Electronic Supplementary Information (ESI)

Multifunctional solvothermal carbon derived from alginate using 'water-in-Deep eutectic solvents' system for enhancing enzyme activity

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Experimental Section

Materials: Cytochrome c (Cyt-c) from equine heart (95%), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (98%), hydrogen peroxide solution (H_2O_2) 30% (w/w) in H_2O purchased from Sigma Aldrich, alginic acid (AA), choline chloride (ChCl; purity >99%), ethylene glycol (EG; purity >99%) were received from SD fine chemicals. Anhydrous sodium phosphate monobasic, sodium phosphate dibasic dehydrate were purchased from Sisco Research Lab (SRL). In experimental process samples were prepared in sodium phosphate buffer pH 7 (10 mM) using deionized water. All the chemicals were used as received without further purification.

Preparation of water-in DES system: Initially, deep eutectic solvent (DES) was prepared by mixing 1:2 molar ratio of ChCl and EG (given density of DES is 1.13 g/ mL).[1,2] This mixture was heated at 80 °C with constant stirring at 500 rpm for 3h.[1]Further, different concentration of DES were made by performing dilutions as mass fraction(wt%) using DI water. In a typical experiment, 90.4 g of DES was dissolved in 20 mL of water to get 81.8 wt% of DES. Calculated using formula-

$$\frac{Mass\ of\ DES}{Mass\ of\ DES + Mass\ of\ Water} \times 100 \text{ (wt\%)}$$

Similarly, 62.8 wt%, and 42.9 wt% aqueous DES systems were prepared by adding 67.8 g, and 45.2 g of DES in 40and 60 mL of DI water, respectively.

Synthesis of alginic acid derived functional carbons (AACs): AA is derived from a naturally occurring brown seaweed and thus it has great potential as a promising renewable resource due to its inherent advantages such as a fast growth rate and easy cultivation. AA has very low solubility in water but an excellent solubility (7.5 wt%) was observed when using the ChCl–EG based DES. The reaction mixture (50 mL) was prepared by dissolving 7.5 wt% of AA to water and different aqueous DES systems prior to solvothermal treatment. The reaction mixture was transferred to 100 mL Teflon-lined stainless steel autoclave which was sealed. Solvothermal carbon was prepared at different reaction conditions (time and temperature). Then the autoclave was heated to a range of temperature 180−220 °C and various time duration of 3−12 h using a hot air oven. The resulting black material was washed with DI water (3 times) and isopropanol (3 times) and was dried at 60 °C for 3 h. The composition of reaction mixture, reaction condition and coding of samples is represented in Table S1. All the obtained carbon material was finely powdered for further characterisation.

Activity measurements: The peroxidase activity of Cyt-c was studied at varying concentrations of AACs (20, 40, 60, 100, 200 µL of 1 mg mL^{-1} stock solution per 600 µL of sample) prepared under different reaction conditions (temperature, time and % DES) in phosphate buffer pH 7. Cyt-c displays peroxidase activity which was performed with ABTS as a substrate in presence of H_2O_2. A green-coloured $ABTS^{+\cdot}$ radical forms as a result of oxidation of ABTS which is initiated after addition of H_2O_2. This formation of $ABTS^{+\cdot}$ radical was monitored by changes in the absorption spectra at 420 nm. The absorption spectra were measured for 2 µM of Cyt-c, 3 mM of ABTS, 1 mM of H_2O_2 different functional carbon. The concentration of different AACs (at different reaction conditions) were 20, 40, 60, 100, 200 µL out of 1mg/mL stock solution of AACs for 600 µL sample in 10 mM phosphate buffer (pH 7). The relative activity was calculated considering 100% of enzyme activity in 10 mM phosphate buffer (pH 7). All the samples were equilibrated for 45 min. The activity was measured three times and average activity is presented.

Spectroscopic studies to probe to stability of Cyt-C: For analyzing the structural stability of Cyt-c in presence of STCs, UV-visible and circular dichroism (CD) spectroscopies were performed. UV-visible spectroscopy of Cyt-c in presence and absence of STCs was investigated on Shimadzu UV-1800 (Japan) spectrophotometer having highest resolution (1 nm) using 1 cm path length quartz cuvette. CD spectroscopic studies of Cyt-c were recorded on Jasco-815 (150-S) spectrophotometer, equipped with a Peltier system for temperature control and wavelength range of 163-900 nm were performed. Far UV-CD spectra were observed in the range 190–250 nm. The response time of 1 s and 1 nm bandwidth was used with a scan speed 100 nm/min. The composition of secondary structures for Cyt-c under different conditions was calculated using online CD analysis program, DICHROWEB, with the help of CONTIN/LL algorithm.[3-6] The enzyme concentration was taken as 0.124 mg/mL in 10 mM phosphate buffer (pH 7) and the concentration of different AACs (at different reaction conditions) were 20, 40, 60, 100, 200 µL out of 1mg/mL stock solution of AACs in 10 mM phosphate buffer (pH 7). The samples were equilibrated for 45 min. All spectra displayed here are procured after blank subtraction. All the AACs were dispersed using ultrasonicator for 30 mins before performing experiment.

Characterization: The chemical composition of AAC material was determined using CHNS analyzer ElementarAnalysensysteme Germany, Model-Vario Micro Cube. To investigate functional groups present, FTIR was carried out using a Perkin Elmer instrument with wavelength range between 4000-600 cm^{-1} by making pellet of AAC material by mixing with KBr. Field emission scanning electron microscopy (FESEM) was used to study surface morphology using a JEOL model-JSM7100F instrument. Raman spectra were recorded using a Renishaw Invia II Laser Raman spectrometer using source of 514 nm. X-Ray

photoelectron spectroscopy (XPS) Physical Electronics Model-PHI 5000 Versa Probe. The zeta potential of STC was determined using dispersed aqueous solution (1 mg/mL) with ZetasizerNano ZS light scattering instrument, Malvern Instruments, having He–Ne laser (633 nm, 4 mW) at 298.15 K.

Table S1. Alginic acid carbons (AACs) preparation at different conditions with their coding.

Sl. No.	New Code	Temp./Time	Composition
01	AAC-1	200/6h	Water + 7.5 wt% Alg. acid
02	AAC-2	200/6h	42.9wt%DES + 7.5 wt% Alg. acid
03	AAC-3	200/6h	62.8wt%+ 7.5 wt % Alg. acid
04	AAC-4	200/6h	81.8wt%DES + 7.5 wt% Alg. acid
05	AAC-5	200/6h	Pristine DES + 7.5 wt% Alg. acid
06	AAC-6	200/3h	81.8wt%DES + 7.5 wt% Alg. acid
07	AAC-7	200/9h	81.8wt%DES + 7.5wt% Alg. acid
08	AAC-8	200/12h	81.8wt%DES + 7.5 wt% Alg. acid
09	AAC-9	180/6h	81.8 wt%DES + 7.5 wt% Alg. acid
10	AAC-10	220/6h	81.8 wt%DES + 7.5 wt% Alg. acid

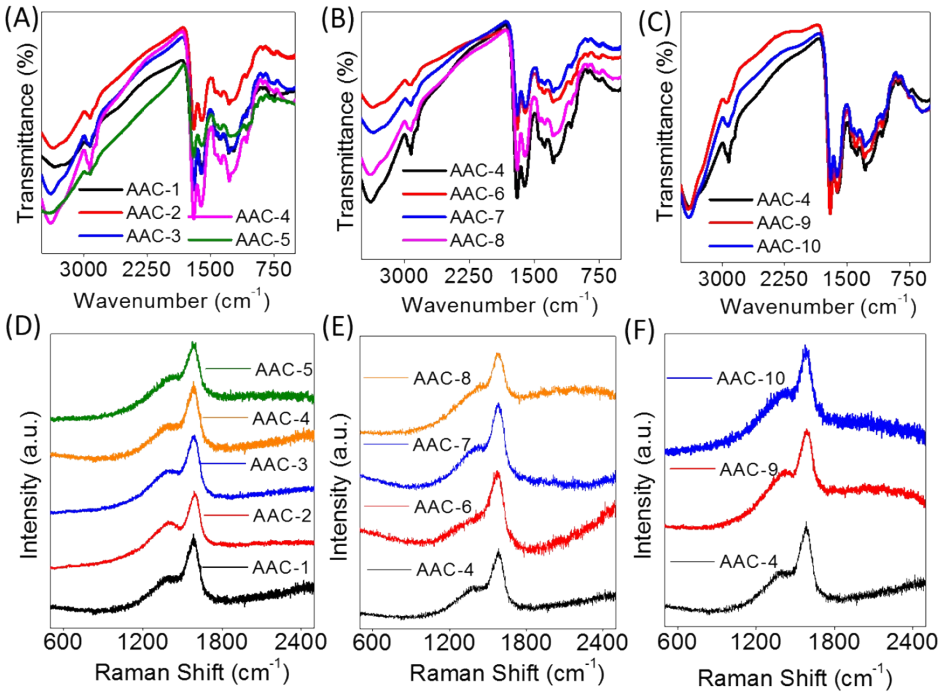

Fig. S1. FTIR spectra of (A) materials prepared using different concentrations of DES, (B) materials prepared at different hydrothermal reaction times and (C) materials prepared at different hypothermal reaction temperatures. And Raman spectra obtained for (D) materials prepared using different concentrations of DES, (E) materials prepared at different hydrothermal reaction times and (F) materials prepared at different hypothermal reaction temperatures.

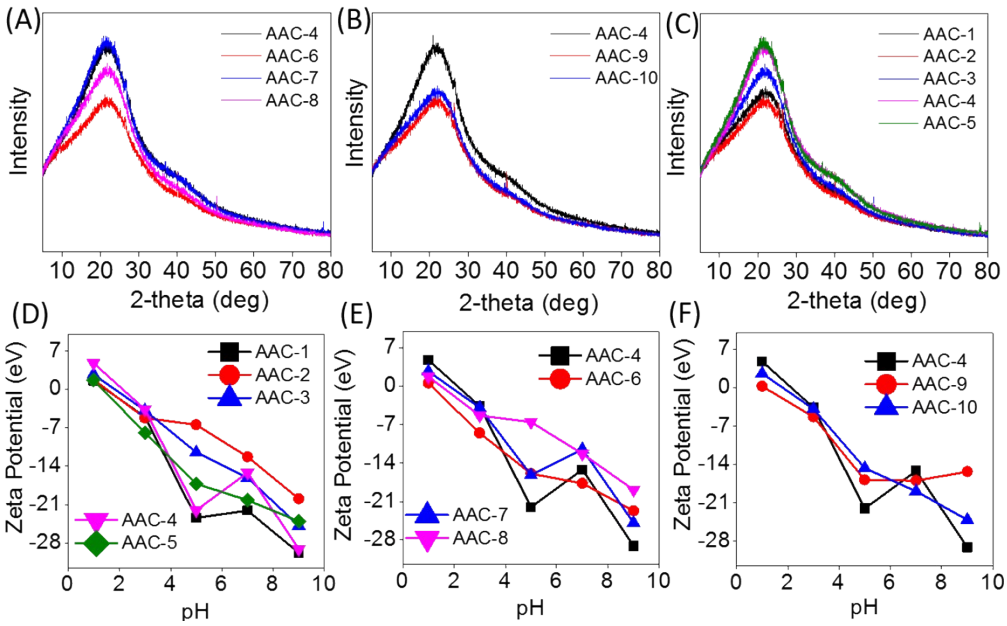

Fig. S2. XRD patterns of (A) materials prepared using different concentrations of DES, (B) materials prepared at different hydrothermal reaction times and (C) materials prepared at different hypothermal reaction temperatures. And zeta potential results obtained for (D) materials prepare during different concentrations of DES, (E) materials prepared at different hydrothermal reaction times and (F) materials prepared at different hypothermal reaction temperatures.

Table S2. Elemental compositions of carbon materials prepared under various conditions.

Name	Yield (%)	N%	C%	H%	O%	O/C ratio
AAC-1	23.27	0.66	62.41	4.474	32.374	0.51
AAC-2	21.04	0.6	61.08	4.612	34.247	0.56
AAC-3	23.13	0.69	62.53	4.647	32.015	0.51
AAC-4	30.08	0.98	62.92	4.728	31.3	0.5
AAC-5	29.67	0.76	60.6	4.545	34.036	0.56
AAC-6	25.16	0.83	58.82	4.488	35.801	0.61
AAC-7	18	1.1	60.31	4.6	33.939	0.56
AAC-8	23.9	0.94	63.05	4.797	31.133	0.49
AAC-9	17.41	0.95	59.49	4.613	34.876	0.59
AAC-10	25.37	0.77	64.7	4.208	30.259	0.47

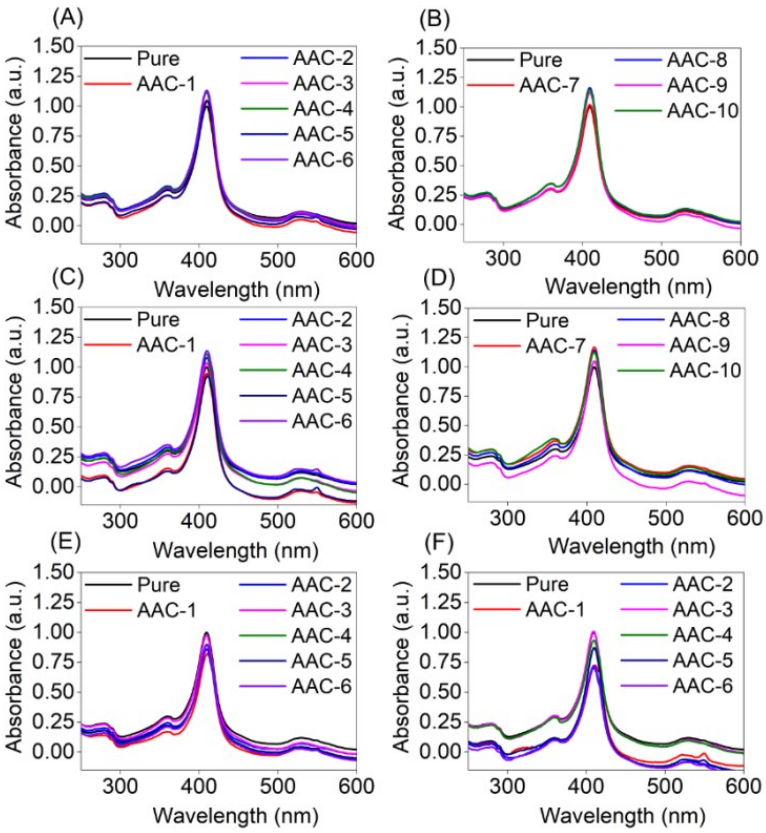

Fig. S3. UV-vis spectra of pure Cyt-c and (A) 20 µL of AAC-1, AAC-2, AAC-3, AAC-4, AAC-5 and AAC-6 (B) 20 µL of AAC-7, AAC-8, AAC-9 and AAC-10 (C). 40 µL of AAC-1, AAC-2, AAC-3, AAC-4, AAC-5 and AAC-6 (D) 40 µL of AAC-7, AAC-8, AAC-9 and AAC-10 (E) 100 µL of AAC-1, AAC-2, AAC-3, AAC-4, AAC-5 and AAC-6 (F) 200 µL of AAC-1, AAC-2, AAC-3, AAC-4, AAC-5 and AAC-6.

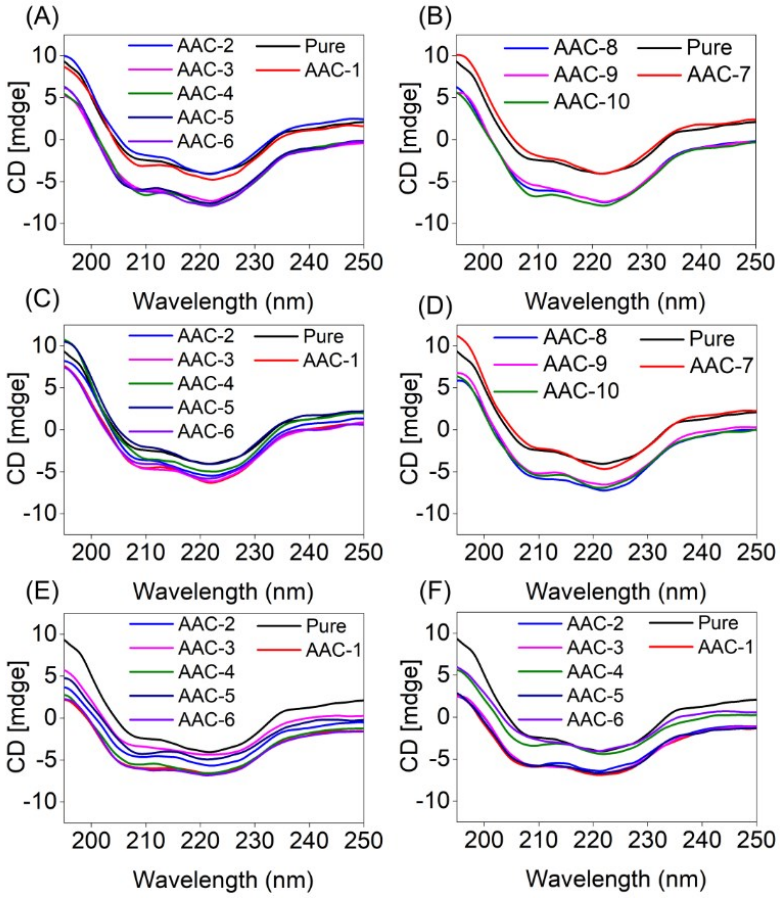

Fig. S4. Far UV-CD spectra of pure Cyt-c and (A) 20 µL of AAC-1, AAC-2, AAC-3, AAC-4, AAC-5 and AAC-6 (B) 20 µL of AAC-7, AAC-8, AAC-9 and AAC-10 (C). 40 µL of AAC-1, AAC-2, AAC-3, AAC-4, AAC-5 and AAC-6 (D) 40 µL of AAC-7, AAC-8, AAC-9 and AAC-10 (E) 100 µL of AAC-1, AAC-2, AAC-3, AAC-4, AAC-5 and AAC-6 (F) 200 µL of AAC-1, AAC-2, AAC-3, AAC-4, AAC-5 and AAC-6.

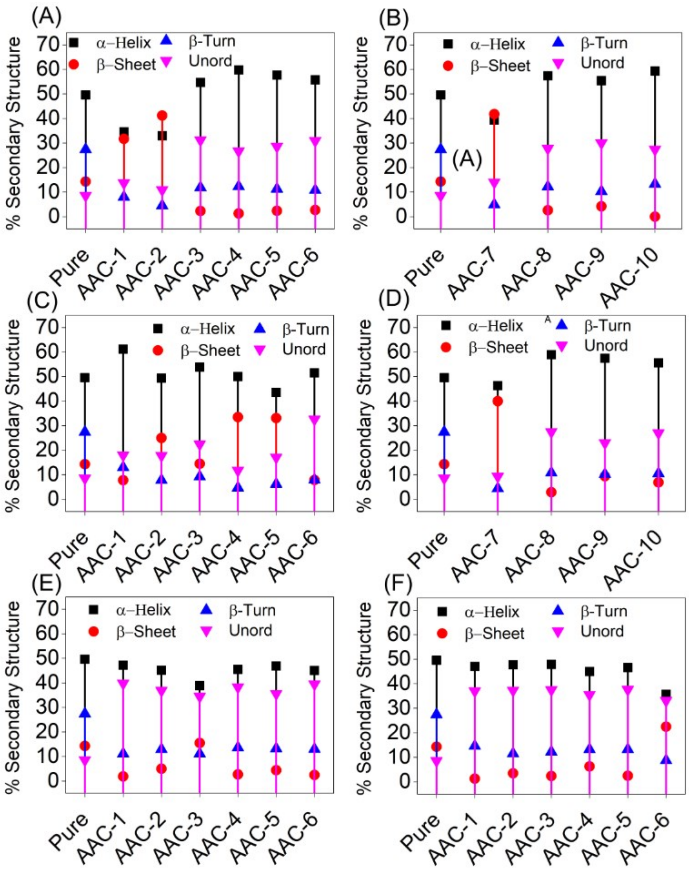

Fig. S5. % Secondary change in pure Cyt-c and (A) 20 µL of AAC-1, AAC-2, AAC-3, AAC-4, AAC-5 and AAC-6 (B) 20 µL of AAC-7, AAC-8, AAC-9 and AAC-10 (C). 40 µL of AAC-1, AAC-2, AAC-3, AAC-4, AAC-5 and AAC-6 (D) 40 µL of AAC-7, AAC-8, AAC-9 and AAC-10 (E) 100 µL of AAC-1, AAC-2, AAC-3, AAC-4, AAC-5 and AAC-6 (F) 200 µL of AAC-1, AAC-2, AAC-3, AAC-4, AAC-5 and AAC-6.

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