Electronic Supplementary Material (ESI) for Nanoscale.

An Insight into Embryogenesis Interruption by Carbon Nitride Dots: Can They Be Nucleobase Analogs?

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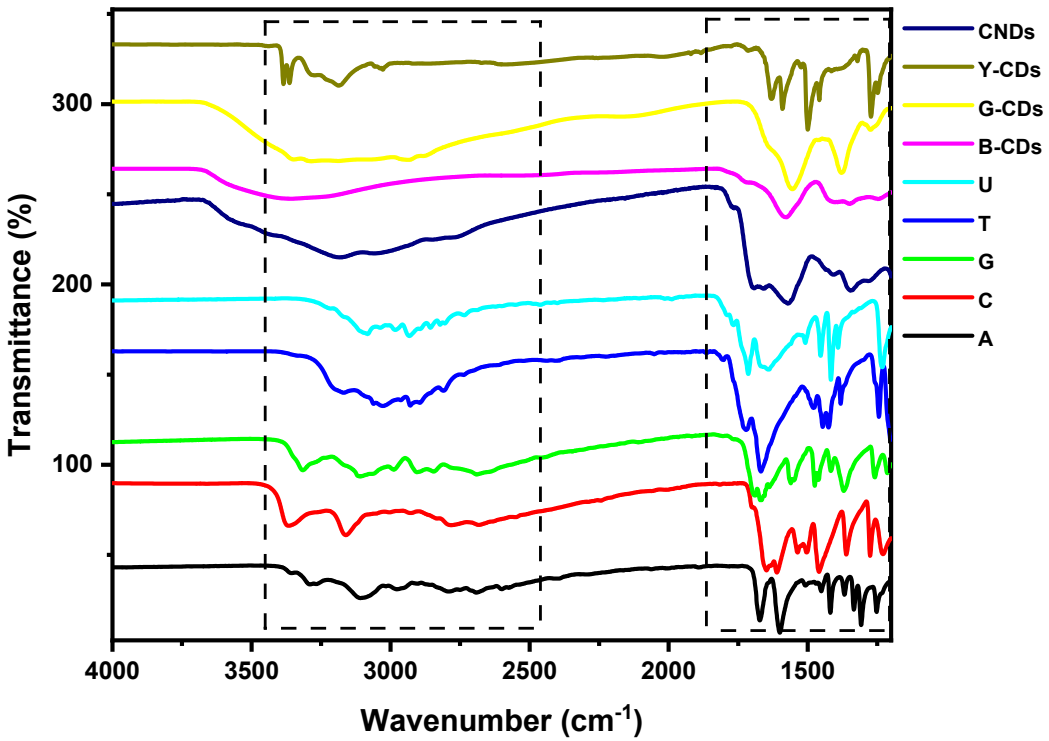

Fig. S1. The FTIR spectra of different CDs including the bare CNDs, Y-CDs, G-CDs, and B-CDs, in comparison to different nucleobases, namely adenine (A), cytosine (C), guanine (G), thymine (T), and uracil (U). Air was used for background subtraction.

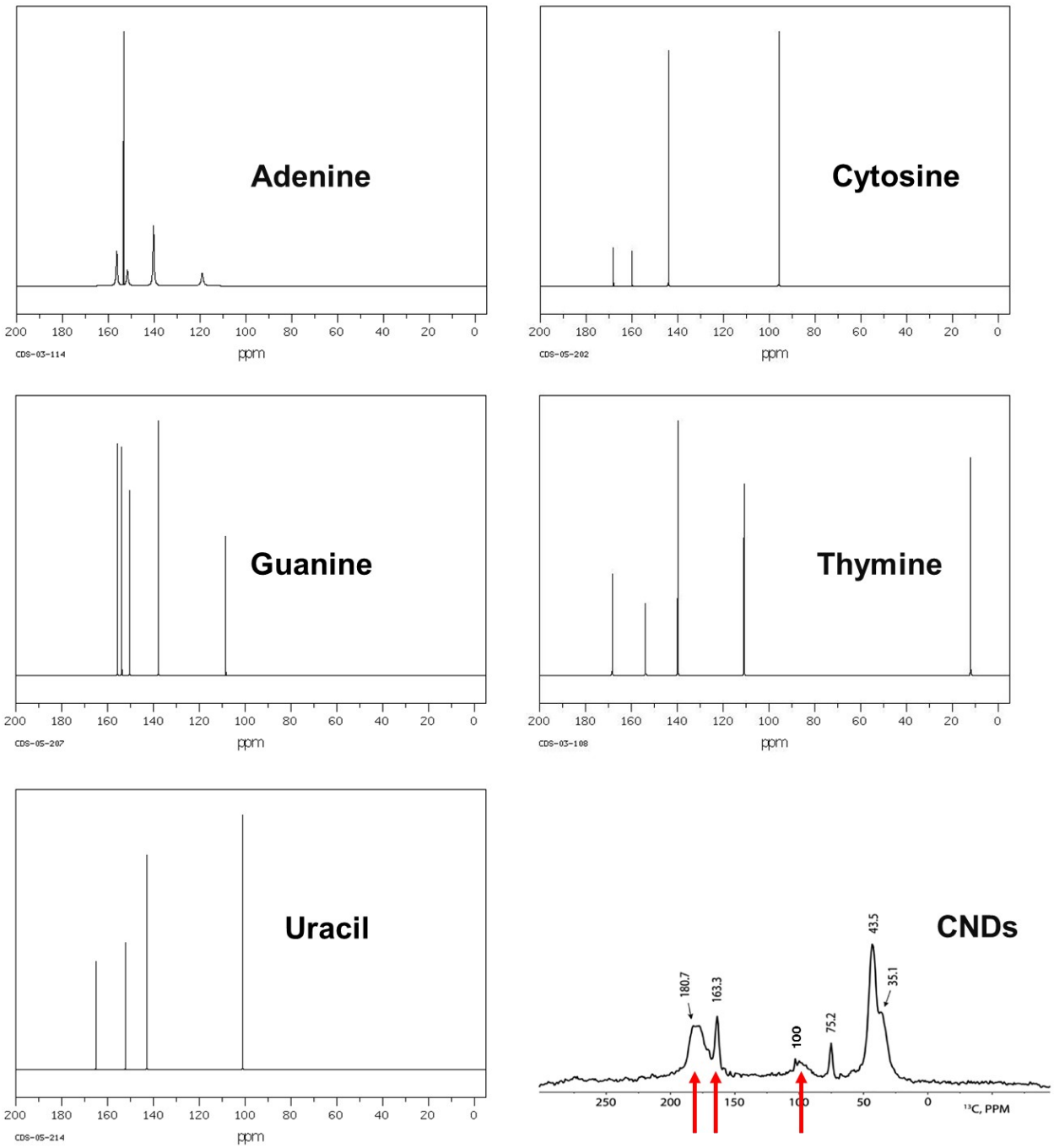

Fig. S2. Solid-state ^{13}C NMR spectra of bare CNDs and different nucleobases, namely adenine, cytosine, guanine, thymine, and uracil. The solid-state ^{13}C NMR spectra of different nucleobases originate from Chemical Book.com. And the spectrum of bare CNDs was adapted from our previous study[1]. Red arrows point to similar chemical shifts shared by CNDs and some nucleobases.

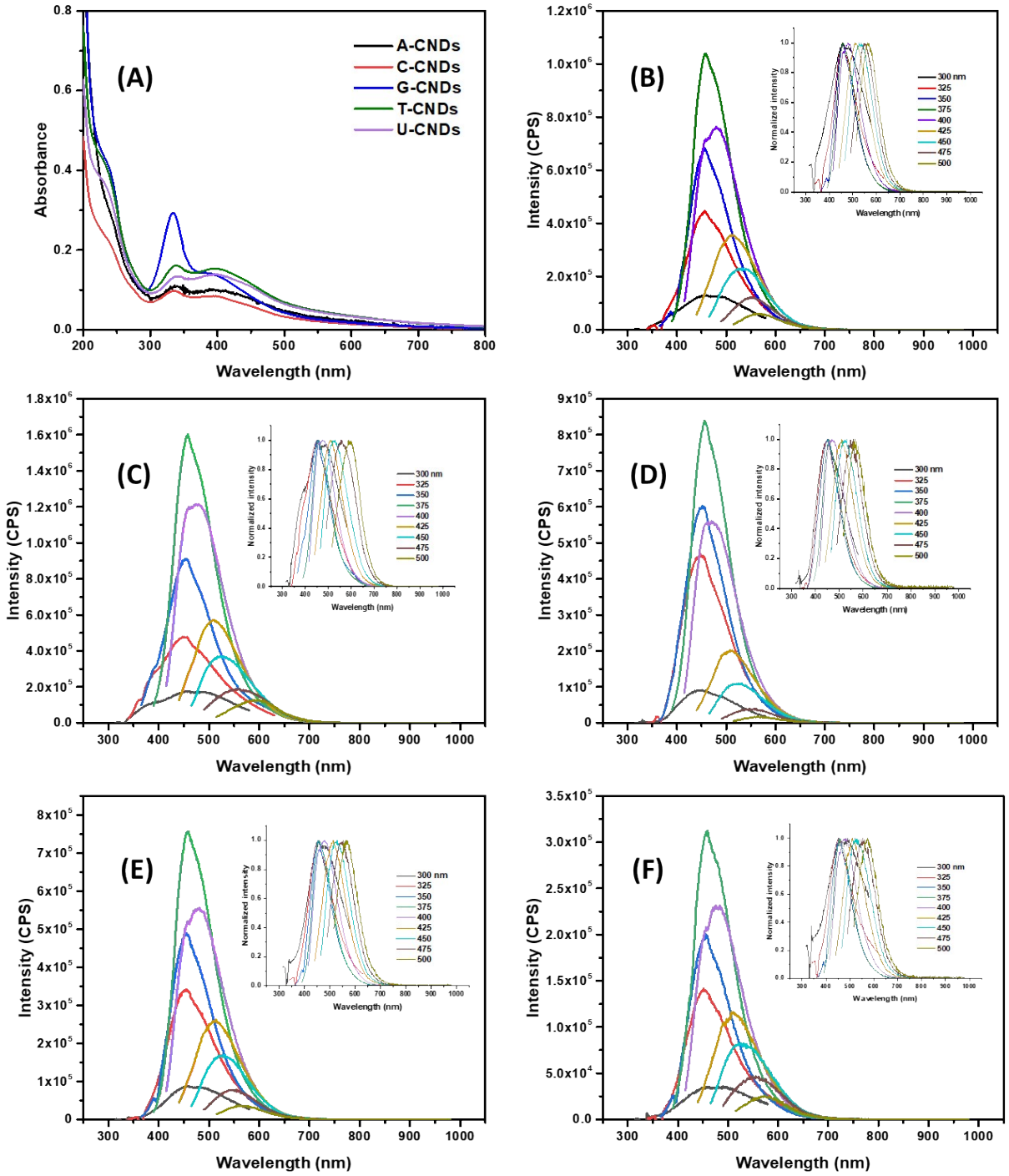

Fig. S3. UV/vis absorption spectra of A-CNDs (1×10^{-2} mg/mL), C-CNDs (5×10^{-2} mg/mL), G-CNDs (2×10^{-2} mg/mL), T-CNDs (2×10^{-2} mg/mL) and U-CNDs (2×10^{-2} mg/mL) **(A)**. Fluorescence emission spectra of distinct CNDs: A-CNDs (1×10^{-2} mg/mL) **(B)**, C-CNDs (2×10^{-2} mg/mL) **(C)**, G-CNDs (5×10^{-3} mg/mL) **(D)**, T-CNDs (5×10^{-3} mg/mL) **(E)** and U-CNDs (5×10^{-3} mg/mL) **(F)**.

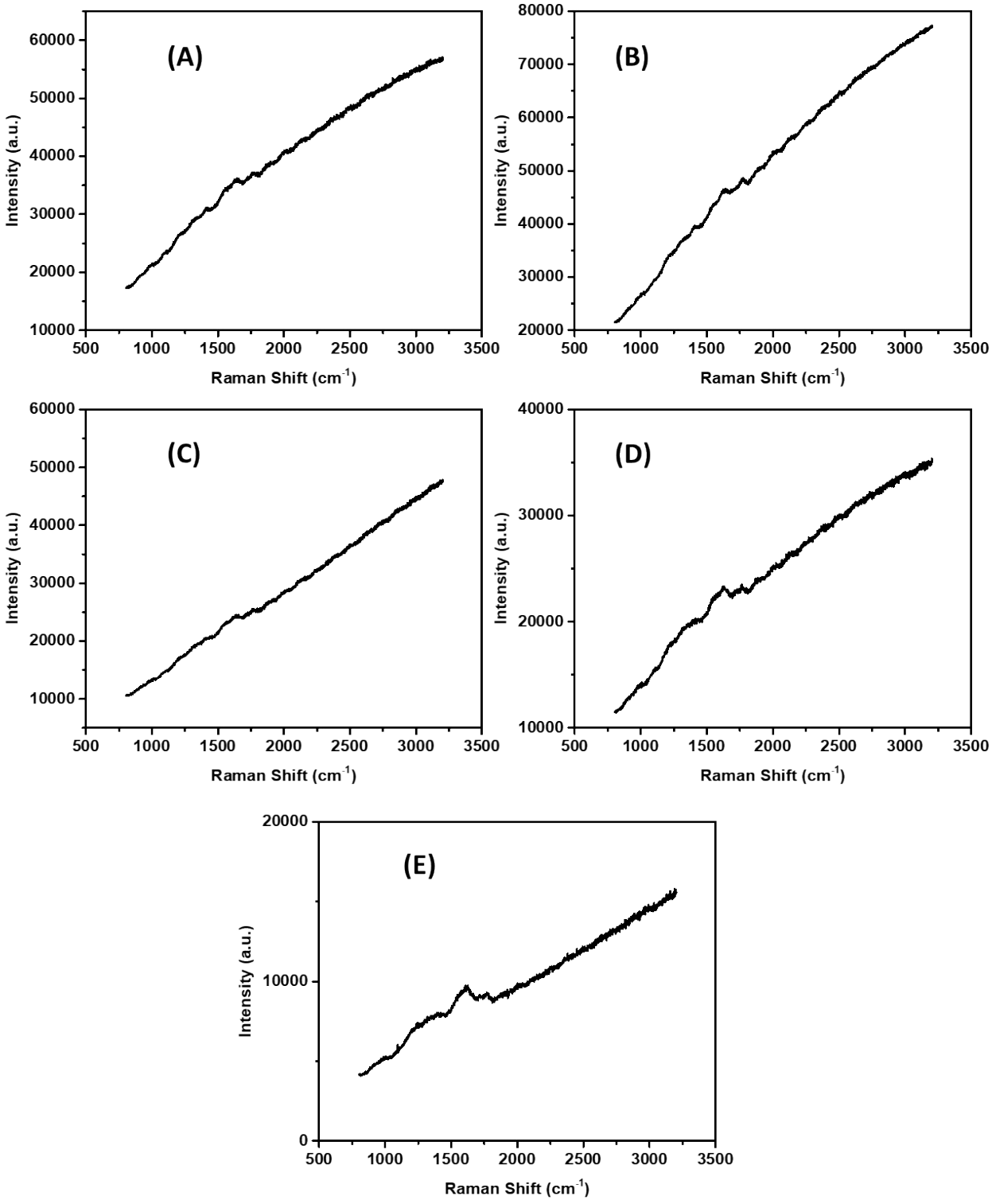

Fig. S4. Raman spectra of different nucleobase-doped CNDs (**A-E**, A-CNDs, C-CNDs, G-CNDs, T-CNDs, U-CNDs, respectively).

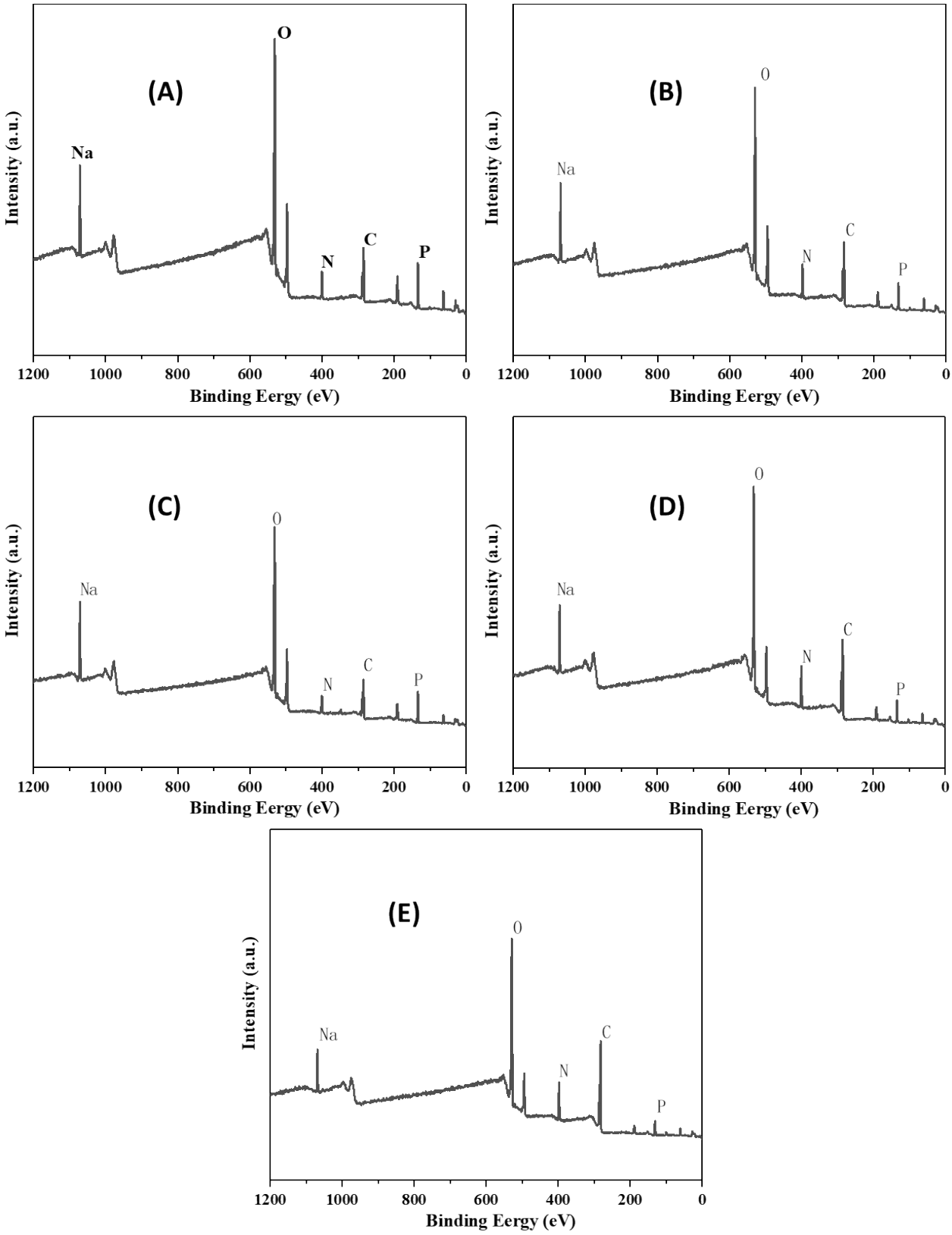

Fig. S5. XPS full spectra of different nucleobase-doped CNDs (**A-E**, A-CNDs, C-CNDs, G-CNDs, T-CNDs, U-CNDs, respectively).

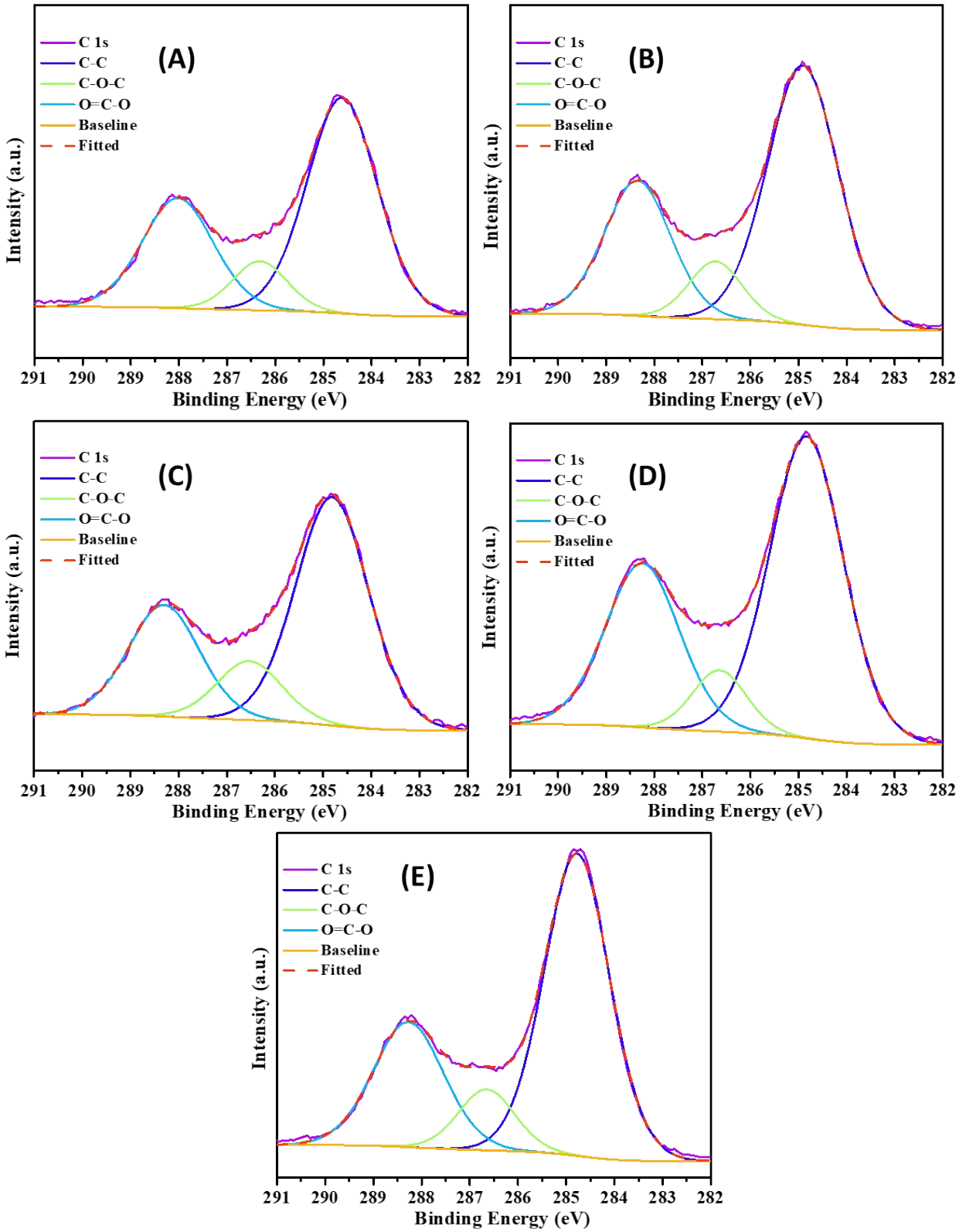

Fig. S6. High-resolution C 1s spectra of different nucleobase-doped CNDs (**A-E**, A-CNDs, C-CNDs, G-CNDs, T-CNDs, U-CNDs, respectively).

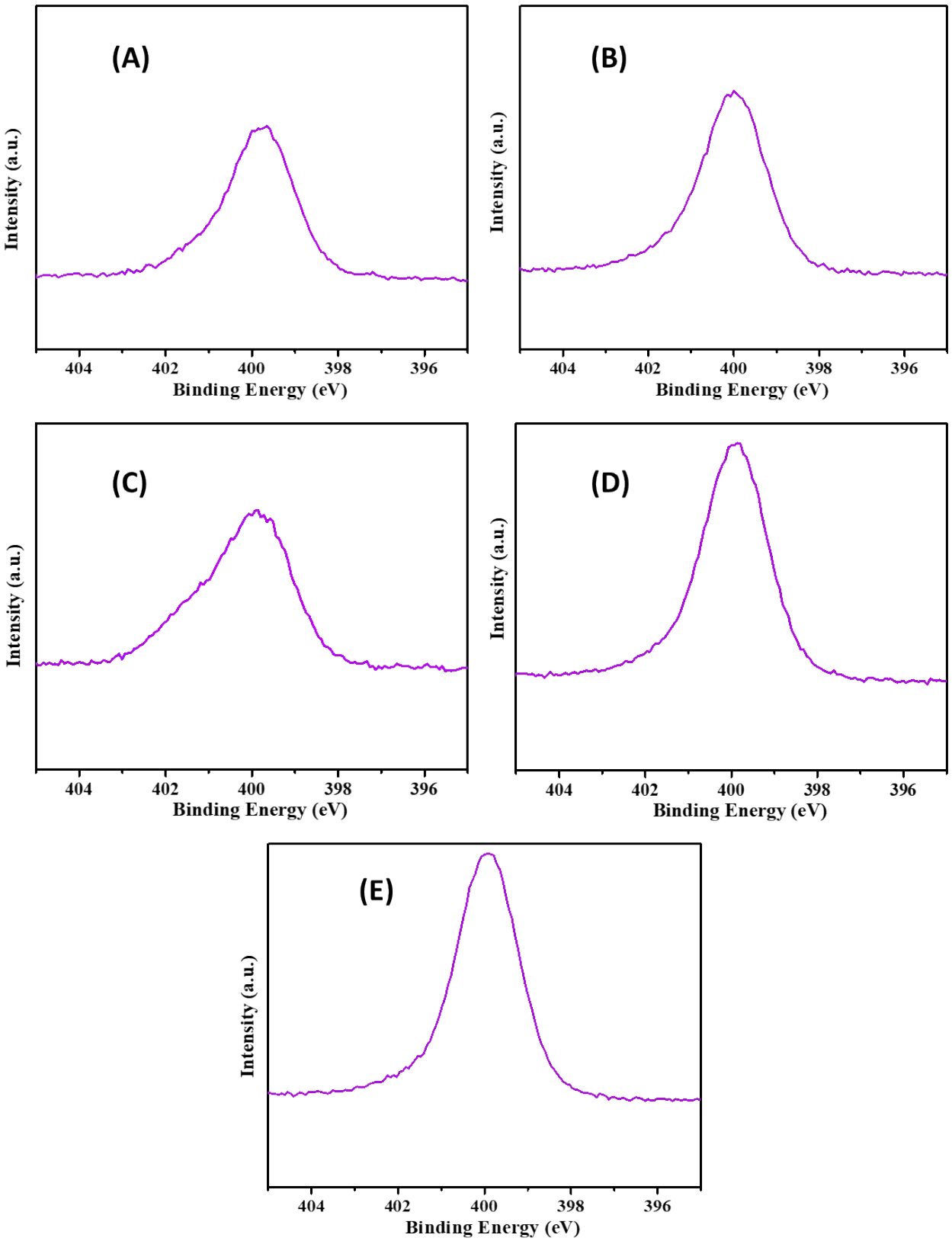

Fig. S7. High-resolution N 1s spectra of different nucleobase-doped CNDs (**A-E**, A-CNDs, C-CNDs, G-CNDs, T-CNDs, U-CNDs, respectively).

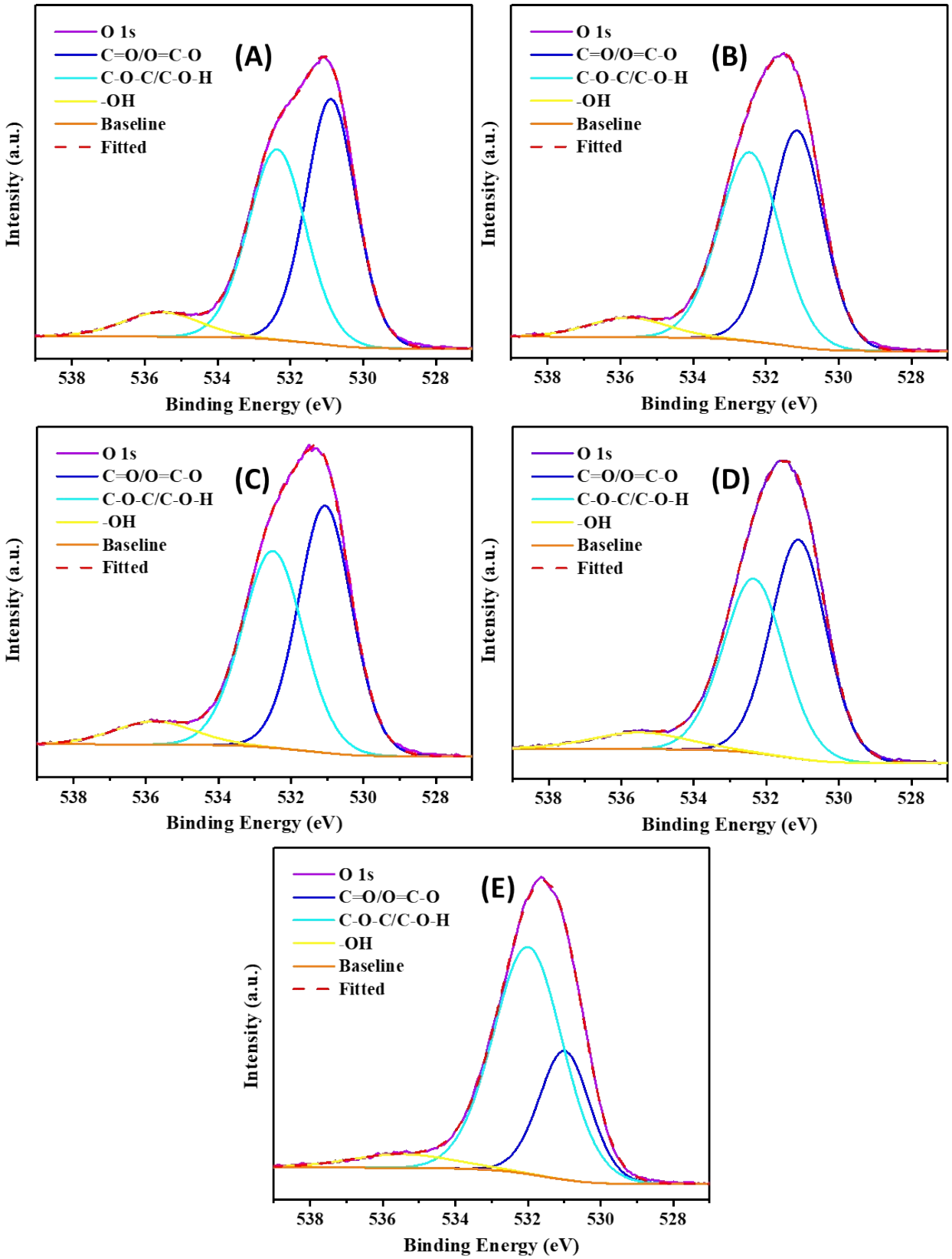

Fig. S8. High-resolution O 1s spectra of different nucleobase-doped CNDs (**A-E**, A-CNDs, C-CNDs, G-CNDs, T-CNDs, U-CNDs, respectively).

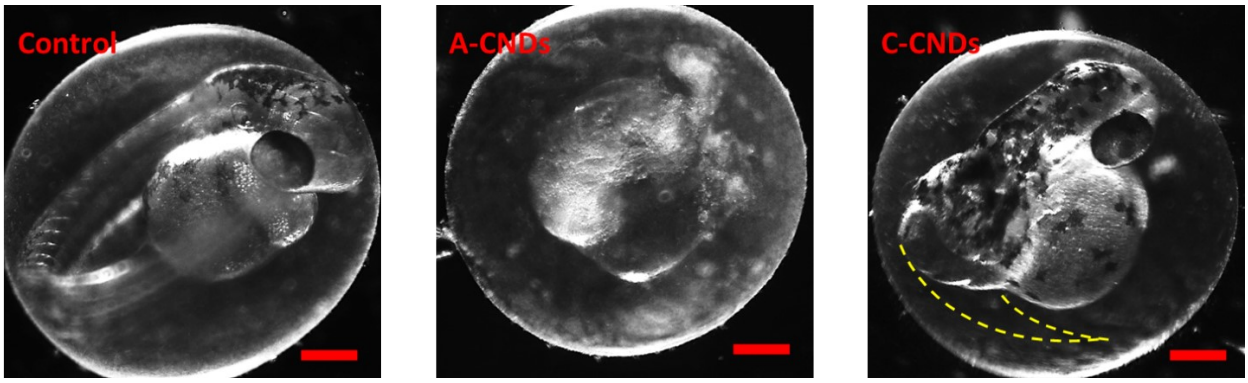

Fig. S9. Abnormal development of zebrafish embryos (48 hpf) treated with a high concentration (100 μg/mL) of A-CNDs and C-CNDs. The area depicted by the yellow dash line represents the tail detachment. The scale bars represent 160 pixels.

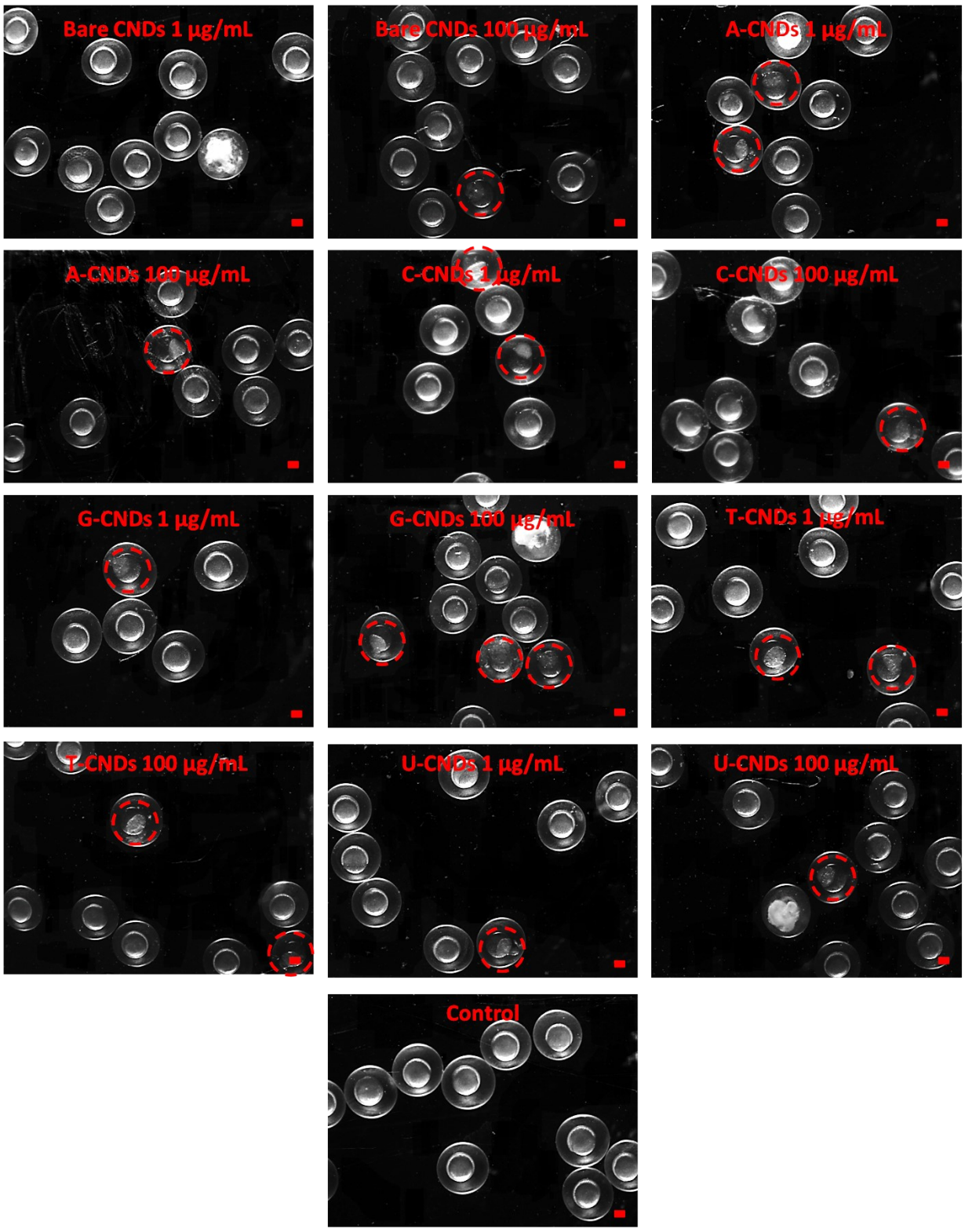

Fig. S10. Morphology of zebrafish embryos (8 hpf) treated with different CNDs at two different concentrations. The scale bars represent 160 pixels. Diseased embryos are circled in red.

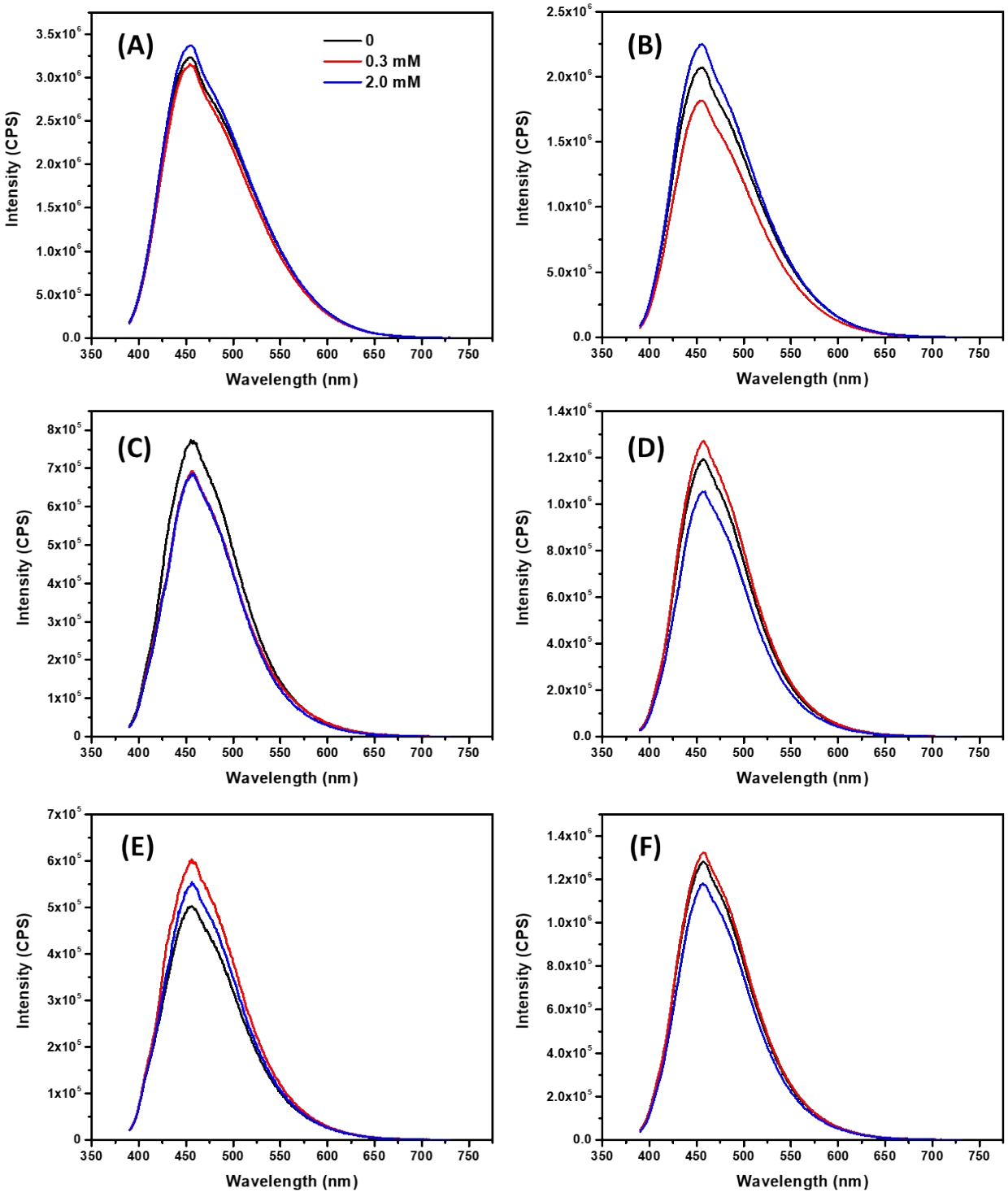

Fig. S11. Fluorescence emission spectra of distinct CNDs (1 µg/mL) in presence of different concentrations of Ca^{2+} (0, 0.3 and 2 mM): Bare CNDs **(A)**, A-CNDs **(B)**, C-CNDs **(C)**, G-CNDs **(D)**, T-CNDs **(E)** and U-CNDs **(F)**.

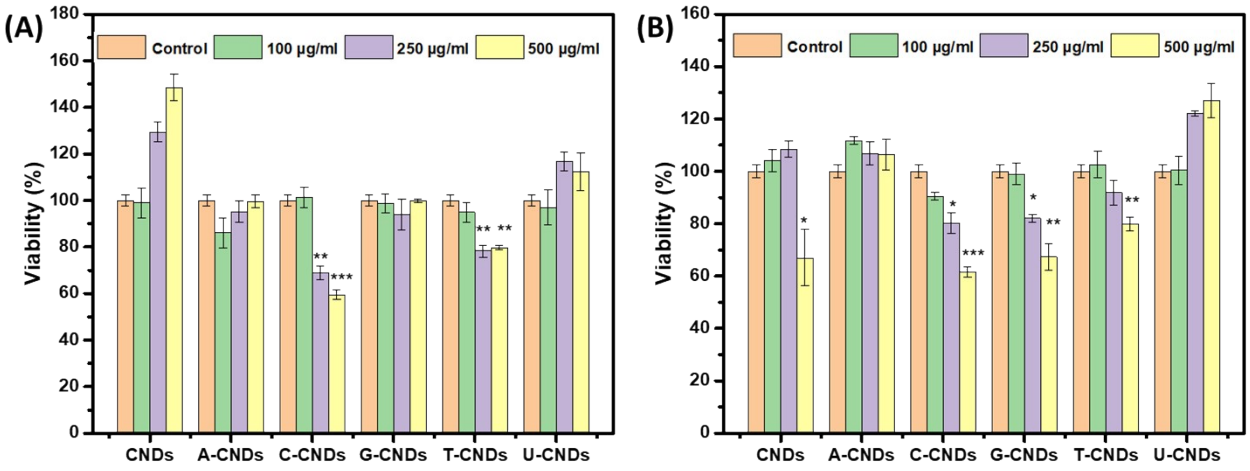

Fig. S12. Viability of PC-3 **(A)** and 4T1 **(B)** cells treated with different concentrations (100, 250 and 500 µg/mL) of bare and various nucleobase-doped CNDs. *p<0.05, **p<0.01, ***p<0.001 compared to non-treated controls.

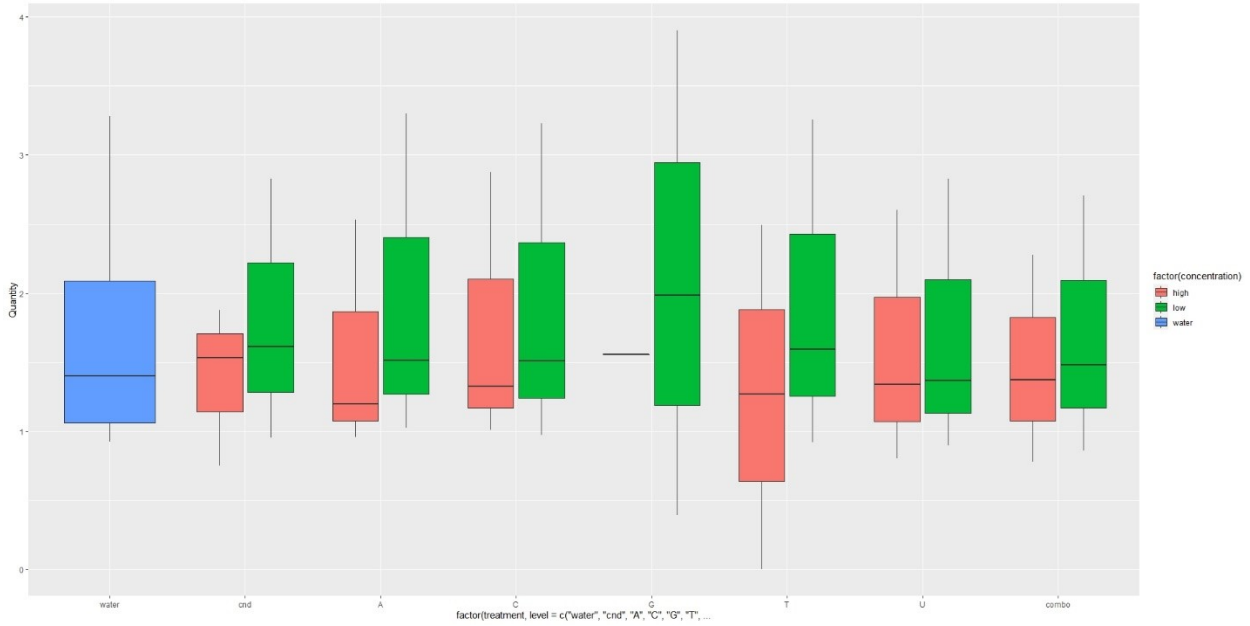

Fig. S13. Amplification plot of the zebrafish polymerase alpha-1 gene in presence of bare and doped CNDs (after omitting two outliers for G-CNDs at high concentrations).

Table S1. FWHM of different nucleobase-doped CNDs.

CND species	Bare CNDs	A-CNDs	C-CNDs	G-CNDs	T-CNDs	U-CNDs
FWHM (nm)	105	91	92	87	89	89

Table S2. XPS atomic percentages of different nucleobase-doped CNDs.

	Peak (eV)	A-CNDs (%)	C-CNDs (%)	G-CNDs (%)	T-CNDs (%)	U-CNDs (%)
Na 1s	1070	6	6	8	5	3
O 1s	531	47	41	46	38	31
N 1s	400	7	9	7	10	11
C 1s	285	29	37	30	42	50
P 2p	134	11	7	10	6	4

Table S3. Binding energies and peak areas for C 1s of different nucleobase-doped CNDs.

Peak (eV)	Bond	A-CNDs (%)	C-CNDs (%)	G-CNDs (%)	T-CNDs (%)	U-CNDs (%)
284.6	C-C	59.09	60.53	55.95	58.97	61.78
286.3	C-O-C	10.43	10.07	12.95	8.81	10.71
288.0	O=C-O	30.48	29.4	26.57	32.23	27.51

Table S4. Binding energies and peak areas for O 1s of different nucleobase-doped CNDs.

Peak (eV)	Bond	A-CNDs (%)	C-CNDs (%)	G-CNDs (%)	T-CNDs (%)	U-CNDs (%)
530.8	O=C-O/C=O	48.87	46.47	48.76	49.84	27.65
532.4	C-O-C/C-O-H	43.59	47.19	44.32	43.70	66.33
535.5	-OH	7.54	6.34	6.93	6.46	6.02

Table S5. Functional moiety identification and quantification by TGA and DTG measurements.

	Original								Recalculated					
	adsorbed H_2O	**-OH, alcohol**	**C=O + COOH + C-O**	**amines**	**shell**	**Core**	**remnant**	**effective mass**	**-OH**	**amine**	**C=O + COOH + C-O**	**shell**	**core**	**OH + NH_2 on the shell**
Bare CNDs	(40-122 °C) 9%	(122-168 °C) 2%	(168-338 °C) 18%	(338-448 °C) 18%	38%	(448-1000 °C) 53%	0	91%	2%	20%	20%	42%	58%	57%
A-CNDs	(40-122 °C) 9%	(122-147 °C) 1%	(147-338 °C) 18%	(338-498 °C) 20%	39%	(498-1000 °C) 13%	39%	52%	2%	38%	35%	75%	25%	53%
C-CNDs	(40-102 °C) 8%	(102-133 °C) 2%	(133-354 °C) 21%	(354-459 °C) 23%	46%	(459-1000 °C) 29%	17%	75%	3%	31%	28%	62%	38%	55%
G-CNDs	(40-121 °C) 8%	(121-147 °C) 1%	(147-338 °C) 18%	(338-508 °C) 33%	52%	(508-1000 °C) 16%	24%	68%	1%	49%	26%	76%	24%	66%
T-CNDs	(40-116 °C) 8%	(116-147 °C) 1%	(147-338 °C) 22%	(338-533 °C) 33%	56%	(533-1000 °C) 6%	30%	62%	2%	53%	35%	90%	10%	61%
U-CNDs	(40-122 °C) 10%	(122-147 °C) 1%	(147-328 °C) 22%	(328-508 °C) 39%	62%	(508-1000 °C) 14%	14%	76%	1%	51%	29%	81%	19%	64%

Table S6. Zeta potentials of various nucleobase-doped CNDs.

CND species	**A-CNDs**	**C-CNDs**	**G-CNDs**	**T-CNDs**	**U-CNDs**
Zeta potential（mV）	-23.6 ± 1.5	-23.8 ± 2.4	-28.6 ± 0.8	-30.0 ± 3.4	-29.1 ± 0.8

Table S7. Rate of zebrafish embryos with reduced cell adhesion.

	Bare CNDs	**A-CNDs**	**C-CNDs**	**G-CNDs**	**T-CNDs**	**U-CNDs**
1 μg/mL	0	20%	20%	10%	20%	10%
100 μg/mL	10%	10%	10%	30%	20%	10%

Table S8. Results of generalized linear model for qPCR comparing amplification of the zebrafish polymerase alpha-1 gene in presence of bare and doped CNDs (after omitting two outliers for G-CNDs at high concentrations).

GLM (formula = value ~ treatment + concentration, family = gaussian (identity), data = zebrafish)

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	1.57931	0.42124	3.749	0.000605 ***
Treatment [T.C]	0.06717	0.55958	0.120	0.905109
Treatment [T. bare CNDs]	-0.16067	0.55958	-0.287	0.775623
Treatment [T. combo]	-0.17567	0.55958	-0.314	0.755342
Treatment [T. G]	0.11866	0.62979	0.188	0.851589
Treatment [T. T]	-0.16400	0.55958	-0.293	0.771102
Treatment [T. U]	-0.11483	0.55958	-0.205	0.838531
Treatment [T. water]	-0.04083	0.55958	-0.073	0.942222
Concentration [T. low]	0.34638	0.28897	1.199	0.238276

References

1. Liyanage, P. Y.; Graham, R. M.; Pandey, R. R.; Chusuei, C. C.; Mintz, K. J.; Zhou, Y.; Harper, J. K.; Wu, W.; Wikramanayake, A. H.; Vanni, S.; Leblanc, R. M., Carbon nitride dots: A selective bioimaging nanomaterial. *Bioconjugate Chem.* **2019,** *30*, 111-123.