

Size-Dependent Interactions of γ H2AX and p53 Proteins with Graphene Quantum Dots.

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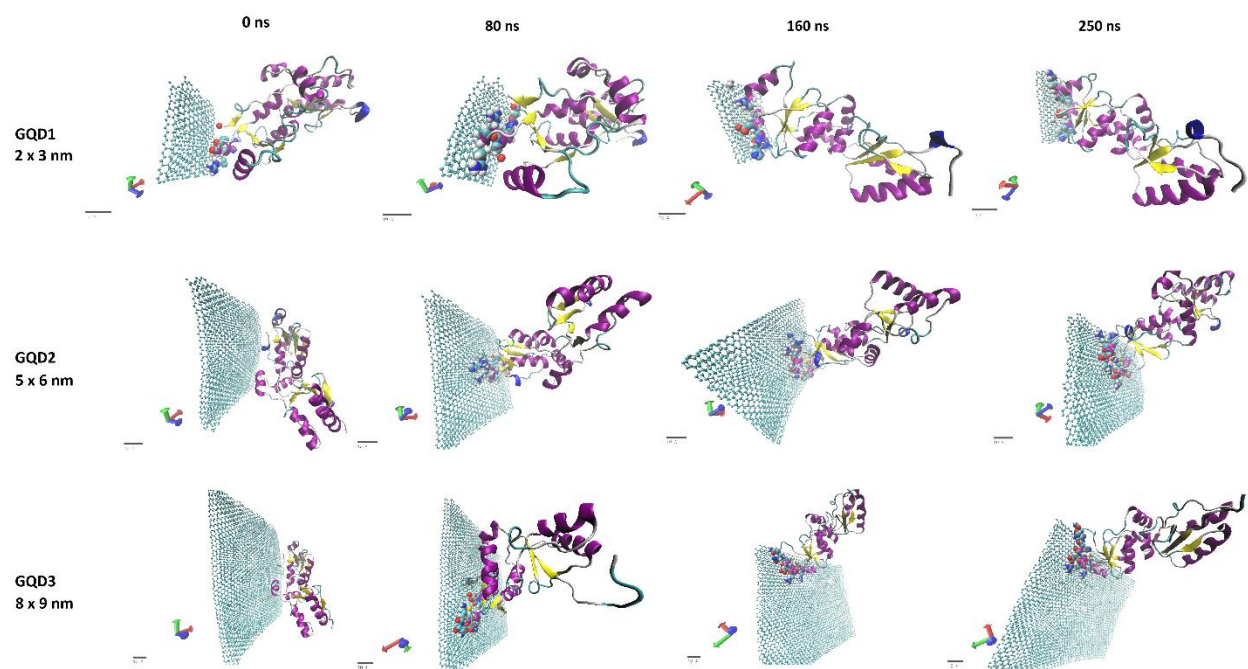


Figure S1. Trajectory screenshots of γ H2AX protein in the presence of the three GQDs at 0 ns, 80 ns, 160ns and 250ns intervals. The protein is represented using 'New Cartoon', the GQDs are represented using 'CPK', and the adsorbed atoms of the protein on the GQD (using the 5Å adsorption criteria) are represented using VDW spheres.

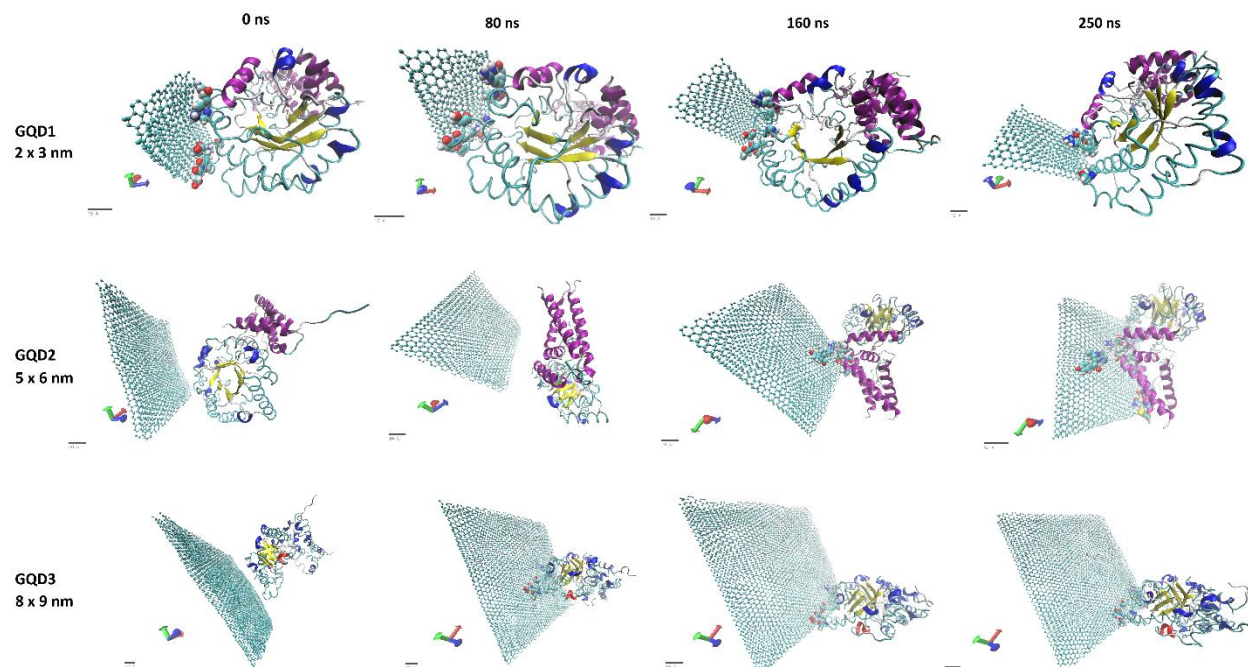


Figure S2. Trajectory screenshots of p53 protein in the presence of the three GQDs at 0 ns, 80 ns, 160ns and 250ns intervals. The protein is represented using 'New Cartoon', the GQDs are represented using 'CPK', and the adsorbed atoms of the protein on the GQD (using the 5Å adsorption criteria) are represented using VDW spheres. It can be clearly seen that in the presence of GQD3 (8 X 9 nm), the protein lost its secondary structure in the form of α -helix indicating the denaturing role of GQD3 towards p53.

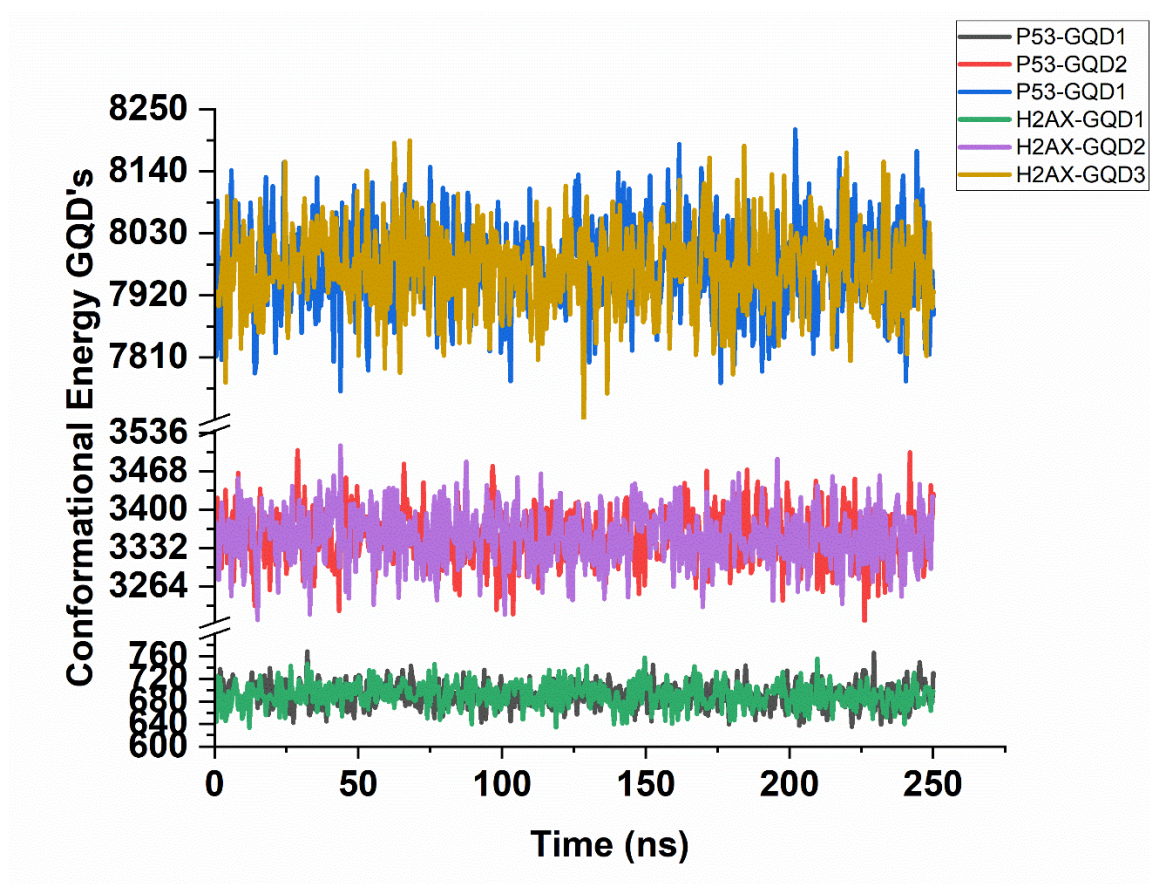


Figure S3. Analysis of the conformational energies of GQD1, GQD2 and GQD3 in the presence of p53 and γ H2AX.

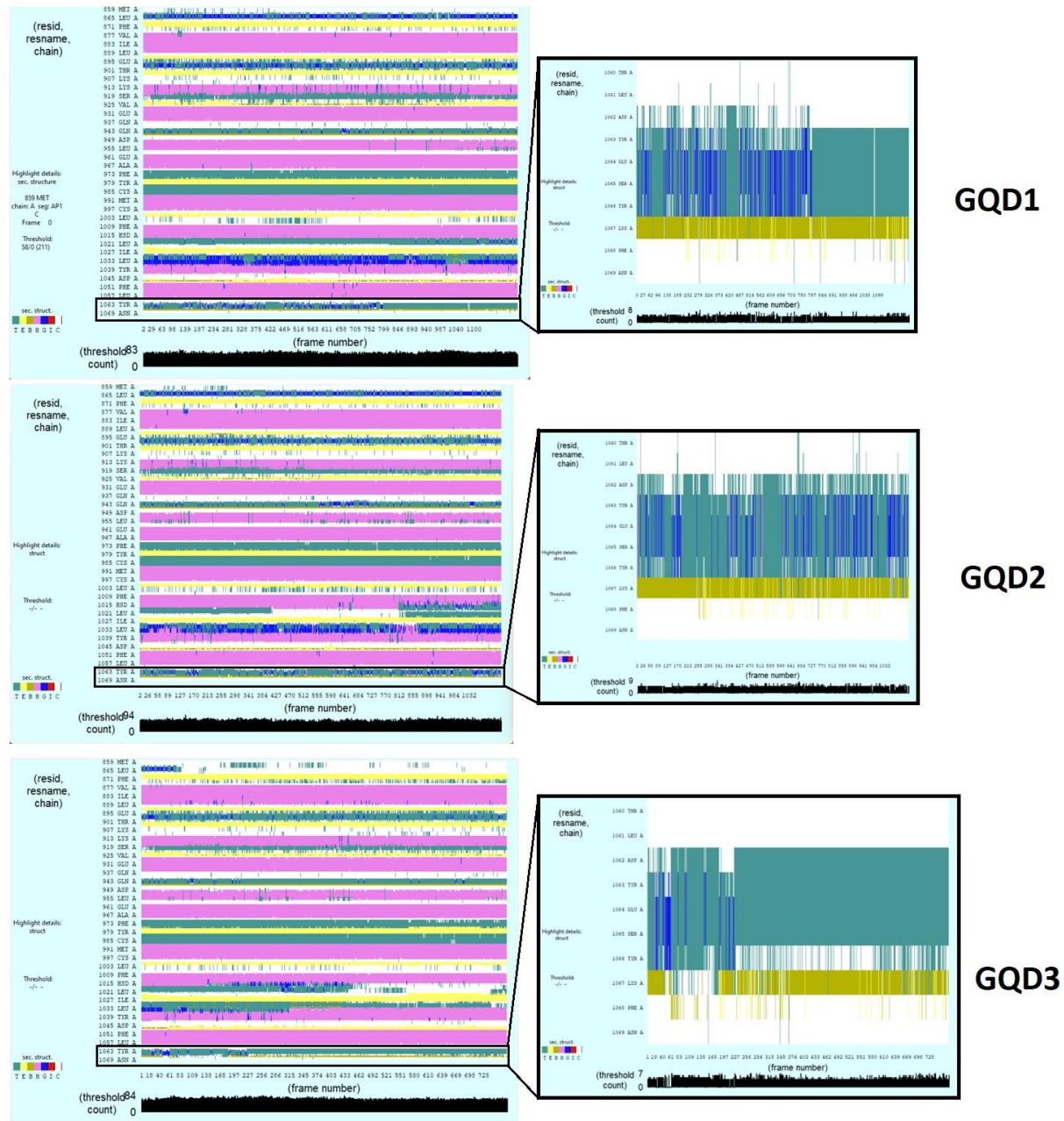
Table S1. Analysis of salt bridges within p53 in the presence of GQD1, GQD2 and GQD3.

Salt Bridges	Salt Bridges P53 with GQD's		
	Formed	Sustained	Broken
saltbr-ASP100-LYS102			GQD1
saltbr-ASP142-ARG108		GQD1, GQD2	
saltbr-ASP174-ARG104	GQD2	GQD3	GQD1
saltbr-ASP174-LYS98			GQD1, GQD2, GQD3
saltbr-ASP279-ARG299		GQD1, GQD2, GQD3	
saltbr-ASP285-LYS98	GQD2, GQD3		GQD1
saltbr-ASP287-ARG290		GQD1, GQD3	
saltbr-ASP287-LYS289	GQD1, GQD3		
saltbr-ASP557-ARG627		GQD1, GQD2, GQD3	
saltbr-ASP563-LYS574	GQD2	GQD1,	GQD3
saltbr-ASP646-ARG682		GQD1, GQD3	GQD2
saltbr-ASP646-LYS289		GQD2	GQD1, GQD3
saltbr-ASP696-ARG580	GQD3	GQD1	
saltbr-ASP700-ARG290		GQD1	GQD3
saltbr-ASP700-ARG682	GQD1, GQD2		
saltbr-ASP700-ARG691	GQD1		GQD2, GQD3
saltbr-ASP700-LYS257			GQD1
saltbr-ASP700-LYS289	GQD1		
saltbr-GLU109-LYS102	GQD1		GQD2
saltbr-GLU115-LYS102		GQD1, GQD3	GQD2
saltbr-GLU128-ARG127			GQD1
saltbr-GLU128-LYS79	GQD2		GQD1
saltbr-GLU130-ARG127			GQD1
saltbr-GLU132-ARG127	GQD2	GQD1	
saltbr-GLU132-ARG167	GQD2, GQD3	GQD1	
saltbr-GLU150-ARG108	GQD3	GQD1, GQD2	
saltbr-GLU150-LYS149	GQD2		GQD1
saltbr-GLU186-LYS185			GQD1
saltbr-GLU201-LYS155		GQD1, GQD2, GQD3	
saltbr-GLU210-ARG127			GQD1
saltbr-GLU239-ARG235	GQD2, GQD3		GQD1
saltbr-GLU239-LYS240		GQD1	
saltbr-GLU239-LYS272		GQD1	GQD2
saltbr-GLU246-LYS308		GQD1, GQD2, GQD3	
saltbr-GLU258-LYS257			GQD1
saltbr-GLU265-ARG306		GQD1, GQD2, GQD3	
saltbr-GLU265-LYS269	GQD1, GQD2		
saltbr-GLU302-ARG306	GQD2		GQD1
saltbr-GLU317-ARG71	GQD2, GQD3	GQD1	

saltbr-GLU319-ARG84		GQD1, GQD2, GQD3	
saltbr-GLU386-LYS87	GQD2, GQD3	GQD1	
saltbr-GLU390-LYS87	GQD1, GQD2		
saltbr-GLU556-LYS574			GQD1, GQD2
saltbr-GLU570-LYS297	GQD3	GQD2	GQD1
saltbr-GLU581-ARG610		GQD1, GQD2, GQD3	
saltbr-GLU605-ARG610	GQD1		
saltbr-GLU606-ARG608	GQD1		
saltbr-GLU606-ARG610			GQD1
saltbr-GLU615-ARG664	GQD1, GQD3		
saltbr-GLU615-LYS655	GQD3	GQD1, GQD2	
saltbr-GLU616-LYS613		GQD1, GQD2, GQD3	
saltbr-GLU688-ARG586	GQD1		GQD3
saltbr-GLU688-ARG691	GQD2, GQD3		GQD1
saltbr-GLU76-LYS79	GQD1, GQD3	GQD2	
saltbr-GLU80-ARG83	GQD3	GQD1, GQD2	
saltbr-GLU80-ARG84		GQD1, GQD2, GQD3	
saltbr-GLU89-LYS333		GQD1, GQD2, GQD3	
saltbr-ASP159-ARG156		GQD2	
saltbr-ASP287-ARG691	GQD2, GQD3		
saltbr-ASP639-ARG71	GQD2		
saltbr-ASP646-ARG290	GQD2		
saltbr-ASP696-ARG691			GQD2, GQD3
saltbr-ASP99-ARG104		GQD2	GQD3
saltbr-ASP99-LYS98	GQD2, GQD3		
saltbr-GLU570-LYS257		GQD2	
saltbr-GLU581-ARG580	GQD2		
saltbr-GLU66-ARG78		GQD2, GQD3	
saltbr-GLU688-ARG290		GQD2	
saltbr-ASP696-ARG290			GQD3
saltbr-ASP700-LYS253	GQD3		
saltbr-GLU266-LYS269	GQD3		
saltbr-GLU390-ARG83	GQD3		
saltbr-GLU390-ARG84	GQD3		

Table S2. Analysis of salt bridges within γ H2AX in the presence of GQD1, GQD2 and GQD3.

Salt Bridges	Salt Bridges γ H2AX with GQD's		
	Formed	Sustained	Broken
saltbr-ASP1045-LYS1016	GQD1		GQD2, GQD3
saltbr-ASP1045-LYS1067		GQD1, GQD2, GQD3	
saltbr-ASP949-LYS907	GQD2, GQD3	GQD1	
saltbr-GLU1014-ARG1010		GQD3	GQD1
saltbr-GLU1014-LYS1011	GQD1, GQD2, GQD3		
saltbr-GLU1023-LYS1016	GQD1	GQD3	GQD2
saltbr-GLU1023-LYS1067	GQD1		
saltbr-GLU1023-LYS976		GQD3	GQD1, GQD2
saltbr-GLU1038-ARG1042			GQD1, GQD2
saltbr-GLU1050-ARG910	GQD1	GQD2, GQD3	
saltbr-GLU1064-LYS1067		GQD1, GQD2	GQD3
saltbr-GLU864-LYS899	GQD1		
saltbr-GLU892-LYS860			GQD3
saltbr-GLU895-LYS899			GQD1
saltbr-GLU895-LYS913	GQD3		
saltbr-GLU928-ARG935			GQD2
saltbr-GLU931-ARG935	GQD1, GQD2		GQD3
saltbr-GLU931-LYS885		GQD1, GQD2	GQD3
saltbr-GLU932-ARG935		GQD1, GQD2	GQD3
saltbr-GLU951-LYS907	GQD1, GQD2, GQD3		
saltbr-GLU953-ARG948	GQD1, GQD3	GQD2	
saltbr-GLU961-ARG948		GQD1, GQD2, GQD3	
saltbr-GLU961-LYS965	GQD1	GQD2	GQD3
saltbr-GLU962-ARG966	GQD2	GQD1	
saltbr-GLU962-LYS965	GQD3		GQD2
saltbr-GLU996-LYS992		GQD1, GQD2, GQD3	
saltbr-GLU1023-LYS1011			
saltbr-GLU1038-LYS1016	GQD2		
saltbr-GLU892-LYS899			GQD2
saltbr-GLU864-LYS860	GQD3		



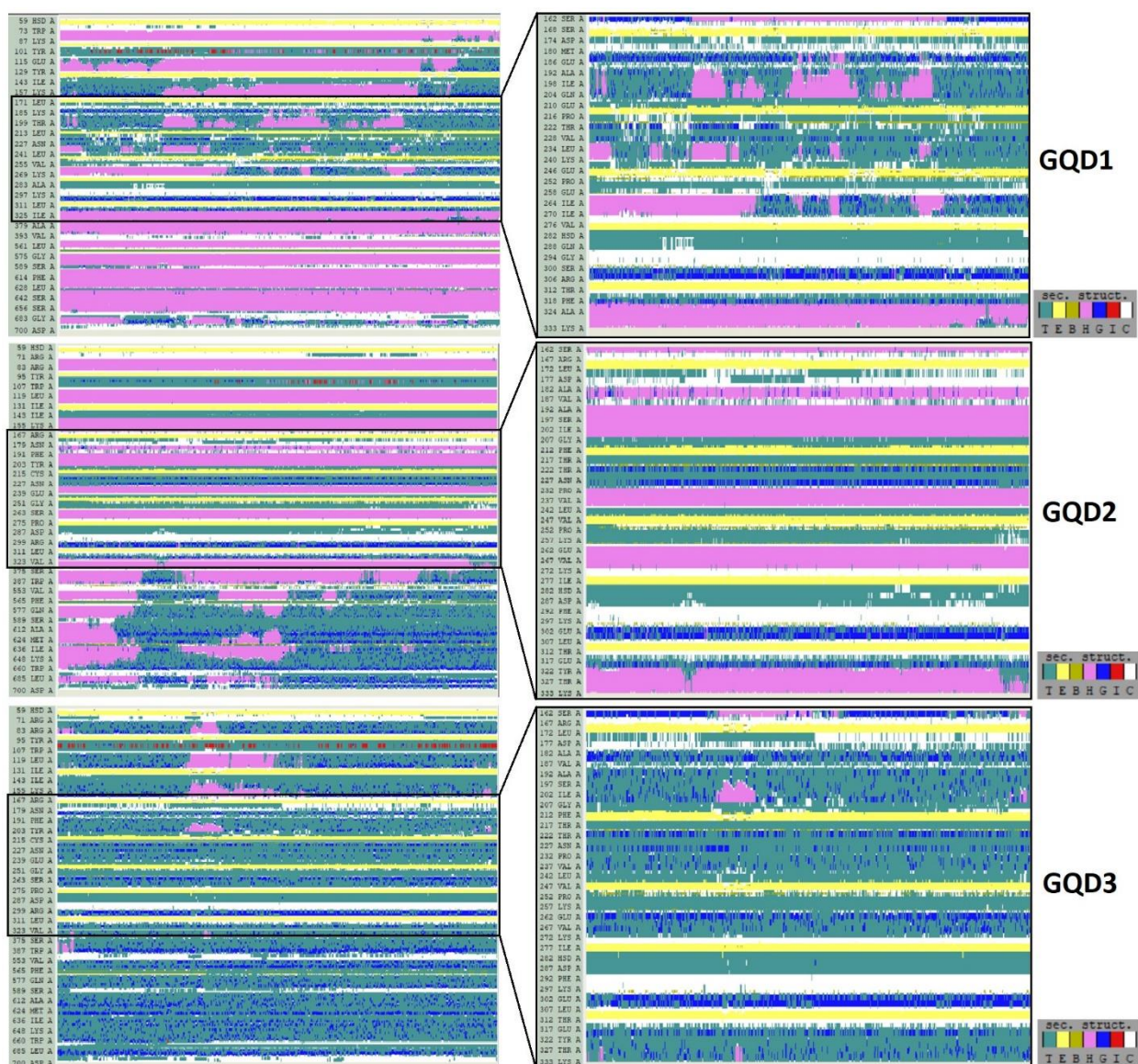


Figure S5. Secondary structure analysis of p53 in the presence of GQD1, GQD2 and GQD3. The reactive site on p53 from SER142 to LYS333 is shown separately as a blowup image indicating the denaturing role of both GQD1 and GQD3 where p53 also underwent an irreversible conformational change where nearly all the α -helices are permanently conformed to a turn.

Background analysis on the optimal adsorption distance between the proteins, p53/ γ H2AX and the three GQDs:

This study investigates the interactions of the proteins p53 and γ H2AX with GQD1, GQD2, and GQD3, focusing on distances up to 12 Å from the surface of the GQDs, which is considered an effective threshold for analyzing long range forces in molecular interactions. As seen in Figs. S6A and S6C, at very short distances (around 1 to 2 Å), no interacting p53 or γ H2AX atoms are observed mainly because the measured distance is shorter than atomic distances. However, as the measured distance between the proteins and GQDs increases, the number of atoms involved in the interactions also rises, with significant increases occurring between 3 to 8 Å. Larger GQDs (GQD2 and GQD3) consistently demonstrate a larger number of interacting atoms suggesting that the size of the GQD influences the number of interacting atoms. For example, at 3 Å, the interaction between P53 and GQD2 involves ~71 atoms, while with GQD3, it involves ~29 atoms, compared to ~19 atoms for P53-GQD1. This indicates that larger GQDs offer more surface area for protein binding. Similarly, γ H2AX demonstrates stronger interactions with larger GQDs. For instance, GQD3 shows ~41 interacting atoms compared to ~24 atoms for GQD1. As the distance increases to 4-6 Å, both proteins exhibit further increases in the number of atoms involved in the interactions, with GQD3 consistently presenting the highest values. At 6 Å, P53-GQD3 shows ~160 atoms, while γ H2AX -GQD3 displays ~98 atoms, indicating substantial interaction compared to GQD1 and GQD2. At longer distances (9 to 12 Å), the number of atoms involved remains relatively high reflecting continued engagement with the GQD surfaces. For instance, at 12 Å, P53-GQD3 has ~287 atoms, while γ H2AX -GQD3 has ~259 atoms, suggesting strong interactions as the distance approaches the threshold for effective molecular binding. These findings highlight that larger GQDs (GQD2 and GQD3) facilitate stronger binding interactions with both P53 and γ H2AX proteins, even in the absence of hydrogen atoms. By analyzing protein-GQD interactions without considering hydrogen atoms, we gain clearer insights into fundamental non-hydrogen-dependent interactions, which is essential for understanding protein behavior when interacting with nanomaterials. This analysis offers valuable insights that can inform the designer of more efficient protein-functionalized nanodevices for applications in nanotechnology, biosensing, and drug delivery.

Furthermore, Fig. S6A shows the number of P53 atoms in contact with different GQDs (GQD1, GQD2, GQD3), both with and without hydrogen, as a function of distance. The red curve (P53-GQD2 with hydrogen) shows the highest number of atomic contacts, peaking at over 1200 atoms, indicating a stronger or more extensive interaction surface. In contrast, systems without hydrogen exhibit generally lower atomic contact counts, suggesting that hydrogen atoms may enhance the surface interaction between P53 and GQDs. Among all combinations, P53-GQD3 without hydrogen (yellow) shows the least number of contacts, reflecting weaker interaction or lower binding affinity in the absence of hydrogen. Similarly, Fig. S6B illustrates the interaction energy (IE) between P53 and GQDs over distance. More negative values denote stronger interactions. P53-GQD2 with hydrogen (red line) again shows the strongest interaction, with IE dropping close to ~180 kcal/mol, suggesting a highly favorable binding. Without hydrogen, the interaction energies are significantly weaker, with all curves lying above their hydrogenated counterparts. This highlights the critical role of hydrogen in stabilizing P53-GQD complexes, particularly enhancing the interaction strength of P53 with GQD2.

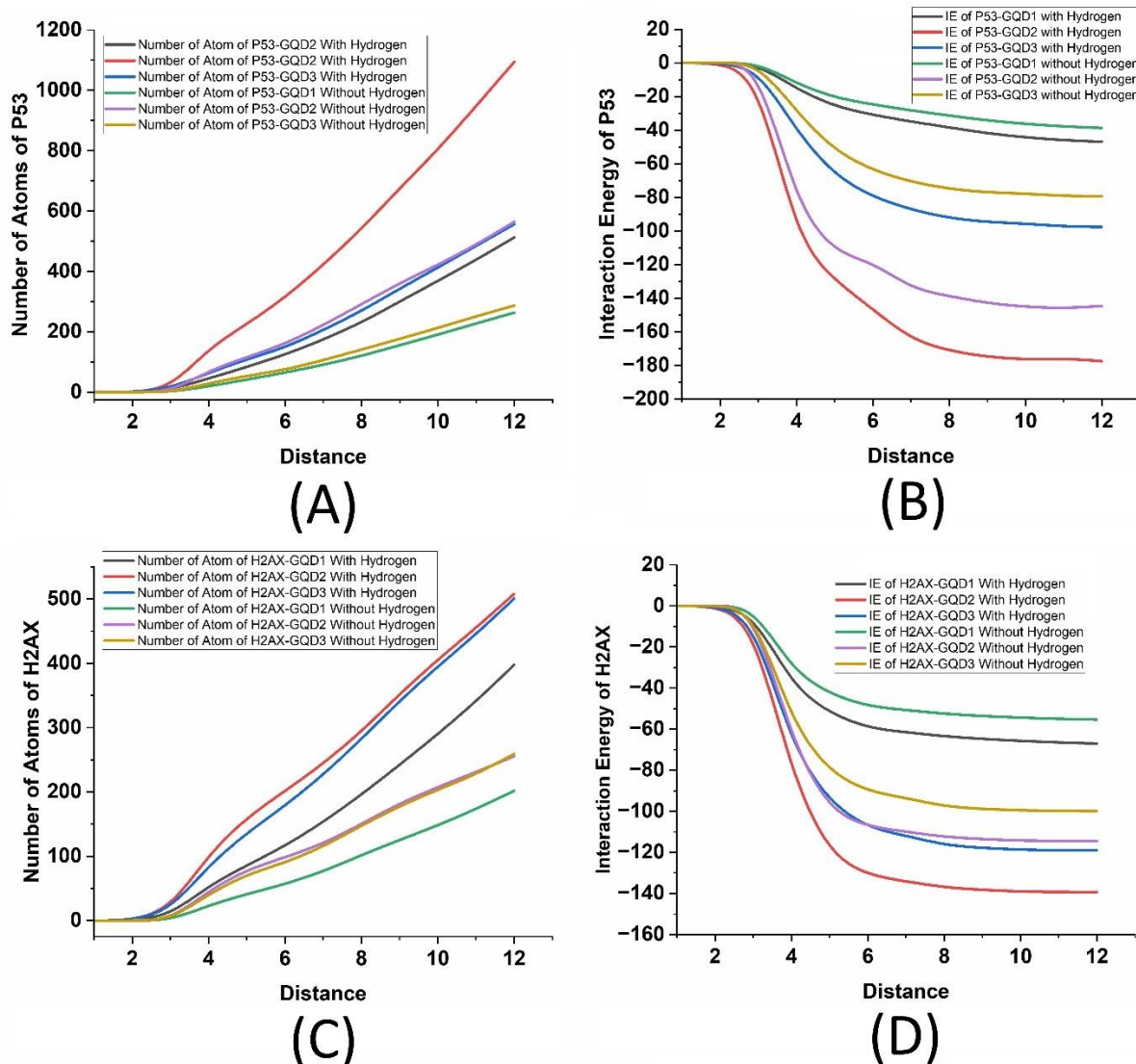


Figure S6. Number of atoms (NoA) and interaction energy (IE) analysis of p53 and γ H2AX in the presence of GQD1, GQD2 and GQD3. (A) NoA of p53 with and without hydrogen in the presence of GQD1, GQD2 and GQD3 across the distances up to 12 Å of the long range forces, (B) IE of p53 atoms within the distances up to 12 Å from the surface of GQD1, GQD2 and GQD3 with and without hydrogen, (C) NoA of γ H2AX with and without hydrogen in the presence of GQD1, GQD2 and GQD3 across the distances up to 12 Å of the long range forces, (D) IE of γ H2AX atoms within the distances up to 12 Å from the surface of GQD1, GQD2 and GQD3 with and without hydrogen.

From Fig. S6B and S6D, it is found that the size of the GQD plays a crucial role in determining the interaction strength, with GQD2 consistently exhibiting the most attractive (VDW) energies across all distances, indicating the strongest interactions with both p53 and γ H2AX compared to GQD1 and GQD3. At very short distances (1–2 Å), no interactive energies are measured owing to the length scale below the range of atomic distances. However, from 3 Å onward, the interaction energies become negative, reflecting increasing attractive forces. At 3 Å, for example, p53-GQD2 has a VDW energy of 8.1785 kcal/mol, which is significantly stronger than p53-GQD1 (0.62 kcal/mol) and p53-GQD3 (3.32 kcal/mol). Similarly, for

γ H2AX, the interaction with GQD2 (7.69 kcal/mol) is more intense than with GQD1 (3.03 kcal/mol) and GQD3 (5.51 kcal/mol). This trend continues across all distances, with GQD2 showing the highest interaction strength. At 6 Å, p53-GQD2 exhibits a VDW energy of 146.51 kcal/mol, much more than p53-GQD1 (30.93 kcal/mol) and p53-GQD3 (79.86 kcal/mol). The same pattern is observed for γ H2AX, where at 6 Å, the interaction energy with GQD2 is 131.11 kcal/mol, significantly more than with GQD1 (59.45 kcal/mol) and GQD3 (108.02 kcal/mol). These values indicate that GQD2 enables the strongest protein interactions, likely due to an optimal balance between surface area and molecular structure that enhances VDW forces. At larger distances (9–12 Å), the interaction energies start to plateau. Comparing the values for each protein-GQD interaction at their plateau will reveal which adsorptions are the strongest. At 12 Å, p53-GQD2 still demonstrated the strongest interaction with a VDW energy of 177.44 kcal/mol, compared to p53-GQD1 (46.94 kcal/mol) and p53-GQD3 (97.58 kcal/mol). Similarly, γ H2AX -GQD2 exhibits an energy of 139.36 kcal/mol, which is more than γ H2AX -GQD1 (67.03 kcal/mol) and γ H2AX -GQD3 (119.12 kcal/mol). Overall, this analysis confirms that in both cases (p53 and V), all GQDs exhibit significant VDW interactions, but GQD2 consistently shows the most negative energies, indicating the strongest binding. This suggests that the size and structure of GQD2 create an optimal environment for protein interactions, making it a more effective candidate for applications requiring strong protein-nanomaterial interactions. Similarly, the interaction energies between the proteins p53/ γ H2AX and the three GQDs were also analyzed in the absence of hydrogen atoms. In line with the with-hydrogen analysis, without hydrogen atoms also tend to show similar trends beyond the distances of 3 Å. Notably, at 4 Å, p53-GQD2 shows a VDW energy of 84.80 kcal/mol, which is considerably stronger than that of p53-GQD1 (12.31 kcal/mol) and p53-GQD3 (29.12 kcal/mol). A similar pattern is observed with γ H2AX, where GQD2 exhibits a stronger interaction than GQD1 and GQD3. At 6 Å, p53-GQD2's energy reaches 118.44 kcal/mol, further highlighting its superior binding strength compared to p53-GQD1 (24.68 kcal/mol) and p53-GQD3 (63.90 kcal/mol). γ H2AX -GQD2 also shows a notable interaction value, reinforcing the trend. GQD2 consistently exhibits the most favorable binding efficiency, likely due to its optimal size and molecular structure. As distances reach 9–12 Å, interaction energies remain negative, indicating sustained protein engagement with the GQDs, but the rate of increase stabilizes. At 12 Å, p53-GQD2 demonstrates the strongest interaction at 144.58 kcal/mol, compared to p53-GQD1 (38.70 kcal/mol) and p53-GQD3 (79.30 kcal/mol). Likewise, γ H2AX -GQD2 surpasses the others in strength. In summary, GQD2 exhibited the most favorable interactions with both p53 and γ H2AX across varying distances, indicating its potential efficacy as a binding partner due to its optimal molecular characteristics.

Fig. S6C displays the number of atoms of γ H2AX in contact with various GQDs (GQD1, GQD2, GQD3), both with and without hydrogen, as a function of distance. The red and blue curves (γ H2AX -GQD2 and γ H2AX -GQD3 with hydrogen, respectively) show the highest atomic contacts, reaching over 500 atoms, indicating enhanced interaction surfaces. In contrast, the corresponding systems without hydrogen exhibit significantly lower atomic contact, with γ H2AX -GQD1 without hydrogen (green) showing the lowest interaction level. Overall, the presence of hydrogen promotes a greater number of interactions between γ H2AX and GQDs, especially for GQD2 and GQD3, suggesting more favorable binding geometries or molecular orientations. Fig. S6D shows the IE between γ H2AX and GQDs over distance. The red curve (γ H2AX -GQD2 with hydrogen) again demonstrates the strongest interaction energy, reaching values close to ~150 kcal/mol, followed closely by γ H2AX -GQD3 with hydrogen (blue). All hydrogenated systems exhibit more negative IE values compared to their non-hydrogenated counterparts, highlighting the role of hydrogen in enhancing the binding affinity. γ H2AX -GQD1 without hydrogen (green) shows the least favorable interaction, consistent with the results from atomic contact.