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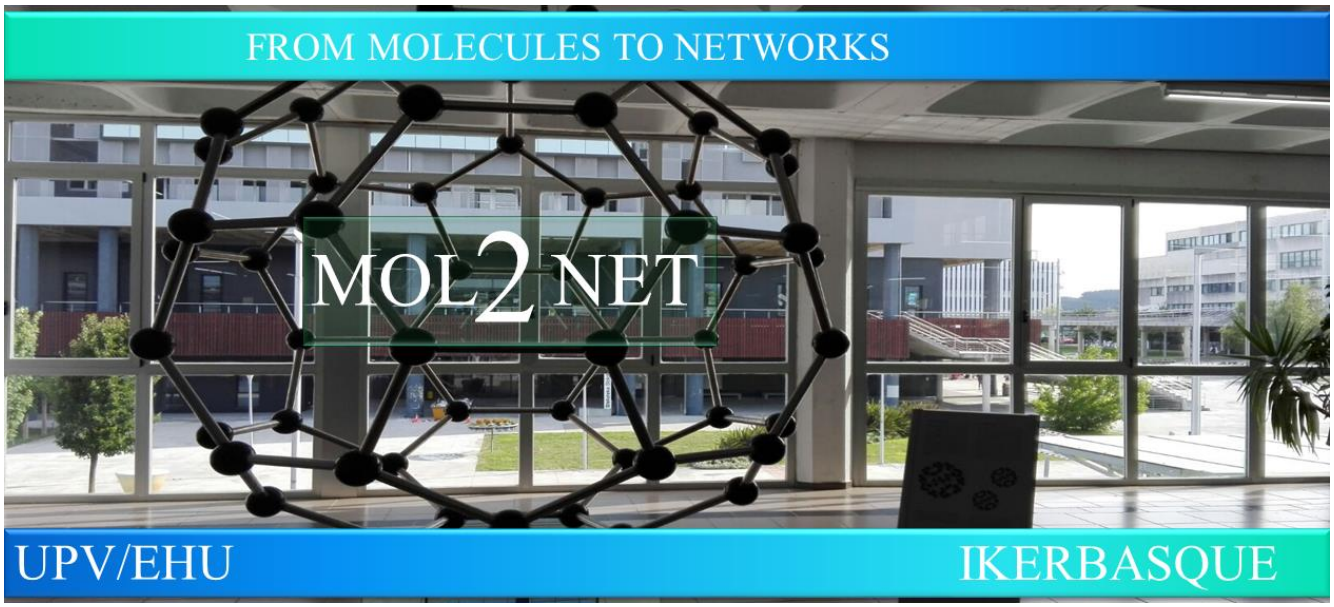


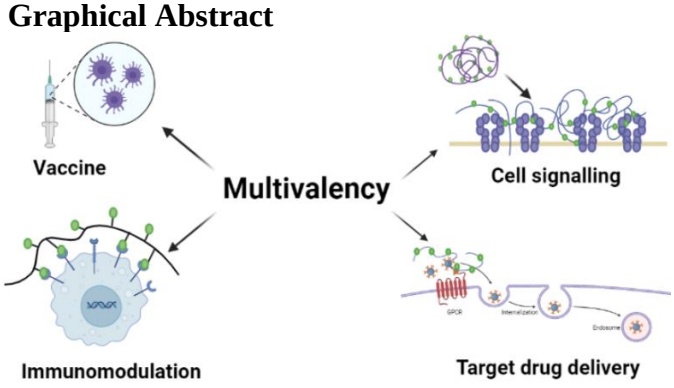
Exploring multivalent interaction in biotechnology

Michael Batsa 1[ab]

[a] Laboratory of Molecular Robotics and Biosensor Materials, Solution Chemistry of Advanced Materials and Technologies (SCAMT) Institute, ITMO University, 197101 Saint Petersburg, Russia

[b] Department of Biotechnology, Faculty of Bioscience, University for Development Studies, 1882, Tamale, Ghana

Graphical Abstract



Abstract.

Multivalent systems are biotechnological tools that utilize multiple, specific interactions to achieve a desired function. These systems often involve the use of multivalent ligands, which are molecules that can bind to multiple target molecules simultaneously, and multivalent receptors. By leveraging the power of multiple, specific interactions, multivalent systems can

Multivalent interactions in biotechnology: using multiple, specific interactions to achieve higher effectiveness and specificity. Multivalent ligands and receptors are key tools in drug delivery, protein engineering, cell signaling, and immune system modulation. In this review, we explore the potential of multivalent systems in nucleic acid therapies	achieve higher levels of effectiveness and specificity than traditional monovalent approaches. Research on multivalency is currently an interplay of the fields of biochemistry and supramolecular chemistry. In biotechnology, multivalent systems have been used in a variety of applications, including drug delivery, protein engineering, and immune system modulation. The use of multivalent systems has the potential to revolutionize the way that biotechnology approaches complex problems and has already led to numerous breakthroughs in a variety of fields. In this review, we focus on the application of multivalent systems in biotechnology and their potential in nucleic acid therapies.

Keywords: multivalent interaction; multivalent ligands; multivalence; multivalent drug design; biotechnology avidity; biosensors; detection; nucleic acid

1 Introduction

In biotechnology, the term "multivalent interactions" [1,2] refers to a molecule's capacity to bind to numerous other molecules at once, such as proteins or small molecules. These interactions are crucial to numerous biological processes and can be used to develop novel medications and treatments (figure 1).

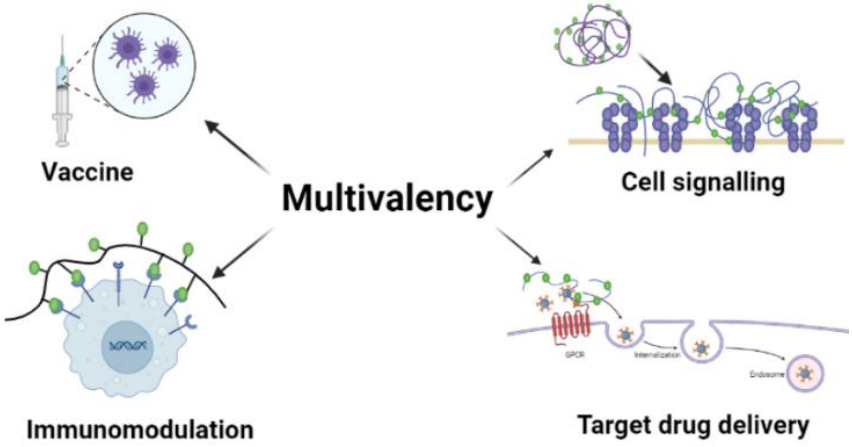


Figure 1: Multivalent interactions. Multiple ligands on one object bind to multiple receptors on another and are commonly found in nature. Designing multivalent ligands to regulate biological interactions is of great interest.

It is frequently possible to achieve a more strong and more specific effect by concurrently targeting multiple sites on a protein or other biomolecule [3]. To overcome weak monovalent and non-covalent interactions and improve their biomolecular interactions so that desirable biological processes can occur, many biomolecular components adopt complicated multivalent interactions [4, 5]. A backbone or scaffold that has a certain number (valency) of the same or various epitopes attached to it makes up a multivalent ligand [6]. There are a lot of protein-carbohydrate interactions [7,8], some of which are involved in host-pathogen interactions, viral entry, and cell surface adhesion. Protein-protein interactions, such as those that facilitate the development of the immunological synapse at the T cell-B cell junction [6], are another type of significant multivalent interaction [9,10]. The interaction of antibodies with antigens is a crucial illustration of multivalent interactions [11,12]. Proteins called antibodies are created by the immune system to identify and combat foreign invaders like viruses and bacteria. They consist of two heavy polypeptide chains and two light polypeptide chains joined together by disulfide bonds [11,12]. Each chain has a variable area that controls antigen binding and recognition and a constant portion that controls immune system activation [13]. The variable region, which contains several binding sites [14,15] that can interact with numerous epitopes (antigenic determinants) on the surface of the antigen, often mediates the binding of an antibody to an antigen. The efficiency of the immune response depends on this multivalent interaction between antibodies and antigens [15,16].

The interaction of lectins with carbohydrates is another significant example of multivalent interactions [17,18]. Proteins or glycoproteins which are lectins can bind exclusively to certain sugars or sugar chains [19]. They participate in the immunological defense, cell-cell communication, and cell adhesion. The sugar-binding domain of the lectin, which has several binding sites and can interact with various sugar residues on the carbohydrate, mediates the binding of lectins to carbohydrates [20,21]. The detection and signaling between cells, as well as the control of immunological responses, depend on this multivalent interaction between lectins and carbohydrates. Multivalent interactions have been used to generate a variety of medicinal medicines in the realm of biotechnology. For the treatment of cancer, autoimmune disorders, and infectious diseases, multivalent antibodies—also known as antibody-based therapeutics—have been created [22–24]. These substances are made to attach to antigens or epitopes on cancer cells or harmful microbes, causing the immune system to attack and kill the target organisms. Additionally, multivalent lectins have been designed to deliver imaging or therapeutic substances to cells or tissues [24,25]. It is possible to selectively distribute medications or imaging agents to the targeted site in the body when the substances contain lectins that bind to carbohydrate moieties on the surface of the target cells. Many naturally occurring lectins and their ligands are multivalent. For instance, membrane-bound glycoproteins can serve as scaffolds for various oligosaccharide determinants [26]. Additionally, oligomeric quaternary configurations of saccharide-binding receptors are common. For cell signaling,

cells utilize a variety of multivalent receptors [27]. These illustrations show that multivalent interactions play a crucial role in biological processes and that they should be recognized and used in biotechnologies. Natural multivalent glycoconjugates [28] can be used to prevent bacteria, bacterial toxins, and viruses from adhering to host cells and to activate the innate and adaptive immune systems [29] in response to these invaders. To create new antiviral medications and immune modulators, it has also been utilized to target dendritic cell absorption of DC-SIGN [29,30]. However, access to ligands that resemble endogenic multivalent arrays can be made possible via chemical synthesis.

To investigate the contributions of different ligand characteristics to a naturally multivalent binding interaction, employ the synthetic ligands [31–33]. Identification of the pertinent underlying molecular pathways is challenging since physiological multivalent ligands are usually in short supply and physically heterogeneous or complicated (for example, on a cell or virus surface) [34]. The flexibility, size, and form of the scaffold from which these binding sites are exhibited, as well as the number (valency) and orientation of receptor binding sites, may all be important factors in binding for these ligands [35]. Synthetic multivalent ligands can have their scaffold structure, the type of binding components they contain, how many there are, and how they are spaced apart systematically changed [27]. Both arrays that resemble natural multivalent displays and those that are unconnected to them have been developed.

2 Application of multivalent interactions in biotechnology

The development of cancer treatments is one area where multivalent interactions have proven particularly beneficial. Numerous anti-cancer medications focus on proteins that are overexpressed or altered in cancer cells. It is frequently possible to achieve a higher inhibitory impact and more efficiently target cancer cells when medicines can bind to several locations on these proteins. For instance, a cancer-associated protein may have many binding sites that can be targeted by monoclonal antibodies, which are proteins that specifically bind to other proteins [36,37]. This may increase the strength and efficiency of cancer treatment [38].

The development of vaccines has also utilized multivalent interactions [39,40]. Numerous vaccines stimulate the immune system by using multivalent molecules like viruses [41,42], or bacteria [43–45]. The development of antibodies that can recognize and bind to numerous locations on the surface of the pathogen can be stimulated by these multivalent molecules, enhancing the efficacy of the vaccination. As an illustration, some vaccines employ virus-like particles, which are artificial constructs that resemble viruses but do not contain any infectious agents [46–48]. These particles can bind to numerous locations on pathogens, activating the immune system and causing it to develop antibodies that can identify and kill the pathogen.

Another illustration is the fact that the flu vaccination comprises a variety of influenza strains [49], each of which has numerous immune system-recognizable epitopes. By exposing the immune system to several

distinct epitopes, the vaccination can stimulate a stronger immunological response and offer broader protection against the flu [50,51]

In addition, the discovery of additional drug classes that target enzymes [52,53], receptors [52,53], and other proteins involved in diverse biological processes has also looked into multivalent interactions. For instance, the activity of enzymes can be inhibited or the binding of a protein to its ligand can be blocked using multivalent small molecules, such as substances that possess several groups capable of binding to a target protein.

Enhancing therapeutic effectiveness, improving affinity, increasing target efficiency and specificity, increasing stability, multiplexing, and controlled release of molecules such as medicines are all benefits of employing multivalent systems in biotechnology [54–56].

The use of multivalent interactions in biotechnology is not without its difficulties and restrictions, though. Designing and synthesizing stable multivalent compounds that can successfully bind to their target is a difficulty [57]. The potential for multivalent compounds to bind to undesired sites, which can result in undesirable side effects, is another difficulty. Additionally, the potency and effectiveness of a multivalent therapy may be impacted by the strength of multivalent interactions, which can vary depending on the precise nature of the interaction and the environment in which it takes place [58]. The complexity of their design and synthesis is one of the difficulties presented by possible applications [57,59]. Toxicity levels and immunological reactions could be additional difficulties.

Table 1 summarises some of the applications of multivalent systems.

Example	Application
Drug design and delivery	The effectiveness and specificity of the medications and their distribution are enhanced through the development of nanoparticles or other delivery systems that specifically target proteins or disease-related pathways.
Diagnostic	Very sensitive and specialized diagnostic tests were developed that enable the precise and reliable diagnosis of illnesses or other disorders.
Protein-protein interaction	This approach is utilized to investigate protein interactions, which can shed light on the mechanisms behind cellular functions and disease pathology.
Antibody-antigen interaction	Designing multivalent antibodies or other compounds that can bind to several antigen epitopes to increase the interaction's affinity and specificity.

Molecular recognition	Design compounds with high affinities that can recognize and bind to targets, including receptors or enzymes.
Gene regulation	Design compounds that can attach to DNA or RNA sequences to control the expression of genes in a precise manner.
Immunotherapy	To increase the efficiency of the treatment, design immunotherapies that target a variety of proteins or pathways implicated in cancer or other disorders.
Biomarker discovery	To find molecules that can act as biomarkers for particular illnesses or situations, allowing for the detection or monitoring of these conditions
Biosensors	Design sensitive and precise biosensors that can identify certain chemicals or analytes in complicated combinations.
Enzyme inhibition	Design compounds that can block enzymes, which can be helpful in the creation of medications to treat conditions like cancer or cardiovascular diseases
Protein engineering	Design proteins that have specific characteristics or capabilities, such as enzymes with increased catalytic activity or antibodies with increased specificity.
Vaccine development	Design vaccines that are more effective and comprehensive by targeting a variety of antigens or epitopes.
Biocatalysts	Design biocatalysts or other enzymes that are more efficient or selective at catalyzing particular processes.
Tissues engineering	Create supports for the development and differentiation of cells or tissues, such as scaffolds or other materials.
Biomaterial design	Create materials with specified features or capabilities, such as enhanced biocompatibility or medication delivery systems.
Biodegradable polymers	Create biodegradable materials for uses such as tissue engineering and medication delivery.
Bioremediation	Design enzymes or other biocatalysts that can break down pollutants or toxins.
Industrial biotechnology	Design enzymes or other biocatalysts for the synthesis of chemicals, fuels, or other goods.

Agriculture biotechnology	Design molecules that can increase crop yields or withstand pests, illnesses, or other agricultural obstacles.

3 Mechanism of multivalent bindings

Interactions between molecules that involve more than two binding sites or valences are known as multivalent interactions [60,61]. Nucleic acids, proteins, carbohydrates, and other molecules can all be involved in these interactions, which can be crucial in a variety of biological processes. The development of protein-carbohydrate complexes [62–65], which are crucial for numerous biological processes such as cell adhesion, immunological recognition, and signaling, is a significant type of multivalent interaction [66].

A crucial characteristic of multivalent interactions is their capacity to produce binding that is both strong and specific [67]. This is because as the number of binding sites grows, so does the number of attractive forces, increasing the binding affinity of a multivalent interaction [68–70]. As a result, very stable complexes may emerge. These complexes are crucial for many biological functions.

The capacity of multivalent interactions to control protein activity is another crucial feature [71]. For instance, a protein's conformation might change as a result of the binding of a multivalent ligand, which can either activate or inhibit the protein's activity [72–74]. This may be a crucial mechanism for controlling the function of proteins and enzymes in cells.

Additionally, it may contribute to the emergence of supramolecular structures such as polymers and aggregates [75–78]. Through the self-assembly of molecules, which can be aided by multivalent interactions, these structures can be created. For instance, multivalent interactions between proteins can be the cause of protein aggregation formation in cells [79,80].

In the immune system, where they are crucial for the identification and activation of immune cells, multivalent interactions are also significant [80–83]. For instance, immune cell-produced proteins called antibodies can interact with antigens in multivalent ways to bind to them [5,84–87]. Immune cells like T cells and B cells may become activated because of these interactions and may then initiate an immune response against the foreign chemical.

Additionally, they participate in the development of viral capsids, which are protein structures that encase the viral DNA [88–90]. Viral proteins self-assemble into these capsids, which can be aided by interactions with other multivalent proteins.

Nature makes use of multivalency to create strong and specific interactions when the binding sites of a protein receptor have just a modest affinity for monovalent ligands [91]. Dysregulation may be responsible for the advancement of diseases, such as cancer metastasis, which need exact spatial orientation of ligands and receptors at the nanoscale [92]. A fundamental biological activity called ligand binding to cell surface receptors starts cellular adhesion, communication, metabolism, and other functions [91,93]. The

strength and specificity with which a given ligand binds to its target receptor determine how well these mechanisms work [94–96].

Numerous ligands have weak binding characteristics when used alone, but when linked together by a scaffold, they can provide a high collective affinity for a corresponding receptor with multiple binding sites [97]. The observed binding increases for multivalent interactions may be attributed to chelation, cross-linking, statistical rebinding, steric shielding, clustering effect, and subsite binding, among other factors (Figure 2) [98,99]. Chelation [100] is the simultaneous binding of numerous ligand molecules to multiple binding sites, which causes a decrease in the rate at which ligands dissociate (off-rate), enhancing the binding affinity, force (also known as avidity), and stability of multimeric ligands (Figure 2A).

The physical size and distribution of ligand molecules may inhibit other competing ligands from binding to receptors in the steric stabilization mechanism [101] when multivalent ligands engage with their receptors (Figure 2D). The increased concentration of local ligands surrounding the receptor causes the statistical effect of multivalent ligands to increase the likelihood that ligands will rebind to their binding site and decrease the off-rate (Figure 2C).

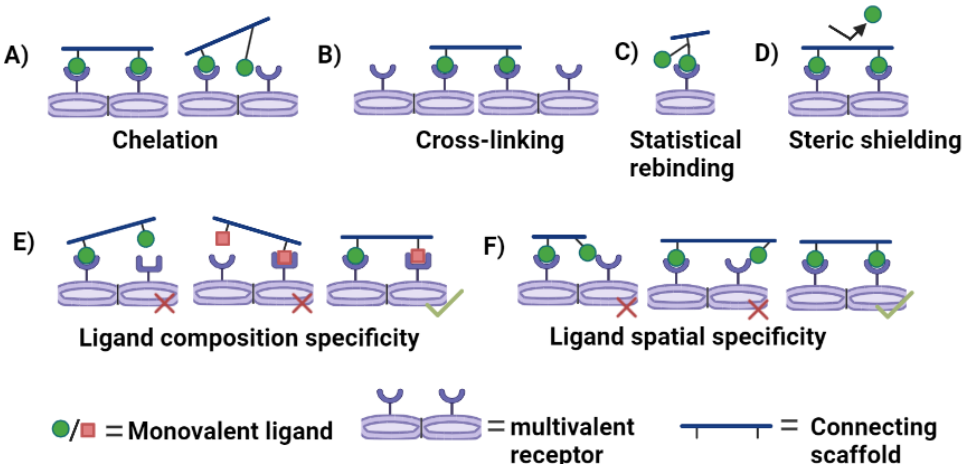


Figure 2: Multivalent ligand-receptor interactions can produce significant affinity (A–D) or specificity (E and F) enhancements relative to monovalent interactions through several contributing mechanisms. A) Chelation bridges either two primary binding sites (left) or a primary and a secondary binding site of a single receptor (right). B) Cross-linking spans binding sites on two different receptor molecules. C) Statistical rebinding involves the rapid exchange of locally clustered ligands. (C) Steric shielding from a large scaffold can hinder the approach to competing for monovalent ligands. Multivalent binding agents must present ligands of the correct composition (E) and spatial context (F) to be recognized by their target receptor.

The structural complexity of a multivalent ligand determines the binding process that attaches it to its receptor [102]. Multivalent ligands may change their closeness and orientation when they bind to receptors, which could affect how well the receptors communicate [27,103,104]. Natural or artificial ligands can function as effective effectors or inhibitors in a range of biological processes in multivalent systems by signal

transduction. Multivalent ligands effectively inhibit or stimulate biological reactions by binding to their receptors with high affinity, avidity, and specificity [105,106]. Furthermore, these complex scaffolds are intended to self-assemble from component strands using Watson-Crick and Hoogsteen base-pairing rules [5]. Site-specific ligand integration into these component strands is possible during solid-phase synthesis, either by directly using ligand-modified monomers or by using post-synthetic conjugations [91].

Van der Waals forces, hydrogen bonds, and electrostatic interactions are only a few of the forces that can influence multivalent complexes [107,108].

Between charged molecules, electrostatic interactions [109,110] are attractive forces that can be positive or negative. These interactions, which depend on the charge and separation between the molecules, have the potential to be potent enough to promote the development of intricate structures [111].

Another form of interaction that can result in multivalent interactions is hydrogen bonding [112,113]. These compounds covalently bind hydrogen atoms to electronegative atoms like oxygen, nitrogen, or fluorine to form this type of bond [114]. Given that carbohydrates contain several hydroxyl groups that can interact with proteins to generate hydrogen bonds, hydrogen bonding can be particularly significant for the creation of protein-carbohydrate complexes.

Due to changes in their electron distributions, molecules can experience Van der Waals forces which are weaker attractive forces [115,116]. These forces may be crucial for the attachment of tiny molecules to proteins and may help multivalent interactions remain stable.

The conformational alterations that take place in interacting molecules upon binding can also affect multivalent interactions [117]. For instance, when a multivalent ligand binds to a protein, conformational changes in the protein can either activate or inhibit the protein's function [73,118]. This may be a crucial mechanism for controlling the function of proteins and enzymes in cells.

4 The Role of Multivalent Ligands in the Development of drug

In recent years, the field of drug development has paid a lot of attention to multivalent ligands. It has been investigated for a range of medicinal uses, including the treatment of infectious illnesses, cancer, and inflammation. The capacity of multivalent ligands to achieve high-affinity binding with target molecules is one of its main advantages [119,120]. Due to the higher binding power between the ligand and the target, this high-affinity binding may have a larger therapeutic effect [121]. Inhibiting the activation of inflammatory pathways has also been investigated as a potential treatment for inflammation [122]. To prevent pathogen growth and spread, multivalent has also been created for the treatment of infectious disorders [123–128].

The development of multivalent ligands for therapeutic use is fraught with difficulties. Due to these ligands' higher binding affinities and broad-spectrum actions, there is a risk of increased toxicity. The necessity for properly crafted ligands that can selectively bind to the chosen target without attaching to

additional proteins or receptors is also present because doing so may have unfavorable side effects. The potential benefits of these ligands make them an appealing target for therapeutic development despite the difficulties associated with their development.

The two main reasons why drugs fail in clinical trials are because they are unsafe and do not work [129]. The development of molecular biology and genomics has had a significant impact on the search for new drugs. The pharmaceutical industry's research division is being established by the biotech sector. Recombinant proteins and monoclonal antibodies have significantly expanded the toolbox of therapeutic agents [130,131]. By analyzing the genetic bases of complicated diseases and determining the most efficient pharmacological targets, genome sciences and bioinformatic methods enable us to widen the spectrum of accessible therapeutic alternatives. The probability of getting efficacy and selectivity improved significantly when a multivalent ligand was used as a homo- or hetero-dimer, making the usage of multivalent ligands a more alluring method for creating novel pharmaceuticals like anti-HIV agents with therapeutic uses [132]. An illustration of the use of multivalent ligands in drug development can be found in the almost universally weak binding of specific carbohydrates by proteins necessary for the regulation of cellular activity, such as fertilization, lymphocyte homing, and endocytosis mediation, but overcome by one entity with multiple ligands binding to another entity, leading to numerous ligand-receptor interactions [133]. To increase the efficacy of carbohydrates as ligands or inhibitors, multivalent carbohydrates are generated in a variety of forms, including dendrimers, polymers, micelles, vesicles, and functional nanotubes. According to Peter, proteins that are complementary to carbs typically only attach to them weakly, and stronger binds and greater inhibitions are frequently attained by numerous interactions with different carbohydrates [134]. He went on to say that the so-called "cluster effect" is widespread in nature and was the driving force behind the development of multivalent inhibitors that block protein-carbohydrate interactions. Some of the most significant multivalency effects, such as the inhibition of AB-5 toxins and other multi-site lectins like the asialoglycoprotein receptor, are likely to be the result of chelation [135]. The fact that multivalent carbohydrates frequently aggregate is also evident, but it is less clear how much this improves affinity or inhibitory potency, or whether the aggregation processes can be distinguished from the statistical rebinding effects [136]. To stimulate the innate and adaptive immune systems, multivalent glycoconjugates have also been employed in vaccines based on carbohydrates [137]. Lipopolysaccharides and S-layers, which are multivalent glycoconjugates that are naturally present on the surfaces of bacteria and can be employed or targeted in anti-infective therapies [29]. With structures that can competitively hinder pathogen and host cell recognition processes, it may be feasible to halt colonization or even the growth of biofilms. Such carbohydrate-based vaccines can prime the immune defense systems in advance of infection or encourage the body to defend itself against an ongoing chronic illness through a lectin-mediated cellular absorption process [138].

Multivalent inhibitors can prevent pathogens from attaching to cells, preventing the development of drug-resistant strains even when they do not kill germs [139]. Pathogens that are coupled to multivalent

ligands can no longer multiply and can either be cleared off by the reticuloendothelial system or destroyed by macrophages. However, the enthalpic and entropic contributions have a significant impact on the effectiveness of this competitive inhibition. However, accurate theoretical modeling of thermodynamic and kinetic parameters is typically limited by the complexity of biological systems. The pharmaceutical industry has not embraced the idea of multivalent inhibitors as pharmaceuticals since the optimization of reproducible polymeric multivalent ligands is practically more challenging than the normal monovalent medications (or prophylactics). Another challenge is the multivalent ligand pathogen binder's capacity to be applied in the latter phases of infection when a pathogen has already started to reproduce within the biological system [139]. In a study, mice infected with the flu virus fared better when given 36 different dosages of an aerosolized glycopolymer made of polyacrylamide on days 2 through 5 following infections. This investigation proved the value of this inhibitor of multivalent virus attachment in the treatment and prevention of influenza. However, there aren't many instances of multivalent glycoconjugates being employed effectively as drugs or vaccinations. To absorb Shiga-like toxins from E. coli, the synthetic glycolipid analog SynsorbPk, which is covalently attached to a silica particle, has become a promising antiadhesive treatment candidate. Recombinant E. coli serves as another example because it has a lipopolysaccharide (LPS) mimicking the Shiga toxin receptor on its surface and has shown improved affinity and efficacy over SynsorbPk. It has been demonstrated that an OPS conjugated to toxin A is immunogenic and effective against Pseudomonas aeruginosa. The challenges and problems with concentrating on multivalent interactions while concentrating on our comprehension of these interactions have been highlighted. The creation of strong anti-infective drugs as well as other effective binders may be facilitated by multivalency.

5 The multivalent nucleic acid construct interactions

Nucleic acids with numerous binding sites, such as DNA and RNA, can interact with other molecules in a process known as multivalent nucleic acid interactions. Numerous biological processes, such as DNA replication, protein synthesis, and gene control, depend on these interactions. The binding of transcription factors to sequences in the promoter region of a gene serves as one example of multivalent nucleic acid interactions. Proteins called transcription factors to attach to DNA and control how genes are expressed. The binding of numerous transcription factors to a gene can activate or repress gene expression because they frequently have multiple binding sites that can interact with specific regions in the promoter region of a gene.

The interaction of ribosomes with mRNA during protein synthesis is another instance of multivalent nucleic acid interactions. Ribosomes are intricate molecular factories in which amino acids are converted into proteins. They attach to mRNA and convert the genetic instructions into an amino acid sequence.

Ribosomes can effectively generate proteins because of their numerous binding sites, which connect with mRNA sequences.

DNA replication also heavily relies on interactions between multivalent nucleic acids. The replisome, a group of proteins that unwinds and divides the double helix's two strands during DNA replication, then uses the data from the template strands to create new strands of DNA. Various proteins in the replisome each have multiple binding sites that interact with distinct DNA sequences to effectively manufacture new DNA strands.

Multivalent nucleic acid interactions perform crucial functions in DNA repair and DNA recombination by permitting the binding of proteins to specific DNA sequences, in addition to their roles in cell signaling, gene regulation, protein synthesis, and DNA replication.

Researchers have noticed that multivalent nucleic acid constructions are compatible with a variety of ligand types and receptor classes and can be used to build high-affinity binding agents for studies in solution and on surfaces to understand the principles behind these interactions [96].

Contrary to ordinary polymers, nucleic acid materials can access a variety of hard, three-dimensional forms via the sequence-programmed self-assembly of component strands. It has also been used to address fundamental multivalency issues to comprehend how the intensity of monovalent contacts, the flexibility of the scaffold, the spacing between interacting sites, and the spatial layout affect the potential affinity increases. Nucleic acid constructs have been used in a slightly different way to study the significance of receptor proximity or to create tools that allow control over biological signal transduction pathways. These constructs have been used as chemical dimerizers of protein receptors [140]. A review by Yeldell in 2020 discusses how nucleic acid constructs can be used to study multivalent protein interactions, which provides context for the other papers. Wu found that multivalent ion-induced aggregation of double-stranded RNAs and DNAs may depend on the topological nature of helices [141].

In his opinion, ion-mediated contact plays a significant role in the structure and stability of nucleic acids. This suggests that nucleic acids' spatial organization may affect how they interact with multivalent ions. Numerous studies demonstrate that nucleic acid assemblies and multivalent nanostructures may both be helpful for cleaving nucleic acids and understanding how multivalency influences binding [142]. Examples of multivalency in nucleic acid, however, are not specifically addressed in any of the studies. Although they don't directly address multivalency, Zhang's discoveries from 2018 and Srinivasan's from 1987 apply to nucleic acids [143,144]. In his research published in 2020, Yeldell claimed that nucleic acid assemblies provide answers to basic issues and produce high-affinity binding substances [145]. Similar to this, Lu discovered in 2019 that nucleic acid detection may be accomplished using a single-step electrocatalytic biosensor with dual-affinity regulation [146]. These publications demonstrate that nucleic acid cleavage and/or binding affinity can be enhanced by multivalent nucleic acid binding. Kim's 2008 article states that biomolecular engineers are increasingly creating novel molecular assemblies using multivalent interactions, while others are designing multifunctional or better-performing ligands using

small molecules or antibody epitopes, leading to new functions for ligands or improved performance of current ligands [147]. There have been a relatively small number of reported attempts to employ nucleic acid aptamers as functional domains. In contrast to the binding of the monovalent ligand, the simultaneous binding of two aptamers after linking produced 16.6 times higher inhibitory efficacy, according to Kim's research, and this improvement was caused by changes in the kinetics of the binding interactions [148]. However, no one has directly addressed in any of the publications how multivalent nucleic acids boost binding affinity [149]. As a result, we can only assume that multivalent nucleic acids may boost the binding affinity without making any assumptions about how. Additional research is needed to answer this question more thoroughly.

6 Multivalent DNAzymes

Multivalent DNAzymes [150] are synthetic nucleic acid enzymes that attach to particular target molecules using numerous binding sites and catalyze chemical processes [97]. They have numerous potential uses, such as in gene therapy, medication delivery, and diagnostics [151–153]. Multiple oligonucleotides that are created to bind to particular target molecules often make up multivalent DNAzymes [67]. They frequently consist of several "arms" that are linked by a central core, which enables them to bind to numerous target molecules at once (figure 3) [154]. Both "sticky ends" and "toeholds," which are brief DNA sequences that can bind to complementary sequences on the target molecule, can serve as binding sites on DNAzymes [71,155,156]. The DNAzyme normally undergoes a conformational shift upon binding to the target molecule, activating the enzyme's catalytic activity [157–159]. Numerous chemical processes, such as small molecule synthesis, nucleic acid cleavage, and nucleic acid ligation, can be catalyzed by multivalent DNAzymes [97,157]. The transfer of a phosphate group from ATP to a protein, for example, illustrates how they might be utilized to facilitate the transfer of chemical groups across molecules [160].

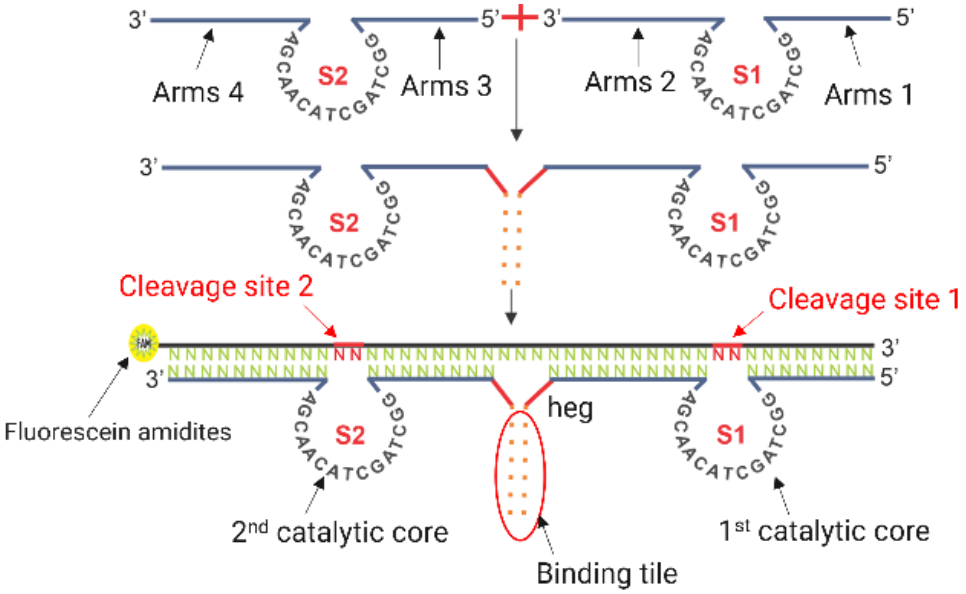


Figure 3: Bivalent DNAzyme systems. S1 and S2 are subunits of Dz 10-23. S1 and S2 are conjugated in the 3' -5' direction using a HEG linker. S1 and S2 are conjugated in the 3' -5' direction using a HEG linker. As a result, the S1-S2 nanostructure's affinity, and ability to hybridize with the targeted mRNA are improved. The likelihood of downregulating the targeted mRNA is increased by the presence of two catalytic centers on the S1-S2 nanostructure.

7 Applications of Multivalent DNAzymes

Diagnostics is one possible use for multivalent DNAzymes [150,161,162]. DNAzymes can be made to attach to certain diseases or biomarkers, and the DNAzyme-target complex can be found using several methods, such as mass spectrometry [163,164], radioactivity [165], or fluorescence [166]. This makes it possible to detect molecules quickly and accurately, which helps diagnose illnesses or locate contaminants in a sample.

Another potential use for multivalent DNA enzymes is drug delivery [153,167,168]. To deliver medications or other therapeutic agents to certain cells or tissues, DNAzymes can be engineered to attach to particular cell surface receptors [169] or intracellular targets [152]. This enables the targeted administration of medications to areas, which may assist in lessening treatment adverse effects and increase the therapy's efficacy.

Gene therapy may potentially benefit from the use of multivalent DNAzymes [55,170,171]. The therapy of genetic abnormalities or the modification of gene expression in other applications may benefit from the use of DNAzymes to target genes and activate or inhibit their expression. The development and application of multivalent DNAzymes involve several difficulties and considerations. Designing DNAzymes with appropriate catalytic activity and specificity for their target molecules is a challenge [157]. This necessitates a thorough knowledge of the target molecule's structure and properties, as well as the DNAzyme's binding and catalytic mechanisms. The delivery of DNAzymes to their target cells or tissues presents another difficulty [152,172]. This necessitates the creation of efficient delivery methods that can keep DNAzymes from degrading while ensuring their delivery to the intended place.

Additionally, there are worries regarding DNAzymes' possible toxicity [173,174], especially if they are given systemically. DNAzymes may cause an immunological response if the immune system perceives them as foreign substances, or they may unintentionally interact with other molecules in the body. Before DNAzymes may be employed in therapeutic settings, these risks must be thoroughly investigated. Similar to numerous biomolecular components, which rely on multivalent interactions to strengthen their biomolecular interactions and overcome weak monovalent and noncovalent interactions to enable the occurrence of desired biological processes [5], multivalent Dzs [175,176] are currently the focus of research to develop effective cleavage agents for gene therapy [177,178]. To address the issue, Unwalla (2001) tested three manufactured monoenzymes and one di-DNA enzyme against TAT and TAT/REV RNA to show

their capacity to precisely cleave target RNA and their effects on HIV-1 gene expression, but the di-DNA enzyme performed poorly [179].

8 Ethical considerations surrounding the use of multivalent interactions in biotechnology.

The application of multivalent interactions in biotechnology has the potential to transform the way we approach a variety of biological issues, including drug delivery and the detection and treatment of disease. The utilization of multivalent interactions in biotechnology does, however, bring up several ethical issues that need to be carefully considered, as with any new technology.

1. Possibility of unexpected repercussions Multivalent interactions have the potential to be very selective and focused, but they also carry the risk of unintended consequences that are challenging to anticipate or manage. For example, multivalent ligands designed to bind to a particular protein may also bind to other proteins, leading to unintended cellular effects [180]. This can be particularly problematic when medicinal applications involving multivalent interactions are involved, as off-target effects may endanger the patient. The off-target effect in multivalent interactions, however, has not been sufficiently demonstrated in the literature.
2. The possibility of biases or inequality. The utilization of multivalent interactions in biotechnology might be utilized to develop individualized treatments that are catered to a person's unique genetic composition [181,182]. However, this might also result in disparities in access to care, with some people being excluded because they lack a specific genetic marker or can't afford individualized treatments.
3. The likelihood of abuse or overuse. Multivalent interactions have the potential to be utilized for evil reasons like making bioterrorism agents or biological weapons. Therefore, strict regulation and oversight of the creation and application of multivalent interactions are necessary to guarantee that they are not utilized for detrimental ends.
4. The possibility for environmental impact. Certain multivalent ligands are made to bind to particular environmental pollutants, like heavy metals or pesticides, and draw those substances out of the environment [183]. These multivalent ligands run the danger of having unintended environmental effects, such as attaching to molecules that are not their intended targets or impairing normal ecological proceses.

In general, several different ethical issues surround the utilization of multivalent interactions in biotechnology. As technology continues to advance and finds new and creative applications, it is crucial to carefully analyze and take these factors into account.

Conclusion

A single molecule can attach to numerous locations on another molecule through multivalent interactions, producing powerful and specific interactions. Numerous biological activities, including the identification and binding of proteins and DNA, the development of cell-cell and tissue-to-tissue adhesions, and the control of enzyme activity, depend on these interactions.

A fundamental idea in biotechnology, multivalent interaction has a wide range of applications in fields like drug delivery, vaccine development, and nanotechnology. If we want to develop therapeutic strategies, diagnostic instruments, and drugs that particularly target specific biological processes, we must have a thorough understanding of the molecular mechanics underlying multivalent interactions.

It is important to note that due to the wide range of variables that might affect the strength and specificity of the contacts, multivalent interactions can be complicated and challenging to research. Because of this, research on multivalent interactions is a lively and quickly developing field of biotechnology, and significant strides have been made in this area recently. Their interactions are a basic and common biological mechanism that is essential for numerous physiological activities.

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