

Supporting Information

Chemically Reactive Protein Nanoparticles for Synthesis of Durable Superhydrophobicity—That performs in Severe Settings

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Experimental section

Materials: Bovine serum albumin ($M_w \sim 66.5$ KDa, fraction V), dipentaerythritol pentaacrylate (5Acl, $M_w \sim 524.51$ gmol⁻¹), octadecylamine, methylene blue, silicon oil, chloroform, ethylacetate were purchased from Sigma-Aldrich (Bangalore, India). Absolute ethyl alcohol (CAS 64-17-5, Lot 17030799) was purchased from TEDIA Company (United States of America). Methanol (CAS 67-56-1) was purchased from RANKEM (Maharashtra, India). Reagent grade THF was purchased from RANKEM (Maharashtra, India). Nile red was purchased from Tokyo Chemical Industry. Dichloroethane was purchased from Loba Chemie Pvt. Ltd., Mumbai, India. Adhesive tape was purchased from Jonson Tapes Pvt Ltd., New Delhi, India. Motor oil (20W-40) was purchased from Castrol India Ltd. Kerosene, petrol, diesel were purchased from Indian Oil petrol pump. Vegetable Oil and medical cotton were purchased from local shops in Guwahati, India. Crude Oil is collected from oil India refinery, Assam.

General considerations: Glass vials (Borosil) used for preparing the solutions were washed thoroughly with acetone and ethanol prior to use. The Zeta potential (ζ) and dynamic light scattering (DLS) analysis was carried out using Zetasizer Nano ZS90 (model no. ZEN3690). Contact angle measurements were taken using a KRUSS Drop Shape Analyser-DSA25 with an automatic liquid dispenser at ambient conditions. FTIR spectra were recorded with a Perkin Elmer instrument at ambient conditions using KBr pellets. Scanning electron microscope images were obtained using a Sigma Carl Zeiss scanning electron microscope (samples were coated with a thin layer of gold prior to imaging). Fluorescence microscopic images of the superhydrophobic cotton before and after loading of Rh-6G and Fluorescein were acquired using a AX10 observer Z1 & AXio Cam MRCS, Carl Zeiss, Germany. Digital images were acquired using a Canon Powershot SX420 IS digital camera. Milli-Q grade water was used for all experiments.

Synthesis of Chemically 'reactive' BSA-Protein nano-complex:

BSA nanoparticles were prepared by following desolvation method. Briefly, 3 ml of ethanol was dropwise (at the rate of 1ml/min) added into 1ml (30mg/ml) of BSA in aqueous solution, under constant stirring (600 rpm). Thereafter, the resulting solution was centrifuged down (12000 rpm for 15mins) and then re-dispersed in 5Acl/methanol (1.325g in 10ml) to introduce chemically reactive covalent cross-linking. The resulting nano-complexes were centrifuged down (12000rpm for 10mins), and re-dispersed in methanol and subsequently centrifuged down to wash (once) off the loosely bound 5Acl molecules. Then, the obtained nano-complexes were re-dispersed in Milli-Q water. The size of the nanocomplex and the surface potential was characterized using DLS and the morphology was examined using field

emission scanning electron microscopy (FESEM). FTIR spectra were recorded for examining the residual chemical functionalities in the synthesized material.

Bovine Serum Albumin (BSA) Derived Superhydrophobicity:

To develop a durable and highly compressible superhydrophobic material from a naturally existing ingredient, following steps were followed 1) fibrous medical cotton of dimension (1.5cm×1.5cm×0.5cm) was dipped in 3ml aqueous solution of bovine serum albumin (BSA) of concentration 10 mg/ml and kept for continuous agitation for 3 hours. 2) 6 ml of ethanol was added dropwise and the BSA solution appeared turbid due to the formation of nano-complex and the whole system was left undisturbed for 1 hour. Subsequently, the cotton was washed thoroughly with ethanol. (3) The BSA soaked cotton was then further treated with 5Acl in methanol (1.325g in 10 ml) for 3 hours, after which it was washed with methanol to remove the loosely adhered 5Acl molecules. (4) To achieve the liquid water anti-wetting property, the chemically reactive BSA coated cotton was treated with octadecylamine solution (5 mg/mL) for overnight. Next, the material was washed thoroughly with ethanol and left to air dry. The anti-wetting property of the fibrous substrate treated with amine containing small molecule was examined through visual inspections and contact angle measurements.

Physical and Chemical Durability Tests: Various severe and practically relevant standard physical and chemical durability tests were adopted to examine the durability of the serum protein derived superhydrophobic cotton. The details of each of these tests have been explained in details below:

Compressive Deformation: The superhydrophobic cotton was manually compressed with 0% to 80% compressive strain and the material restored its shape after releasing the applied manual stress. The manual compressive strain (80 %) was repetitively incurred for 1000 times. The water wettability was examined at regular intervals to account for the durability of the embedded superhydrophobicity in the BSA derived superhydrophobic cotton.

Adhesive Tape Test: Adhesive tape was fixed onto the BSA derived superhydrophobic cotton and peeled out to arbitrarily expose the interior of the synthesized material. The contact angle and digital images were thereafter acquired to examine the anti-wetting property on the freshly exposed interior of the as-synthesized material.

Sand Paper Abrasion : Sand paper abrasion was executed by fixing the protein coated cotton (2cm x 2cm) onto a glass slide using adhesive tape and thereafter, rubbed onto a sand paper placed beneath it in a back and forth motion with 200g load atop the substrate. Subsequently, the anti-wetting property was examined using contact angle measurement and digital images.

Finger Wiping Abrasion : The filter paper abrasion test was carried out by fixing the serum based cotton substrate (2cm X 2cm) onto a glass slide and subsequently, rubbing it with finger multiple times in a to and fro motion. Thereafter, the extreme water repellent property was examined through contact angle measurements and digital images.

Physical Manipulations: Bending, Creasing, Twisting and Winding was performed on the BSA coated superhydrophobic cotton for 25 times to examine the durability of the material. The anti-wetting property was further validated after these physical manipulations through contact angle measurements and digital images.

UV Irradiation Test: The serum protein derived superhydrophobic cotton was exposed to both short (254 nm) and long (365 nm) wavelengths UV radiation for 30 days. The water wettability was regularly examined by measuring the water contact angle and visual inspections.

Chemical Durability Tests: The as synthesized BSA protein based superhydrophobic cotton was exposed to various severe and practically relevant aqueous media including acidic (pH 1), alkaline (pH 12), anionic (SDS, 1mM)/cationic (DTAB, 1mM) surfactant contaminated aqueous phases, artificial sea and river (Brahmaputra, Assam, India) water for 30 days. The artificial sea water was prepared by mixing MgSO_4 (0.325g), MgCl_2 (0.226g), CaCl_2 (0.112g) and NaCl (2.673g) in 100mL deionized water in a volumetric flask. The water wettability was examined at regular intervals by measuring the water contact angle and visual inspections.

Selective Absorption Based Remediation of Oil Spills:

Model light oils including motor oil and silicon oil were separated from air/water interfaces successfully using the BSA protein derived superhydrophobic cotton. Briefly, 1ml (dyed with Nile red in case of silicon oil for visual inspection) of the oil was placed in a petri-dish filled with water. The superhydrophobic cotton was then placed next to the floating oil and the superhydrophobic cotton immediately started absorbing the respective oil phase selectively from the oil contaminated aqueous phase. Thereafter, the oil can be recovered by manually squeezing of the synthesized superhydrophobic material. Further, heavy oil—that is dichloroethane (model heavy oil) settled at the bottom of the water could be successfully separated using the superhydrophobic cotton. Briefly, 5ml of the oil (dyed pink for visual inspection) was placed in a beaker filled with 25 ml of water (dyed blue for visual inspection) and thereafter, the cotton brought in contact with the oil/water mixture, the superhydrophobic cotton instantly soaked the oil selectively and the oil could be recollected by squeezing the oil off the substrate manually.

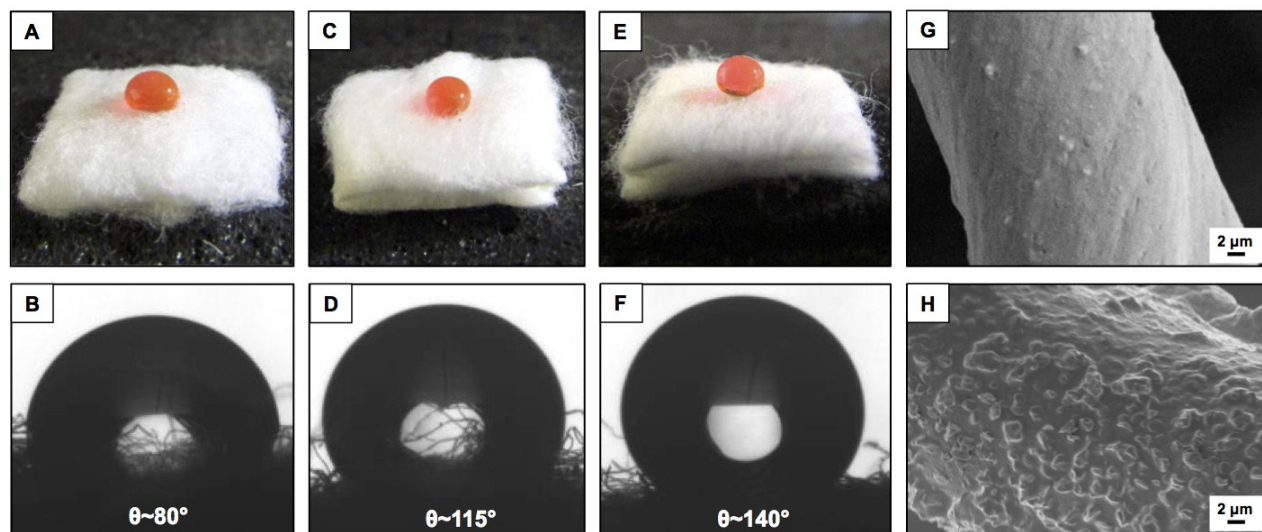


Figure S1. A-F) Digital images (A,C,E) and contact angle images (B,D,F,) of beaded water droplet on bovine serum albumin (BSA) coated (post modified with ODA) cotton that were prepared by varying the concentrations of BSA solution i.e., 3 mg/mL (A-B), 5 mg/mL(C-D) and 7 mg/mL (E-F). FESEM images of pristine cotton (G) and BSA coated cotton (H) that prepared with protein concentration of 7mg/mL.

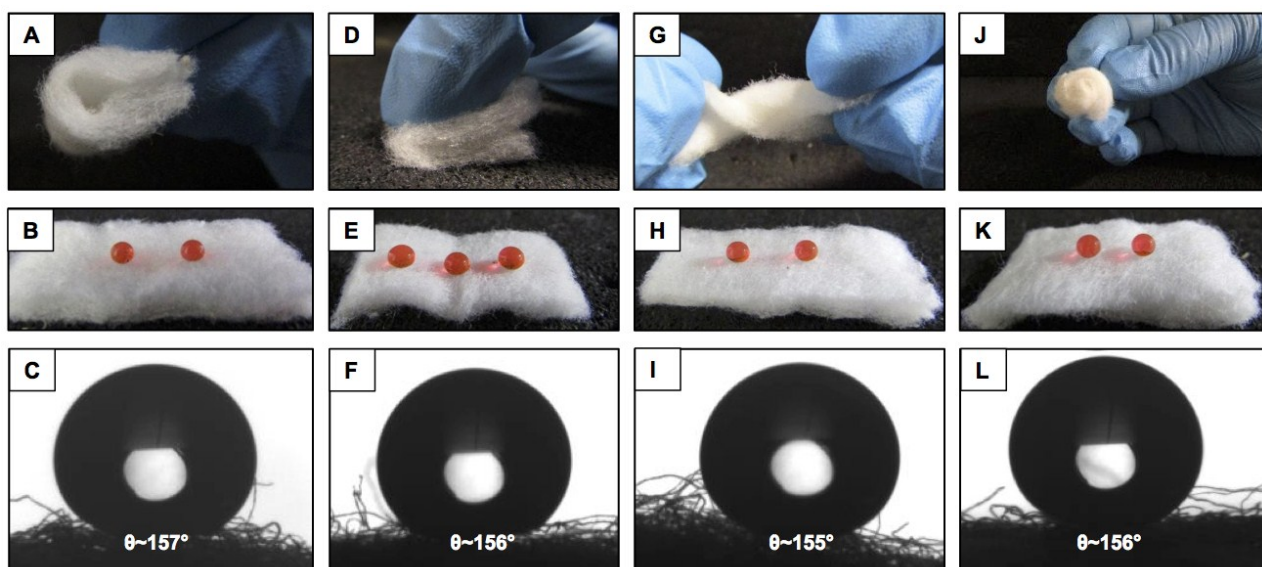


Figure S2. Digital Images (A-B, D-E, G-H, J-K) and water contact angles (C, F, I, L) demonstrating the impact of various physical manipulations—including bending (A-C), creasing (D-F), twisting (G-I) and winding (J-L) on the BSA coated superhydrophobic cotton.

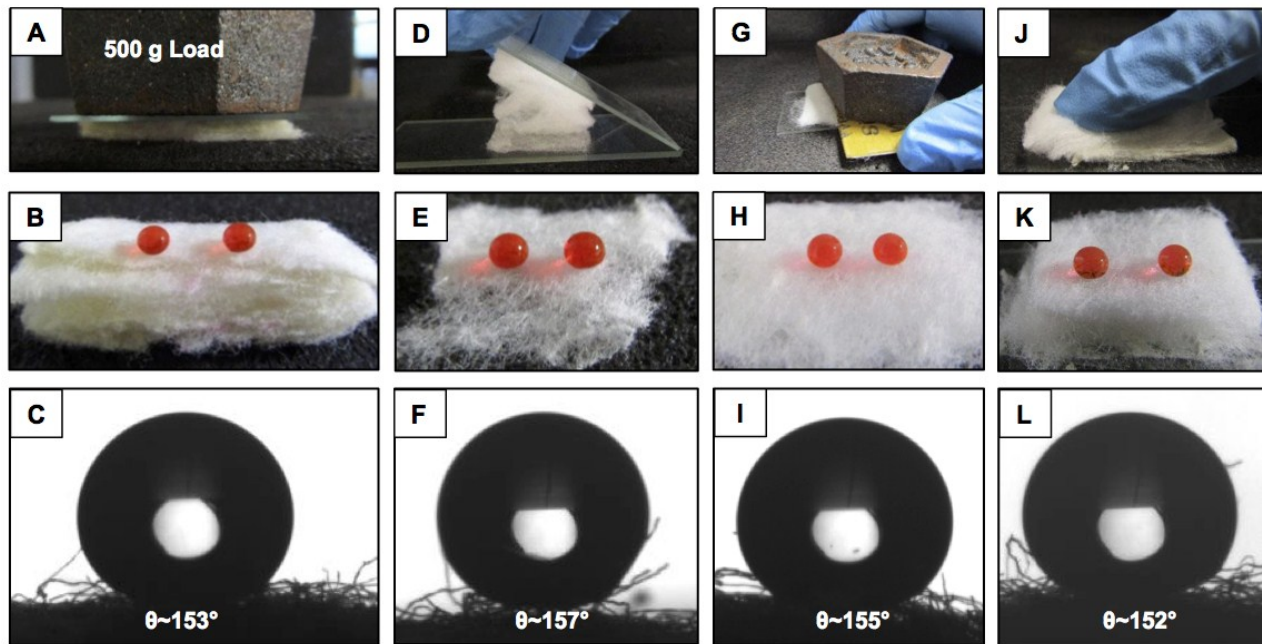


Figure S3. Digital images (A, D, G, J) illustrating the process of various physical abrasions that were performed on the superhydrophobic cotton, 500 g of load was applied to perform the compressive deformation. The substrate recovered its dimensions on releasing the load. The superhydrophobic property remained intact, after successive application of this load for 500 times as confirmed by the digital image (B) and contact angle image (C) of the beaded water on the treated interface. Digital images (D-E) accounting for the adhesive tape test, the contact angle (F) revealed the existence of unperturbed superhydrophobic in the cotton even after arbitrarily tearing the cotton during adhesive tape test. Digital image (G) demonstrating the set-up of the abrasive sand paper test under applied load, whereas the digital image (H) and contact angle (J) image showing the wettability of beaded water droplets on the abrasive sand paper treated interfaces. Digital image (J) illustrating the finger wiping abrasion test, digital (K) and contact angle (L) images confirmed the presence of intact superhydrophobicity in the BSA derived nature-inspired coating.

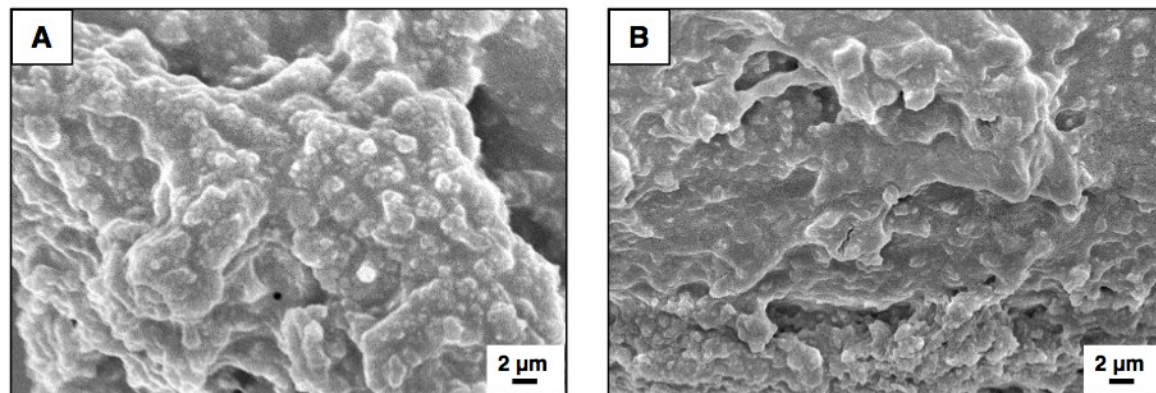


Figure S4. FESEM images of BSA derived superhydrophobic cotton after performing adhesive tape test (A), sand paper abrasion (B).



Table S1

No. of Pieces	$\theta_{\text{Static}} (^{\circ})$		$\theta_{\text{Hys}} (^{\circ})$	
	Water	Oil	Water	Oil
1	157.6±1.1	0	7.4±0.7	0
2	158.1±1.7	0	8.1±1.0	0
3	155.9±1.2	0	7.7±1.1	0
4	156±2.3	0	8.5±0.2	0

Figure S5. (A) Digital image of superhydrophobic cotton that was arbitrarily torn into 4 pieces and water droplets (red dyed) beaded onto the substrate to examine both water (red in color) and oil (yellow) wettability throughout the material. Table S1 accounting for the detailed oil and water wettability on the superhydrophobic cotton.

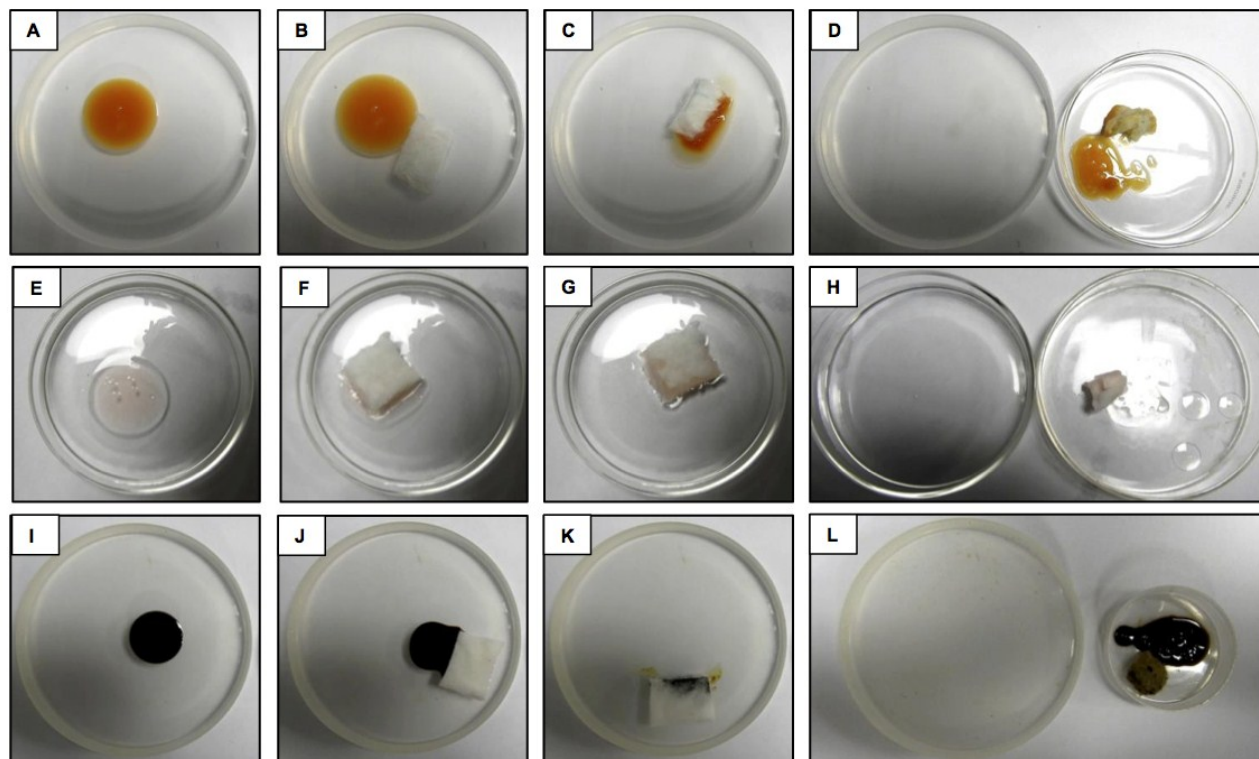


Figure S6. A-L) Digital images illustrating the separation of motor oil (A-D), silicon oil (E-H) and crude oil (I-L) from aqueous phase using superhydrophobic cotton through selective absorption of oil and the separated oil was recovered by squeezing the material.

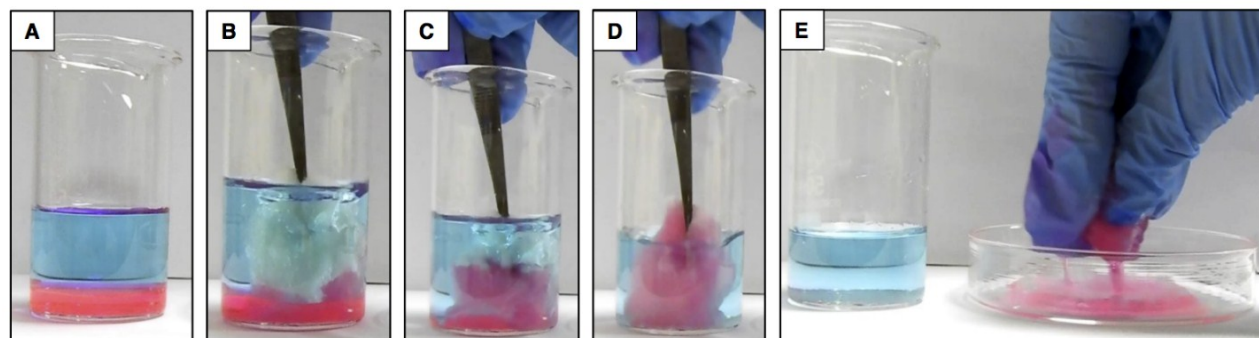


Figure S7. A-E) Digital images illustrating the separation of model heavy oil (dichloroethane,DCE; dyed pink for visual inspection) using BSA derived superhydrophobic cotton, the sediment oil/oily phase at the bottom was selectively absorbed by the synthesized material.

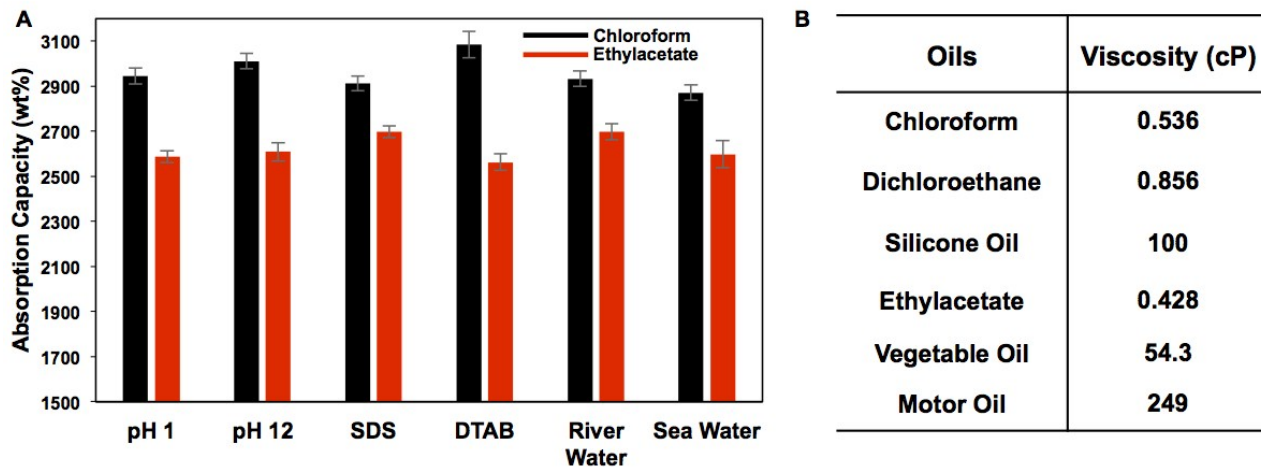


Figure S8. Plot accounting for the absorption capacity of oil from a mixture of oil/water for model oil ethylacetate (red) and chloroform (black) in different chemically severe settings i.e. acidic and basic water, surfactant contaminated water (SDS,DTAB), artificial seawater and river (Brahmaputra river, Assam) water. B) the table accounting for the viscosity values of different oils used for oil/water separation demonstrations.

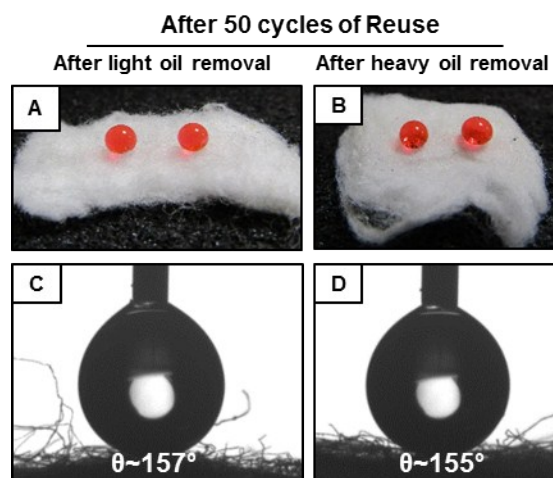


Figure S9. A-D) Digital images (A-B) and contact angle images (C-D) illustrating the water wettability on the BSA derived superhydrophobic cotton after 50 cycles of repetitive removal of both light (ethylacetate, A, C) and heavy (chloroform, B, D) oils.

