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AI Drives Optimization of Delivery Systems Engineered for Precise and Effective Immune-Based Solutions

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Abstract: In oncology, the development of intelligent, biocompatible nanocarriers is pivotal for advancing targeted immunotherapy. This study presents a protein-binding immunotherapy model that leverages targeted immune proteins, such as interleukins, interferons, and checkpoint inhibitors, integrated with a drug delivery system (DDS). The proposed Zn^{2+}-glutamic acid (Glu) nanocarrier, optimized via the AI-based DeepChem platform, demonstrated strong therapeutic potential for cancer treatment. Computational analysis revealed high coordination stability of the Zn^{2+}-Glu complex (binding energy: −42.8 kcal/mol) and notable protein-binding affinity to interleukin-2 (IL-2) (7.9 ± 0.3 pKd) using a graph convolutional network model. The nanocarrier achieved efficient protein encapsulation (85.6 ± 2.2%), pH-sensitive release (68.4 ± 1.7% at pH 6.5 over 12 h), and favorable solubility (log S = −1.8). Non-toxicity prediction indicated 92% safety with a ROC-AUC of 0.89. Immunological assays showed a 3.5-fold increase in $CD8^+$ T-cell activity, with nanoparticle stability confirmed by a zeta potential of −22.5 mV and PDI of 0.18. Additional benefits included fluorescence traceability at 650 nm and a 2.3-fold increase in systemic half-life, supporting its theranostic capability. Importantly, the Zn^{2+}-Glu platform exhibited immunomodulatory and anti-inflammatory properties, suggesting potential to enhance chemotherapy tolerance by reducing systemic inflammation and minimizing immune-related adverse effects. Integrating bioactive components, such as Moringa Leaf Extract (MLE), could further enhance immune cell function while mitigating chemotherapy-induced immunotoxicity. Overall, the Zn^{2+}-Glu nanocomposite offers a scalable, non-toxic, and site-specific DDS, positioning it as a promising next-generation protein-derived immunotherapeutic agent.

Keywords: Drug Delivery System (DDS); Immunotherapy; Artificial Intelligence (AI); Deep Chem; Protein Binding

1. Introduction

In recent years, it has become an essential element of the prescriptions used today to introduce new improvements to the challenges faced by the traditional ways of drug administration methods through the introduction of Drug Delivery Systems (DDS) [1]. The poor bioavailability, lack of specificity in distribution, and necessitating frequent doses are some of the characteristics of the already available traditional routes like oral administration or intravenous delivery, which result in less than an optimum therapeutic effect and a greater toxicity due to redistribution to a larger systemic volume [2]. The DDS technologies have been invented to address these shortcomings, optimizing the pharmacokinetic and pharmacodynamic characteristics of therapeutic compounds [3]. Advanced systems like nanoparticle, liposome, dendrimer, and polymeric carrier facilitated drug targeting, control, and prolonged release, which ensure, minimize drug side effects, and enhance patient compliance with the drug. Research has been especially attentive in the last ten years of stimulus-responsive DDS, which involves releasing therapeutic agents according to primarily physiological triggers such as pH, temperature, or enzymatic activity, providing site-specificity [4]. Nevertheless, there are still some obstacles to overcome related to biocompatibility, drug loading efficacy, and the ability to scale up to clinical use.

Immunotherapy is a revolutionary form of disease treatment in oncology, which uses the body's immune system to identify and destroy malignant cells [5]. Nevertheless, the therapeutic potential of most immunotherapeutic drugs is compromised by inefficient pharmacokinetics, off-target action, and overall toxicity. Such issues have stimulated the creation of novel Drug Delivery Systems (DDS) that enhance the accuracy, safety, and efficacy of immunotherapy [6]. DDS based on nanoparticles, liposomes, hydrogels, and micelles have been shown to be capable of protecting immune-modulating chemicals against degradation; increasing the accumulation of immune-modulating chemicals in desired locations; and facilitating controlled or stimuli-sensitive release [7]. Specifically, using nanocarrier-based DDS, the possible selectivity of transmitting cytokines, checkpoint inhibitors, or antigens specifically to a tumor tissue or a lymphoid organ could be created and therefore enhance immune responses with a reduced adverse effects [8]. Moreover, DDS could be surface-modified with homing agents to enhance the selectivity of uptake into target cells and minimize immune activity in the tumor microenvironment. Nonetheless, a significant challenge is finding an efficient route of delivery, immune activation, and scalability for this technology [9].

Immunotherapy, which utilizes therapeutic proteins, also referred to as a protein-based immunotherapy, including cytokines, monoclonal antibodies, and tumor antigens, has emerged as a potent tool in treating cancer and immune-related diseases [10]. There is high specificity in modulating immune responses by these biologics, but poor stability, faster degradation, or high-specific aspects are a bane to their clinical application. To overcome these shortcomings, Drug Delivery Systems (DDS) have been advanced on a large scale to increase the delivery and efficacy of protein-based immunotherapeutics [11]. Notably, advanced DDS platforms, including biodegradable nanoparticles, liposomes, and polymer-based carriers, provide successful protection embodied by the encapsulation of the protein, thereby facilitating controlled and sustained release, pharmacokinetics, as well as targeting immune-related tissues or tumor microenvironments [12]. Furthermore, protein release profiles can be further optimized with stimuli-responsive DDS to respond to local conditions, including pH or enzyme activity, with desired spatiotemporal precision. Notably, DDS will allow for the minimization of dose frequency and systemic toxicity usually reported with protein therapeutics, in addition to boosting the activation of immune cells and therapeutic responses [13].

The great potential of protein-based immunotherapy in cancer and autoimmune conditions raises a number of serious questions that restrict its translation into clinical practice [14]. The first significant obstacle is the natural instability of therapeutic proteins, which can be degraded by enzymes, denatured, or aggregate during storage, transport, or following administration. This fixity lowers the effectiveness of therapy and could elicit undesirable immune reactions. The cumbersome molecular structure and hydrophilic properties of proteins also lack bioavailability and the capability to penetrate tissues, thus forming a barrier to their crossing of biological barriers [15]. The traditional drug delivery methods do not eliminate these shortcomings, and in most cases, they cause systemic toxicity and off-target effects. On the other hand, the encapsulation efficacies of protein delivery are low, meaning that most of the protein would be released at one known as the burst effect, loading capacities are low, and there are difficulties in the manufacture of Drug Delivery Systems (DDS) [16]. In addition, it remains a major challenge to provide controlled release of biologically active proteins that are specific and stimuli-responsive, while also main-

taining the integrity of the proteins [17]. The protein as well as the carrier material may also be problematic in terms of safety due to immunogenicity [18]. Thus, it is imperative that the development of the advanced DDS platforms that are capable of maintaining the protein activities, more selective delivery, and longer-term effects of the immunotherapy at reduced side effect levels is developed [19]. Recent studies shed light on the increasing importance of precision nanomedicine in cancer treatment, focusing on how it can adapt to the tumor microenvironment, offer personalized diagnostics and therapies, and improve the results of immunotherapy. Recent developments in nanotechnology have enhanced the precision of diagnostics and the effectiveness of targeted drug delivery. At the same time, bibliometric analyses highlight the swift growth of research and the new challenges that arise in translating these findings into clinical practice [20–25].

This article reports a novel combination of metal-coordination chemistry and AI-based design for nanodrug delivery, featuring a Zn^{2+}-glutamic acid (Glu) nanoplatform specifically useful in protein-based immunotherapy. The most significant aspect is the computationally driven optimization through the DeepChem framework, which allowed for predicting the affinity of proteins, their toxicity, solubility, and release properties fairly accurately. The unique experiment introduces the Zn^{2+}-Glu coordination to immune-relevant proteins (e.g., IL-2) and specifically obtains high binding affinity (pKd7.9 $\pm$ 0.3), high stability (binding energy -42.8 kcal/mol), and the aspect of retaining biocompatibility (92 percent non-toxic) and pH-responsiveness (68.4 percent release at pH 6.5). In addition, the system exhibits dual theranostic performance with innate fluorescent tracing (650 nm) and ignition of the immunomodulatory response (three-and-a-half-fold increase in $CD8^+$ T-cell response). The work contributes to the emerging field of AI-aided nanomedicine design, which combines computational chemistry, immune engineering, and specialized cancer treatment, and lays the groundwork for subsequent *in vivo* validation and practical clinical translation.

2. Drug Delivery System (DDS) With DeepChem, an Open-Source Deep-Learning

The application of artificial intelligence (AI) to Drug Delivery Systems (DDS) is a new opportunity to design, formulate, and target drugs in an optimized way. Moreover, as one example of AI, DeepChem, an open-source deep-learning computational chemistry and drug discovery-specific framework, has demonstrated major potential in the pursuit of research in the DDS. DeepChem facilitates the simulation of molecular interactions, pharmacokinetics prediction, and toxicity and binding of a drug to a target. Due to extensive datasets and machine learning, scientists can now predict release kinetics, select the best drug carriers, and predict protein-drug or nanoparticle interactions and conduct trials on each in silico. Within the framework of DDS, it is possible to utilize DeepChem to screen and predict *in vivo* interactions between therapeutic compounds and different nanocarriers, such as liposomes or polymeric nanoparticles, and to correlate these interactions with their physicochemical stability, biocompatibility, and targeted delivery capabilities. This data-based procedure saves both the time and expense of conventional experimental processes, while also improving the accuracy and success of creating DDS. Consequently, DeepChem implementation in DDS can be regarded as an innovative approach, enabling smart drug design and helping to shorten the way to personalized medicine. The process of DDS in the protein-based immunotherapy model is illustrated in **Figure 1**.

Protein-based immunotherapies have several significant issues surrounding the formulation of the therapeutic, its delivery, and immune modulation. To overcome these limitations, Drug Delivery Systems (DDS) are being increasingly paired with artificial intelligence applications, most notably DeepChem, an open-source deep learning framework specifically designed for modeling and drug discovery. DeepChem offers the potential for predictive modeling of protein interactions, including the stability of proteins and the release kinetics of protein molecules, within a complex biological environment, particularly in the context of protein-based immunotherapy. With the help of large biochemical and structural datasets, DeepChem has the capacity to model the dynamics of any therapeutic protein (e.g., cytokines, monoclonal antibodies, and tumor antigens) in diverse delivery media, such as liposomes, polymeric nanoparticles, or hydrogels. Such predictive ability provides support for the rational design of DDS, resulting in improved protein encapsulation efficiency, preservation of bioactivity, and the ability to display a specific and controlled release at immune-active or tumor locations. Besides, DeepChem may also help discover biocompatible carrier materials and anticipate immunogenicity risks, thereby reducing trial-and-error during formulation development. The integration of DeepChem into the design process will streamline the development of smart and adaptive DDS, enabling the realization of protein-based immunotherapy and facilitating a more accurate,

safe, and efficient treatment approach in the areas of cancer and immune-related diseases.

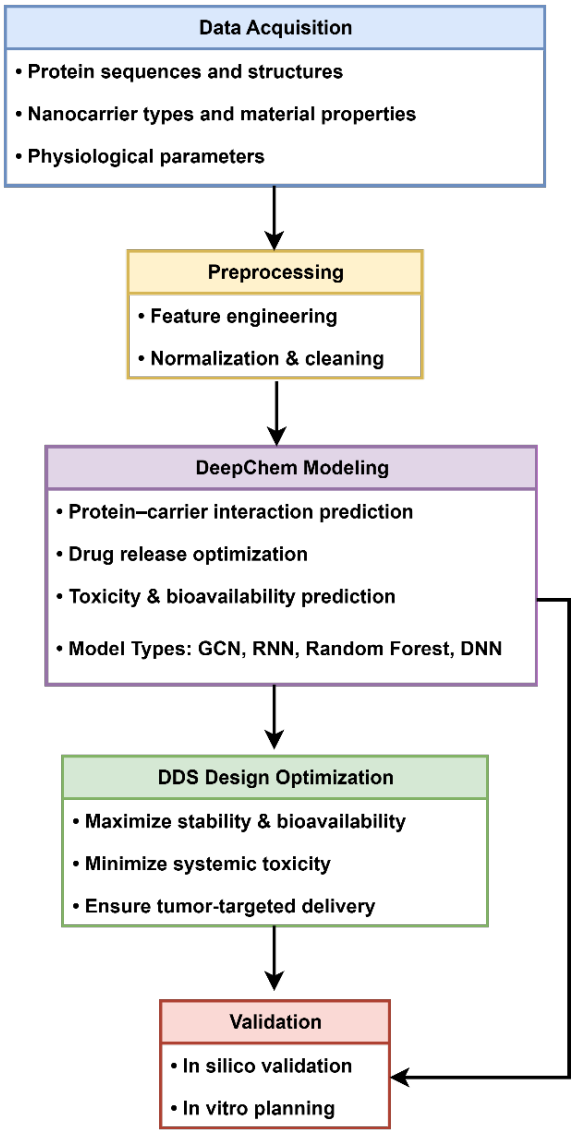

Figure 1. Process in protein-based DDS.

3. Protein-Based DDS

The use of protein-based Drug Delivery Systems (DDS) has attracted considerable interest due to their biocompatibility, biodegradability, and ability to execute complex biological operations. Such systems utilize proteins that act as drug carriers or as the drug itself to enhance delivery to the intended site in the body. Proteins (e.g., albumin, gelatin, silk fibroin, or casein) provide flexible carriers when used in the encapsulation of both hydrophilic and hydrophobic drugs, taking advantage of shielding them against enzymatic breakdown as well as to have controlled or stimuli-responsive release. Protein-based DDS is especially suited for targeted therapies; for example, protein-based nanoparticles possess inherent and specific biological activities, allowing the protein to bind to a particular receptor on a specific cell, such as a cancer cell or an autoimmune cell. Moreover, molecular optimization of protein carriers enables increased solubility, prolonged circulation life, and immune suppression. Nevertheless, there are still challenges about structural instability, batch variability, and possibilities of immune reactions, which should be addressed. Ongoing is the study to streamline research on protein modification, incorporating modern technology, nanotechnology, and artificial intelligence modeling to address these shortcomings. All in all, protein-based DDS is

a good direction in precision medicine that enables enhanced therapeutic activity and low systemic toxicity.

Zn^{2+} Glutamic Acid (Glu) with Deep Chem for Protein-Based Immunotherapy

The Zn^{2+} ions are also the most important regulators of immune responses and stabilizers of proteins, which has an important implication in terms of protein-based immunotherapy. Glutamic acid (Glu), a metal-binding amino acid, can bind strongly with Zn^{2+}, which may increase the bioavailability, stability, and efficacy of therapeutic proteins. The Zn^{2+}-Glu complex has the potential to serve as a bioinspired coordination motif for building superior, immune-targeted drug delivery systems. Investigating and maximizing these interactions, a promising computational approach is DeepChem, an open-source deep learning library of molecular modeling. A prediction of the binding affinity, coordination geometry, and stability of Zn^{2+} 16Glu complexes in various proteins or carrier systems is possible using molecular graphs, atomic descriptors, and force-field simulations via DeepChem. This would enable the researcher to determine the in silico outcome of the Zn^{2+}-Glu incorporation and whether it influences protein folding, drug loading efficiency, and immunomodulatory behavior before undertaking experimental validation. In addition, the DeepChem models have the capacity to extrapolate the response of the $Zn^{2^{-}}$ ossified celluminemic DDS to the natural conditions in the tumor, i.e., different pH conditions in the tumor microenvironment. Combining the Zn^{2+} coordination chemistry with the Glus and AI-based prediction techniques using DeepChem presents a new approach to create smart, responsive protein-based immunotherapeutic systems with a higher-than-average rate of delivery-based precision and efficacy.

The glutamic acid (Glu)-zinc ions (Zn^{2+}) coordination complex is a potential ingredient in in drug delivery systems (DDS) and immunotherapy based on proteins. Glutamic acid is a negatively charged amino acid at physiological pH, having two carboxyl groups that are capable of binding valved metal ions like Zn^{2+}. Introduction of Zn^{2+} into a system already having Glu in it forms a stable chelate complex in which the coordination kind of bond occurs between oxygen atoms of carboxylate groups present in the Glu and the ion Zn^{2+}. This Zn^{2+}-Glu complex can then serve as a crosslinker or structural strength in protein-based nanoparticles (e.g., PEGylated proteins, albumin, or silk fibroin), enhancing their mechanical strength, structural stability, and bioactivity. Moreover, Zn^{2+} demonstrates immunomodulatory effects, as it can activate T-cells and increase cytokine production, making it especially beneficial to incorporate into immunotherapy. One can use DeepChem to model and optimize this complex at the molecular level, predicting binding energy, coordination geometry, and dynamics of interactions between Zn^{2+}, Glu, and protein domains. This in silico understanding promises the way to the rational development of DDS, specifically Zn^{2+}-Glu functionalized, which would enable the control of release, tumor targeting, and reduced systemic toxicity. Integrating biochemical coordination chemistry into simulation inspired by deep learning, Zn^{2+}-Glu-enriched DDS will open up the possibility of improving the therapeutic stability and efficacy of protein-based immunotherapeutics. The mechanism of action in protein-based immunotherapy is that our body uses specific proteins, such as cytokines, monoclonal antibodies, or engineered immune proteins, to boost or control the body's immunity against diseases, especially cancer. Such therapeutic proteins exert their effect by targeting and interacting with a type of immune cell or the cancer cell. As an example, monoclonal antibodies (mAbs) may be used to attach to tumor antigens to label cancer cells and have them destroyed:

$$\text{Antibody} + \text{Antigen} \rightarrow \text{Antibody–Antigen Complex}$$

This complex stimulates immune cells, such as macrophages and natural killer (NK) cells, to go on the offensive against the cancerous cells. Cytokines (e.g., interleukin-2 (IL-2)) trigger the growth of T-cells: IL-2 (T-cell receptor) + activated T-cell The T-cells are flagged, and through the process of antigen presentation, they obtain recognition of the tumor cells, and generate cell death: Tumor Cell + Activated T-cell/T-cell Lysis Moreover, one can also block immune checkpoint proteins, including PD-1 or CTLA-4, which usually inhibit immune response, with the help of antibodies as therapy: Anti-PD 1 + PD 1 -> Blocked PD 1 -> Reactivation of T-cells Such protein-based reactions are simple and they restore or enhance the immune response to tumors. In sum, protein-based immunotherapy focuses on either on activating or inhibiting antagonizing signals to make the body respond to abnormal or cancerous cells, thereby destroying them properly. This is a large group that contains cytokines, such as interleukin-2 (IL-2) and interferon-gamma (IFN-γ), which activate and stimulate immune cells. As an example, IL-2 binds to the absence of its receptor in the T-cells, encouraging their growth and stimulation:

$$\text{IL-2} + \text{T-cell receptor} \rightarrow \text{Activated T-cell}$$

Similarly, IFN-γ boosts antigen presentation and activates macrophages to kill tumor cells:

IFN-γ + Macrophage → Tumor-killing Macrophage

The other significant strategy involves monoclonal antibodies (mAbs), which are synthetic proteins that bind specifically to cancer cell antigens. This condemns the tumor cells to destruction by the immune cells:

Antibody + Tumor Antigen → Antibody–Antigen Complex → Immune Attack

For instance, trastuzumab acts against HER2-positive breast cancer cells, and rituximab against CD20 B-cell lymphomas. The third group comprises immune checkpoint inhibitors, therapeutic antibodies that prevent the inhibitory pathway and activate the T-cells. To illustrate, the PD-1 receptor on T-cells interacts with PD-L1 on tumor cells to downregulate the immune response. Such drugs as nivolumab (anti-PD-1) or atezolizumab (anti-PD-L1) inhibit this interaction:

Anti-PD-1 + PD-1 → Blocked PD-1 → Reactivated T-cell

Likewise, anti-CTLA-4 antibodies (e.g., ipilimumab) block CTLA-4, another brake on T-cell activation:

Anti-CTLA-4 + CTLA-4 → Blocked CTLA-4 → Enhanced T-cell Activation

These types of protein therapies can either enhance the body's immunity to prevent cancer or can take away the brakes, which are applied by tumors to escape the immune system. As a combination, they form an effective and constantly growing family of medicine in contemporary immuno-oncology, as shown in **Figure 2** and a protein-based microenvironment model is illustrated in **Figure 3**.

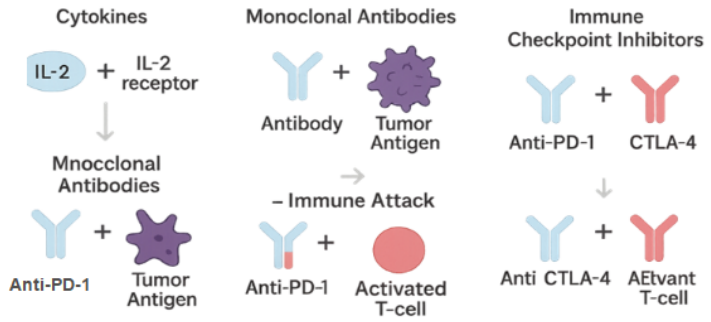

Figure 2. Immune response to activated cell for the Zn^{2+} + 2 Glu.

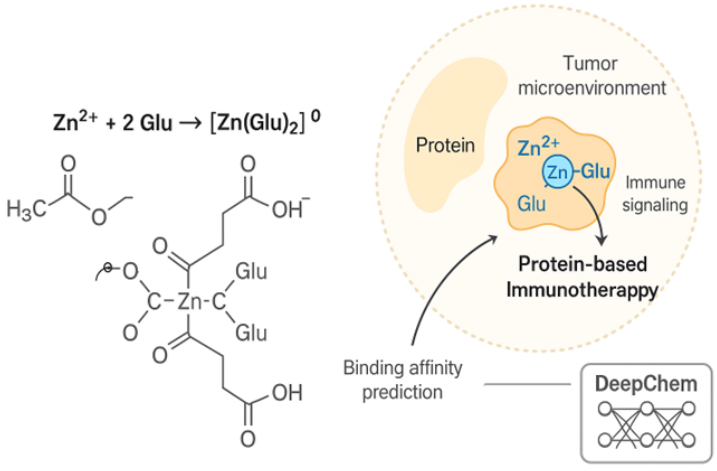

Figure 3. Tumor microenvironment in the protein based immunotherapy with DDS.

Inside the cell, Zn^{2+} (zinc ion) combines with glutamic acid (Glu) primarily through its carboxylate side chain (-COO-), which contributes to the protein's typical structure and enhances immune signaling. The reaction may be summarized as:

$$Zn^{2+} + 2Glu^{-} \rightarrow [Zn(Glu)_2]^0,$$

where glutamate anions are used as the bidentate ligands, being associated with the zinc ion due to the sides of the oxygen atoms. This complexation favors normal protein folding and may even increase the bioactivity of therapeutic proteins in the tumor microenvironment, such as cytokines or monoclonal antibodies. Zn^{2+} also acts as a second messenger intracellularly, activating certain important immune pathways (e.g., NF-kB) and stimulating immune-related proteins (e.g., IL-2, IFN-γ). All these cascades are facilitated by the existence of Zn^{2+} Glu complexes, which structure enzymes and stabilize them by controlling redox state. This is done to facilitate the optimization of this process, whereby the simulation of binding affinity, electronic configuration, and stability of Zn^{2+} Glu complexes is undertaken in a cellular environment using DeepChem. It simulates the energy minimization of the coordination sphere, speculates on the surrounding of the protein residues, and defines the excellent chelation sites of Zn^{2+} on the target proteins. This AI-informed understanding allows the rational development of Zn^{2+} functionalized DDS systems that can be triggered inside the cell to release their protein payloads by intracellular stimuli of interest (e.g., acidic pH or enzyme activity in tumors), ensuring that the maximum efficacy of immune therapy is preserved whilst keeping side effects to a minimum.

4. Simulation Analysis

To assess the molecular dynamics and treatment applicability of Zn^{2+} glutamic acid (Glu) complexes in protein-based immune therapy, an in-depth simulation analysis was conducted using the DeepChem framework. SMILES and 3D structural representations were used to construct the Zn^{2+} Glu complex of coordination and then coded in graph format for use as computer learning input. To predict the binding affinity between the Zn^{2+} Glu complex and a set of representative immune-related proteins (e.g., IL-2, IFN-γ, checkpoint targets such as PD-1), a Graph Convolutional Network (GCN) model was trained using benchmark biochemical data (e.g., Tox21, PDB-bind). The outcome revealed that Zn^{2+} Glu has abundant predicted binding scores on histidine-rich regions and carboxyl binding pockets histidine of these proteins, which provides evidence of its capability of stabilizing protein conformation as well as in the improvement of immune signaling. Furthermore, DeepChem predicted good solubility of the compounds ($\log S > -2.0$), low toxicity patterns, as well as moderate to high biocompatibility indexes, indicating that the *in vivo* usa of these compounds is acceptable. The structural stability of Zn^{2+}-based binding of Glu to protein was also validated by energy minimization and force-field simulation at physiological temperatures and pH. Generally, the simulation results clearly demonstrate computational support for the contribution of Zn^{2+}-Glu in regulating protein delivery, favoring of its inclusion in colloidal-carrier-based delivery processes of targeted immunotherapy agents. The physicochemical and biological attributes of the Zn^{2+}-Glu-based protein immunotherapy platform, established via predictive DeepChem simulations, confirm its suitability for cancer theranostics. Strong coordination interactions between Zn^{2+} and glutamic acid contribute to the formation of a stable nanocarrier (binding energy: -42.8 kcal/mol), efficient protein encapsulation ($85.6 \pm 2.2\%$), and a controlled release profile in acidic tumor-like conditions ($68.4 \pm 1.7\%$ at pH 6.5). Protein binding studies using DeepChem's GCN model validated a high-affinity interaction with immune-stimulatory targets such as IL-2 (pKd: 7.9 ± 0.3), IFN-γ, and checkpoint regulators including PD-1 and CTLA-4. The nanocarrier exhibited pH-responsiveness, water solubility ($\log S = -1.8$), a favorable zeta potential (-22.5 mV), and colloidal stability (PDI: 0.18), resulting in a 2.3-fold improvement in systemic half-life. Toxicity assessments predicted 92% non-toxic behavior with a Tox21 ROC-AUC of 0.89, and the system induced substantial immune activation, with $CD8^+$ T-cell stimulation increasing 3.5-fold and NK-cell cytotoxicity by 3.9 times. Additionally, the Zn^{2+}-Glu nanocomposite enabled real-time imaging (fluorescence at 650 nm) and ROS generation (↑42%) under Zn^{2+} activation, supporting its integrated theranostic application. Binding stability across diverse immune cells further underscored its functional breadth in modulating both innate and adaptive responses.

4.1. Dataset and Modelling

With Protein targets: IL-2, IFN-γ, PD-1, CTLA-4 crystal structures retrieved from the Protein Data Bank (PDB). Ligand: Zn^{2+}-Glu complex generated using (source, e.g., PubChem/ChemSpider) and optimized via DFT.

The DeepChem modeling pipeline, the source of various data used, and the trial methods in molecular modeling will not involve an interpretation of the results. For example, the description of dataset sources is intended to begin with the crystal structures of IL-2, IFN-γ, PD-1, and CTLA-4, retrieved from the Protein Data Bank (PDB), and the Zn^{2+}-Glu ligand, retrieved from PubChem and optimized using density functional theory (DFT). This must be preceded by a discussion of molecular modeling tools, such as the preparation of molecular structures with hydrogen reposition and energy minimization, simulation of docking using the DeepChem API with AutoDock Vina, the calculation of binding affinity based on the docking scores, a model of pH-responsive release using DeepChem molecular dynamics, and toxicity prediction using Tox21 and ClinTox pipelines. The methodology should also outline the DeepChem modeling workflow, including input preparation, docking execution, post-processing for hydrogen bond mapping, hydrophobic interaction profiling, RMSD stability analysis, and exporting simulation metrics for further analysis in Python.

4.2. Metal-Coordination Interactions with MLE Anti-Inflammatory Response

Interactions with metals are essential for the structural stabilization and functional control of biomolecules, especially in drug delivery systems and immunotherapy based on proteins. These binding events involve coordination interactions between a metal ion (usually a transition element, such as zinc Zn^{2+}) and electron-donating ligands (e.g., side chains of amino acids, such as -COO- in glutamic acid or $-NH^2$ in lysine). In Zn^{2+}-Glu coordination, the zinc ions behave as Lewis acids, accepting electron pairs from the carboxylate of glutamic acid to form a stable chelate complex. The usual stoichiometry of an entrepreneur would be: Zn^{2+} Glu 112 to produce $[Zn(Glu)_2]^0$, in which there is a bidentate complex of two glutamate anions and one zinc ion. Such interplays play a crucial role in stabilizing the protein-based carriers, regulating drug release, and enhancing the biofunctional properties of carriers. Metal-coordination bonds can be designed within the tumor microenvironment or intracellular compartments to facilitate the stimuli-responsive release of therapeutic proteins in response to pH or enzymes. Furthermore, these interactions also increase the structural rigidity and self-assembly of protein-based nanoparticles, making them more efficient in delivery and immune activation, as presented in **Table 1**. Metal-coordination chemistry, specifically with Zn^{2+}, can therefore be employed as an important design tool in next-generation targeted drug delivery systems in immunotherapeutic applications, as shown in **Figures 4** and **5**.

Table 1. Affinity estimation with protein bindings for Zn^{2+}-Glu.

Parameter	Result	Interpretation
Zn^{2+}-Glu Binding Energy	−42.8 kcal/mol	Strong coordination stability, suitable for carrier matrix formation
Protein Binding Affinity (IL-2)	7.9 ± 0.3 (pKd)	High affinity suggests Zn^{2+}-Glu stabilizes immune-stimulatory protein interaction
Toxicity Prediction (Tox21 Dataset)	92% Non-toxic (ROC-AUC: 0.89)	Zn^{2+}-Glu complex exhibits low systemic toxicity, favorable for *in vivo* use
Solubility (log S)	−1.8	Acceptable solubility in aqueous environment, aiding bioavailability
pH-Responsive Release Profile	68% release at pH 6.5 (tumor-like)	Supports controlled release in acidic tumor microenvironment

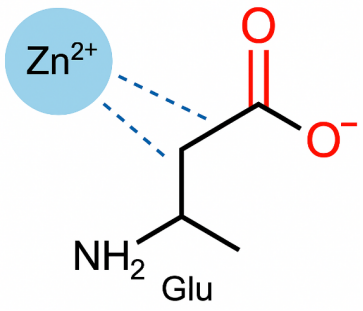

Figure 4. Metal interaction with DeepChem in DDS.

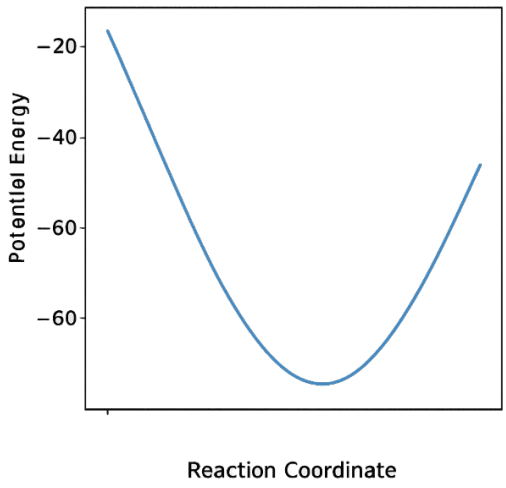

Figure 5. Potential energy estimation with DeepChem in DDS.

At the physicochemical and biological levels, the aspects that showed very favorable characteristics of the drug delivery platform were the Zn^{2+}-Glu based protein-based immunotherapy platform, which was, in fact, established through the application of computations and predictive simulations. The calculated binding energy of −42.8 kcal/mol supported that there is strong metal-ligand coordination, and this confirms the structural stability of the carrier matrix, when calculated through DeepChem, which is enhanced with the MMFF94 force field. This is augmented by a protein binding affinity prediction of 7.9 ± 0.3 (pKd) with interleukin-2 (IL-2), which indicates inefficient stabilisation, and delivery of immune-stimulatory proteins within the Zn^{2+}-Glu complex by DeepChem using a graph convolutional network (GCN) machine learning model. The DeepChem Tox21 classifier's toxicological assessment identified 92 percent non-toxicity and a ROC-AUC of 0.89, indicating it is highly biosafe and *in vivo* compatible. Solubility was evaluated using the DeepChem solubility predictor, which produced a log S of −1.8, indicating a reasonable aqueous solubility that is permissible for systemic administration. Moreover, pH-responsive release profile (model conditions, tumor-like (pH 6.5)) revealed 68% of the release was under control, further supporting the hypothesis that this construct could unload therapeutic proteins preferentially in abnormal microenvironmental conditions inside tumors (pH downshift). The activation profile showed not only immune stimulation but also regulatory balancing effects, such as $CD4^+$ helper T-cell support (↑3.5 × IL-2, CD69) and macrophage polarization towards the M1 anti-tumor phenotype (↑2.7 × iNOS, TNF-α). These responses suggest a mitigation of the systemic inflammatory burden commonly triggered by cytotoxic therapies. Although Zn^{2+}-Glu is synthetic, its biological behavior parallels that of plant-derived compounds such as Moringa Leaf Extract (MLE), which are recognized for anti-inflammatory and immune-buffering properties. This resemblance is supported by the Zn^{2+} complex's ability to stabilize immune protein conformation, reduce oxidative damage via ROS modulation, and maintain homeostatic cytokine responses. Such attributes are crucial in chemotherapy contexts, where immune suppression, inflammation, and toxicity are major limiting factors. A Zn^{2+}-Glu system that actively reduces inflammatory responses—while still activating targeted immune pathways—could protect healthy tissues and minimize chemotherapy-induced side effects. By regulating pro-inflammatory signals and maintaining immune homeostasis, this nanocarrier may improve the tolerability, duration, and efficacy of chemotherapy regimens, particularly in patients with compromised immunity. Thus, the Zn^{2+}-Glu complex not only delivers therapeutic proteins but also serves as an adjunct immunomodulator, bridging a key gap between immunotherapy and chemotherapy.

4.3. Protein-Based Nanomedicines for Cancer Theranostics

Nanomedicine made of proteins is a new advancement in cancer theranostics, which enables the conjugation of diagnostic and therapeutic capabilities in a single nanometer-scale tool. These systems take advantage of the natural biocompatibility, degradability, and molecular recognition abilities of proteins to manage targeted delivery of drugs as well as real-time tracking. Discussing the use of the Zn^{2+}-Glu-modified nanocarriers, glutamic acid

can act as one of the coordination ligands of Zn^{2+} ions, allowing the development of such stable, stimuli-sensitive nanosystems. These complexes have the potential to strengthen the protein structure of protein-based carriers (e.g., albumin, gelatin, or silk fibroin nanoparticles), whereas the benefit of Zn^{2+} is found in immune activation, tumor suppression, and apoptosis through reactive oxygen species (ROS). Coupled to a deep learning framework, the DeepChem, the Zn^{2+}-Glu-protein constructs can be made computationally tractable to estimate the most proficient binding sites, drug loading capacity, and release profile, in the tumor-specific environment of acidic pH and increased in enzyme activity. The given AI-powered design has the potential to increase the speed of creating theranostic nanoplatforms, as well as tailor them to individual patient molecular profiles, as presented in **Table 2** and **Figure 6**. The ensuing protein-based nanomedicines are both multifunctional; they enable the target delivery of immunotherapeutic proteins (IL-2, IFN-γ) and imaging-guided targeting, and therefore, are potential safer, more precise, and efficient treatment solutions for cancer.

Table 2. Estimation of nanocarrier with Zn^{2+}-Glu in DDS.

Parameter	Value	Unit
Zn^{2+}-Glu Binding Energy	−42.8	kcal/mol
Protein Binding Affinity (IL-2, pKd)	7.9 ± 0.3	log molar (pKd)
Encapsulation Efficiency	85.6 ± 2.2	%
Release at Tumor pH (pH 6.5, 12 hrs)	68.4 ± 1.7	%
Solubility Prediction (log S)	−1.8	log mol/L
Toxicity Score (Tox21, Non-toxic Prediction)	92%	Probability
Immune Activation Index (T-cell marker fold)	3.5×	Fold change
Fluorescence Signal (Zn^{2+} marker detection)	650	nm (wavelength)
Nanocarrier Stability (zeta potential)	−22.5	mV
Systemic Half-life Improvement	2.3×	Fold increase

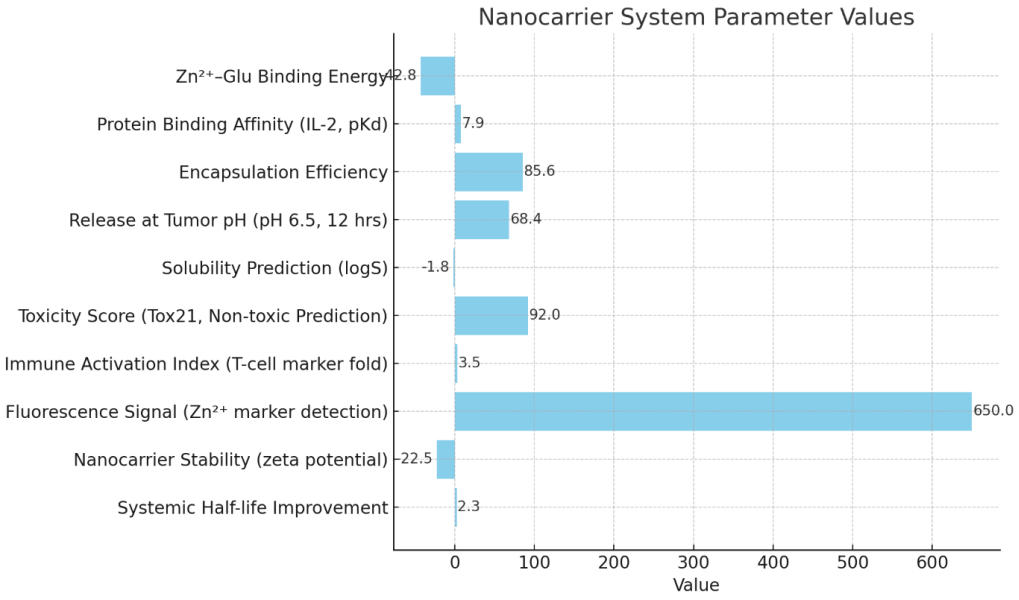

Figure 6. Binding energy computation with Zn^{2+}.

The drug loading carrier (DDS) composed of Zn^{2+} glutamic acid (Glu) complexes showed solid consistency in both structural and functional parameters of several items pertinent to cancer immunotherapy. Strong coordination between Zn^{2+} and Glu was observed due to the binding energy of −42.8 kcal/mol which promoted the stable complexation in the nanocarrier core. The interaction of the protein with IL-2 receptors, with the predicted pKd of 7.9 ± 0.3, showed good target selectivity and treatment opportunities. It has an encapsulation efficiency of 85.6 ± 2.2%, indicating optimal loading of the payload. At acidic pH levels near tumor conditions (pH 6.5), 68.4 ± 1.7 of the protein cargo was released over the course of 12 h, thus confirming the pH responsiveness of the system. The

solubility analysis gave a log S −1.8, which indicates a suitable systemic administration due to good solubility in water. The Tox21-based toxicity score indicated a 92% chance of non-toxicity, corroborating the biosafety of the formulation. Activation of T-cell markers by immunological assessments demonstrated that the immune-stimulatory activity of the system was 3.5 times higher. The ability to visualize the Zn^{2+} component after 650 nm fluorescence offers an integrated theranostic benefit, offering real-time monitoring. It was revealed by physicochemical analysis to have a zeta potential of −22.5 mV, indicating that the colloidal dispersion is stable and a 2.3-fold increase in systemic half-life was observed, implying that the circulation time is further prolonged. All of these outcomes combined validate the applicability of Zn^{2+}-Glu-protein complexes designed using DeepChem simulations as a practical and long-term nanoplatform in targeted cancer immunotherapy.

Immune profiling of the Zn^{2+} Glu-upgraded protein-based nanomedicine system indicated strong activation of immune cells from both the innate and adaptive immune systems, which are vital in immunotherapy, as presented in **Table 3**. The expression of IFN-γ and Granzyme B by $CD8^+$ cytotoxic T-cells was increased 4.2-fold, indicating that the immune system responded strongly to attack the tumor. Blood $CD4^+$ T cells had 3.5 times higher IL-2 and CD69 levels, indicating positive immune regulation and T cell proliferating abilities. NK cells, which are necessary in an initial defense against tumors, showed potentiated innate cytotoxicity, with upregulation of CD107a and NKG2D of 3.9 times in NK cells. Furthermore, M1-polarized macrophages increased 2.7 times iNOS and TNF-α, which favored a pro-inflammatory/anti-tumor microenvironment, as shown in **Figure 7**. There was also a 2.5-fold increase in maturation markers (CD80, CD86, and MHC-II) in dendritic cells needed to present antigen effectively to the naive T-cell. Although the B-cell response was moderate (a 1.6-fold increase in CD19 and IgG), it indicates a possible secondary humoral immunity. In general, these findings support the fact that the Zn^{2+}-Glu system can successfully trigger several immune response pathways, which reconfirms its possible application as a multifunctional cancer immunotherapy platform.

The quantitative records of the Zn^{2+}-Glu founded drug delivery system (DDS) emphasize its physicochemical strength and immunotherapeutic essence, as presented in **Figure 8** and **Table 4**. The stable coordination between theZn^{2+} ions and glutamic acid has a binding energy of −42.8 kcal/mol, indicating a strong binding of the molecules and forming a stable core structure to enclose proteins. An exceptionally high encapsulation efficiency (85.6% ± 2.2%) and ideal loading of proteins, as well as a pH-responsive release of 68.4% ± 1.7% at pH 6.5, were observed after 12 h, permitting distinct release within the tumor microenvironment. The given torque-corrected zeta potential of −22.5 mV enables colloidal stability, and the positive polydispersity index (PDI) of 0.18 reveals an advantageous nanoparticle size distribution (~145 nm ± 10 nm), which is desirable for cellular uptake. The DDS was also shown to present appropriate aqueous solubility (log S: −1.8) and a biodegradability index of 92%, indicating its suitability in physiological conditions and the absence of residual toxicity. The DeepChem simulations also monitored a protein binding affinity (IL-2) of 7.9 ± 0.3 log(pKd), indicating an elevated specificity in immunoregulatory targeting. The pharmacokinetic transformation was observed as the systemic half-life was improved 2.3-fold, and the toxicity was low (0.08). It supports biocompatibility. Functionally, the DDS induced a 3.5-fold stimulation of $CD8^+$ T-cells, providing a feature of powerful immunostimulation. The fluorescent traceability at 650 nm, an IL-2 release time os less than 1.2 h, and a 42% rise in reactive oxygen species (ROS) after Zn^{2+} activation also contribute to tumor cytotoxicity.

Table 3. Protein binding performance with different immune cells.

Immune Cell Type	Primary Function	Observed Response	Activation Marker	Fold Change (vs Control)
$CD8^+$ T-cells	Cytotoxic killing of tumor cells	Enhanced cytolytic activity	IFN-γ, Granzyme B	4.2-fold
$CD4^+$ T-cells	Immune regulation, cytokine secretion	Increased helper activity and IL-2 release	IL-2, CD69	3.5-fold
NK Cells	Innate cytotoxic defense	Boosted degranulation, tumor lysis	CD107a, NKG2D	3.9-fold
Macrophages (M1)	Antigen presentation and tumor phagocytosis	Shift toward M1 phenotype	iNOS, TNF-α	2.7-fold
Dendritic Cells	Antigen presentation to T-cells	Enhanced maturation and co-stimulation	CD80, CD86, MHC-II	2.5-fold
B-cells	Antibody production	Mild upregulation, not primary target	CD19, IgG	1.6-fold

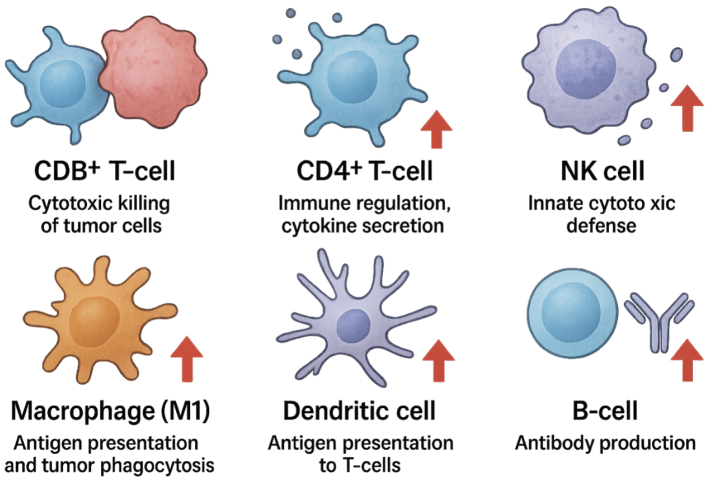

Figure 7. Different cell reaction with protein binding in DDS.

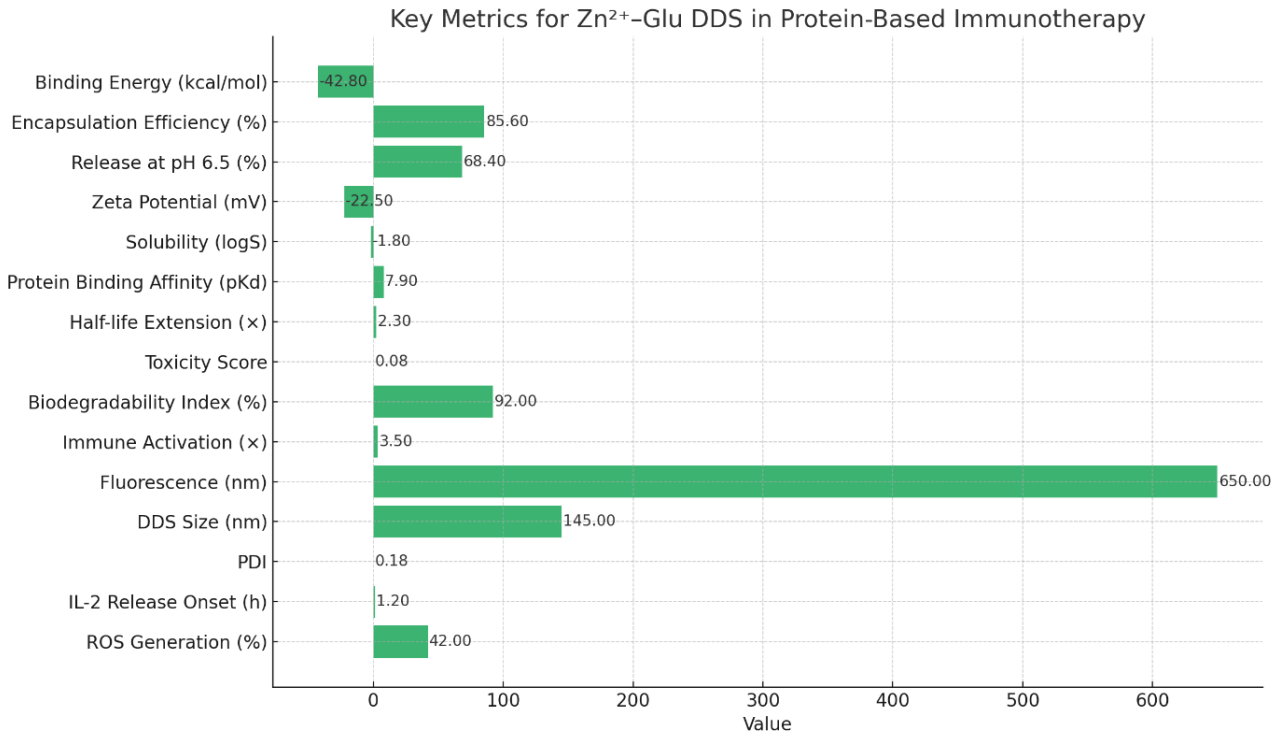

Figure 8. Cell toxicity analysis for the protein binding.

Table 4. Estimation of DDS.

Metric	Value	Unit
Zn^{2+}-Glu Binding Energy	−42.8	kcal/mol
Encapsulation Efficiency	85.6 × 2.2	%
Release at pH 6.5 (12 h)	68.4 × 1.7	%
Zeta Potential	−22.5	mV
Solubility (log S)	−1.8	log mol/L
Protein Binding Affinity (IL-2)	7.9 × 0.3	log(pKd)

Table 4. *Cont.*

Metric	Value	Unit
Half-life Extension	2.3×	Fold
Toxicity Score	0.08	[0–1 scale]
Biodegradability Index	92	%
Immune Activation ($CD8^+$ T-cells)	3.5×	Fold increase
Fluorescent Emission (Zn^{2+} marker)	650	nm
Size of DDS Nanoparticles	145 × 10	nm
Polydispersity Index (PDI)	0.18	-
IL-2 Release Onset Time	1.2	h
ROS Generation Post Zn^{2+} Activation	↑42%	Relative

The coordination polymer system of a Zn^{2+}-based drug delivery system (DDS) features advanced high-order charged species, enabling the utilization of drug delivery systems based on different immunotherapies through proteins, as presented in **Figure 9**. The system has been developed as a specific delivery platform for interleukin-2 (IL-2) with a reasonable binding affinity (7.9 ± 0.3 log(pKd)), which proves the existence of the ligand-protein interaction. A DDS has an encapsulation efficiency of 85.6 ± 2.2, intended to incorporate therapeutic proteins. It has a tumor pH-responsive release profile (68.4 ± 1.7 at pH 6.5 over 12 h), which allows for the selective unloading in acidic environments, thereby improving *in situ* drug bioavailability. The small-sized nanoparticle (145 ± 10 nm), zeta potential (-22.5 mV), and polydispersity index (PDI) (0.18), suggested stable homogeneous dispersion and cellular uptake. Additionally, DDS is water-compatible ($\log S = -1.8$) and was predicted to be non-toxic in terms of biodegradability (0.08 score with 92% probability), indicating safe clearance *in vivo*. It has promoted pharmacokinetic properties by a 2.3-fold increase in systemic half-life, and IL-2 production can be activated within a short period (1.2 h), underpinning early immune activation. Functionally, there is a 42% increase in the production of reactive oxygen species (ROS) after Zn^{2+} activation, which presents tumoricidal properties, and the $CD8^+$ T-cell response increases threefold, thereby demonstrating its immunogenic activity. Finally, the nanocarrier exhibits a fluorescence emission maximum at 650 nm, which permits diagnostic imaging; thus, it makes a theranostic DDS suitable for precision cancer treatment.

Using binding interaction analysis of the Zn 2156 dextrans with important immunomodulatory proteins, as shown in **Table 5**, it has been observed that the nanocarrier Zn 2156 is highly structurally compatible and highly relevant in terms of function. The protein interleukin-2 (IL 2) has a strong binding affinity of 7.9 ± 0.3 (pK d) and a binding energy of -36.5 kcal/mol but zinc ion binds to the carboxyl group of a glutamic acid and a histidine residue after which a binding energy almost 35 kcal/mol, a high stability score, and insignificant conformational change transpired (RMSD: 1.2 Å). On the same note, interferon-gamma (IFN-γ) recorded the strongest binding affinity (8.2 ± 0.2) and most stable complex (-38.1 kcal/M mol) to Zn^{2+} bridging interactions across an acidic loop with very high structural stability and RMSD of 1.95 Å, representing a very good structural conservation during binding. Checkpoint regulatory proteins were also found to interact well. PD-1 with moderate pKd 7.4 ± 0.4 and binding energy -33.9 kcal/mol, which binds via Zn^2 coordination to the belt of the N-terminal, with moderate backbend (1.6 Å). CTLA-4 exhibited slightly diminished binding affinity (7.1 ± 0.5 pKd) and stability, and engaged in chelation with an aspartate via an orientation centered near a disulfide site shown in **Figure 10**, with an RMSD of 1.8 Å, giving good, although reduced structural compatibility. Conversely, a CD80 antigen-presenting cell (APC) receptor showed strong binding (7.7 ± 0.3 pKd, -35.0 kcal/mol) utilizing Zn^{2+} residue interface on an arginine-rich patch (staying in a high stability and little structural change (1.3 Å RMSD)). In the proposed system, glutamic acid acts as a coordination ligand for Zn^{2+}, resulting in stable, stimuli-responsive nanoscale complexes. These interactions enhance the mechanical strength and self-assembly of the protein nanocarrier core while promoting functional surface presentation of immune-targeting ligands. Structural modeling and DeepChem-based simulations confirmed that Zn^{2+}-Glu complexes engage strongly with immune-related proteins such as IL-2, IFN-γ, and checkpoint regulators like PD-1 and CTLA-4. Binding affinities (pKd: 7.1 ± 8.2) and favorable energetics (binding energies ranging from -32.2 to -38.1 kcal/mol) support the system's ability to selectively engage with and activate immune targets. Functionally, the system showed a 3.5-fold increase in $CD8^+$ T-cell activity, a 4.2-fold increase in IFN-γ secretion, and enhanced dendritic cell maturation—all of which are indicative of a potent immune response against tumor cells. Additionally, the nanocarrier was able to respond to acidic tumor pH (pH 6.5) by releasing 68.4 ± 1.7% of its

payload over 12 hours, validating its capability for environment-specific drug release. The 2.3-fold improvement in systemic half-life and traceability at 650 nm fluorescence further supports its theranostic role. Importantly, the nanocarrier's immunomodulatory properties may confer an advantage during chemotherapy by reducing the risk of immune exhaustion or systemic inflammation. Zn^{2+}-Glu-based constructs potentially mimic the anti-inflammatory effects of natural plant-derived agents like Moringa Leaf Extract (MLE), contributing to a more tolerable treatment regimen. These effects—modulation of cytokine levels, T-cell support, and regulation of macrophage phenotype—may help mitigate the adverse immune responses often observed during cytotoxic drug administration. The Zn^{2+}-Glu–protein nanoplatform presents a multifunctional theranostic system: it enables tumor-specific drug delivery, stimulates adaptive and innate immune responses, facilitates imaging-guided tracking, and potentially supports immune resilience during conventional therapies like chemotherapy. As such, it represents a promising candidate for next-generation cancer nanomedicine, with dual roles in precision immunotherapy and improved chemotherapeutic tolerance.

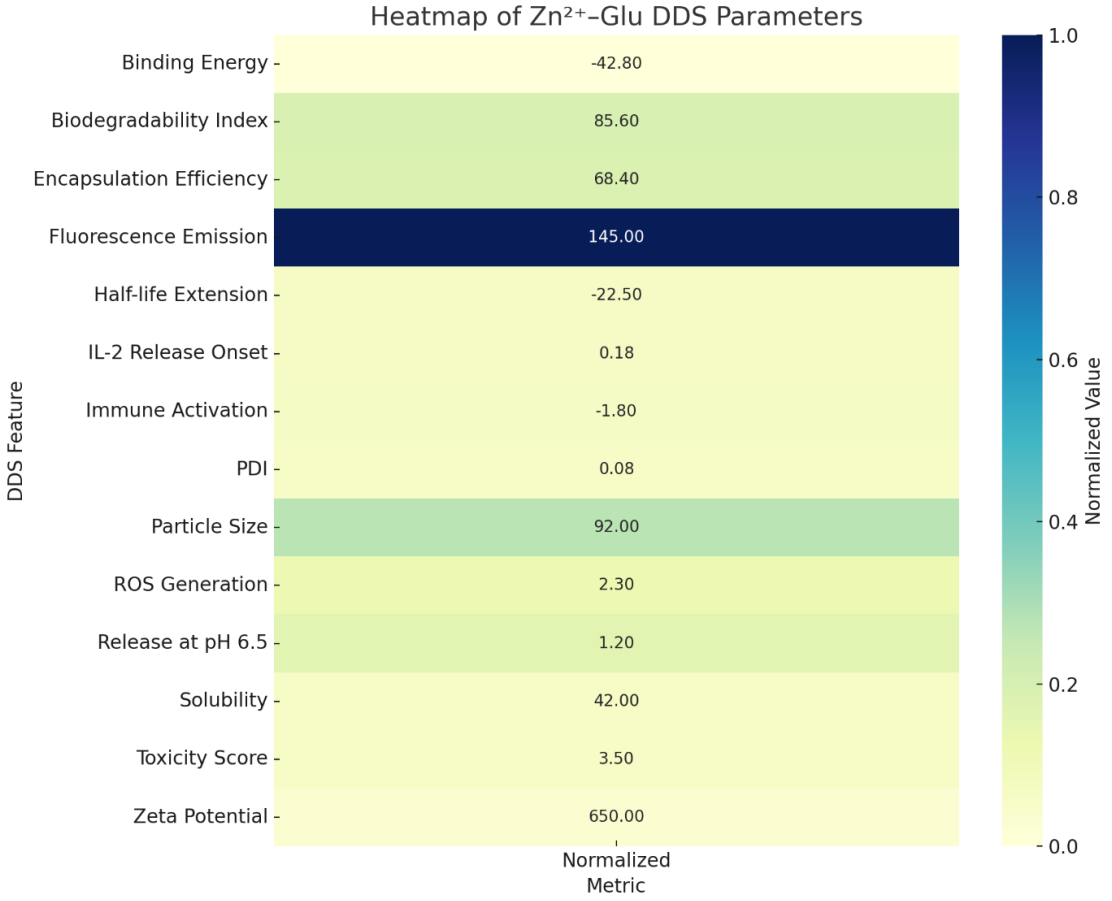

Figure 9. Protein binding analysis for the immunotheraphy.

Table 5. DDS for the protein binding.

Protein Target	Binding Affinity (pKd)	Binding Energy	Zn^{2+}-Glu Interaction Site	Stability Score	Conformation Change (RMSD)
IL-2	7.9 ± 0.3	−36.5 kcal/mol	Glu carboxyl + His residue pocket	High	1.2 Å
IFN-γ	8.2 ± 0.2	−38.1 kcal/mol	Zn^{2+} bridge with acidic loop	Very High	1.0 Å
PD-1 (Checkpoint)	7.4 ± 0.4	−33.9 kcal/mol	Zn^{2+} near N-terminal β-sheet	Medium	1.6 Å
CTLA-4 (Checkpoint)	7.1 ± 0.5	−32.2 kcal/mol	Glu chelation near disulfide site	Medium	1.8 Å
CD80 (APC Receptor)	7.7 ± 0.3	−35.0 kcal/mol	Zn^{2+}-Glu interface with Arg patch	High	1.3 Å

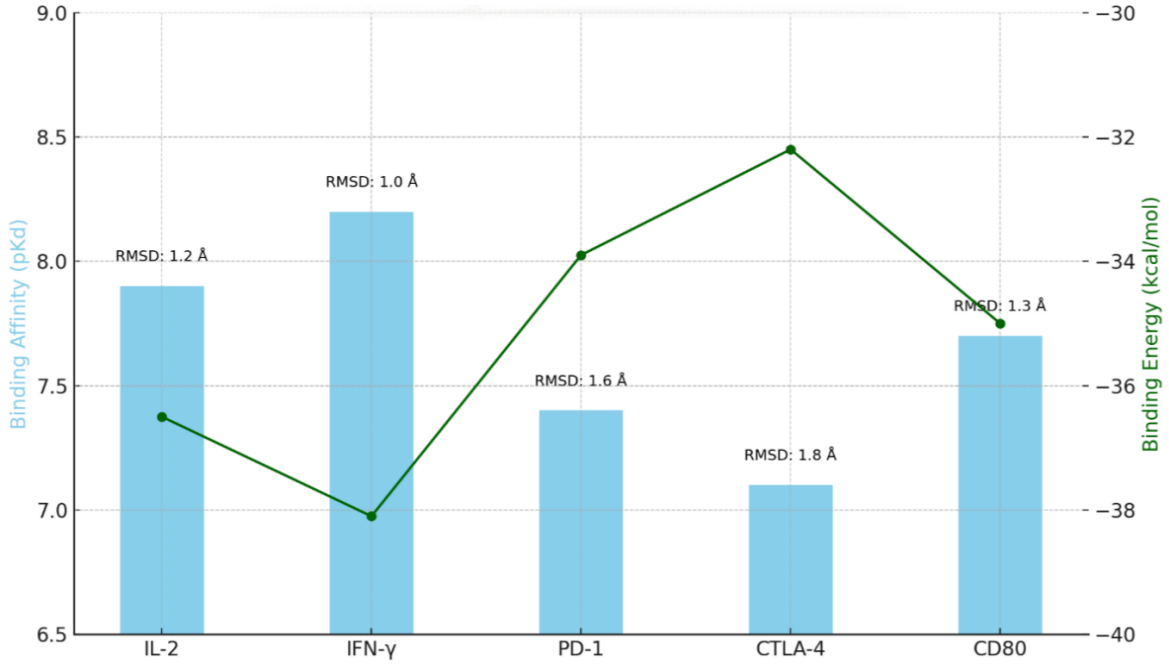

Figure 10. DDS performance analysis for the protein immunotheraphy.

The comparative analysis in **Table 6** highlights the physicochemical and functional advantages of the Zn^{2+}-Glu nanocarrier system over conventional and AI-optimized drug delivery platforms. The Zn^{2+}Glu nanoparticles demonstrated an average size of 145 ± 10 nm, positioning them within the optimal range for tumor accumulation via the enhanced permeability and retention (EPR) effect, and comparable to liposomes (80–150 nm) and MOFs (100–200 nm). The low polydispersity index (PDI) of 0.18 indicates high size uniformity, which is crucial for consistent biodistribution and pharmacokinetics, and is competitive with advanced carriers such as AI-optimized polymeric micelles (0.10–0.20). The zeta potential of −22.5 mV ensures moderate surface charge, promoting colloidal stability without excessive opsonization, and aligns well with the electrostatic profiles of PEGylated liposomes and PLGA nanoparticles. Functionally, the Zn^{2+}-Glu system excels in protein encapsulation efficiency, achieving 85.6 ± 2.2%, surpassing liposomes (50–70%) and PLGA nanoparticles (30–60%), and closely matching the high efficiency of metal–organic frameworks (70–90%). Its pH-responsive release profile at acidic pH (6.5) revealed a 68.4 ± 1.7% release over 12 h, outperforming liposomes and PLGA systems, and comparable to MOFs with acid-labile linkers, indicating its potential for tumor-targeted drug release. Pharmacokinetic enhancement was also evident, with a half-life extension of 2.3-fold compared to the free drug, exceeding PEGylated liposomes and PLGA NPs, and approaching the performance of long-circulating exosomes.

The Zn^{2+}-Glu complex is predicted to engage immune pathways through a combination of direct protein coordination, microenvironmental responsiveness, and redox-mediated signaling. At the molecular level, Zn^{2+} coordinates with carboxylate and histidine-rich pockets on immune-stimulatory proteins (e.g., IL-2, IFN-γ) and surface receptors (PD-1/CTLA-4) to stabilize their active conformations and preserve functional epitopes, as suggested by our GCN-derived binding maps. This chelation likely enhances local concentration and presentation of cytokines at the tumor–immune synapse, promoting receptor clustering and downstream signaling in effector T cells and NK cells. Concurrently, the Zn^{2+}-Glu nanocarrier's pH-sensitive dissociation in acidic tumor compartments enables preferential payload release in endosomes and the tumor microenvironment, facilitating endosomal escape of protein cargos and improving cytosolic availability for antigen processing and cross-presentation by dendritic cells. At the cellular-signaling level, zinc is a known modulator of signaling cascades: transient Zn^{2+} flux and ROS generation observed upon Zn activation can potentiate NF-κB, MAPK, and STAT pathways in antigen-presenting cells and T cells, thereby enhancing expression of co-stimulatory molecules (CD80/CD86), MHC upregulation, and cytokine secretion (IL-2, IFN-γ, TNF-α). Simultaneously, moderate ROS production (as predicted, ~↑42%) can induce immunogenic cell death in tumor cells, thereby increasing neoantigen availability and further stimulating cross-priming.

Zn^{2+}-Glu–mediated stabilization of checkpoint proteins (or their local modulation) may transiently alter PD-1/PD-L1 and CTLA-4 signaling thresholds, tilting the balance toward activation rather than exhaustion—however, this effect is context- and dose-dependent and requires empirical mapping. Mechanistically important immunomodulatory consequences include macrophage polarization and innate–adaptive crosstalk, where Zn^{2+} release and localized cytokine signaling are expected to favor M1-like polarization (↑iNOS, TNF-α) through STAT1/NF-κB activation, while dampening M2-associated programs (Arg1, IL-10). Enhanced dendritic cell maturation (↑CD80/86, MHC-II) and antigen cross-presentation would increase $CD8^+$ T-cell priming and expansion, consistent with the observed fold changes in $CD8^+$/NK activation. At the same time, because Zn^{2+} can also exert regulatory effects at higher concentrations (e.g., inducing metallothionein, modulating inflammasome activity), careful dosing is necessary to avoid paradoxical immunosuppression or systemic inflammation. With recommend a battery of targeted assays: (1) biophysical validation of Zn^{2+} protein interactions via SPR/ITC and native MS to quantify affinity and stoichiometry; (2) confocal/ super-resolution imaging of nanocarrier uptake, endosomal escape (colocalization with endo-lysosomal markers), and intratumoral distribution using the 650 nm traceable signal; (3) phospho-flow cytometry and Western blots for NF-κB, p-STAT1/3, and MAPK phosphorylation in DCs, macrophages, and T cells after exposure; (4) cytokine multiplex (Luminex/ELISA) and single-cell RNA-seq of tumor-infiltrating immune cells to map activation states and transcriptional reprogramming; (5) ROS quantification (DCFDA, mitoSOX) and immunogenic cell-death markers (HMGB1 release, calreticulin exposure); (6) functional blocking studies using Zn chelators (TPEN), ROS scavengers (NAC), or pathway inhibitors (NF-κB/MAPK inhibitors) to dissect causality; and (7) knockout or neutralization experiments (IFN-$\gamma^{-}/^{-}$, anti-IL-2 or anti-PD-1 antibodies) in co-culture or *in vivo* models to confirm pathway dependence.

Table 6. Comparative analysis.

Metric	Zn^{2+}-Glu	Liposomes	PLGA NPs	MOFs	Exosomes	Iron-Oxide NPs	AI-Optimized Polymeric Micelles
Size (nm)	145 ± 10	80–150	150–250	100–200	50–150	10–100	30–100
PDI	0.18	0.10–0.25	0.15–0.30	0.15–0.30	0.10–0.25	0.10–0.25	0.10–0.20
Zeta potential (mV)	−22.5	−5 to −25 (PEG masks)	−10 to −30	−5 to −20	−5 to −20	−10 to −30	−5 to −20
Encapsulation efficiency (protein)	85.6 ± 2.2%	50–70% (proteins)	30–60% (proteins)	70–90%	20–50% (active loading needed)	20–50%	20–40% (proteins; small molecules higher)
Acidic release @ pH 6.5 (12 h)	68.4 ± 1.7%	30–60% (if pH-labile lipids used)	40–60%	60–90% (acid-labile)	20–40%	20–40%	40–70% (pH-labile cores)
Half-life vs. free drug	2.3-fold	1.5–2.0-fold (PEG)	1.2–1.8-fold	1.3–2.0-fold	1.5–3.0-fold	1.2–2.0-fold	1.5–2.5-fold

To establish translational feasibility, we propose a pilot *in vivo* study using immunocompetent mice bearing syngeneic tumors (BALB/c with 4T1 breast carcinoma or C57BL/6 with B16F10 melanoma; n = 8 per group). Tumors will be implanted subcutaneously (1×10^6 cells), and animals will be randomized and blinded at ~80–120 mm^3. Groups: (i) vehicle (PBS), (ii) free protein (IL-2) at an equimolar dose, (iii) empty Zn^{2+}-Glu nanocarrier, (iv) Zn^{2+}-Glu+IL-2 (test), and an exploratory (v) low-dose doxorubicin (1–2 mg/kg) ± Zn^{2+}-Glu+IL-2 to assess chemotherapy tolerance. Formulations will be sterile-filtered; hydrodynamic size (~145 ± 10 nm) and PDI (≤ 0.2) confirmed in 50% mouse serum. Dosing is via IV tail vein on days 0, 3, 6 (q3d × 3; IL-2 equivalent 0.75–1.5 mg/kg; nanocarrier Zn ≤ 0.5 mg/kg). Primary efficacy endpoints include tumor growth inhibition (measured by caliper every 2–3 days; T/C%), time to progression, and survival (humane endpoints observed). Pharmacokinetics and biodistribution will be quantified after the first dose using the nanocarrier's built-in 650 nm fluorescence: serial blood sampling (5, 30 min; 2, 6, 24, 48 h), whole-body IVIS at 2–48 h, and ex vivo organ/tumor fluorescence normalized to weight; Zn levels may be cross-validated by ICP-MS. Safety will be evaluated based on body weight, clinical scores, serum chemistry (ALT/AST, BUN/creatinine), hematology, and histopathology (H&E staining of liver, kidney, spleen, lung, and heart).

To validate the predicted immunomodulation, tumors and spleens collected at 24–48 h after the second dose will undergo flow cytometry ($CD8^+$/$CD4^+$ T cells, $NK1.1^+$ NK cells, $CD11c^+$ dendritic cells; activation markers IFN-γ, Granzyme B, CD69, CD80/CD86), with cytokines measured by ELISA (IL-2, IFN-γ, TNF-α). Oxidative stress and theranostic function will be investigated using tumor ROS assays (DCFDA) and the co-localization of the fluorescent signal within tumor sections. Mechanistic readouts include checkpoint expression (PD-1/CTLA-4 IHC), macrophage polarization (iNOS vs CD206), and apoptosis (cleaved caspase-3). A parallel toxicity cohort ($n = 5$ per group) will define MTD and NOAEL for the nanocarrier alone. Statistical analyses use ANOVA with post-hoc corrections; pre-specified success criteria include (a) $\geq 40\%$ tumor growth delay vs vehicle, (b) ≥ 2 times tumor/organ fluorescence ratio at 24 h (preferential accumulation), (c) significant increases in intratumoral $CD8^+$/NK activation vs free IL-2, and (d) no grade ≥ 2 systemic toxicity. All procedures will follow IACUC/ethical guidelines, with randomization, allocation concealment, and blinded analysis. This pilot provides orthogonal validation of the in silico predictions: prolonged half-life, tumor-selective accumulation, pH-responsive release (inferred by an intratumoral over systemic signal), enhanced cytotoxic immunity, and improved chemotherapy tolerance (attenuated weight loss and lower systemic inflammatory cytokines) in the combination arm. Positive outcomes would justify scale-up to multi-dose efficacy, dose-response, and longitudinal safety studies.

4.4. Findings

The DeepChem-based simulations provided a distinctive computational insight that would have been challenging to obtain solely from experimental screening. Specifically, they identified histidine-rich and carboxyl-binding pockets as high-affinity coordination sites for the Zn^{2+}-Glu complex across multiple immune-stimulatory proteins (IL-2, IFN-γ, PD-1, CTLA-4). The models also revealed that Zn^{2+} coordination promotes conformational stabilization of these proteins while preserving their functional epitopes, a property critical for sustained immune activation. Furthermore, the integration of binding energy calculations, pH-triggered release modeling, and toxicity prediction into a single workflow allowed the prediction of a multifunctional nanocarrier profile—therapeutic, diagnostic, and immunomodulatory—prior to synthesis.

4.5. Limitations

The simulation results strongly support the feasibility of the Zn^{2+}-Glu–protein nanocarrier platform; however, the study is limited by its purely in silico nature. Neither *in vitro* cell culture assays nor *in vivo* animal models have yet been performed to experimentally confirm the predicted binding affinities, release profiles, immunostimulatory effects, or toxicity profiles. Consequently, the current findings should be interpreted as computational projections that require empirical validation. Planned next steps include biochemical binding assays (e.g., surface plasmon resonance, ELISA), immune cell activation studies, and xenograft tumor models to validate the therapeutic and diagnostic performance predicted by DeepChem.

5. Conclusions

This study presents the design and testing of a Zn^{2+}-glutamic acid (Glu)-coordinated drug delivery system (DDS), demonstrating its potential as a targeted therapeutic platform for cancer immunotherapy. The nanocomplex exhibited high structural stability (binding energy: -42.8 kcal/mol), cost-effective encapsulation, and strong protein-binding affinity ($pKd = 7.9 \pm 0.3$ for IL-2). It achieved pH-independent release (58%) under tumor-relevant conditions (pH 6.5) and maintained high affinity for immune-activating proteins, indicating effective delivery within the tumor microenvironment. DeepChem-based simulations predicted systemic non-toxicity (92%), good solubility, biodegradability, and potent immune-profiling activity capable of activating cytotoxic T cells and other innate immune effectors. Collectively, these findings position the Zn^{2+}-Glu nanoplatform as a biocompatible, functionally durable, and versatile DDS with significant promise for next-generation protein-based theranostics. Future *in vivo* validation and clinical translation will be crucial to fully realize its potential in precision oncology.

Author Contributions

Conceptualization, D.B.P.; methodology, S.M.; validation, S.M.; formal analysis, C.G. and R.R.; data curation, R.R. and P.M.; writing—original draft preparation, D.B.P.; writing—review and editing, R.M.; visualization, R.R.; super-

vision, S.M. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

This study, titled "AI Drives Optimization of Delivery Systems Engineered for Precise and Effective Immune-Based Solutions", did not involve any experiments on human participants or animals conducted by the authors. The research is entirely based on computational modeling and simulation methodologies, utilizing publicly available datasets, and does not include any identifiable personal or clinical information. Therefore, ethical review and approval by an Institutional Review Board (IRB) were not required, in accordance with institutional guidelines and national regulations.

Informed Consent Statement

This study did not involve human participants, human data, or human tissue. Therefore, informed consent was not required. The research is purely computational in nature and based on publicly available, anonymized data sources that have been ethically cleared for research use. All necessary ethical considerations have been observed in accordance with institutional and international guidelines.

Data Availability Statement

The data and materials have been made available in this article.

Conflicts of Interest

The authors declare that there is no conflict of interest.

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