Analyzing the origins of receptor–ligand adhesion forces measured by the scanning force microscope

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Enthalpic approaches have been shown to be of value in the simulation of scanning force microscope (SFM) force–distance experiments. We show that for streptavidin, adiabatic mapping with lenient minimization convergence criteria can produce useful data for the comparative analysis of different ligands. The lenient mapping protocol profiles the undocking pathway in a fraction of the time required for other methods presented in the literature. Unbinding pathways and hydrogen bonding patterns for three ligands are predicted in a total of 74 computer-hours using a single processor of a Hewlett-Packard J-210. Hence this method allows the analysis of SFM ligand rupture pathways with a low computational overhead and also importantly suggests further avenues of biophysical experimental investigation and data interpretation.

Introduction

Molecular interactions are key to the processes of life. It is important to understand the fundamental mechanisms of such interactions to further our comprehension of biological processes. One technique to study the strength of these interactions is the scanning force microscope (SFM) force–distance experiment,[1] which measures the force required to rupture the bonds between receptor and ligand. Utilising this technique intermolecular forces as low as 10 pN, corresponding to individual hydrogen bonds, have been resolved.[2] Complementary techniques can be used to elucidate the origin of particular interactions, for example those between amino acid side chains and ligand chemical moieties. This is achieved by experimental techniques such as site directed mutagenesis (*e.g.* ref. 3) and comparative studies examining the behaviour of ligand analogues (*e.g.* ref. 4). Computer simulations of these interactions allow the investigation of atomistic behaviour, revealing unique insights into the contributions of individual atoms and bonds to complex formation. However, current techniques for these simulations suffer from an unacceptably heavy demand on computer time to model real systems (multimeric proteins, over millisecond time scales). Here we present a fast computational methodology for the rapid prediction and evaluation of molecular interactions in the scanning probe microscopy force–distance experiment. The methodology also has broad applications for the study of receptor–ligand interactions, and will be a useful aid in a milieu of biophysical investigations, such as suggesting useful candidate amino acids for site-directed mutagenesis, and aiding in the design of novel drug–receptor interactions.

Avidin is a glycoprotein obtained from egg white, streptavidin is a non-glycosylated protein derived from *Streptomyces avidinii*; these proteins are structurally similar and both bind the vitamin biotin, shown in Fig. 1(a), with very high affinity.[5] The streptavidin–biotin system has an affinity constant of 10^{15} l mol^{-1},[6] the highest known: it contains four binding sites for biotin, one on each of the monomeric subunits. This high

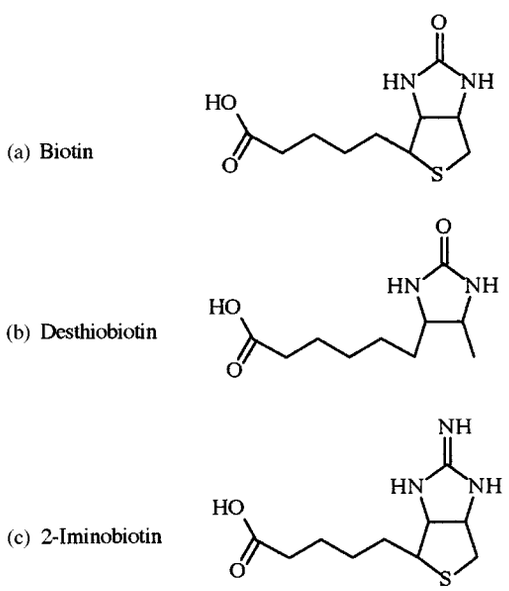

Fig. 1 The structure of biotin and two of its analogues. (a) Biotin (BTN). (b) Desthiobiotin (dBTN), where the lower ring is now open due to the lack of a sulfur atom. (c) Iminobiotin (iBTN), where the carbonyl oxygen of the ureido group is replaced by a nitrogen.

specificity and the ability to bind multiple ligands, coupled with the small size of biotin, has led to it being extensively utilised in bioscience applications. Weber *et al.*[7] have derived the structure of the complex using X-ray crystallography and the binding pocket was shown to involve a number of direct biotin–amino acid hydrogen bonds. This extensive ligand–receptor hydrogen bond network is seen as the main contributor to the extraordinarily high affinity of the system. Numerous analogues of biotin exist naturally and many more have been generated through chemical modification. Two are shown in Fig. 1(b) and (c) respectively; desthiobiotin, which lacks the

sulfur atom, and 2-iminobiotin, which has an imino group replacing the keto group of the ureido ring of biotin. The affinity constant for streptavidin–desthiobiotin is 5×10^{13} l mol^{-1} [8] and for iminobiotin it is approximately 10^{8} l mol^{-1}.[3] The iminobiotin was modelled unprotonated, as this is reported to be the only form bound by streptavidin.[9]

It is desirable to both predict the rupture forces for a series of ligands and explore the underlying mechanisms. Several workers have investigated experimental ligand rupture data through simulation. This has often been performed through a molecular dynamics (MD) protocol. Grubmüller *et al.* demonstrated a wide variety of biotin–side chain interactions in their MD investigations of biotin interacting with a water saturated streptavidin monomer.[10] Also using MD, Izrailev predicted a slip-stick process of unbinding between avidin and biotin.[11] Molecular dynamics have also been used to study the stretching by the SFM of dextran molecules,[12] and similarly xanthan. Despite these successes the molecular dynamics approach is not an ideal technique to simulate the rupture experiment since the time scales of simulation and experiment differ by at least five orders of magnitude.

We have previously used adiabatic mapping to simulate the forced undocking of biotin from the streptavidin monomer.[13] In adiabatic mapping the separation between receptor and ligand is incrementally increased and then the resulting structure minimized, the minimization being terminated according to a set convergence criterion. The value of the convergence criterion controls the amount of minimization performed. Previously we have used a very strict convergence criterion, demanding a high degree of minimization of the conformation before termination, which necessitates a high computational overhead. Here we further explore the adiabatic technique by investigating the effects of relaxing the convergence criterion, thus lowering the computational overhead, but also leading to less minimized conformers. This allows the rapid exploration of unbinding phenomena in days, rather than the months of computational time necessitated by other techniques.

Results and discussion

With the strict convergence criterion the adiabatic mapping of the biotin rupture experiment took a total of 900 hours. With the less strict criterion the simulation took 25 hours. The adiabatic measurements of the rupture pathways of the desthiobiotin and iminobiotin ligands took 25 and 24 hours, respectively.

In order to compare the behaviour of the biotin ligand during undocking with different minimization conditions we calculated the root mean square difference (RMSD) of the ligand conformations after each increase in separation. For the strict minimization conditions the biotin quickly reaches a maximum RMSD of 0.32 nm at a separation increase of 1.39 nm before decreasing to 0.27 nm at 2 nm, after which it slowly rises to a final value of 0.28 nm. In contrast, with lenient minimization conditions the RMSD of biotin conformation is approximately linear, rising slowly to a maximum of 0.15 nm at the end of the simulation. These results demonstrate the greater degree of conformational flexibility allowed by stricter minimization conditions, as much more of the conformational space and energy landscape is explored. In fact, the RMSD results for the strict run show some correlation with the energy profile of the undocking, which would suggest that the biotin conformation provides a significant contribution to the undocking process. The difference in the results suggest that an alternative reaction coordinate was explored using the two different conditions. This may in part be due to the extra degrees of freedom mentioned above, allowing the biotin and streptavidin to rearrange their conformations so that the initial contacts in the binding pocket are maintained for longer in the strict run. As the undocking protocol increases the separation between the constrained atoms it may be that there is a degree of accommodation in the strict run, resulting in both an elongation of the beta barrel of the streptavidin monomer and the alkyl chain of the biotin.

In order to investigate the differences in the number of iterations needed to undock the biotin from the binding pocket and the conformational freedom of the biotin during undocking, the position of the biotin ring carbonyl and carboxyl moieties relative to the backbone C_α carbon of Phe[130] was calculated for both the strict and lenient runs and can be seen respectively in Fig. 2(a) and (b). Comparing the two graphs we can see that the distance from carboxyl and carbonyl (the shaded area) is always greater in the strict run. This indicates that the biotin in the strict run is more extended. There is greater variance in position with respect to separation increase in the strict run, especially comparing the carbonyl atom positions, which shows greater conformational flexibility. We also see that in the lenient run the separation between the biotin and the binding pocket increases at a faster rate than in the strict run. This is illustrated by the difference in constrained separation increase that is necessary to increase the separation between the carbonyl oxygen of the ureido ring, O3, and the binding pocket. In the strict run this is 0.84 nm, whilst in the lenient run it is 0.62 nm. This again indicates that a different reaction coordinate was traversed, or that changes in the conformation of the structures in the strict run were made to accommodate the distance restraints, allowing the persistence of initial binding pocket contacts.

To assess whether the same interactions occur in each of the undocking pathways followed, we can compare the energy profiles of the two runs. The strict run energy can simply be plotted against the increase in separation, however due to the difference of the conformational flexibility between this and the lenient run, it is necessary to have a consistent point of reference for the pathway. As we have demonstrated previously that the O3 moiety dominates the interaction energetics of this pathway,[13] we chose to synchronize the pathways by adjusting for the difference in the position of the carbonyl oxygen (O3) of biotin. The lenient run energy was plotted against this adjusted value. The x axes of the two runs are displayed from the initial position to the moment of undocking for each run. This comparison is shown in Fig. 2(c). It can be seen from this comparison that there are many similar features in both energy profiles, such as the two peaks at 1 and 1.3 nm in the strict run and at 0.6 and 1 nm in the lenient run. The actual values of the energy profiles can be seen to be roughly comparable over the first two-thirds of the graph, but markedly different at the end. We would expect to see the lenient energies being slightly higher due to the reduced minimization applied, and this is indeed the case. The large deviation at the end of the run is due to the streptavidin adopting a higher energy resting conformer, due to the less strict minimization criterion used. These results suggest that the same undocking pathway is followed using both convergence criteria.

The hydrogen bonding interactions between the ligands and steptavidin during the unbinding simulations were calculated and can be seen in Fig. 3. If we first compare the strict and lenient biotin runs, we can see that the initial interactions of the carbonyl oxygen (O3) of the biotin ureido group to the binding pocket amino acids break in the same order, and that the interaction between the carboxyl group (O2) and Ser[88] is almost identical. The O3–Ser[45] interaction is also seen in both runs, however, the last two interactions of the strict run, between O3 and Ser[88], and S1 and Thr[115] are not seen. This may again indicate a different reaction coordinate in the latter third of the simulation. In general, the O3 interactions break at a lower separation increase in the lenient run. As can be seen by a comparison of the carbonyl atom positions in Fig. 2(a) and (b), this is because the leniently minimized system does not allow the biotin to fully relax and stretch back into the binding pocket

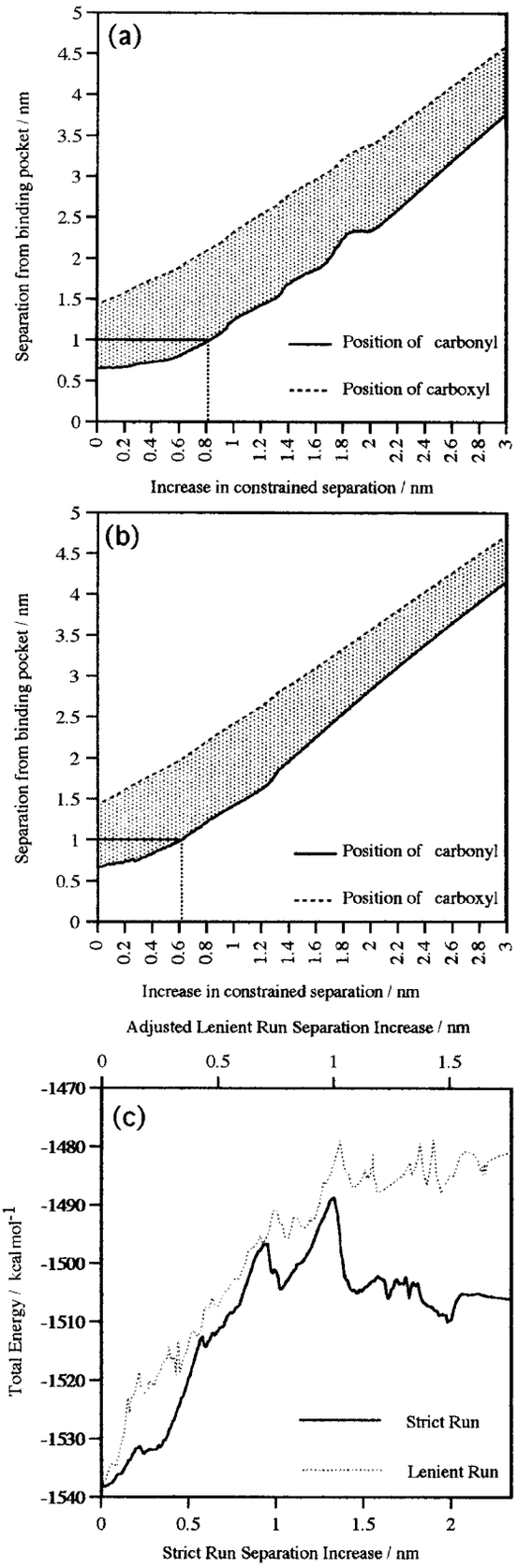

Fig. 2 Comparison of strict and lenient biotin position and energies. The position of biotin relative to the distal end of the binding pocket in the strict (a) and lenient (b) runs. The position of the ureido carbonyl oxygen (O3) is indicated by solid lines, the position of the carboxyl moiety of the alkyl (O2) by dashed lines. The shaded area indicates the elongation of the biotin. A dotted line indicates the point where the separation between O3 and the binding pocket reaches 1 nm. The positions are all relative to the C_α of Phe[130]. (c) The system energy for the strict and lenient runs. The x axes are adjusted to cover the same unbinding pathway.

after the ligand has been moved. These findings lead us to conclude that we are exploring approximately the same unbinding pathway in both runs.

It can be seen from Figs. 3(b) and 3(c) that the interactions of biotin and desthiobiotin are very similar, as would be expected due to the fact that the O3 carbonyl atom is present in both. The fact that some hydrogen bonds break at a higher separation increase in the desthiobiotin undocking may be due to the extra degrees of freedom of rotation of the ureido group, due to the lack of the ring-closing sulfur atom. The interactions of iminobiotin [Fig. 3(d)] have many similarities to that of the other two ligands, but the initial structure of the binding pocket is different, in that the main contacts made by the ureido ring are between Asp[128] and the imino and N2 moieties. Initial contacts are also made between Ser[27] and the ureido ring and Ser[88] and the carboxyl group, as in the other ligands. During undocking we see contacts between N2 and Ser[45] and Val[47], and N3 and Tyr[43], comparable to those made in the other ligands, but no contact between the imino group and Ser[45].

Due to the distortion of the energy curves for the lenient runs' by the convergence criteria used, it is not possible to predict a maximum rupture force for the ligands.[14] In order to gain some insight into the forces during the unbinding event we have investigated the energetic contributions of the hydrogen bonding interactions. The hydrogen bond interaction energy was modelled as a Lennard–Jones potential, *i.e.* a sixth power decaying field [eqn. (1)].

$$E_{\text{hbond}} = \sum \frac{1}{(\text{Donor} - \text{Acceptor Separation})^6} \qquad (1)$$

The sum of the hydrogen bonding energies for each ligand were calculated and are shown in Fig. 3(e). The curves show that, as expected, the biotin and desthiobiotin curves are quite similar, with biotin generally having a higher interaction energy, and a significantly greater energy at the end of the undocking. Iminobiotin has a lower interaction energy, with the hydrogen bonds generally being of shorter duration and having higher donor–acceptor separations. The total sum of interaction energies for biotin, desthiobiotin and iminobiotin were calculated and found to be in the ratio 1 : 0.7 : 0.5 respectively. The ratio of rupture forces found in experiment are 1 : 0.8 : 0.5,[15] using avidin as a receptor. This data then shows qualitative agreement in the ratios, although a different receptor is used they are structurally similar. The total time for this prediction of the three rupture pathways was 74 hours, a significant improvement in computational overhead.

Previously we have shown that adiabatic mapping can successfully predict the rupture force for a ligand–receptor interaction. The results presented here indicate that a comparable undocking pathway can be explored using less strict convergence criteria and therefore be analyzed with much reduced computational overhead. Analysis of molecular interactions enforces the importance of hydrogen bonding in the rupture forces for the streptavidin system. The results also show that it is not possible to use this technique to directly predict the rupture force of an SFM experiment.

Conclusions

We have shown that adiabatic mapping with lenient minimization convergence criteria profiles the same rupture pathway as the slower, strict protocol. However, the energies determined are distorted by the magnitude of the criteria. The lenient mapping protocol profiles the undocking pathway with a much reduced computational overhead compared to other methods presented in the literature. Subsequent analysis of the hydrogen bonding patterns permits the qualitative prediction of interaction energies for the streptavidin protein, but not the prediction of the SFM rupture force. Relative rupture energies for

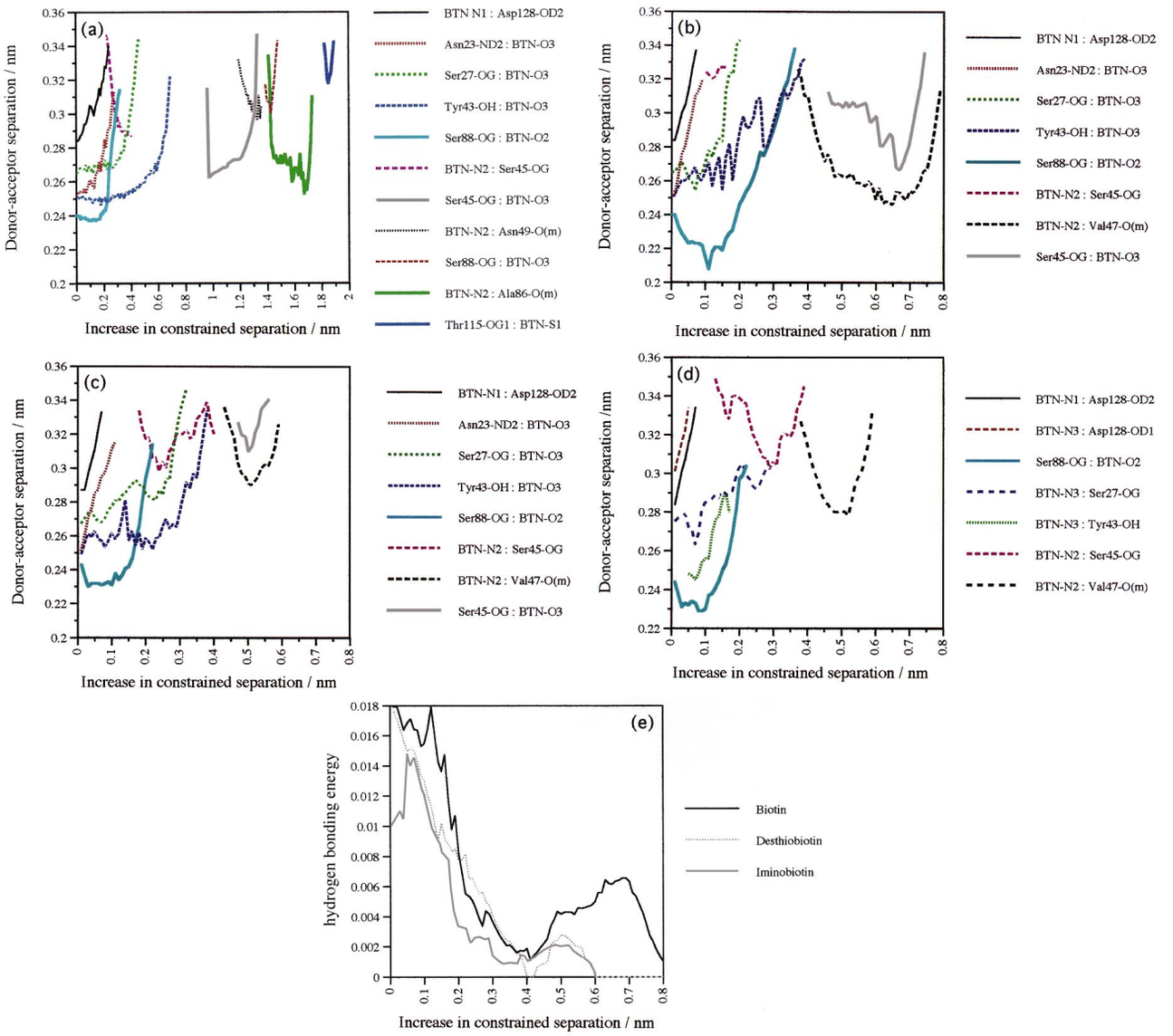

Fig. 3 Hydrogen bonding patterns and energies. (a) Streptavidin–biotin undocking with strict convergence criterion. (b) Streptavidin–biotin undocking with lenient convergence criterion. (c) Streptavidin–desthiobiotin undocking with lenient convergence criterion. (d) Streptavidin–iminobiotin undocking with lenient convergence criterion. (e) The hydrogen bonding energy of the three ligands undocked with lenient convergence criterion.

three different ligands were predicted in a total simulation time of 74 computer-hours.

We have demonstrated a method of improving the significant computational overhead involved in the analysis of SFM ligand rupture experiments. The method is a useful aid to suggest further avenues of experimental investigation and in the interpretation of the results. The techniques are also valid for studying the fundamental process of structural recognition.

Experimental

From the X-ray crystallographic, or other suitable starting structure, the ligand is undocked from its binding site by increasing the separation between it and its host in increments. After each increment the system is energy minimized with constraints applied to maintain the ligand/receptor separation. The convergence criterion for these minimizations during the undocking is specified as eqn. (2), where D is the current

$$D = \begin{cases} 0.1D_{\max} & \text{for } I \le 0.1I_{\max} \\ D_{\max}\left(\dfrac{I}{I_{\max}}\right) & \text{for } I > 0.1I_{\max} \end{cases} \quad (2)$$

convergence criterion, $D_{\max}$ is the target maximum criterion, I is the iteration number and $I_{\max}$ is a target limit of iterations permitted. D is also constrained with a lower limit of $D_{\max}/10$. Convergence is measured as the maximum of the derivatives of the atomic energies (the forces). After each minimization the energy of the system and position of the atoms is calculated and recorded for analysis. The PULMIN algorithm and full details of the constraint terms and general method for this adiabatic mapping are detailed in our first report.[13]

The starting structures used here are based on the crystallographic data of the streptavidin (monomer)/biotin complex.[7,16] Streptavidin/iminobiotin and streptavidin/desthiobiotin, structures were generated by substitution of the biotin ligand in the crystallographic data. The biotin, desthiobiotin and iminobiotin complexes were energy minimized within the COSMIC(90)[17] forcefield using 1461, 341 and 517 iterations, respectively, of a conjugate gradient minimization procedure.

Using the PULMIN procedure with the COSMIC(90) forcefield the ligands were removed from their receptors and the forces recorded. Biotin was pulled from streptavidin using two

convergence criteria; with $D_{max} = 5$ kcal mol^{-1} Å^{-1}† and $I_{max} = 500$, and $D_{max} = 100$ kcal mol^{-1} Å^{-1} and $I_{max} = 1000$. Desthiobiotin and aminobiotin were analyzed using only the second, less strict criterion. The increment used for each simulation was 0.01 nm and the undocking performed over a total of 3 nm. The mappings were performed using one processor of a Hewlett Packard J-210 workstation (HPUX 10.20).

The hydrogen bonding interactions of all runs were analyzed using HBPlus.[18]

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† 1 Calorie = 4.184 Joules (by definition).

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