# Paper-based chemiresistor for detection of ultralow concentrations of protein

Marta Pozuelo, Pascal Blondeau*, Marta Novell, Francisco J. Andrade, F. Xavier Rius and Jordi Riu*

Department of Analytical and Organic Chemistry. Universitat Rovira i Virgili

Campus Sescelades, c/ Marcel·lí Domingo s/n, 43007 - Tarragona, Catalonia-Spain.

e-mail: pascal.blondeau@urv.cat and jordi.riu@urv.cat

**Abstract.** A new paper-based chemiresistor composed of a network of single-wall carbon nanotubes (SWCNTs) and anti-human immunoglobulin G (anti-HIgG) is reported herein. SWCNTs act as outstanding transducers because they provide high sensitivity in terms of resistance changes due to immunoreaction. As a result, the resistance-based biosensor reaches concentration detection as low as pM. The resulting paper-based biosensor is sensitive, selective and employs low-cost substrate and simple manufacturing stages. Since chemiresistors require low-power equipment and are able to detect low concentrations with inexpensive materials, the present approach may pave the way for the development of resistive biosensors at very low-cost with high performances.

**Keywords:** carbon nanotubes, chemiresistor, protein detection, paper substrate

## 1. INTRODUCTION

Affinity-based biosensors are one type of sensors with most promising potential applications in several fields: health care, food contamination, environmental safety and security, among others. For instance, detecting a human disease or infection in its early stage would represent a considerable advantage for the medical treatment to be effective. Ideally, part of this progress would be given by the development of simple, portable and low-cost biosensors. Importantly, these latter factors could extend the diagnosis to non-developed countries (Mabey et al., 2004; Peeling and Mabey, 2010).

Resistance-based biosensors emerged as an attractive complement to other electrochemical methods of detection. These label-free devices can measure small changes of the target analyte concentration with high precision and low power instrumentation (Esser et al., 2012; Das et al., 2011).

Initial chemiresistors based on nanostructured materials are composed of interdigitated microelectrodes containing one-dimensional nanostructures as transducer elements for gas sensing (Li et al., 2003). In addition to these nanostructured materials, the incorporation of molecular recognition elements, which confer selectivity to the system, considerably broaden the available sensing analytes from typical volatile organic compounds (VOC) to bacteria for instance (García-Aljaro et al., 2010). Such sensors are able to detect therefore chemicals, biomolecules and microorganisms of interest (Li et al., 2003; García-Aljaro et al., 2010; Cella et al., 2010; Wang et al., 2008). They provide high versatility since several nanostructured materials (e.g. carbon nanotubes or nanowires), and various receptors (e.g. aptamers, artificial receptors or antibodies), have been combined together with high reliability. Although the detection technique (i.e. measure of electrical resistance or intensity of electrical current) displays simplicity of operation, the conventional device construction still limits the real applications the sensors can address. These chemiresistors are commonly built on $SiO_2$/Si surfaces with manufacturing approaches that involve specialized equipment such as lithography techniques and trained personnel so that both the substrate and the construction strategy considerably raise the final cost of production. Other drawbacks of these devices are the lack of estimation of some performance parameters in most of the reported chemiresistors and the need to obtain better reproducibility, both in the measurement and the construction stages, which hinder the ultimate goal of these sensing devices: real sample measurement (Yáñez-Sedeño et al., 2010).

The possible integration of sensors in daily materials such as yarns, rubber or paper materials (Shim et al., 2008; Sekitani et al., 2008; Wang et al, 2009) emerged as an attempt to solve the cost-related issues. In this context, single-wall carbon nanotubes (SWCNTs) have proven to be suitable candidates to be used in sensing devices due to both excellent electronic transduction properties and effective deposition characteristics using carbon nanotubes-based ink. For instance, Shim et al. have

taken advantage of cotton yarns to build a SWCNT based chemiresistor for protein detection (Shim et al., 2008). Recently, Ammu et al. reported on flexible chemiresistor vapour sensors based on cellulosic substrates and plastics (Ammu et al., 2012). Paper retains attention not only because it affords simplicity, low-cost and disposability but also because it allows developing very sensitive diagnostic systems. Although paper-based sensors operate for several detection techniques with relevant performances (Martinez et al., 2007, 2010; Jokerst et al., 2012; Miranda et al., 2011; Yu and White, 2010; Novell et al., 2012; Ge et al., 2012), to the best of our knowledge, such paper-based chemiresistors have not been developed so far for biomolecules of interest.

In this communication, we aim to demonstrate the development of a novel paper-based chemiresistor for rapid, direct and sensitive detection of proteins. We selected SWCNTs as efficient transducers and as a proof-of-concept for immunoreaction, we have used human immunoglobulin G (HIgG) as a target analyte. The conductive paper was generated by simply painting paper filter with SWCNT ink. Notably, this procedure is among the simplest, low-cost mass manufacturing methodologies (Gonzalez-Macia et al., 2010). The analytical performances of the resulting biosensors afford pM detection of HIgG. Cost effective, facile use and easiness of construction are additional operational features that add on the performance parameters of the novel biosensor reported here.

## 2. EXPERIMENTAL PART

The conductive paper (obtained painting filter papers with SWCNT ink) was cut into strips of 2 mm width and 10 cm long. Each strip was further trimmed so that a thinner part (1 mm width and 5 mm long) was left in the centre of the strip. Then, each strip was coated with glue at 1.5 cm from the centre in both sides keeping free the ending parts of the SWCNT-deposited paper where the clamps will be connected. The glue addition was necessary to avoid any water migration through the paper to the electrical connections. Finally an adhesive cover was incorporated on the upper side of the chemiresistor and sealed with glue to avoid any direct contact with the solution (Figure 1a). Details about materials, methods and construction of the biosensor can be found in the Supporting information.

## 3. RESULTS AND DISCUSSION

The construction of the biosensor started by painting the filter paper with SWCNT-ink to convert it into conductive paper. The fast adhesion of the SWCNTs could be detected at naked eye due to a rapid colour change of the paper from white to grey-black after a single painting. After each painting cycle, we removed most of the sodium dodecylbenzenesulfonate used in the ink by washing with water because the surfactant may interfere in the conduction path of the electrons (Hu et al., 2009) and in the further SWCNTs functionalization steps. The conductive paper obtained was folded and bended while the resistance monitored. However, these mechanical manipulations did not alter the electrical conductivity accounting for very robust conductive papers without specific requirements for particular maintenance or conditioning. We functionalized the SWCNTs by absorption of anti-HIgG followed by incubation with Tween 20 to avoid non-specific interactions, following an already reported procedure (Cid et al., 2008).

The chemiresistor was electrochemically characterized after each functionalization step. We recorded the current *vs.* voltage (I-V) curves for SWCNT immobilization onto the paper, anti-HIgG physisorption onto the SWCNT, Tween 20 blockage of the SWCNTs gaps and 62 pM HIgG addition (Figure 1b). The current decreased after each functionalization step (from bare SWCNT to Tween 20) accounting for suitable functionalization of the paper-based biosensor. The immobilized molecules over the SWCNTs act as electron donators providing negative charges to the p-type SWCNT in presence of air, which lead to a reduction of the charge carriers so that the current value decreases (Wang et al., 2008; Star et al., 2003; Heller et al., 2008; Salehi-Khojin et al., 2011). The change in the addition of 62 pM concentration of HIgG corresponds to a change of about 18 nA in the response signal from the Tween 20 curve, which cannot be appreciated in Figure 1b due to the scale. This change in intensity, however, can be clearly differentiated from other additions of HIgG (see Figure 3a and further discussion below).

The instrumental response of chemiresistors depends on the change of the recorded resistance upon the addition of the target molecule. Therefore, among other factors, the sensitivity depends on the SWCNTs density (which is tuned by increasing or decreasing the number of painting cycles). Therefore, we selected this factor to optimize the biosensor performance. A batch of four biosensors with decreasing amounts of deposited SWCNTs, and therefore with increased resistances from 4 to 360 kΩ, was tested to select the optimal sensitivity. Figure 2 shows the instrumental response (in terms of normalized resistance of the four biosensors 100 x $(R - R_0)/R_0$, where R and $R_0$ are the resistances of the biosensor after the exposure to 6.3 pM of HIgG and to buffer, respectively) as a function of the biosensor resistance (related to the density of the immobilized SWCNT). A resistance around 200 kΩ gives the optimal response, which corresponds to three painting cycles, and this resistance was selected for the next experiments. This behaviour agrees with previous chemiresistors based on SWCNTs (although using different substrates than paper) where an intermediate resistance affords the optimum sensitivity (Wang et al., 2008). Noteworthy, the paper-based chemiresistor requires a denser network of SWCNTs compared to the perfectly flat $SiO_2$/Si surfaces where higher resistances have been reported (1 to 10 MΩ) (Salehi-Khojin et al., 2011). In our case, the formation of the network of SWCNTs is clearly altered by the 3D structure of the paper while in a flat surface -such as $SiO_2$/Si- the SWCNTs are easier to locate and interweave. This reason may explain the fact that the optimal resistance values for $SiO_2$/Si surfaces correspond to less dense networks of SWCNTs and hence to higher resistance values.

The intensity of current of a single biosensor was measured upon addition of different concentrations of HIgG (Figure 3a). We can distinguish two distinct phenomena: the sensitivity for the range 0-6.3 pM is -1.73 ± 0.85 nA/pmols·$L^{-1}$, which may correspond to the detection of the HIgG, while the sensitivity for the range 6.3-62 pM is -70.8 ± 58.7 pA/pmols·$L^{-1}$. This second range may correspond to the increasing saturation of the recognition site availability (Das et al., 2011). Besides, the measurements were achieved at different times (15 and 25 minutes and afford similar results, data not shown). Interestingly, the paper substrate did not alter the required incubation time for protein

sensing since the latter characteristics was comparable to conventional systems (Salehi-Khojin et al., 2011).

The calibration curve corresponding to three different biosensors is shown in Figure 3b. For the sake of comparability between the different sensors Figure 3b shows the normalized response of the three biosensors. Despite the comparable initial resistance of the biosensors, a significant standard deviation was observed when monitoring the target HIgG. This fact could be attributed to several factors. First, these biosensors were hand-made, what decreased the construction reproducibility between different biosensors in parameters such as the area over the layer of SWCNTs where the functionalization was done, or the uniformity of the 3D network of SWCNTs. Nevertheless, the biosensor construction was based on a painting process which is one of the simplest and low-cost methodologies so that mass manufacturing could be easily achieved. Second, the functionalization process was achieved by adsorption of anti-HIgG onto SWCNTs. Ideally the recognition domain should be accessible to the antigen but it should be noted that due to random adsorption of anti-HIgG over SWCNTs, a fraction of anti-HIgG might not be accessible for the biorecognition event with HIgG, decreasing therefore the detection ability (Chevalier et al., 2010). Further work is indeed required to improve the reproducibility of the paper-based biosensor: the generation of conductive paper, the SWCNT–ink density, the surfactant and its concentration are some of the parameters that could improve the biosensor construction as well as its performances. Ideally, an easy and simple mechanical process should be incorporated in the biosensor construction in order to ensure a constant and exact value of the biosensor size, particularly in the sensing part where the anti-HIgG is placed. Here, we were able to detect concentrations as low as pM in less than 15 minutes, concentrations in liquid samples significantly lower than other chemiresistors based on $SiO_2$/Si with limits of detection of 98 nM of salivary $\alpha$-amylase for instance (Tlili et al., 2010). Currently, HIgG levels are usually measured in blood tests through nephelometry, which is based on light diffraction. Although this technique could be easily automated, an alternative such as the one introduced here, i.e. the construction of low cost small-size biosensors that could be used in decentralized measurements, would represent a considerable progress.

To assess the selectivity of the biosensor the response was measured for increasing concentrations of bovine serum albumin, BSA, the most abundant protein in human serum (Figure 3b). Several papers in the literature claim that proteins are strongly and non-specifically adsorbed onto carbon nanotubes (Byon et al., 2006; So et al., 2005), and by using BSA we pretend to check the nonspecific binding of proteins in our paper-based device. The additions of BSA did not afford any significant response: 1.0 % at 6.6 pM BSA *vs* 6.0 % at 6.6 pM HIgG. In addition, the response was measured for increasing concentrations of HIgG without previous functionalization with anti-HIgG. Again, a reduced response was given in comparison with the one from the functionalized sensor (1.9 % *vs* 6.0 % at 6.6 pM HIgG, respectively). This may correspond to unspecific HIgG/SWCNTs interactions due to a non-totally homogeneous coating of the SWCNTs by Tween 20.

## 4. CONCLUSIONS

In this work we show for the first time the development of a disposable and low-cost chemiresistor for HIgG detection. Compared to conventional systems, the biosensor construction was significantly simplified and the cost was dramatically reduced. Even though the sensitivity of the sensor was not maintained as high as for conventional systems, this paper-based biosensor allowed pM detection of proteins in a selective manner and with a significantly reduced cost. Further optimization should be required to improve the reproducibility of the biosensor construction. Point-of-care applications with integration of this paper-based biosensor come as the next step in the elaboration of early diagnosis out of the laboratory.

## ACKNOWLEDGEMENTS

Financial support from the Spanish Ministry of Science and Innovation (MICINN) through project Grant CTQ2010-18717 is gratefully acknowledged.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/to be completed.

## REFERENCES


Ammu, S., Dua, V., Agnihotra, S.R., Surwarde, S.P., Phulgirkar, A., Patel, S., Manohar, S.K., 2012. Journal of the American Chemical Society 134, 4553-4556.

Byon, H.R., Choi, H.C., 2006. Journal of the American Chemical Society 128, 2188-2189.

Cella, L.N., Chen, W., Myung, N.V., Mulchandani, A., 2010. Journal of the American Chemical Society 132, 5024-5026.

Chevalier, S., Cuestas-Ayllon, C., Grazu, V., Luna, M., Feracci, H., de la Fuente, J. M., 2010. Langmuir 26, 14707-14715.

Cid, C.C., Maroto, A., Riu, J., Rius, F.X., 2008. Analyst 133, 1005-1008.

Das, B.K., Tlili, C., Badhulika, S., Cella, L.N., Chen, W., Mulchandani, A., 2011. Chemical Communications 47, 3793-3795.

Esser, B., Schnorr, J.M., Swager T.M., Esser, B., 2012. Angewandte Chemie International Edition 51, 5752–5756.

García-Aljaro, C., Cella, L.N., Shiraleb, D.J., Park, M., Muñoz, F.J., Yates, V.M., Mulchandani, A., 2010. Biosensors and Bioelectronics 26, 1437-1441.

Ge, S., Ge., L., Yan, M., Song, X., Yu, J., Huang, J., 2012. Chemical Communications 48, 9397-9399.

Gonzalez-Macia, L., Morrin, A., Smyth, M.R., Killard A.J., 2010. Analyst 135, 845-867.

Heller, I., Janssens, A.M., Mannik, J., Minnot, E.D., Lemay, S.G., Dekker, C., 2008. Nano Letters 8, 591-595.

Hu, L., Choi, J.W., Yang, Y., Jeong, S., Mantia, F.L., Cui, L.F., Cui, Y., 2009. Proceedings of the National Academy of Sciences 106, 21490-21494.

Jokerst, J.C., Adkins, J.A., Bisha, B., Mentele, M. Goodridge, L.D., Henry, C.S., 2012. Analytical Chemistry 84, 2900-2907.

Li, J., Lu, Y., Ye, Q., Cinke, M., Han, J., Meyyappan, M., Li, J., 2003. Nano Letters 3, 929-933.

Mabey, D., Peeling, R.W., Ustianowski, A., Perkins, M.D., 2004. Nature Reviews Microbiology 2, 231-240.

Martinez, A.W., Phillips, S.T., Butte, M.J., Whitesides, G.M., 2007. Angewandte Chemie International Edition 46, 1318-1320.

Martinez, A.W., Phillips, S.T., Whitesides, G.M., Carrilho, E., 2010. Analytical Chemistry 82, 3-10.

Miranda, O.R., Li, X., Garcia-Gonzalez, L., Zhu, Z.-J., Yan, B., Bunz, U.H.F., Rotello, V.M., 2011. Journal of the American Chemical Society 133, 9650-9653.

Novell, M., Parrilla, M., Crespo, G.A., Rius, F.X., Andrade F.J., 2012. Analytical Chemistry 84, 4695-4702.

Peeling, R.W., Mabey, D., 2010. Clinical Microbiology and Infection. 16, 1062–1069.

Salehi-Khojin, A., Khalili-Araghi, F., Kuroda, M.A., Lin, K.Y., Leburton, J.-P., Masel, R.I., 2011. ACS Nano 5, 153-158.

Sekitani, T., Noguchi, Y., Hata, K., Fukushima, T., Aida, T., Someya, T., 2008. Science 321, 1468-1472.

Shim, B.S., Chen, W., Doty, C., Xu, C., Kotov, N.A., 2008. Nano Letters 8, 4151- 4157.

So, H.M., Won, K., Kim, Y.H., Kim, B.K., Ryu, B.H., Na, P.S., Kim, H., Lee, J.O., 2005. Journal of the American Chemical Society 127, 11906-11907.

Star, A., Gabriel, J.C.P., Bradley, K., Gruner, G., 2003. Nano Letters 3, 459-463.

Tlili, C., Cella, L.N., Myung, N.V., Vivek Shetty, V., Mulchandani, A. 2010., Analyst 135, 2637-26421.

Wang, F., Yang, Y., Swager T.M., Wang, F., 2008. Angewandte Chemie International Edition 47, 8394-8396.

Wang, L., Chen, W., Xu, D., Shim, B.S., Zhu, Y., Sun, F., Liu, L., Peng, C., Jin, Z., Xu, C., Kotov, N.A., 2009. Nano Letters 9, 4147-4152.

Yáñez-Sedeño, P., Riu, J., Pingarrón, J.M., Rius, F.X., 2010. Trends in Analytical Chemistry, 29, 939-953.

Yu, W.W., White, I.M., 2010. Analytical Chemistry 82, 9626-9630.

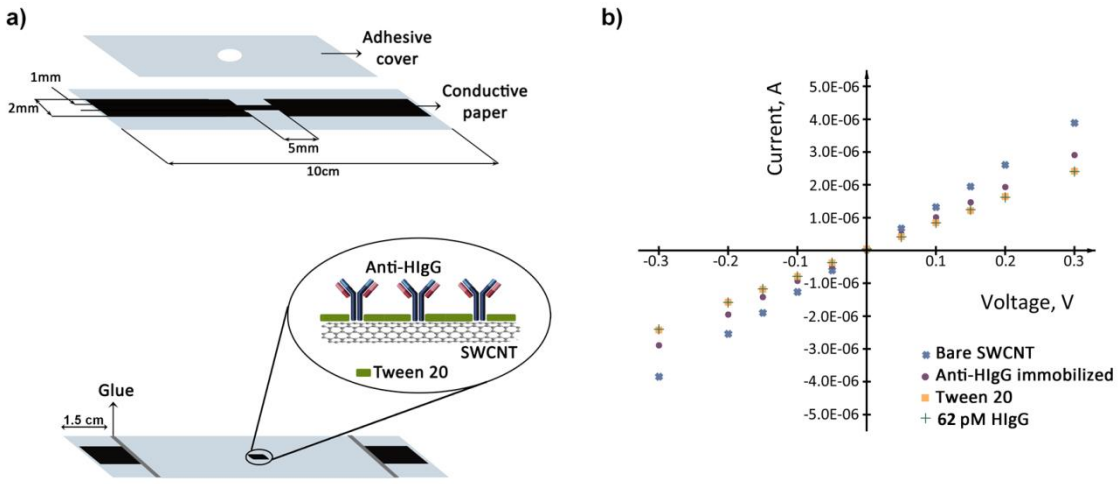


**Figure 1.** a) Schematic representation of the chemiresistor with the ideal attachment of the anti-HIgG onto the SWCNTs. b) I – V characterization of the paper-based chemiresistor for the different construction steps and HIgG sensing.

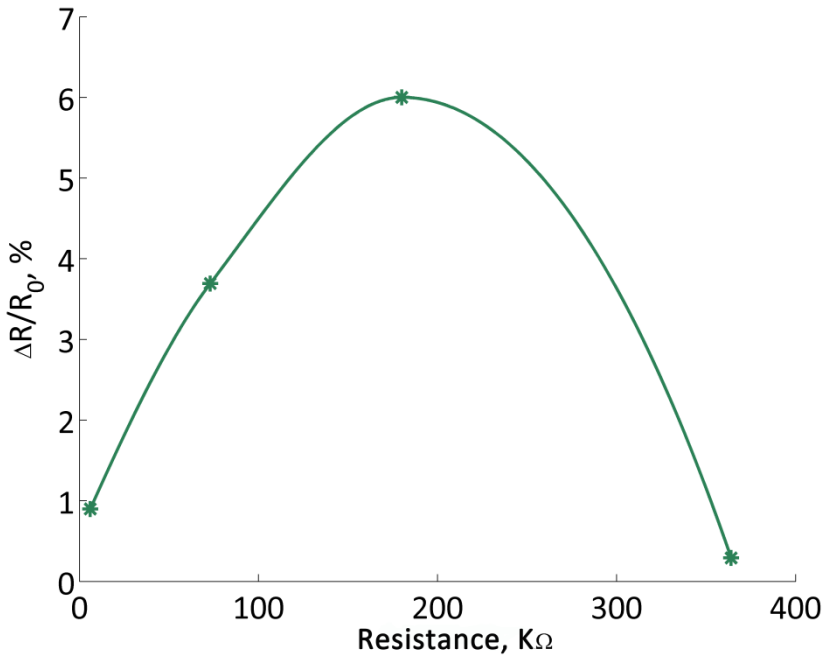


**Figure 2.** Dependence of the instrumental response on the resistance of the paper-based chemiresistor for 6.3 pmols·L-1 of HIgG. The plot was adjusted using a spline curve.

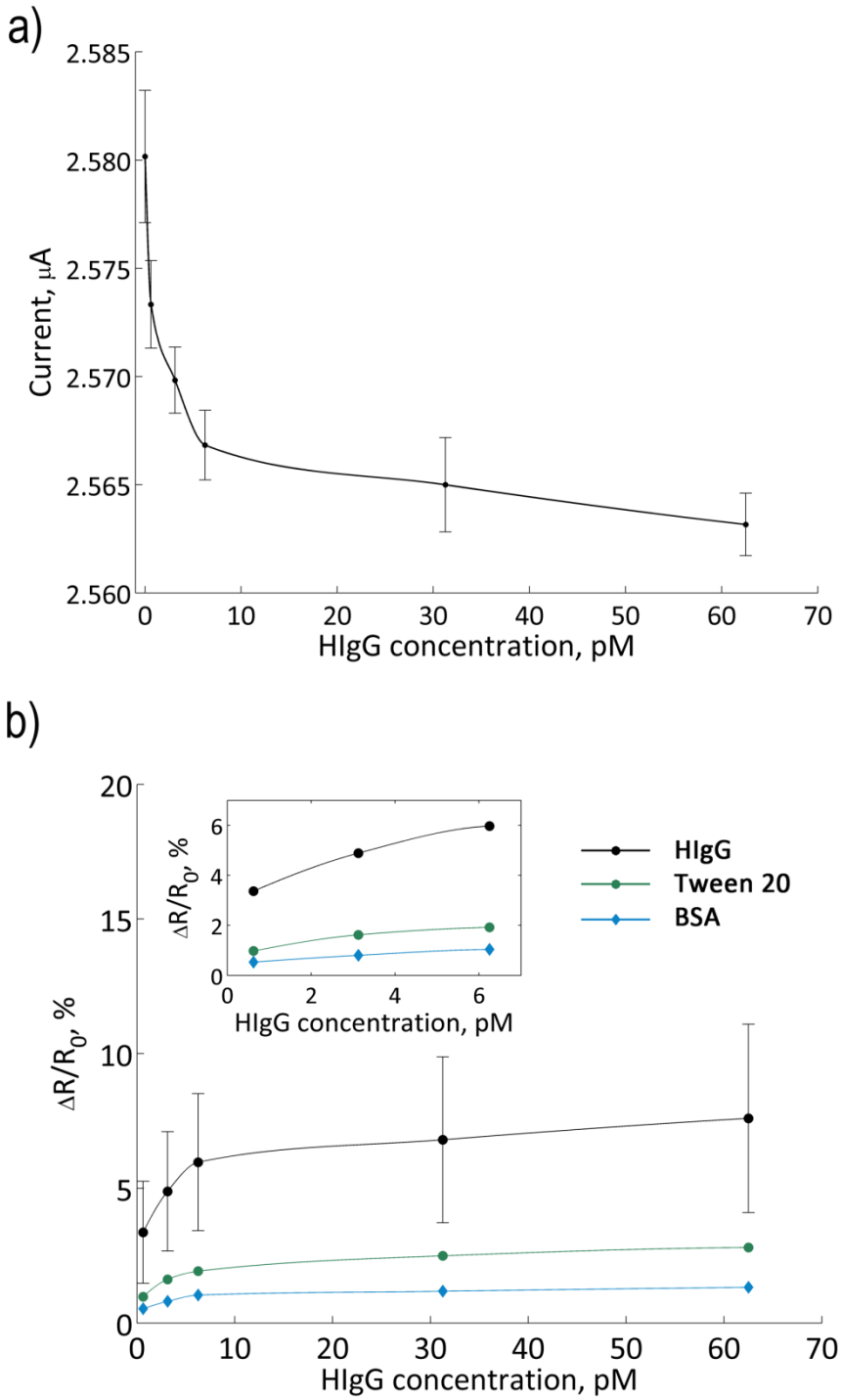


**Figure 3.** a) Response of one biosensor in 2 mM PBS. Each data point is an average of three measurements with the same sensor. The error bars correspond to the standard deviations of the three measurements. b) Biosensor calibration for HIgG in 2mM PBS (black), negative control with BSA (blue), and the Tween 20 control experiment where no antibody was immobilized onto SWCNTs control (green). Data points of HIgG calibration are averages of three different biosensors with the corresponding standard deviation. The inset shows a magnification (without error bars) of the low concentration range.