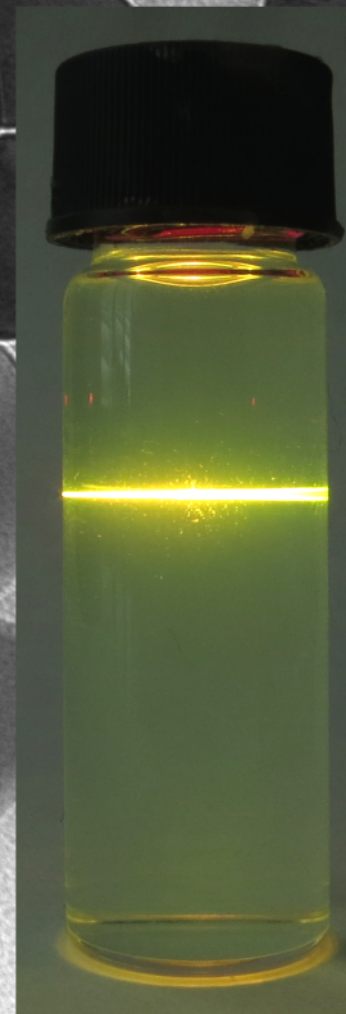


Příprava diskrétních biokonjugátů photon-upconversion nanočástic

LD15023 COST CZ MŠMT ČR

Antonín Hlaváček

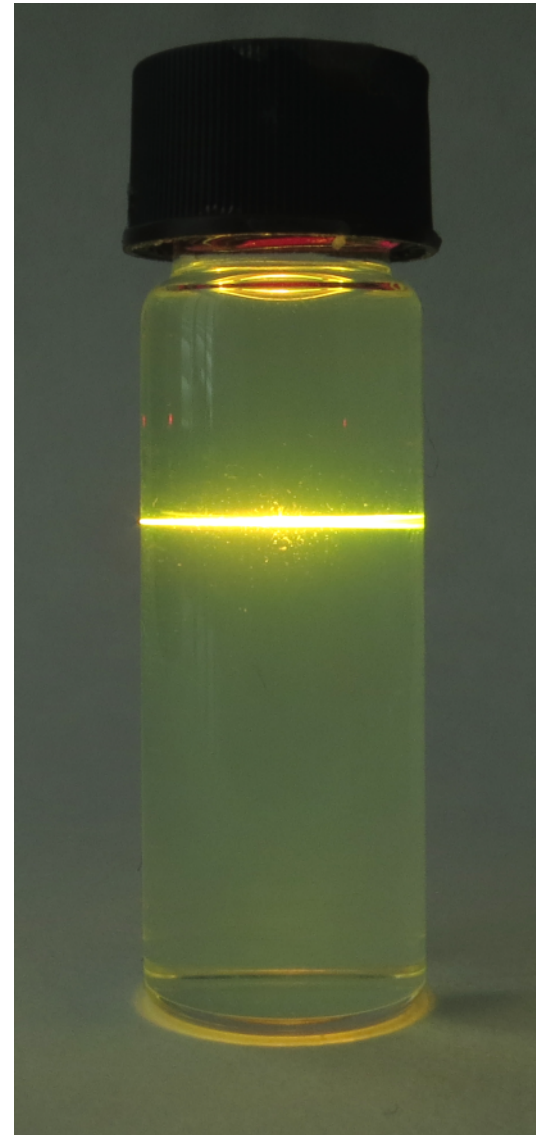
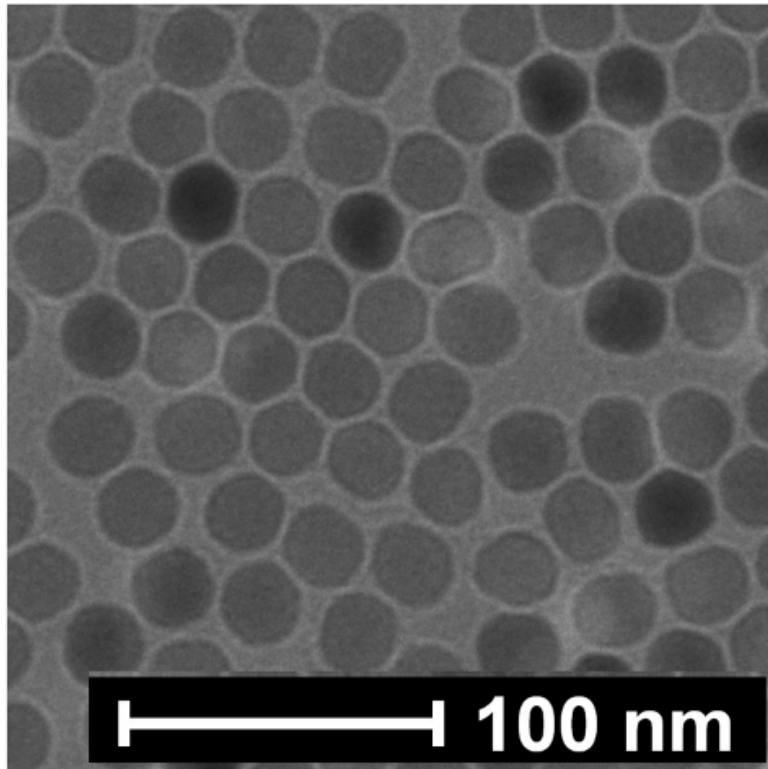
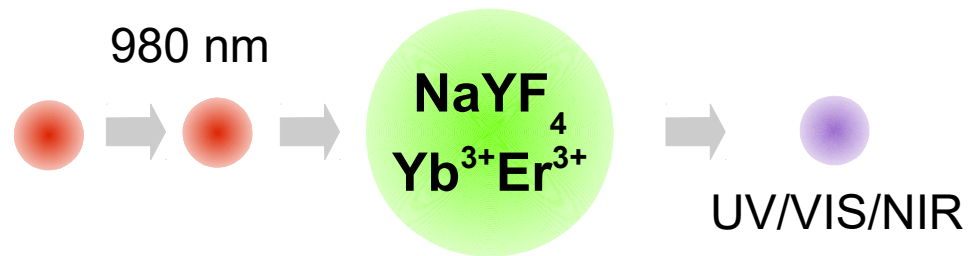
100 nm



Obsah

- Prezentace řešení
 - Foton up-konverzní nanočástice (UCNP)
 - Diskrétní biokonjugáty UCNP
 - Využití pro imunochemické detekce
- Oponentní posudky
- Zápis, podpisy..

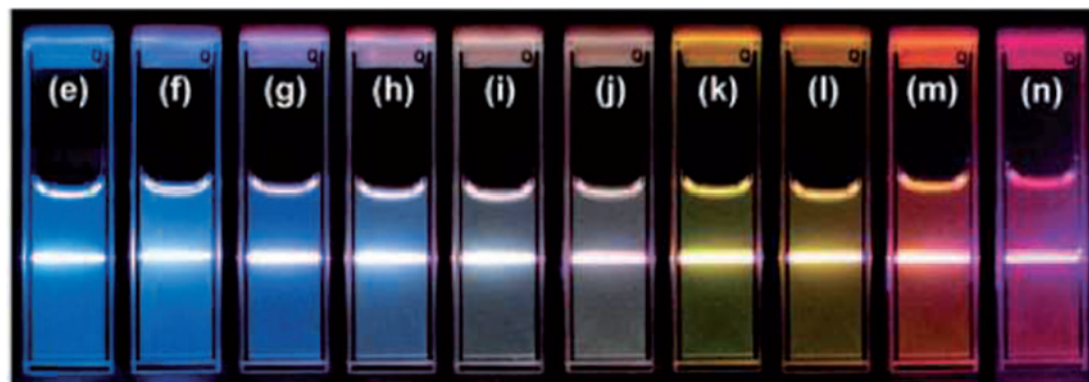
Foton up-konverzní nanočástice



Foton up-konverzní nanočástice

$\text{YLiF}_4\text{:Yb,Tm}$
 $\text{NaYF}_4\text{:Yb,Er}$
 $\text{NaYF}_4\text{:Yb,Er}$
 $\text{NaYF}_4\text{:Yb,Er}$
 $\text{NaYF}_4\text{:Yb,Er}$
 $\text{NaYF}_4\text{:Yb,Er}$
 $\text{NaYF}_4\text{:Yb,Tm}$
 $\text{NaGdF}_4\text{:Yb,Er}$
 $\text{NaGdF}_4\text{:Yb,Tm}$
 NaGdF_4
 $\text{NaLuF}_4\text{:Yb,Er}$
 $\text{NaLuF}_4\text{:Gd,Yb,Tm}$
 BaLuF_5
 $\text{KGdF}_4\text{:Yb,Tm}$
 $\text{KYb}_2\text{F}_7\text{:Er}$
 $\text{LiLuF}_4\text{:Yb,Er}$
 $\text{KMnF}_3\text{:Yb,Er}$
 $\text{YOF}\text{:Yb,Er}$
 $\text{CaF}_2\text{:Yb,Er}$
 $\text{YF}_3\text{:Yb,Er}$

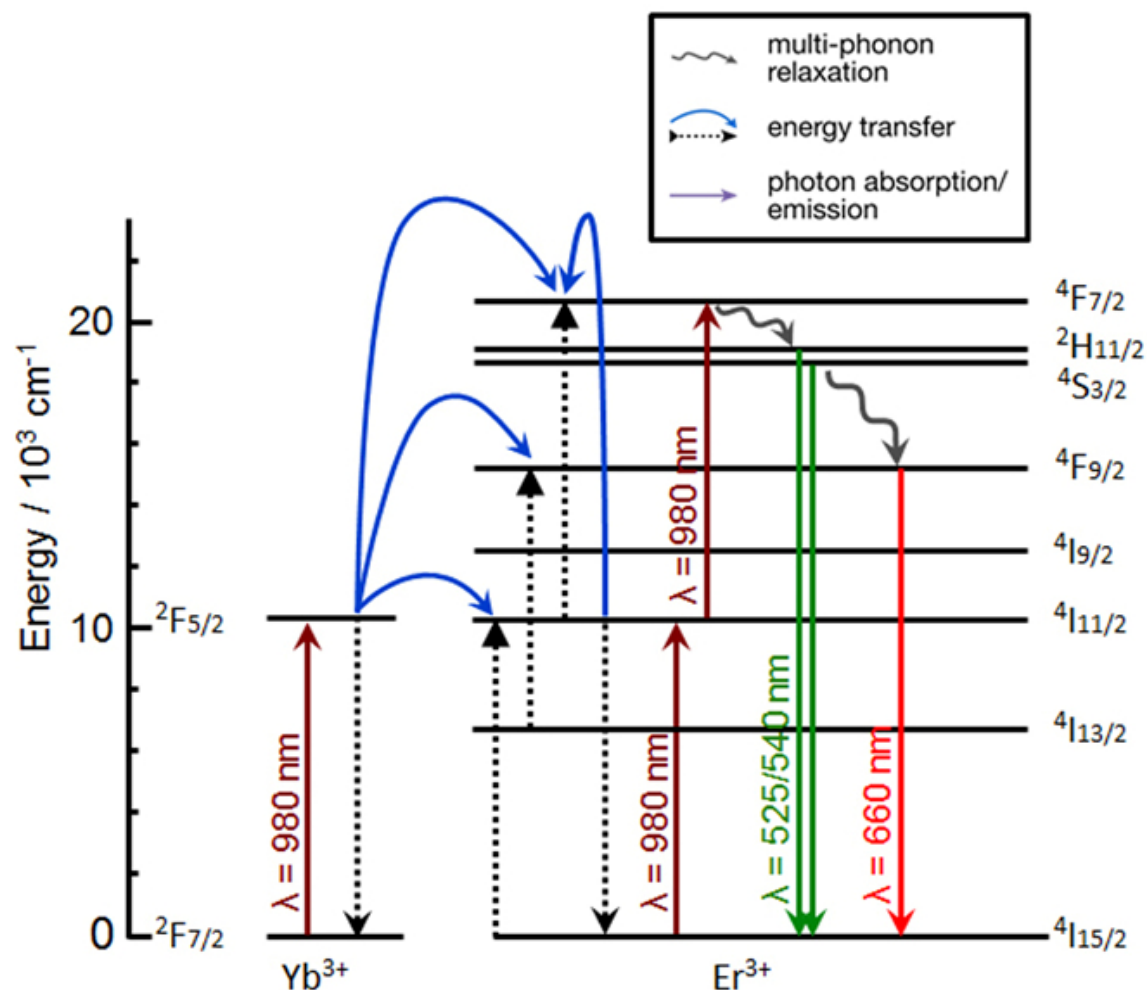
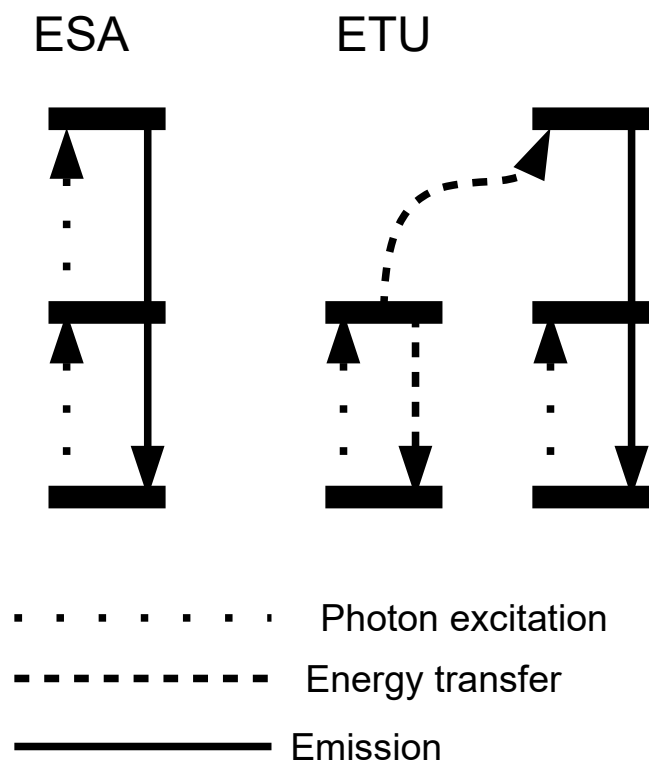
1 H Hydrogen 1.00794	2 He Helium 4.002602																	13 B Boron 10.811	14 C Carbon 12.011	15 N Nitrogen 14.0064	16 O Oxygen 15.9994	17 F Fluorine 18.9984	18 Ne Neon 20.1797												
3 Li Lithium 6.941	4 Be Beryllium 9.012182																	19 K Potassium 39.0983	20 Ca Calcium 40.078	21 Sc Scandium 44.95591	22 Ti Titanium 47.88	23 V Vanadium 50.9415	24 Cr Chromium 51.9961	25 Mn Manganese 54.938	26 Fe Iron 55.847	27 Co Cobalt 58.9332	28 Ni Nickel 58.6934	29 Cu Copper 63.546	30 Zn Zinc 65.39	31 Ga Gallium 69.723	32 Ge Germanium 72.64	33 As Arsenic 74.921595	34 Se Selenium 78.96	35 Br Bromine 79.904	36 Kr Krypton 83.80
11 Na Sodium 22.989768	12 Mg Magnesium 24.305	3 III 3B	4 IV 4B	5 V 5B	6 VI 6B	7 VII 7B	8 VIII	9 VIII	10 VIII	11 IB 1B	12 II 2B							49 In Indium 114.818	50 Sn Tin 118.71	51 Sb Antimony 121.760	52 Te Tellurium 127.6	53 I Iodine 126.90447	54 Xe Xenon 131.29												
19 K Potassium 39.0983	20 Ca Calcium 40.078	21 Sc Scandium 44.95591	22 Ti Titanium 47.88	23 V Vanadium 50.9415	24 Cr Chromium 51.9961	25 Mn Manganese 54.938	26 Fe Iron 55.847	27 Co Cobalt 58.9332	28 Ni Nickel 58.6934	29 Cu Copper 63.546	30 Zn Zinc 65.39	49 In Indium 114.818	50 Sn Tin 118.71	51 Sb Antimony 121.760	52 Te Tellurium 127.6	53 I Iodine 126.90447	54 Xe Xenon 131.29																		
37 Rb Rubidium 85.4678	38 Sr Strontium 87.62	39 Y Yttrium 88.90585	40 Zr Zirconium 91.224	41 Nb Niobium 92.90638	42 Mo Molybdenum 95.94	43 Tc Technetium 98.9072	44 Ru Ruthenium 101.07	45 Rh Rhodium 102.9055	46 Pd Palladium 106.42	47 Ag Silver 107.8662	48 Cd Cadmium 112.411	81 Tl Thallium 204.3833	82 Pb Lead 207.2	83 Bi Bismuth 208.98037	84 Po Polonium [209]	85 At Astatine [210]	86 Rn Radon [222]																		
55 Cs Cesium 132.90543	56 Ba Barium 137.327	57-71	72 Hf Hafnium 178.49	73 Ta Tantalum 180.9479	74 W Tungsten 183.85	75 Re Rhenium 186.207	76 Os Osmium 190.23	77 Ir Iridium 192.22	78 Pt Platinum 195.08	79 Au Gold 196.96655	80 Hg Mercury 200.59	113 Uut Ununtrium [288]	114 Uuq Ununquadium [289]	115 Uup Ununpentium [290]	116 Uuh Ununhexium [291]	117 Uus Ununseptium [292]	118 Uuo Ununoctium [293]																		
87 Fr Francium 223.017	88 Ra Radium 226.0254	89-103	104 Rf Rutherfordium [261]	105 Db Dubnium [262]	106 Sg Seaborgium [266]	107 Bh Bohrium [264]	108 Hs Hassium [265]	109 Mt Meitnerium [268]	110 Ds Darmstadtium [269]	111 Rg Roentgenium [270]	112 Cn Copernicium [277]																								
Lanthanide Series												57 La Lanthanum 138.9055	58 Ce Cerium 140.115	59 Pr Praseodymium 140.90768	60 Nd Neodymium 144.24	61 Pm Promethium 144.9127	62 Sm Samarium 150.36	63 Eu Europium 151.9654	64 Gd Gadolinium 157.25	65 Tb Terbium 158.92534	66 Dy Dysprosium 162.50	67 Ho Holmium 164.93032	68 Er Erbium 167.26	69 Tm Thulium 168.93421	70 Yb Ytterbium 173.04	71 Lu Lutetium 174.967									
Actinide Series												89 Ac Actinium 227.0277	90 Th Thorium 232.0377	91 Pa Protactinium 231.03688	92 U Uranium 238.02891	93 Np Neptunium 237.04817	94 Pu Plutonium 244.0642	95 Am Americium 243.06136	96 Cm Curium 247.07545	97 Bk Berkelium 247.07125	98 Cf Californium 251.07958	99 Es Einsteinium 252.0832	100 Fm Fermium 257.10	101 Md Mendelevium 258.10	102 No Nobelium 259.10	103 Lr Lawrencium 262.10									



F. Wang, X. G. Liu, J. Am. Chem. Soc. 2008, 130, 5642 – 5643.

Mechanismus fotonové up-konverze

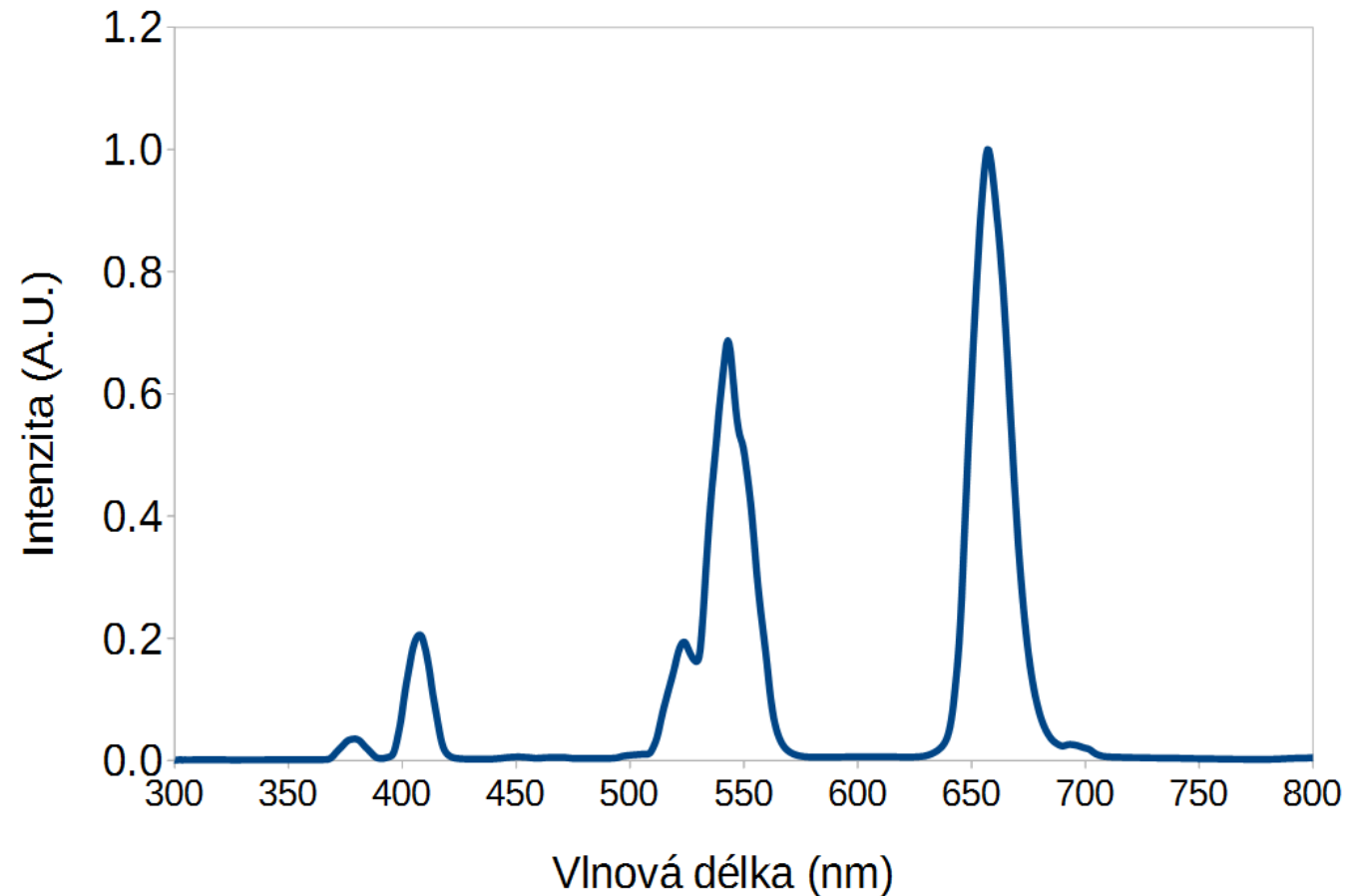
- ESA: excited-state absorption
- ETU: energy transfer upconversion



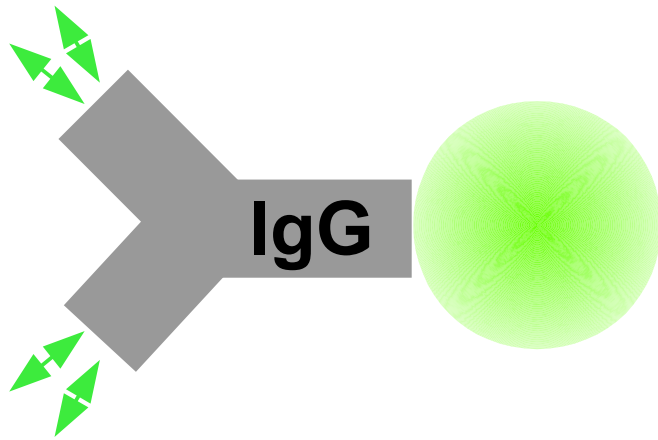
Energetické přechody v $\beta\text{-NaYF}_4$ nanokrystalech, které jsou dopované Yb^{3+} (sensitizer; obsah 20 %) a Er^{3+} (emitters; 2 %) ionty

Gargas DJ, Chan EM, Ostrowski AD, Aloni S, Altoe MVP, Barnard ES, Sanii B, Urban JJ, Milliron DJ, Cohen BE, Schuck PJ (2014) Nature Nanotechnology, DOI: 10.1038/NNANO.2014.29

Mechanismus fotonové up-konverze

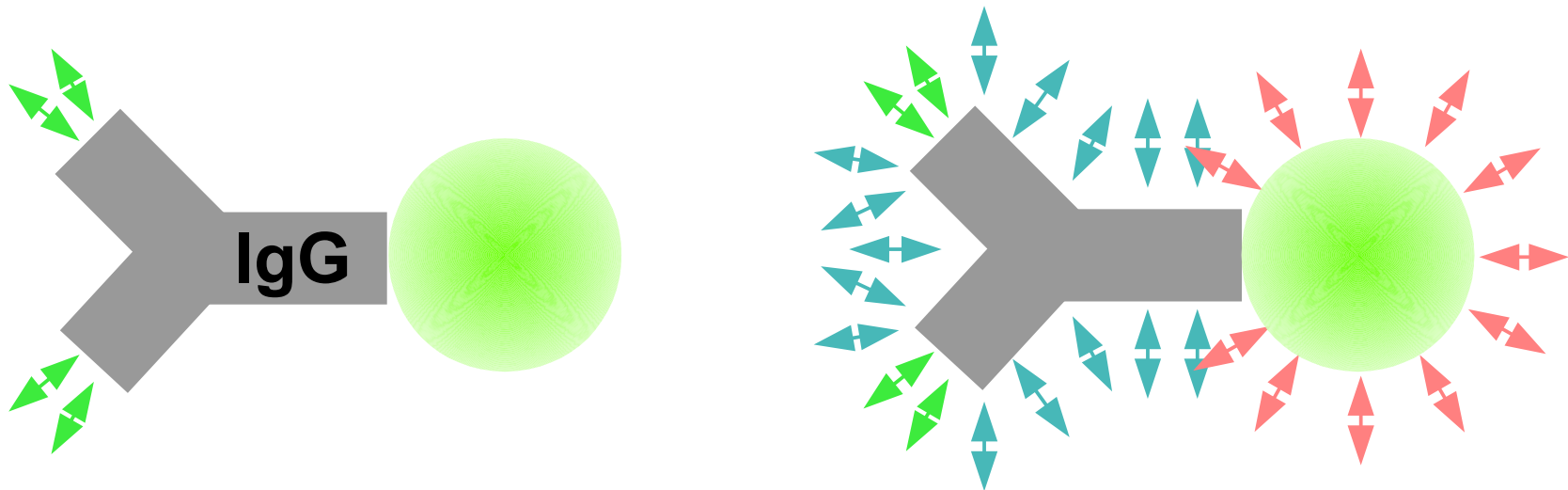


Biokonjugáty UCNP



 **Interactions**
Specific

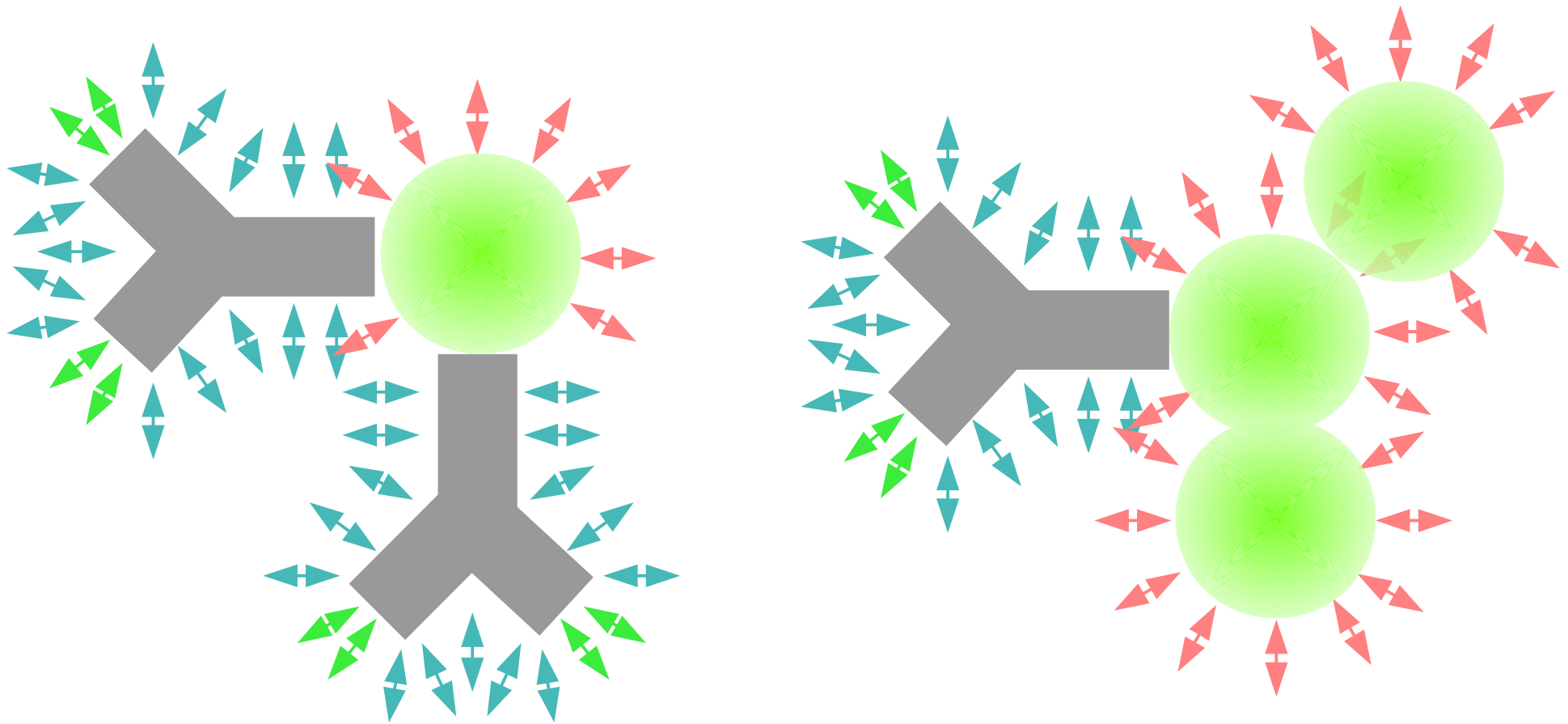
Biokonjugáty UCNP



Interactions

- Specific
- Biomolecule non-specific
- Nanoparticle non-specific

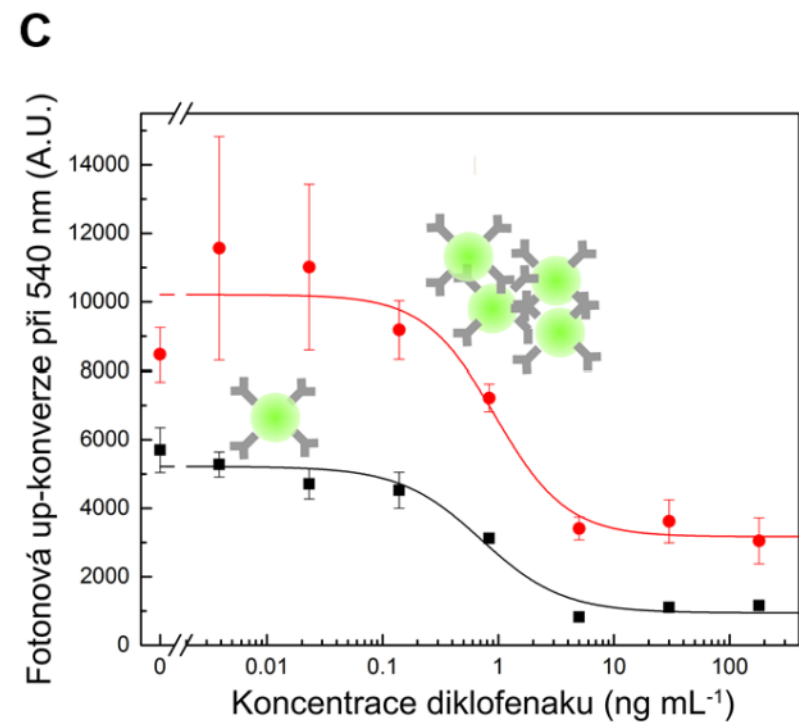
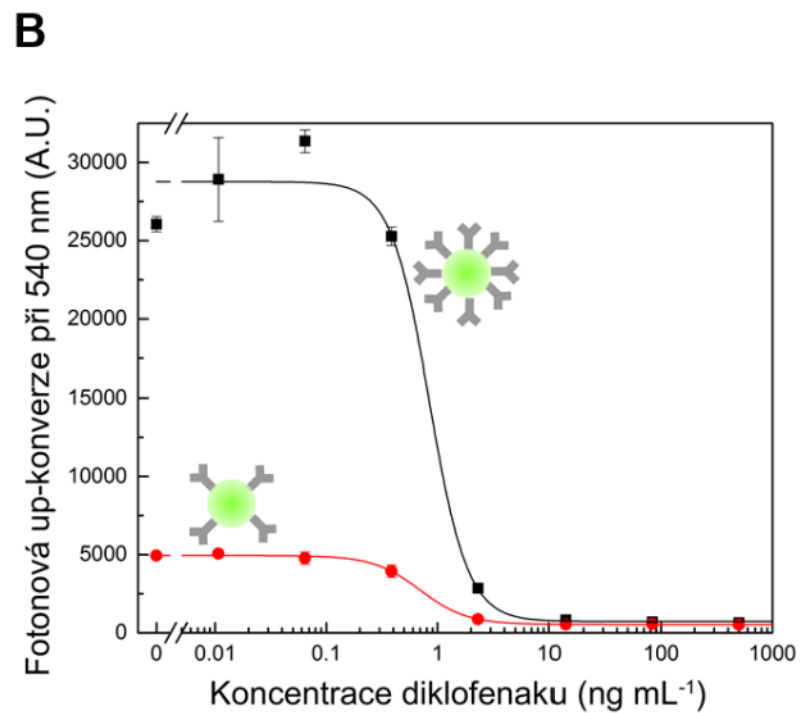
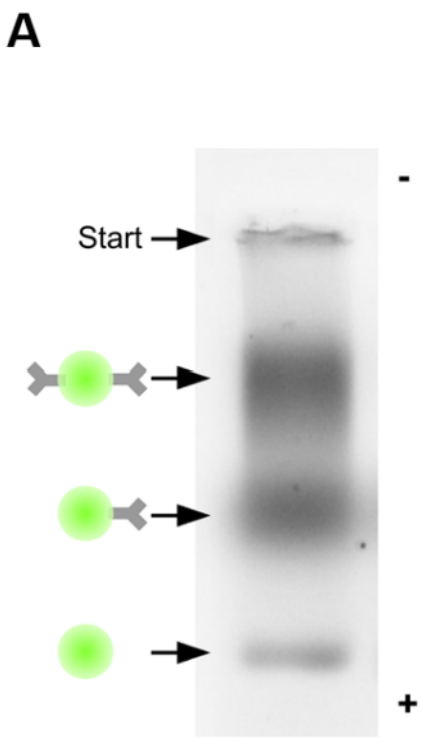
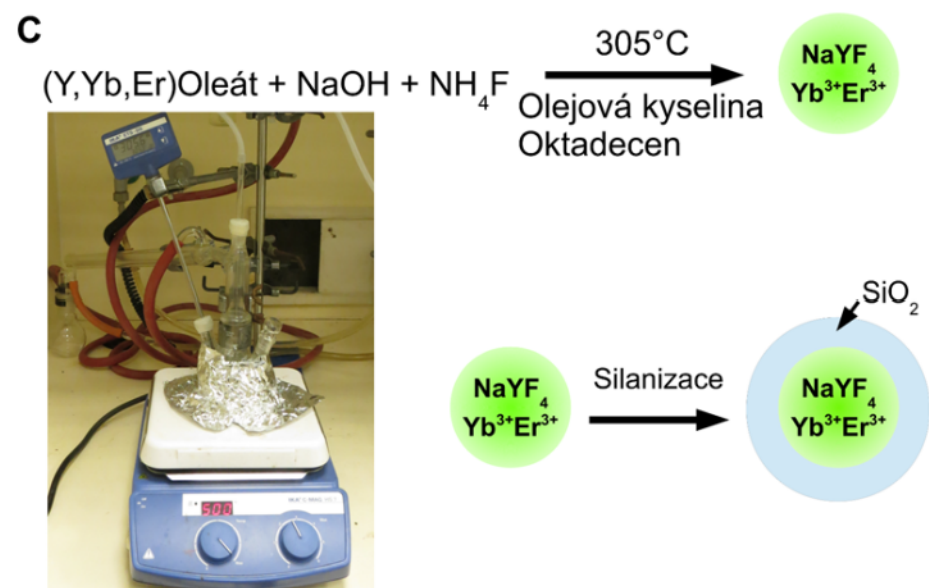
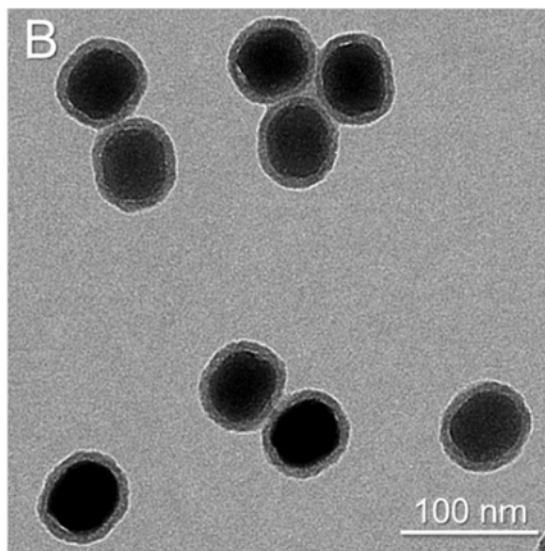
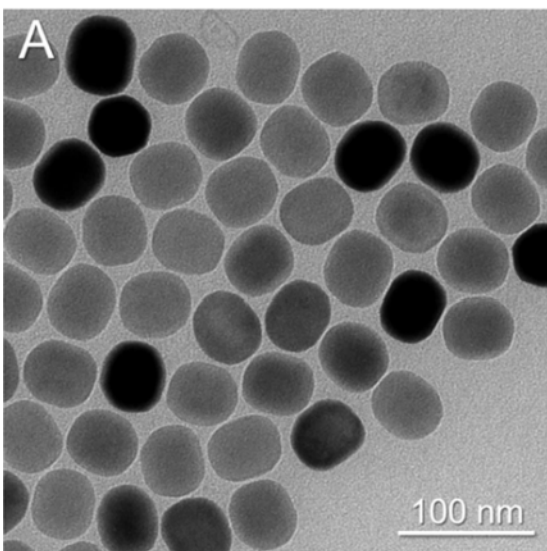
Biokonjugáty UCNP



Interactions

- Specific
- Biomolecule non-specific
- Nanoparticle non-specific

Syntéza



Purifikace

A

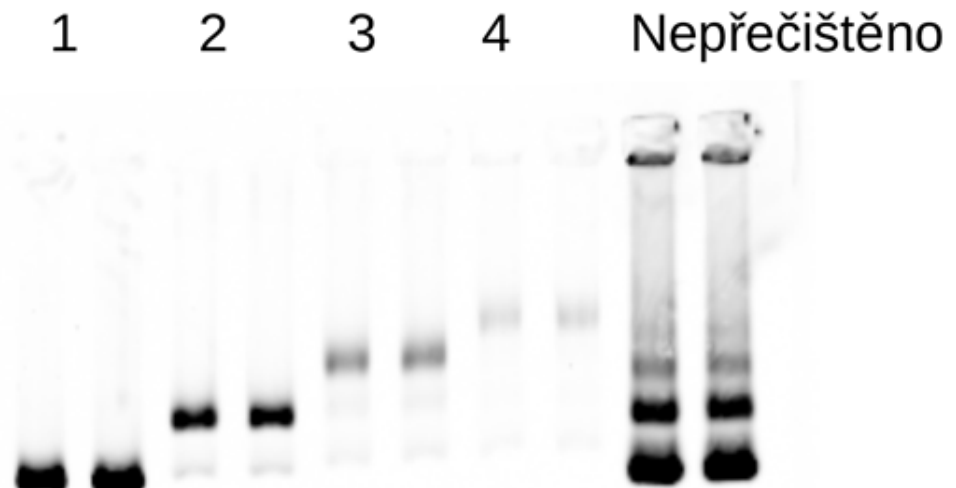
Start



4
3
2
1

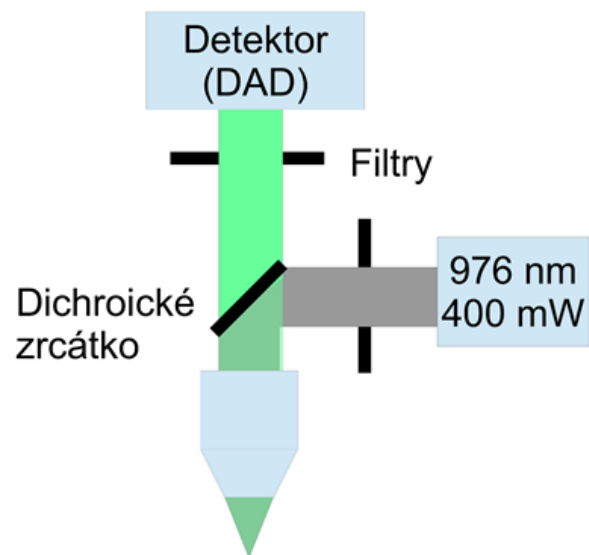
B

Start

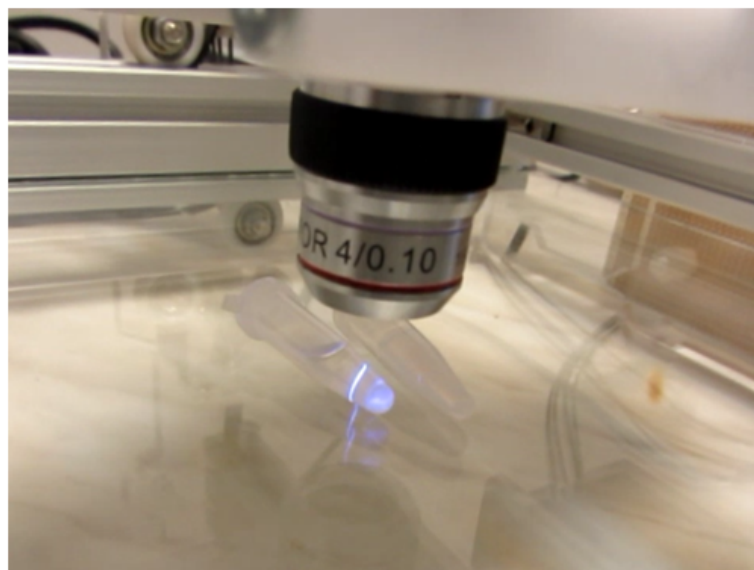


Purifikace

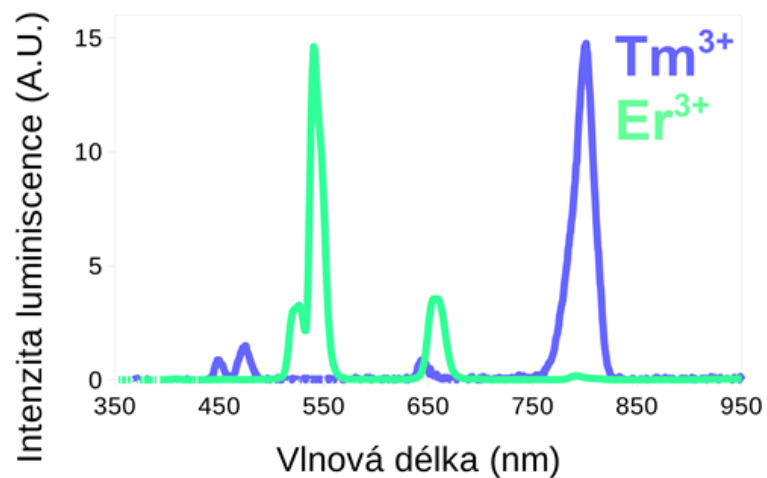
A



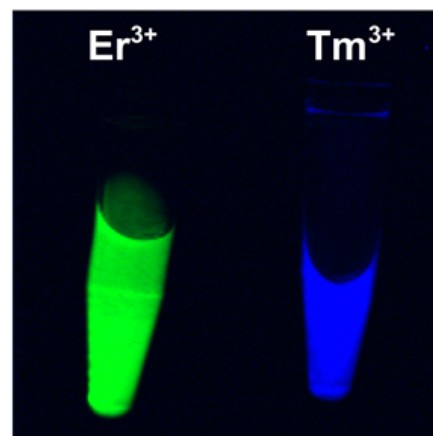
B



C



D



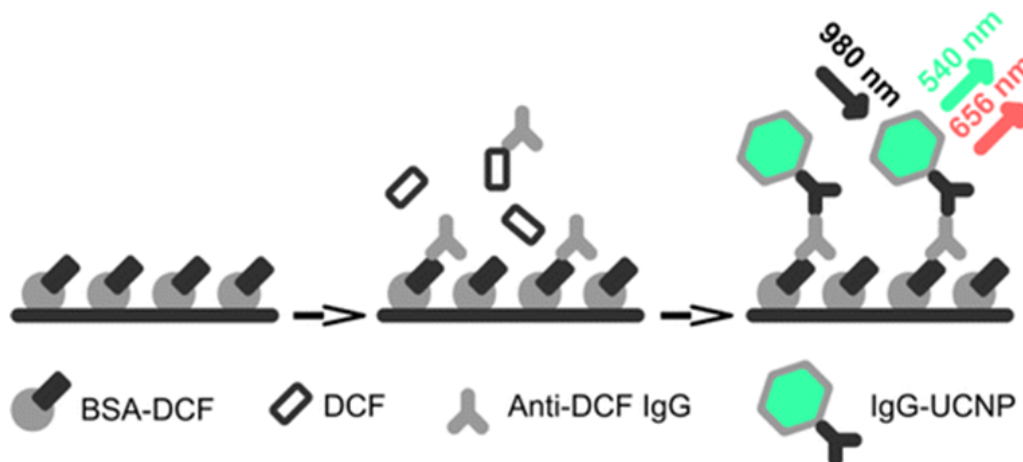
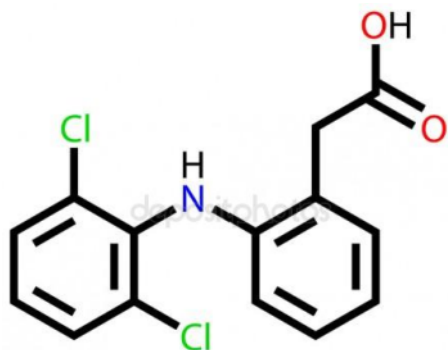
R -; G 520-540 nm; B 468-479 nm

E



Využití-Imunochemické detekce

● Diklofenak



ULISA □

IC_{50} 0.23 ng mL⁻¹

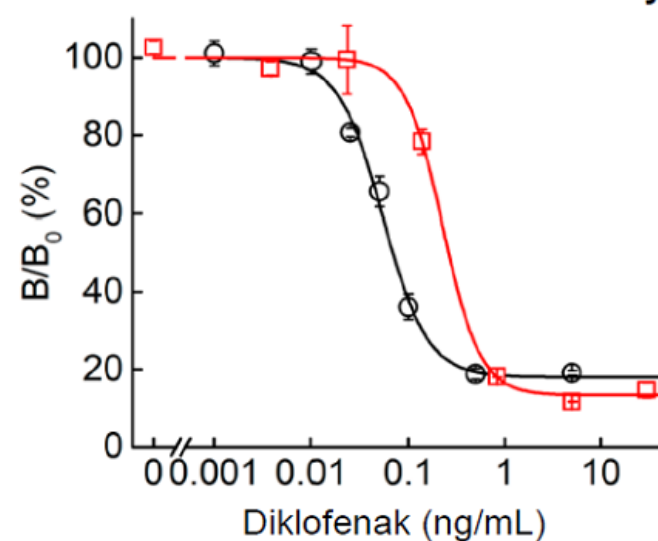
LOD 0.05 ng mL⁻¹

ELISA ○

IC_{50} 0.05 ng mL⁻¹

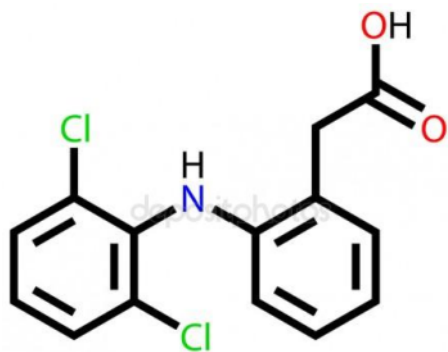
LOD 0.01 ng mL⁻¹

Normalizované kalibrační křivky

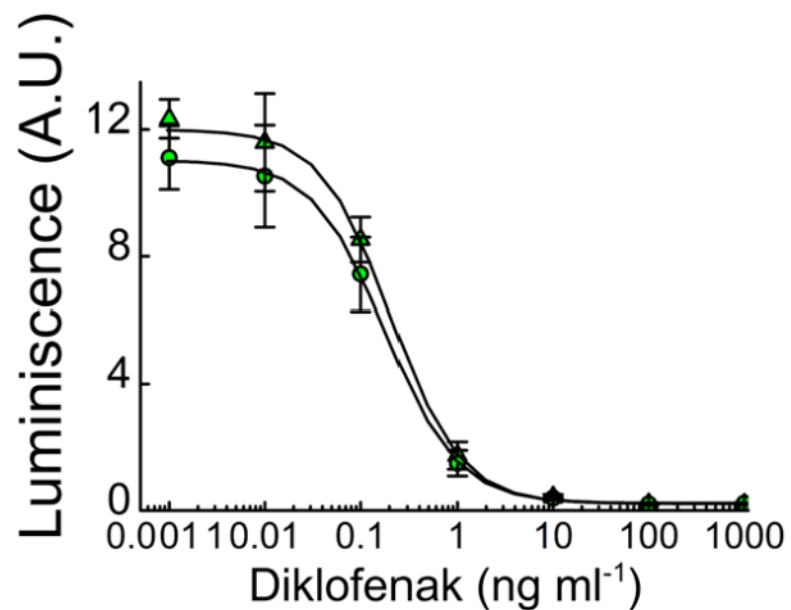
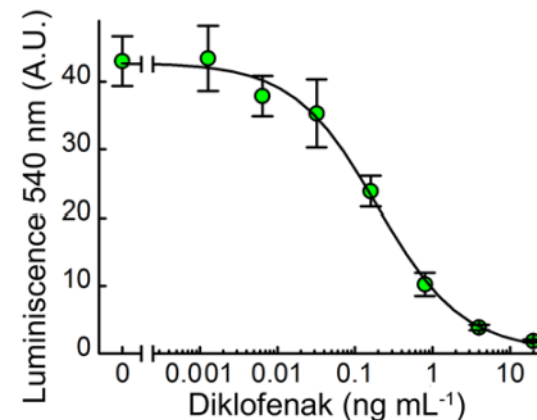
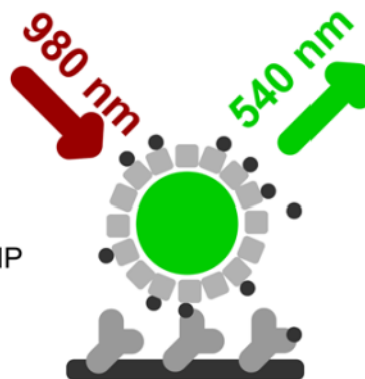


Využití-Imunochemické detekce

● Diklofenak



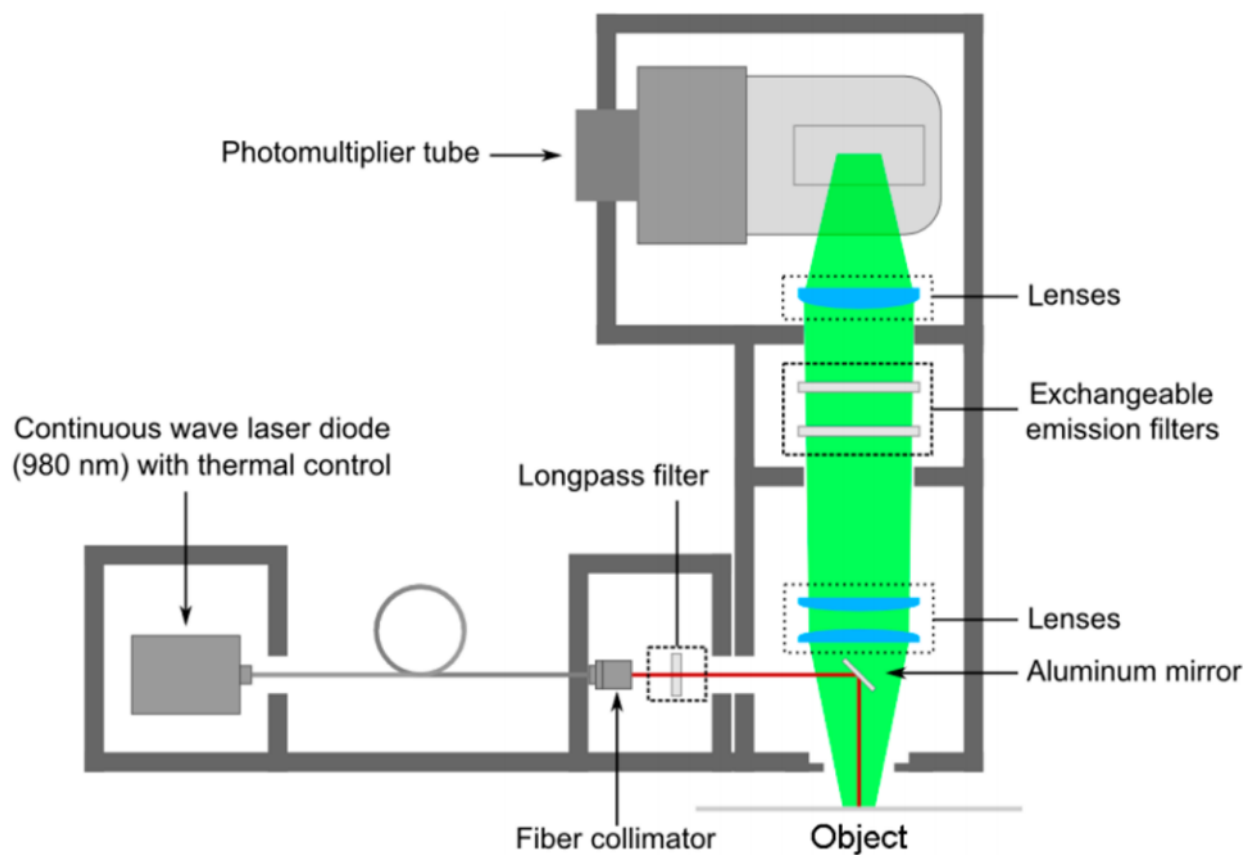
- Diklofenak
- Hovězí γ -globulin
- Karboxylované UCNP
- Anti-diklofenak IgG



Detekce UCNP na površích

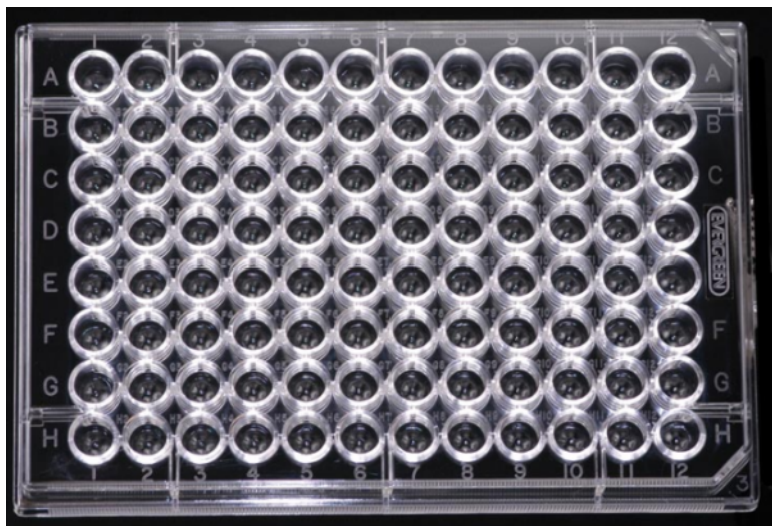


- Epifluorescenční mikroskop
 - Excitace 980 nm
 - Emise 535 nm
- Intenzita excitačního záření 560 W/cm^2
- Fotonásobič

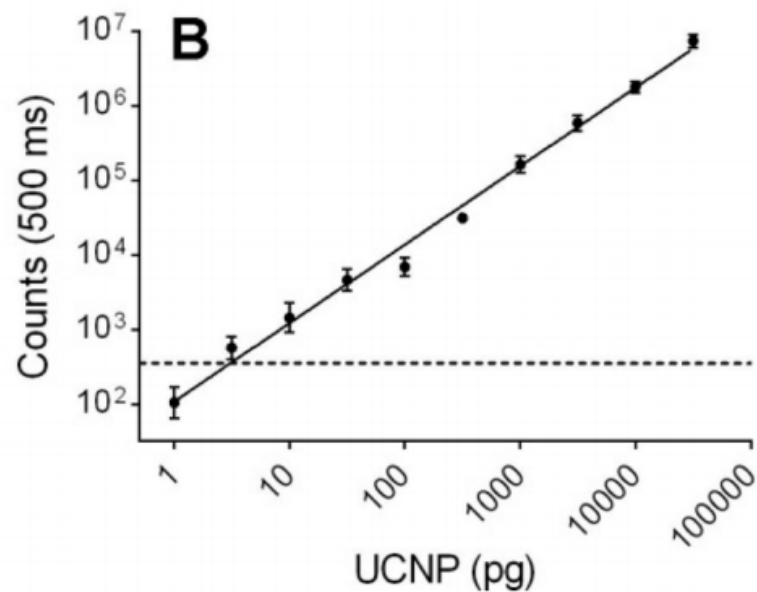
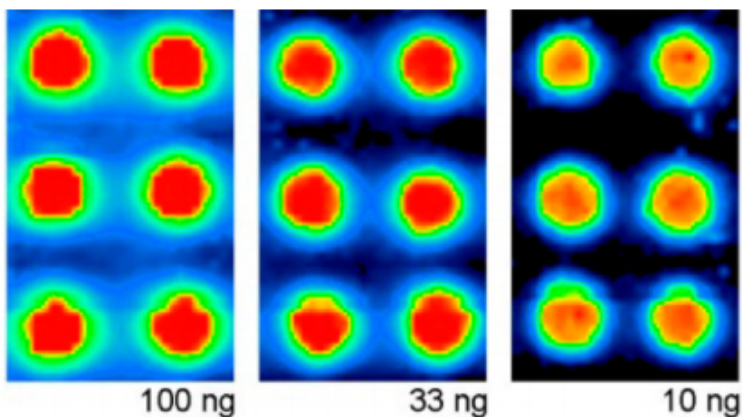


Sedlmeier, A., Hlaváček, A., Birner, L., Mickert, M.J., Muhr, V., Hirsch, T., Corstjens, P.L.A.M., Tanke, H.J., Soukka, T., Gorris, H.H. (2016) Analytical Chemistry, 88 (3), pp. 1835-1841

Detekce UCNP na površích



- Epifluorescenční mikroskop
 - Excitace 980 nm
 - Emise 535 nm
- Intenzita excitačního záření 560 W/cm^2
- Fotonásobič

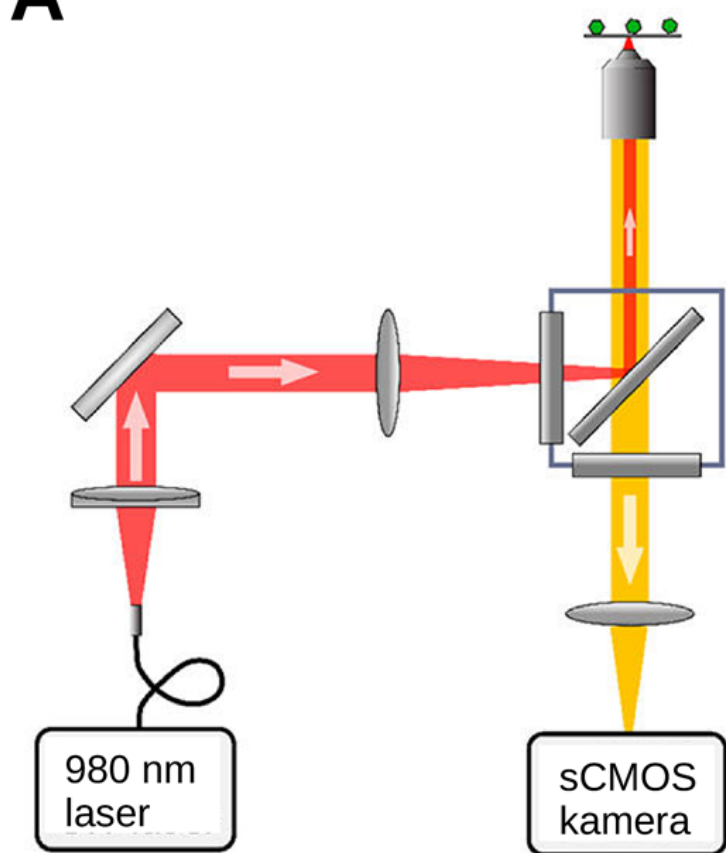


Sedlmeier, A., Hlaváček, A., Birner, L., Mickert, M.J., Muhr, V., Hirsch, T., Corstjens, P.L.A.M., Tanke, H.J., Soukka, T., Gorris, H.H. (2016) Analytical Chemistry, 88 (3), pp. 1835-1841

Využití-Digitální imunochemické detekce

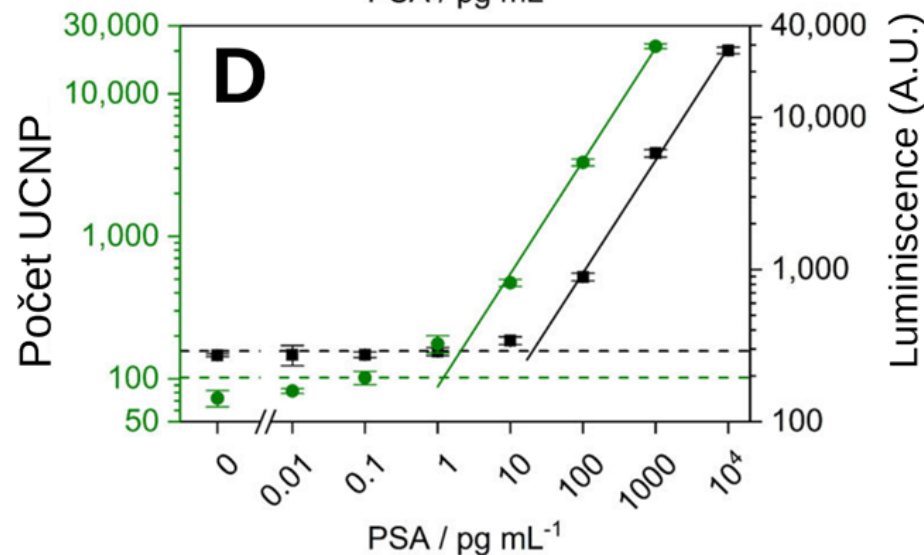
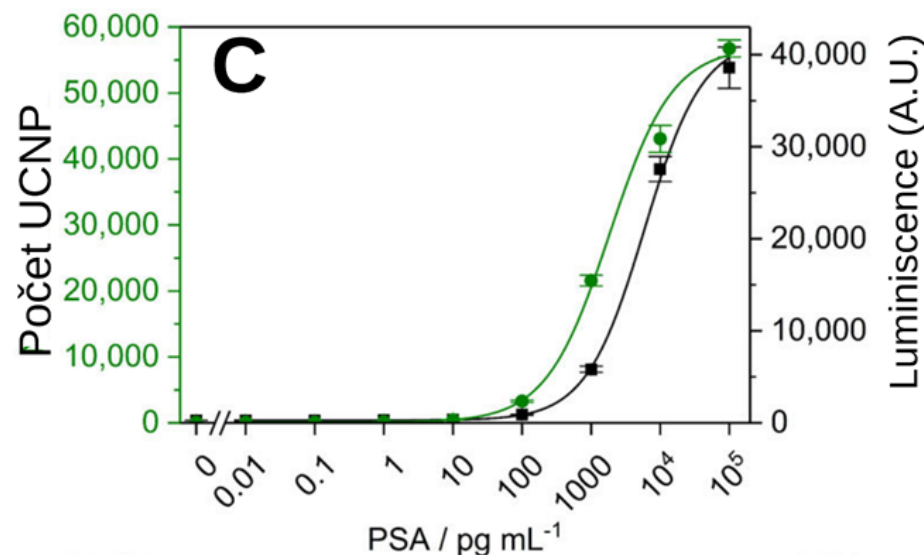
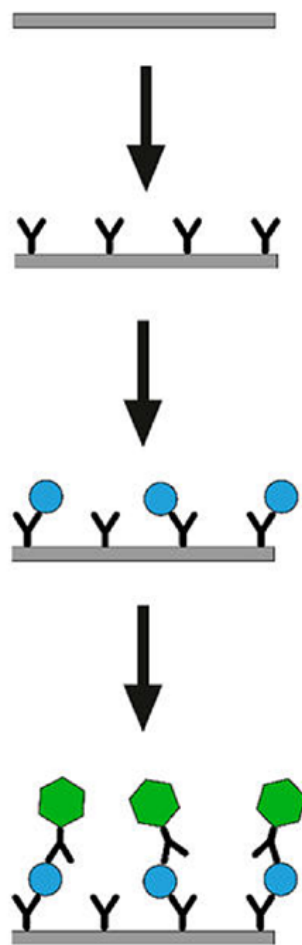
● Prostate-specific antigen

A



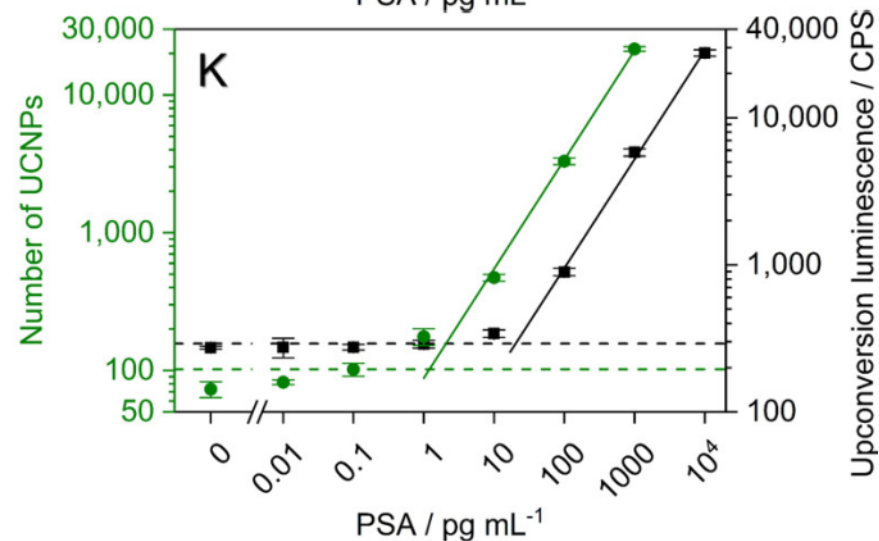
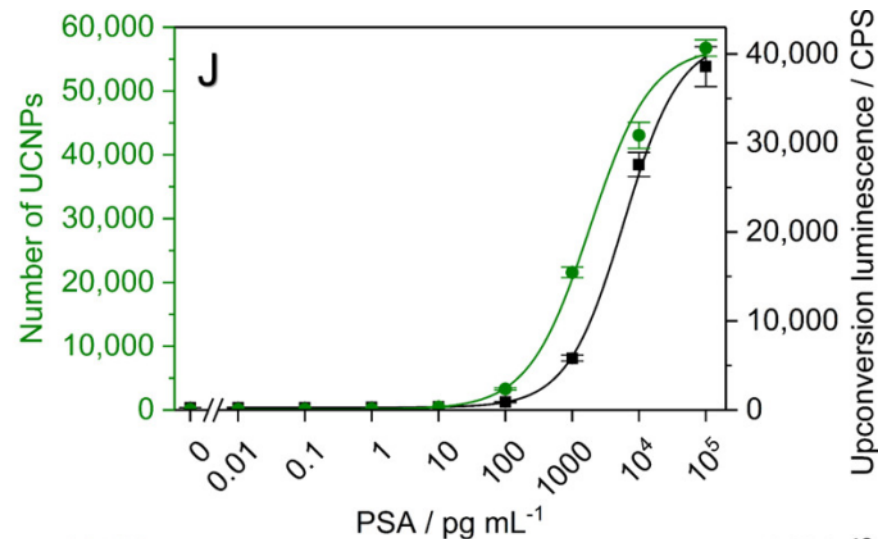
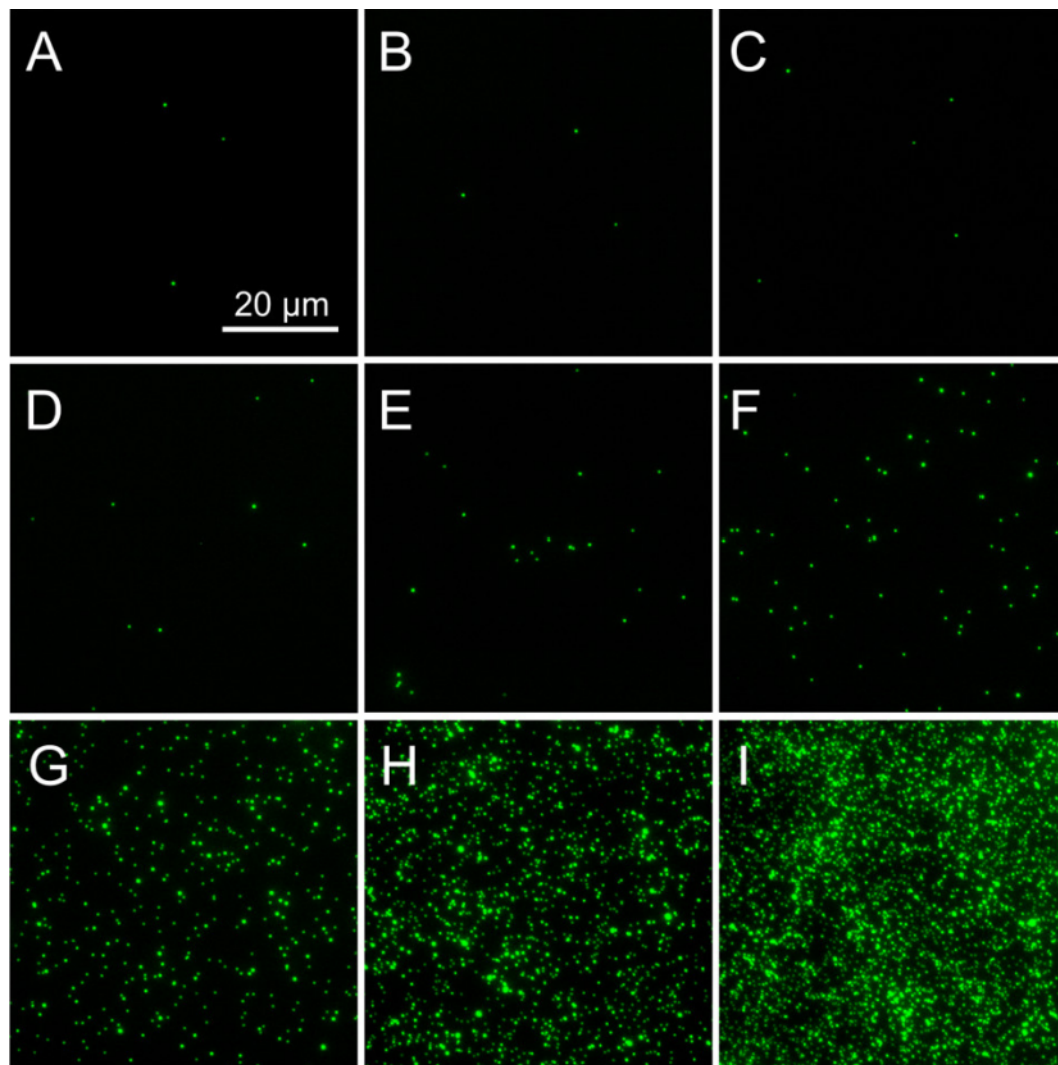
● UCNP ● PSA Y Protilátka

B



Využití-Digitální imunochemické detekce

● Prostate-specific antigen



Závěr-výsledky

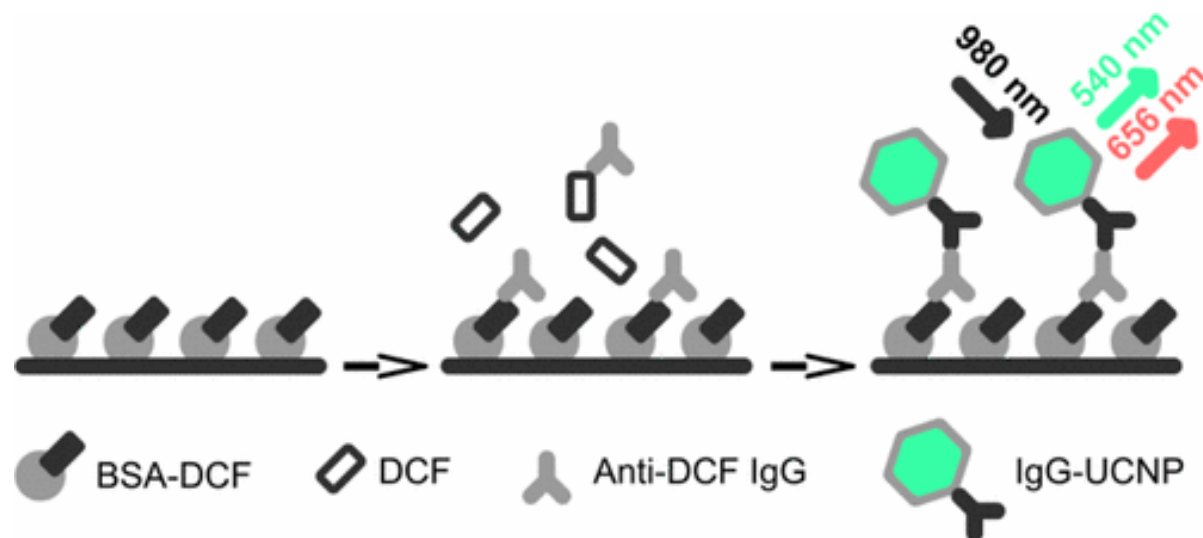
● 3x prezentace na konferencích

- Přednáška; Hlaváček, A., Přikryl, J., Foret, F.; ELECTROPHORETIC CHARACTERIZATION OF PHOTON UPCONVERTING NANOPARTICLES AND THEIR BIOCONJUGATES; UP-CON 2016, 1st Conference and Spring School on Properties, Design and Applications of Upconverting Nanomaterials, Wroclaw, Poland, 23-27 May 2016.
- Poster; Hlaváček, A., Farka, Z., Huebner, M., Horáková, H., Němeček, D., Skládal, P., Knopp, D., Gorris, H-H.; Determination of the Pharmaceutical Micropollutant Diclofenac in Fresh Water by Upconversion-Linked Immunosorbent Assay (ULISA), EUROPT(R)ODE XIII - Conference on Optical Chemical Sensors and Biosensors, Graz, Austria, 20-23 March 2016
- Poster; Hlaváček, A., Farka, Z., Mickert, M., Foret, F., Gorris, H. H.; Electrophoretic Purification Of Photon-Upconversion Nanoparticle Bioconjugates For A Single Molecule Upconversion-Linked Immunosorbent Assay; The 15th Conference on Methods and Applications in Fluorescence. Bruges, Belgium, 10-13 September, 2017

● 3x J-recenzovaný odborný článek

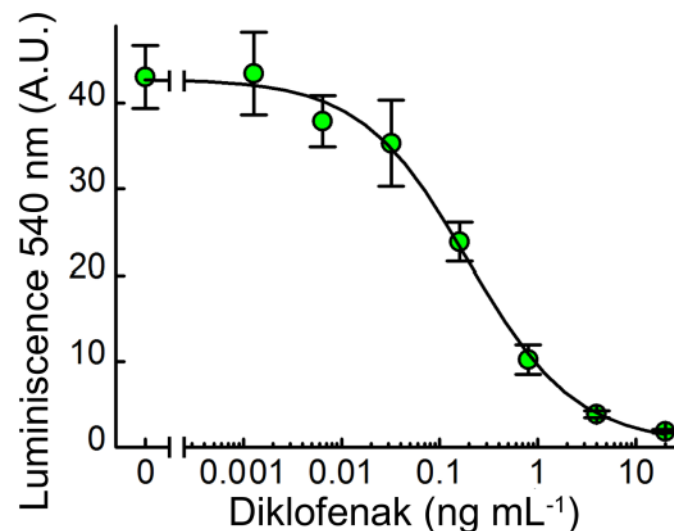
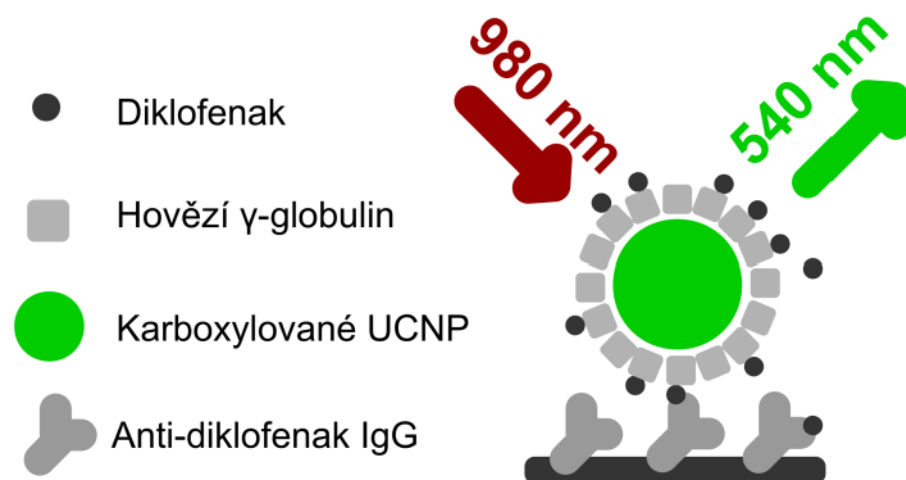
Závěr-výsledky

- Hlaváček, A., Farka, Z., Hübner, M., Horňáková, V., Němeček, D., Niessner, R., Skládal, P., Knopp, D., Gorris, H.H. Competitive Upconversion-Linked Immunosorbent Assay for the Sensitive Detection of Diclofenac (2016) *Analytical Chemistry*, 88 (11), 6011-6017; IF₂₀₁₆ **6,320**



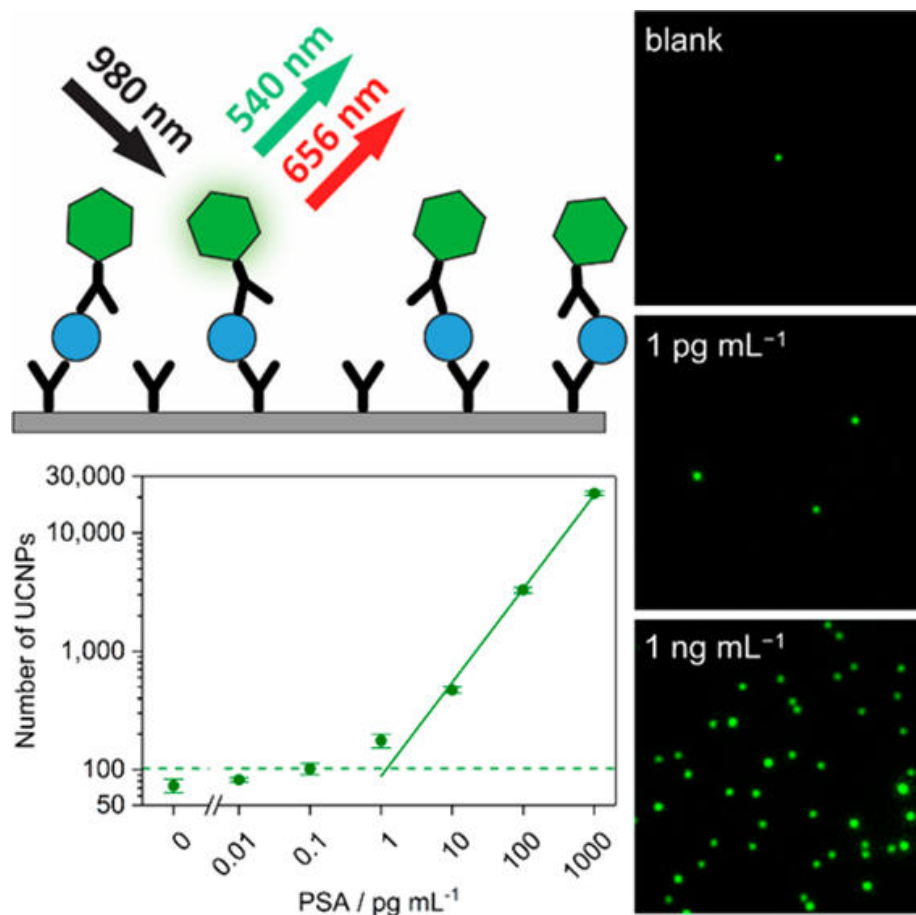
Závěr-výsledky

- Hlaváček, A., Peterek, M., Farka, Z., Mickert, M.J., Prechtel, L., Knopp, D., Gorris, H.H. Rapid single-step upconversion-linked immunosorbent assay for diclofenac (2017) *Microchimica Acta*, 184 (10), pp. 4159-4165; IF₂₀₁₆ 4,580



Závěr-výsledky

- Farka, Z., Mickert, M.J., Hlaváček, A., Skládal, P., Gorris, H.H. Single molecule upconversion-linked immunosorbent assay with extended dynamic range for the sensitive detection of diagnostic biomarkers (2017) *Analytical Chemistry*, 89 (21) 11825–11830; IF₂₀₁₆ 6,320



Děkuji za pozornost