Soft Matter

RSC Publishing

HIGHLIGHT

The entropic impact of tethering, multivalency and dynamic recruitment in systems with specific binding groups

Cite this: *Soft Matter*, 2013, **9**, 3213

Francisco J. Martinez-Veracoechea[a] and Mirjam E. Leunissen*[b]

This Highlight Article discusses the important yet frequently overlooked entropic effects in soft matter systems that have one or more tethered binding groups. We show that the effective interactions depend sensitively on a combination of configurational, combinatorial and translational entropy factors, which have to do with the tethering of the binding groups, the binding state multiplicity in multivalent systems, and the dynamic recruitment of surface-mobile binding groups. Importantly, these entropic effects can give rise to qualitatively new behavior, *e.g.* in the phase behavior of DNA-functionalized colloids or in the targeting of ligand-functionalized nanoparticles to cell receptors. A better understanding of the thermodynamics of tethered (multivalent) bond interactions thus impacts a wide range of fields, including soft materials science, biophysics, nanomedicine and biosensing, supramolecular and colloidal self-assembly, and nanofabrication.

Received 30th November 2012
Accepted 18th January 2013

DOI: 10.1039/c3sm27766f

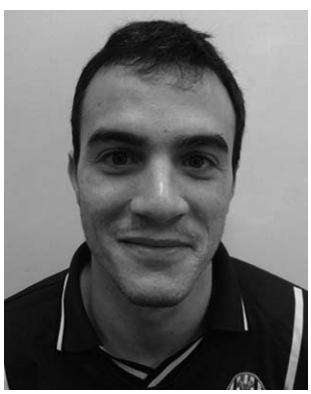
www.rsc.org/softmatter

1 Introduction

The present Highlight Article deals with a broad class of synthetic and biological systems that involve one or more tethered binding groups. Examples of such systems that have recently attracted a lot of attention are DNA-functionalized colloids and synthetic multivalent host–guest systems for self-assembly and nanofabrication purposes, as well as ligand-functionalized nanoparticles for targeted drug delivery. In the context of these systems, we will discuss at a very general level a number of important yet frequently overlooked entropic effects. To be precise: the configurational (or 'conformational') entropy, the combinatorial entropy and the translational entropy factors, which have to do with the tethering of the binding groups, the

[a]*Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK*

[b]*FOM Institute AMOLF, Science Park 104, 1098 XG, Amsterdam, The Netherlands. E-mail: m.e.leunissen@amolf.nl*



Francisco J. Martinez-Veracoechea obtained his PhD in 2009 from the School of Chemical and Biomolecular Engineering at Cornell University. He received the Austin Hooey Graduate Research Recognition Award for his contributions to the understanding of diblock copolymer phase behavior. Currently, he works as a Postdoctoral Research Associate in the Chemistry Department of the University of Cambridge with Prof. Daan Frenkel. His research interests involve the use of statistical mechanics and computer simulations to obtain design principles for targeted nanoparticles and self-assembling systems.

Mirjam Leunissen obtained her PhD with distinction in 2007, in the Soft Condensed Matter group at Utrecht University (The Netherlands). As a postdoctoral fellow, she performed experiments and numerical simulations on DNA-functionalized colloids, at the Center for Soft Matter Research (New York University), the University of Cambridge (UK) and FOM Institute AMOLF (The Netherlands). In 2011, she started the Supramolecular Interactions research group at AMOLF, which studies interactions, structures and self-organization processes that are the result of weak specific bonds in soft matter and bio-inspired systems. Recently, she was awarded a prestigious personal 'Vidi' grant and was elected to the Young Academy of the Royal Dutch Academy of Sciences.

binding state multiplicity in the case of multivalency and, in some systems, the dynamic recruitment of surface-mobile binding groups. The same concepts also apply to other systems, *e.g.* the binding of multivalent ligand–receptor complexes or supramolecular structures in bulk solution, and we will discuss these when particularly relevant. In general, we have aimed at selecting recent publications that clearly illustrate the key concepts, instead of providing a comprehensive review. Furthermore, we only discuss equilibrium aspects of the interactions and not the kinetics of their formation, which, for flexibly tethered binding groups and multivalent systems, can be interesting in their own right. In particular, we will here shed light on the current debate in the literature about the configurational, combinatorial and translational entropies, showing how one should properly account for these contributions and that they depend sensitively on the exact properties of the system under consideration, so that they should never be discarded *a priori*. An incomplete understanding of the thermodynamic aspects of multivalency and the tethering and recruitment of binding groups actually hinders the advancement in many areas of science and technology, including supramolecular, colloidal and nano-scale self-assembly, responsive materials, biosensing, immunology and disease treatment. We therefore also highlight briefly the biomedical targeting of ligand-functionalized nanoparticles and give some striking examples of how the entropic effects can give rise to entirely new behavior that could benefit the targeting selectivity of drugs, the proper self-assembly of DNA-coated colloids, or other applications.

2.1 Configurational entropy

When a binding group is tethered to a surface through a molecular construct with a certain degree of conformational freedom (Fig. 1a) the free energy that is released upon forming a bond with a complementary binding group on an opposing surface ($\Delta G_{\text{tethered}}$) will usually be smaller than the binding free energy of the same binding groups when they are free in solution ($\Delta G^0_{\text{solution}}$):

$$\Delta G_{\text{tethered}} = \Delta G^0_{\text{solution}} - T\Delta S_{\text{conf}}$$

The difference is due to an entropic cost that arises from the reduced configurational space that is available to the tethered groups after bond formation, as compared to the situation before a bond is formed between the two surfaces. This entropic cost is commonly referred to as the 'configurational' or 'conformational' entropy cost and depends on the basic properties of the tethers (length and flexibility), their relative positions on the two surfaces and the surface separation.

It was shown for systems of DNA-functionalized colloids that the quantitative description of interactions mediated by surface-tethered binding groups requires the inclusion of a significant configurational entropy cost.[1] For a typical system, the configurational entropy cost is similar in magnitude to the hybridization free energy of a free pair of DNA 'sticky ends' in solution (~5–10 k_BT) and it can thus considerably change the effective strength of these binding groups.[2] It was further demonstrated that for identical sticky ends the length and flexibility of the tether construct can indeed have a significant impact on the association properties.[3] As the configurational entropy cost only depends on the tether conformational properties and not on the character of the binding groups that are attached to their ends, insights obtained with one type of system can easily be translated to other types of systems. Ref. 4 thus gives general expressions for the configurational entropy cost incurred by rigid tethers and also shows how polymer statistics can be used to estimate the cost for more complicated tethers, such as flexible freely jointed chains.

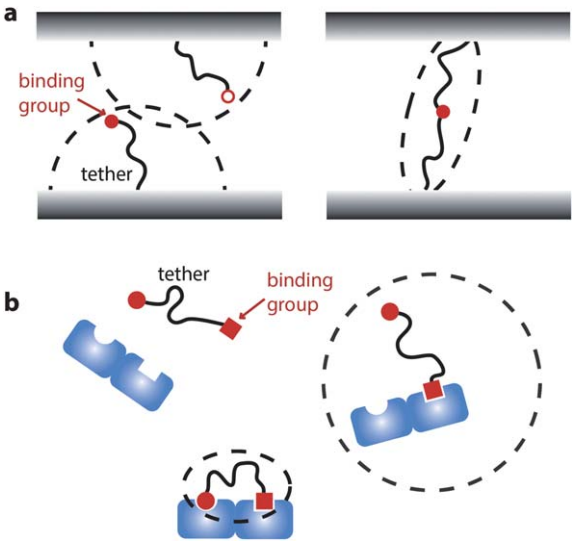


Fig. 1 Configurational entropy effects due to tethering of the binding groups. (a) The configurational space (dashed envelopes) available to surface-tethered binding groups is reduced upon bond formation, giving rise to a configurational entropy cost that can considerably change the effective binding strength. (b) Similar configurational entropy considerations apply to binding groups in bulk solution that are tethered together to form multivalent constructs, *e.g.* flexibly connected pairs of ligands that can bind to divalent receptor molecules.

Already in 1998, it was pointed out that configurational entropy effects should also play an important role when binding groups are tethered together to form poly- and multivalent constructs that exist in bulk solution[6] – a simple example is shown in Fig. 1b. Surprisingly though, there is still an ongoing controversy in the literature about the exact impact of the tether entropy, despite a host of experimental and theoretical studies on mostly divalent model systems with different types of tethers between a pair of ligands. Ref. 6 predicted that flexible tethers are unfavorable, but they are frequently found to be better than rigid tethers which supposedly have a much smaller configurational entropy cost. Recent experiments further indicate that the divalent ligand–receptor binding is not so sensitive to the length of flexible tethers, provided that they are long enough to bridge the receptor sites.[7,8] It is nevertheless difficult to draw conclusions from this about the entropic effects of the tethers, because the separate enthalpic and entropic contributions to the binding cannot be directly extracted from these experiments on protein-based receptors. Moreover, for variable tether length, the

interpretation of the binding data is further convoluted by the changing 'effective concentration' of the second ligand when it is confined within a certain volume near its binding site upon formation of the first ligand–receptor bond. The elegant experiments in ref. 5 form an exception, as the thermal profiles recorded for the temperature-dependent hybridization of synthetic DNA 'tile' nanostructures provide direct access to divalent binding enthalpies and entropies. As expected, rigid DNA scaffolds have the smallest configurational entropy cost and the highest thermal stability (Fig. 2). However, it also appears that flexible scaffolds can perform better than expected, because their increased freedom to properly orient the ligands for binding can result in a more favorable enthalpic gain that can (partially) compensate for the higher entropic cost. The controversy is further resolved by a general theoretical treatment of the tether thermodynamics,[9] which shows that multivalent binding can indeed have a weak dependence on the tether length, even if there is a significant configurational entropy cost. This treatment also clarifies how the various entropic contributions relate to the often used 'effective concentration' concept.[10]

Overall, one can conclude that configurational entropy effects will play a role in any system with tethered binding groups, but that the exact outcome will depend on the precise features of the system under consideration. The tether properties will alter the effectivity of multivalent drugs, and the interactions of particle-immobilized ligands and DNA sticky ends are also clearly affected by the additional configurational entropy cost for bond formation. In some cases, small changes in the configurational entropy cost due to stretching of the tethers are expected to cause large changes in the onset of interparticle binding[11] and computer simulations suggest that branched tethers with multiple binding groups at their ends can enhance the cell surface targeting strength of nanoparticles by a sharing of the tether's configurational entropy cost.[12] The purely entropic modulation of the effective binding strength without any structural changes in the ligand itself offers interesting possibilities for practical purposes, but it can also be a pitfall. Binding groups are frequently tethered to surfaces through polymeric linkers to facilitate their investigation with force spectroscopy and single molecule measurements. Somewhat surprisingly, the effect of tether stretching on the applied loading rate has long been worked out,[13] but the tether-dependent change of the effective binding free energy still is rarely fully considered.[14]

2.2 Combinatorial entropy

The early review of biologically relevant multivalent interactions in ref. 6 suggests design strategies for more effective pharmaceutical agents based on collections of binding groups that are tethered together. Usually, the collective binding strength of such multivalent constructs is larger than that of an individual monovalent unit, even after discounting the configurational entropy cost of the tethers. This 'multivalency effect' was first put on a proper thermodynamic footing in ref. 15 for multivalent complexes in solution. Importantly, it was shown that the multivalent binding strength is enhanced beyond a simple linear addition of the binding free energies of the monovalent constituents, due to the large number of possible permutations in the binding pattern of multivalent complexes. The larger the degeneracy in the partition function of the bound states, the larger the 'combinatorial' entropy (or 'avidity' entropy[15]) contribution to the total free energy, and the stronger the overall interaction. Collections of surface-tethered binding groups fall between the two limits depicted in Fig. 3. If $\Omega(N_1, N_2, N_B)$ is the number of distinct binding arrangements corresponding to a total of N_B bonds formed in a system with N_1 (N_2) binding groups on surface 1 (2), then when all binding groups on each of the two surfaces can interact with each other in limit I (*e.g.* long or surface-mobile tethers):

$$\Omega(N_1, N_2, N_B) = \frac{N_1!N_2!}{(N_1 - N_B)!N_B(N_2 - N_B)!}$$

and when each binding group can interact with only one of the binding groups on the other surface in limit II (*e.g.* short, immobile tethers):

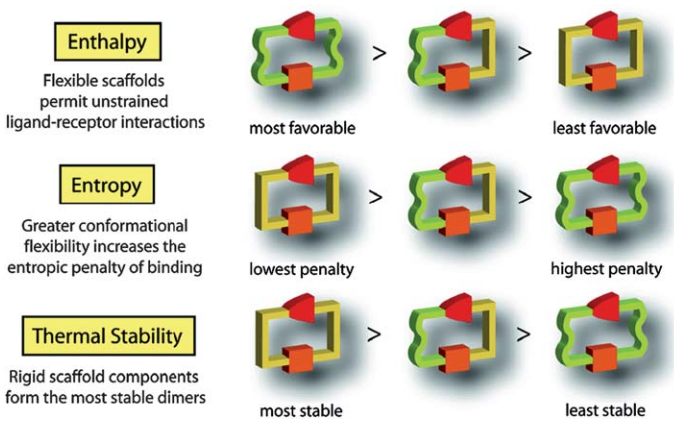


Fig. 2 Entropic and enthalpic factors for divalent DNA scaffolds with different degrees of conformational flexibility. Experiments show that rigid scaffolds have the smallest configurational entropy cost, whereas flexible scaffolds maximize the binding enthalpy by optimizing the orientation of the interaction sites. For the constructs used, the trade-off between the enthalpic and entropic contributions provides the rigid scaffolds with the largest thermal stability. Figure reprinted with permission from ref. 5. Copyright 2011 American Chemical Society.

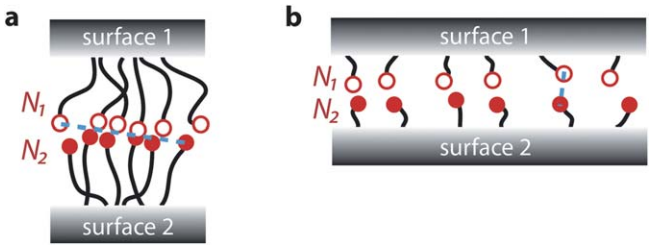


Fig. 3 The two binding limits for collections of surface-tethered binding groups. (a) Limit I: all binding groups on surface 1 can interact with all binding groups on surface 2, the bonds are inter-dependent. The dashed blue line indicates one of the most extremal bond pairs. (b) Limit II: each binding group on surface 1 can interact with only one of the binding groups on surface 2, the bonds are independent. The dashed blue line indicates one of the unique bond pairs. For both limits, the large number of possible permutations in the binding pattern gives rise to a combinatorial entropy gain that strengthens the overall interaction. Many systems lie between the two limits, such that each binding group on surface 1 can interact with a certain subset of the binding groups on surface 2.

$$\Omega(N_1, N_2, N_B) = \binom{\min(N_1, N_2)}{N_B}$$

Note that especially in the first limit the number of binding arrangements, and thus the combinatorial entropy, grows rapidly with the density of the binding groups on the surfaces. The combinatorial entropy gain further depends on the tether length and surface separation, as is illustrated by numerical studies of DNA-functionalized colloids.[2] When the surface separation decreases, several tens of possible binding partners come within reach of each DNA sticky end. The result is a stronger attraction even though the overall number of available binding groups remains the same, due to a significant combinatorial entropy gain that can effectively amount to several k_BT per bond formed. Ref. 4 explains how one can analytically calculate ligand- and DNA-mediated particle interactions while properly accounting for both the combinatorial and configurational entropy contributions.

In spite of the fact that the combinatorial entropy contribution arises naturally from a statistical thermodynamics point of view, its existence is often overlooked. This has led to a lot of confusion in the literature and to the invocation of all sorts of unlikely 'cooperativity' scenarios.[16] In order to explain the more than additive enhancement of multivalent interactions, such scenarios assume that the formation of one bond somehow changes the intrinsic binding properties for subsequent bonds, making them more favorable. Several years ago, however, it had already been observed that 'the majority of multivalent systems reported in the literature can be analyzed using a statistical, non-cooperative binding mode'.[17] Possibly, the only clear example of real positive allosteric cooperativity is that of oxygen binding to hemoglobin, where the protein changes its conformation upon binding of the first molecule. The sharp dissociation transitions of the currently very popular DNA-functionalized particle systems are also sometimes incorrectly taken as a hallmark of cooperativity, as is explained in ref. 2. Additionally, the contributions of multivalency and cooperativity in the adsorption of multivalent molecules to interfaces are deconvoluted in ref. 18, using an effective concentration concept.

Importantly, combinatorial entropy effects can give rise to qualitatively new behavior, other than the establishment of strong interactions with collections of weak ligands. For instance, colloidal particles connected by long DNA tethers[19] or emulsion droplets bridged by telechelic polymers[21] phase separate into dilute and dense phases (Fig. 4). Whereas the overall number of bonds remains the same, there are many more ways to arrange these bonds in the dense network phase. The increase in combinatorial entropy (or 'bond disorder'[19]) thus overcompensates the loss of translational entropy upon demixing. Such entropy-dominated phase behavior is likely to be quite robust with respect to the properties of the constituents, which is advantageous for applications. In the context of drug targeting, it was suggested that nanoparticles with multiple weak ligands are more selective, because a stable connection requires a certain minimum number of ligands to be bound.[22] Even more powerful is an approach that explicitly takes advantage of the highly non-linear increase in the number of possible binding arrangements – and thus the combinatorial entropy – as the surface concentration of receptors increases.[20] In this case, a subtle interplay of valency, strength and concentration gives a sharp transition in the fraction of bound ligand-functionalized nanoparticles as a function of the receptor concentration (Fig. 5). This 'super-selective' targeting method could be used for diseased cells that overexpress certain receptors as compared to healthy cells.

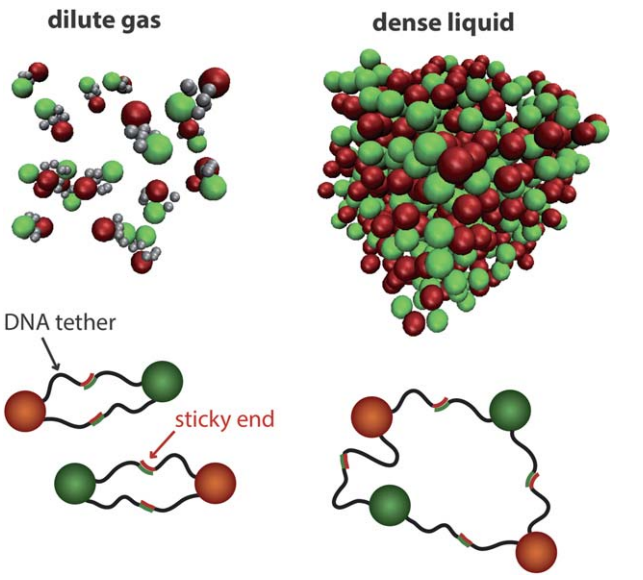


Fig. 4 Colloidal phase separation driven by combinatorial entropy effects. Simulation snapshots of low-density (left) and high-density (right) phases of colloidal 'hard spheres' (red and green) coated with long complementary DNA tethers (shown as small grey spheres in left snapshot). As is schematically illustrated below the snapshots, there are many more ways to arrange the same number of bonds in the dense phase, leading to a significant combinatorial entropy gain and spontaneous phase separation. Figure adapted with permission from ref. 19. Copyright 2008 APS Journals.

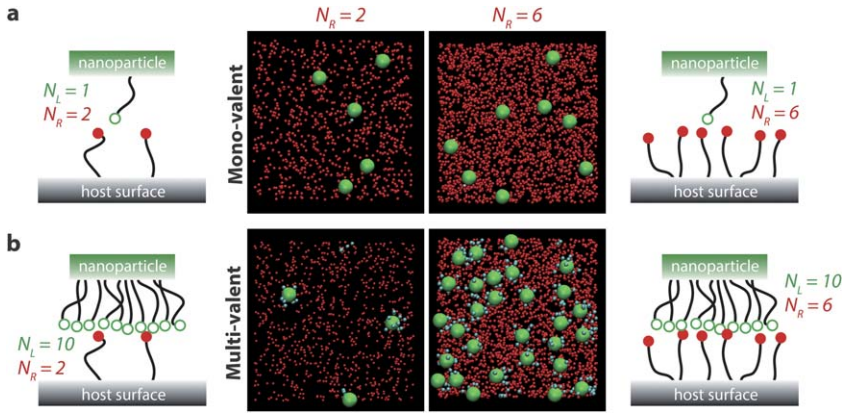


Fig. 5 Combinatorial entropy based 'super-selective' targeting of multivalent nanoparticles. Simulation snapshots comparing the surface adsorption of (green) monovalent nanoparticles ($N_L = 1$, top row (a)) and multivalent nanoparticles carrying $N_L = 10$ ligands, which each are 5 k_BT weaker than the monovalent ligand (bottom row (b)). The receptor density on the host surface was varied by a factor of three, such that $N_R = 2$ (left column) or $N_R = 6$ (right column) receptors (red) were available for bond formation in the nanoparticle contact area. (a) The monovalent nanoparticles provide little selectivity with respect to the host surface receptor density: the average number of bound nanoparticles only increases by a factor of 1.8 when the receptor coverage is three times higher. (b) The multivalent nanoparticles discriminate super-selectively between low and high receptor densities: a three times higher receptor density gives on average a 10-fold increase of the number of bound nanoparticles. This sharp transition is due to the strong increase in the number of possible ligand–receptor bond arrangements, and hence the combinatorial entropy, with increasing receptor density. Figure adapted with permission from ref. 20. Copyright 2011 National Academy of Sciences.

2.3 Translational entropy

When the binding groups on a surface are mobile, instead of being tethered at fixed positions, the loss in translational entropy of these groups upon bond formation also needs to be taken into account (Fig. 6), in addition to the combinatorial and configurational entropy contributions discussed above. A typical example is the interaction between binding sites on nanoparticles or viruses and mobile receptors on the fluid cell membrane. Clearly, scenarios involving mobile binding groups are of great relevance for biology and nanomedicine and a proper understanding of the underlying physics can bolster the continuous fight against disease. Moreover, research on B-type white blood cells shows that binding group mobility can cause complex behavior. In this particular case, an immune response could be induced by exposing the cells to a multivalent polymeric construct that 'recruits' mobile receptors on the cell membrane.[23]

Some progress has been made in unraveling the respective roles of the translational and configurational entropy effects in the adsorption of proteins onto membranes.[24–27] Charged groups on the proteins interact with collections of oppositely charged lipids, which thus effectively act as mobile receptors in the fluid membrane. It appears that adsorbed proteins can influence the spatial arrangement of mobile receptors dramatically. Interestingly, at low protein coverage the receptor mobility is invariably seen to increase the adsorption strength compared to immobile receptors,[28] but at higher protein coverage the adsorption is increasingly hindered, thus preventing full coverage of the membrane.[25] In spite of these protein model studies and the importance of systems with mobile binding groups in the area of nanomedicine as well as in numerous other fields, many general aspects still remain to be uncovered. There is a need for simple theoretical models that simultaneously account for the configurational, combinatorial

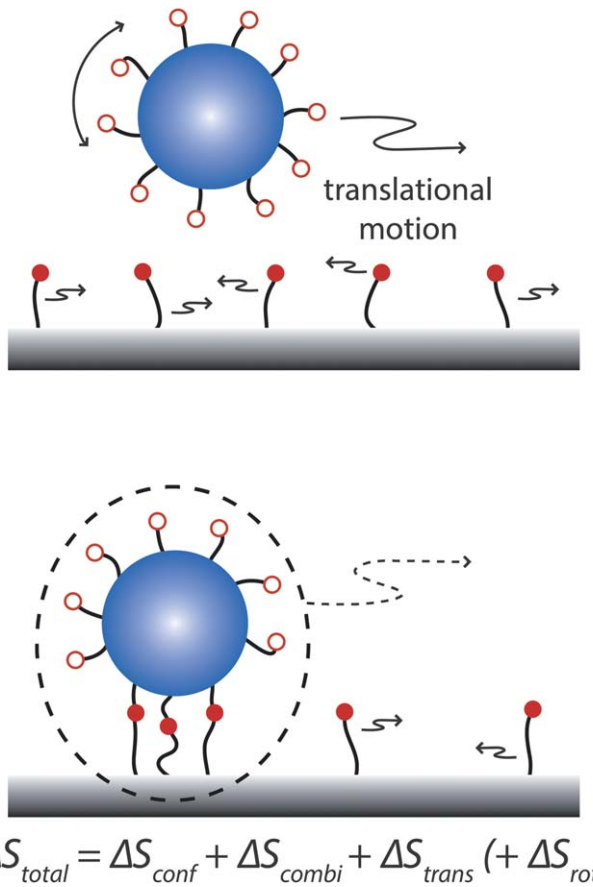


Fig. 6 Translational entropy effects due to binding group mobility. When a multivalent object (*e.g.* a ligand-functionalized nanoparticle) binds to a number of mobile binding groups on a surface (*e.g.* receptors on the cell membrane), the latter lose their translational independence. This gives rise to a translational entropy loss (ΔS_{trans}) that is associated with the surface binding groups and the nanoparticle, in addition to the configurational (ΔS_{conf}) and combinatorial (ΔS_{combi}) entropy contributions of the bonds. The nanoparticle itself may also lose some rotational entropy upon binding (ΔS_{rot}, not discussed any further here).

and translational entropy effects. Such models are bound to reveal qualitatively new behavior. From a practical point of view, this could mean more powerful binding-group-recruiting pharmaceutical or self-assembly agents, drug delivery vehicles that are more sensitive to the receptor concentration, surfaces that self-limit the number of adsorbed particles, and possibly even particles that can auto-regulate their effective valency.

3 Recent examples from the literature

We conclude this Highlight with a selection of recent research papers and reviews that contain examples of what can be done with multivalency and the tethering and dynamic recruitment of binding groups, in addition to the examples that were already used to illustrate the key concepts above.

Ref. 29 and 30 respectively use the difference in configurational entropy cost of intra- and inter-particle bonds, and the difference in combinatorial entropy gain for different tether lengths to improve the self-assembly properties of DNA-functionalized colloids. The reviews of ref. 31–33 provide a multitude of examples of multivalent systems in supramolecular chemistry, biomimetic architecture and nanofabrication. Both ref. 34 and 35 study the binding of multivalent species to multivalent substrates, using combinatorial entropy effects in a multivalent 'dendrimer display' format,[34] or a format that discriminates between different-valency species.[35] Ref. 36 focusses on the idea of 'self-assembled multivalency' in which ligands are dynamically recruited and organized instead of being attached to prefabricated scaffolds. Finally, ref. 37 and 38 provide elegant examples of multivalent therapeutics for selective tumor cell targeting, while ref. 39 illustrates the power of drug targeting through the active functionalization of multivalent nanoparticles. We point out that it is actually believed that multivalent ligand-coated nanoparticles that target specific cell receptors can revolutionize the medical field, as it enables the treatment of cells at the individual level.[40,41] Tumor cells frequently overexpress many types of receptors and this strategy is thus particularly attractive for the treatment of cancer.[42,43] Additionally, targeted nanoparticles carrying drugs can be combined with imaging agents to provide 'theranostic' medicine, in which the diseased tissue is simultaneously imaged, diagnosed and treated.[44–46]

In conclusion, we expect that a better understanding of the thermodynamics of tethered (multi-)bond interactions will have an immediate impact on the advancement of many fields, ranging from nanomedicine and biosensing, to supramolecular and colloidal self-assembly, and to nanotechnology in general.

Acknowledgements

FJMV was supported by the ERC (Advanced Grant agreement 227758). MEL was supported by the research program of the Foundation for Fundamental Research on Matter (FOM), which is part of the Netherlands Organization for Scientific Research (NWO). We thank Yves L. A. Rezus for a critical reading of the manuscript.

References

1 R. Dreyfus, M. E. Leunissen, R. Sha, A. V. Tkachenko, N. C. Seeman, D. J. Pine and P. M. Chaikin, *Phys. Rev. Lett.*, 2009, **102**, 048301.
2 M. E. Leunissen and D. Frenkel, *J. Chem. Phys.*, 2011, **134**, 084702.
3 M. E. Leunissen, R. Dreyfus, R. Sha, N. C. Seeman and P. M. Chaikin, *J. Am. Chem. Soc.*, 2012, **132**, 1903–1913.
4 P. Varilly, S. Angioletti-Uberti, B. M. Mognetti and D. Frenkel, *J. Chem. Phys.*, 2012, **137**, 094108.
5 J. Nangreave, H. Yan and Y. Liu, *J. Am. Chem. Soc.*, 2011, **133**, 4490–4497.
6 M. Mammen, S.-K. Choi and G. M. Whitesides, *Angew. Chem., Int. Ed.*, 1998, **37**, 2754–2794.
7 E. T. Marck, P. W. Snyder, R. Perez-Castillejos and G. M. Whitesides, *J. Am. Chem. Soc.*, 2011, **133**, 11701–11715.
8 E. T. Marck, P. W. Snyder, R. Perez-Castillejos, B. Bilgicer, D. T. Moustakas, M. J. Butte and G. M. Whitesides, *J. Am. Chem. Soc.*, 2012, **134**, 333–345.
9 R. S. Kane, *Langmuir*, 2010, **26**, 8636–8640.
10 D. J. Diestler and E. W. Knapp, *Phys. Rev. Lett.*, 2008, **100**, 178101.
11 C.-Z. Zhang and Z.-G. Wang, *Langmuir*, 2007, **23**, 13024–13039.
12 S. Wang and E. E. Dormidontova, *Soft Matter*, 2011, **7**, 4435–4445.
13 E. Evans and K. Ritchie, *Biophys. J.*, 1999, **76**, 2439–2447.
14 N. W. Moore and T. L. Kuhl, *Biophys. J.*, 2006, **91**, 1675–1687.
15 P. I. Kitov and D. R. Bundle, *J. Am. Chem. Soc.*, 2003, **125**, 16271–16284.
16 M. Reynolds and S. Perez, *C. R. Chim.*, 2011, **14**, 74–95.
17 A. Mulder, J. Huskens and D. N. Reinhoudt, *Org. Biomol. Chem.*, 2004, **2**, 3409–3424.
18 J. Huskens, A. Mulder, T. Auletta, C. A. Nijhuis, M. J. W. Ludden and D. N. Reinhoudt, *J. Am. Chem. Soc.*, 2004, **126**, 6784–6797.
19 B. Bozorgui and D. Frenkel, *Phys. Rev. Lett.*, 2008, **101**, 045701.
20 F. J. Martinez-Veracoechea and D. Frenkel, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 10963–10968.
21 A. Zilman, J. Kieffer, F. Molino, G. Porte and S. A. Safran, *Phys. Rev. Lett.*, 2003, **91**, 015901.
22 S. Wang and E. E. Dormidontova, *Biomacromolecules*, 2010, **11**, 1785–1795.
23 A. H. Courtney, E. B. Puffer, J. K. Pontrello, Z.-Q. Yang and L. L. Kiessling, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 2500–2505.
24 A. Hinderliter and S. May, *J. Phys.: Condens. Matter*, 2006, **18**, S1257–S1270.
25 V. P. Zhdanov and B. Kasemo, *Biophys. Chem.*, 2010, **146**, 60–64.
26 R. S. Dias and P. Linse, *Biophys. J.*, 2008, **94**, 3760–3768.
27 S. Loew, A. Hinderliter and S. May, *J. Chem. Phys.*, 2009, **130**, 045102.
28 S. Tzlil and A. Ben-Shaul, *Biophys. J.*, 2005, 2972–2987.

29 M. E. Leunissen, R. Dreyfus, F. C. Cheong, D. G. Grier, R. Sha, N. C. Seeman and P. M. Chaikin, *Nat. Mater.*, 2009, **8**, 590–595.
30 B. M. Mognetti, M. E. Leunissen and D. Frenkel, *Soft Matter*, 2012, **8**, 2213–2211.
31 J. D. Badjic, A. Nelson, S. J. Cantrill, W. B. Turnbull and J. F. Stoddart, *Acc. Chem. Res.*, 2005, **38**, 723–732.
32 J. Huskens, *Curr. Opin. Chem. Biol.*, 2006, **10**, 537–543.
33 C. Fasting, C. A. Schalley, M. Weber, O. Seitz, S. Hecht, B. Koksch, J. Dernedde, C. Graf, E.-W. Knapp and R. Haag, *Angew. Chem., Int. Ed.*, 2012, **51**, 10472–10498.
34 M. M. C. Bastings, B. A. Helms, I. van Baal, T. M. Hackeng, M. Merkx and E. W. Meijer, *J. Am. Chem. Soc.*, 2011, **133**, 6636–6641.
35 T. Andre, A. Reichel, K.-H. Wiesmuller, R. Tampe, J. Piehler and R. Brock, *ChemBioChem*, 2009, **10**, 1878–1887.
36 A. Barnard and D. K. Smith, *Angew. Chem., Int. Ed.*, 2012, **51**, 6572–6581.
37 A. Joshi, D. Vance, P. Rai, A. Thiyagarajan and R. S. Kane, *Chem.–Eur. J.*, 2008, **14**, 7738–7747.
38 C. B. Carlson, P. Mowery, R. M. Owen, E. C. Dykhuizen and L. L. Kiessling, *ACS Chem. Biol.*, 2007, **2**, 119–127.
39 Y. J. Liu, *et al.*, *J. Nucl. Med.*, 2011, **52**, 1956–1963.
40 Y. Yan, G. K. Such, A. P. R. Johnston, J. P. Best and F. Caruso, *ACS Nano*, 2012, **6**, 3663–3669.
41 J. R. Heath and M. E. Davis, *Annu. Rev. Med.*, 2008, **59**, 251–265.
42 S. B. Willingham, J.-P. Volkmer, A. J. Gentles, D. Sahoo, P. Dalerba, S. S. Mitra, J. Wang, H. Contreras-Trujillo, R. Martin, J. D. Cohen, P. Lovelace, F. A. Scheeren, M. P. Chao, K. Weiskopf, C. Tang, A. K. Volkmer, T. J. Naik, T. A. Storm, A. R. Mosley, B. Edris, S. M. Schmid, C. K. Sun, M.-S. Chua, O. Murillo, P. Rajendran, A. C. Cha, R. K. Chin, D. Kim, M. Adorno, T. Raveh, D. Tseng, S. Jaiswal, P. O. Enger, G. K. Steinberg, G. Li, S. K. So, R. Majeti, G. R. Harsh, M. van de Rijn, N. N. H. Teng, J. B. Sunwoo, A. A. Alizadeh, M. F. Clarke and I. L. Weissman, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 6662–6667.
43 J. D. Heidel and M. E. Davis, *Pharm. Res.*, 2011, **28**, 187–199.
44 M. Shokeen, E. D. Pressly, A. Hagooly, A. Zheleznyak, N. Ramos, A. L. Fiamengo, M. J. Welch, C. J. Hawker and C. J. Anderson, *ACS Nano*, 2011, **5**, 738–747.
45 T. Lammers, S. Aime, W. E. Hennink, G. Storm and L. L. Kiessling, *Acc. Chem. Res.*, 2011, **44**, 1029–1038.
46 K. Fan, C. Cao, Y. Pan, D. Lu, D. Yang, J. Feng, L. Song, M. Liang and X. Yan, *Nat. Nanotechnol.*, 2012, **7**, 459–464.