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# Binding kinetics and multi-bond: Finding correlations by synthesizing interactions between ligand-coated bionanoparticles and receptor surfaces

Wenjing Wang [a, *], Andreas Voigt [b], Michael W. Wolff [a, c], Udo Reichl [a, c], Kai Sundmacher [a, b]

[a] Max Planck Institute for Dynamics of Complex Technical Systems, D-39106 Magdeburg, Germany
[b] Chair for Process Systems Engineering, Otto-von-Guericke University Magdeburg, D-39106 Magdeburg, Germany
[c] Chair for Bioprocess Engineering, Otto-von-Guericke University Magdeburg, D-39106 Magdeburg, Germany

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ABSTRACT

The number of bonds formed between one single bionanoparticle and many surface receptors is an important subject to be studied but is seldom quantitatively investigated. A new evaluation of the correlation between binding kinetics and number of bonds is presented by varying ligand density and receptor density. An experimental system was developed using measurements with surface plasmon resonance spectroscopy. A corresponding multi-site adsorption model elucidated the correlation. The results show that with the increase of the receptor density, the adsorption rate first decreased when the number of bonds was below a maximum value and then increased when the number of bonds stayed at this maximum value. The investigation on ligand density variation suggests that the coating density on top of the bionanoparticle surface may have a particular value below which more ligand will accelerate the adsorption rate. The ratio of ligand amount bound by the receptors to the total ligand amount associated with a single bionanoparticle will remain constant even if one attaches more ligands to a bionanoparticle. We envision that the bionanoparticle desorption will not depend on density changes from either ligand or receptor when the number of bonds reaches a specific efficient value.



Promising biological performances and functionalities can be achieved with manmade synthetic systems. Synthetic systems often consist of different and versatile ligands attached to selected nanomaterial scaffolds and corresponding receptors. Researchers have already created exciting smart entities and introduced new materials such as glyconanomaterial [1,2], nanoparticle drug carriers [3,4], and multivalent vaccines [5]. One very important direction of current investigations is the improved understanding of the multivalent interaction between the entities with the ligands and the receptors. Many valuable inspirations have been triggered and various application fields have been opened up with the help of new chemical synthesis methods [6–11]. Systematic investigations have been started in multivalent binding systems such as glycosylated nanoparticles for carbohydrate–protein interaction [12], multiple-ligand functionalized microbeads for identifying extracellular protein interactions [13], and heterobivalent ligand targets for tumor treatment [14]. In general, the main advantage of a multivalent interaction in comparison with a monovalent interaction is that it improves the functionality with respect to binding affinity [15], inhibition potency [16], potent antiviral effects [17], and pharmacokinetic profiles [18].

To use the superiority of multivalent interactions under practical circumstances, one needs a comprehensive understanding of all effects involved in the multivalent interactions. State-of-the-art research includes experimental studies implementing new chemical synthesis methods [19–21] or theoretical studies driven by thermodynamic and kinetic aspects [22]. Apart from the biologically associated complexities of (mono)valent interactions, it is

*Abbreviations used:* IgG, immunoglobulin G; BioNP, bionanoparticle; SPR, surface plasmon resonance; Mes, 2-(*N*-morpholino)ethanesulfonic acid monohydrate; EDC, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; NHS, *N*-hydroxysuccinimide; MWCO, molecular weight cutoff; DLS, dynamic light scattering; UV–Vis, ultraviolet–visible; RB, running buffer; MW, molecular weight.

* Corresponding author.
*E-mail address:* wangw@mpi-magdeburg.mpg.de (W. Wang).

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more demanding to investigate multivalent interactions. In such interactions, one of the important characteristics is the number of bonds among the components, the chosen ligands, and the corresponding receptors. The number of bonds is also called multivalency in this work. Experimental data has shown that the effective multivalent interactions are mostly influenced by two factors: the ligand density [23,24] and the receptor density [25]. Besides, theoretical research has revealed that the number of bonds is growing under increased ligand density [26,27]. It seems that the densities of ligand and receptor will affect the bond formation as well as the multivalency. In previous studies researchers evaluated binding curves and rate constants to elucidate multivalent interactions, but so far little emphasis has been put on the number of bonds. Indeed, it is difficult to detect the number of bonds experimentally with current methods, and hence a quantitative correlation of bond number with ligand and receptor densities is hard to find. Currently, the binding kinetics of multivalent interactions is mostly investigated by the classical monovalent kinetic model, the Langmuir model [25,28–30]. However, it has been argued that simulation data of a Langmuir model may deviate from experimental data by disregarding multivalence [31]. It is obvious that improvements in the field can be made if the knowledge about the binding kinetics includes the correlation between the number of bonds and changes in both ligand density and receptor density.

The number of bonds will play a key role in the performance assessment of multivalent interactions. Previous experimental studies concerning multivalency discovered some specific criteria about the valency, for example, threshold valency [29] and optimal valency [21]. In addition, the number of bonds is very important to understand, and it is important to develop a data bank of multivalent design correlated with avidity, enhancement factor, cooperativity, and thermodynamics. The bond design for different applications can be influenced via smart ligand-coating strategies for various nanomaterials. The current focus is on a low range of the bond number within up to several tens of bonds, for example, in the case of a dendrimer conjugate. There are only a few studies [22,24] at a high range of several hundred bonds. This work illustrates the number of bonds even beyond the high range with the application of antibody conjugates.

The current work aimed to elucidate the correlation between the number of bonds and the binding kinetics by varying the ligand and receptor densities. It is hoped that this will lead to a more profound understanding and comprehension of multivalent effects related to the multivalency.

To achieve the goal, obtaining experimental data on a well-chosen model system is required. Polystyrene nanoparticles with a diameter of 110 nm were selected as the scaffold and coated with a particular ligand, polyclonal human immunoglobulin G (IgG). As the receptor, Protein A was immobilized at a specific sensor surface. The coating with ligand and immobilization of receptors were both achieved with the help of a carbodiimide reaction. The multivalent interactions of ligand-conjugated bionanoparticles (BioNPs) at the receptor surface were measured by surface plasmon resonance (SPR) spectroscopy. SPR was extensively used in the detection of the multivalent interactions [19,20,25,28–30,32]. In the past, the antibody-coated polymeric nanoparticles were shown to provide a fundamental platform, for example, to investigate the nanomedicine targeting to diseased tissues [33,34]. The systematic evaluation of the multivalent interactions of human IgG-coated nanoparticles may reveal reasons for the potential toxicity of antibody-related nanomedicine.

Having obtained data with variations of ligand and receptor densities, we can try to find useful correlations of multivalency on various system parameters. This finding was done by fitting the data to a suitable adsorption model. A related multi-site kinetic model has already been used for a study about the adsorption of influenza virus to a surface with immobilized lectin receptor [35]. The proposed quantitative identification of the number of bonds from experimental data by changing ligand density and receptor density using synthetic design methods has not been done previously.

The approach of combining experiments and model evaluation will help to examine the binding kinetics with respect to the number of bonds in addition to previous studies on binding curves and rate constants. Moreover, the multivalent effects can be revealed directly from the interaction between two partners instead of the prediction based on the characteristics of the respective partner. With respect to the ligand distribution, Mullen and coworkers proposed a Poisson distribution among dendrimer conjugates [36]. But here we go beyond this statistical method and take into account the average number of ligands per particle [37]. With this average assumption, we studied the multivalency using quantitative estimations of ligand density per particle and the amount of ligands binding to the receptors. By performing specifically designed synthetic experiments in terms of ligand density and receptor density in a wide range, our approach will add to the knowledge of multivalent effects by revealing the active number of bonds between two interacting entities.

## Materials and methods

### *Materials*

Polystyrene nanoparticles were purchased from Life Technologies, Thermo Fisher Scientific. Mes (2-(*N*-morpholino)ethanesulfonic acid monohydrate) was purchased from Carl Roth (Karlsruhe, Germany). Polyclonal human IgG, EDC (*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride), NHS (*N*-hydroxysuccinimide), glycine, and all other chemicals were purchased from Sigma–Aldrich. The dialysis membranes were purchased from Spectrum Labs (Frankfurt, Germany). Vivaspin 20 centrifugal concentrators with a molecular weight cutoff (MWCO) of 1000 kDa and the syringe filters were purchased from Sartorius (Göttigen, Germany). Protein A was purchased from Vector Laboratories. The sensor chips C1 and amine coupling kit were bought from GE Healthcare Biosciences (Sweden). All of the buffers were filtered using a filter with a pore size of 0.22 μm, and all of the glass accessories were autoclaved.

### *Synthesis of BioNPs*

First of all, the nanoparticle solution was adjusted to 10 mM Mes (pH 6.0) by dialysis using a 1000-kDa MWCO membrane. Nanoparticle solution (1 ml) was dialyzed against 1 L of 10 mM Mes (pH 6.0), three times with a stirring of 600 rpm (2 h at room temperature for the first two times and finally overnight at 4 °C). As shown in Scheme 1, the first step in the BioNP synthesis was to activate the carboxylic groups at the nanoparticle surface by EDC/NHS. Nanoparticle solution (1 ml) was injected into one glass tube and diluted by 2.75 ml of 10 mM Mes (pH 6.0). Then, 1 ml of 1 M EDC and 0.25 ml of 1 M NHS were mixed and injected into 3.75 ml of nanoparticle solution under the condition of sonication. Next, the mixture was incubated for 2 h at 25 °C by intermixing (30 s at 600-rpm rotation and 30-s pause; Thermomixer, Eppendorf). After the activation, the unreacted EDC and NHS were removed from the activated nanoparticles by dialyzing (1000-kDa MWCO) 1 ml of mixture against 1 L of 10 mM sodium phosphate and 150 mM NaCl (pH 7.4). The dialysis buffer was changed three times (2 h at room