Computational Analysis of RNA-Based Aptamers Targeting Epidermal Growth Factor Receptor (EGFR) for Diagnostic and Therapeutic Applications in Glioblastoma

Saanvi Talwar[1,2], **Edha Talwar**[1,2], **Gaurav Sharma**[2]

Received February 24, 2025
Accepted June 29, 2025
Electronic access July 31, 2025

Glioblastoma is a fast-growing and aggressive malignant brain tumor that is hard to treat and cure. Epidermal Growth Factor Receptor (EGFR) is a protein overexpressed in glioblastoma that drives tumor growth through autocrine signaling and causes resistance to therapies. A mutation in the EGFR could also activate it, resulting in uncontrolled cell proliferation. An aptamer is a short, single-stranded DNA or RNA molecule that can bind a target molecule (usually a protein with high specificity). Therefore, these aptamers can be designed to bind to the binding site of the EGFR and can be used to detect and inhibit GBM cells. Given the critical role of EGFR in promoting tumor growth, we propose that targeting its active site could inhibit its function. We hypothesize that aptamer A1 binds strongly to the active site of the EGFR and could block its activity. In this research work, we have employed various in silico methods to understand the mechanism of aptamer binding to the EGFR. A deep learning algorithm, AlphaFold 3, was used to predict the 3D structure of the EGFR for docking studies. UNAfold was employed to predict the aptamer secondary structure, while the aptamer 3D configuration was refined by FARFAR 2 for accurate structural representations. Molecular docking simulations were performed using the HDOCK software to predict the binding affinity and orientation between the aptamer and EGFR. Utilizing PLIP, the docking results were analyzed, showing prominent protein-ligand interactions, including hydrogen bonds and hydrophobic contacts that contribute to the stability of the aptamer-EGFR complex. These findings may pave the way for creating aptamer-based therapeutics for glioblastoma EGFR targeting.

Keywords: Aptamer, Glioblastoma, Epidermal Growth Factor Receptor (EGFR), Molecular Docking.

Introduction

Glioblastoma is a deadly brain cancer that starts in astrocytes, the star-shaped cells found in the brain and spinal cord.(Alifieris & Trafalis, 2015)[1] It ranks as the most common and fatal primary brain tumor in adults. Its fast growth and spreading ability make it hard to remove through surgery.(Alifieris & Trafalis, 2015)[1] People with this cancer often experience bad headaches, feel sick, have seizures, think less, and struggle with brain functions based on where the tumor sits.(Alifieris & Trafalis, 2015)[1] Doctors treat it with a mix of surgery, radiation, and drugs, but the outlook remains grim. Most patients live 12-18 months after diagnosis.(Alifieris & Trafalis, 2015)[1] Cancer's resistance to standard care and its habit of coming back show that we need new and better ways to fight it. Figure 1a shows the overexpressed EGFR. Normal cells have fewer EGFR, while cancer cells overexpress EGFR, leading to uncontrolled growth.

Aptamers are short, single-stranded nucleic acid molecules (DNA or RNA) capable of high affinity and specific binding with target molecules, such as proteins, small molecules, or cells.(Keefe, Pai, & Ellington, 2010)[2] Because of their versatility and ability to bind precisely, they find extensive applications in diagnostics, drug delivery, and targeted therapeutics.(Keefe et al., 2010)[2] They have proved especially useful in implementing biosensors for diagnosing diseases, cancer therapies, and treatments against viruses. Usually, the technology applied for developing an aptamer is a method entitled SELEX—Systematic Evolution of Ligands by Exponential Enrichment—in which a vast library of random nucleic acid sequences undergoes multiple exposure cycles to the target molecule.(Dunn, Jimenez, & Chaput, 2017)[3] These binding sequences are selected, amplified, and refined through numerous rounds of selection, yielding aptamers with strong and specific binding properties. Aptamers targeting the overexpressed EGFR could be used to detect and kill the glioblastoma cells, Figure 1b. Figure 1b illustrates how aptamers aid in detecting and treating glioblastoma. The aptamer binds to EGFR in cancer cells. For detection, it is conjugated with a fluorescent dye to highlight tumor cells and can be used in their detection. For treatment, it delivers an anti-cancer drug that kills glioblastoma cells while minimizing harm to healthy tissue.

Peng et al. have developed promising aptamer-bound gold

nanoparticles that target EGFRvIII, inhibit the EGFR-related signaling pathway, and prevent DNA damage repair in GBM cells.(Peng et al., 2020)[4] Similarly, Affinito et al. developed Ephrin Receptor Tyrosine Kinase A2 (EPHA2)-selective aptamers targeting GBM stem cells.(Affinito et al., 2020)[5] On the other hand, DNA aptamers have been made by Kichkailo et al. for visualization of glial brain tumors and can be used to detect circulating tumor cells.(Kichkailo et al., 2023)[6] Similar to this, Silva et al. have demonstrated that nucleic-acid aptamers targeting EGFR and integrins can detect GBM heterogeneity in human tumor sections.(Cruz Da Silva et al., 2022)[7]

Molecular docking is a set of computer programs that predict the binding of a low-molecular-weight compound, usually a drug, to a target protein or another macromolecule.(Fan, Fu, & Zhang, 2019)[8] The procedure that stimulates the binding process is considered the common origin for the parameters such as the shape, electrostatic interactions, and chemical properties of both the ligand and the receptor.(Fan et al., 2019[8]) Its primary purpose is to determine the most stable binding structure, which can be used to understand the mechanism of a particular drug or therapeutic compound. Docking is a widely used approach in drug discovery, where large libraries of drug candidates are screened against disease-related targets to identify which candidates can bind efficiently. (Fan et al., 2019)[8] It can also optimize existing drugs, improving their binding affinity and specificity. In addition to drug design, docking has applications in studying protein-protein interactions, enzyme function, and vaccine development. By providing a medium for molecular interaction tests, docking accelerates discovery and reduces the number of costly experimental tests.(Fan et al., 2019)[8]

Despite existing research on aptamers targeting EGFR in GBM, few studies have conducted a comprehensive in silico comparison of multiple RNA aptamers specifically binding to the EGFR active site. Previous studies have focused on the aptamers conducting wet-lab experiments without extensive in silico modeling to predict aptamer-EGFR interactions and understanding the binding mechanism. In this paper, we tested and analyzed various aptamers to develop a more effective and targeted treatment for glioblastoma. We hypothesized that aptamer A1 binds to the active site of EGFR and could block its activity. We found that aptamer A1 binds effectively to the active site of EGFR, showing promising potential for targeting and blocking its activity. This suggests that aptamer A1 could be a valuable tool for diagnosing and treating glioblastoma by targeting EGFR. Further experiments could confirm its effectiveness in living organisms, paving the way for its use as a targeted treatment for glioblastoma.

Method

In this study, the amino acid sequence of EGFR was accessed from the UniProt database, a central comprehensive repository containing protein sequences and functional information.(Consortium, 2014)[9] Indeed, UniProt is a highly resourceful database that provides comprehensive information about proteins, including their sequences, structures, and annotations, and is therefore used for molecular research. AlphaFold 3 is a deep learning based method created by DeepMind.(Abramson et al., 2024)[10] AlphaFold 3 predicts three-dimensional protein structures with optimal accuracy from their amino acid sequences.(Abramson et al., 2024)[10] There have been three AlphaFold versions: (a) AlphaFold 1: predicted distances between parts of a protein; however, it needed other tools to turn those distances into a 3D shape; (b) AlphaFold 2: Predicted the full 3D shape of a protein directly from its sequence; and (c) AlphaFold 3: Can predict how proteins interact with other proteins, DNA, RNA, or small molecules. Multiple sequence alignment was carried out using MMseqs2, and template selection was set to automatic. The predicted Local Distance Difference Test (pLDDT) scores averaged 91.3 across the full structure, while the binding site region (residues 345–490) showed a high-confidence score of 93.8. Predicted Aligned Error (PAE) maps further validated the domain-level accuracy of the model, particularly around the ligand-binding region used for docking simulations.

Molecular docking is an computational method used to predict the interaction of a ligand (such as an aptamer or small–molecule drug) with a target protein at the molecular level. This method provides valuable insights into the strength of binding affinity and intermolecular interactions, enabling a more comprehensive analysis of the ligand's biological activity and therapeutic implications. We have used HDOCK software

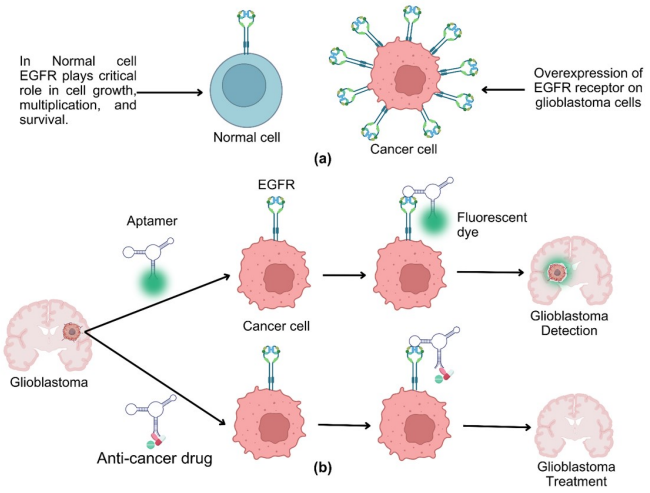

Fig. 1 Conceptual representation of EGFR expression and aptamer function in glioblastoma. (a) Comparison of EGFR expression levels between normal and glioblastoma cells; and (b) Illustration of aptamer targeting for detection: aptamers are conjugated with fluorescent dyes; for treatment, they deliver anti-cancer drugs to EGFR-expressing

to perform molecular docking simulations. Molecular docking simulations were performed using the HDOCK server, which applies a hybrid algorithm combining template-based modeling and free docking. The receptor was treated as rigid, while the aptamer was treated as flexible during the docking process. Default parameters were used, which include an automatic grid box centered on the predicted binding site, with the grid size adaptively set by the server. The docking protocol generated the top 100 binding conformations ranked by a knowledge-based scoring function, and the most favorable conformation based on binding energy and interface analysis was selected for further study.

We used various methods to understand and visualize the aptamer's structure, starting with the Mfold RNA Folding Form for the aptamer's secondary structure.(Markham & Zuker, 2008)[11] This image was created using Canva and matched the downloaded .ct file, which would guide the next step through the connectivity map. The output should now provide respective dot-bracket notation using CT to Dot-Bracket Converter. The .ct file has been converted to yield dot-bracket notation, simplifying the structure into something interpretable. Finally, the sequence and notation were submitted to Rosie RNA Structure Modeling, resulting in a 3D model—an description of the aptamer's elaborate design, ready to further the research.(Watkins & Das, 2023)[12] In the FARFAR2 protocol, 5,000 RNA models were generated per aptamer using Rosetta's Monte Carlo-based sampling with default temperature (0.8) and fragment assembly strategies. Both low-resolution and high-resolution Rosetta RNA scoring functions were applied to rank the models, and the top 10 structures were selected based on energy scores. The final aptamer conformations were chosen based on the lowest Rosetta energy scores (ranging from –110 to –125) and structural convergence within 2.0 Å RMSD. Visualization and validation were performed using ChimeraX (v1.9), prior to docking with EGFR using HDOCK, where the receptor was treated as rigid and the aptamers as flexible ligands. The aptamer used in this research is shown in Table 1.

The following criteria were used to select the aptamer. First, we used the predicted binding site shown in Figure 2b, highlighted in red. The aptamer should bind to this site so that it can compete with the natural epidermal growth factor ligand. All four aptamers used in this study met this first criterion. For the second criterion, the interactions formed between the aptamers and the EGFR receptor were analyzed using BLEP software. The interaction graph, shown in Figure 4, indicates that Aptamer 1 forms the highest number of strong interactions. Therefore, Aptamer 1 was selected as the most appropriate candidate.

Results

In this research work, we conducted a computational analysis to evaluate RNA-based aptamers targeting EGFR for potential diagnostic and therapeutic applications in glioblastoma.

Surface properties of the EGFR: Figure 2a shows the 3D structure of the EGFR. The predicted binding site of the EGFR was identified using a combination of P2Rank(9) and ScanNet(10), computational tools for binding site prediction (Figure 2b). P2Rank analyzed the receptor's 3D structure and identified pockets with high potential for ligand binding based on geometric and physiochemical properties. To fine-tune this expectation, ScanNet was utilized to determine the predicted binding site on the protein surface. Knowing the information about the predicted binding site is crucial for aptamer design, as these sites are key to identifying effective candidates. If an aptamer binds to the predicted site, it can be selected for further development and may potentially inhibit the cancer receptor. ChimeraX stimulated the electrostatic surface potential (ESP) of the EGFR as displayed in Figure 2c (Meng et al., 2023)[13]. The resulting ESP map revealed the positive and negative electrostatic charge areas, primarily those in the ligand-binding site, which are key elements in the interaction between EGFR and aptamer A3. This reveals the charge of the protein. A positive or negative charge is crucial for aptamer binding because aptamers are highly negatively charged. In this study, the EGFR receptor was found to be more negatively charged; therefore, specific strategies will be required for aptamer transport. For instance, positively charged proteins like protamine can be used to facilitate the transport of aptamers to the tumor microenvironment.

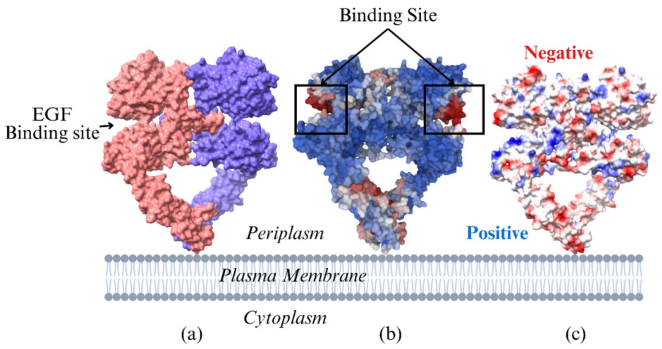

Fig. 2 Structural and Electrostatic Characterization of the Membrane-Bound Protein. (a) Surface model showing the EGF (Epidermal Growth Factor) binding site in pink, highlighting the periplasmic domain of the receptor; (b) Predicted binding sites (boxed) on the extracellular domain based on structural analysis; and (c) Electrostatic surface potential map with red indicating negatively charged regions and blue indicating positively charged regions, suggesting areas of charge complementarity that are critical for ligand or antibody interaction.

The molecular docking studies were performed using HDOCK as shown in Figure 3. The Figure 3 shows the results of the molecular docking simulation, where all aptamers (A1, A2, A3, and A4) successfully bind to the predicted binding site of the

Table 1 RNA Aptamer Sequences A1 to A4: Nucleotide sequences and corresponding dot-bracket notation.

Label	Sequence	Dot-Bracket Notation
A1	UUGUGUUGGUCCUAAAUG	....(((.....))).
A2	CAUUUAGGACCAACACAA	...((.....)).....
A3	ACGCACCAUUUGUUUAAUAUGUUUUUUAAUUCCCCUUGUGGUGUGU	(((((((((....((((.....))))........)))))))))
A4	ACGCACCAUUUGUUUAAUAUGUUUUUUAAUUCCCCUUGUGGUGUGUCAUUUAGGACCAACACAA	(((((((((....((((.....))))........))))))))).....

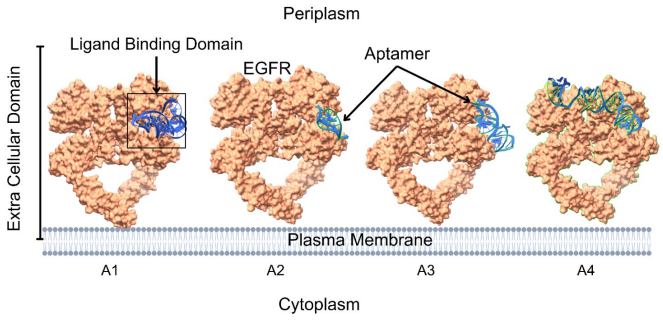

Fig. 3 Aptamer Binding to EGFR Extracellular Domain. A1–A4 depict various conformations of the aptamer (blue/green) interacting with the extracellular domain of the EGFR receptor (orange). A1 highlights the aptamer binding at the ligand binding domain, while A2–A4 demonstrate additional aptamer orientations on the receptor's surface. These structural models suggest potential inhibitory aptamer interactions with EGFR for therapeutic targeting.

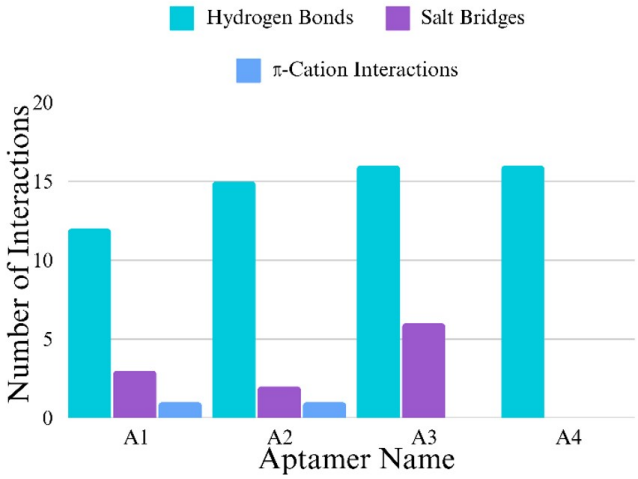

Fig. 4 Comparative Analysis of Molecular Interactions Between Aptamers and EGFR. The bar graph illustrates the number of hydrogen bonds, salt bridges, and π-cation interactions formed by each aptamer (A1–A4) with the EGFR receptor. Aptamers A3 and A4 exhibit the highest number of hydrogen bonds, with A3 also showing the most significant number of salt bridges, indicating strong and diverse binding potential.

receptor, as identified in Figure 2. This consistent binding confirms the accuracy of the binding pocket predicted by P2Rank in Figure 2b. Consequently, the docking outcomes serve to validate the P2Rank prediction, and in turn, the reliability of the docking simulation itself is reinforced. It displays the extracellular and ligand-binding domains of the EGFR protein, illustrating the aptamer-specific binding to its target site. The main goal of the diagram is to highlight specific components—particularly how the aptamer interacts with the EGFR (Epidermal Growth Factor Receptor). To study these interactions, the researchers used a tool called PLIP (Protein-Ligand Interaction Profiler), which can identify the types of bonds and interactions between a protein and a ligand (in this case, the aptamer). Figure 4, and Table 1 visually represents these interactions. By analyzing the docking results using PLIP, the study concluded that among all the aptamers tested, aptamer A3 showed the strongest binding to EGFR, indicating it may be the most effective for diagnostic or therapeutic applications.

Discussion

Current treatments for glioblastoma, including MRI & biopsy, surgery, radiotherapy, and chemotherapy, have limitations such as late detection, invasiveness, tumor variability, and severe side effects.(Silantyev et al., 2019)[14] Surgery cannot remove all cancer cells, radiotherapy damages healthy tissue, and chemotherapy faces resistance and side effects.(Silantyev et al., 2019)[14] These methods are insufficient due to rapid tumor spread, resistance development, and reduced quality of life. Our aptamer-based approach targets glioblastoma cells specifically, offering more precise treatment with fewer side effects and addressing the key limitations of existing therapies.(Silantyev et al., 2019)[14]

Limitations and Future Work: The lack of experimental validation restricts the application and verification of these findings in real–life situations. To address this, future studies could investigate the use of controlled in vitro and in vivo experiments to support the theoretical results. Moreover, using different assay techniques, such as fluorescence or surface plasmon resonance, could yield direct experimental evidence supporting the effectiveness of the suggested approach. The limited number of aptamers restricts diversity and limits the potential for wide-ranging applications across various biological systems. To broaden this library, techniques such as SELEX (Systematic Evolution of Ligands by Exponential Enrichment) could enhance the approach's versatility, making it possible to target a broader range of biomarkers. (Darmostuk, Rimpelova, Gbelcova, &

Table 2 Docking and Binding Evaluation of Aptamers Targeting EGFR. The table summarizes docking score, confidence score, ligand RMSD, and binding energy for four aptamers (Apt 1–4) interacting with the EGFR receptor. Aptamer 4 shows the most favorable docking score (–348.46) and highest confidence (0.9815), while Aptamer 1 demonstrates the strongest binding energy (–16.5 kcal/mol), suggesting a balance between stability and binding strength across different aptamer candidates.

Rank	Docking Score	Confidence Score	Ligand RMSD (Å)	Binding Energy (kcal/mol)
Apt 1	-276.87	0.9267	72.04	-16.5
Apt 2	-302.3	0.9546	138.56	-12.4
Apt 3	-307.01	0.9585	158.08	-14.5
Apt 4	-348.46	0.9815	95.48	-12.3

Ruml, 2015)[15] Moreover, applying computational methods to predict how aptamers interact could further streamline the selection process. Dealing with aptamers by nucleases poses a big challenge, undermining their stability and effectiveness over time. This issue might be addressed by modifying the aptamers with nuclease–resistant modifications, such as phosphorothioate backbones or 2'-O-methyl substitutions, which would help them resist enzymatic breakdown. Finally, molecular docking simulations also lack water molecules in the simulations. Since, the human body contains 65-70% water, it plays a major role in the protein movement and flexibility. Therefore, in future studies we will be performing molecular dynamics simulations to added the dynamical effect in the protein-aptamer complex.

Applications: Aptamers can be specifically designed to bind to glioblastoma cells, enabling more targeted treatment or the delivery of therapeutic agents.(Chandola, Kalme, Casteleijn, Urtti, & Neerathilingam, 2016)[16] This method not only enhances the effectiveness of the treatment but also minimizes harm to surrounding healthy tissues, offering a more precise alternative to traditional therapies. Aptamers are helpful in non–invasive diagnostic methods, such as liquid biopsies, which identify biomarkers in blood or urine.(Chandola et al., 2016)[16] This capability enables the early detection of conditions such as cancer, resulting in improved outcomes and a reduced need for invasive procedures. Advanced imaging techniques, including MRI and CT scans, as well as novel approaches such as liquid biopsies and genetic profiling, are utilized for glioblastoma detection, facilitating the early identification of tumors and assessment of treatment responses. In this study, we employed computational methods to identify aptamers targeting the EGFR of glioblastoma. Based on our results, we have identified aptamer A3 as a strong binder to the EGFR, which can be utilized in glioblastoma detection and treatment.

Conclusion

In conclusion, our study demonstrates that RNA-based aptamers, particularly aptamer A3, show strong predicted binding to the EGFR based on computational analysis. These findings suggest potential for aptamer A3 in glioblastoma detection and treatment. However, it is important to note that these results are solely based on in silico simulations and do not establish clinical efficacy or biological activity. Without experimental validation, the therapeutic or diagnostic applicability of these aptamers remains speculative. Future in vitro and in vivo studies are essential to confirm the binding affinity, specificity, and stability of aptamer A3 under physiological conditions. Only then can its true potential in clinical applications be evaluated.

References

1 C. Alifieris and D. Trafalis, *Glioblastoma multiforme: Pathogenesis and treatment*.

2 A. Keefe, S. Pai and A. Ellington, *Aptamers as therapeutics*.

3 M. Dunn, R. Jimenez and J. Chaput, *Analysis of aptamer discovery and technology*.

4 L. Peng, L. Yanling, Z. Xinxin, L. Zhiman, T. Yinghong, L. Shuji and X. Zhang, *Aptamer-Conjugated Gold Nanoparticles Targeting Epidermal Growth Factor Receptor Variant III for the Treatment of Glioblastoma*.

5 A. Affinito, C. Quintavalle, C. Esposito, G. Roscigno, C. Giordano, S. Nuzzo and G. Condorelli, *Targeting Ephrin Receptor Tyrosine Kinase A2 with a Selective Aptamer for Glioblastoma Stem Cells*.

6 A. Kichkailo, A. Narodov, M. Komarova, T. Zamay, G. Zamay, O. Kolovskaya and M. Berezovski, *Development of DNA aptamers for visualization of glial brain tumors and detection of circulating tumor cells*.

7 E. Silva, S. Foppolo, B. Lhermitte, M. Ingremeau, H. Justiniano, L. Klein and L. Choulier, *Bioimaging Nucleic-Acid Aptamers with Different Specificities in Human Glioblastoma Tissues Highlights Tumoral Heterogeneity*, `https://www.mdpi.com/1999-4923/14/10/1980`, Retrieved from.

8 J. Fan, A. Fu and L. Zhang, *Progress in molecular docking*.

9 T. Consortium, *UniProt: a hub for protein information*.

10 J. Abramson, J. Adler, J. Dunger, R. Evans, T. Green, A. Pritzel and J. Jumper, *Accurate structure prediction of biomolecular interactions with AlphaFold 3*.

11 N. Markham and M. Zuker, *UNAFold*.

12 A. Watkins and R. Das, *RNA 3D Modeling with FARFAR2, Online*.

13 C. Meng, D. Goddard, F. Pettersen, S. Couch, J. Pearson, H. Morris and E. Ferrin, *¡scp¿UCSF ChimeraX¡/scp¿: Tools for structure building and analysis*.

14 A. Silantyev, L. Falzone, M. Libra, O. Gurina, K. Kardashova, T. Nikolouzakis and A. Tsatsakis, *Current and future trends on diagnosis and prognosis of glioblastoma: from molecular biology to proteomics*.

15 M. Darmostuk, S. Rimpelova, H. Gbelcova and T. Ruml, *Current approaches in SELEX: An update to aptamer selection technology*.

16 C. Chandola, S. Kalme, M. Casteleijn, A. Urtti and M. Neerathilingam, *Application of aptamers in diagnostics, drug-delivery and imaging*.