

Supplementary materials

Chirality governs the structure and activity changes of *Photinus pyralis* firefly luciferase induced by carbon quantum dots

Mehrnaz Rad-Faraji^{1†}, Marziyeh Mousazadeh^{1†}, Maryam Nikkhah^{1*}, Sajad Moradi², Mohabbat Ansari³, Klara Cepe⁴, Saman Hosseinkhani⁵, Aram Rezaei^{2*}

1. Department of Nanobiotechnology, Faculty of Biological Sciences, Tarbiat Modares University, P. O. Box: 14115-154, Tehran, Iran.
2. Nano Drug Delivery Research Center, Health Technology Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran.
3. Department of Tissue Engineering and Applied Cell Science, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
4. Regional Centre of Advanced Technologies and Materials, Palacký University, Olomouc, Czech Republic.
5. Department of Biochemistry, Faculty of Biological Sciences, Tarbiat Modares University, P. O. Box: 14115-154, Tehran, Iran.

Corresponding authors:

* Maryam Nikkhah m_nikkhah@modares.ac.ir

* Aram Rezaei aram.rezaei@gmail.com, aram.rezaei@kums.ac.ir

† These authors contributed equally to this work.

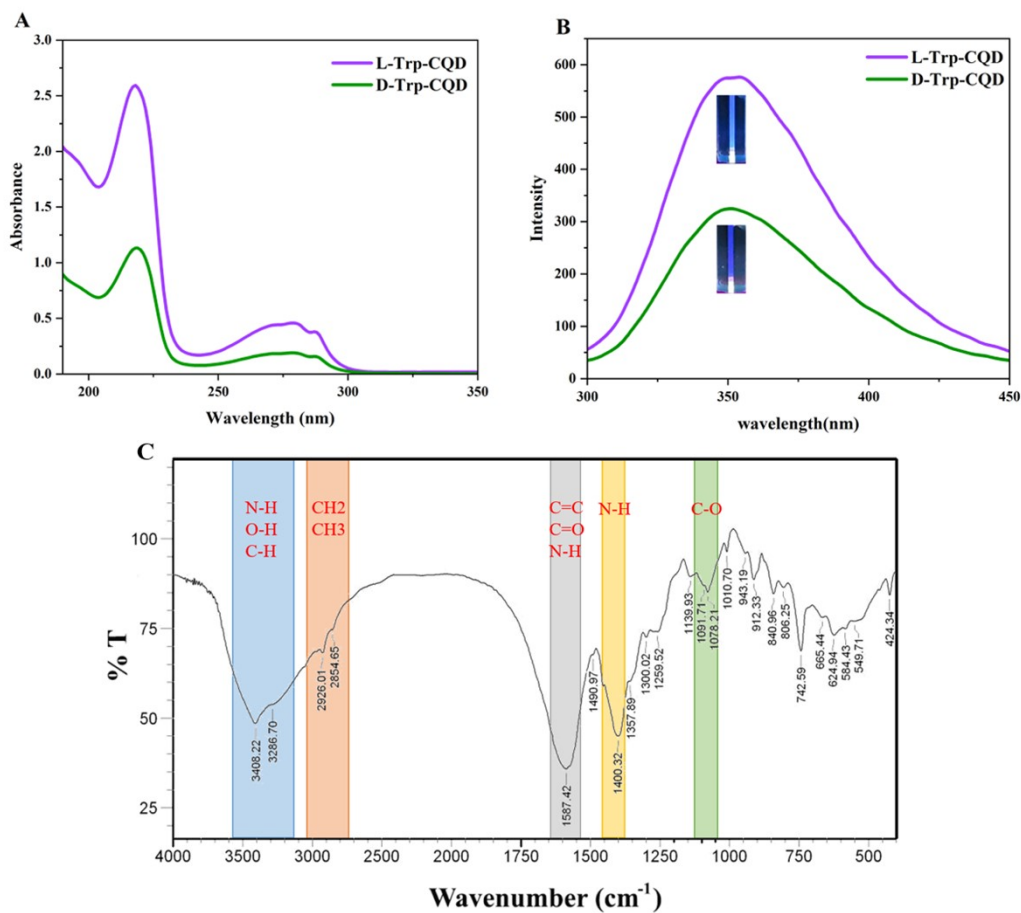


Figure S1. **A)** UV-vis absorbance spectra of L- and D-Trp-CQDs (2 $\mu g/ml$), **B)** Fluorescent spectra of L- and D-Trp-CQDs (2 $\mu g/ml$) in the $\lambda_{ex} = 270$ nm. All were done in ambient condition, **C)** The FTIR analysis of L/D-Trp-CQDs

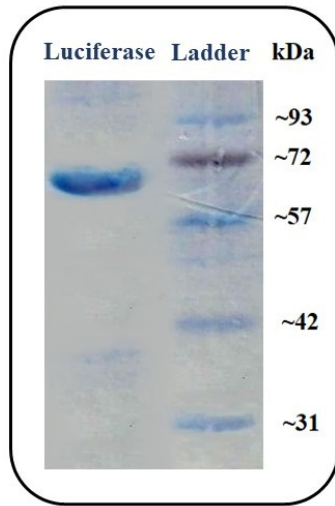


Figure S2. SDS page of luciferase enzyme which is 62 kDa

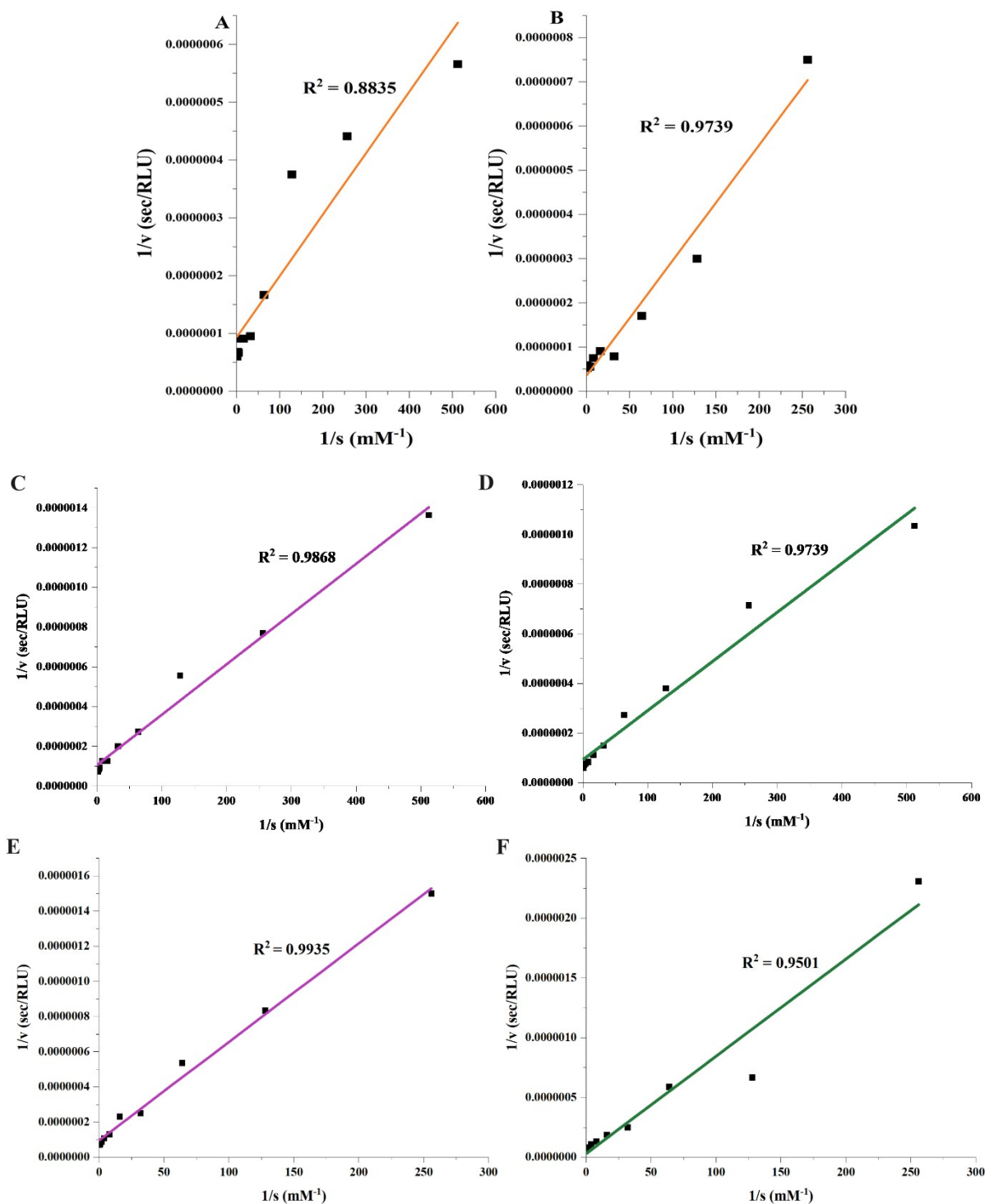


Figure S3. The line Weaver-Burk plot of luciferase enzyme for: **A)** luciferin in the absence of CQDs, **B)** ATP in the absence of CQDs, **C)** luciferin in the presence of L-Trp-CQD, **D)** luciferin in the presence of D-Trp-CQD, **E)** ATP in the presence of L-Trp-CQD, and **F)** ATP in the presence of D-Trp-CQD

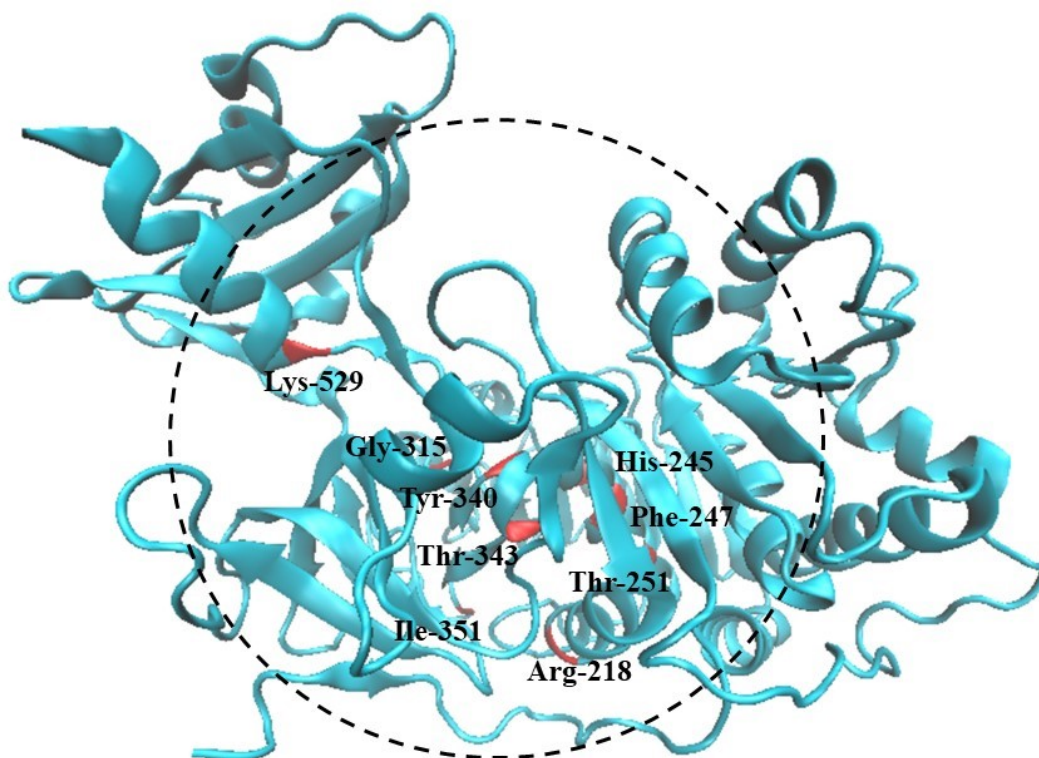


Figure S4. The simulated structure of luciferase (PDB code:1Lci) representing the nine important amino acids in luciferase active site. The model was generated using VMD 1.9.2 software.

Table S1. The changes in luciferase secondary structures upon interaction with L/D -Trp-CQD.

	Range of amino acids	DSSP (Secondary structure)	RMSF	Changed amino acids in the active site	Changed amino acids involved in activity
D-Trp-CQDs	80-90	Coil to Bend, Turn to α -helix, β -sheet to Coil	Reduce	-	-
	100-110	Bend to Turn & Coil	Reduce	-	-
	120-130	Bend to β -sheet,	Increase	-	-
		3-10 Helix			

	&Turn to α - Helix			
140-150	Coil to Bend & β -sheet	Reduce	-	-
150-160	Turn to Bend	Reduce	-	-
190-200	Bend to Turn, β - sheet to Coil	Increase	-	Asn197, Ser199
200-210	Bend to Turn, β - sheet to Coil	Increase	-	-
220-230	Turn to Bend	Increase	-	-
240-250	Turn to Bend	Reduce	His245, Phe247	-
270-280	β -sheet to B- bridge	Reduce	-	-
290-300	Turn & 3-10 helix to α -helix	Reduce	-	-
300-310	β -sheet to Coil, Turn to Bend	Reduce	-	-
310-320	Turn to Bend, Coil & Turn to β -sheet	Reduce	Gly315	Ala317
330-340	B-bridge to Coil, Coil to Bend & β -sheet	Increase	-	Arg337
340-350	3-10 Helix to Turn	Reduce	Tyr 340, Thr343	Ala348

	350-360	Turn to Bend, Coil to Bend	Reduce	Ile351	Pro353, Gly354, Gly355, Asp356, Pro359
	430-440	β -sheet to Coil, Bend to Turn & 3-10 Helix	Reduce	-	-
	440-450	Coil to β -sheet, Coil to Bend	Reduce	-	-
	470-480	Bend to Turn	Reduce	-	-
	520-530	Bend to Turn & Coil	Increase	Lys529	-
L-Trp- CQDs	0-10	Turn to Bend	Reduce	-	-
	10-20	Coil to Bend	Increase	-	-
	40-50	β -sheet to Coil	Reduce	-	-
	80-90	Bend & Coil to Turn	Reduce	-	-
	90-100	Turn to β -sheet	Reduce	-	-
	100-110	β -sheet & Bend to Coil	Reduce	-	-
	120-130	3-10 Helix to Turn, Turn & Coil to Bend	Reduce	-	-
	140-150	Turn & B-sheet to Coil, Turn & α -Helix to Bend	Reduce	-	-

180-190	Bend to Turn	Reduce	-	-
190-200	β -sheet to Coil & B-bridge	-	-	Asn197, Ser199
200-210	β -sheet to Coil, Bend to Coil & B-bridge	Reduce	-	-
280-290	α -Helix to 3-10 Helix & Turn	Reduce	-	-
290-300	α -Helix to 3-10 Helix & Turn & Bend	Reduce	-	-
300-310	Turn to 3-10 Helix & Bend	Reduce	-	-
310-320	Coil to β -sheet, Turn to Bend	Reduce	Gly315	Ala317
330-340	β -sheet to Coil & B-bridge	Reduce	-	-
350-360	Turn to Bend, β - sheet to Coil	Reduce	Ile351	Gly354
360-370	β -sheet to B- bridge	Reduce	-	-
420-430	Bend to Turn	Reduce	-	-
430-440	β -sheet to Coil, Bend & Turn to α -Helix & 3-10	Reduce	-	-

Helix					
440-450	Coil & B-bridge	Reduce	-	-	
	to β -sheet, Bend				
	to Turn				
450-460	α -helix to Turn	Reduce	-	-	
470-480	Turn to Bend	Reduce	-	-	
520-530	Bend to Turn	Reduce	Lys529	-	

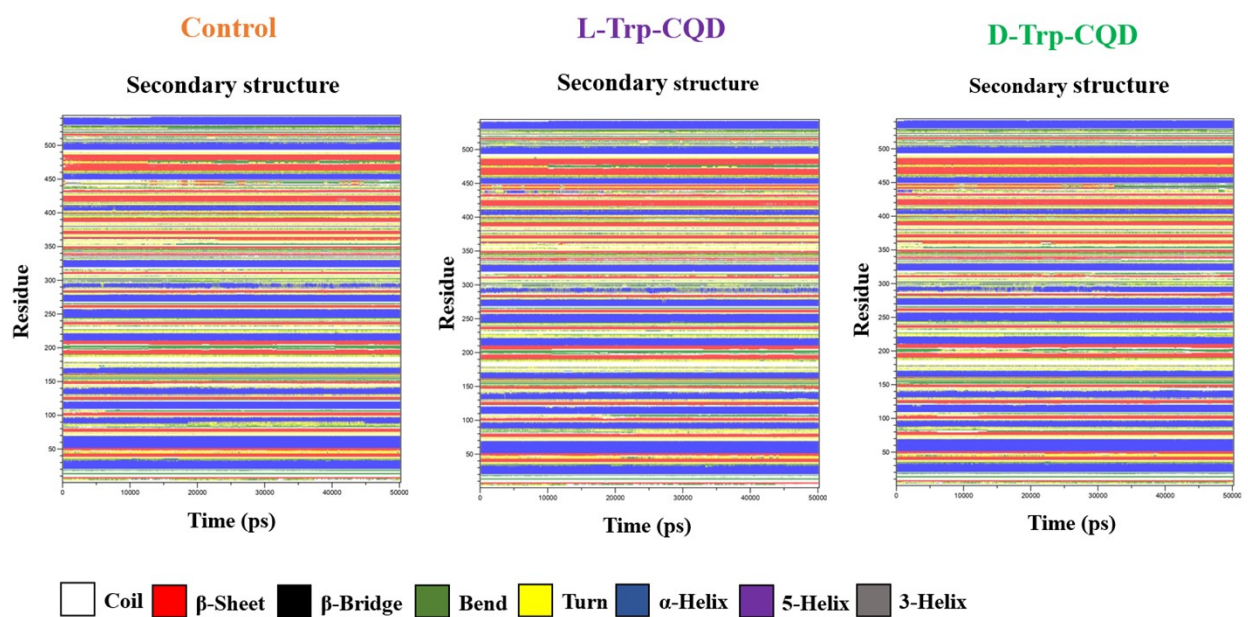


Figure S5. The DSSP diagram of free luciferase (control) and the luciferase interacting with L-Trp-CQD and D-Trp-CQD during 50 ns.

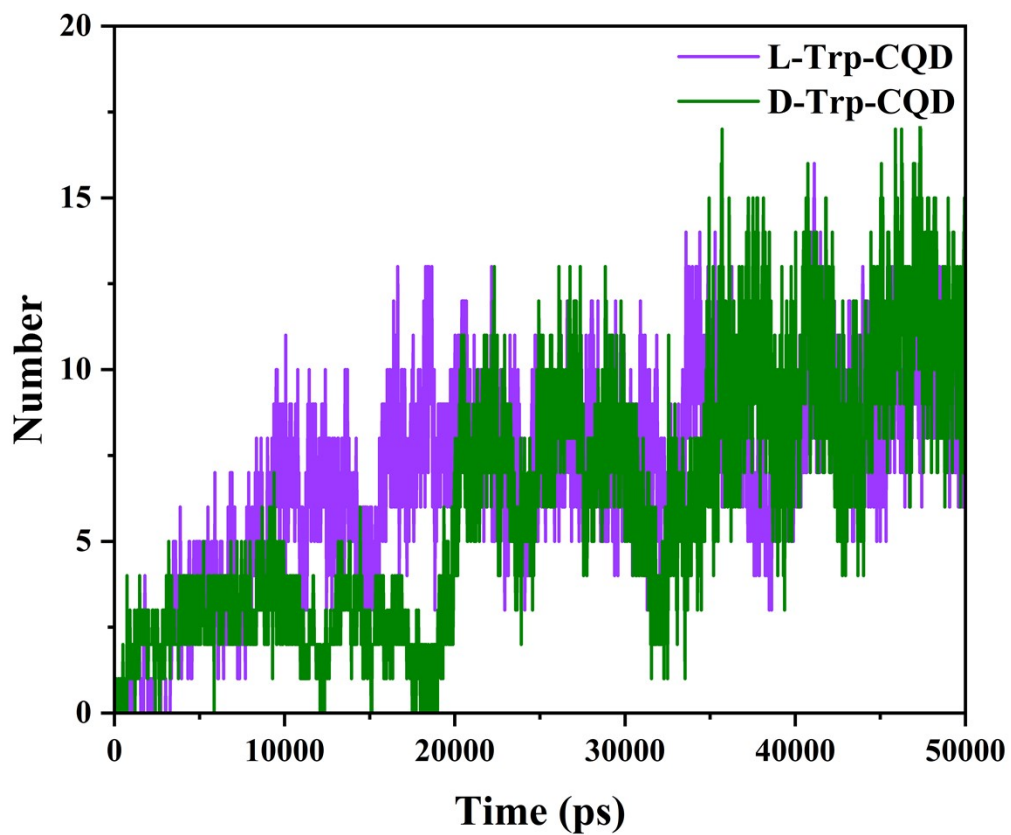


Figure S6. Total number of hydrogen bonds of luciferase enzyme interacting with L-Trp-CQD and D-Trp-CQD during 50 ns