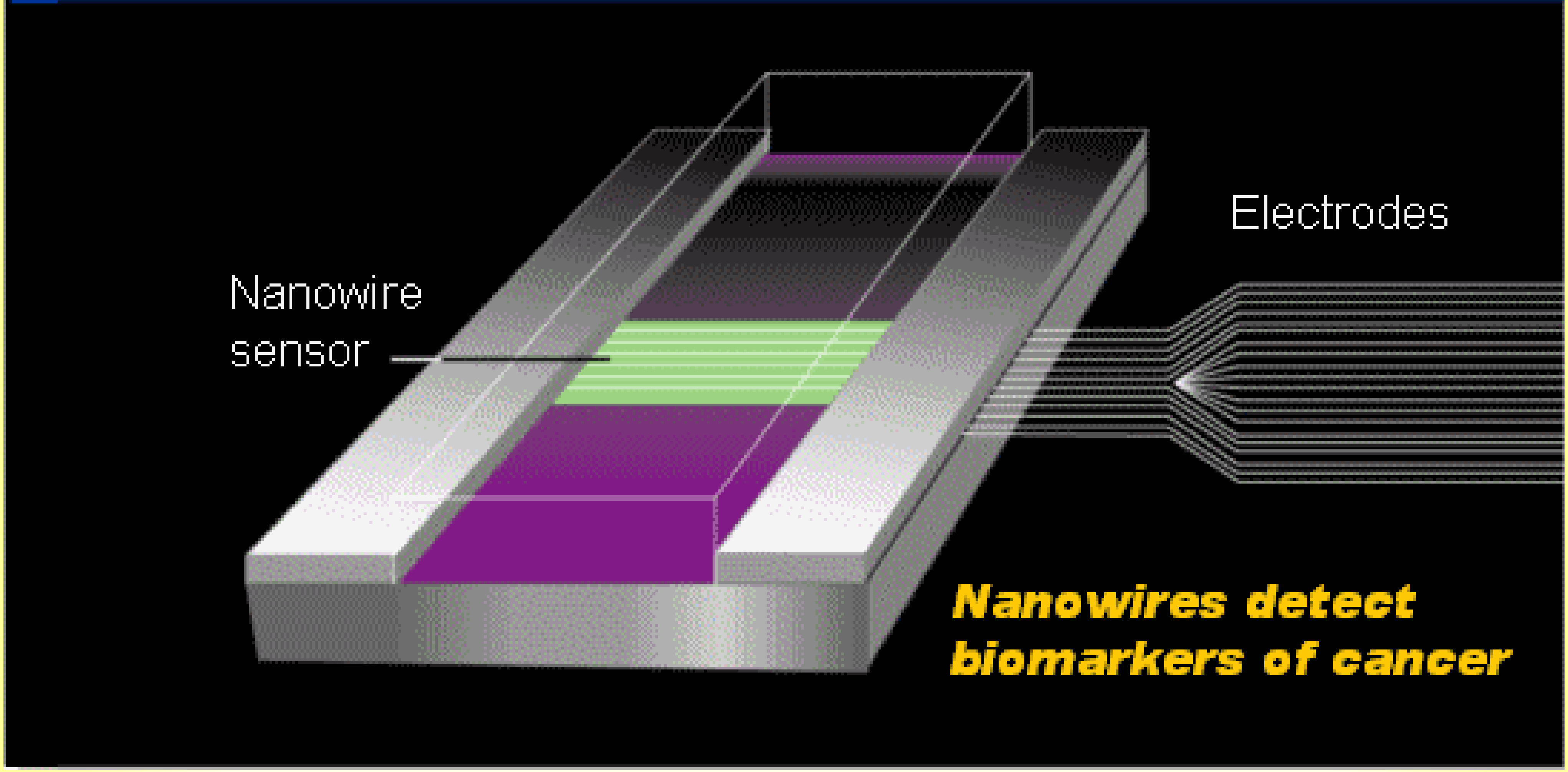
„Nanowires"



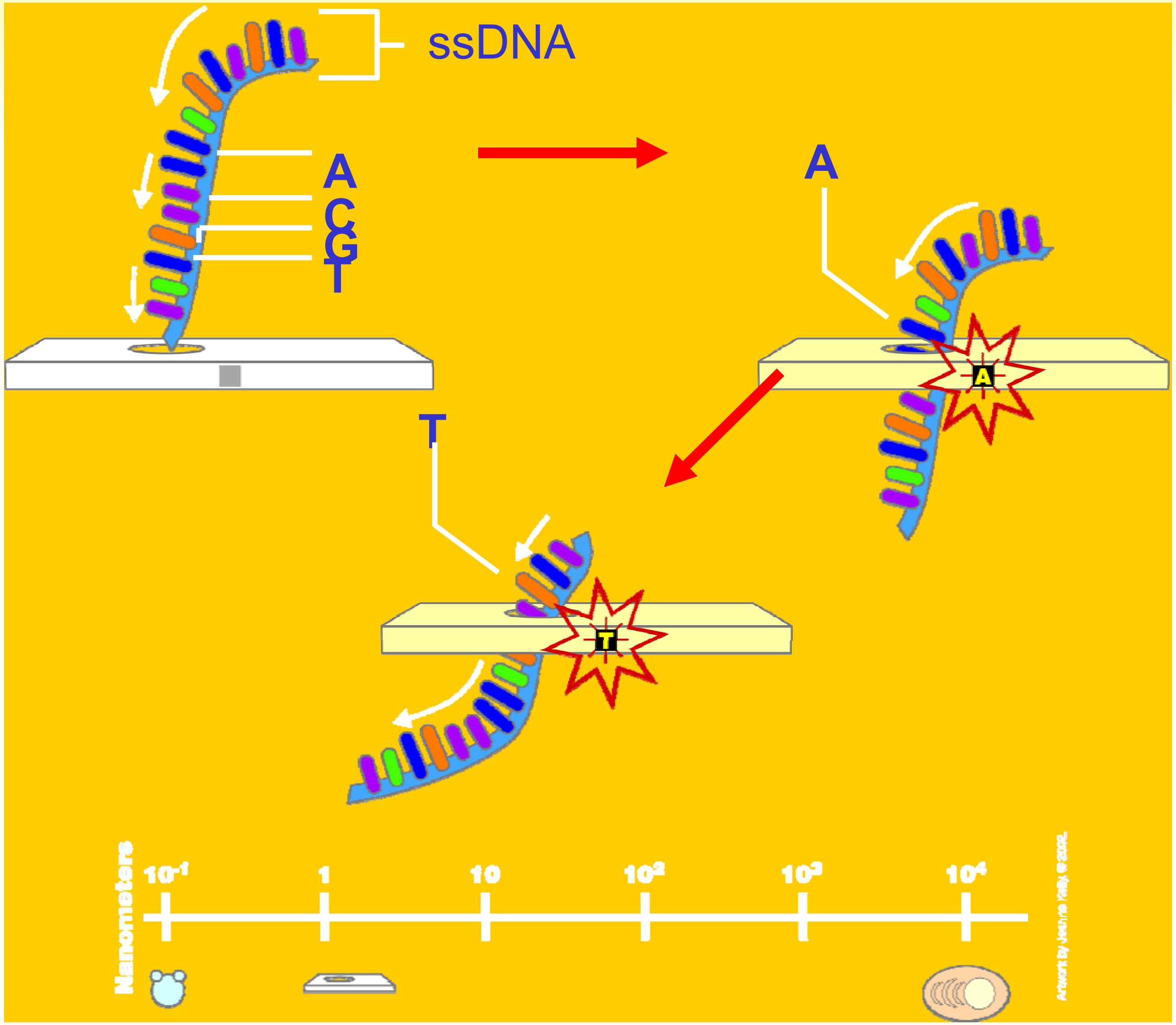
Biologické makromolekuly (NA, proteíny) nesú vo vodných roztokoch náboj a ich možno detegovať pomocou kremíkového "nanowire field effect device“ s vhodnými receptormi na povrchu. Výhodou je priamy prenos elektrického signálu v reálnom čase, vysoká citlivosť a selektivita.

Princíp merania s „nanowire“ senzorom spočíva v meraní elektrickej vodivosti. Jej zmena závisí od množstva DNA.

Nanowire Sensor

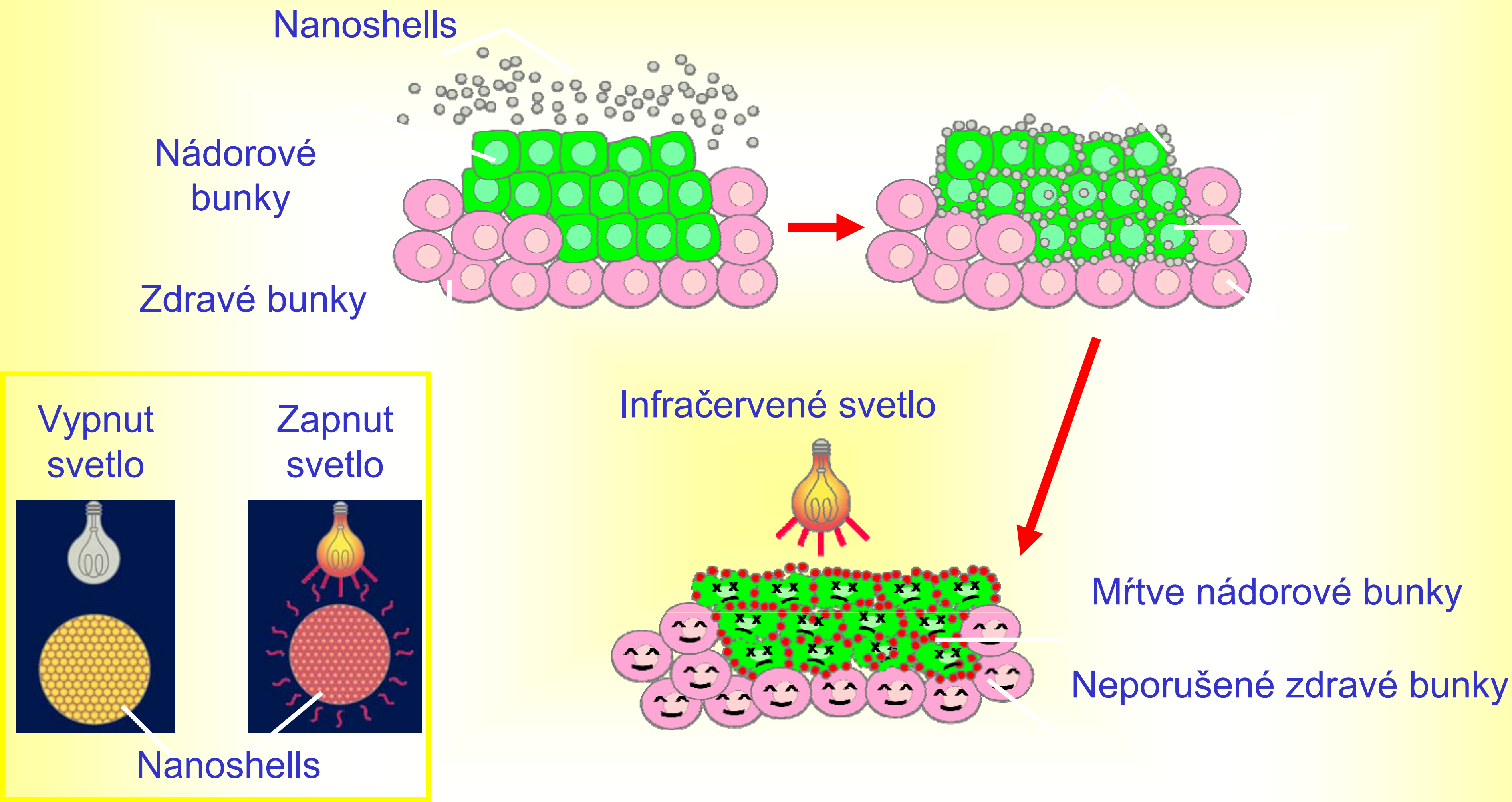


"Nanopores"



Pri pohybe molekuly cez kanály v membráne dochádza k charakteristickým zmenám elektrickej vodivosti. Pomocou „nanopórov“ v membráne sme schopní detegovať zmeny v sekvencii jednovláknovej (ss) DNA.

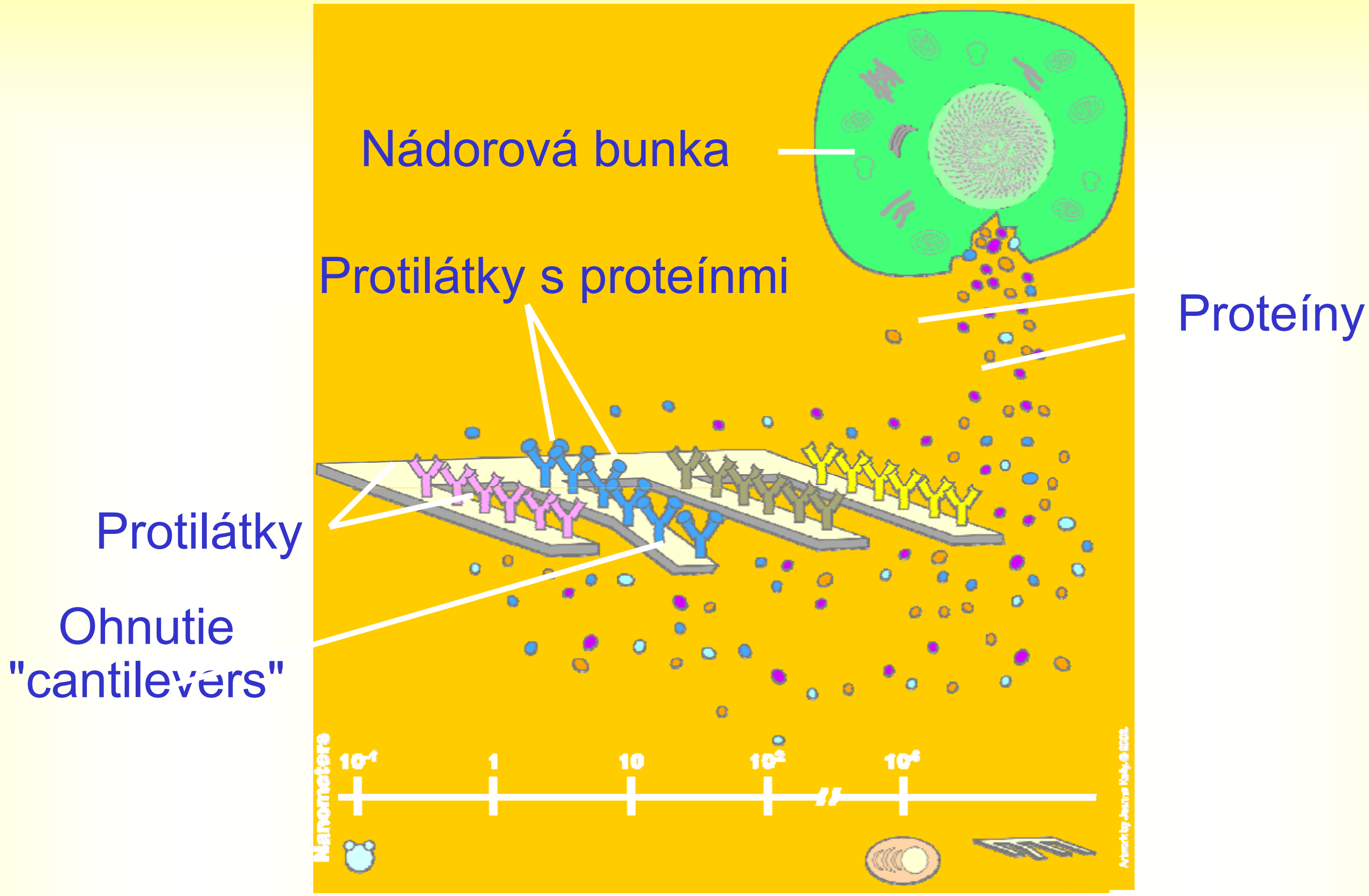
"Nanoshells"



Zlaté „nanoshells“ (nanoobaly) okolo dielektrického jadra vykazujú optickú rezonanciu, ktorú môžno kontrolovať pomerom hrúbky obalu a priemeru nanočastíc.

Ag/Au (jadro/obal) nanočastice po tiolácii sa použili ako sondy pri hybridizácii DNA.

"Cantilevers"

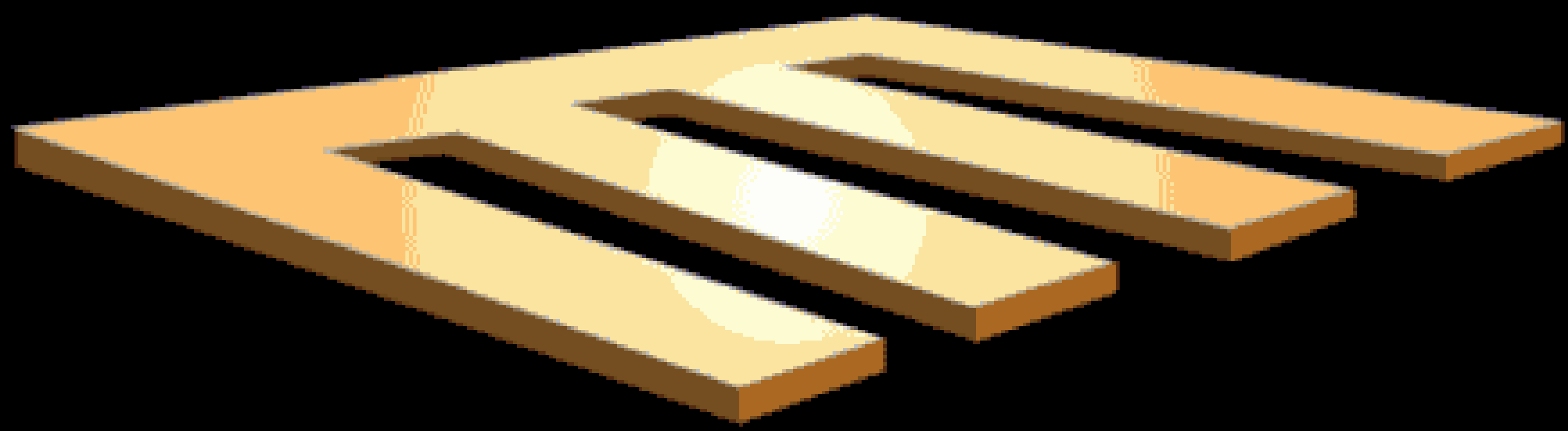


"Cantilevers" (konzoly) sú zodpovedné za difúziu analytu na vrstve senzora a chemickú reakciu s vrstvou senzora. Ak je vrstva senzora vystavená analytu, „cantilevers“ odpovedajú mechanicky ohnutím sa.

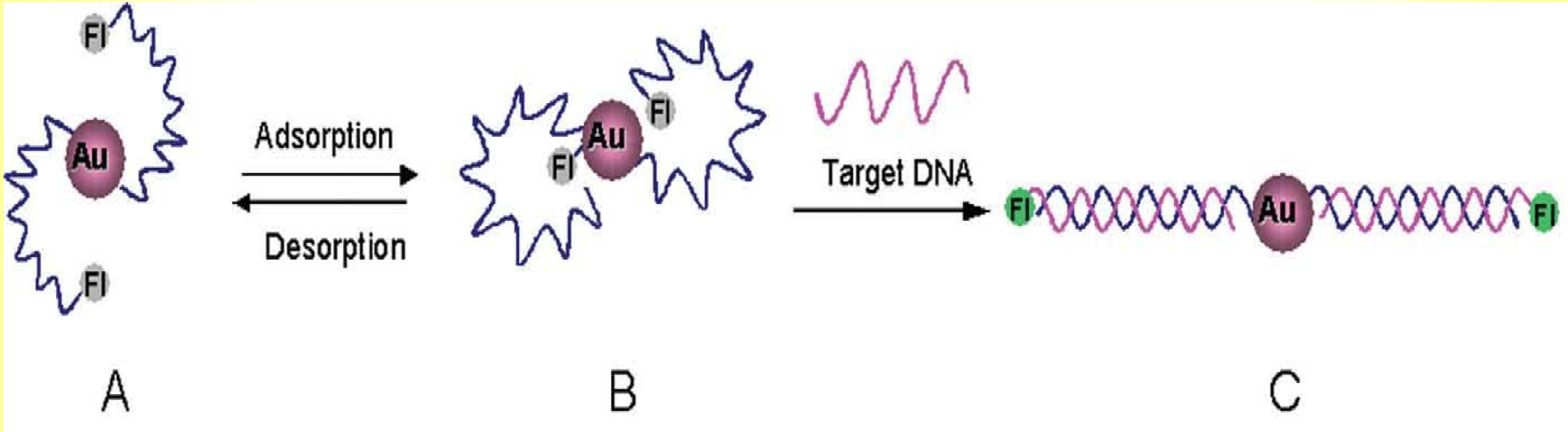
Vlastná detekcia spočíva v meraní rezonančnej frekvencie. "Cantilevers" zrýchľujú a zvyšujú citlivosť nádorových testov.

Nanoscale Cantilevers

Cantilevers detect biomarkers of cancer

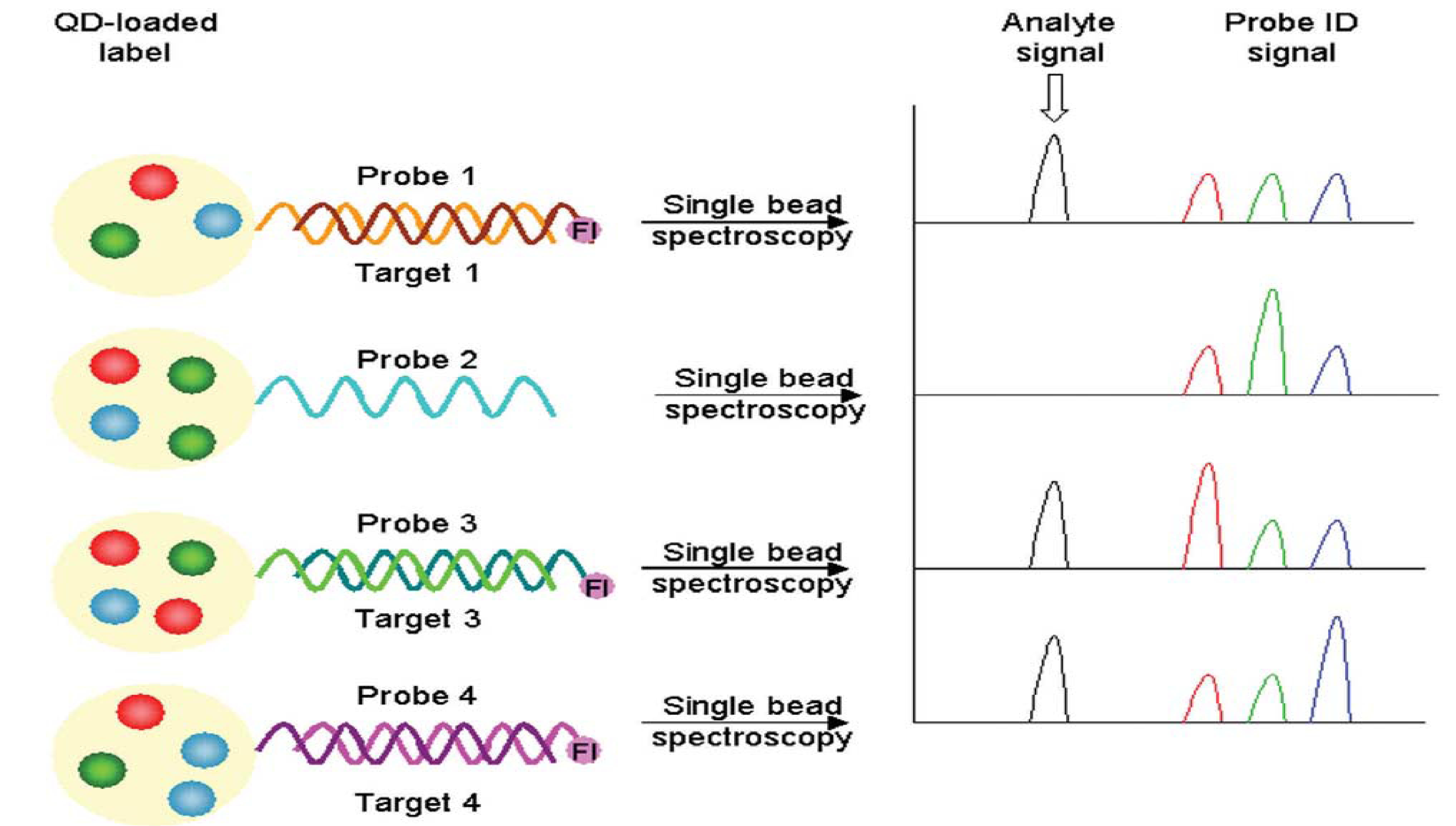


Optical detection



Au nanoparticles as fluorescent quenchers. (a, b) Owing to the conformation, the fluorescence labels are in close proximity to the Au nanoparticles and their signals are quenched. (c) Upon hybridization, the rigid double-helical DNA molecules ‘open up’ their conformation and fluorescence is restored.

Ref.: Maxwell, D. J., J. Am. Chem. Soc.(2004) 124, 9606

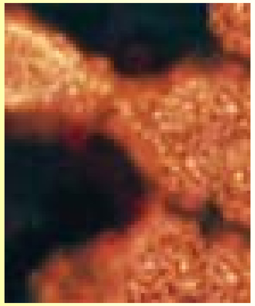


Wavelength-and-intensity multiplexed detection of DNA sequence using QD-loaded beads. The unique probe identification (ID) signal is used to identify individual sequences, while the analyte signal is generated by an attached fluorophore.

Ref.: Han, M. et al., Nat. Biotechnol.(2001) 19, 631

Au nanoparticles target cancer

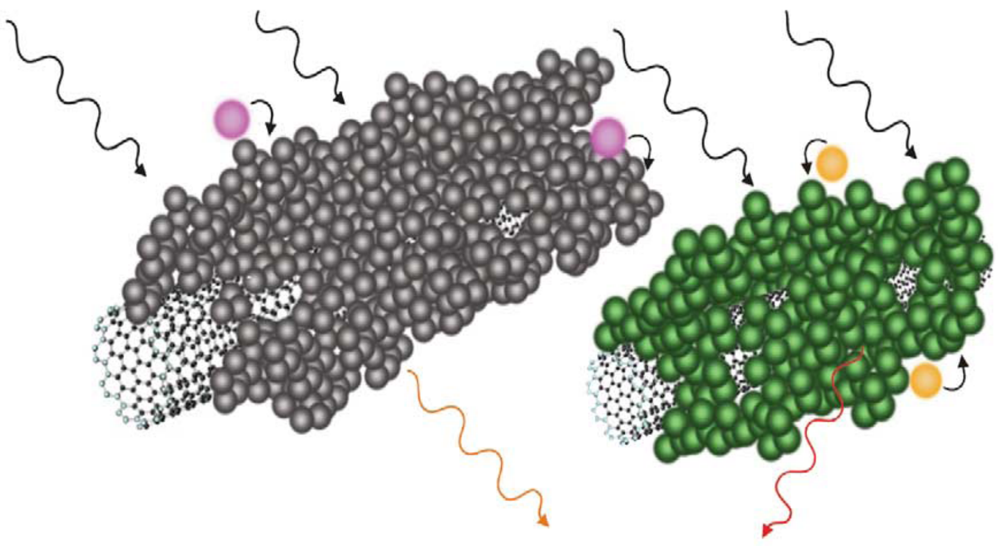
P. K. Jain, I. H. El-Sayed, and M. A. El-Sayed

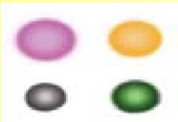


Nanoparticles are generating great enthusiasm in medicine. Au nanoparticles, in particular, have immense potential for cancer diagnosis and therapy on account of their surface plasmon resonance (SPR) enhanced light scattering and absorption.

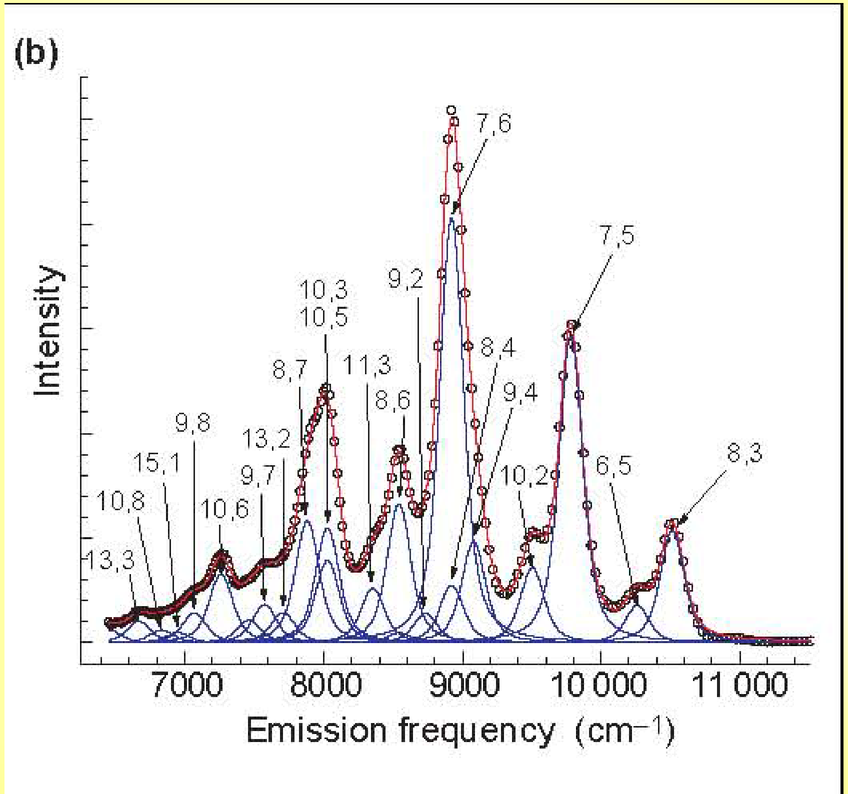
Conjugation of Au nanoparticles to ligands specifically targeted to biomarkers on cancer cells allows molecular-specific imaging and detection of cancer.

Ref.: Nanotoday 2, 18 (2007)





Analytes **A** and **B**
Enzymes **1** and **2**

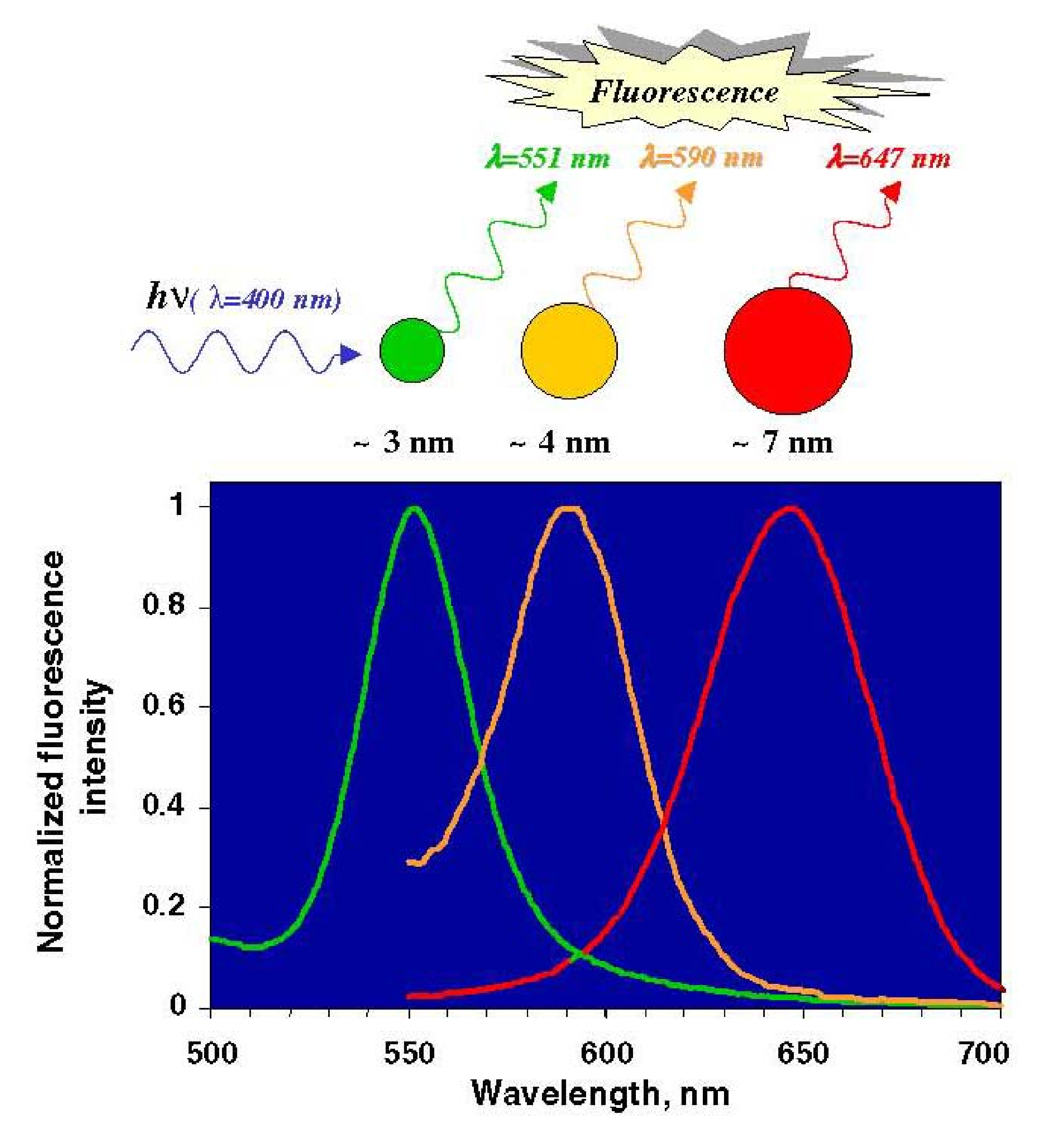


SWNT-based multi-analyte biosensors.

(a) Nanotubes of a given (n,m) type are surrounded by a specific enzyme designed to interact with a specific analyte. These enzymes modulate the transfer of electrons in and out of the nanotube, thereby affecting the fluorescence intensity. Each (n,m) type will give a characteristic nIR fluorescence with narrow band emission that can be collected with a multichannel detector.

(b) Deconvolution of the emission spectrum by (n,m) type will then enable simultaneous measurement of the concentration of each analyte.

Ref.: Kirk J. Ziegler, Trends in Biotechnology 23 (9) 2005

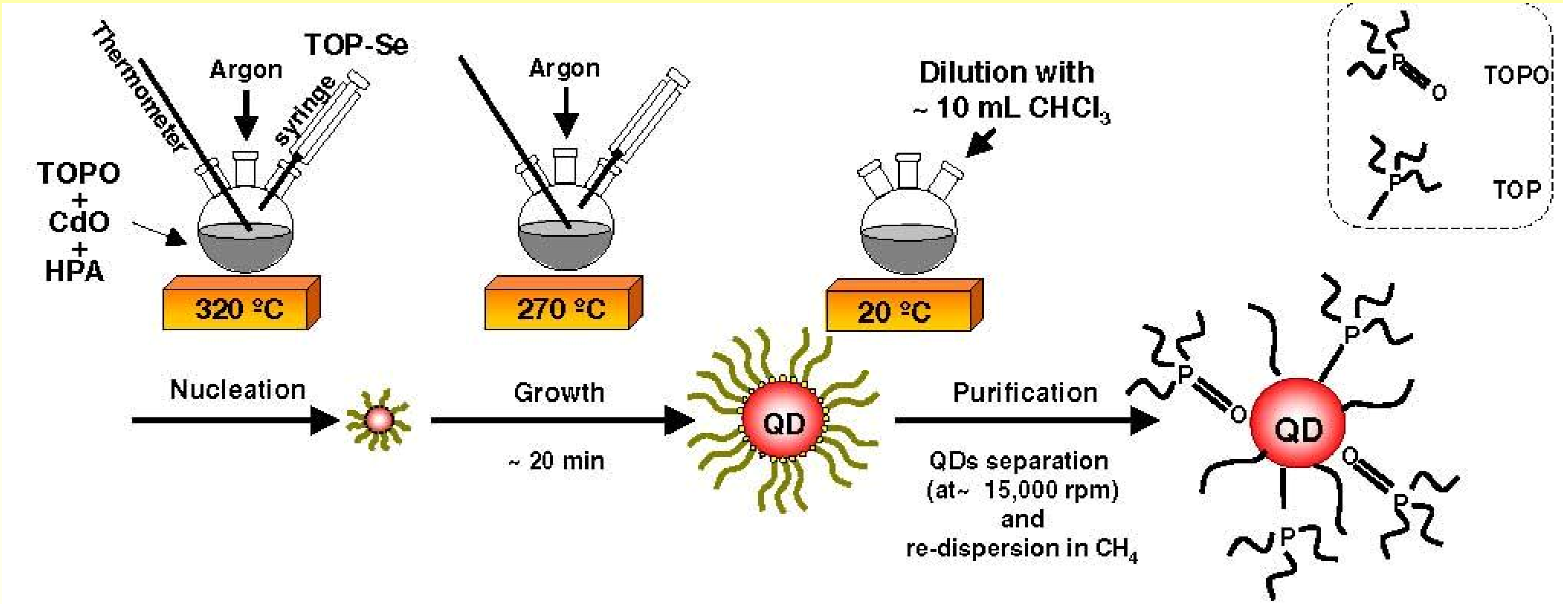


Size-tuneable fluorescence spectra of CdSe QDs.

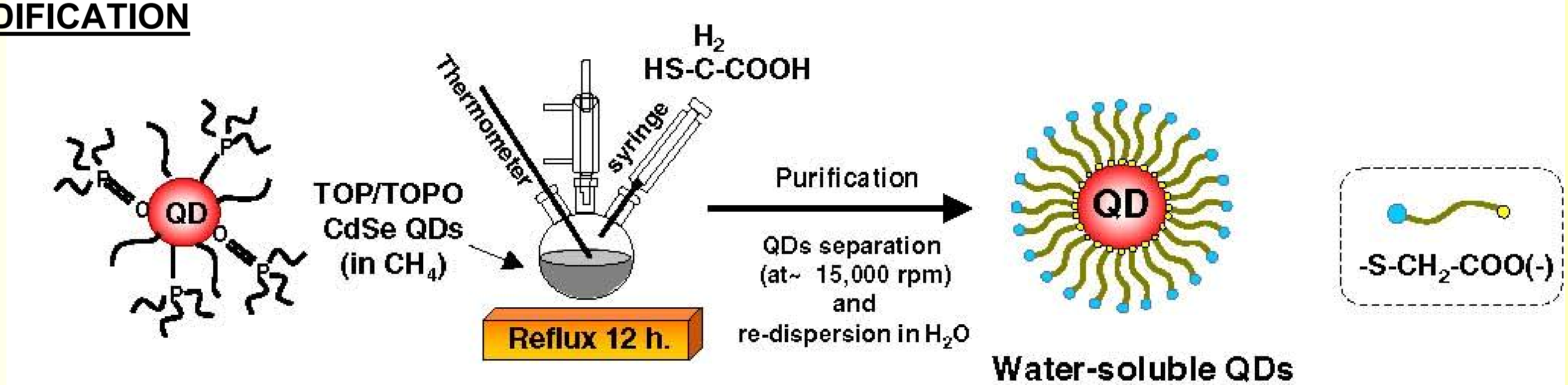
The diameter sizes of the nanoparticles are shown over the fluorescence spectrum.

Ref.: J. M. Costa-Fernandez et al., Trends Anal. Chem. 25, 2006, 207.

(a) NANOPARTICLE SYNTHESIS



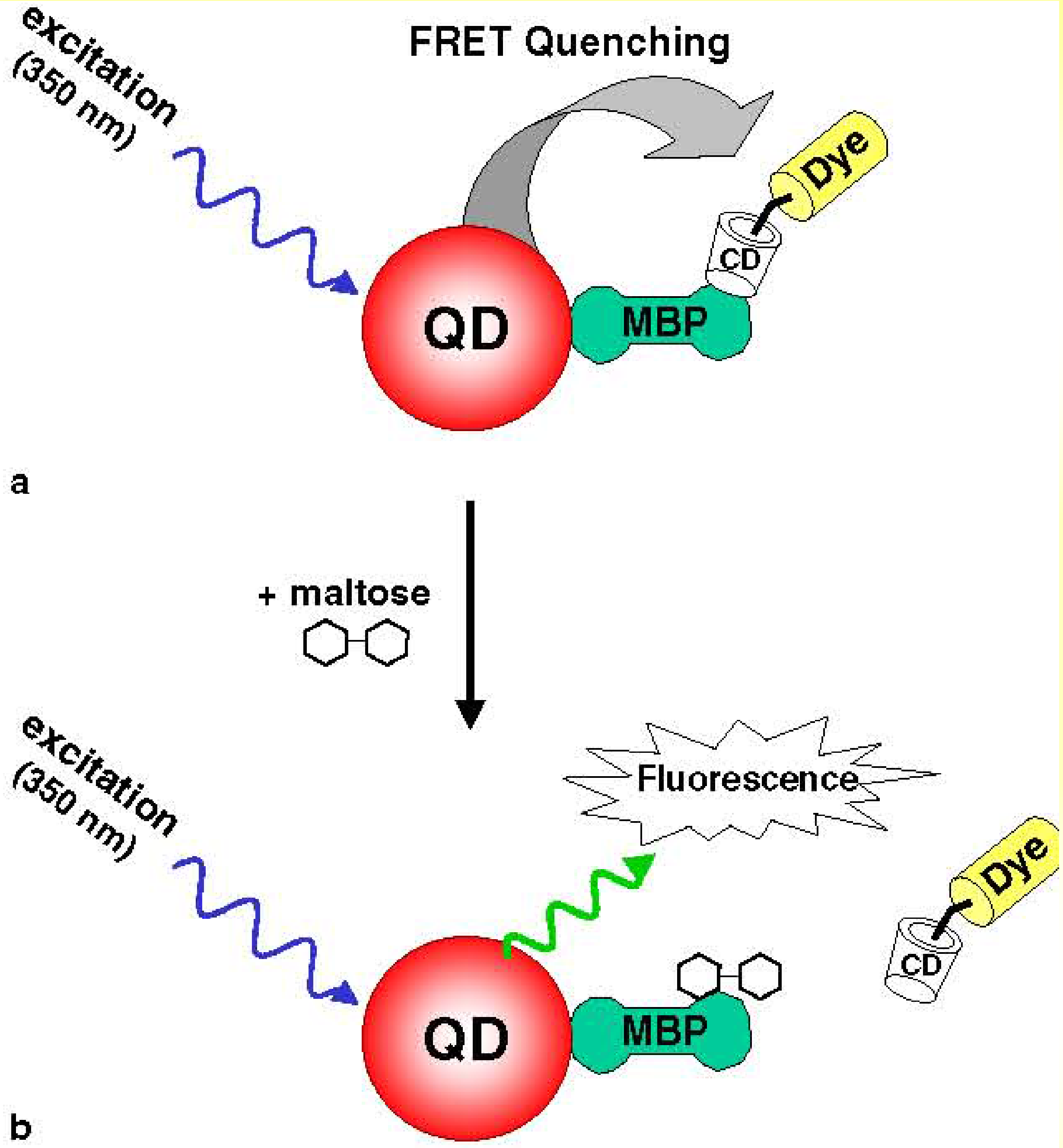
(b) SURFACE MODIFICATION



Schematic illustration of a typical synthesis process and surface-modification of a luminescent QD based on the use of CdS as precursor.

TOP: trioctylphosphine; TOPO: trioctylphosphine oxide; HPA: hexylphosphonic acid.

Ref.: J. M. Costa-Fernandez et al., Trends Anal. Chem., 25, 2006, 207.



Schematic diagram of the quantum-dot based FRET maltose sensor.

QDs conjugated to around 10 maltose-binding proteins (MBP) function as the FRET donors. Non-fluorescent dyes bound to a cyclodextrin serve as the acceptors and in the absence of maltose are filling the protein binding sites resulting in a quenching of the luminescence. When maltose is present, it removes the cyclodextrin (CD)-dye complex and the fluorescence is recovered.

Ref.: J. M. Costa-Fernandez et al., Trends Anal. Chem., 25, 2006, 207

Mikro/nano elektródy

Výhody:

- Ustálený difúzny tok vysokej hustoty
- Malá kapacita elektrickej dvojvrstvy
- Malé množstvo indiferentného elektrolytu
- Vysoký pomer signálu a šumu

Nevýhoda:

- Malá hodnota prúdu

$\Rightarrow$ Zoskupenie do súboru IDA (interdigitated array)

- Zberná účinnosť
- Redoxné cyklovanie

Fabrication of Nanopatterned Electrodes

Y. H. Lanyon and D. W. M. Arrigan,

Tyndall National Institute, Lee Maltings, University College, Cork, Ireland

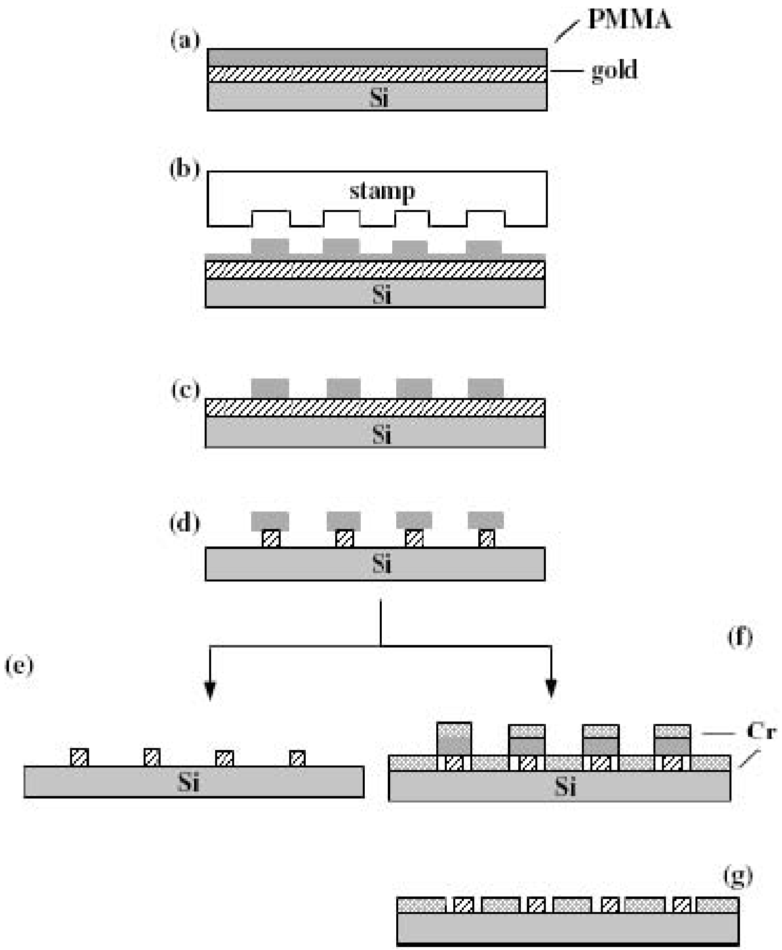
The development of ultrasmall electrodes for various applications, including sensing and electroanalysis, is of interest because of the enhanced sensitivity which is obtained (enhanced mass transport) together with the opportunity to place more nanoscale devices within a sensor array footprint.

Furthermore, the drive to emulate the biological nanopores used in nature for transmembrane ion transport provides challenges in device construction.

Ref.: Eftekhari (Ed.): Nanostructured Materials in Electrochemistry, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, 2007.

Lithographic methods such as electron-beam, focused ion beam and nanoimprint lithography for the preparation of individual nanoelectrodes or nanopores as well as arrays of these are reviewed.

The fabrication of nanoelectrodes below the 10 nm scale is increasingly more difficult to prepare reproducibly, hence the fabrication of nanogap electrodes has become a promising alternative in approaching the dimensions of the single molecules they are designed to detect.



Use of negative nanoimprint lithography (N-NIL) for preparation of nanoelectrodes

(a) silicon substrate precoated with a metal film and a thin poly-methylmethacrylate (PMMA) film is spin-coated on the metal;
(b) after imprinting, the stamp pattern is replicated in the PMMA film;
(c) residual PMMA in the trenches is removed by reactive ion etching;
(d) the exposed metal film is dissolved with wet etchant;
(e) metallic nanostructures after lift-off of residual PMMA;
(f) a second metal is deposited using a PMMA pattern as a mask;
(g) the bimetallic nanostructures which result, after removing PMMA.

Ref.: L. Jiao et al., Nanotechnology, **2005**, *16*, 2779-2784.

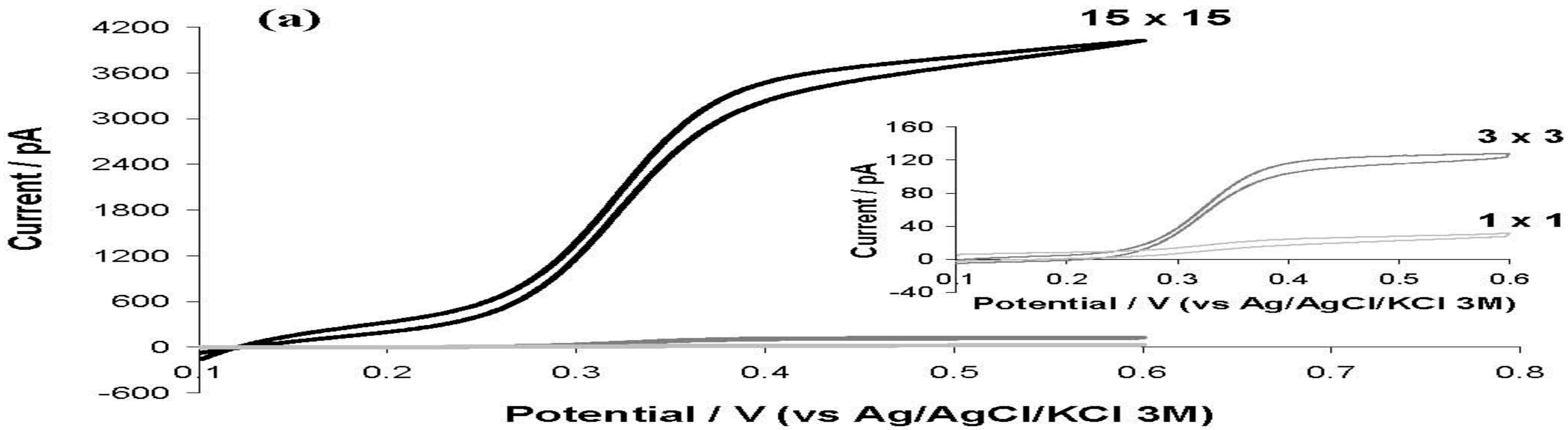
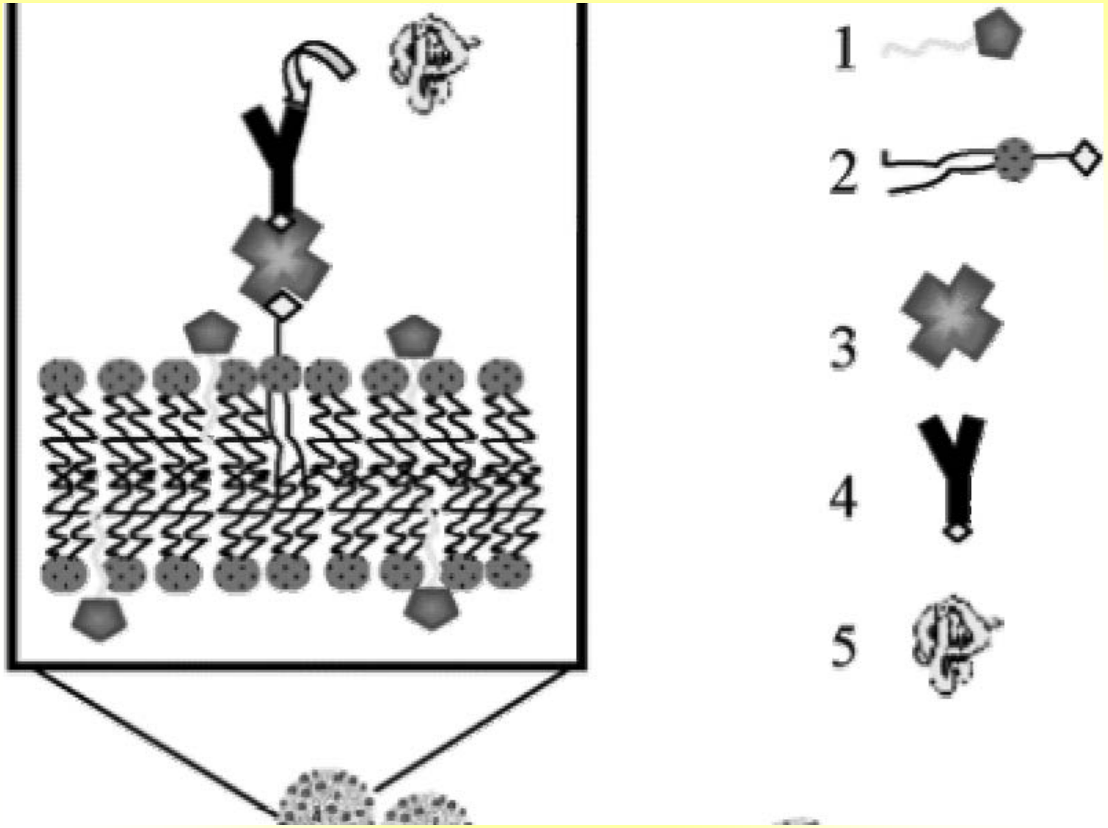


Figure 7. Voltammetric characterisation of selected nanopore electrode arrays using 1 mM FcCOOH in PBS. The influence of increasing nanoelectrode numbers on the CV response at 5 mV s^{-1} based on arrays with pore radii of 225 nm. Reprinted with permission from *Analytical Chemistry*, **2007**, DOI 10.1021/ac061878x. Copyright 2007 American Chemical Society.



Nanoelectrode-based approach to functional lipid vesicles (FLV) immunosensing.

(1) N-(10,12-pentacoasdiynoic) acetylferrocene (Fc-PDA); (2) 1,2-dioleoylphosphatidyl-ethanolamine-N-caproly-amine (Cap-PE); (3) streptavidin;
(4) biotinylated capture antibody; (5) target protein.

Ref.: Langmuir **2005**, *21*, 6025-6029.

Nanotechnology: Assessing the Risk

Nanotechnology is seen as a transformative technology, which has the potential to stimulate scientific innovation while greatly benefiting society.

However, the enthusiasm with which the scientific and technical communities are embracing the technology is being tempered by concerns over possible downsides, including risks to human health.

“Are these concerns valid?” is a question being asked by many, but frequently from differing perspectives. Given the increasingly complex interface between nanotechnology and society, relevant answers will be built on solid science and framed within a societal context.

Ref.: Andrew D. Maynard, Nanotoday 1, 2006

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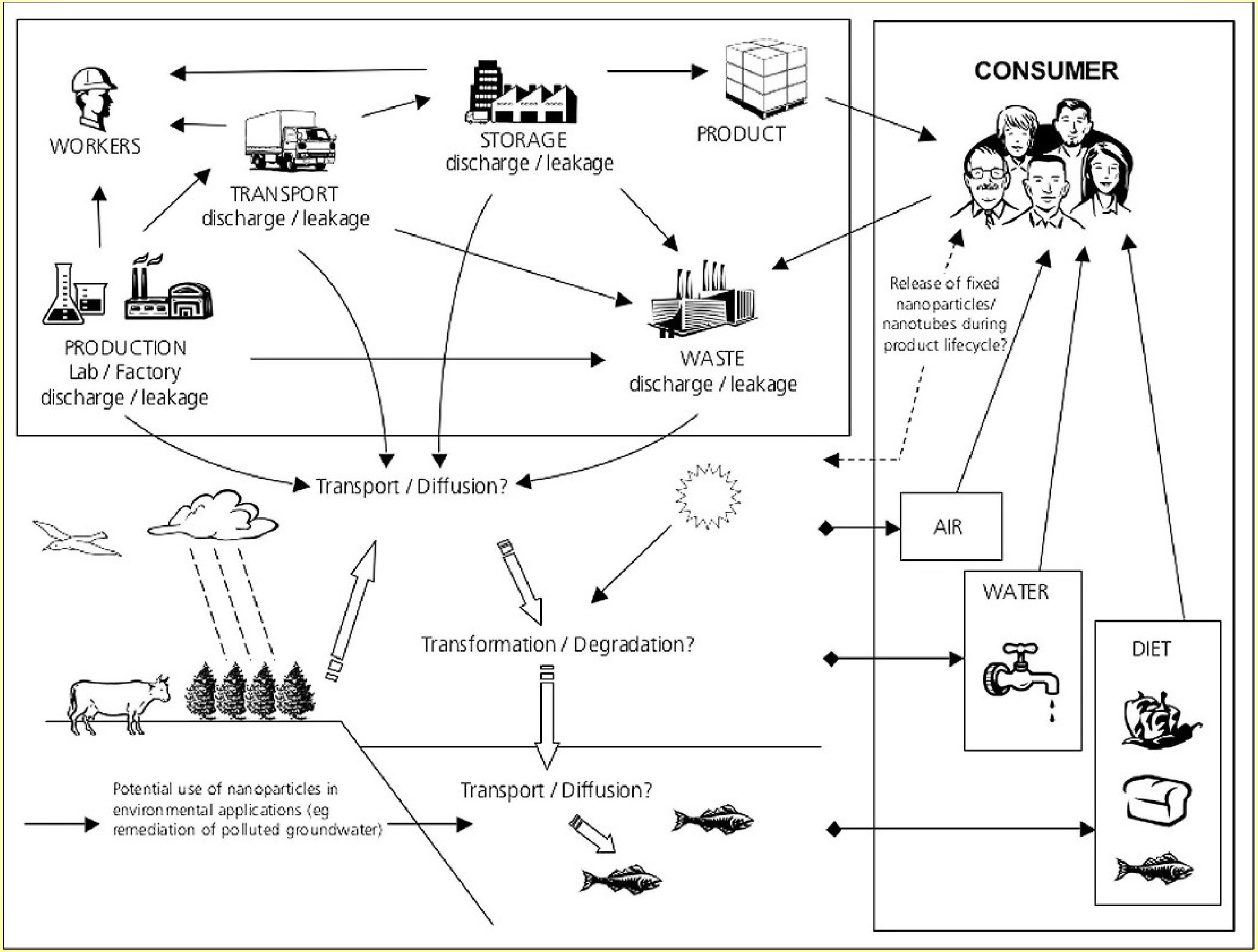
Toxicology of carbon nanomaterials: Status, trends, and perspectives on the special issue

This lead article draws from the ten contributed articles to discuss overall status, trends, and research needs in this new field. Common themes in the contributed articles are highlighted and a special appeal is made for collaboration between toxicologists and materials scientists in the joint development of "green" nanomaterial formulations - those cooptimized for function and minimal health impact.

Ref.: Robert H. Hurt, Marc Monthioux, Agnes Kane, Carbon 44 (2006) 1028–1033

Cesty, ktorými môžu nanočastice vplývať na ľudský organizmus



Účinok SWNT (single wall nanotubes) na respiračný systém

Warheit D.B.: Nanoparticles: Health impacts? Materialstoday 2 (2004) 32-35

- Suspenzia nanočastíc bola vpravená do pľúc myší a potkanov cez tracheu. Dávka 5 mg/kg spôsobila smrť zvierat.
- SWNT vytvorili agregáty, ktoré blokovali respiračný trakt.

Renwick L.C., Brown A., Clouter A., Donaldson K.: Increased inflammation and altered macrophage chemotactic responses caused by two ultrafine particle types. Occup. Environ. Med. 61 (2004) 442-447

- SWNT spôsobili zápal pľúc potkanov.
- Záviselo však od veľkosti samotných nanočastíc ako aj od spôsobu podávania. U potkanov, ktoré inhalovali SWNT samovoľne nebolo výrazne dokázané zhoršenie zdravotného stavu.

Účinok SWNT na pokožku

Shvedova A.A., Castranova V., Kisin E., et al.: Exposure to carbon nanotube material: Assessment of nanotube cytotoxicity using human keratynocyte cells, J. Toxicol. Environ. Health Part A 66 (2003) 1909-1926

- SWNT vyvolali zápal kožných buniek v kultúre *in vitro.*
- Tieto závery však nie je možné aplikovať in vivo, pretože SWNT sa nedostanú ku kožným bunkám (keratinocytom). Nie sú schopné prekonať bariéru, ktorá je tvorená odumretými bunkami na povrchu kože.

Smart S.K., Cassady A.I., LU G.Q., Martin D.J.: The biocompatibility of carbon nanotubes, Carbon 44 (2006) 1034-1047

- Nepurifikované CNT spôsobovali určitý stupeň toxicity *in vivo* aj *in vitro.*
- CNT v prítomnosti kovov tak isto indukovali určitý stupeň poškodenia.
- Purifikované CNT aj pri vyššej koncentrácii nespôsobovali poškodenie *in vivo* ani *in vitro.*

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Carbon nanotubes as nanomedicines: From toxicology to pharmacology

L. Lacerda, A. Bianco, M. Prato, K. Kostarelos

Most of these applications will involve the administration or implantation of carbon nanotubes and their matrices into patients. The toxicological and pharmacological profile of such carbon nanotube systems developed as nanomedicines will have to be determined prior to any clinical studies undertaken.

Ref.: Advanced Drug Delivery Reviews 58(2006)1460– 1470

Applications of carbon nanotubes in drug delivery

A. Bianco, K. Kostarelos and M. Prato

Carbon nanotubes (CNT) have emerged as a new alternative and efficient tool for transporting and translocating therapeutic molecules. CNT can be functionalised with bioactive peptides, proteins, nucleic acids and drugs, and used to deliver their cargostocells and organs. Because functionalised CNT display low toxicity and are not immunogenic, such systems hold great potential in the field of nanobiotechnology and nanomedicine.

Ref.: Current Opinion in Chemical Biology 9 (2005) 674 – 679

Targeted nanoparticles for cancer therapy

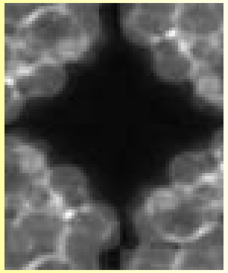
Omid C. Farokhzad *et al.*

The increasing interest in using nanoparticles for cancer therapy has led to the development of smart targeted nanoparticles. These can deliver drugs at a sustained rate directly to cancer cells and may provide improved efficacy and lower toxicity.

Ref.: Nanotoday 2, 14 (2007)

Magnetic nanoparticles for drug delivery

Jesús Santamaría *et al.*



Magnetic nanoparticles show potential for drug delivery by combining a magnetic core and a suitable coating for drug loading. This review looks at the problems and recent advances in their development.

Ref.: Nanotoday 2, 22 (2007)

Záver: Trendy vývoja

Nanomateriály / nanoštruktúry

- CNT: závislosti štruktúra - topológia - vlastnosť
- fyzikálna a chemická modifikácia CNT
- samo-organizácia CNT s molekulami DNA
- nové uhlíkové materiály
- nové anorganické nanorúrky (Au, MoS_2, WS_2, ZnO, ZrO_2, WO_3, Fe_7S_8, etc.) a nanodrôty (Ga_2O_3, Si_3N_4, atď.)
- nanočastice (Au, kovové clustery, magnetické Fe oxidy)
- porézny silikagél - porous silicon (schopný emisie svetla)

Nanoprípravy

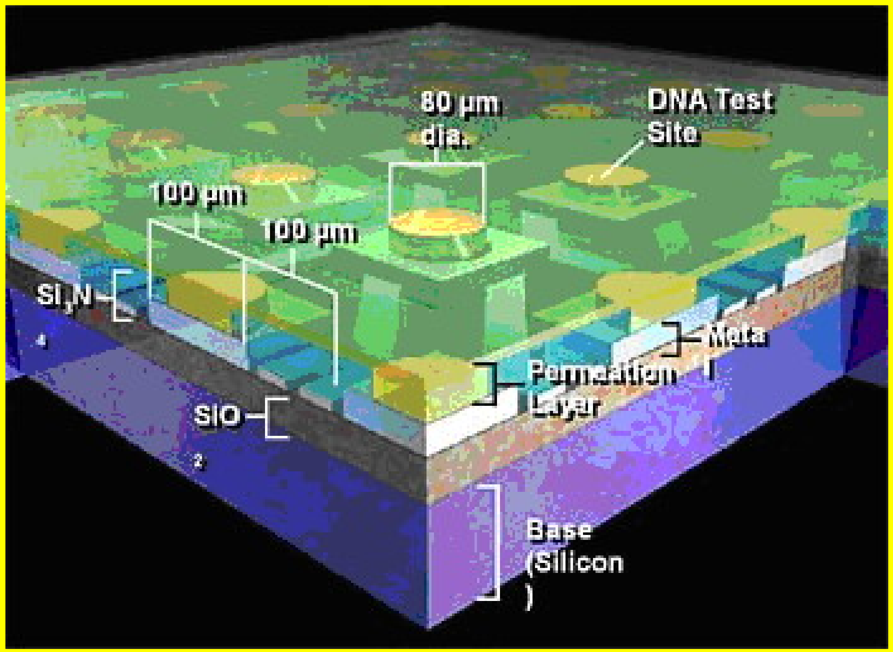
(fotolitografia, vylúčenie tenkých filmov, tvorba nanodrôtov, atď.)

- interdigitované elektródové súbory (arrays) v nano-škále
- cantilewers, súbory cantilewers
- intergrácia v rámci biočipov
- DNA súbory založené na nanočasticiach,
- DNA ako templát pre spontánne súbory (nanodrôty)
    - klastre kovových častíc
    - vodivé polyméry (polyanilín)
    - polovodivé nanokryštály

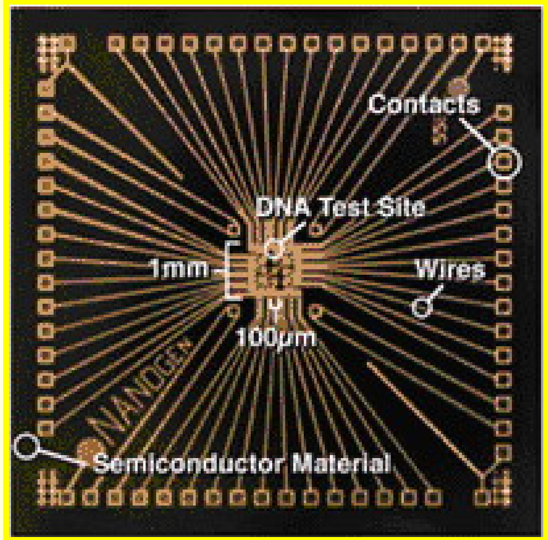
Aplikácie

- bezreagentové (bio)senzory s ultranízkym DL (hybridizácia DNA)
- merania v individuálnych bunkách, “drug delivery”

"Lab-on-chips"



Nanogénový polovodič testujúci DNA



Nanogénová prestupová vrstva je rozhranie medzi povrchom mikročipu a biologickým prostredím

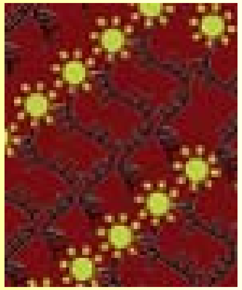
Miniaturizácia objemného laboratórneho zariadenia a jeho integrácia do jednotlivého mikročipu.

Nanogén zahŕňa elektronickú separáciu buniek, elektronický transport vzorky, elektronicky urýchlenú hybridizáciu DNA a elektronickú denaturáciu DNA.

Využíva sa na určenie množstva oligonukleotidových hybridov s rôznymi väzbovými silami (vrátane úplného porovnávania a zistenia jednobázových nezhôd) a na vylúčenie nehybridizovanej DNA.

DNA-based nanodevices

Tim Liedl, Thomas L. Sobey, and Friedrich C. Simmel



DNA plays an increasingly important role as a building block for nanoscale materials and devices. Possible applications of DNA-based nanodevices include nanoscale motors and actuators, and novel biosensors.

Ref.: Nanotoday 2, 36 (2007)

Functional devices from DNA and proteins

Christof M. Niemeyer

Natural proteins and synthetic nucleic acid building blocks can be combined to produce nanoscale functional devices. These semisynthetic DNA-protein conjugates can be used in the biofunctionalization of planar surfaces for micro- and nanoarray technologies.

Ref.: Nanotoday 2, 42 (2007)