

## *Supplementary Information*

# A Sustainable One-Pot Synthesis of Chiral Carbon Dots Using Response Surface Methodology: Elucidating the Mechanistic Pathways through Biological Responses

Arezoo Setayesh,<sup>ac</sup> Laura Domínguez Mercado,<sup>a</sup> Adryanne Clermont-Paquette,<sup>abc</sup> Brandon L. Findlay,<sup>ab</sup> Rafik Naccache<sup>ac\*</sup>

<sup>a</sup>*Department of Chemistry and Biochemistry and Center for NanoScience Research, Concordia University, Montreal, QC, Canada, H4B 1R6.*

<sup>b</sup>*Department of Biology and the Centre for Microscopy and Cellular Imaging, Concordia University, Montreal, QC, Canada, H4B 1R6.*

<sup>c</sup>*Quebec Centre for Advanced Materials, Department of Chemistry and Biochemistry, Concordia University, Montreal, QC, Canada, H4B 1R6.*

\* Corresponding author e-mail: [rafik.naccache@concordia.ca](mailto:rafik.naccache@concordia.ca)

**Table S1.** Experimental matrix for the synthesis of L-pro.CA CDots using BBD. Independent variables are  $X_1$ = time,  $X_2$ = temperature and  $X_3$ = molar ratio of proline to CA and their dependent response is Y= Chiral integration.

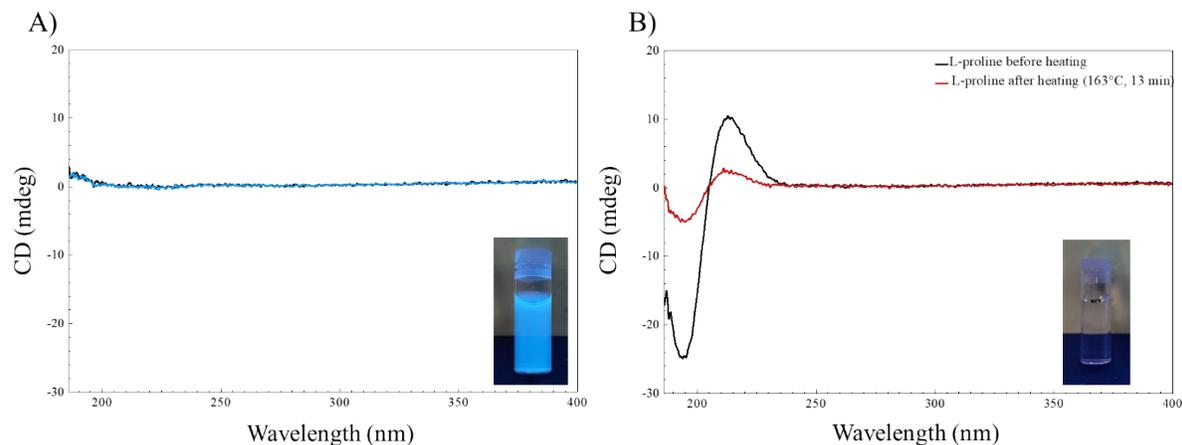
Run	Factors			Response
	$X_1$	$X_2$	$X_3$	Y
1	17.5	160.0	3.0	547.6
2	10.0	175.0	3.0	528.6
3	17.5	175.0	5.0	411.8
4	17.5	160.0	7.0	410.5
5	10.0	175.0	7.0	305.2
6	17.5	175.0	5.0	360.9
7	25.0	175.0	7.0	310.0
8	17.5	190.0	3.0	307.6
9	17.5	175.0	5.0	423.7
10	17.5	175.0	5.0	393.7
11	25.0	160.0	5.0	444.5
12	10.0	160.0	5.0	420.7
13	10.0	190.0	5.0	349.5
14	17.5	190.0	7.0	243.3
15	25.0	190.0	5.0	159.9
16	17.5	175.0	5.0	400.4
17	25.0	175.0	3.0	321.0

**Table S2.** The result for sequential model sum of squares. The quadratic model, which offers a polynomial of higher order than a linear model, is employed. In addition, the quadratic model avoids aliasing, distinguishing it from the cubic model.

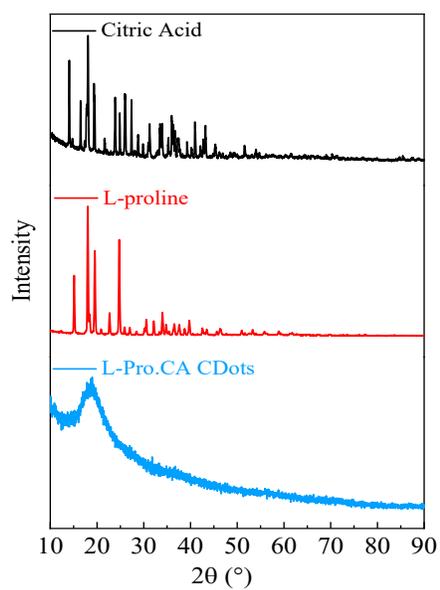
Source	Sum of Squares	df	Mean Squares	<i>F</i> -Value	<i>P</i> -Value	
Linear	1	3	0.33	11.67	0.0005	
2FI	0.24	3	0.08	6.20	0.01	
Quadratic	0.11	3	0.03	10.23	0.0059	Suggested
Cubic	9.370E – 003	3	3.123E – 003	0.84	0.5367	Aliased

**Table S3.** ANOVA results for the modified quadratic model.(X<sub>1</sub>= time, X<sub>2</sub>= temperature and X<sub>3</sub>= molar ratio of proline to CA)

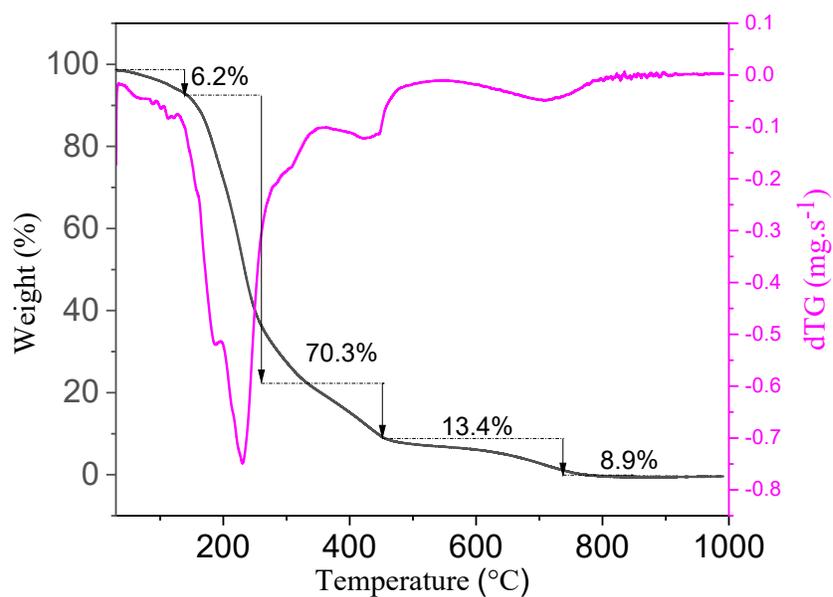
Source	Sum of Squares	df	Mean Squares	F-Value	P-Value Prob >F
Model	1.35	7	0.19	69.52	< 0.0001
X <sub>1</sub>	0.18	1	0.18	66.03	< 0.0001
X <sub>2</sub>	0.67	1	0.67	240.12	< 0.0001
X <sub>3</sub>	0.15	1	0.15	55.26	
X <sub>1</sub> X <sub>2</sub>	0.18	1	0.18	63.19	.0001
X <sub>1</sub> X <sub>3</sub>	0.06	1	0.06	19.97	.0016
X <sub>1</sub> <sup>2</sup>	0.05	1	0.05	16.10	.0031
X <sub>2</sub> <sup>2</sup>	0.04	1	0.04	5.48	.0473
Residual	0.02	9	2.77E – 003		
Lack of fit	0.01	5	2.03E – 003	0.55	0.7373
Mean	5.88		R-Squared	0.98	
C.V%	0.89		Adj. R-Squared	0.96	
Adeq. precision	32.95		Pred. R-Squared	0.91	



**Figure S1.** Absence of chiral signal in citric acid precursor before and after microwave in CD spectra; Inset: fluorescence of citric acid sample dissolved in water, indicating the formation of CDots (A). Heat treatment does not affect exciton couplet in L-proline, as demonstrated by CD spectra analysis; Inset: Fluorescence emission of L-proline after heating and dissolution in water demonstrating no sign of photoluminescence properties (B).



**Figure S2.** XRD pattern of L-Pro.CA CDots. Upon formation, the L-Pro.CA CDots evidence an amorphous halo centered at  $17^\circ 2\theta$  along with the absence of all crystalline reflections from the precursors.



**Figure S3.** TGA profile of L-Pro.CA CDots reveals multiple weight losses with increasing temperature. The predominant weight loss of 70.3% occurs at 260°C, indicating the thermal stability of L-Pro.CA CDots up to this temperature.