Molecular dynamics approach to identification of new OGG1 cancer-associated somatic variants with impaired activity

Received for publication, May 19, 2020, and in revised form, December 22, 2020 Published, Papers in Press, December 23, 2020, https://doi.org/10.1074/jbc.RA120.014455

Aleksandr V. Popov1,2,*, Anton V. Endutkin1,2, Dylan D. Yatsenko1,2, Anna V. Yudkina1, Alexander E. Barmatov1, Kristina A. Makasheva2, Darya Y. Raspopova1, Evgeniia A. Diatlova1, and Dmitry O. Zharkov1,2,‡

From the 1Laboratory of Genome and Protein Engineering, SB RAS Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russia; 2Department of Natural Sciences, Novosibirsk State University, Novosibirsk, Russia

Edited by Patrick Sung

DNA of living cells is always exposed to damaging factors. To counteract the consequences of DNA lesions, cells have evolved several DNA repair systems, among which base excision repair is one of the most important systems. Many currently used antitumor drugs act by damaging DNA, and DNA repair often interferes with chemotherapy and radiotherapy in cancer cells. Tumors are usually extremely genetically heterogeneous, often bearing mutations in DNA repair genes. Thus, knowledge of the functionality of cancer-related variants of proteins involved in DNA damage response and repair is of great interest for personalization of cancer therapy. Although computational methods to predict the variant functionality have attracted much attention, present strategies are mostly based on sequence conservation and make little use of modern capabilities in computational analysis of 3D protein structures. We have used molecular dynamics (MD) to model the structures of 20 clinically observed variants of a DNA repair enzyme, 8-oxoguanine DNA glycosylase. In parallel, we have experimentally characterized the activity, thermostability, and DNA binding in a subset of these mutant proteins. Among the analyzed variants of 8-oxoguanine DNA glycosylase, three (I145M, G202C, and V267M) were significantly functionally impaired and were successfully predicted by MD. Alone or in combination with sequence-based methods, MD may be an important functional prediction tool for cancer-related protein variants of unknown significance.

This article contains supporting information.

* These authors contributed equally to this work.

‡ For correspondence: Aleksandr V. Popov, apopov@niboch.nsc.ru; Dmitry O. Zharkov, dzharkov@niboch.nsc.ru.

© 2021 THE AUTHORS. Published by Elsevier Inc on behalf of American Society for Biochemistry and Molecular Biology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by/4.0/).
Inactive OGG1 mutants identified by molecular dynamics

At present, the majority of algorithms used for the prediction of the effects of mutations on the protein functions are based on the analysis of evolutionary conservation, changes in the amino acid physicochemical properties, and the available static structures rather than on the modeling of the dynamic behavior of the protein molecule (16–26). Some modern prediction protocols use neural network and machine learning methods to integrate various kinds of information (27–29). Molecular dynamics (MD), due to its much higher computational power demands and the lack of experimentally determined 3D structures for many proteins, had seldom been used for the prediction of protein variant functionality. However, with the number of known structures growing quickly, and processor capabilities and computational algorithms improving rapidly, this approach is gaining in popularity (30, 31). For example, MD was used to predict the inhibitor binding with 12 commonly found somatic variants of BCR-ABL1 oncogenic protein kinase (32). In another recent study, structures of 79 naturally occurring nonsynonymous SNPs of human triose phosphate isomerase were analyzed, and some were found to destabilize the protein fold (33). There is little doubt that MD will soon become a powerful approach for the prediction of mutation effects on the protein functionality either on its own or as an important part of combined procedures integrating structural and evolutionary information.

In this study, we have developed a semiautomated pipeline to model the structures of somatic mutant variants of proteins found in clinical samples of human tumors and predict the effects of the mutations. As a proof of principle, we have applied the procedure to mutants of 8-oxoguanine DNA glycosylase (OGG1). DNA glycosylases, as many other BER enzymes, modulate the resistance of cancer cells to chemotherapy and radiotherapy. OGG1 initiates the repair of oxidized purines, mostly 8-oxoguanine (oxoG) and formamidopyrimidines. In cell cultures, OGG1 significantly attenuates the toxicity of bleomycin (34), thiotepa (35, 36), carmustine (36, 37), mafosfamide (36), cisplatin, and oxaliplatin (38). Thus, somatic mutations in the OGG1 gene in tumor cells could affect their sensitivity to therapeutic interventions. Using the computational pipeline, we have identified three novel clinical variants of OGG1 with impaired activity.

Results

Predicting the mutation effect in OGG1 variants

At the beginning of this study, the Catalogue Of Somatic Mutations In Cancer (COSMIC) database (39) held 39 non-synonymous single-nucleotide mutations in the OGG1 gene. We have used four popular software packages for mutation effect prediction from sequence conservation—SIFT (18), FATHMM (24), MutationTaster (25), and PROVEAN (22)—to analyze the functionality of these mutations. All of them produce a binary output: whether the protein function is expected to be affected or not affected in some way. Of 39 substitutions, 36 were included and three (all located in exon 8, the last exon of the human gene) were discarded because of the insufficient number of homologous sequences (Table S1). Cohen’s κ coefficient (40) was used to measure the agreement between the prediction methods (−1 ≤ κ ≤ 1; where κ = −1 for complete disagreement, κ = 1 for complete agreement, and κ = 0 for agreement expected from random coincidence). As can be seen from Table 1, the agreement between different algorithms was rather low in most cases. For a comparison, we took the gene for another human DNA glycosylase, UNG, and repeated the analysis for nonsynonymous single-nucleotide mutations found in the COSMIC database its coding region (44 total, all included; Table S1). Neither the UNG mutation set nor the combined OGG1/UNG set demonstrated better performance than the OGG1 set (Table 1). Given the discordance in the predictions by sequence-based algorithms, we set out to apply MD for estimating the mutation effects in the OGG1 coding sequence.

MD of OGG1 variants

We have designed a computational pipeline that retrieves all missense variants of the gene of interest from the COSMIC database (or, with an appropriate setup of the connection module, from any database that can be automatically parsed), maps them on the selected protein sequence and structure, randomly selects a given number of mutants, and prepares them for MD. The script is available as an open source code from https://doi.org/10.5281/zenodo.3828057. Mapping is an important part of the procedure because many protein isoforms are known for OGG1 (41, 42), and the COSMIC database uses the longest polypeptide, the major mitochondrial isof orm OGG1-2a, as a reference, whereas the vast majority of biochemical and all structural data in the literature are for the shorter major nuclear isoform OGG1-1a. Of the randomly selected 20 mutants, only one (R229Q) turned out to be studied before with respect to its biochemical properties, and for another one (R131G), a substitution of Gln for the same Arg131 residue was characterized as inactive (Table 2). The location of the mutants in the sequence and structure of OGG1 protein is shown in Figure 1. All 20 mutants and WT OGG1 were subjected to MD in explicit water.

Forty ns trajectories were essentially equilibrated after 20 ns (Fig. S1), so the last 20 ns were analyzed. To extract functionally relevant differences between the mutants, we focused on the enzyme’s active site, defined as all residues in protein

| Table 1 | Cohen’s κ for agreement between the predictions of the effect of mutations in OGG1 and UNG by different algorithms |
|---------|----------------------------------------------------------------------------------------------------------------------------------|
|         | FATHMM | MutationTaster | PROVEAN |
| SIFT    | 0.163  | 0.385         | 0.780   |
|         | 0.068  | 0.323         | 0.178   |
|         | 0.087  | 0.428         | 0.490   |
| FATHMM  | 0.192  | 0.289         | 0.049   |
|         | 0.049  | 0.117         | 0.177   |
|         | 0.079  | 0.177         | 0.503   |
| MutationTaster | 0.503   | 0.371         | 0.464   |

* First number is Cohen’s κ for the OGG1 mutation set, second, for the UNG mutation set, third, for the combined set.
and DNA, at least one atom of which is within 5 Å of 8-oxo-2′-deoxyguanosine (oxoG) in the OGG1–DNA complex X-ray structure (1EBM (43)) that served as a starting point for our models. Thus defined, the active site encompasses 23 amino acid residues and three nucleotides: oxoG and its 5′ and 3′ neighbors. Of all studied mutations, only V267M affected a residue in the active site, which includes N and Cα atoms of Val267. In addition, we analyzed a narrower set of key geometric parameters for the catalytic dyad Lys249–Asp268, namely the Nζ[K249]–C1[oxoG] and Oδ1/Oδ2[D268]–O4′.

Table 2

| OGG1 variant | SIFT   | FATHMM | MutationTaster | PROVEAN | Combined score | Experimental | Properties reported in the literature |
|--------------|--------|--------|----------------|---------|----------------|-------------|---------------------------------------|
| R87K         | No     | No     | No             | No      | +4             |             | R131Q: small-cell lung carcinoma; inactive (86, 87) |
| P88L         | No     | No     | Yes            | No      | +2             |             |                                       |
| E92D         | No     | No     | Yes            | No      | +2             | No          |                                       |
| R97C         | Yes    | No     | No             | Yes     | 0              |             |                                       |
| H111R        | No     | No     | No             | No      | +4             | No          |                                       |
| S115F        | Yes    | No     | No             | Yes     | 0              |             |                                       |
| S118F        | Yes    | No     | Yes            | Yes     | −2             | No          |                                       |
| R131G        | Yes    | Yes    | Yes            | Yes     | −4             |             |                                       |
| I145M        | Yes    | No     | Yes            | Yes     | −2             | Yes         |                                       |
| A153T        | No     | Yes    | No             | No      | +2             |             |                                       |
| R161W        | Yes    | Yes    | Yes            | Yes     | −4             | Yes/No      | Leukemia; active but thermolabile at 37 °C; cells radiation sensitive (88–90) |
| G202C        | Yes    | Yes    | Yes            | Yes     | −4             |             |                                       |
| R206C        | Yes    | Yes    | Yes            | Yes     | −4             |             |                                       |
| Q226H        | No     | No     | Yes            | No      | +2             |             |                                       |
| R229Q        | No     | No     | No             | Yes     | +2             |             |                                       |
| Q263H        | Yes    | No     | Yes            | Yes     | 0              | No          |                                       |
| V267M        | Yes    | No     | Yes            | No      | −2             | Yes         |                                       |
| T285M        | No     | No     | No             | No      | +4             | No          |                                       |
| P291Q        | Yes    | No     | No             | No      | +2             | No          |                                       |
| S292N        | No     | No     | No             | No      | +4             | Yes/No      |                                       |

* Binary output from individual algorithms, coded as the following: Yes: SIFT “affects protein function,” FATHMM “damaging,” MutationTaster “disease causing,” PROVEAN “deleterious”; No: SIFT “tolerated,” FATHMM “tolerated,” MutationTaster “polymorphism,” PROVEAN “neutral.” Combined score: sum of the four prediction, with +1 for “No” and −1 for “Yes”; negative values indicate an effect on the protein function.

Figure 1. Location of the studied mutations in the primary structure (A) and the tertiary structure of OGG1 (B). In the primary structure, AlkA_N-like domain is homologous to the N-terminal domain of Escherichia coli 3-methyladenine–DNA glycosylase AlkA, and the HhH domain contains a helix–hairpin–helix motif found in many DNA glycosylases. In the tertiary structure (1EBM, (43)), the mutated residues are shown as colored spheres and the everted oxoG nucleotide as colored sticks. OGG1, 8-oxoguanine DNA glycosylase.
[oxoG] distances and the N[K249]–C1’[oxoG]–N9[oxoG] angle. These three parameters reflect correct positioning of the catalytic amine nucleophile of Lys249 and the damaged nucleotide (43, 44) and were earlier shown to be good predictors for the OGG1 activity on a limited set of substrates (oxoG:C, oxoG:A, and AP:C) and active site–obstructing mutants (45, 46).

**Trajectory analysis and clustering**

Principal component analysis (PCA) is often used to reduce dimensionality of multivariate data with minimal information loss. We have first performed the PCA using the dihedral covariance matrix taking into account all torsion angles of the active-site residues for all analyzed variants. Principal components 1 and 2 for all OGG1 mutants are illustrated in Figure 2A. To cluster the full trajectories rather than their means (e.g., as presented in Fig. 2D), we have calculated Bhattacharya similarity coefficients in the PC1 versus PC2 plane for all pairs of trajectories (see Experimental procedures) and presented the results as a tree (Fig. 2G). The variants formed several tight clusters; P88L and R229Q were closest to the WT enzyme, and I145M was close to this group. Q263H and P291Q lied farthest from WT OGG1 and were widely separated from all other proteins.

---

**Figure 2. Principal component analysis of OGG1 mutants.** A–C, location of all OGG1 mutants (means of principal components 1 and 2) in the PC1 versus PC2 plane. D–F, all trajectory points of WT OGG1 (black dots) and one of the mutants, OGG1 G202C (white dots) in the PC1 versus PC2 plane. G–I, unrooted distance tree derived from the Bhattacharya similarity coefficients in the PC1 versus PC2 plane. The PCA was performed for the whole active site (A, D, and G), only the torsions within the active site whose mean deviated >36° from WT OGG1, and at least one mutant (B, E, and H), or for three critical geometric parameters (C, F, and I). OGG1, 8-oxoguanine DNA glycosylase; PCA, Principal component analysis.
High dimensionality of multivariate data introduces large dispersion into principal components, lowering the ability of the PCA to separate the distributions coming from different samples. We tried to reduce the number of dimensions by including only those torsions within the active site that demonstrated large differences (>36° difference in the means) between WT OGG1 and at least one mutant (Fig. 2, B, E and H). The trajectories were better separated (Fig. 2E), and, after tree building, two clusters were evident, one containing WT OGG1 and E92D, R87K, R97C, S118F, and T285M mutants, the other consisting of P88L, S115F, R131G, A153T, R161W, R206C, Q226H, and R229Q. Of the remainder, I145M grouped together with G202C, and Q263H, with S292N.

When we repeated the PCA with the three key active site geometric parameters as the only variables, the principal component projections were considerably different (Fig. 2, C and F). Owing to a lower number of variables, the clustered structure of the population of WT OGG1 active site conformations, noted in the earlier OGG1 models (45, 46), was more evident in the first two PC dimensions than with the 5-Å active site. In Figure 2F and Fig. S2, the conformations of the WT OGG1 with an optimal geometry for an attack at C1’ (45, 46) crowd around the origin of coordinates in the PC1 versus PC2 plane. The tree grouped WT OGG1 with E92D, H111R, S118F, R229Q, Q263H, T285M, and S292N mutants, whereas R87K, P88L, R97C, S115F, R131G, A153T, R161W, R206C, and Q226H produced another cluster. I145M, G202C, and V267M formed a cluster remote from most of other mutants, whereas P291Q was an isolated branch. Comparing individual trajectories in the PC1 versus PC2 plane, one can notice that I145M, G202C, and V267M are very different from WT OGG1 and other mutants, while P291Q and other variants are close to the “optimal” cluster.

**Biochemical characterization of OGG1 variants**

Of twenty OGG1 mutants analyzed by MD, 13 plus the WT were successfully overproduced and purified (Fig. S3), whereas nine were either very poorly induced or insoluble upon induction in *Escherichia coli*. All polypeptides carried an N-terminal His6-tag, which, according to the known OGG1 structure (43), lies in an unstructured part remote from the active site and is unlikely to affect the protein function. The effect of the mutations on the protein stability was estimated from their Trp fluorescence melting profiles (Fig. 3). All mutants and the WT protein demonstrated a single transition point with *T* < sub > m </ sub > ranging between 55.9 °C and 59.4 °C, indicating no significant destabilization (Table 3). The mutations did not appreciably change the F<sub>350</sub>/F<sub>330</sub> ratio at the physiological temperature, suggesting that all mutants have the same general conformation as WT OGG1.

The overall activity of OGG1 variants was assessed in a single time-point assay using an oligonucleotide duplex containing an oxoG:C pair, the natural substrate for this enzyme (47) (Fig. 4A). Two variants, I145M and G202C, displayed the activity ~10- to 20-fold lower than the WT enzyme, whereas the cleavage by R161W, V267M, and S292N was ~1.5- to 2-fold lower. These findings were corroborated by enzyme titration experiments, which demonstrated very low cleavage by I145M even at the highest enzyme concentrations (Fig. 4B). G202C activity was more than 10-fold lower, whereas R161W and S292N mutations showed a milder effect. Interestingly, some of the mutants, such as H111R and R206C, were apparently more active than WT OGG1.

To get a deeper insight into possible reasons of the variation in the activity of OGG1 mutants, we have determined the apparent rate constants *k*<sub>2</sub> and *k*<sub>3</sub>. OGG1 is characterized by fast oxoG excision and slow dissociation from the DNA product, so *k*<sub>2</sub> reflects the base excision rate, whereas *k*<sub>3</sub> is a combined constant accounting for the nascent AP site cleavage and product release (48, 49). Single-turnover and burst phase experiments are used to determine *k*<sub>2</sub> and *k*<sub>3</sub> for OGG1 and

### Table 3

| OGG1 variant | *T*<sub>m</sub> °C | F<sub>350</sub>/F<sub>330</sub> at 35 °C |
|-------------|----------------|---------------------------------|
| WT          | 58.4 ± 0.1     | 0.67 ± 0.01                     |
| E92D        | 58.1 ± 0.2     | 0.66 ± 0.01                     |
| H111R       | 58.4 ± 0.1     | 0.67 ± 0.01                     |
| S118F       | 56.2 ± 0.1     | 0.67 ± 0.01                     |
| I145M       | 57.2 ± 0.1     | 0.67 ± 0.01                     |
| R161W       | 55.9 ± 0.2     | 0.72 ± 0.02                     |
| G202C       | 57.1 ± 0.1     | 0.67 ± 0.01                     |
| R206C       | 56.8 ± 0.2     | 0.70 ± 0.01                     |
| Q226H       | 58.1 ± 0.1     | 0.66 ± 0.01                     |
| Q263H       | 55.9 ± 0.1     | 0.66 ± 0.01                     |
| V267M       | 56.2 ± 0.1     | 0.67 ± 0.01                     |
| T285M       | 56.4 ± 0.1     | 0.69 ± 0.01                     |
| S292N       | 59.4 ± 0.1     | 0.66 ± 0.01                     |

OGG1, 8-oxoguanine DNA glycosylase.

---

**Figure 3. Thermal denaturation of a representative OGG1 mutant, OGG1 H111R.**

*Panel A* Trp fluorescence (F<sub>350</sub>/F<sub>330</sub>) melting profile. *Panel B* differential Trp fluorescence melting profile (two averaged melting curves). OGG1, 8-oxoguanine DNA glycosylase.
other slow-turnover DNA glycosylases (48, 50). For comparison with the WT OGG1, we have selected four mutants with low activity (I145M, R161W, G202C, and S292N), two high-activity variants (H111R and R206C), and an apparently neutral Q263H. The results are summarized in Table 4 and Figs. S4 and S5. The low activity of I145M did not allow reliable determination of the kinetic constants. For G202C, no reliable \( k_2 \) and \( k_3 \) values could be obtained: although the apparent \( k_2 \) was decreased at least 50-fold, and \( k_3 \) was 4.5-fold lower compared with the WT enzyme, as reported in Table 4, the actual constants may be even more affected, given that at the highest enzyme concentration, the reaction with G202C did not reach the endpoint (Fig. S4). V267M was also affected, with \( k_2 \) showing a ~12-fold decrease. R161W and S292N, which demonstrated mild overall effect of the mutation (Fig. 4), were also the least affected in kinetic terms, showing only a ~2-fold \( k_2 \) decrease. Although some of the affected mutants may not fully bind the substrate at 800 nM (see below), the reported values provide a useful lower estimate of \( k_2 \). In WT OGG1, H111R, R206C, Q263H, and P291Q variants, we could provide only lower estimates for \( k_2 \) because the cleavage was very fast even at 15 °C, the temperature at which the experiment was performed. With this precaution, the “hyperactive” H111R and R206C showed a 2- to 2.5-fold increase in the apparent \( k_2 \) with about the same decrease in \( k_3 \), whereas the “neutral” Q263H had unaffected \( k_2 \) and the highest \( k_3 \) of all studied variants. Overall, it seems that in this particular set of variants, the activity measured in single time-point experiments better correlates with the base excision rate constant. Based on these experiments, we classified I145M, G202C, and V267M as “affected” and R161W and S292N as “possibly affected.”

Loss of the ability to bind DNA can also be a reason for inactivation of OGG1. We have used the microscale thermophoresis technology (51, 52) to compare the affinity of WT OGG1 and four affected or partially affected mutants for a DNA duplex containing (3-hydroxytetrahydrofuran-2-yl) methyl phosphate (THF) and uncleavable AP site analog. As shown in Figure 5 and Fig. S6, WT OGG1 efficiently formed a complex with THF–DNA (\( K_d = 0.14 ± 0.03 \) μM), with the affinity within the range of values reported in the literature (tens to hundreds nM) that were determined by the gel shift assay and fluorescence titration (49, 53–55). For I145M, G202C, and V267M, we measured a 2- to 2.5-fold increase in the apparent \( k_2 \) with about the same decrease in \( k_3 \), whereas the “neutral” Q263H had unaffected \( k_2 \) and the highest \( k_3 \) of all studied variants. Overall, it seems that in this particular set of variants, the activity measured in single time-point experiments better correlates with the base excision rate constant. Based on these experiments, we classified I145M, G202C, and V267M as “affected” and R161W and S292N as “possibly affected.”

Loss of the ability to bind DNA can also be a reason for inactivation of OGG1. We have used the microscale thermophoresis technology (51, 52) to compare the affinity of WT OGG1 and four affected or partially affected mutants for a DNA duplex containing (3-hydroxytetrahydrofuran-2-yl) methyl phosphate (THF) and uncleavable AP site analog. As shown in Figure 5 and Fig. S6, WT OGG1 efficiently formed a complex with THF–DNA (\( K_d = 0.14 ± 0.03 \) μM), with the affinity within the range of values reported in the literature (tens to hundreds nM) that were determined by the gel shift assay and fluorescence titration (49, 53–55). For I145M, G202C, and V267M, we measured a 2- to 2.5-fold increase in the apparent \( k_2 \) with about the same decrease in \( k_3 \), whereas the “neutral” Q263H had unaffected \( k_2 \) and the highest \( k_3 \) of all studied variants. Overall, it seems that in this particular set of variants, the activity measured in single time-point experiments better correlates with the base excision rate constant. Based on these experiments, we classified I145M, G202C, and V267M as “affected” and R161W and S292N as “possibly affected.”
R161W, and S292N, we could not reach binding saturation, but their estimated $K_d$ values were 7- to 10-fold higher (0.90 ± 0.24 μM, 1.06 ± 0.51 μM, and 1.39 ± 0.45 μM, respectively) than that of the WT. The G202C mutant produced a low signal that could not be reliably quantified, possibly because of fluorescence quenching. It appears that loss of DNA binding at least partly contributes to the decreased activity of OGG1 variants.

**Comparison of the experimental data with predictions**

MD does not immediately produce binary results in terms of the mutation effect prediction. We have categorized the mutants according to their position in the distance trees relative to the three-branch point (Fig. 2, G–I) in two ways: either considering all variants on the distal (i.e., containing the models most remote from the WT) branch as “affected” or all variants on the WT-containing (proximal) branch as “unaffected.” Cohen’s $κ$ was used to compare the classification results with the experimental data (Table 5). The best agreement was observed when the tree was built based on the key geometry parameters, and the longest branch was classified as affected ($κ = 0.512$ or 0.811 depending on whether the mild R161W and S292N mutants were classified as experimentally affected or not). This came for a price of falsely predicting P291Q as a deleterious mutation. Predictions based on the affected distant branch generally agreed with the experimental data better than predictions based on the unaffected proximal branch because of a lower number of false positives.

It was also interesting to compare the experimental data with the sequence-based predictions. All four tested algorithms tended to overpredict the number of affected mutants; PROVEAN showed less overprediction and demonstrated better agreement with the experiment (Table 5). Using a combined score from all four algorithms did not improve the overall prediction accuracy. MD-based prediction was superior if R161W and S292N were considered active; if they were counted as affected, PROVEAN and combined sequence–based methods fared better. However, adding the best MD-based method to the combined sequence–based methods improved the prediction accuracy regardless of the R161W/ S292N status (Table 5).

| Prediction method | R161W and S292N neutral | R161W and S292 affected |
|-------------------|-------------------------|-------------------------|
| (1) Active site PCA (all), distal branch affected | 0.317 | 0.378 |
| (2) Active site PCA (all), proximal branch unaffected | 0.143 | 0.429 |
| (3) Active site PCA (different from WT), distal branch affected | 0.429 | 0.429 |
| (4) Active site PCA (different from WT), proximal branch unaffected | 0.197 | 0.364 |
| (5) Geometric PCA, distal branch affected | 0.811 | 0.512 |
| (6) Geometric PCA, proximal branch unaffected | 0.426 | 0.429 |
| SIFT              | 0.340 | 0.314 |
| FATHMM            | 0.152 | 0.317 |
| MutationTaster    | 0.263 | 0.208 |
| PROVEAN           | 0.533 | 0.553 |
| Combined sequence–based methods | 0.519 | 0.530 |
| Combined sequence–based methods and (5) | 0.533 | 0.553 |

OGG1, 8-oxoguanine DNA glycosylase; PCA, principal component analysis.

**Discussion**

The rapid progress of algorithms and hardware for MD has opened the possibility for application of its methods for prediction of effects of mutations on protein function. Although still computationally more expensive than sequence-based methods, MD gives an option of assessing the structural consequences of mutations in detail, which, in turn, allows better segregation of amino acid changes into neutral and deleterious. However, as with any other prediction method, MD results should be benchmarked against real experimental data to ensure their reliability.

We have applied MD to analyze the structures of somatic tumor variants of OGG1, an important DNA repair protein, and to predict their consequences. OGG1 is well characterized structurally, with an extensive set of X-ray structures illuminating its catalytic cycle from the discrimination between normal and damaged DNA to the postexcision steps (43, 44, 56–64). Based on these structures, several MD and quantum mechanics/molecular mechanics exercises were attempted to investigate the process of dynamic damaged base recognition (45, 46, 65–67) and catalytic steps (58, 68–72). Experimental consequences of many site-directed mutations were characterized as part of mechanistic studies on OGG1 activity (43–46, 62, 73, 74). Good understanding of OGG1 mechanism at both experimental and computational levels makes this enzyme a suitable candidate for attempts to predict the effects of mutations of unknown significance.

In our effort, we have reasoned that the main criterion of the “effect” categorization should be dissimilarity between MD trajectories of the WT protein and a mutant. However, there are many possible ways both to quantify this difference and to limit it to functionally important regions. Knowledge of the mechanism of the protein under study is critical to define such regions. Although we have used PCA and three versions of the “important region,” other metrics and definitions can certainly apply and may aid in better clustering of MD trajectories. However, two observations that we made may be useful to consider when searching for optimal mutation categorization. First, an increase in the number of analyzed variables (the whole active site) produced less-pronounced trajectory resolution and worse correspondence with the experimental data. Second, categorization based on the most distant populations was better than that based...
Inactive OGG1 mutants identified by molecular dynamics

on clustering with the WT protein, mostly due to lower over-prediction. Over-prediction of deleterious effects was also the major factor decreasing the accuracy of sequence-based methods. In our study, the best consistency with the experiment was achieved by MD taking into account three key active site parameters, although this might change with more mutants tested experimentally. As MD improved the output of combined sequence-based methods, it seems likely that MD can indeed be considered a useful addition to the repertoire of tools used to predict functional consequences of amino acid changes in proteins. Our simulation time, 40 ns, is comparatively short by today’s supercomputer standards yet attainable in reasonable time for medium-size molecular systems even with relatively low-end hardware such as graphical processors and thus represents a trade-off between the modeling quality and accessibility.

We ran our MD pipeline with 20 OGG1 mutants, of which 19 were never characterized before. To enable the prediction accuracy estimate, we analyzed the activity of 13 of these mutants (plus the WT protein) and obtained more detailed kinetics and binding data for the variants that deviated significantly from the WT. No significant correlation was found between MD predictions and success or failure to produce the mutant protein. Three somatic variants demonstrated significantly impaired activity: I145M, G202C, and, to a lesser degree, V267M. Notably, all these mutants produce very distinct MD trajectories, in which the region conformationally optimal for catalysis was barely populated (Fig. S2). In the OGG1 structure (Fig. 1B), Ile145 and Gly202 all lie in a protein lobe that extends into the minor groove, widening it and assisting the insertion of the intercalating residues (Asn149 and Tyr203) that trigger oxoG eversion from the double helix into the enzyme’s active site. Gly202 is located in the αH/αI turn and presses against the sugar ring of the orphaned cytosine, while Ile145 forms part of the hydrophobic core of the lobe. Although these groups do not fall within 5 Å of the oxoG residue, their mutations affect the dynamics of the active site; the most pronounced changes (>36° in the mean angle, \( p < 0.001 \)) were observed for the catalytic Lys249 and oxoG with its adjacent nucleotides (Fig. 6, A–B). The damaged base lost all stabilizing interactions with Cys253, Gln315, and Phe319 of the damage recognition pocket and was shifted from its position in the WT protein. Locally, the G202C mutation destabilized the cytosine opposite the lesion, as well as two flanking bases, pressing them toward the minor groove (Fig. 6D). Val267 is located next to the catalytic Asp268, which stabilizes the positive charge developing on the deoxyribose during base excision, and the V267M mutation causes the Asp268 carboxyl to turn away from the oxoG sugar ring and also disrupting the interactions of oxoG with the base recognition pocket (Fig. 6, C and E).

Figure 6. Effect of mutations on the active site and local conformation of OGG1. A–C, overlay of the active site of WT OGG1 (green) with I145M (A, cyan), G202C (B, magenta), and V267M (C, yellow). D, overlay of the region including cytosine opposite the lesion, two flanking bases, and the Tyr203 wedge in WT OGG1 (green) and G202C (magenta). E, overlay of the region including oxoG and the catalytic Asp268 in WT OGG1 (green) and V267M (yellow). The structures represent the MD snapshots closest to the center of mass of the clusters in the PC1 versus PC2 plane (Fig. S2) and thus may be regarded as “typical” for each variant. OGG1, 8-oxoguanine DNA glycosylase; oxoG, 8-oxoguanine; MD, molecular dynamics.
Inactivating mutations also significantly impaired DNA binding. Compared with active site disorder, reliable MD prediction of the affinity of protein variants for DNA is more resource-demanding (75). However, as active site disorder contributes to destabilization of the enzyme–substrate pre-catalytic complex, active site–based predictions are not necessarily limited to the effects on catalytic steps but can be partly related to DNA binding efficiency. Careful selection of the regions of importance outside of the immediate active site may improve the overall prediction reliability.

From the biological standpoint, the “hyperactive” OGG1 mutants we observed are perhaps even more interesting than the inactivating mutants because of their somatic cancer origin. Although the magnitude of the effect is not great, such mutations in DNA repair genes might arise and get fixed because of treatment-driven selection in tumors. The ability of such variants to protect human cells from genotoxicity of cancer drugs merits further attention.

Experimental procedures

Model preparation

The atomic structure of human OGG1 (protein data bank [PDB] ID: 1EBM) (43) containing a 15-mer DNA duplex was taken as a reference model. The K249Q inactivation mutation present in 1EBM was manually reverted to Lys249 by editing the PDB file. The backbone of missing residues 80 to 82 was taken from the free OGG1 structure (PDB ID: 1K09), aligned for the best fit using the Kabsch algorithm, and incorporated into the PDB file. Subsequent modeling was performed in explicit water, so all water molecules found in the crystal unit cells were removed. The final model preparation and refinement was performed in LEaP, a part of the AMBER Tools Package, and the model was minimized in 2500 steps of the steepest descent method followed by 2500 steps of the conjugate gradient method using the SANDER module of the AMBER MD suite (76).

Automatic mutation selection

The list of sample missense OGG1 mutations was prepared using the custom Python script M3R-PDB (Missense mutation mapper and randomizer for PDB files), available as an open source code from https://doi.org/10.5281/zenodo.3828057. The script connected to the COSMIC database (39) and fetched the full list of missense mutations in the OGG1 gene. Then, it mapped the mutations onto the known FASTA sequences fetched from the NCBI Reference Sequence Database (77) and determined the original protein sequence, which was then aligned with the OGG1 sequence in 1EBM. Finally, the script randomly picked 20 mutations and output 20 PDB files based on the reference model, each containing a single mutation.

MD

The PDB model output by M3R-PDB script, as well as the reference model, were prepared, solvated, and verified in LEaP. The explicit TIP3P solvent model was used, and Na⁺ counter ions were added to ensure neutrality. The models were optimized using the SANDER module of the AMBER MD suite in two steps. At the first step, the protein–DNA complex was restrained with the harmonic constant 500 kcal/mol, and only the energy of solvent molecules was minimized in 1000 steps of the steepest descent followed by 1000 steps of conjugate gradients. At the second step, the restraints were removed and the whole system was minimized in 2500 steps of the steepest descent followed by 2500 steps of conjugate gradients. Then the system was gradually heated to 310 K during 200 ps with a 2-fs time step using the f99SB force field (78) with parmbsc0 corrections (79) using the pmdm module of the AMBER MD suite. The SHAKE algorithm (80) was applied to constrain the bonds involving hydrogens. Heating was performed in Langevin thermostat with a collision frequency of 2 ps⁻¹; solvation effects were modeled explicitly using particle mesh Ewald electrostatics algorithm (81). The production run was performed for 40 ns under the same conditions, with snapshots captured every 8 ps, thus producing trajectories of 5000 snapshots each. The trajectories in the mdcrd format were processed by the custom script Mdcrd2pdb (available as an open source code from https://doi.org/10.5281/zenodo.3828060), stripping most of the solvent except the water molecules located within 5.0 Å of the oxoG residue for at least 10% of the trajectory.

Trajectory analysis

The resulting all-atom trajectories were processed with the MDTRA software package (82) to extract the required geometric parameters, which were further analyzed using R scripts. The last 2500 snapshots were included in the analysis. The PCA was performed for the combined set of all trajectories. The PC1 versus PC2 plane was partitioned into a 10 × 10 equidistant grid, and Bhattacharyya coefficients $BC = \sum_{i=1}^{n} \sqrt{p_i q_i}$ (n, total number of bins; p, and q, numbers of occurrences of two samples in the ith bin) were calculated over this partition for every pair of trajectories to provide a measure of the overlap between them (83). Because by definition $0 \leq BC \leq N$ ($N = 2500$, the total number of points in any trajectory), $N − BC$ was taken as the distance measure. The pairwise distance matrix was converted to a tree using the unweighted PGMA approach and visualized using Interactive Tree Of Life (84).

Oligonucleotides and enzymes

Oligonucleotides 5′-CTCTCCCCCTCXTCTCTTCTCCTCT-3′ (X = oxoG or THF) and the complementary strand 5′-AGAGGAAGGAGCAGGAGAGGAGGAGGAGGAGG-3′ were synthesized in-house from commercially available phosphoramidites (Glen Research, Sterling, VA). The oxoG-containing strand was 5′-labeled using γ[32P]ATP (SB RAS ICBFM Laboratory of Biotechnology) and phage T4 polynucleotide kinase (SibEnzyme, Novosibirsk, Russia) according to the manufacturer’s protocol, purified on NENSORB C18 sorbent (DuPont, Wilmington, DE) and annealed to an 1.5-fold molar excess of the complementary strand. The THF-containing oligonucleotide...
Inactive OGG1 mutants identified by molecular dynamics

carried a fluorescein residue at the 5' end. Full-length OGG1 mutants carrying an N-terminal His6-tag were produced using the Q5 Site-Directed Mutagenesis Kit (New England Biolabs, Beverly, MA) with the pET-15b-OGG1 plasmid (53) as a template. The target mutations were confirmed by Sanger sequencing. The plasmids were transformed into Rosetta 2(DE3) E. coli (Merck Millipore, Burlington, MA). WT and mutant OGG1 were overexpressed and purified essentially as described (53). Seven of 20 selected mutants were refractory to mutant OGG1 were overexpressed and purified preparations was determined using the calculated extinction coefficient at 280 nm (85). The concentration of the active WT enzyme and, when possible, the mutants was estimated from burst-phase experiments as outlined below; the fraction of the active enzyme varied from ~35% to ~90%. Recombinant human APEX1 was purified as described (48).

Enzyme kinetics

For single time-point experiments and turnover rate constant (kₜ) determination, the reaction mixture contained 100-nM ³²P-labeled substrate duplex, 50-mM Tris HCl (pH 7.5), 100-mM NaCl, 1-mM EDTA, 1-mM DTT, and 10- to 15-nM OGG1 (WT or mutant). The reaction was allowed to proceed at 37 °C for 30 min (single time-point assay), 0 to 60 min (kₜ experiments for H111R, G202C, R206C, S292N), or 0 to 20 min (kₜ experiments for all other variants). To determine the glycosidic bond cleavage rate constant (kₐ), the substrate concentration was 20 nM, and the OGG1 concentration, 800 nM (additionally, 5000 nM for G202C), and the reaction was carried out at 15 °C for 0 to 120 min (G202C), 0 to 60 min (R161W, V267M, and ArcticExpress), two induction temperatures (15 °C and 37 °C), two IPTG concentrations (50 and 1000 μM), and two induction times (3 h and overnight). The total protein concentration in the purified preparations was determined using the calculated extinction coefficient at 280 nm (85). The concentration of the active WT enzyme and, when possible, the mutants was estimated from burst-phase experiments as outlined below; the fraction of the active enzyme varied from ~35% to ~90%. Recombinant human APEX1 was purified as described (48).

### Protein thermal denaturation

OGG1 melting profiles were analyzed by native differential scanning fluorimetry, following the ratio of tryptophan fluorescence at λₑₓ = 350 nm and λₑₘ = 330 nm (λₑₘ = 280 nm) using the Tycho NT.6 capillary fluorimeter (NanoTemper Technologies, Munich, Germany). The change in the intrinsic fluorescence was monitored in the temperature range 35 to 95 °C at a rate of 30 °C/min. All protein solutions contained OGG1 (WT or mutant), 20-mM Na phosphate buffer (pH 7.5), 400-mM NaCl, 1-mM DTT, 1-mM EDTA, and 50% glycerol (v/v).

### Protein–DNA binding

Binding of OGG1 and its mutants to a fluorescent THF-containing duplex was measured by microscale thermophoresis (51). All reaction mixtures with a final volume of 10 μl consisted of labeled DNA duplex (100 nM), 0.0185- to 3.28-μM unlabeled protein, and 10-mM Na phosphate (pH 7.5). Glycerol concentration in all reaction mixtures was adjusted to 7%. Measurements were carried out using standard capillaries in the Monolith NT.115 device (NanoTemper Technologies) equipped with a red/green detection channel and medium infrared laser power. The binding constants were calculated from two independent experiments using SigmaPlot v11.0.

### Data availability

The software is available as a source code from https://doi.org/10.5281/zenodo.3828057 (M3R-PDB) and https://doi.org/10.5281/zenodo.3828060 (mdcrd2pdb). MD trajectories are available from Dmitry O. Zharkov upon request (dzharkov@nibochnsc.ru). All other data are contained within the manuscript.

### Acknowledgments

DNA sequencing was performed at the SB RAS Genomics Core Facility. MD simulations were performed at the Supercomputing center of the Novosibirsk State University. Partial salary support from the Russian Ministry of Science and Higher Education (state funded budget projects AAAA-A17-117020210023-1 and FSUS-2020-0035) is acknowledged.

### Author contributions

A. V. P. and D. O. Z. conceptualization. A. V. P., A. V. Y., and D. O. Z. data curation. A. V. P., A. V. E., A. V. Y., and D. O. Z. formal analysis. A. V. P. and D. O. Z. funding acquisition. A. V. P., A. V. E., D. Y. R., E. A. D. Y., and E. A. D. Y. investigation. A. V. P., A. V. E., and D. O. Z. methodology. A. V. P. and D. O. Z. project administration. A. V. P. and D. O. Z. resources. A. V. P. and A. V. Y. software. D. O. Z. supervision. A. V. P. and D. O. Z. visualization. A. V. P., A. V. E., and D. O. Z. writing—original draft. D. O. Z. writing—review and editing.

### Funding and additional information

This research was supported by the Russian Foundation for Basic Research (grant 17-00-00261/
Conflict of interest—The authors declare that they have no conflicts of interest with the contents of this article.

Abbreviations—The abbreviations used are: AP, apurinic/apyrimidinic; BER, base excision repair; COSMIC, Catalogue Of Somatic Mutations In Cancer; MD, molecular dynamics; OGG1, 8-oxoguanine DNA glycosylase; oxoG, 8-oxo-2′-deoxyguanosine; oxoG, 8-oxoguanine; PCA, Principal component analysis; THF, (3-hydroxytetrahydrofuran-2-yl)methyl phosphate.

References
1. Friedberg, E. C., Walker, G. C., Siede, W., Wood, R. D., Schultz, R. A., and Ellenberger, T. (2006) DNA Repair and Mutagenesis, ASM Press, Washington, DC
2. Zharkov, D. O. (2008) Base excision DNA repair. Cell Mol. Life Sci. 65, 1544–1565
3. Lawley, P. D., and Phillips, D. H. (1996) DNA addsucts from chemotherapeutic agents. Mutat. Res. 355, 13–40
4. Feng, P. C., Ross, D. S., Yap, T. A., Tutt, A., Wu, P., Mergui-Roelvink, M., Mortimer, P., Swaisland, H., Lau, A., O'Connor, M. J., Ashworth, A., Carmichael, I., Kaye, S. B., Schellen, J. H. M., and de Bono, J. S. (2009) Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N. Engl. J. Med. 361, 123–134
5. Curtin, N. J., and Szabo, C. (2013) Therapeutic applications of PARP inhibitors: anticancer therapy and beyond. Mol. Aspects Med. 34, 1217–1256
6. Kaina, B., Margison, G. P., and Christmann, M. (2010) Targeting O6-methylguanine-DNA methyltransferase with specific inhibitors as a strategy in cancer therapy. Cell Mol. Life Sci. 67, 3663–3681
7. Blumenthal, D. T., Rankin, C., Stelzer, K. J., Spence, A. M., Sloan, A. E., Raza, M., and Kaina, B. (2012) Targeting O(6)-methylguanine-DNA methyltransferase with specific inhibitors as a strategy in cancer therapy. Cell Mol. Life Sci. 69, 6660–6667
8. Kumar, P., Henikoff, S., and Ng, P. C. (2009) Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat. Protoc. 4, 1073–1081
9. Gonzalez-Perez, A., and Lopez-Bigas, N. (2011) Improving the assessment of the outcome of nonsynonymous SNVs with a consensus deleteriousness score, condel. Am. J. Hum. Genet. 88, 440–449
10. Bevan, B., Antipin, Y., and Sander, C. (2011) Predicting the functional impact of protein mutations: application to cancer genomics. Nucleic Acids Res. 39, e118
11. Cho, Y., Sims, G. E., Murphy, S., Miller, J. R., and Chan, A. P. (2012) Predicting the functional effect of amino acid substitutions and indels. PLoS One 7, e46688
12. Sim, N.-L., Kumar, P., Hu, J., Henikoff, S., Schneider, G., and Ng, P. C. (2012) SIFT web server: predicting effects of amino acid substitutions on proteins. Nucleic Acids Res. 40, W452–W457
13. Shihab, H. A., Gough, I., Cooper, D. N., Stenson, P. D., Barker, G. L. A., Edwards, K. I., Day, I. N. M., and Gaunt, T. R. (2013) Predicting the functional, molecular, and phenotypic consequences of amino acid substitutions using hidden Markov models. Hum. Mutat. 34, 57–65
14. Schwarz, J. M., Cooper, D. N., Schuelke, M., and Seelow, D. (2013) MutationTaster2: mutation prediction for the deep-sequencing age. Nat. Methods 11, 361–362
15. Wong, K.-C., and Zhang, Z. (2016) SNPtryd: predicting deleterious non-synonymous human SNPs using only orthologous protein sequences. Bioinformatics 30, 1112–1119
16. Raimondi, D., Gazzo, A. M., Rooman, M., Lenoarts, T., and Vranken, W. F. (2016) Multilevel biological characterization of exomic variants at the protein level significantly improves the identification of their deleterious effects. Bioinformatics 32, 1797–1804
17. Pejaver, V., Mooney, S. D., and Radivojac, P. (2017) Missense variant pathogenicity predictors generalize well across a range of function-specific prediction challenges. Hum. Mutat. 38, 1092–1108
18. Dehghanpoor, R., Ricks, E., Hursh, K., Gunderson, S., Farhoodi, R., Haspel, N., Hutchinson, B., and Jagodzinski, F. (2018) Predicting the effect of single and multiple mutations on protein structural stability. Molecules 23, 251
19. Kucukkalk, T. G., Petukh, M., Li, L., and Alexov, E. (2015) Structural and physico-chemical effects of disease and non-disease missense variants on proteins. Curr. Opin. Struct. Biol. 32, 18–24
20. Smejkal, P., and Donato, N. (2015) Molecular dynamics: new frontier in personalized medicine. Adv. Protein Chem. Struct. Biol. 102, 181–224
21. Gibbons, D. L., Prlic, S., Bosco, P., Laurini, F., Femia, M., Sun, H., Telpaz, M., Donato, N., and Quintás-Cardama, A. (2014) Molecular dynamics reveal BCR-ABL1 polypeptides as a unique mechanism of resistance to PAN-BCR-ABL1 kinase inhibitor therapy. Proc. Natl. Acad. Sci. U. S. A. 111, 3550–3555
22. Oliver, C., and Timson, D. J. (2017) In silico prediction of the effects of mutations in the human triose phosphate isomerase gene: towards a predictive framework for TPI deficiency. Eur. J. Med. Genet. 60, 289–298
23. Wu, M., Zhang, Z., and Che, W. (2008) Suppression of a DNA base excision repair gene, hOGG1, increases bleomycin sensitivity of human lung cancer cell line. Toxicol. Appl. Pharmacol. 228, 395–402
24. Kobune, M., Xu, Y., Baum, C., Kelsey, M. R., and Williams, D. A. (2001) Retrovirus-mediated expression of the base excision repair proteins, formamidopyrimidine DNA glycosylase and human oxoguanine DNA glycosylase, protects hematopoietic cells from N,N,
Inactive OGG1 mutants identified by molecular dynamics

N\textsuperscript{3}-triethylimiethiophosphoramidate (thioTEPA)-induced toxicity in vitro and in vivo. Cancer Res. 61, 5116–5125

36. Xu, Y., Hansen, W. K., Rosenquist, T. A., Williams, D. A., Limp-Foster, M., and Kelley, M. R. (2001) Protection of mammalian cells against chemotherapeutic agents thiotaope, 1,3-N\textsubscript{3}-bis(2-chloroethyl)-N-nitrosourea, and mafosfamide using the DNA base excision repair genes Fpg and α-hOGG1: implications for protective gene therapy applications. J. Pharmacol. Exp. Ther. 296, 825–831

37. He, Y.-H., Xu, Y., Kobune, M., Wu, M., Kelley, M. R., and Martin, W. J., II (2009) Structural and oxoguanine–B flavin mononucleotide interactions under previously challenging conditions. Methods 59, 301–315

38. Preston, T. J., Henderson, J. T., McCullum, G. P., and Wells, P. G. (2009) Base excision repair of reactive oxygen species-initiated 7,8-dihydro-8-oxo-2′-deoxoguanosine inhibits the cytotoxicity of platinum anticancer drugs. Mol. Cancer Ther. 8, 2015–2026

39. Tate, J. G., Bamford, S., Jubb, H. C., Sundak, Z., Beare, D. M., Bindal, N., Boutselasikis, H., Cole, C. G., Creature, C., Dawson, E., Fish, P., Harsha, B., Hathaway, C., Jupe, S. C., Kok, C.-Y., et al. (2019) COSMIC: the catalogue of somatic mutations in cancer. Nucleic Acids Res. 47, D941–D947

40. McHugh, M. L. (2012) Interrater reliability: the kappa statistic. Biochem. Med. 22, 276–282

41. Kohno, T., Shinmura, K., Tosaka, M., Tani, M., Kim, S.-R., Sugimura, H., Nohmi, T., Kasai, H., and Yokota, J. (1998) Genetic polymorphisms and DNA repair mechanism of human DNA glycosylase.

42. Boldinova, E. O., Khairullin, R. F., Makarova, A. V., and Zharkov, D. O. (2019) Isoforms of base excision repair enzymes produced by alternative splicing.

43. Bruner, S. D., Norman, D. P. G., and Verdine, G. L. (2000) Structural basis for recognition and repair of the endogenous mutagen 8-oxoguanine in DNA. Nature 403, 859–866

44. Norman, D. P. G., Chung, S. J., and Verdine, G. L. (2003) Structural and biochemical exploration of a critical amino acid in human 8-oxo-7,8-dihydroguanine glycosylase. Biochemistry 42, 1564–1572

45. Lukina, M. V., Popov, A. V., Koval, V. V., Vorobiev, Y. N., Fedorova, O. S., and Zharkov, D. O. (2013) DNA damage processing by human 8-oxoguanine DNA glycosylase mutants with the occluded active site. J. Biol. Chem. 288, 28936–28947

46. Popov, A. V., Yudkina, A. V., Vorobiev, Y. N., and Zharkov, D. O. (2020) Catalytically competent conformation of the active site of human 8-oxo-7,8-dihydroguanine DNA glycosylase. Biochemistry (Mosc) 85, 192–204

47. Bjerås, M., Luna, L., Johnsen, B., Hoff, E., Haug, T., Rognes, T., and Haug, T., Rognes, T., and Haug, T. (2010) Mecha- nistic and conformational dynamics in the human base excision repair enzyme interrogating undamaged DNA elucidates recognition of damaged DNA. Nature 434, 612–618

48. Bjerås, A., and Verdine, G. L. (2006) A nucleobase lesion remodels the interaction of its normal neighbor in a DNA glycosylase complex. Proc. Natl. Acad. Sci. U. S. A. 103, 15020–15025

49. Rudov, C. T., Barnerje, A., and Verdine, G. L. (2007) Structural characterization of human 8-oxo-7,8-dihydroguanine DNA glycosylase variants bearing active site mutations. J. Biol. Chem. 282, 9182–9194

50. Dalhus, B., Forsbrin, M., Helle, H. I., Vik, E. S., Forstrom, R. J., Backe, P. H., Alseth, I., and Bjørås, M. (2011) Separation-of-function mutants unravel the dual-reaction mode of human 8-oxo-7,8-dihydroguanine DNA glycosylase.

51. Crenshaw, C. M., Nam, K., Oo, K., Kurutchian, P. S., Bowman, B. R., Karplus, M., and Verdine, G. L. (2012) Enforced presentation of an extrahelical guanine to the lesion recognition pocket of human 8-oxoguanine DNA glycosylase. J. Biol. Chem. 287, 24916–24928

52. Lee, S., Rudom, C. T., and Verdine, G. L. (2008) Trapping and structural elucidation of a very advanced intermediate in the lesion-extrusion pathway of hOGG1. J. Am. Chem. Soc. 130, 7784–7785

53. Li, H., Endutkin, A. V., Bergonzo, C., Fu, L., Grollman, A. P., Zharkov, D. O., and Simmerling, C. (2017) DNA deformation-coupled recognition of 8-oxoguanine: conformational kinetic gating in human DNA glycosylase.

54. Sowlati-Hashjin, S., and Wetmore, S. D. (2018) Structural insight into the discrimination between 8-oxo-7,8-dihydroguanine conformers by DNA repair enzymes: a molecular dynamics study of human oxoguanine glycosylase 1 and formamidopyrimidine-DNA glycosylase. Biochemistry 57, 1144–1154

55. Schyma, P., Danielsson, J., Pinak, M., and Laaksonen, A. (2005) Theoretical study of the human DNA repair protein HOG1 activity. J. Phys. Chem. A 109, 1713–1719

56. Calvaresi, M., Bottone, A., and Garavelli, M. (2007) Computational clues for a new mechanism in the glycosylase activity of the human DNA repair protein hOGG1. A generalized paradigm for purine-repairing systems? J. Phys. Chem. B 111, 6557–6570

57. Kellie, J. L., and Wetmore, S. D. (2012) Mechanistic and conformational flexibility of the covalent linkage formed during β-lyase activity on an AP-site: application to hOGG1. J. Phys. Chem. B 116, 10786–10797

58. Kellie, J. L., Wilson, K. A., and Wetmore, S. D. (2015) An ONIOM and MD investigation of possible monofunctional activity of human 8-oxoguanine–DNA glycosylase (hOGG1). J. Phys. Chem. B 119, 8013–8023

59. Sadeghian, K., and Ochsenfeld, C. (2015) Unraveling the base excision repair mechanism of human DNA glycosylase. J. Am. Chem. Soc. 137, 9824–9831
73. Lu, R., Nash, H. M., and Verdicone, G. L. (1997) A mammalian DNA repair enzyme that excises oxidatively damaged guanines maps to a locus frequently lost in lung cancer. *Curr. Biol.* **7**, 397–407.

74. Tyugashev, T. E., Vorobjev, Y. N., Kuznetsova, A. A., Lukina, M. V., Kuznetsov, N. A., and Fedorova, O. S. (2019) Roles of active-site amino acid residues in specific recognition of DNA lesions by human 8-oxoguanine-DNA glycosylase (OGG1). *J. Phys. Chem. B* **123**, 4878–4887.

75. Liu, L. A., and Bradley, P. (2012) Atomistic modeling of protein–DNA interaction specificity: progress and applications. *Curr. Opin. Struct. Biol.* **22**, 397–405.

76. Case, D. A., Darden, T. A., Cheatham, T. E., III, Simmerling, C. L., Wang, J., Duke, R. E., Luo, R., Walker, R. C., Zhang, W., Merz, K. M., Roberts, B., Wang, B., Hayik, S., Roitberg, A., Seabra, G., et al. (2010) AMBER 11, University of California, San Francisco.

77. Geer, L. Y., Marchler-Bauer, A., Geer, R. C., Han, L., He, J., He, S., Liu, C., Shi, W., and Bryant, S. H. (2010) The NCBI BioSystems database. *Nucleic Acids Res.* **38**, D492–D496.

78. Hornak, V., Abel, R., Okur, A., Strockbine, B., Roitberg, A., and Simmerling, C. (2006) Comparison of multiple Amber force fields and development of improved protein backbone parameters. *Proteins* **65**, 712–725.

79. Pérez, A., Marchán, I., Svozil, D., Sponer, J., Cheatham, T. E., III, Laughton, C. A., and Orozco, M. (2007) Refinement of the AMBER force field for nucleic acids: improving the description of α/γ conformers. *Biophys. J.* **92**, 3817–3829.

80. Ryckaert, J.-P., Ciccotti, G., and Berendsen, H. J. C. (1977) Numerical integration of the Cartesian equations of motion of a system with constraints: molecular dynamics of n-alkanes. *J. Comput. Phys.* **23**, 327–341.

81. Darden, T., York, D., and Pedersen, L. (1993) Particle mesh Ewald: an N*\log(N) method for Ewald sums in large systems. *J. Chem. Phys.* **98**, 10089–10092.

82. Popov, A. V., Vorobiev, Y. N., and Zharkov, D. O. (2013) MDTRA: a molecular dynamics trajectory analyzer with a graphical user interface. *J. Comput. Chem.* **34**, 319–325.

83. Deza, M. M., and Deza, E. (2014) *Encyclopedia of Distances*, 3rd Ed, Springer, Heidelberg – NY – Dordrecht – London.

84. Letunic, I., and Bork, P. (2016) Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Res.* **44**, W242–W245.

85. Gill, S. C., and von Hippel, P. H. (1989) Calculation of protein extinction coefficients from amino acid sequence data. *Anal. Biochem.* **182**, 319–326.

86. Chevillard, S., Radicella, J. P., Levalois, C., Lebeau, J., Poupon, M.-F., Oudard, S., Dutrillaux, B., and Boiteux, S. (1998) Mutations in OGG1, a gene involved in the repair of oxidative DNA damage, are found in human lung and kidney tumours. *Oncogene* **16**, 3083–3086.

87. Anderson, P. C., and Daggett, V. (2009) The R46Q, R131Q and R154H polymorphs of human DNA glycosylase/β-lyase hOgg1 severely distort the active site and DNA recognition site but do not cause unfolding. *J. Am. Chem. Soc.* **131**, 9506–9515.

88. Hyun, J.-W., Choi, J.-Y., Zeng, H.-H., Lee, Y.-S., Kim, H.-S., Yoon, S.-H., and Chung, M.-H. (2000) Leukemic cell line, KG-1 has a functional loss of hOGG1 enzyme due to a point mutation and 8-hydroxydeoxyguanosine can kill KG-1. *Oncogene* **19**, 4476–4479.

89. Hyun, J.-W., Cheon, G.-J., Kim, H.-S., Lee, Y.-S., Choi, E.-Y., Yoon, B.-H., Kim, J.-S., and Chung, M.-H. (2002) Radiation sensitivity depends on OGG1 activity status in human leukemia cell lines. *Free Radic. Biol. Med.* **32**, 212–220.

90. Hill, J. W., and Evans, M. K. (2007) A novel R229Q OGG1 polymorphism results in a thermolabile enzyme that sensitizes KG-1 leukemia cells to DNA damaging agents. *Cancer Detect. Prev.* **31**, 237–243.