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Ultra-sensitive binding response: Superselectivity and the role of combinatorial entropy in binding

Commentary by

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on

Designing super selectivity in multivalent nano-particle binding
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Statement of Significance

Multivalency refers to the possibility of both synthetic and natural constructs, e.g., nanoparticles or viruses, to bind their target via multiple ligand-receptor bonds. Super-selectivity is a peculiar property of multivalent binding, first rationalised by Martinez-Veracoechea and Frenkel (MVF) in Ref. [1], and refers to the almost step-like response of the binding probability with respect to the number of receptors on the target. This commentary discusses the physical origins of this phenomenon, and how (combinatorial) entropy provides a fruitful point of view to understand ultra-sensitive responses involving multivalent agents.

Multivalent particles can form multiple bonds to a substrate, leading to strong binding interactions even when each individual bond is weak on its own. Not only can multivalency increase binding affinity, but it has also been long appreciated that selectivity can be enhanced through *geometric matching* between the position of ligands on the binding agent and the position of cognate receptors on the target.

Much more recently, however, we came to understand how multivalency can also increase binding selectivity *without* relying on any ordered pattern of receptor and geometric matching, and instead purely based on the receptors' average density. To give a clear example, imagine the following problem: we have two cells, one expressing a high and one expressing a low density of otherwise equal receptors on their surface, randomly distributed. Properly designed, multivalent agents can be made to strongly bind to the former, but not the latter. Quite curiously, however, this type of selectivity is enhanced, and actually even requires, some form of positional disorder in the receptors and ligands involved [2], quite the opposite of geometric matching!

The aforementioned type of binding selectivity is what we will call super-selectivity, a term introduced by Martinez-Veracoechea and Frenkel (MVF) in a milestone paper in 2011 [1], the article we discuss in this commentary. In this paper, MVF used a coarse-grained model together with Monte Carlo simulations to look at the equilibrium adsorption of a solution of multivalent, ligand-decorated nanoparticles to a receptor-decorated surface. The coarse-grained model employed is quite general and strips away any possible source of complexity that might otherwise confuse the picture. The surface and the nanoparticle core are represented as rigid, impenetrable objects via a hard-core potential. Ligands and receptors are represented as complementary binding sites that can form a bond with a given, fixed energy. To respect the valence-limited nature of typical ligand-receptor bonds [3], each ligand and receptor is allowed to form at most one bond (this constraint can be

alleviated by increasing the receptor valency, while still keeping it to a finite value [4]). The bond (free) energy is a constant and does not depend on the state of other ligands or receptors in the vicinity, thus ruling out cooperative allosteric effects by construction. The last ingredient of the model considers that the binding sites of ligands and receptors are connected to the surface via a flexible, molecular linker and accounts for excluded volume effects and entropic stretching of the linker.

The model used by MVF is rather minimal. However, even within this simple coarse-grained model, Monte Carlo simulations clearly show that the response of the binding of a nanoparticle to a receptors-coated surface upon a change in receptors density can be made very sharp (see the original figure from Ref. [1], reproduced here in Fig. 1). The simulations by MVF further show that one can achieve a super-selective response only with multivalent nanoparticles, but not by using a monovalent binder (Fig. 1). Importantly, this feature explains previous experimental observations by Carlson et al. [5] where it was shown that a multivalent vector loaded with a drug had a sharp response to the number of receptors, becoming significant only above a certain threshold, whereas the same drug loaded on a monovalent carrier had an almost indiscriminate profile, basically killing all cells. This behaviour could have arisen from different mechanisms, in particular, cooperative binding as assumed by Carlson et al., or some complicated signalling network. Instead, the simulations by MVF point to the fact that no complex mechanism needs to be invoked, and the sharp response is a consequence of combinatorial entropy of multivalent interactions.

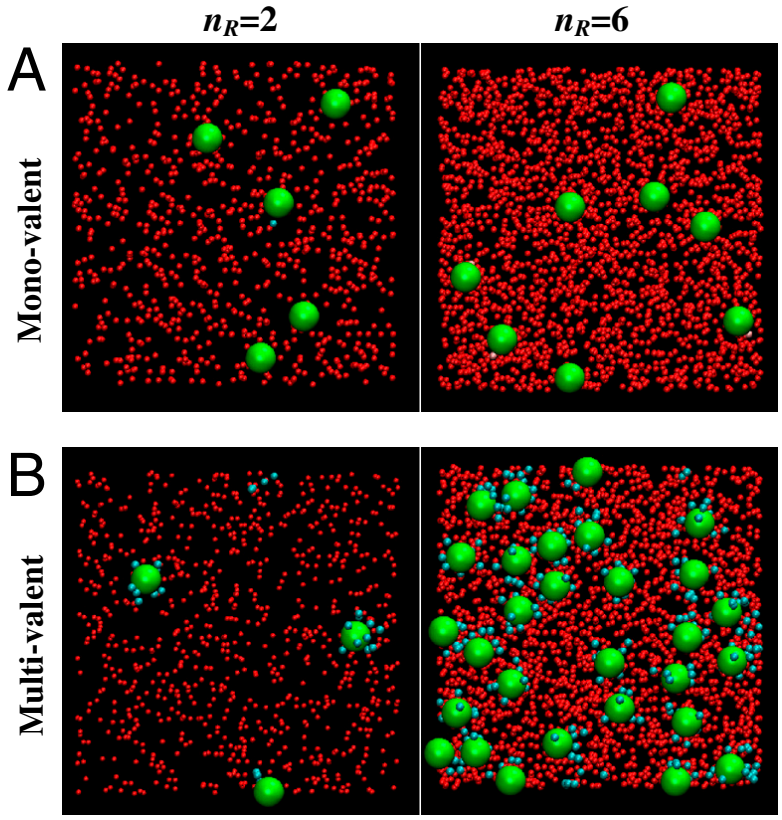

Figure 1: Simulation snapshots comparing the targeting selectivity of monovalent and multivalent guest nano-particles. The two host surfaces have receptor concentrations differing by a factor of three. (A) The monovalent guests provide little selectivity: increasing by three times the receptor coverage (colored red) just increases the average number of bound particles (colored green) by ≈ 2. (B) The multivalent nano-particles behave super selectively: an increase of the receptor coverage by a factor of three causes an almost 10-fold increase in the average number of adsorbed particles. The multivalent guests have ten ligands per particle. Figure reproduced from Ref. [1].

In order to further rationalise what was observed in simulations, in the same paper MVF also looked at recapitulating the simulation results using simple statistical mechanical models of binding, previously introduced by Kitov and Bundle [6], aimed at a simplified description of the entropy of binding based on the average geometry. Using these models, the bound partition function can be expressed as:

$$Z_{\mathrm{bind}} = \sum_{N=1}^{N_{\max}} \Omega(N) \exp(-\beta N \Delta G_{\mathrm{bond}}) \ , \tag{1}$$

where $\Omega(N)$ is the number of combinations in which a nanoparticle can form N bonds with the surface, linked to the binding entropy by Boltzmann's formula $S = -k_{\mathrm{B}} \log \Omega(N)$ with k_{B} the Boltzmann's constant, ΔG_{bond} the ligand–receptor binding free energy, and $\beta = 1/(k_{\mathrm{B}}T)$ the inverse temperature with T the absolute temperature. For a typical case where any ligand can bind to any receptor below the footprint of the nanoparticle, or receptors are mobile, Eq. (1) can be approximated as a binomial sum

$$Z_{\mathrm{bind}} = (1 + n_{\mathrm{R}} e^{-\beta \Delta G_{\mathrm{bond}}})^k - 1 \ . \tag{2}$$

This simplified partition function can be intuitively understood in that each of the k ligands can be independently either unbound or bound to any of the n_{R} receptors. Moreover, at least one bond must be present for the nanoparticle as a whole to be considered bound to the surface (hence the -1 term). MVF combined this expression with the Langmuir adsorption model, finding that the surface area fraction occupied by nanoparticles is

$$\theta = \left\langle \frac{a Z_{\mathrm{bind}}}{1 + a Z_{\mathrm{bind}}} \right\rangle , \tag{3}$$

where a is the nanoparticle activity in solution (approximately equal to the volume fraction). The theory shows that multivalent particles have a non-linear response to the surface receptor density [Eq. (1)] leading to a sharp, "super-selective" response, whereas monovalent particles exhibit only a weak response (see Fig. 2). The sharpness of the response is quantified using what MVF called the selectivity parameter $\alpha \equiv \frac{d \log \theta}{d \log n_R}$, in other words, the slope of the binding curve calculated on a log–log plot. For monovalent binding we find $\alpha \leq 1$, while multivalent binding allows a supra-linear response $\alpha > 1$, which MVF denoted as "super-selectivity".

A major result is that models that explicitly exclude allosteric cooperativity can well explain the super-selective response to a change in receptor concentration and open a potential new paradigm. From the seminal paper of Hill discussing the sharp response of oxygen binding to haemoglobin in terms of allosteric binding cooperativity [7], dating back more than a hundred years, the go-to explanation has often been to explain any sharp response with allosteric cooperativity. This approach has been criticized by others in the field [8], but still remained the paradigm. In fact, a common procedure is to fit the data to a Hill function:

$$\mathrm{Output} = \frac{\mathrm{Input}^n}{C^n + \mathrm{Input}^n} \ , \tag{4}$$

and interpret the Hill coefficient n as a sign of positive $n > 1$ or negative $n < 1$ cooperativity (with $n = 1$ characterizing independent binding). Instead, MVF showed that this sharp response can arise purely from combinatorial entropy. In essence, super-selectivity arises because of the rapid growth of the binding degeneracy $\Omega(N)$ when the number of receptors increases.

Interestingly, the statistical mechanical framework used by MVF shows that the symmetric relation between n_{R} and $\exp(-\beta \Delta G_{\mathrm{bond}})$ in Eq. (2) means that the binding response is sharp not only in terms of a change in the number of receptors, but also in the bond affinity, which in turn depends on parameters such as temperature and pH. Thus, multivalency and combinatorial entropy also provide a simple route to explain an ultra-sensitive and highly non-linear dependence of the binding strength on these latter parameters, not just on the number of receptors [9].

The practical importance of the message from the MVF paper cannot be overstated: the current paradigm of powerful biologics such as Antibody-Conjugated Drugs requires the existence of specific biomarkers, e.g., a protein or glycan, that is unique to the targeted cell type, and considerable effort is directed to find antibodies

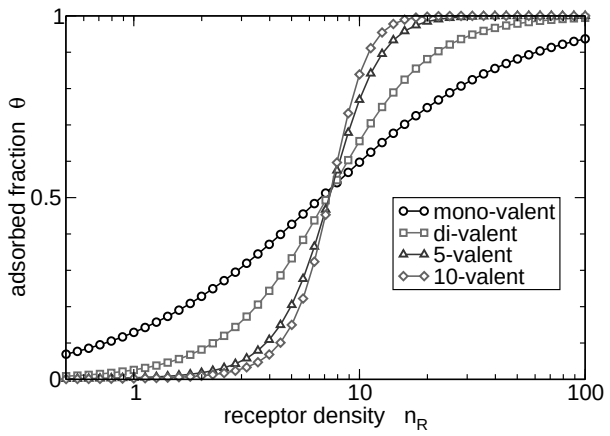

Figure 2: Adsorption profile of mono- vs multi-valent particles. Monovalent adsorption (circles) yields the familiar Langmuir isotherm. In contrast, multivalent particles display a steep, sigmoidal response. In the case shown, the binding affinity of individual bonds decreases as the valency increases from monovalent to 10-valent, such that the onset of adsorption remains roughly constant for all valencies. (Reproduced with permission from Ref. [2])

that strongly bind to a given biomarker. The physics of superselectivity outlined by MVF shows this might not be a fruitful choice if over-expression is what distinguishes target vs non-target, which is actually a very typical situation in cancer cells (e.g., the CD44 or the folic receptor). In such cases, an antibody that strongly binds to the marker will bind and kill cells indiscriminately. This insensitivity might be one of the reasons why chemotherapy is still strongly harmful to our body.

On a broader level, and in terms of long-term impact, a final crucial contribution of the MVF paper is that it spurred a wealth of activity culminating in the formulation and validation of a robust, quantitative framework to think about binding interactions and binding selectivity in multivalent systems. These systems span from functionalized polymers [10, 11] to colloids [12–14], membranes [15, 16] and even viruses [17, 18].

Moreover, by extending the statistical mechanical theory of multivalent interactions valid for arbitrary scenarios we can now quantitatively study systems with arbitrary numbers of competing ligand-receptor pairs [3, 19], off-target interactions [20], receptors that can bind to multiple ligands at once [4], grafted or mobile ligands [15, 21], or systems where binding is mediated by linkers or cofactors in solution [13, 14, 22], as well as consider the effect of different spatial distributions of receptors [3, 19, 23] and their fluctuations [24].

These extensions have facilitated the analysis of intricate scenarios under more realistic conditions pertinent to real-world applications [25]. Notably, they have demonstrated that multivalent systems are particularly advantageous when the objective is to differentiate between target and non-target entities based on disparities of populations of multiple receptor types [16, 24, 26], rather than focusing solely on individual receptors, as has been the traditional approach in the development of targeting technologies, exemplified by antibodies.

Before we conclude, we would like to provide some words of warning, highlighting the limitation of the MVF paper and its "offspring". In general, these studies and the underlying theory focus on the *equilibrium* binding behaviour of multivalent agents. Especially when applied in a biological setting, e.g., when considering targeted drug-delivery, important quantities influencing the bond-energy such as temperature, pH, nanoparticle concentration or even the population of receptors, are not constant in time. Moreover, strong forces might be present due to hydrodynamic interactions, e.g., because of blood flow. Under these conditions, whether or not local equilibrium is achieved is an important question that should be addressed on a case-by-case basis.

In practice, attaining equilibrium in terms of multivalent binding means that enough time has passed for bonds to have broken and reformed multiple times, providing the possibility to explore most if not all of the

potential bonding configurations. For weak bonds of a few $k_{\mathrm{B}}T$ in strength (including the entropic penalty), bond formation and breaking times are milli- or even micro-seconds. If the coupled biological process is on the order of seconds or more, e.g., endocytosis or cellular membrane wrapping via ligand-receptor mediated attraction, (quasi-) equilibrium is probably attained, but in other cases this might not be possible. There remains a significant gap in our understanding of kinetics of multivalent interactions. We expect that future research will focus primarily on understanding how to kinetically control selectivity, an undertaking that we anticipate will be as fruitful as the original MVF paper.

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