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INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Název: Isolation and detection of influenza Haemagglutinin labelled by quantum dots CdS a CdTe

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Reg.č.projektu: CZ.1.07/2.4.00/31.0023

Název projektu: Partnerská síť centra excelentního bionanotechnologického výzkumu

NanoBioMetalNet

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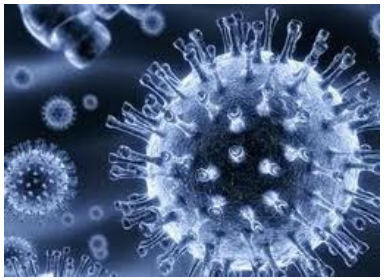
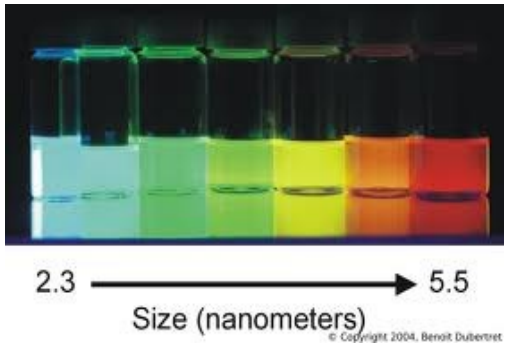


- Preparation of CdS an CdTe quantum dots
- Labelling of HA by CdS and CdTe QDs
- Isolation of QDs labelled HA using MPs
- Electrochemical detection of HA-QDs complex
- Conclusion

http://en.wikipedia.org/wiki/Image:EM_of_influenza_virus.jpg

Preparation of CdS an CdTe quantum dots



QDs

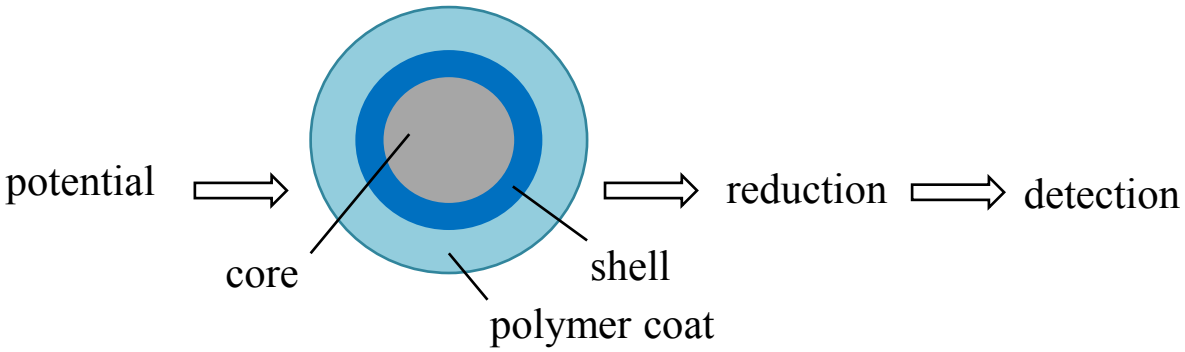
- nanoparticles, size range 2–10 nm
- three basic part (core, shell, polymer coat)
- cadmium sulfid CdS and cadmium teluride (CdTe)

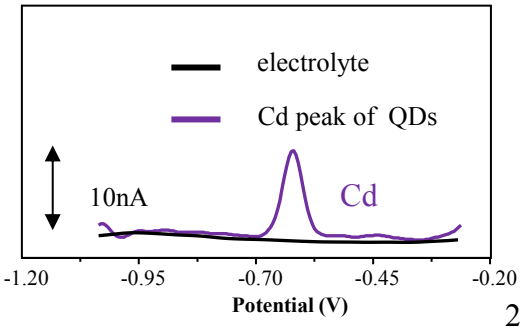
CdS QDs preparation:

- $Cd(NO_3)_2 \cdot 4H_2O$ (0.1 mM) was dissolved in ACS water
- 3-mercaptopropionic acid was slowly added
- $Na_2S \cdot 9H_2O$ in ACS water was poured (stirring)
- yellow solution was stirred for 1 h
- prepared CdS QDs were stored in the dark at 4 ºC

CdTe QDs preparation:

- $Cd(OAc)_2 \cdot 2H_2O$ was dissolved in ACS water and trisodium citrate dihydrate was added
- Na_2TeO_3 was poured into the first solution followed by 3-mercaptopropionic acid
- $NaBH_4$ was added with vigorous stirring
- after 30 min of stirring 2 ml of solution was heated in glass vial in Multiwave Microwave reaction conditions: power 300 W, 120 ºC and time 18 min
- CdTe QDs were stored in dark at 4 ºC



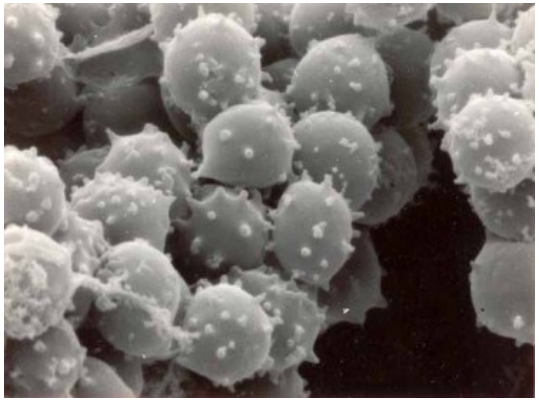


Labelling of vaccine HA by QDs (CdTe and CdS)

- Vaxigrip ®
- inactivated and split virions
- strands: A/California/7/2009 (H1N1), A/Perth/16/2009 (H3N2) and B/Brisbane/60/2008.
- Strains was propagated in fertilised hens´eggs
- Vaxigrip contain 15 micrograms of all of three HA per 0.5ml

- Vaxigrip® (500 µl, 45µg HA) was reduced (filter device – Amicon Ultra 3K)
- mixed with a QDs solution (shaken for 24 h)
- the volume of solution was reduced to 100 µl (Amicon Ultra 3k)
- sample was diluted to 1 ml by ACS water, used for measurements

Isolation of QDs labelled HA using MPs

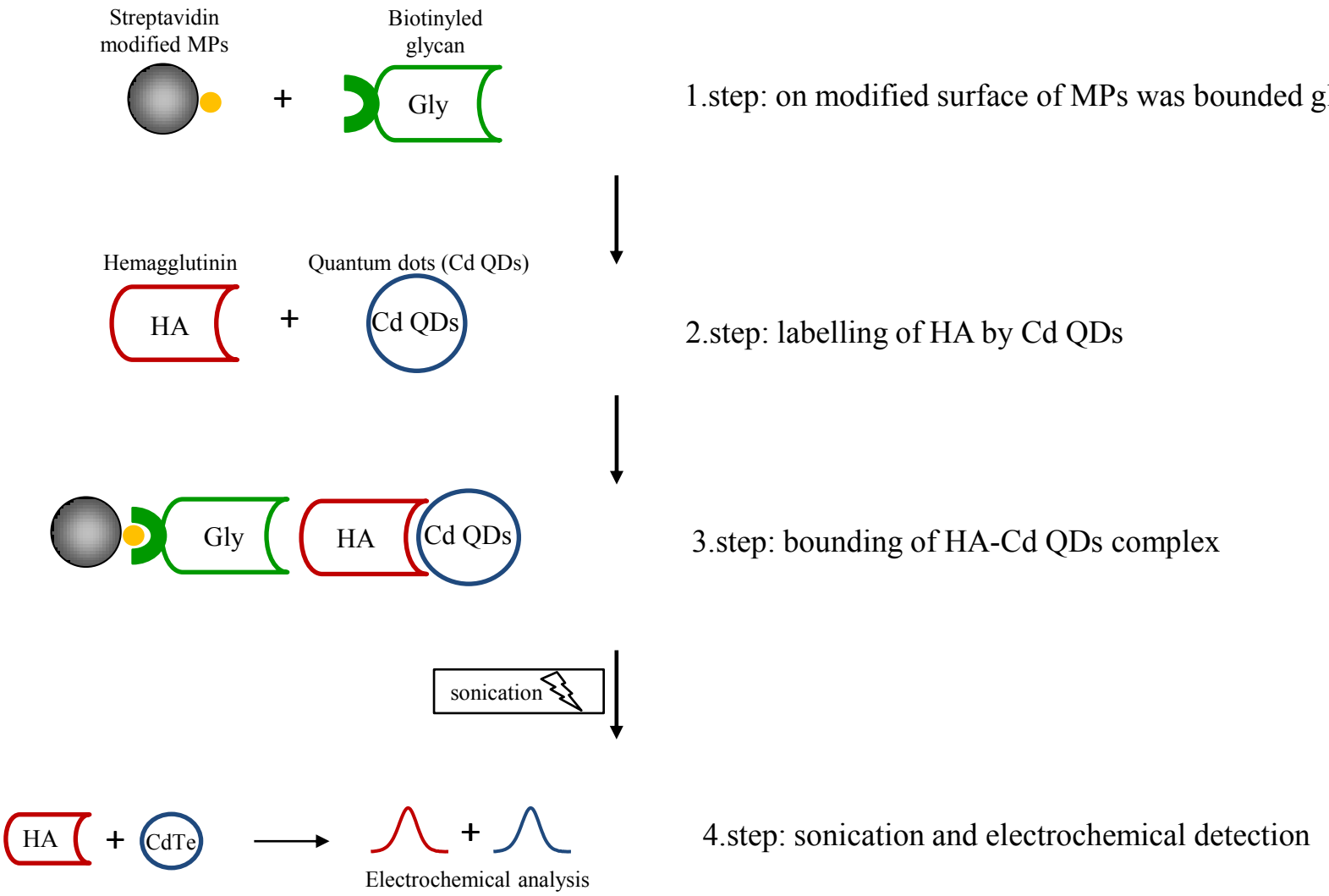


Magnetic particles (MPs)

- Dynabeads® Streptavidine
- Streptavidine modification for catchig of biotinylated glycan

- mRNA poly T modified DNA capture on mobile magnetic beads
- Rapid and gentle magnetic handling procedures
- No mRNA/poly T DNA lost during high g-force spins
- No mRNA/poly T DNA trapped in column membranes during elution

Isolation of QDs labelled HA using MPs - protocol



Electrochemical detection of isolated complex HA-Cd QDs

Detection of HA

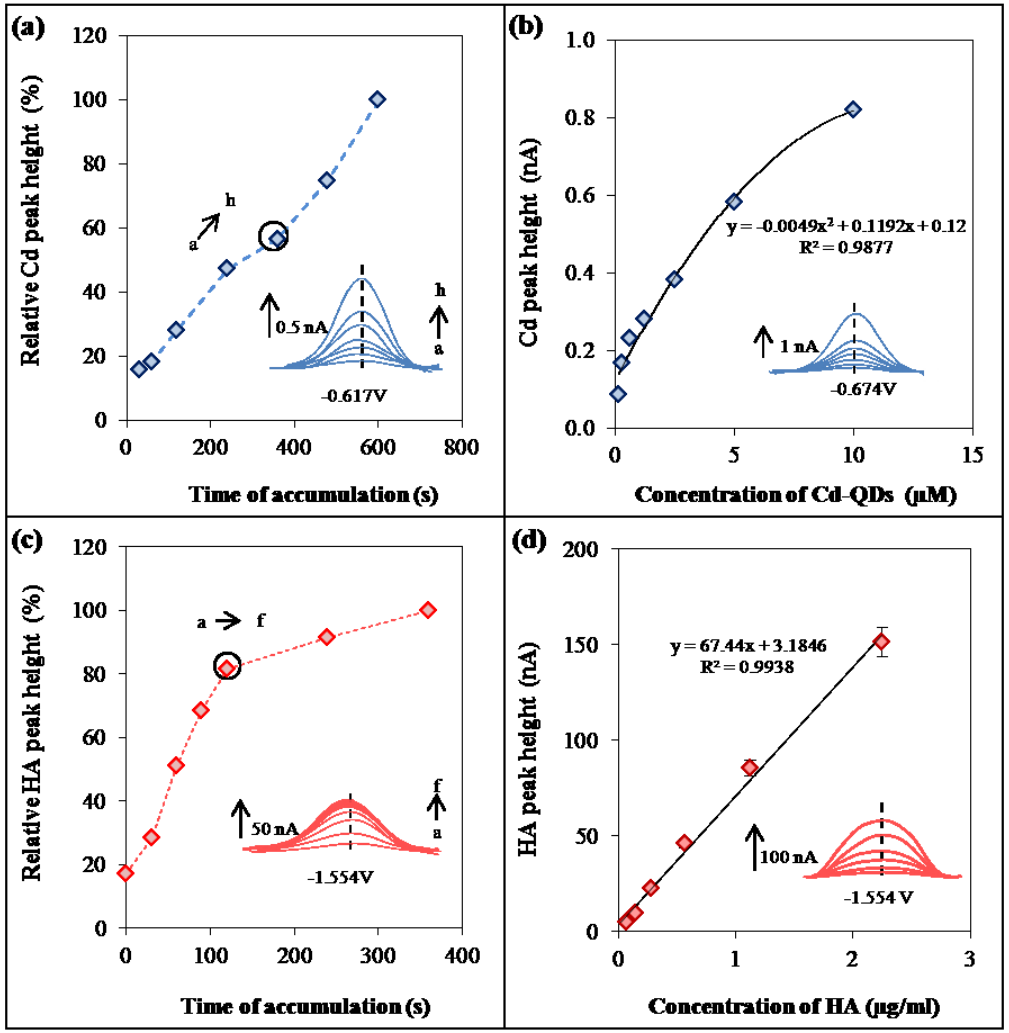
- AdT DPV was used
- Brdicka bufer was used as the bacground electrolyte
- Parameters were folows:

purge time 30 s, initial potential -0.7 V; end potential -1.8 V; potential step 0.002 V; amplitude 0.025 V.

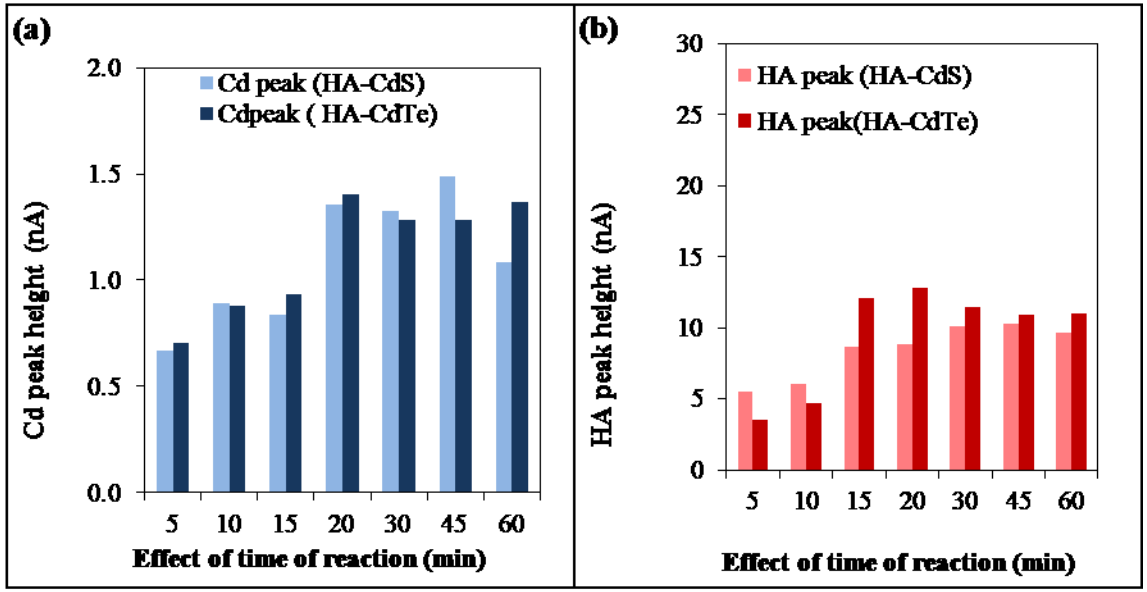
Detection of Cd (from CdTe and CdS QDs)

- ASV DPV was used
- Aceate buffer pH 5.0 was used as the bacground electrolyte
- Parameters were folows:

initial potential -0.8 V; end potential -0.5 V ; deposition potential -0.8 V ; equilibration time 5 s; modulation time 0.06 s; time interval 0.2 s; potential step 0.002 V; modulation amplitude 0.025 V.



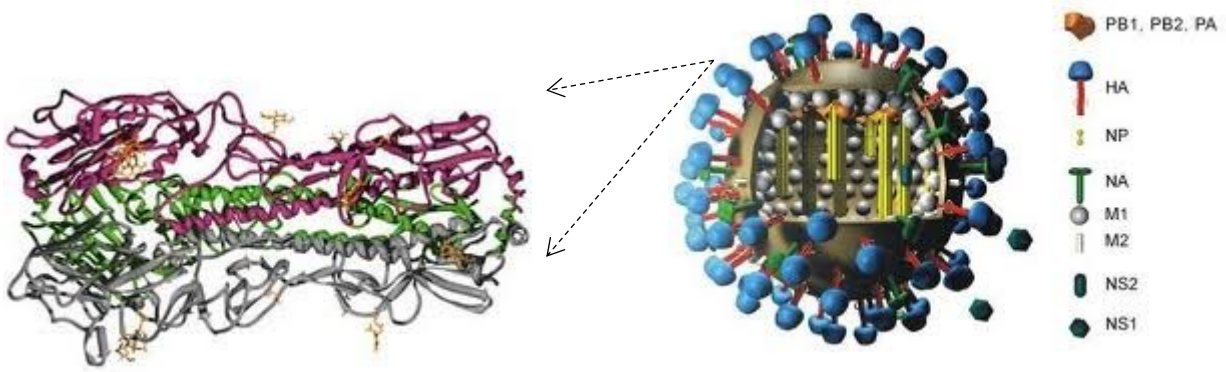
Characterization of HA-QDs complex by electrochemical analysis. **(a)** + **(b)** Characterization of metal part of HA-QDs complex. **(a)** Dependence of Cd peak height on time of accumulationon(s) of HA-QDs complex, **(light colour)** HA-CdS and **(dark colour)** HA-CdTe. **(b)** Dependence of Cd peak height on concentrtion of QDs (µM) in HA-QDs complex: **(light colour)** HA-CdS and **(dark colour)** HA-CdTe. **(c)** + **(d)** Characterization of HA peak from HA-QDs complex. **(c)** Dependence of HA peak height on time of accumulationon (s) of HA-QDs complex, **(light colour)** HA-CdS and **(dark colour)** HA-CdTe. **(d)** Dependence of HA peak height on HA (µg/ml) from HA-QDs concentrtion : **(light colour)** HA-CdS and **(dark colour)** HA-CdTe.

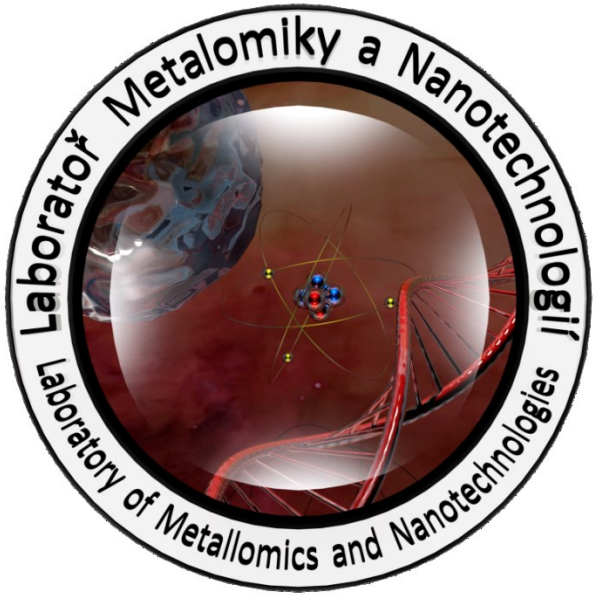


Optimization of time of reaction (binding HA-QDs complex to MPs modified by glycan). Effect of timeof reaction (min) was detected by electrochemical analysis of Cd peak **(a)** and HA peak **(b)**.
(a) Dependence of Cd peak height (nA) on time of reaction (min) and binding of HA-QDs on glycan. All measurements of Cd peak was used ASV DPV.
(b) Dependence of HA peak height (nA) on time of reaction (min) . For all measurements was used AdT DPV.

Conclusion

- Method for isolation and detection of influenza haemagglutinine was designed
- Two different QDs were fabricated and influenza HA was labelled by them
- Complex HA-Cd QDs was isolated by glycan conjugated MPs
- Isolated complex was detected by two different voltammetry methods





Acknowledgements

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Thank you for your attention

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