Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information: Histone Tail Electrostatics Modulate E2-E3 Enzyme Dynamics: A Gateway to Regulate Ubiquitination Machinery

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Table S1: Structural similarity between the target and the template [a]

Chain	RMSD (Å)	Template modeling score	% sequence identity	% sequence similarity	Alignment coverage (%)	Compared with PDB ID
N (H2A)	0.08	0.98	92.8	96.8	100.0	1KX5 [b]
n (H2A)	0.07	0.98	92.8	96.8	100.0	1KX5 [b]
O (H2B)	0.10	0.99	93.4	98.4	100.0	1KX5 [b]
o (H2B)	0.09	0.99	93.4	98.4	100.0	1KX5 [b]
P (H3.2)	0.13	1.00	98.5	98.5	100.0	1KX5 [b]
p (H3.2)	0.08	1.00	98.5	98.5	100.0	1KX5 [b]
Q (H4)	0.11	1.00	100.0	100.0	100.0	1KX5 [b]
q (H4)	0.08	1.00	100.0	100.0	100.0	1KX5 [b]
DNA	1.39					1KX5 [b]
UbcH5c	1.81					5EGG [c]
BRCA1/BARD1	1.95					1JM7 [d]

[a] Homology modeling generated the missing structure of histone tails and DNA by "fitting" their sequence (target) into the 1KX5 (template) structure. Template modeling score = 1 describes a perfect match. Modeled UbcH5c and BRCA1/BARD1 structures were compared with PDBID 5EGG and 1JM7 models.
[b] Davey *et al.*[S1]
[c] Wu *et al.*[S2]
[d] Brzovic *et al.*[S3]

Table S2: Total simulation time

System	Trial No	Production time (ns)
WT	1	1050
WT	2	500
WT	3	1100
WT	4	500
WT	5	500
K all R	1	1000
K all R	2	500
K all R	3	500
K all A	1	800
K all A	2	800
K all A	3	800
K486A	1	500
K487A	1	500
K493A	1	500
K495A	1	500
K497A	1	500
Total time (ns)		10550

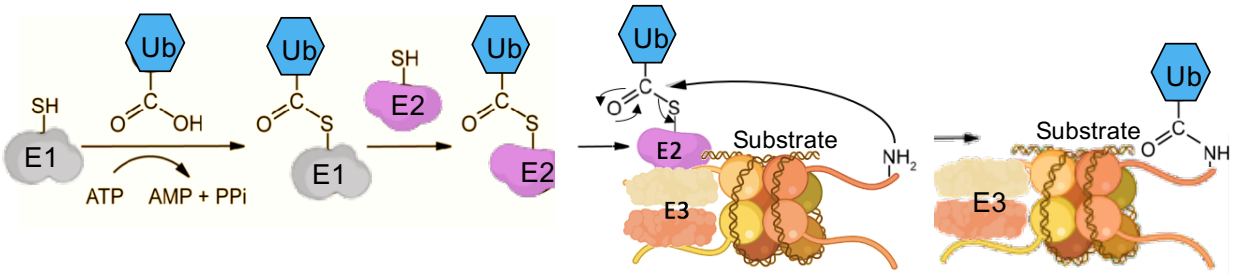


Figure S1: Ubiqutination mechanism. E1, E2 and E3 are called ubiqutin activating, conjugating and ligase enzymes, respectively. In this study BRCA1/BARD1 is E3, and UbcH5c is E2.

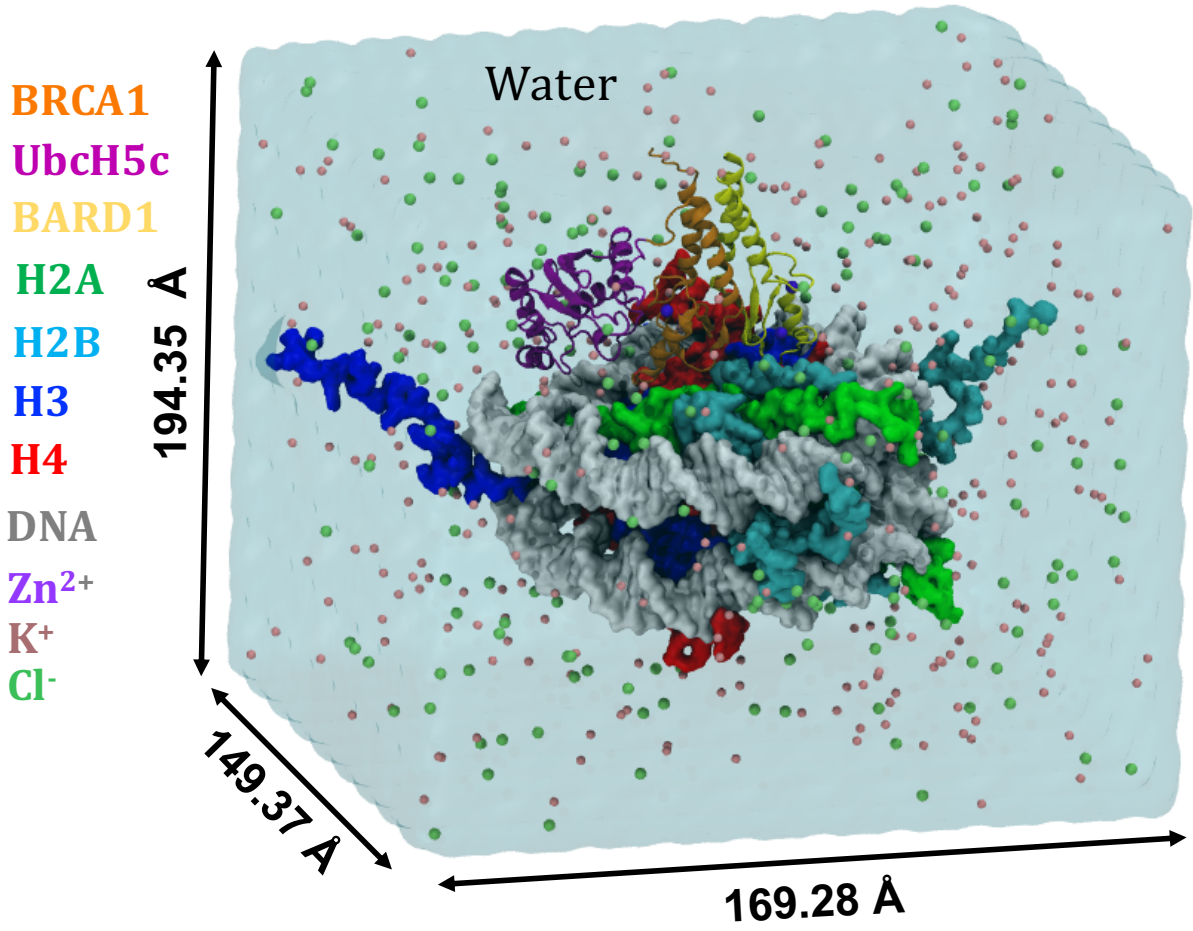


Figure S2: The MD simulation setup.

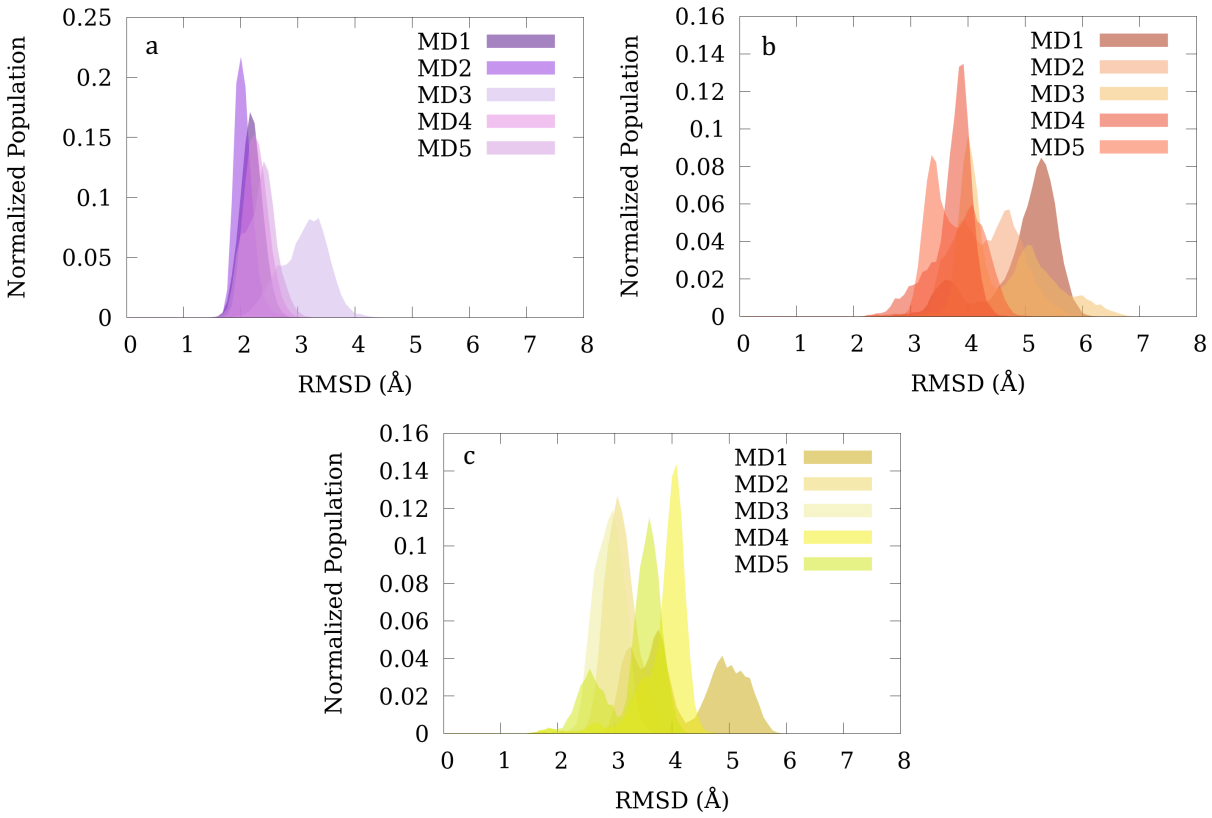


Figure S3: Structural fluctuations of BRCA1, BARD1 and UbcH5c. Normalized RMSD populations of a. UbcH5c, b. BRCA1, and c. BARD1 domains in 5 different WT MD trials with respect to the initial configuration.

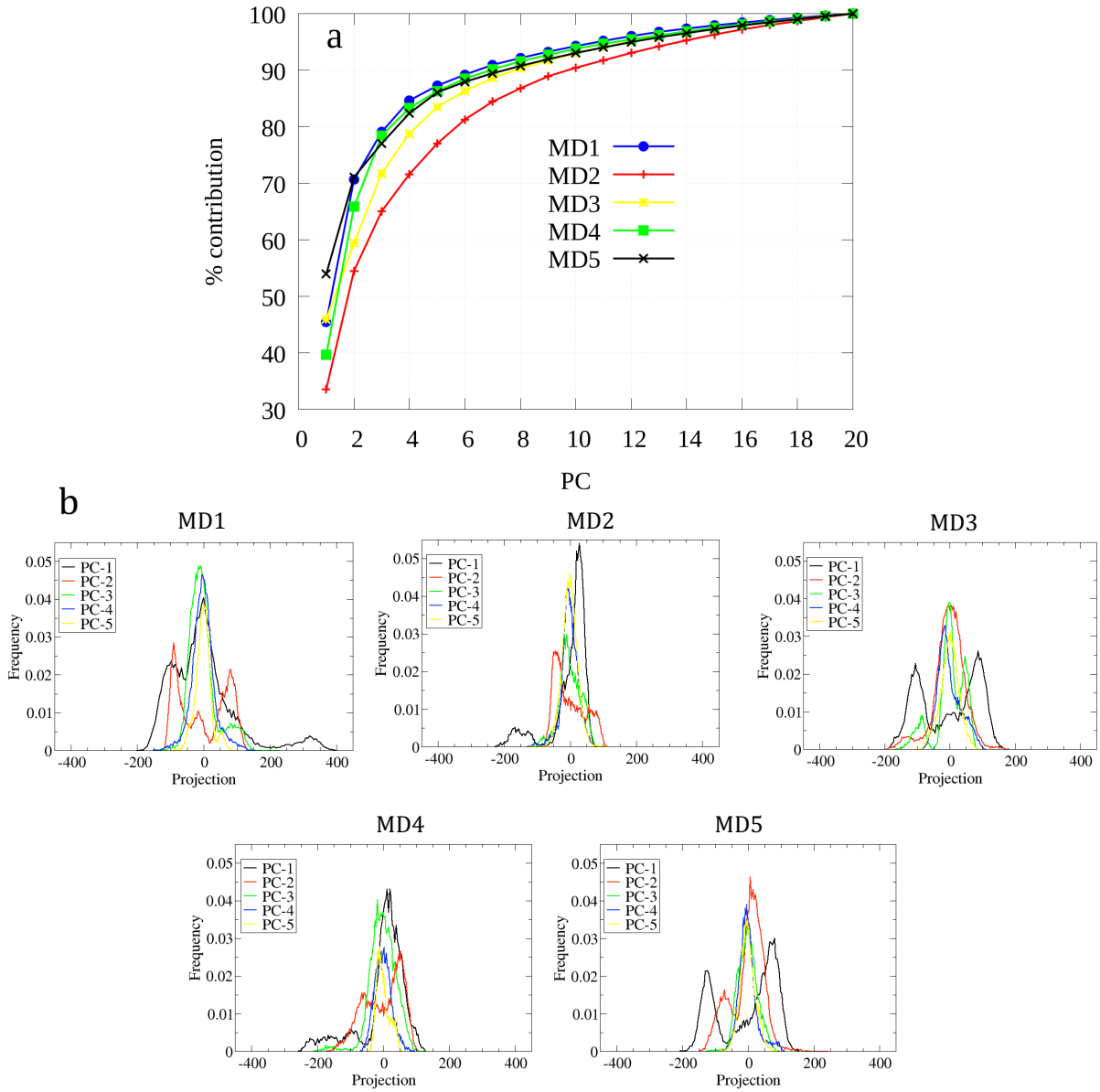


Figure S4: PCA results to characterize conformational variability of BRCA1/BARD1-UbcH5c. a. The cumulative contribution to variance in the BRCA1/BARD1-UbcH5c principal components obtained for all WT simulations (variance of a PC as a percentage of the total variance of the data). The eigenvalue of a PC divided by the sum of all eigenvalues multiplied by 100 will give the "% contribution" of the eigen vector towards the motion of the molecule. The first six PCs account for $> 80\%$ of the total variance and represent $> 80\%$ of the overall conformational variability of BRCA1/BARD1-UbcH5c. Therefore, we focused on these top-ranking 6 PCA modes which reflect the dominant features (shown as modes A-F in Figure S5). b. Normalized histograms of the calculated projections of first 5 PCs of different WT MD runs.

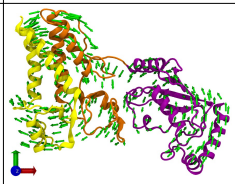
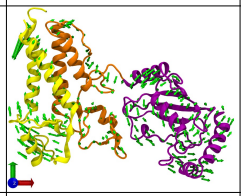
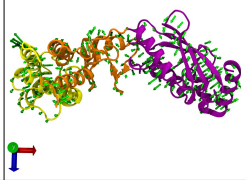
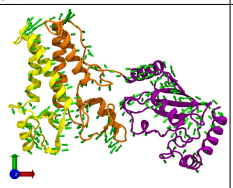
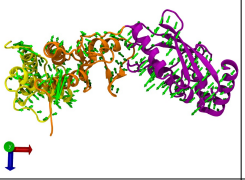
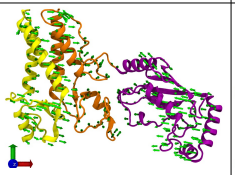
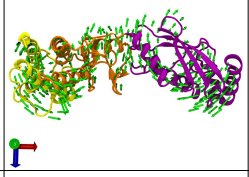
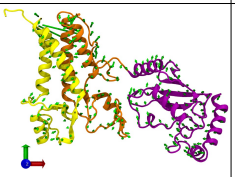
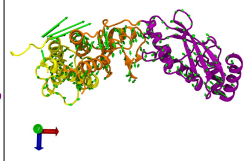
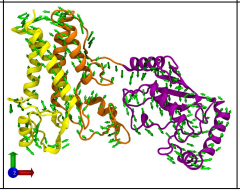
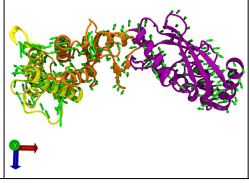
Mode			MD1	MD2	MD3	MD4	MD5	Sum of %contribution
A			PC1 45%	PC4 6%	PC1 34%	PC3 12%	PC1 40%	137%
B			PC2 25%	PC1 33%	PC2 19%	PC1 44%	PC3 8%	128%
C			PC3 8%	PC2 20%	PC6 5%	PC4 7%	PC4 4%	44%
D			PC4 6%	PC5 5%	PC4 6%	PC2 13%	PC2 26%	56%
E			PC5 3%	PC3 10%	PC3 10%	PC5 5%	PC6 4%	32%
F			PC6 3%	PC9 2%	PC7 4%	PC7 4%	PC5 4%	17%

Figure S5: PCA of BRCA1/BARD1-UbcH5 recognizes major conformational movements. PCA data obtained from five WT MD trials. Porcupine plots are shown on the left to visualize the movements of the eigenvectors obtained from the PCA analysis. For ease of visualization, only BRCA1/BARD1-UbcH5c are shown here. The NCP is placed parallel to the XZ plane on the trajectories. The green cones depict the direction of movements of protein subdomains and the length of a cone represents the magnitude of the movement. Six different modes (A-F) correspond to the PC numbers of the MD trials shown to the right of the table. The modes represent dominant movements experienced by the system. The PCs between different MD trials overlap considerably and the order of the PCs is permuted. Therefore, for the convenience of explaining, we labeled the first 6 PCs as "Mode A-F". The percentage contribution of each PC to the total variance taken from Figure S4a is written in blue. Considering the sum of the percentage contributions of PCs measured in the 5 MD trials, the contribution of Modes A-F to the overall conformational variability of BRCA1/BARD1-UbcH5c is calculated as "Sum of % contribution".

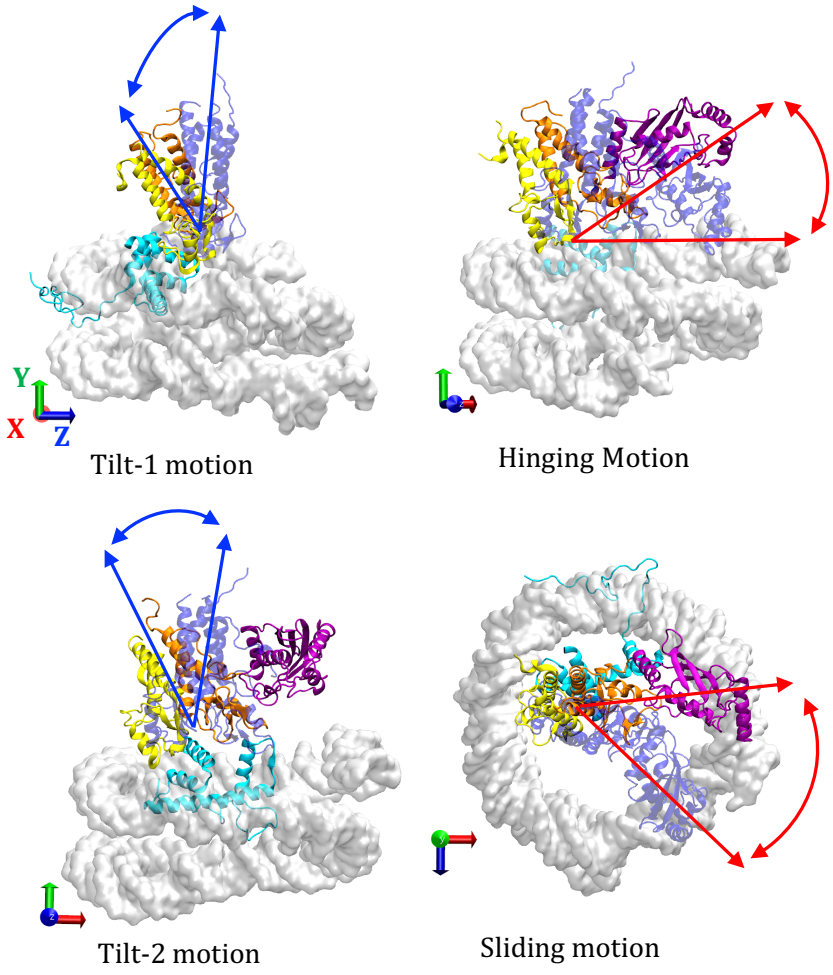


Figure S6: BRCA1/BARD1 and UbcH5c movements on the NCP surface recognized by PCA (appears in mode A and B) compared to their initial location. For ease of visualization, only DNA, H2B and BRCA1/BARD1-UbcH5c were shown. Initial configurations are colored in transparent navy blue. In tilt-1 motion BRCA1/BARD1 tilt is directed towards the H2B αC helix (labeled in Figure S7d) and in tilt-2 motion BRCA1/BARD1 tilts towards the YZ plane parallel to XY plane. In "hinging motion", UbcH5c displays a hinge around its interface with BRCA1. In "sliding motion", BRCA1-connected UbcH5c slides on the nucleosome surface parallel to the ZX plane. Coloring as follows: DNA (gray), BARD1 (yellow), BRCA1 (orange), UbcH5c (purple), and H2B (cyan).

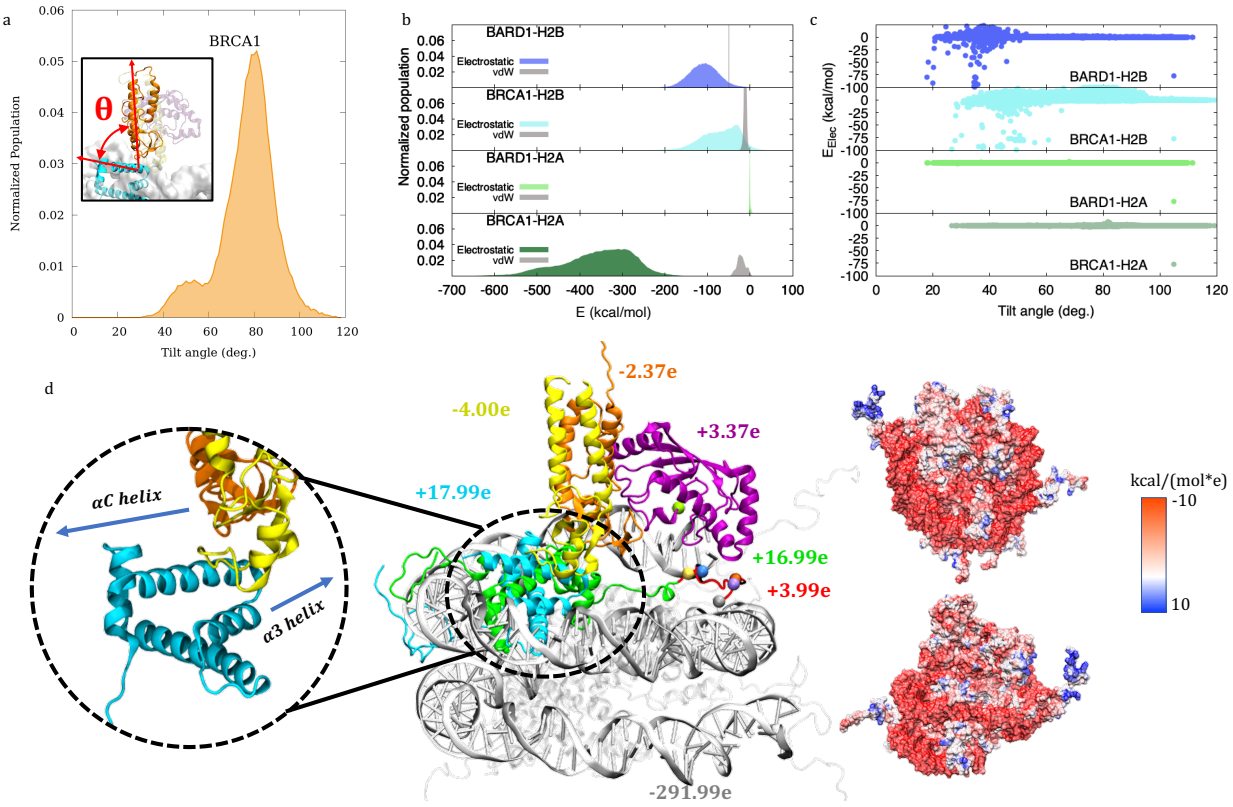


Figure S7: BRCA1/BARD1 tilting motion and interactions. a. BRCA1 tilt angle distribution for WT trajectories. BARD1 tilt angle distribution is shown in Figure 4a. b. Electrostatic and vdW interaction energy between the entire BRCA1 and BARD1 domains with H2B and H2A for WT. The BRCA1/BARD1 interaction energy with DNA is significantly smaller compared to BRCA1/BARD energy with H2B or H2A. c. Electrostatic energy between BRCA1 and BARD1 helical region with H2B and H2A compared to BRCA1 and BARD1 tilt angle. d. The left panel shows a zoomed-in view of a part of the histone H2B with the α helices labeled. The middle and right panels show the net charges of different domains and Coulombic surface coloring. Coloring as follows: BARD1 (yellow), BRCA1 (orange), UbcH5c (purple), and H2B (cyan), H2A (green), H2A C-tail (red), and other NCP domains (gray). C_α atoms of H2A C-tail lysines and UbcH5c active site are colored as follows: K197 (yellowish-green), K497 (gray), K495 (orange), K493 (purple), K487 (blue) and K486 (yellow).

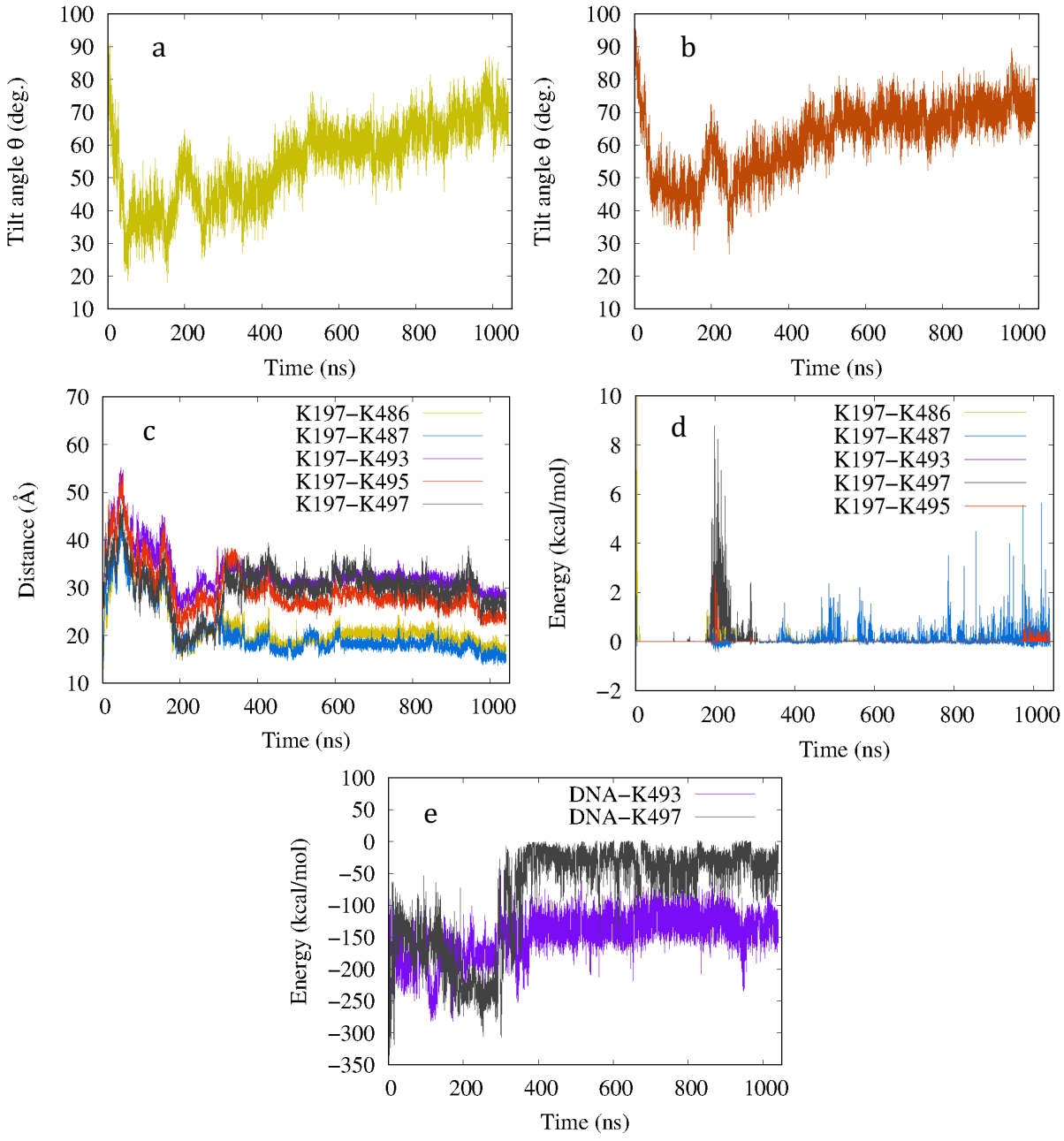


Figure S8: BRCA1/BARD1, UbcH5c and H2A C-tail conformational dynamics and interactions with respect to time. WT MD trial 1 results. a. BARD1 and b. BRCA1 tilt angle change with time. c. Changing C_α-C_α distance between K197 active site and H2A lysines with time. d. Interaction energy between 5 lysine residues in the H2A C-tail and active site K197 as a function of time. e. H2A C-terminal K493 and K497 residues interaction energy with DNA.

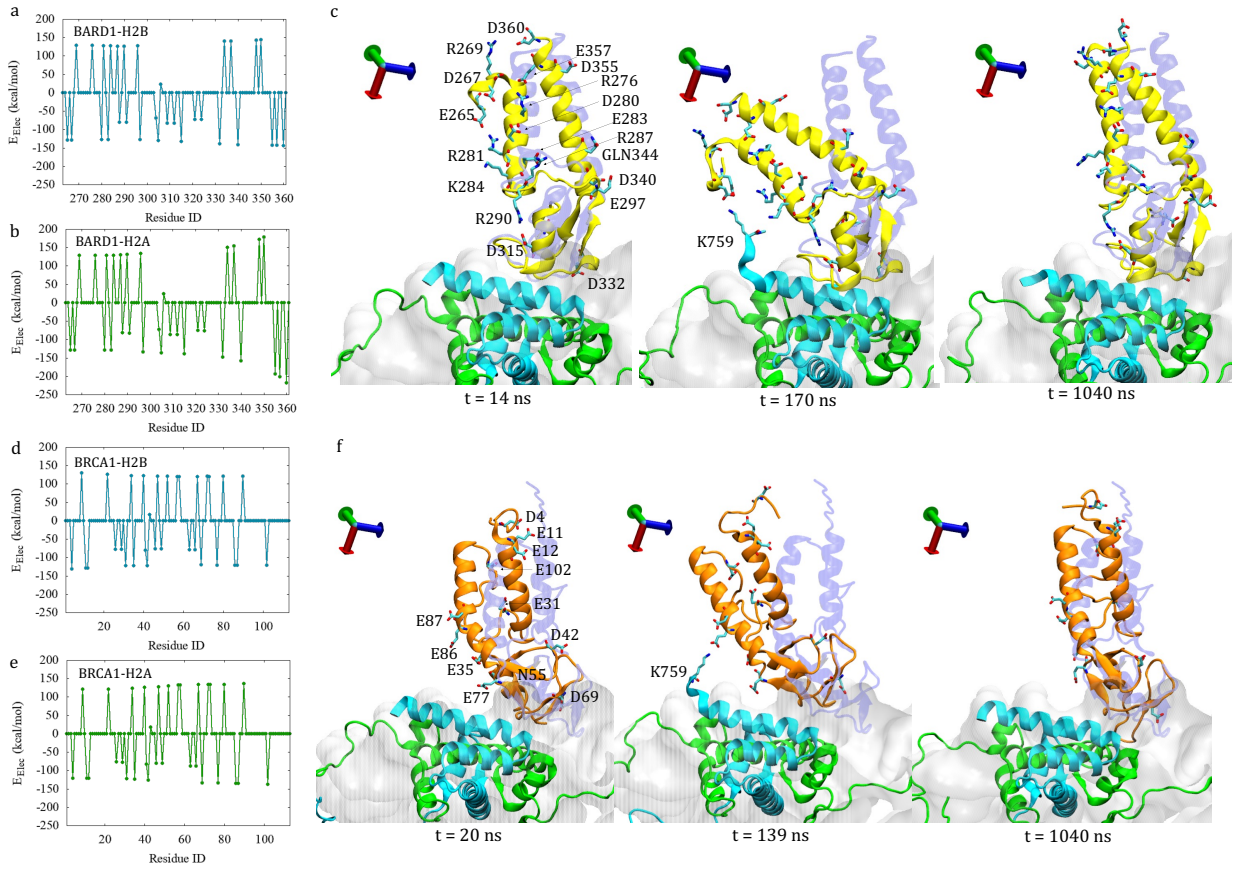
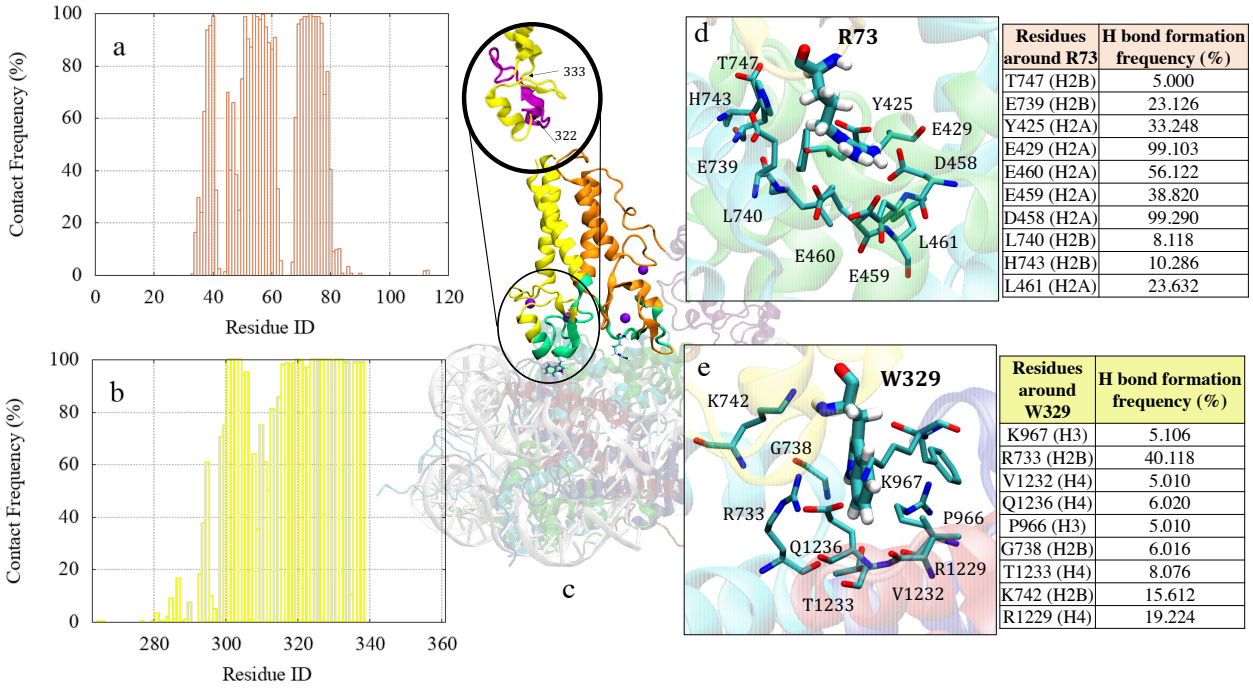


Figure S9: Average electrostatic interaction energies of residues on BARD1/BRCA1 with H2B and H2A for all WT simulations. EDA results of a. BARD1 residues with H2B, b. BARD1 residues with H2A, c. BRCA1 residues with H2B and d. BRCA1 residues with H2A. The residues in e. BARD1 and f. BRAC1 (with electrostatic energy higher than -100 kcal/mol in Figures a,b,d,e) that contribute to the tilting motion relative to its initial configuration are labeled. Coloring as follows: DNA (gray), BARD1 (yellow), BRCA1 (orange), H2B (cyan), H2A (green), initial structure (transparent blue), carbon atoms (dark cyan), oxygen (red), and nitrogen (dark blue).



Residues around R73	H bond formation frequency (%)
T747 (H2B)	5.000
E739 (H2B)	23.126
Y425 (H2A)	33.248
E429 (H2A)	99.103
E460 (H2A)	56.122
E459 (H2A)	38.820
D458 (H2A)	99.290
L740 (H2B)	8.118
H743 (H2B)	10.286
L461 (H2A)	23.632

Residues around W329	H bond formation frequency (%)
K967 (H3)	5.106
R733 (H2B)	40.118
V1232 (H4)	5.010
Q1236 (H4)	6.020
P966 (H3)	5.010
G738 (H2B)	6.016
T1233 (H4)	8.076
K742 (H2B)	15.612
R1229 (H4)	19.224

Figure S10: Residues that retain BRCA1/BARD1 on the NCP surface. a. Residues in a. BRCA1 (orange) and b. BARD1 (yellow) that can be found close to (<7 Å) the NCP surface and their contact frequency in WT simulations. Contact frequencies explain how often these residues are found within 7 Å of the NCP surface. c. Residues 51, 54-57, 69-77, 301-304, 316-334, and 336-338 338 in BRCA1 and BARD1 that are most frequently (more than 95% of the simulation time) found within 7 Å from the NCP surface are highlighted (in mint green). The enlarged panel of the BARD1 RING domain shows residues in the 322-333 region colored in magenta. d. BRCA1 R73 and e. BARD1 W329 anchoring motifs and their surrounding residues with hydrogen bond frequencies. Hydrogen bond frequencies show more residues from H2A and H2B tightly bind to the BRCA1 and BARD1 RING domains to keep BRCA1/BARD1 on the surface. Simulation trajectories show in 20% of the WT simulations, the loop containing W329 moved along H2B due to the BARD1 RING translation motion, while R73 remained trapped on the NCP surface for 100% of the simulation time. Coloring as follows: BARD1 (yellow), BRCA1 (orange), UbcH5c (purple), and H2B (cyan), H2A (green), H3 (blue), H4 (red), DNA (gray), carbon (dark-cyan), oxygen (red), and nitrogen (blue).

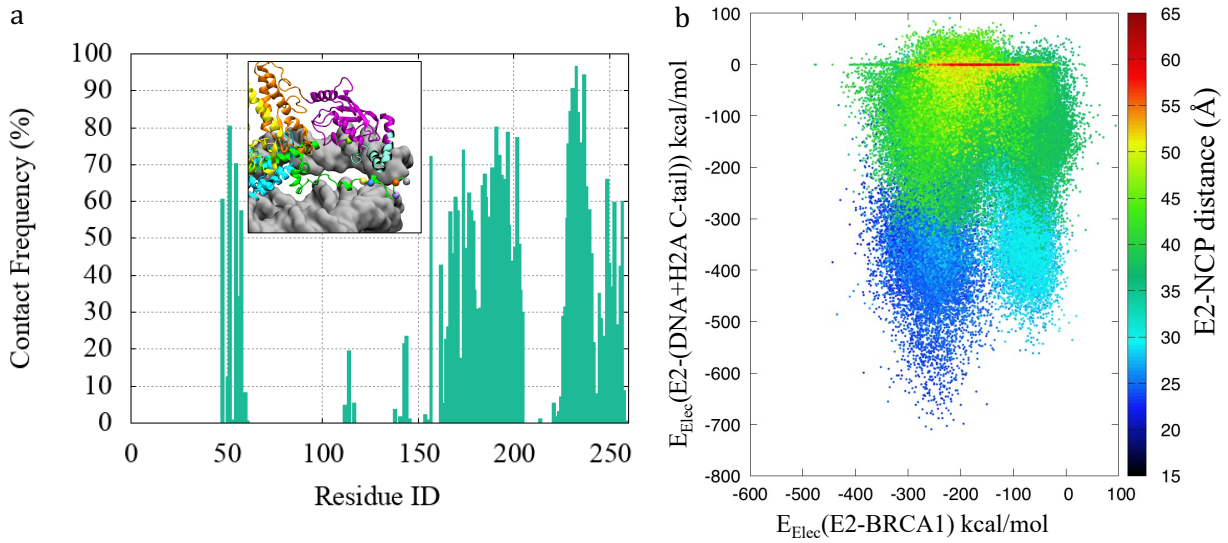


Figure S11: Charged residues play a critical role in the movement of UbcH5c. a. Residues in BRCA1/UbcH5c that can be found close to (<7 Å) DNA and their contact frequency in WT simulations. Contact frequencies explain how often these residues are found within 7 Å of the DNA. Residues K52(BRCA1), P230(UbcH5c), L231(UbcH5c), V232(UbcH5c), P233(UbcH5c) and R237(UbcH5c) were found >80% of the time close to the DNA. Coloring as follows: DNA (gray), BARD1 (yellow), BRCA1 (orange), UbcH5c (purple), and H2B (cyan), H2A (green), H2A C-tail (red) and UbcH5c region that contacts H2A C-tail/DNA is colored in mint green. C_α of H2A C-tail lysines are shown in spheres. b. The electrostatic interaction energy between UbcH5c(E2) and (DNA+H2A C-tail) versus electrostatic interaction energy between UbcH5c(E2) and BRCA1. Colored as a function of UbcH5c-NCP distance.

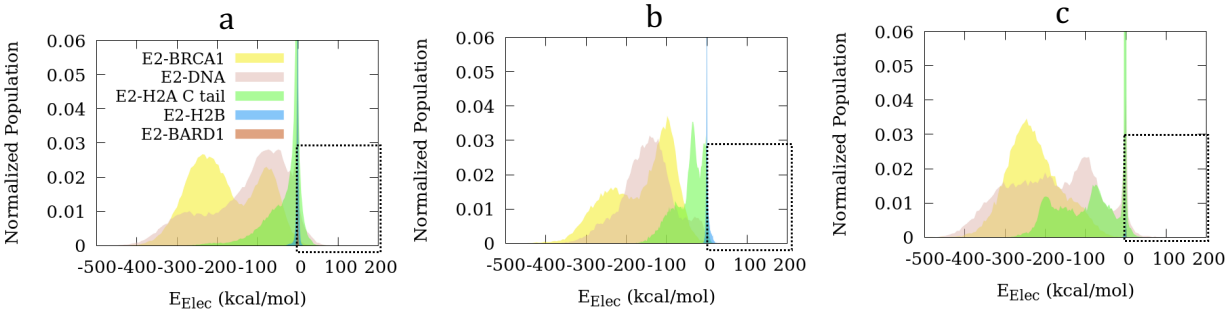


Figure S12: The effect of H2A C-tail lysine electrostatics on UbcH5c motility. The electrostatic interaction energy of UbcH5c with H2A C-terminal tail, DNA, BRCA1, BARD1, and H2B for a. WT, b. all-ALA and c. all-ARG simulations.

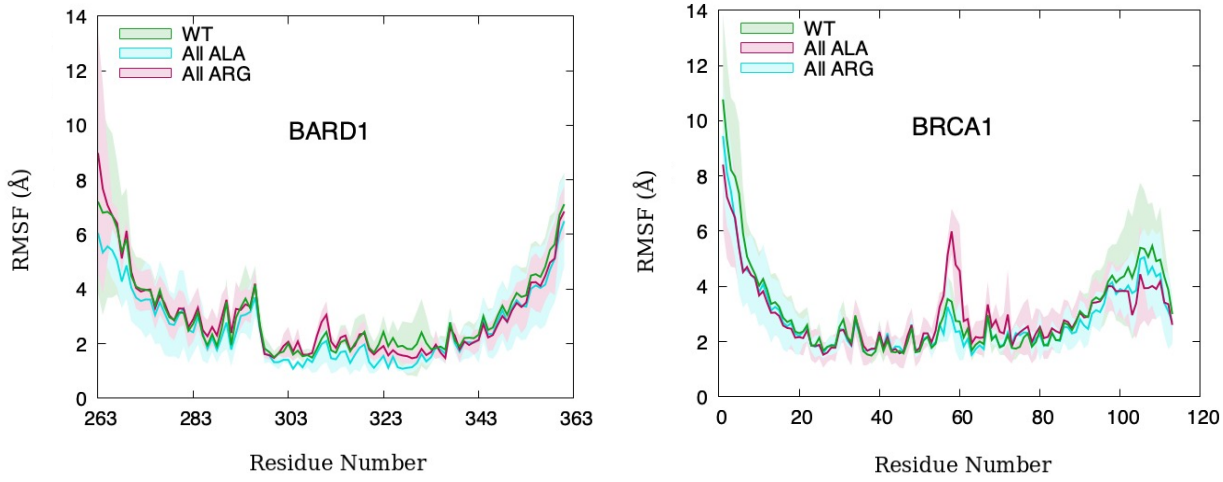


Figure S13: The effect of H2A C-tail lysine electrostatics on BRCA1/BARD1 motility. RMSF of BRCA1 and BARD1 from WT, all-ALA and all-ARG simulations.

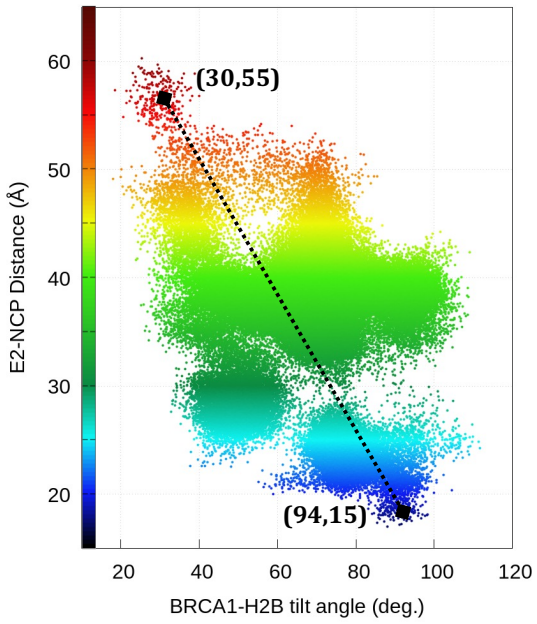


Figure S14: BRCA1/BARD1-UbcH5c shows a seesaw-like dynamics. UbcH5c (E2) and NCP distance versus BRCA1-H2B tilt angle data of all WT simulations. Colored according to the values in Y axis (E2-NCP distance). The dotted line shows a negative correlation. When the distance decreases from 55 Å to 15 Å the angle increases from 30° to 94° with some outliers due to other simultaneous movements (such as sliding, rotation, tilt-2).

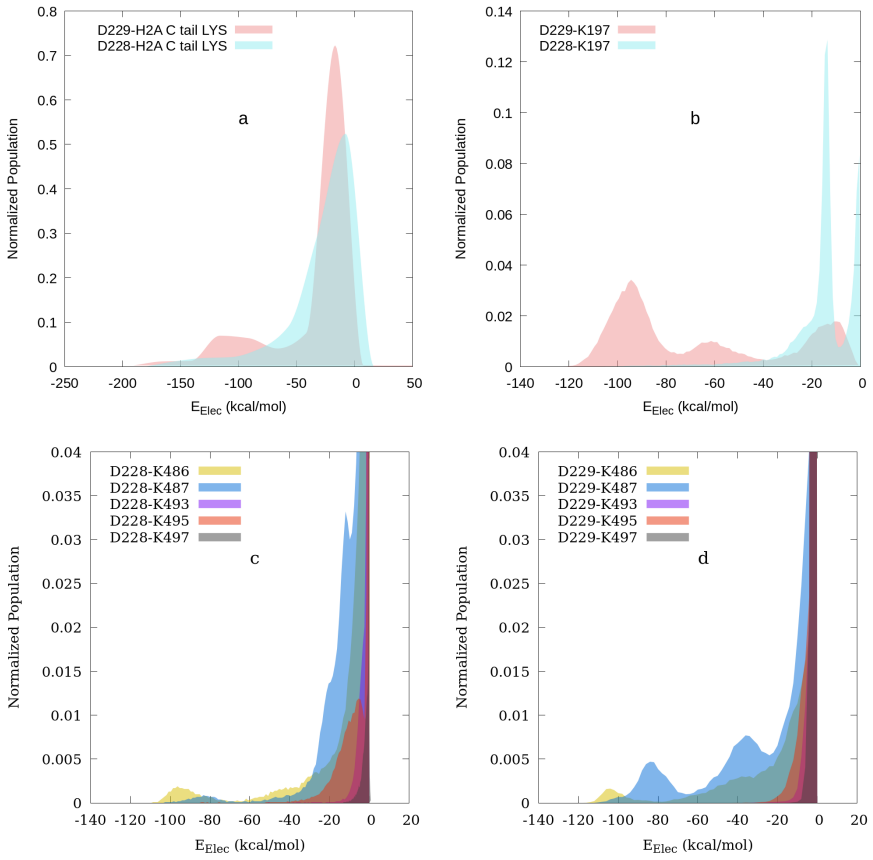


Figure S15: D228 and D229 contribute to bring the active site closer to H2A lysines. Electrostatic interaction energy between, a. UbcH5c D228 and D229 residues with H2A C-tail lysines. b. UbcH5c D228 and D229 residues with UbcH5c active site K197. c. D228 with H2A C-tail lysine residues individually. d. D229 with H2A C-tail lysines individually.

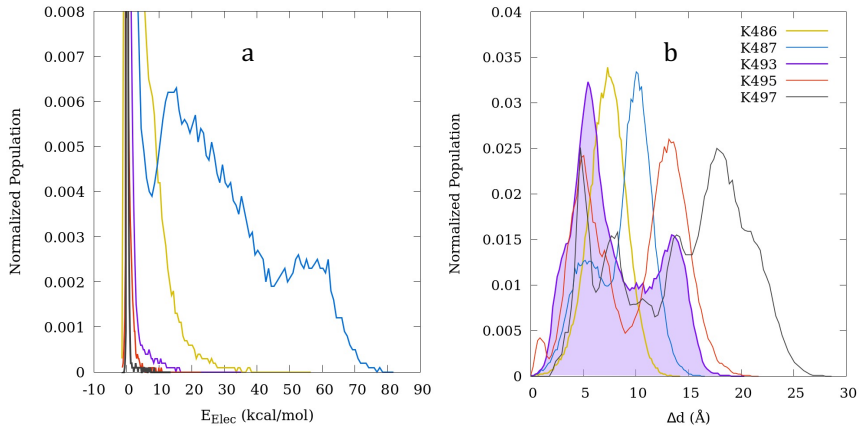


Figure S16: H2A C-tail motion towards UbcH5c. a. Normalized interaction energy between 5 lysine residues in the H2A C-tail and active site K197 for all WT simulations. b. Normalized change in distance between 5 lysine residues (C_α atoms) in the H2A C-tail and P atoms of the 1443 DNA residue to explain the H2A C-tail upward motion.

References

(S1) Davey, C. A.; Sargent, D. F.; Luger, K.; Maeder, A. W.; Richmond, T. J. Solvent Mediated Interactions in the Structure of the Nucleosome Core Particle at 1.9Å Resolution. *Journal of Molecular Biology* **2002**, *319*, 1097–1113.

(S2) Wu, F.; Zhu, J.; Li, H.; Zhu, L. Structural analysis of recombinant human ubiquitin-conjugating enzyme UbcH5c. *Acta Pharmaceutica Sinica B* **2017**, *7*, 390–394.

(S3) Brzovic, P. S.; Rajagopal, P.; Hoyt, D. W.; King, M.-c.; Klevit, R. E. Structure of a BRCA1 – BARD1 heterodimeric RING – RING complex. *Nature* **2001**, *8*, 833–837.