# Supporting Information

## Multifaceted Interaction Studies between Carbon Dots and Proteins of Clinical Importance for Optical Sensing Signals

Smita Das, Roshika Kaushik, Pranab Goswami*

Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati-781039, Assam, India. *Correspondence: Fax: +91 361 2582249; Tel: +91 361 2582202; E-mail: pgoswami@iitg.ac.in (P. Goswami).

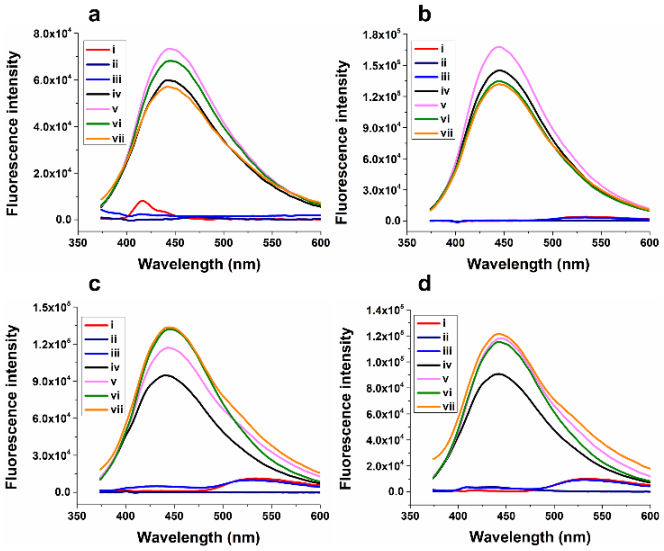


**Fig. S1: (a)** Fluorescence change of different reaction systems at the CD excitation wavelength ($\lambda_{354\ nm}$): (i) ChOx, (ii) Chol, (iii) ChOx+Chol, (iv) CD, (v) CD+ChOx (after 5 minutes), (vi) CD+Chol, (vii) CD+ChOx+Chol.
**(b)** (i) GOx, (ii) GL, (iii) GOx+GL, (iv) CD, (v) CD+GOx (after 5 minutes), (vi) CD+GL, (vii) CD+GOx+GL. **(c)** (i) AOx , (ii) MeOH, (iii) AOx+MeOH, (iv) CD, (v) CD+AOx (after 5 minutes), (vi) CD+MeOH, (vii) CD+AOx+MeOH. **(d)** (i) AOx , (ii) EtOH, (iii) AOx+EtOH, (iv) CD, (v) CD+AOx (after 5 minutes), (vi) CD+EtOH, (vii) CD+AOx+EtOH.

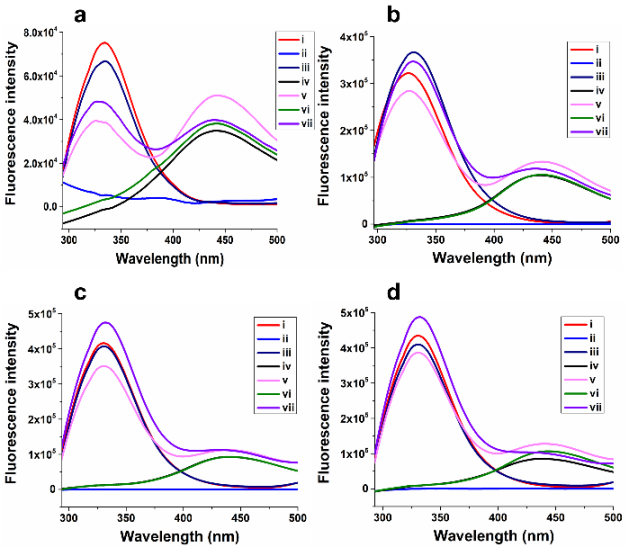


**Fig. S2: (a)** Fluorescence change of different reaction systems at the ChOx excitation wavelength ($\lambda_{274\ nm}$): (i) ChOx, (ii) Chol, (iii) ChOx+Chol, (iv) CD, (v) CD+ChOx (after 5 minutes), (vi) CD+Chol, (vii) CD+ChOx+Chol. **(b)** Fluorescence change of different reaction systems at the GOx excitation wavelength ($\lambda_{274\ nm}$): (i) GOx, (ii) GL, (iii) GOx+GL, (iv) CD, (v) CD+GOx (after 5 minutes), (vi) CD+GL, (vii) CD+GOx+GL. **(c)** Fluorescence change of different reaction systems at the AOx excitation wavelength ($\lambda_{274\ nm}$): (i) AOx , (ii) MeOH, (iii) AOx+MeOH, (iv) CD, (v) CD+AOx (after 5 minutes), (vi) CD+MeOH, (vii) CD+AOx+MeOH. **(d)** Fluorescence change of different reaction systems at the AOx excitation wavelength ($\lambda_{274\ nm}$): (i) AOx , (ii) EtOH, (iii) AOx+EtOH, (iv) CD, (v) CD+AOx (after 5 minutes), (vi) CD+EtOH, (vii) CD+AOx+EtOH.

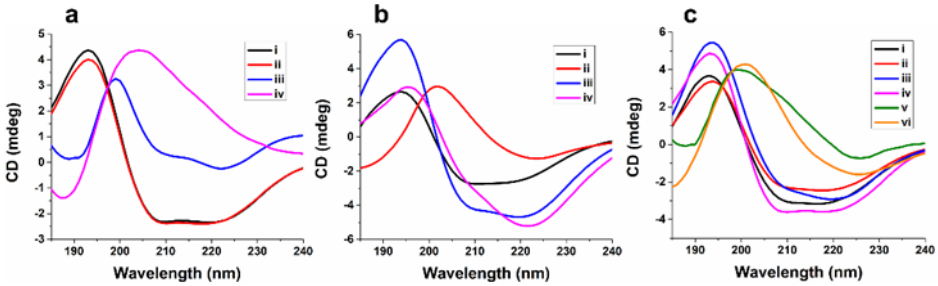


**Fig. S3. (a)** Circular dichroism spectra of (i) ChOx, (ii) ChOx+CD, (iii) ChOx+Chol, (iv) ChOx+Chol+CD, **(b)** Circular dichroism spectra of (i) GOx, (ii) GOx+CD, (iii) GOx+GL, (iv) GOx+GL+CD. **(c)** Circular dichroism spectra of (i) AOx, (ii) AOx+CD, (iii) AOx+EtOH, (iv) AOx+MeOH, (v) AOx+EtOH+CD, (vi) AOx+MeOH+CD.

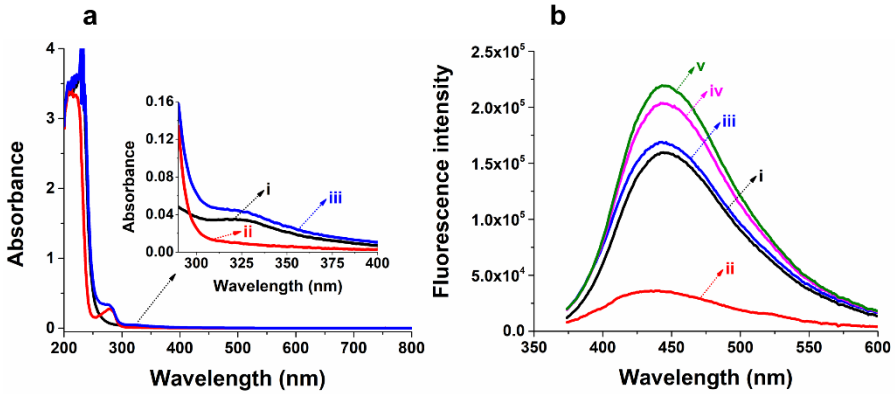


**Fig. S4. (a)** Absorbance of different reaction systems: (i) CD, (ii) HSA, and (iii) CD+HSA, **(b)** Fluorescence emission (i) CD, (ii) HSA, and CD+HSA at (iii) 2 mins, (iv) 6 mins, and (v) 10 mins at excitation of CD (at $\lambda_{ex\ 354\ nm}$) (Inset: Fluorescence intensity of CD+HSA at different time intervals. Experiment was carried out using 50 µL of stock 10.3 mg/mL HSA and 10 µL of stock CD solution (100 mg/mL).

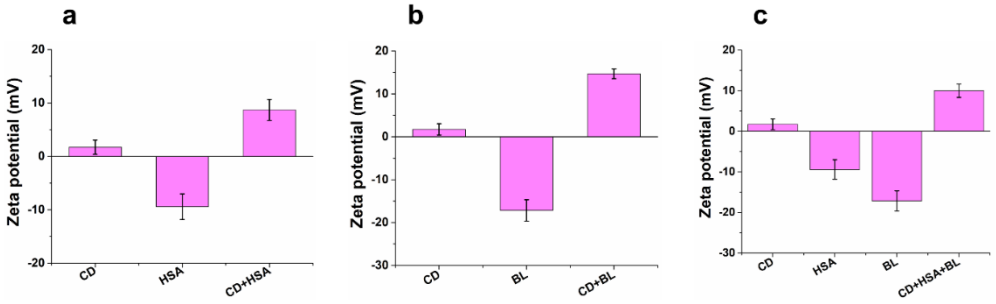


**Fig. S5.** Zeta potential of **(a)** CD, HSA, and CD+HSA, **(b)** CD, BL (Bilirubin), and CD+BL, and **(c)** CD, HSA, BL, and CD+HSA+BL. The concentration of CD (1 mg/mL), HSA (0.52 mg/mL), and BL (2 µM) used in the reaction mixture are shown in the bracket.

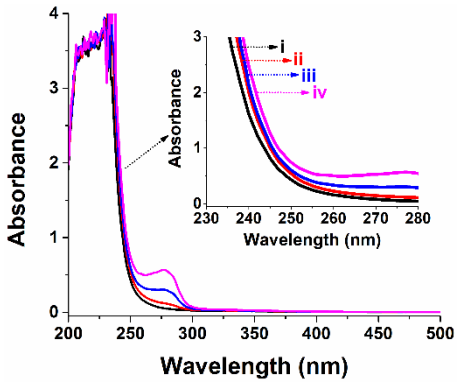


**Fig. S6.** UV-vis spectra of CD and increasing concentrations of HSA. (i) CD, (ii) CD+103 µg/mL HSA, (iii) CD+515 µg/mL HSA, (iv) CD+1.03 mg/mL HSA. The concentration of CD used in the experiment is 1 mg/mL.

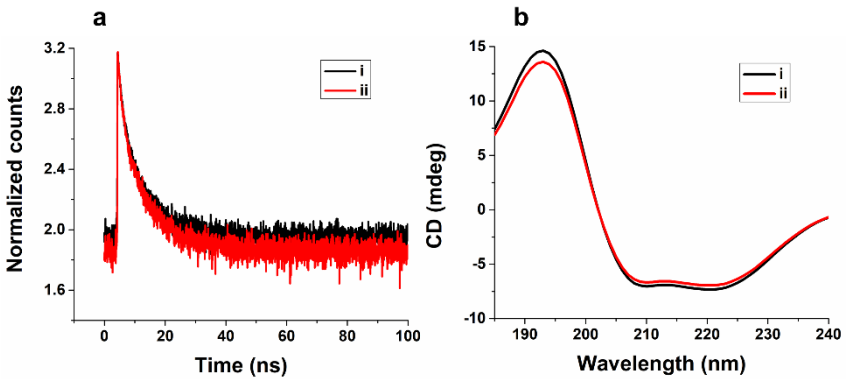


**Fig. S7. (a)** Time resolved fluorescence spectra of (i) CD, and (ii) CD+HSA, **(b)** Circular Dichroism spectra of (i) HSA, (ii) CD+HSA.

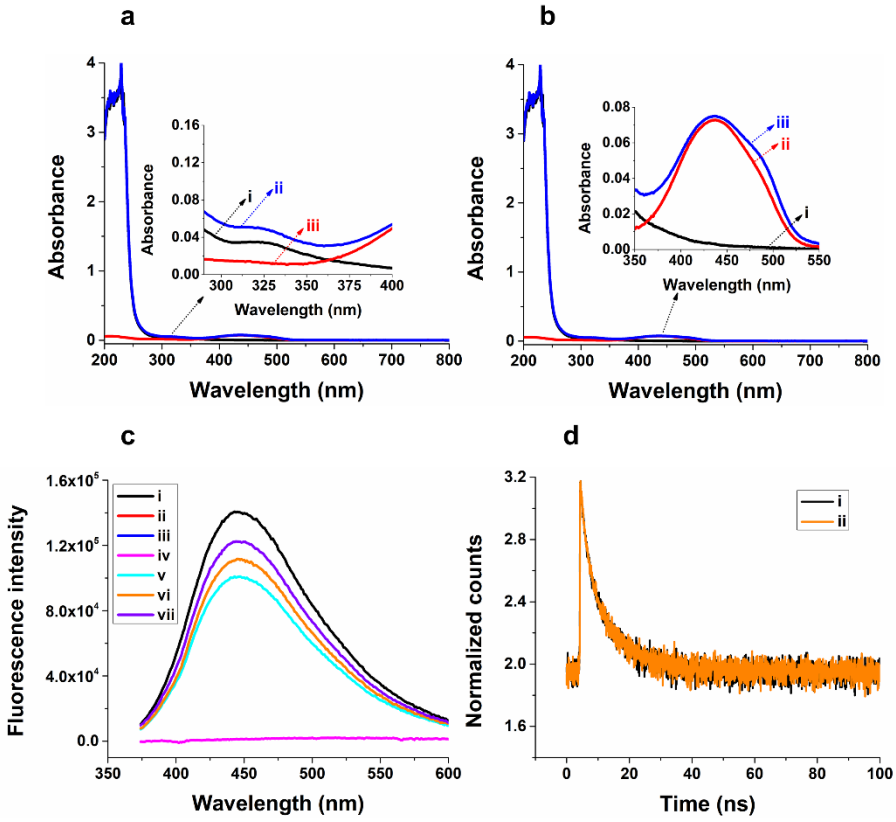


**Fig. S8. (a)** Effect of BL on absorbance of CD. (i) CD, (ii) BL, and (iii) CD+BL, **(b)** Effect of CD on the absorbance of BL. (i) CD, (ii) BL, (iii) CD+BL, **(c)** Fluorescence of (i) CD, (ii) 0.02 µM BL, (iii) 0.3 µM BL, (iv) 2.43 µM BL, (v) CD+0.02 µM BL, (vi) CD+0.3 µM BL, (vii) CD+2.43 µM BL, **(d)** Time resolved fluorescence spectra of (i) CD, and (ii) CD+BL. The concentration of CD used in the experiment is 1 mg/mL.

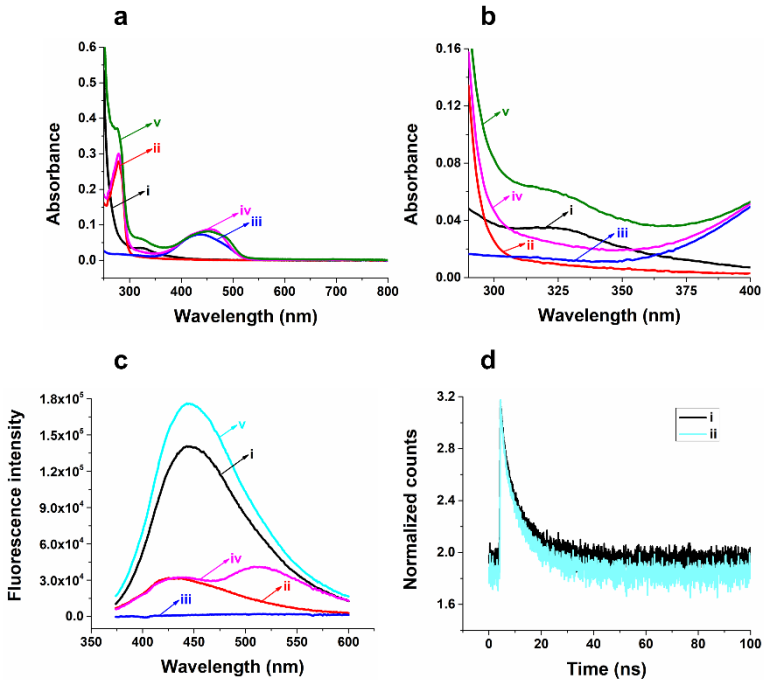


**Fig. S9. (a)** Effect of CD on the absorbance of HSA+BL. (i) CD, (ii) HSA, (iii) BL, (iv) HSA+BL, (v) CD+HSA+BL, **(b)** Effect of HSA+BL on the absorbance of CD. (i) CD, (ii) HSA, (iii) BL, (iv) HSA+BL, (v) CD+HSA+BL, **(c)** Effect of HSA+BL on the fluorescence of CD (CD excitation wavelength: 354 nm). (i) CD, (ii) HSA, (iii) BL, (iv) HSA+BL, (v) CD+HSA+BL, **(d)** Time resolved fluorescence spectra of (i) CD, and (ii) CD+HSA+BL. Experiment was carried out using 50 µL of stock 10.3 mg/mL HSA, 10 µL of stock CD solution (100 mg/mL), and 20 µL of stock 100 µM bilirubin.

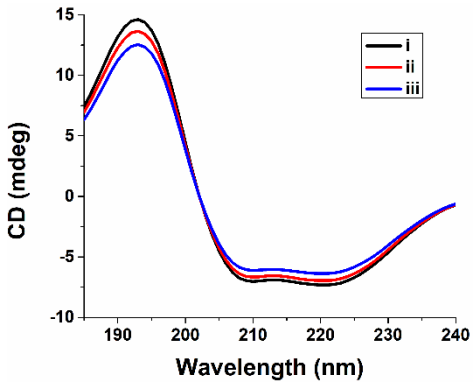


**Fig. S10.** Circular Dichroism spectra of (i) HSA, (ii) HSA+BL, (iii) HSA+BL+CD.

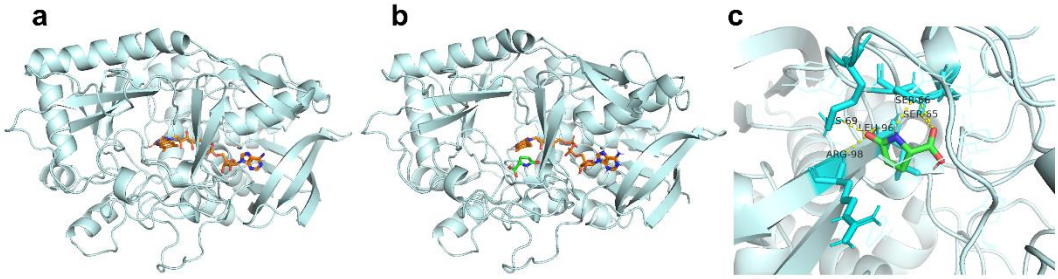


**Fig. S11. (a)** Crystallographic structure of ChOx with the FAD (orange), **(b)** Best binding pose for PGA (green) with ChOx; **(c)** Amino acid residues in ChOx that form hydrogen bond with PGA.

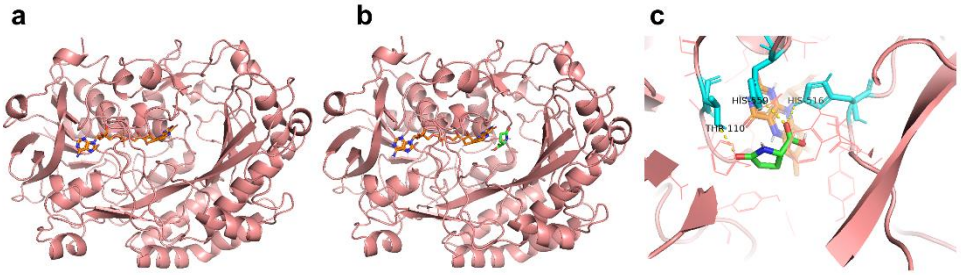


**Fig. S12. (a)** Crystallographic structure of GOx with the FAD (orange), **(b)** Best binding pose for PGA (green) with GOx; **(c)** Amino acid residues in GOx that form hydrogen bond with PGA.

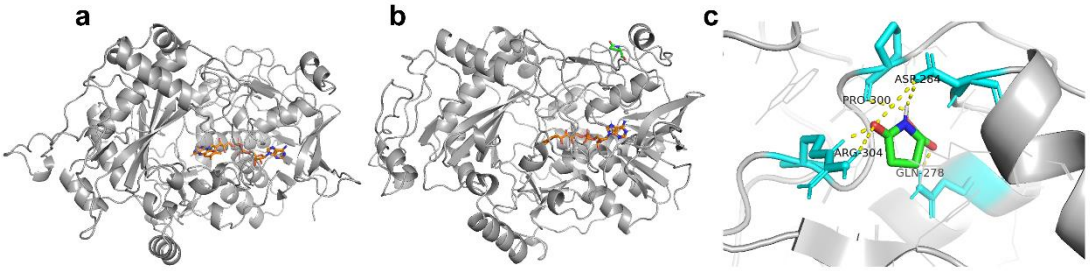


**Fig. S13. (a)** Crystallographic structure of AOx with the FAD (orange), **(b)** Best binding pose for PGA (green) with AOx; **(c)** Amino acid residues in AOx that form hydrogen bond with PGA.

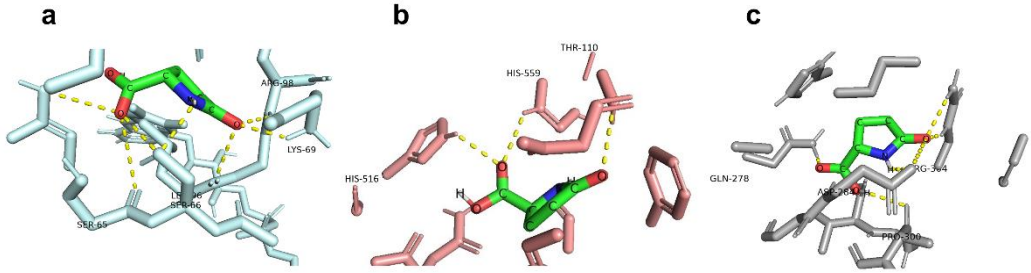


**Fig. S14.** Different functional groups of PGA involved in the binding with amino acids of **(a)** ChOx, **(b)** GOx, and **(c)** AOx.

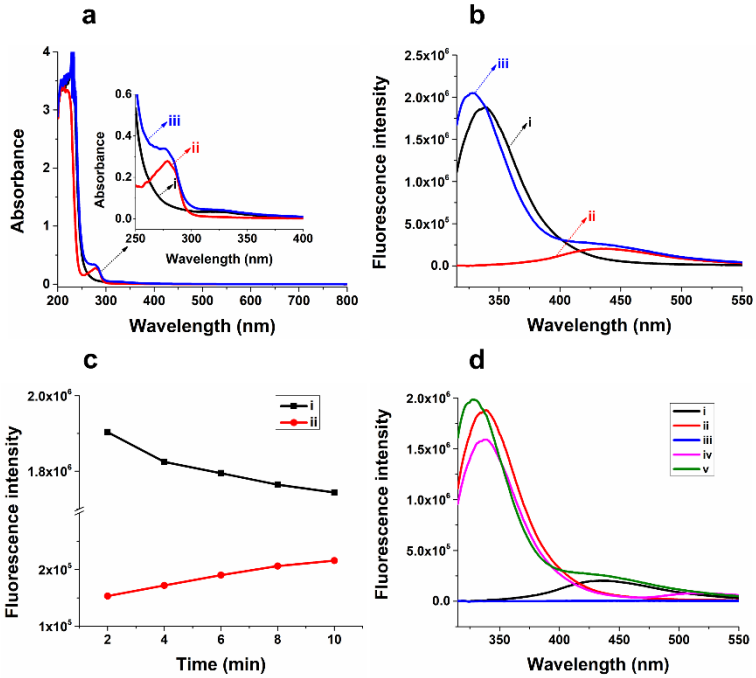


**Fig. S15. (a)** Absorbance of different reaction systems: (i) CD, (ii) HSA, and (iii) CD+HSA, **(b)** Fluorescence of (i) HSA, (ii) CD, and (iii) CD+HSA (after 5 minutes) at the HSA excitation wavelength ($\lambda_{ex\ 295\ nm}$), **(c)** Fluorescence change of (i) HSA and (ii) CD at different incubation time at HSA excitation wavelength ($\lambda_{ex\ 295\ nm}$), **(d)** Effect of CD on the fluorescence of HSA+BL (HSA excitation wavelength: 295 nm) . (i) CD, (ii) HSA, (iii) BL, (iv) HSA+BL, (v) CD+HSA+BL. Experiment was carried out using 50 µL of stock 10.3 mg/mL HSA and 10 µL of stock CD solution (100 mg/mL).

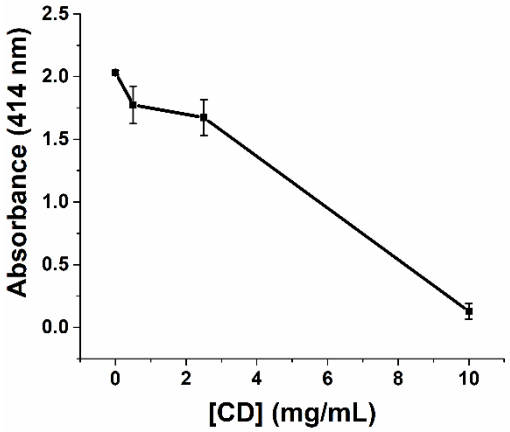


**Fig. S16.** Influence of interaction of CD on the catalytic activity of GOx. Experiment was performed using different concentrations (0 mg/mL, 1 mg/mL, 2.5 mg/mL, and 10 mg/mL) of stock 100 mg/mL CD solution, 10 µL of 1 mg/mL stock GOx, 10 mM GL, and 5 mM ABTS.

**Table S1.** The content of different secondary structures of HSA in the absence and presence of CD

| Reaction system | Helix (%) | β strand (%) | Turns (%) | Unordered (%) |
|---|---|---|---|---|
| HSA | 48.1 | 10.2 | 17.2 | 25.1 |
| HSA+CD | 48.0 | 11.4 | 17.8 | 25 |

**Table S2.** The content of different secondary structures of HSA in different reaction systems

| Reaction system | Helix (%) | β strand (%) | Turns (%) | Unordered (%) |
|---|---|---|---|---|
| HSA | 48.1 | 10.2 | 17.2 | 25.1 |
| HSA+BL | 47.7 | 12.9 | 17.5 | 25.1 |
| HSA+CD+BL | 45.1 | 13.7 | 19.7 | 26.4 |

**Table S3.** Binding of the different functional groups of the PGA to the amino acid residues of the enzymes

| Functional groups | ChOx | GOx | AOx |
|---|---|---|---|
| -C=O | LYS69, LEU96, ARG98 | THR110 | ARG304, ASP284 |
| -COOH | SER65, SER66, 219TYR | HIS516, HIS559 | GLN278, PRO300 |
| -NH | SER66 | | ASP284 |

**Table S4.** Binding site of the substrates with their respective enzyme

| Enzyme | Substrate | Interacting amino acids (hydrophobic bond) | Interacting amino acids (hydrogen bond) |
|---|---|---|---|
| **Cholesterol oxidase** | Cholesterol | 70ASN, 71ARG, 72THR 73GLU, 74ALA, 75PRO, 76LEU, 78SER, 79PHE, 86ASN, 98ARG, 107TYR, 432LEU, 433PHE, 434GLY | 77GLY |
| **Glucose oxidase** | Glucose | 80TYR, 94ILE, 95ARG, 103SER, 289ALA, 290GLY, 293VAL, 294SER, 328ASP, 450TYR, 515TYR, 516HIS, 518VAL, 548ASP, 550SER | 96SER, 291SER, 292ALA, 517GLY, 549GLY |
| **Alcohol oxidase** | Methanol | 6LYS, 87ALA, 269GLY, 270THR, 271ILE, 568LYS | - |