

Immunosensor Incorporating Anti-His (C-term) IgG F(ab') Fragment Attached to Gold Nanorods for Detection of His-Tagged Proteins in Culture Medium

Michał Wąsowicz¹, Małgorzata Milner², Dorota Radecka², Krystyna Grzelak² and
Hanna Radecka^{1,*}

¹ Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Tuwima 10, 10-747 Olsztyn, Poland; E-Mail: m.wasowicz@pan.olsztyn.pl

² Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5a, 02-106 Warsaw, Poland; E-Mails: gosiam@ibb.waw.pl (M.M.); dorota.radecka@student.uw.edu.pl (D.R.); kg@ibb.waw.pl (K.G.)

* Author to whom correspondence should be addressed; E-Mail: hanna.radecka@pan.olsztyn.pl; Tel.: +48 89 523 46 36; Fax: +48 89 524 0124;

Fabrication of Nanorods (according to procedure reported by B. Bushbee, S. Obare, C. Murphy, *Advanced Materials* 2003,15, 414-416)

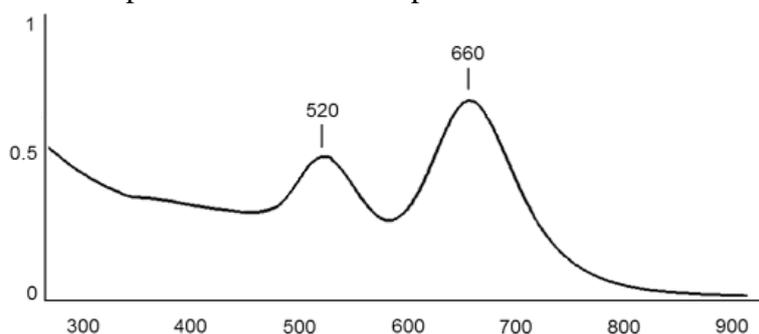
For gold-seed production, 0.5 mL of 0.01 M HAuCl₄ trihydrate in water and 0.5 mL of 0.01 M sodium citrate in water were added to 18 mL of deionized water and stirred. Next, 0.5 mL of freshly prepared 0.1 M NaBH₄ was added and the solution color changed from colorless to orange. Stirring was stopped and the solution was left undisturbed for 2 h. The resulting spherical nanoparticles were used as gold seed.

For gold nanorod growth from the seeds, three flasks were labeled A, B and C.

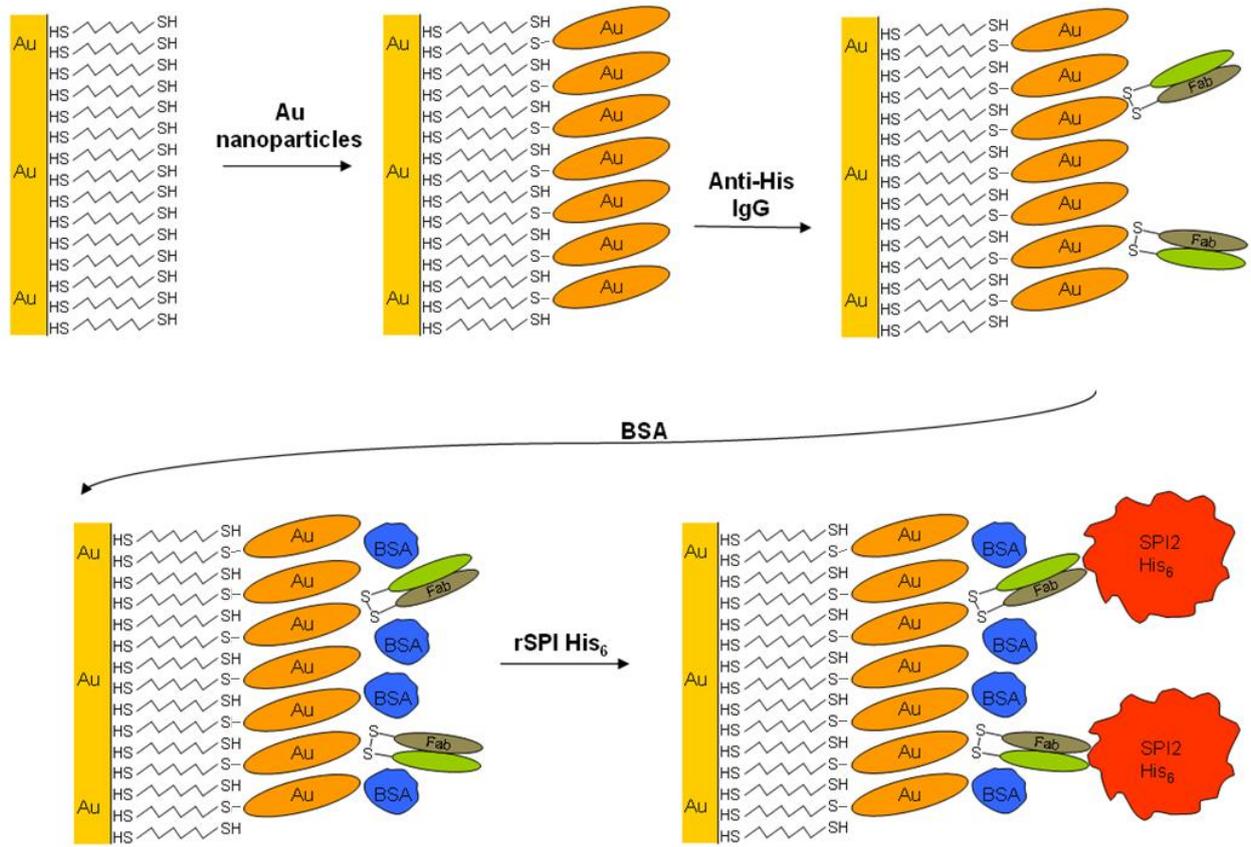
Growth solutions A and B consisted of 9 mL of 0.1 M cetyltrimethylammonium bromide (CTAB) in water, 0.25 mL of 0.01 M HAuCl₄ trihydrate in water, 50 μL of 0.1 M ascorbic acid.

Growth solution C consisted of 90 mL 0.1 M CTAB, 2.5 mL 0.01 M HAuCl₄, 0.5 mL 0.1 M ascorbic acid.

The seed solution was added to growth solution A and shaken for 3 s. Then it was poured into solution B, shaken for 4s and added to solution C. The mixture turned reddish in several minutes and was left overnight at room temperature. Excess CTAB was removed by centrifugation (Eppendorf MiniSpin, 1,000 rpm 5 min). Supernatant was removed and the pellet resuspended in water and stored in +4 °C. The typical UV-Vis spectra of nanorods is presented below.



Scheme S1. Schematic representation of successive modification steps of immunosensor fabrication.



Scheme S2. Schematic representation of IgG2 antibody digestion by papain.

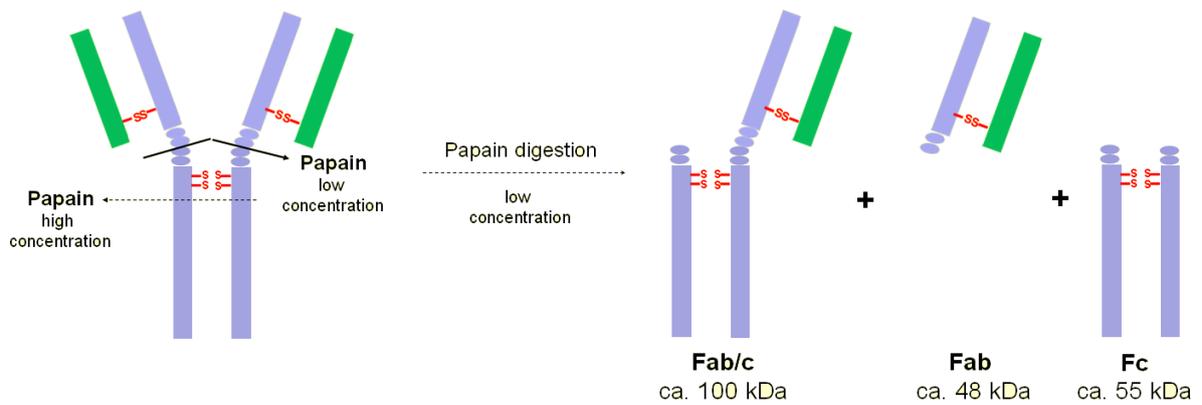


Figure S1. Electrophoretic separation of digestion products of papain catalysed hydrolysis of IgG2b. Polyacrylamide gel (10% gel) at non-reducing condition: lane 1, uncut IgG2b; lane 2, IgG2b digested for 1 h; lane M, molecular weight standard. The size of the standard protein bands is indicated.

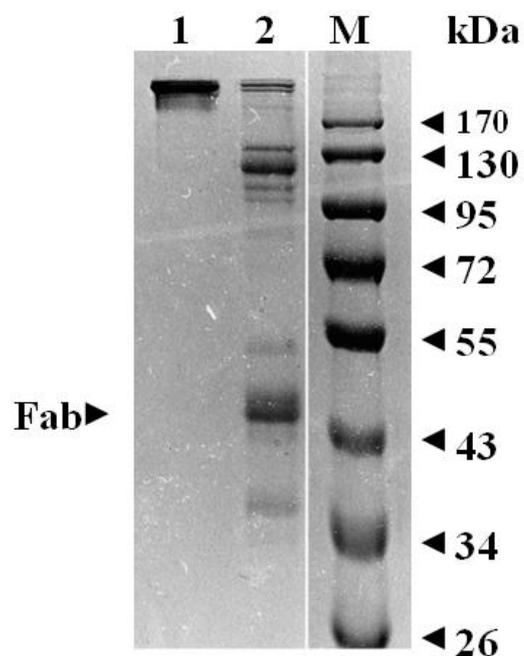


Figure S2. Cyclic voltammograms of (A) clean gold electrode surface; (B) 1,6-hexanedithiol/Au electrode; (C) gold nanorods/1,6-hexanedithiol/Au electrode; (D) Fab/gold nanorods/1,6-hexanedithiol/Au electrode; (E) bovine serum albumin/Fab/gold nanorods/1,6-hexanedithiol/Au electrode. Electrochemical cell setup: Au (BAS, USA) as the working electrode, Ag/AgCl as reference electrode, Pt as counter electrode. Solution composition: of 0.1 M PBS pH 7.4 and $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (0.5 mM each).

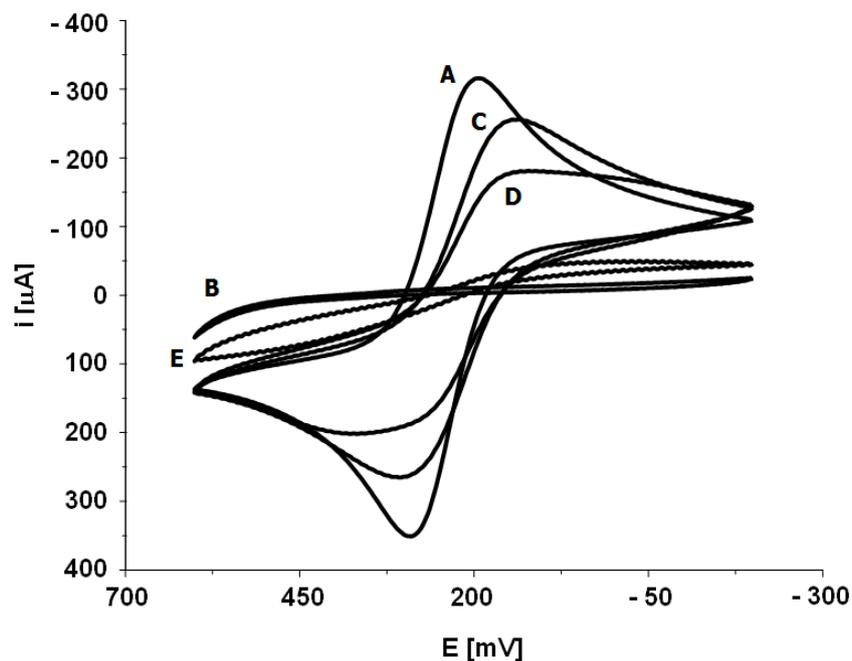


Figure S3. Impedance spectra of (A) clean gold electrode surface; (B) 1,6-hexanedithiol/Au electrode; (C) gold nanorods/1,6-hexanedithiol/Au electrode; (D) Fab/gold nanorods/1,6-hexanedithiol/Au electrode; (E) bovine serum albumin/Fab/gold nanorods/1,6-hexanedithiol/Au electrode. Circuit model used for fitting Nyquist plots in inset. R_s —solution resistance, R_{et} —electron transfer resistance, C_{dl} —double layer capacitance. Electrochemical cell setup: Au (BAS, USA) as the working electrode, Ag/AgCl as reference electrode, Pt as counter electrode. EIS setup: frequency range 10 kHz to 100 mHz, formal potential 0.170 V. Solution composition: of 0.1 M PBS pH 7.4 and $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (0.5 mM each).

