

Cancer Related BRCA-1 and BRCA-2 Mutations as Analysed by the Resonant Recognition Model

Irena Cosic^{1,2*}, Drasko Cosic² and Katarina Lazar²

¹RMIT University, La Trobe Street, Melbourne, 3000, Victoria, Australia

²AMALNA Consulting, 46 Second St, Black Rock, 3193, Victoria, Australia

Email: irena.cosic@rmit.edu.au

Abstract. It is documented that the large number of mutations within BRCA-1 and BRCA-2 genes are related to development of breast cancer, ovarian cancer, as well as prostate cancer and pancreatic cancer. However, it is not known which mutations are the most critical for formation of these cancers. We have analysed human BRCA-1, BRCA-2 and related RAD51 protein functions and functional mutations using our previously developed Resonant Recognition Model (RRM). The RRM is capable to analyse protein biological functions/interactions, predict bioactive mutations and design *de novo* bioactive peptides with desired biological function. The most critical mutations for formation of cancer in human BRCA-1, BRCA-2 and RAD51 proteins have been predicted and compared with experimental results. The predicted mutations within 3D structures have been presented and discussed. Such findings can lead to development of much simpler and more relevant tests for genetic predisposition to breast, ovarian, prostate and pancreatic cancers.

Keywords: Genetic predisposition to cancer; BRCA-1, BRCA-2 and RAD51; resonant recognition model; molecular modelling.

1 Introduction

BRCA-1 is a human tumor suppressor gene [1,2] and related protein is also called by the synonym breast cancer type 1 susceptibility protein. BRCA-1 protein is responsible for repairing DNA [3]. BRCA-1 and BRCA-2 are normally expressed in the cells of breast and other tissue, where they help repair damaged DNA or destroy cells if DNA cannot be repaired. Thus, these proteins are involved in DNA repair, particularly in repair of double strand breaks [3]. If BRCA-1 or BRCA-2 is damaged by mutations, then they are not able to repair DNA properly and thus there is increased risk for breast and some other types of cancer.

BRCA-1 proteins are directly involved in DNA repair, while BRCA-2 proteins are also involved in DNA repair, but through binding and assembling with RAD51 proteins [4].

Certain mutations of BRCA-1 and BRCA-2 proteins are found to lead to increased risk of breast cancer, ovarian cancer, prostate cancer and pancreatic cancer. It is possible that these mutations are diminishing BRCA-1 and BRCA-2 ability to repair DNA and consequently induce cancer development.

Mutations related to cancer development in BRCA-1 and BRCA-2 proteins are found to be inherited and are related to the ethnicity. These mutations have been spread all along protein sequences. Within human BRCA-1 protein, cancer related mutations are mostly clustered between amino acids in ranges of: 460-555, 800-960, 1020-1220 and 1660-1840 [4].

BRCA-2 protein activity is more complex, as it involves in DNA repair through binding and assembling with RAD51 proteins. Experimentally the large number of critical mutations have been found, which are spread along the whole human BRCA-2 protein, which is extremely long protein built up of 3418 amino acids [4].

Although, there have been a large number of mutations experimentally found in human BRCA-1 and BRCA-2 proteins to be potentially related to cancer development, it is still not well known which mutations are the most critical for the formation of these cancers. If it is possible to identify these critical mutations, it would create a possibility for much simpler and more relevant genetic tests for cancer predisposition.

We have applied here our previously developed Resonant Recognition Model (RRM) [5-7] to analyse biological functions of human BRCA-1, BRCA-2 and RAD51 proteins and predict the most critical mutations, which can diminish their DNA repair activity. These predictions have been compared with the experimental results and predicted mutations within 3D structures have been presented and discussed.

2 Methods

2.1 Resonant Recognition Model (RRM)

The Resonant Recognition Model (RRM) is physico-mathematical model, which is capable to analyse protein biological functions/interactions, predict bioactive mutations and design *de novo* bioactive peptides with desired biological function [5-7].

The RRM model is based on findings that certain periodicities/frequencies within the distribution of energies of free electrons along the protein are strongly correlated with the protein biological functions/interactions. The RRM enables these characteristic frequencies for each biological function/interaction to be identified by presenting each amino acid by its free electron energy and then analysing such distribution of free electron energies along the protein using Fourier Transform and finding the common frequencies for proteins with common biological functions/interactions. In our previous extensive research, we have identified frequencies characterising large number of biological functions/interactions [5-8], as presented in Figure 1. It has been observed that the number of biological functions/interactions can be clustered into super families [8], for example proteins involved in: uncontrolled growth, growth, enzymatic activity, DNA binding, etc. The RRM model has been described in detail in our previous publications [5-7].

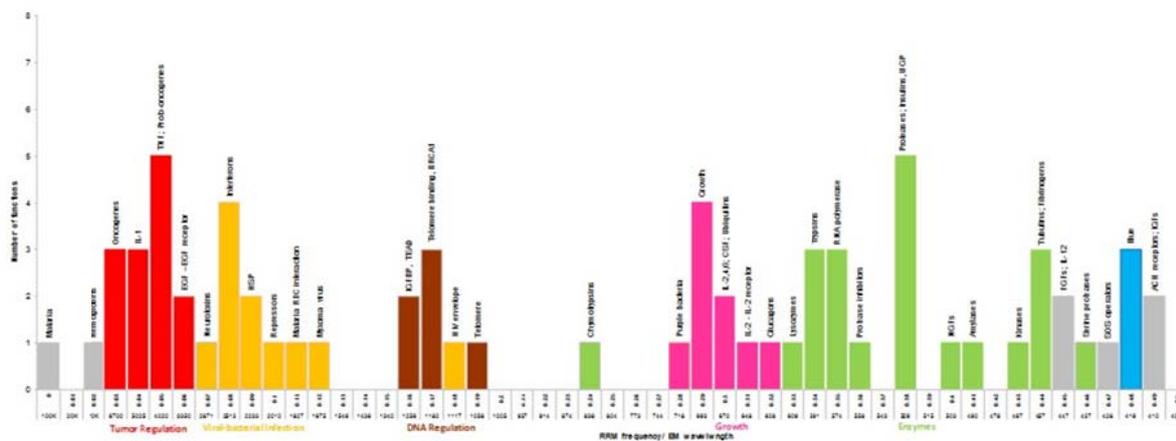


Figure 1. The spectrum of biological functions/interactions, as determined so far, versus RRM frequencies. The clustered families are presented in different colours and named below the graph.

2.2 Prediction of the Key Amino Acids – “Hot Spots”

As described in earlier publication [7]: ‘Knowing the characteristic frequency of particular protein function creates the possibility to predict which amino acids prevail in the sequence and predominantly contribute to this frequency and consequently to the observed function. This could be achieved by small alternations of amplitude in single protein spectrum at characteristic frequency and then observing which amino acids are mostly sensitive to this alternation [5-6, 9-11]. These sensitive amino acids (“hot spots”) are related to characteristic frequency and consequently to the corresponding biological function. The “hot spots” predictions, using the RRM, have been applied already to number of protein and DNA examples including: interleukin-2, SV40 enhancer, epidermal growth factor EGF, Ha-ras p21 oncogene product, glucagons, haemoglobins, myoglobins and lysozymes [5-6, 9-11].

It has been experimentally documented at the example of influenza virus that such predicted amino acids denote residues crucial for protein function [12]. In addition, these “hot spots” amino acids are found to be spatially clustered in the protein tertiary structure and to be positioned in and around the protein active site [9-11].”

2.3 Identification of Oncogene Vs Proto-Oncogene Characteristics

The RRM analysis of oncogene proteins, which are involved in oncogene transformation of the cell, has shown the most prominent characteristic frequency of 0.0322, with less prominent characteristic frequency of 0.0537 [5-7]. The RRM analysis of proto-oncogene proteins, which are very homologous to oncogene proteins, but are not producing cell transformation and are possibly involved in control of cell growth without transformation, has shown the most prominent characteristic frequency of 0.0537, with less prominent characteristic frequency of 0.0322. The conclusion from this earlier work was that the frequency of 0.0322 characterises the process of cell transformation, while the frequency of 0.0537 characterises the cell growth without transformation [7], as presented in Figure 2 and Figure 3.

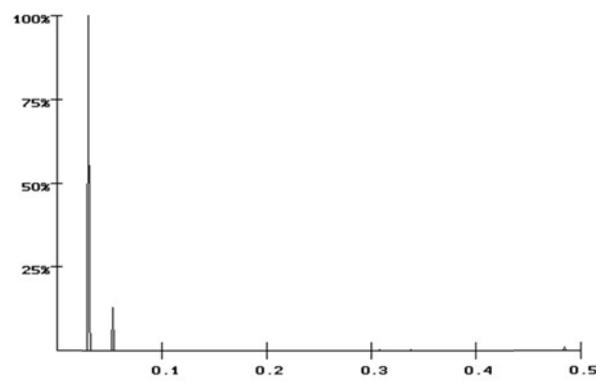


Figure 2. The RRM spectrum for oncogene proteins.

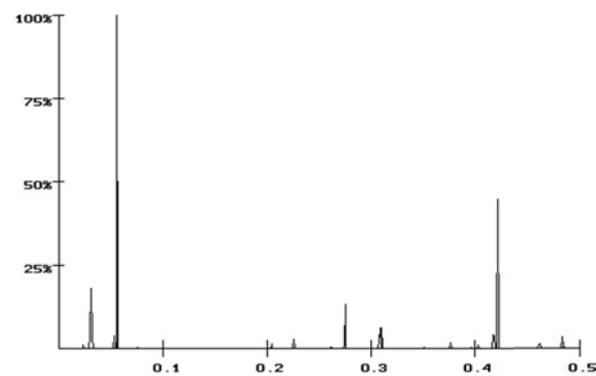


Figure 3. The RRM spectrum for proto-oncogene proteins.

2.4 Protein Sequences

Here, we have analysed the following protein sequences from UniProt database [4]:
nine BRCA-1 proteins:

- P38398 - BRCA1_HUMAN Breast cancer type 1 susceptibility protein;
- Q864U1 - BRCA1_BOVIN Breast cancer type 1 susceptibility protein homolog;
- Q95153 - BRCA1_CANLF Breast cancer type 1 susceptibility protein homolog;
- Q6J6I8 - BRCA1_GORGO Breast cancer type 1 susceptibility protein homolog;
- Q6J6I9 - BRCA1_MACMU Breast cancer type 1 susceptibility protein homolog;

P48754 - BRCA1_MOUSE Breast cancer type 1 susceptibility protein homolog;
 Q9GKK8 - BRCA1_PANTR Breast cancer type 1 susceptibility protein homolog;
 Q6J6J0 - BRCA1_PONPY Breast cancer type 1 susceptibility protein homolog;
 O54952 - BRCA1_RAT Breast cancer type 1 susceptibility protein homolog.

three BRCA-2 proteins:

P51587 - BRCA2_HUMAN Breast cancer type 2 susceptibility protein;
 P97929 - BRCA2_MOUSE Breast cancer type 2 susceptibility protein homolog;
 O35923 - BRCA2_RAT Breast cancer type 2 susceptibility protein homolog.

four human RAD51 proteins:

Q06609 - RAD51_HUMAN DNA repair protein RAD51 homolog 1;
 O15315 - RA51B_HUMAN DNA repair protein RAD51 homolog 2;
 O43502 - RA51C_HUMAN DNA repair protein RAD51 homolog 3;
 O75771 - RA51D_HUMAN DNA repair protein RAD51 homolog 4.

We have also used 3D structures from PDB database [13]:

1. PDBe>1jnx -Crystal structure of the BRC repeat region from the breast cancer associated protein, BRCA1 (source: homo sapiens);
2. PDBe>1n0w – Crystal structure of a RAD51-BRCA2 BRC repeat complex (source: homo sapiens).

3 Results and Discussion

3.1 Prediction of BRCA-1 Critical Mutations

When nine BRCA-1 proteins from different species were compared, the common characteristic frequency was found to be at 0.1685, as presented in Figure 4. This frequency could be characteristic of the DNA repair function common for BRCA-1 proteins.

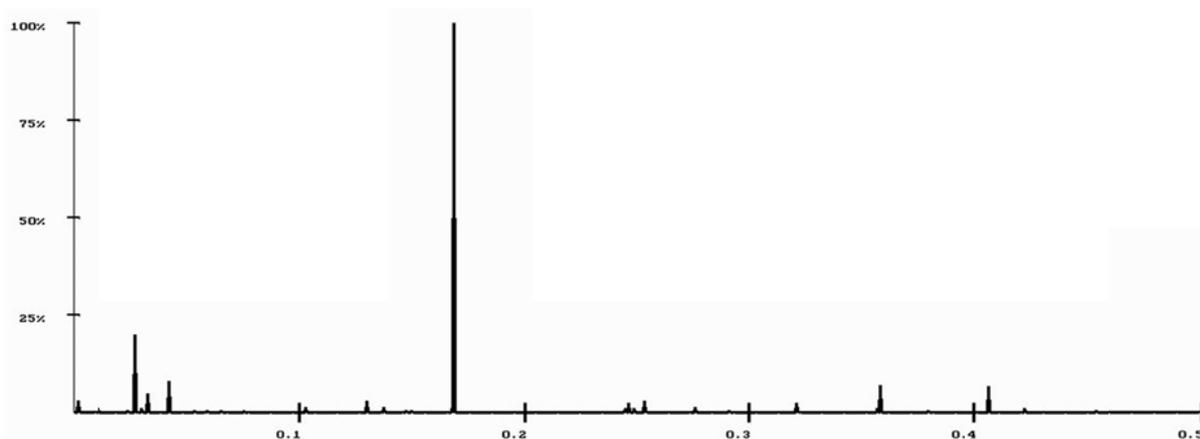


Figure 4. The RRM spectrum for nine BRCA-1 proteins.

The second prominent frequency common for all analysed BRCA-1 proteins is overlapping with previously identified oncogene frequency, so we can identify two distinct RRM characteristics within the BRCA-1 proteins. Based on our earlier research, we propose that the frequency of 0.0322 is related to cell transformation and that the frequency of 0.1685 is related to the DNA repair. Based on these characteristics, we propose related functional mutations in human BRCA-1 protein (P38398) and compare predicted mutations with already experimentally identified ones [4].

The six most significant functional mutations related to the oncogene frequency are at the following positions (in green) below:

57, 1232, 1492, 1706, 1709 and 1710.

The six most significant functional mutations related to the DNA repair frequency are at the following positions (in red) below:

1662, 1668, 1704, 1706, 1748 and 1801.

The amino acid at the position: 1706 is critical for both oncogene and DNA repair frequencies and is highlighted in yellow above.

Our findings show the majority of predicted functional mutations, that are related to both oncogene and DNA repair functions, are within region experimentally found to be DNA binding region between sequence positions 1646 and 1859, confirming the ability of RRM model to predict critical mutations for biological function/interaction. Specifically, we propose that:

1. the region between sequence positions 1704 and 1710 is related to both oncogene and DNA repair functions;
2. the region between sequence positions 1232 and 1492 is more related to oncogene function;
3. the region between sequence positions 1662 and 1668 is more related to DNA repair function.

All predicted functional mutations are positioned within the 3D structure of human BRCA-1 DNA binding segment (from PDB database) and highlighted with CPKs in red for DNA repair, green for oncogene function and yellow for amino acids involved in both functions, as presented in Figure 5.

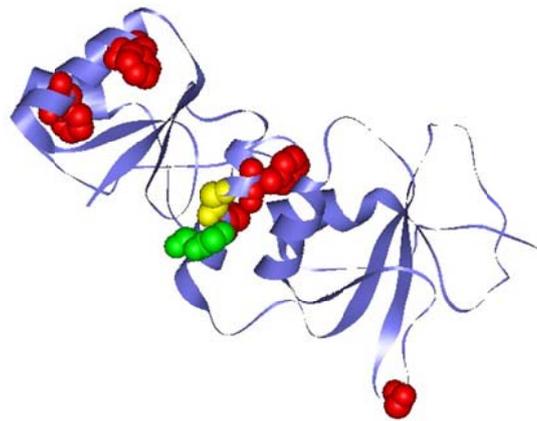


Figure 5. 3D structure of human BRCA-1 DNA binding segment with predicted functional mutations highlighted with CPKs in red for DNA repair, green for oncogene function and yellow for amino acids involved in both functions.

From these results, it can be observed that all the most critical amino acids for both oncogene and DNA repair functions are within the BRC repeat region [13], which is considered and experimentally proven to be the most critical for normal functioning of human BRCA-1 protein. Therefore, the mutations in this region could be the most critical for disturbing the normal functioning of the human BRCA-1 protein and consequently formation of cancer. It is important to note that the RRM predicted not only the critical BRC repeat region, but also the most critical single amino acids within that region.

3.2 Prediction of BRCA-2 Critical Mutations

The BRCA-2 proteins are analysed in conjunction with RAD51 proteins, as their DNA repair function is expressed through binding and assembling with RAD51 proteins [4]. The RRM spectrum of three BRCA-2 proteins and four RAD51 proteins has been presented in Figure 6. From this result, it can be observed that there is one significant common frequency for analysed BRCA-2 and RAD51 proteins at frequency of 0.2561, as presented in Figure 6. According, to RRM principles this common frequency for BRCA-2 and RAD51 proteins is characterising biological function for analysed protein complex.

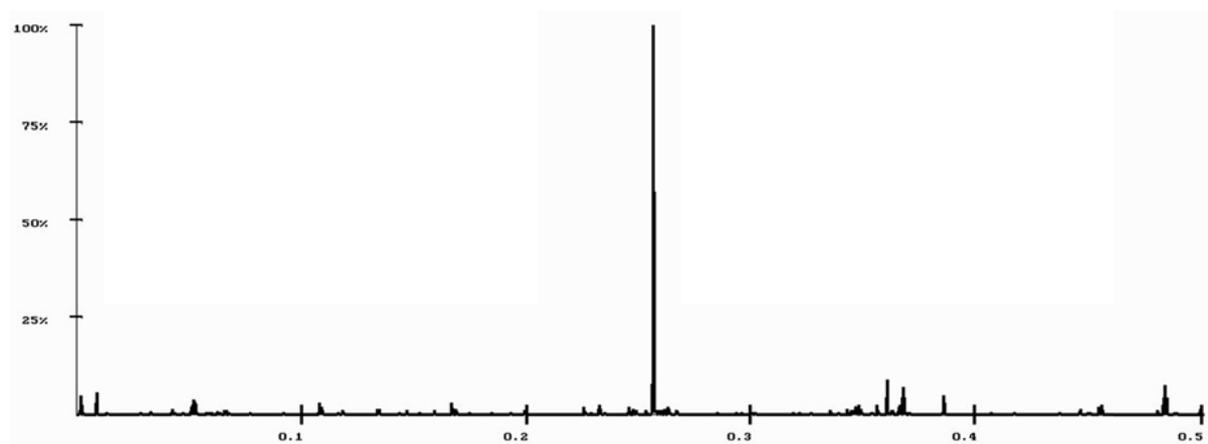


Figure 6. The RRM spectrum for three BRCA-2 and four RAD51 proteins.

Based on the RRM principles, we propose that the frequency of 0.2561 characterises involvement of BRCA-2 protein in double-strand break repair and/or homologous recombination through its binding with the RAD51 protein. Therefore, it is possible that the critical mutations in human BRCA-2 protein can diminish DNA repair function of BRCA-2/RAD51 complex and consequently enable cancer development. We have applied here the RRM model to predict the key mutations in human BRCA-2 (P51587) and RAD51 (Q06609) proteins, that can significantly modify frequency of 0.2561 and are consequently proposed to diminish DNA repair function of human BRCA-2/RAD51 complex. These mutations could be the most critical for cancer formation.

The five most significant functional mutations related to DNA repair frequency in human BRCA-2 protein are at positions (in black) below:

1523, 3090, 3210, 3325 and 3358.

From these results, we propose that the region from 3090 to 3358 is the most significant for human BRCA-2 DNA repair function and thus mutations in this region could be the most critical for cancer development. In addition, amino acid: 1523 is at the binding position between BRCA-2 and RAD51 proteins, highlighted in green above and presented with green CPKs in 3D structure of their complex in Figure 7. Thus, this amino acid at position: 1523 could be critical for recognition between BRCA-2 and RAD51 proteins and consequently could diminish DNA repair function of BRCA-2/RAD51 complex and thus could be critical for cancer development as well.

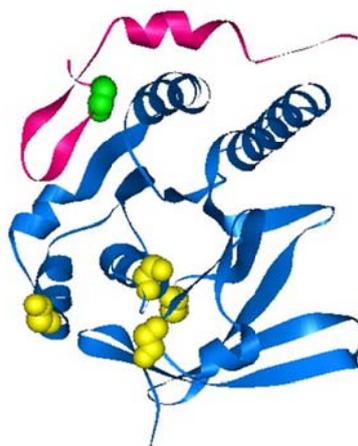


Figure 7. Crystal structure of BRCA-2/RAD51 BRC repeat complex (PDBe>1n0w) with proposed 'hot spots' in RAD51 (blue ribbon) highlighted with CPKs in yellow and 'hot spot' at position 1523 in BRCA-2 (red ribbon) highlighted with CPKs in green.

When the RRM approach was applied to human RAD51 protein to identify amino acids mostly related to DNA repair frequency the four most significant functional mutations are found to be at positions below:

105, 132, 152 and 179.

When these amino acids are positioned in 3D structure of BRCA-2/RAD51 complex (from PDB database), as presented with proposed 'hot spots' in RAD51 (blue ribbon) highlighted with CPKs in yellow and 'hot spot' at position 1523 in BRCA-2 (red ribbon) highlighted with CPKs in green in Figure 7, it can be observed that they are clustered together around the DNA binding site. Thus, they could be critical for binding to DNA. Consequently, these amino acids could be critical for DNA repair and therefore, these mutations could be critical for cancer development. It is important to note that the RRM predicted the amino acids within BRCA-2/RAD51 complex, which could be the most critical for their DNA repair function and could lead to the cancer development.

4 Conclusions

Here we utilised the Resonant Recognition Model (RRM), the physico-mathematical model which can analyse protein function and functional mutations, to predict the critical mutations in human BRCA-1, BRCA-2 and RAD51 proteins. All predicted mutations are within the regions in these proteins that have been experimentally found to be critical for their DNA repair function and consequently development of cancer.

It is important to note that there are a large number of mutations, experimentally found to be related to development of cancer, but there is no evidence which is the most critical. The RRM can provide an advantage of identifying small number of the most critical mutations. These findings could lead to simpler and more relevant genetic tests of mutations related to predisposition for cancer development.

Acknowledgments. This work was supported by AMALNA Consulting.

References

1. J.A. Duncan, J.R. Reeves and T.G. Cooke, "BRCA1 and BRCA2 proteins: roles in health and disease", *Molecular Pathology*, 1998, vol. 51, no. 5, pp. 237-247, doi:10.1136/mp.51.5.237.
2. K. Yoshida and Y. Miki, "Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription and cell cycle in response to DNA damage", *Cancer Science*, 2004, vol. 95, no. 1, pp. 866-871, doi: 10.1111/j.1349-7006.2004.tb02195.x.
3. L.M. Starita and J.D. Parvin, "The multiple nuclear functions of BRCA1: transcription, ubiquitination and DNA repair", *Current Opinion in Cell Biology*, 2003, vol. 15, no. 3, pp. 345-350, doi: 10.1016/S0955-0674(03)00042-5.
4. UniProt Database, 2017.
5. I. Cosic, 'Macromolecular bioactivity: is it resonant interaction between macromolecules? -Theory and applications', *IEEE Trans. on Biomedical Engineering*, 1994, vol. 41, pp. 1101-1114.
6. I. Cosic I, "The Resonant Recognition Model of Macromolecular Bioactivity: Theory and Applications", Birkhauser Verlag, Basel, 1997.
7. I. Cosic, D. Cosic and K. Lazar, "Analysis of Tumor Necrosis Factor Function Using the Resonant Recognition Model", *Cell Biochemistry and Biophysics*, 2016, vol. 74, no. 2, pp. 175-180, doi: 10.1007/s12013-015-0716-3.
8. I. Cosic, D. Cosic and K. Lazar, "Environmental Light and Its Relationship with Electromagnetic Resonances of Biomolecular Interactions, as Predicted by the Resonant Recognition Model", *International Journal of Environmental Research and Public Health*, 2016, vol. 13, no. 7, pp. 647, doi: 10.3390/ijerph13070647.
9. I. Cosic and M.T.W. Hearn, "Hot Spot Amino Acid Distribution in Ha-ras Oncogene Product p21: Relationship to Guanine Binding Site", *J. Molecular Recognition*, 1991, vol. 4, pp. 57-62.
10. I. Cosic and M.T.W. Hearn, "Studies on Protein-DNA Interactions Using the Resonant Recognition Model: Application to Repressors and Transforming Proteins", *Eur. J. Biochem*, 1992, vol. 205, pp. 613-619.
11. I. Cosic, A.N. Hodder, M.I. Aguilar and M.T.W. Hearn, "Resonant Recognition Model and protein topography: model studies with myoglobin, haemoglobin and lysozyme", *Eur. J. Biochem*, 1991, vol. 198, pp. 113-119.

- 12.S. Schmier, A. Mostafa, T. Haarmann, N. Bannert, J. Ziebuhr, V. Veljkovic, U. Dietrich and S. Pleschka, “In silico prediction and experimental confirmation of HA residues conferring enhanced human receptor specificity of H5N1 influenza A viruses”, *Scientific Reports*, 2015, vol. 5, doi: 10.1038/srep11434.
- 13.PDB Database, 2017.