

Bio Nano Ink for Printing Membrane Proteins

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1. Experimental

1.1:- Carbon nanotube Functionalization

Single-walled CNTs are purchased from “Nanointegris Technologies Inc.” Canada. To make CNT hydrophilic, their surfaces were treated with acid as described previously. Briefly, 5 g of CNTs were refluxed in 100 mL of 98% H₂SO₄ and 68% HNO₃ (1:3 v/v) for 40 min at 110°C. The treated CNTs were centrifuged and then thoroughly washed several times by sonication with ultrapure water. Finally, the CNTs were dispersed in ultrapure water (~1 mg/mL) by probe sonication at room temperature for several hours.

1.2:- Synthesis of bR protein wrapping peptides

The polymerizable peptide, acetyl-(octyl)GSLSL(*N*-1-octene)D(octyl)GD-NH₂ was synthesized using an infinity 2400 peptide synthesizer (AAPPtec) according to the 9-fluorenylmethoxycarbonyl (Fmoc) approach using Rink Amide AM resin (0.46 mmol/g loading). HCTU (2-(6-chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate) was used as the amino acid coupling reagent. All the Fmoc-protected amino acids were purchased directly from AAPPtec Inc. Peptides were acetylated at the N-terminus, cleaved from resin in the presence of 95% trifluoroacetic acid (TFA) and 2.5% triisopropylsilane, and then purified by reverse-phase HPLC (solvents CH₃CN/H₂O, 0.1% TFA). The molecular weight of each peptide was verified by liquid chromatography-mass spectrometry. The purity of peptide was found to be above 95% according to HPLC analysis.

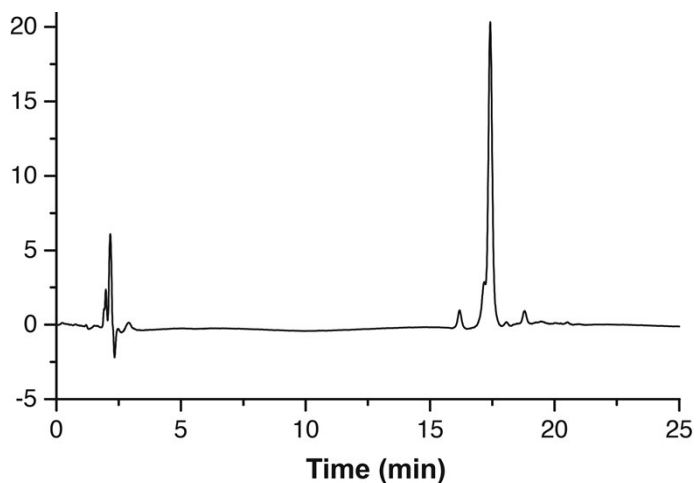


Figure S1:- HPLC chromatogram of polymerizable peptide.

The peptide mass analysis was performed on an Agilent 1100 LC/MSD Trap system with an electrospray ionization (ESI) interface. 20 μ L of peptide samples were injected into the LC/MSD system through an Agilent 1100 series autosampler. Separations were carried out on a 5 μ m Agilent ZORBAX SB-C18 column (4.6 \times 150 mm), with a flow rate of 1 mL/min using HPLC method II or I. Methanol used as mobile phase solvent used in these methods. The flow was directed to the electrospray source after passing through an Agilent 1100 series diode array multiple wavelength detector (detection at 250 nm). The LC-MSD-Trap-00045 was operated at ESI negative ion mode (ES⁻) with a capillary voltage of 3500 V. Data were acquired over the m/z range of 100–1600 under standard mode with normal scan resolution (13 000 m/z per s).

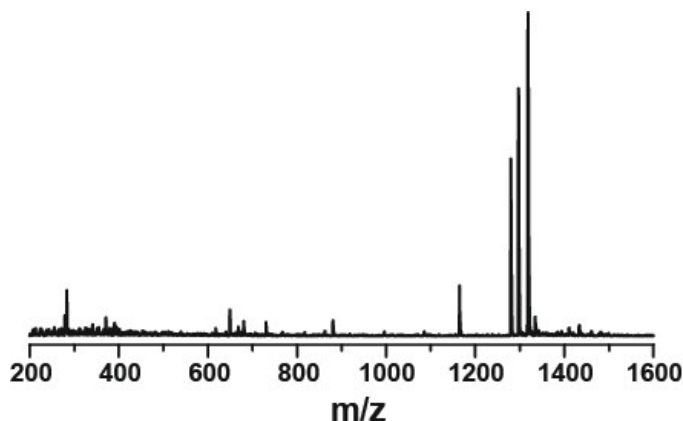


Figure S2:- LCMS spectrum of polymerizable peptide.

1.3:- Wrapping of bR protein with Beta sheet peptide

Wrapping of bR protein with polymerizable beta sheet peptide was carried out in a 40:1-molar ratio (peptide to protein). The excess peptides were removed by dialysis against 50 mM PBS buffer (6 kDa cutoff membrane) at 4 °C in dark for 3 days. Regular exchange of PBS buffer was carried out in order to complete the unbound peptide exchange. The peptide wrapping studies were carried out on a circular dichroism (CD) spectra were recorded on a JASCO CD spectrometer at 25 °C using a quartz sample cell with 1 mm path length (3 s integration per step, an average of three scans). Samples of β -sheet peptides (BPs) were prepared at concentrations of 0.5 mM in 50 mM PBS buffer. Molar ellipticity $[\theta]$ was calculated from the equation $[\theta] = (100 \times \theta)/(c \times l)$, where θ = observed ellipticity (in degrees), c = concentration (in moles per liter), l = cell path length (in centimeters).

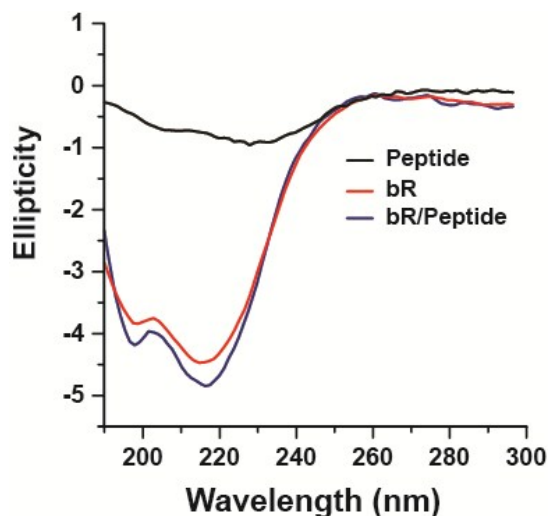


Figure S3:- CD spectra of the polymerizable peptide (beta sheeted peptide) wrapped on bR protein.

1.4:- UV-vis Analysis of bR protein (Light-induced proton transport activity)

Absorption spectra of bR protein were conducted on PerkinElmer Lambda 1050 spectrometer. The bR solution (3.8×10^{-5} M in 100 mM PBS buffer) was placed in standard 1 cm pathlength quartz cuvette and was irradiated with 535 nm light (long band pass filter) the absorption spectra were scanned at regular interval. The λ_{\max} at 560 nm decreased significantly and a slight increase in λ_{\max} at 365 nm, which may attribute to the bathochromic shift of chromophore band bR solution. This photo-reaction analysis provide direct evidence of proton-pumping of bR protein with absorbance changes at 560 nm representing the recovery of the original Trans, 365 nm representing the M-decay and 560 nm representing the N(O)-decay.

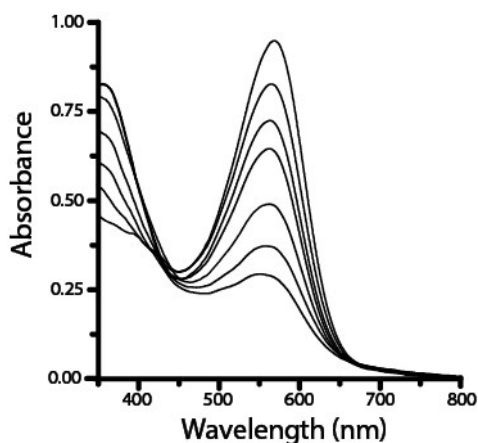


Figure S4:- UV-vis spectra of peptide wrapped bR protein irradiated with 535 nm light.

1.5:- Synthesis of Silver nanoparticles

The silver nanoparticles were prepared by a one-step synthetic method using ethylene glycol and glucose as reducing agents. In a typical procedure, 156 mg of AgNO_3 and 5g of polyvinyl pyrrolidone (PVP) dissolved in 100 mL of Ethylene glycol in a 150 mL round bottom flask and heated it at $50^\circ\text{-}70^\circ\text{C}$ under an inert atmosphere. The conversion of silver nanoparticle was monitored by the precipitation test with sodium chloride solution. Formation of turbidity in the reaction solution indicates the presence of ionic silver while a clear solution confirms completion of the reaction. The Transmission electron microscope image of the silver nanoparticle reveals the size of around 6 nm.

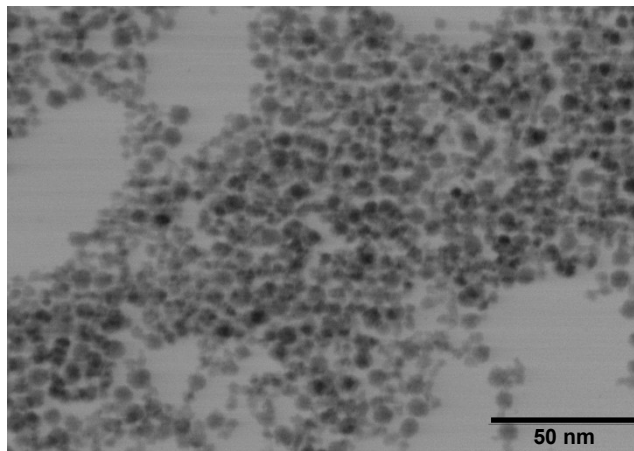


Figure S5:- TEM image of silver nanoparticle.

1.6:- X-Ray Diffraction (XRD) Analysis

Wide angle X-Ray diffraction measurements were performed using a Bruker D8 advance system with the diffraction patterns were recorded using a copper $\text{Cu K}\alpha$ irradiation ($\lambda = 1.5406 \text{ \AA}$) X-ray source with scanning angle range of $10^\circ\text{-}90^\circ$ at a scanning rate of $10^\circ/\text{min}$ at 40 mA and 40 kV. The diffractometer was used in transmission mode. The XRD patterns of hydrogel and Hydrogel/CNT/bR/AgNP shown in Figure S# indicate the crystalline structure of the 3D printed samples. The diffraction peaks from 38.24° to 44.4° , 64.5° , 77.5° , and 83.5° of the hydrogel correspond to the face-centered cubic cell of silver nanoparticle unit cell. The diffraction peaks at 27.5° due to the (002) plane of CNTs and other peaks may be due to the NaCl. Furthermore, no distinct diffraction peak observed on the hydrogel, indicating that the amorphous nature of the 3D printed hydrogel without silver nanoparticle.

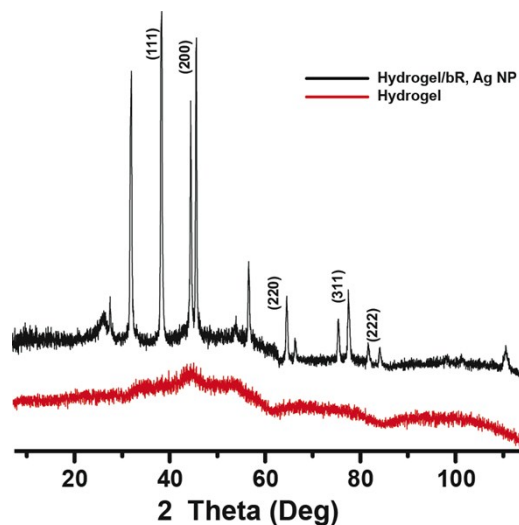
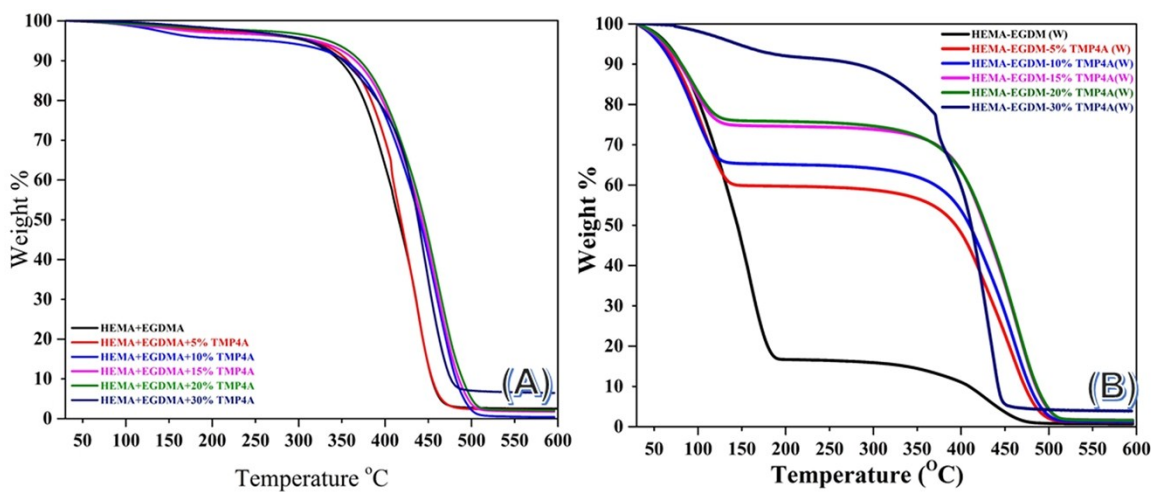


Figure S6:- XRD pattern of the 3D printed hydrogel (-) and hydrogel/bR, Ag nanoparticles (-).

1.7. Thermogravimetric Analysis



Sample	T _g	T _g (w)	Water absorbed
HEMA-EGDM	375.6°C	370.8°C	83%
HEMA-EGDM-5% TMP4A	383.9°C	385.4°C	63%
HEMA-EGDM-10% TMP4A	391.8°C	392.0°C	35%
HEMA-EGDM-15% TMP4A	396.9°C	397.3°C	25%
HEMA-EGDM-20% TMP4A	399.9°C	400.1°C	23%
HEMA-EGDM-30% TMP4A	392.1°C	399.5°C	14%

Figure S7: Thermogravimetric curves of 3D printed discs with different crosslinker amount (A) before and (B) after overnight soaking in buffer. Table shows the water uptake with varying amount of crosslinker.

1.8. Electrochemical Analysis

Electrochemical analyses were done on a Gamry Reference 3000 electrochemical analyzer in a three-electrode setup using 3D printed hydrogel, an Ag/AgCl electrode, and a platinum wire as working, reference and counter electrode, respectively. Polarization curves were obtained by linear sweep voltammetry (LSV) method with and without light irradiation (535 nm light, 50 mW cm⁻²) with a scan rate of 2 mV/s. Hydrogel electrode. Tafel plot of the without silver nanoparticle embedded and with Silver nanoparticle/bR/CNT hydrogel electrodes was also obtained and the reference electrode was calibrated to the reversible hydrogen electrode scale in all measurements as follows: $E \text{ (RHE)} = E \text{ (SCE)} + 0.655 \text{ V}$ in 1.0 M phosphate buffered saline (PBS) solution.

The hydrogel was placed in a quartz cell contains 1.0 M phosphate buffered saline (PBS) solution at pH 7.4 and the solution was purged with N₂ gas for 30 min to remove the dissolved oxygen. The hydrogel electrode was irradiated with 535 nm light (intensity 50mW/cm²) to activate the bR protein. The hydrogen generated was collected into a tube and were collected from the cell using a gas-tight syringe and finally analyzed by gas chromatography. The hydrogen evolution was quantified by gas chromatography (Agilent Technologies 6890N Network GC system, TCD detector, 5 Å molecular sieve column).

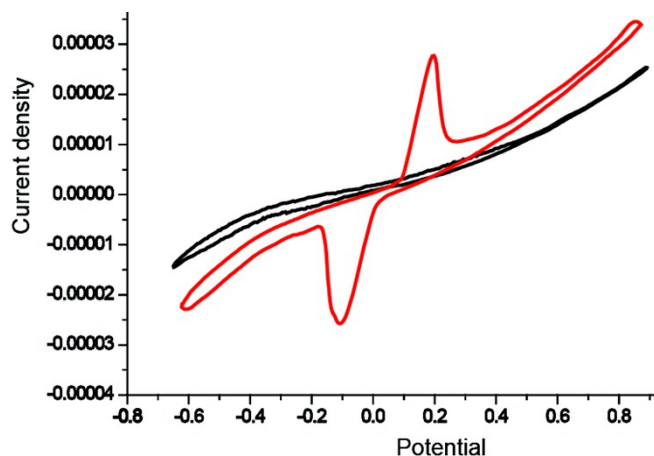


Figure S8:- Cyclic voltammograms of the Silver nanoparticle embedded on CNT hydrogel and (b) hydrogel electrode without Silver nanoparticles in 1M PBS solutions at pH 7.4 at a scan rate of 25 mV/s. The peaks are due to electrochemical dissolution of silver nanoparticles on the electrode surface, at ~ +0.18 V and reduction of the generated silver ions in the oxidative part of cycle from the solution at ~ -0.09 V. The cyclic voltammogram peaks appear broader, due to that silver oxide and silver phosphate are both involved in the redox reactions

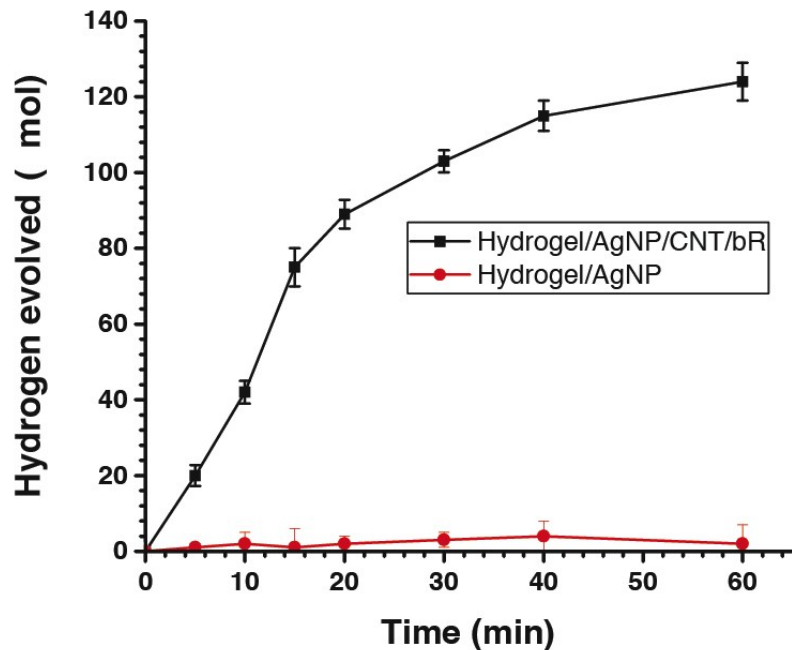


Figure S9:- H₂ evolution rates of Hydrogel electrode incorporated with silver nanoparticles, bR protein and CNT (black) and hydrogel with silver nanoparticles only (red) in an PBS (1M at pH 7.4) irrigating the electrode with 535 nm light (intensity 50mW/cm²) for an hour.