# The selectivity features of a biological functionalized carbon nanotubes complex towards blood

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## ABSTRACT

The present work has studied the selectivity process of a biological complex based on Carbon Nanotubes and a specific amino-acid sequence for interacting specifically with blood. In this work, Human Serum Albumin (HSA) has been targeted as an indicator of blood, and a short chain peptide, GAQGHTVEK (GK-1) has been used as the biological system for binding the HSA. Indeed, the GK-1 has shown to specifically bind to serum albumin over a wide range of bodily fluids and everyday liquid media such as urine, water, juice, and coffee. Here, the GK-1 has also been covalently attached to the surface of carboxylated multi-wall carbon nanotubes through a carbodiimide catalyzed amide linkage, and the binding of HSA by the MWCNT-GK-1 complex has been confirmed. The selectivity of the investigated semi-conductor nano-bio complex, can serve as a technical platform for developing biomarkers, or bio-sensing textiles where, the CNT-protein complex can act as the stable and robust solid-state detecting core material.

***Keywords***: carbon nanotubes, blood sensors

## 1 INTRODUCTION

Carbon nanotubes, (CNTs), are nanomaterials that have been widely investigated over the last twenty years in the research areas of Science, Engineering and Materials. Along with their distinctive structural features, it has been determined that CNTs have exceptional chemical, mechanical, thermal, optical, optoelectronic and electrical properties [1]. A particular arena that attempts to highlight the electro-chemical features of CNTs is the sensing technology. Indeed, due to their charge sensitive conductance properties, high current density and high thermal stability, CNTs have been identified as promising sensing platforms [2]. The ability of specifically functionalize their structure allows their use as a very selective bio-chemical detecting element. These sensing features can potentially be incorporated into textile fabrics to act as electronic textiles (e-textiles) with biological or chemical monitoring capabilities [3]. Indeed, the ability to incorporate such technologies into the uniforms of persons working in hazardous or dangerous environments would be a great advance in occupational safety. Specifically, the ability to electronically detect the presence of blood on a law enforcement officer or a soldier, could allow for an emergency signal to be dispatched automatically, with no physical action needed by the subject. Some success has been achieved in the development of e-textiles by incorporating conducting polymers [4]. However, polymer based textile fibers are mechanically weak, are susceptible to humidity, and lack the electrical current carrying capacity of conducting materials [5]. To introduce strength and higher conductivity, researchers have included conductive metal wires and other rigid materials into fabrics, which typically result on bulky composites with limited mobility [6,7]. In contrast, the incorporation of carbon nanotubes into textile has been an appealing subject, since CNTs can provide attractive specific strength, flexibility as well as semi-conducting features. Zhu et al [8] reported the development of a CNT based bio-sensing textile fiber. However, bio-functionalization performed through passive absorption could result in relatively easy desorption of enzymes from the surface of the CNTs when placed in aqueous systems. Shim et al [6] also designed a blood detecting smart fabric by coating cotton yarn with carbon nanotubes and antibodies toward human serum albumin (HAS), the essential protein in human blood. However, the usable lifetime of the fibers was dependent upon the lifetime of the antibody, and its denaturation could become a problem in environments with variable climatic conditions [9]. Thus, a more robust sensing complex needs to be developed in order to yield a stable blood sensing system. However, before such development can take place, it is critical to identify a steady, enduring, robust and selective bio-moiety towards human blood that can be subsequently attached to nanotubes for enhancing their nanosensitivity capabilities. The present research program studies an amino acid with a specific affinity towards HSA, as well as its incorporation into nanotubes for establishing the selective sensing platform to potentially yield a human blood sensing textile fiber.

## 2 EXPERIMENTAL METHODS

Based on the work performed by Adams et al [10] and Pignali et al [11], the GK-1 (GAQGHTVEK > 94% purity) peptide from NEO Biolabs was used as the specific binding element towards HSA, and its specificity was investigated using an Enzyme-Linked ImmunoSorbent Assay (ELISA) testing. Here, the target was initially immobilized on polyvinyl chloride (PVC) wells and coated with a 5% of instant nonfat dry milk (NFDM from SACO mix'n drink) block in 1x PBS buffer. The wells were then exposed to $1.207x10^{4}$ mmol/ml of biotinylated GK-1 (1:10 of B-GK-1 in sample buffer), and after the binding period, the unbound B-GK-1 was removed and horseradish peroxidase streptavidin (HRP-Streptavidin) was added. Following the removal of the unbound HRP-Streptavidin, 3.3',5.5'-tetramethylbenzidine (TMB) colorimetric substrate was added to the wells, and the colorimetric change quantified (see figure 1).

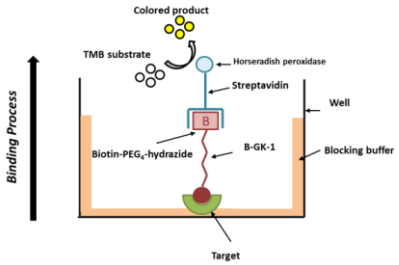


Figure 1. Indirect ELISA method for determining GK1 specificity.

The specificity of the GK-1was also studied against other typical extraneous solutions that a human subject may come in contact with on a daily basis was also investigated. Table 1 lists the individual targets tested through the indirect GK-1 ELISA specificity trials. The biotinylation of the analytes investigated was carried out with the purpose of quantify the amount proteins bound to them. Indeed, it is well known that Biotin has a high affinity towards avidin and streptavidin and therefore, a biotinylated molecule can be identified and quantified using an avidin based colorimetric assay. The biotinylation was performed using a EZ-link Biotin-PEG4-hydrazide in dimethyl sulfoxide (Thermo Scientific #21360). For the specificity testing, 100 µg/mL of HSA were here used; on average, blood has 40 mg/mL HSA, thus 100 µg/mL HSA is indicative of a 1:400 dilution of human blood [6]. In contrast 1:400 dilutions (from the original stock sample) of apple juice, orange juice, coffee, saliva, urine, and sodium chloride solution were examined. Indeed, positive and negative controls were considered in the study.

After corroborating the selectivity of the GK-1 sequence peptide towards HSA, GK-1 as well as B-GK-1 were covalently attached to carboxylate multi-walled carbon nanotubes (COOH-MWCNTs, from Cheap Tubes, Inc. Vermont, US. Purity >99%wt, ~4nm ID, 13-18nm OD, 1-12µm) through a condensation process using 1-Ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC). The purpose of using B-GK-1 as reference sample was to corroborate the covalent bonding between the $NH_2$ groups of the GK-1 and the COOH moieties present on the CNTs. The protein attachment upon the nanotubes was corroborated using a HABA/avidn [4'-hydroxyazobenzene-2-carboxylic acid, (MW 242.23, from Thermo Scientific #28010) / avidin (Thermo Sci 21121)] complex. Indeed, Biotin has a higher affinity toward avidin than HABA, thus in the Biotin–HABA/avifin interaction, HABA was displaced, and easily quantified through visible spectroscopy. It is worth noting, that due to the solid phase of the CNT-peptide complex, only the supernatant containing the HABA indicator was assayed. After corroborating the attachment of B-GK-1 onto the CNTs, direct attachment of GK-1 to the surface of MWCNT-COOH was performed, and the ability of the MWCNT-GK-1conjugate to bind HSA was investigated. Here, the HSA was biotinylated in order to once again quantify the HSA bound to the CNTs-GK-1 complex (see figure 2). In this process, equal volumes of MWCNT-GK-1 solution (27.4 mg/mL) and B-HSA (6.6 mg/mL or 9.851 x $10^{-5}$ mmol/mL) were placed together in a reaction test tube at ambient conditions and mixed gently for 2 minutes to allow for molecule interaction. The tube was then placed at rest and the MWCNT-GK-1-B-HSA conglomerates were allowed to fall out of suspension. After precipitation, the supernatant, representing the unbound B-HSA, was removed and quantified, allowing the calculation of B-HSA bound to the MWCNT-GK-1. The MWCNT-GK-1 were also exposed to an ultra-sonication process in a 2510 Branson sonicator for 30 minutes to investigate the robustness of the CNT-GK-1 binding.

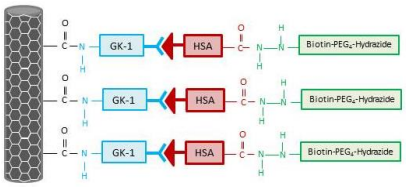


Figure 2. Schematic of the binding process between B-HSA and the CNT-GK-1 complex.

## 3 RESULTS AND DISCUSSION

The affinity of the GK-1 towards different analytes is shown in Figure 3. From the figure, it is observed that when GK-1 is subjected to comparable concentrations of HSA and everyday liquids, it binds with a distinctly higher specificity towards HSA. Included in figure 3, is the GK-1 binding results subtracting the absorbance negative control (see figure 3b). The figure shows that a normalized absorbance of 0.464 was observed for HSA, followed by absorbance of 0.081 for Orange juice. These results confirm that GK-1 binds approximately six times more to HSA than Orange Juice and over ten times more to HSA

than to saliva, urine or salt water. Figure 3b also shows a negative absorbance for the coffee and milk, suggesting the some binding process took place between the GK-1 and the non-analyte 5% NFDM block (negative control). However, this NFDM block-protein binding is much less than 30% than that shown between the protein and the HSA. It can also been determined that GK-1 has a lower binding affinity toward apple juice, coffee and milk than towards the 5% NFDM block.

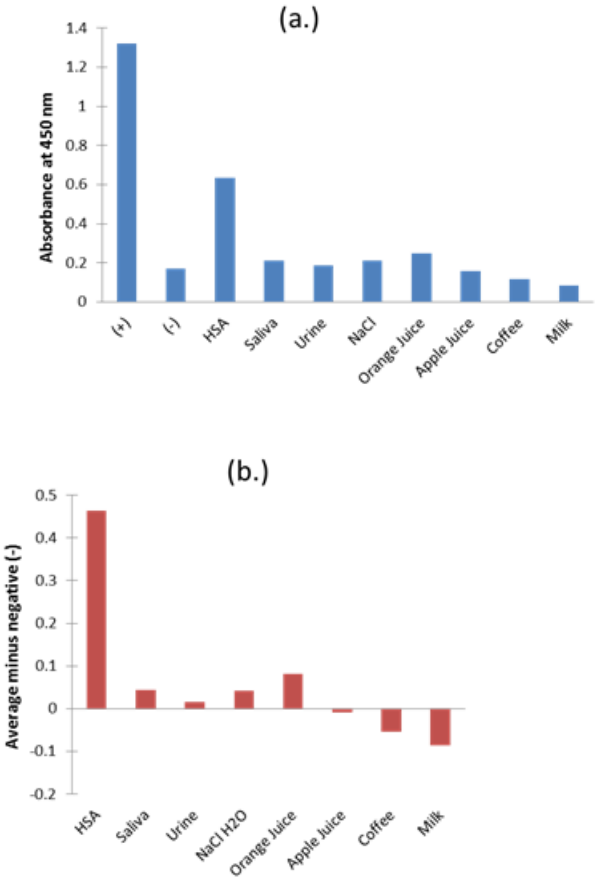


Figure 3. Absorbance results of 1:10 B-GK-1 against 1:400 HSA (100 µg/mL) in comparison to 1:400 dilutions of saliva, urine, salt water, orange juice, apple juice, coffee and milk (a) as-read (b) minus the negative control.

Following the investigation of the affinity of the GK-1 towards blood, the protein was incorporated onto CNTs in order to establish a semi-conductive platform for potentially developing a blood sensor. Here, the GK-1 was also biotinylated in order to corroborate and quantify the attachment. Hence, a mass balance was performed on the system to calculate the amount of peptide material bound to the CNTs. From the initial mass balance, it was determined that a maximum of $1.114 \times 10^{-4}$ mmol of B-GK-1 can be attached to $4.440 \times 10^{-3}$ mmol of COOH groups under the conditions studied, implying that approximately 40 moles of COOH interact with one mole of B-GK-1. However, an ideal external graphene spatial analysis, suggest that one mole of B-GK-1 could interact with up to 5 moles of COOH-CNT (see figure 4). This suggests that a physical carrying capacity on the surface of the CNTs is achieved before the chemical capacity. Indeed, even by taking into account the spatial constraints of the B-GK-1 binding, it can be noted that a complete surface binding still does not seem to take place in the reaction. Thus, in addition to the limited active surface area, the steric hindrance between B-GK-1 molecules, the nonlinear binding of the B-GK-1, and the presence of individual GK-1 and/or Biotin-$PEG_4$-hydrazide could be factors that limit the B-GK-1 binding. By considering the previous spatial analysis as well as the size difference between the B-GK-1 and GK-1 (GK-1 is approximately two thirds the size of B-GK-1), it can be ideally predicted that about 27 moles of GK-1 are bound to 1 mol of COOH-CNT. These results indicate that the binding process between the protein and the CNT requires some degree of optimization to further increase the amount of bound GK-1 on the nanotubes. Indeed, a higher amount of present GK-1 on the CNTs would result in a bio-sensing platform with a superior sensitivity.

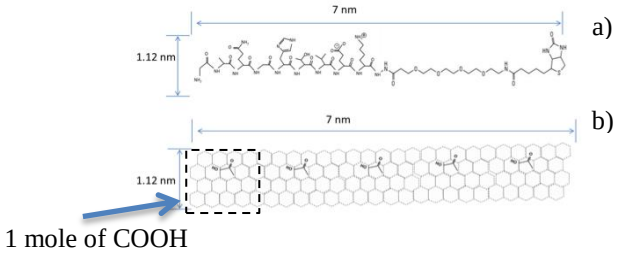


Figure 4. Dimensional analysis of the B-GK-1 and COOH-CNT used. The dashed area encloses one mole of COOH-CNT, by containing 1 COOH unit per approximately 20 carbon rings.

An appropriate mole-weight transformation needs to be taken into account on MWCNTs, by considering either external and/or internal-external layers within the nanotubes. Besides the surface COOH-CNT-GK-1 crowding aspects, it has been shown that a specific protein towards blood can be attached to the surface of CNTs. The corroboration of the binding between the HSA and GK-1 was carried out by biotinylating the HSA and exposing 5.96E-6 mmol of serum to 6.78E-6 mmol of biological functionalized CNTs. Thus, the amount of B-HSA bound to the MWCNT-GK-1 system was determined through the HABA/avidin assay, resulting in a 3.36E-6 mmol of HSA bound on the biologically functionalized CNTs. Here, a negative control, consisting of plain MWCNTs was also incorporated in the research program. Thirty minutes ultrasonicated MWCNTs-GK-1 were also exposed to B-HSA, and it was found that 1.81E-6 mmol of HSA was bound to the protein based nanotubes. Ultrasonicated plain MWCNTs were also subjected to a reaction with B-HSA; however, it was found that no presence of HSA was able to be quantified in the HABA/avidin assay. These results

suggest that the biological functionalization is required to bind the HSA. Once again, this is an encouraging outcome for potentially developing an e-textile based CNTs capable of detecting blood. Current work on the inclusion of MWCNTs-GK-1 on textile threads is under investigation.

## 4 CONCLUSION

The present research has investigated the selectively process of a specific peptide sequence towards HSA, which in this research program has been considered as an indicator of blood. The results have shown that the GK-1 peptide has a marked specificity toward HSA over other miscellaneous liquids that a person may come in contact with on a daily basis such as orange juice, coffee, and milk. Here, the corroboration was performed through an indirect ELISA testing. Following the assessment of the affinity of GK-1 towards HSA, it was also demonstrated that a robust covalent bonding of the protein onto carboxylated multi-wall carbon nanotubes through a carbodiimide catalyzed amide linkage can be achieved. The attachment of GK-1 onto the MWCNTs was corroborated by biotinylating the protein, followed by their quantification using a HABA/avidin process. An ideal calculation of the ration between the CNT functional groups COOH and the GK-1 suggest that a synthesis optimization is required in order to increase the number of GK-1 moles on the nanotubes. The interaction between HSA and MWCNTs-GK1 was also here investigated, and it was shown that the HAS effectivly binds the biological functionalized nanotubes. These preliminary results suggest a promising path for the development of selective blood sensors such as smart textiles.

## REFERENCES


[1] Tománek, D. et al. In *Carbon Nanotubes: Advanced Topics in the Synthesis, Structure, Properties and Applications*; Jorio, A., Dresselhaus, G., Dresselhaus, M.S., Eds.; Topics in Applied Physics; Springer: Berlin, **2008**, *111*, 1-12.

[2] Lin, J. Ng, H.T. Carbon Nanotube Sensors. In *Encyclopedia of Nanoscience and Nanotechnology*; Nalwa, H.S., Ed.; America Scientific Publishers. **2004**, *1*, 591-604.

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

[4] McGraw. S.K. et al. *Nanotech.* **2011**, *3*, 56-59.

[5] Olszewski , A. *Nanotech.* **2012,** 1, 212, 256-259.

[6] Shim, B.S. et al. *Nano Letters.* **2008**, *8* (12), 4151-4157

[7] Panhuis, M. Wu, J. Ashraf, S.A. Wallace, G. *Synthetic metals*. **2007,** *157*, 358-362.

[8] Zhu, Z. Song, W. Burigapalli, K. Moussy, F. Li, Y. Zhong, X. **2001**, *21*, 165501-165511.

[9] P. Cortes et al. *Proc. of the Annual AIChE meeting.* **2014,** Paper 345517.

[10] Adams, P.A. et al. *J. Inorganic Biochem*. **1989**, *37*, 91-109.

[11] Pingali, A. et al. *J. Molec. Recog*. **1996**, *9*, 426-432.