

# Sequence Specificity in the Entropy-Driven Binding of a Small Molecule and a Disordered Peptide

Gabriella T. Heller<sup>1</sup>, Francesco A. Aprile<sup>1</sup>, Massimiliano Bonomi<sup>1</sup>, Carlo Camilloni<sup>2</sup>, Alfonso De Simone<sup>3</sup> and Michele Vendruscolo<sup>1</sup>

<sup>1</sup> - Department of Chemistry, University of Cambridge, Cambridge CB2 1EW, UK

<sup>2</sup> - Dipartimento di Bioscienze, Università degli Studi di Milano, 20133 Milan, Italy

<sup>3</sup> - Division of Molecular Biosciences, Imperial College London, London SW7 2AZ, UK

Correspondence to Michele Vendruscolo: [mv245@cam.ac.uk](mailto:mv245@cam.ac.uk)

<http://dx.doi.org/10.1016/j.jmb.2017.07.016>

Edited by Richard W. Kriwacki

## Abstract

Approximately one-third of the human proteome is made up of proteins that are entirely disordered or that contain extended disordered regions. Although these disordered proteins are closely linked with many major diseases, their binding mechanisms with small molecules remain poorly understood, and a major concern is whether their specificity can be sufficient for drug development. Here, by studying the interaction of a small molecule and a disordered peptide from the oncogene protein c-Myc, we describe a “specific-diffuse” binding mechanism that exhibits sequence specificity despite being of entropic nature. By combining NMR spectroscopy, biophysical measurements, statistical inference, and molecular simulations, we provide a quantitative measure of such sequence specificity and compare it to the case of the interaction of urea, which is diffuse but not specific. To investigate whether this type of binding can generally modify intermolecular interactions, we show that it leads to an inhibition of the aggregation of the peptide. These results suggest that the binding mechanism that we report may create novel opportunities to discover drugs that target disordered proteins in their monomeric states in a specific manner.

© 2017 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Disordered proteins [1–8] represent a major untapped potential for drug discovery [9–12]. The identification of small molecules that interact with the monomeric forms of these proteins, however, is hindered by a limited understanding of the corresponding mechanisms of binding and by the uncertainty about whether specificity is possible for these interactions. Traditional drug discovery methods, which have been developed mainly to target globular proteins, focus on the optimization of interactions between small molecules and binding pockets with well-defined structures [13,14]. Disordered proteins do not readily lend themselves to this type of binding [1–7,9–12,15,16] as they lack stable conformations and are better represented as ensembles of many structures with relatively low populations. Consequently, disordered proteins often do not adopt suitable binding pockets amenable to traditional drug discovery programs. Therefore, developing a greater understanding of the

mechanisms by which small molecules can interact with disordered proteins, and hence potentially alter their disease-promoting behavior, could have profound implications for the search of new drugs. However, the identification of small molecules that interact with disordered proteins is particularly challenging because their highly dynamic nature makes it extremely difficult to study them experimentally [17], and notably, few monomeric disordered proteins have been successfully targeted by small molecules [9–12,16–20].

In order to investigate how small molecules bind disordered proteins, here we characterized the interaction between a small molecule and the disordered protein, c-Myc. High-throughput screening approaches have already yielded several compounds able to prevent the oncogenic dimerization of c-Myc to its partner Max, identifying in particular a small molecule, 10058-F4, that binds the 11-residue region comprising residues 402–412 (c-Myc<sub>402–412</sub>)

[10,21–23]. In this study, we combined experimental thermodynamic techniques with all-atom NMR-restrained metadynamic metainference simulations to determine how c-Myc<sub>402–412</sub> binds 10058-F4, revealing a “diffuse-specific” mechanism, which is sequence-specific while being of predominantly entropic nature.

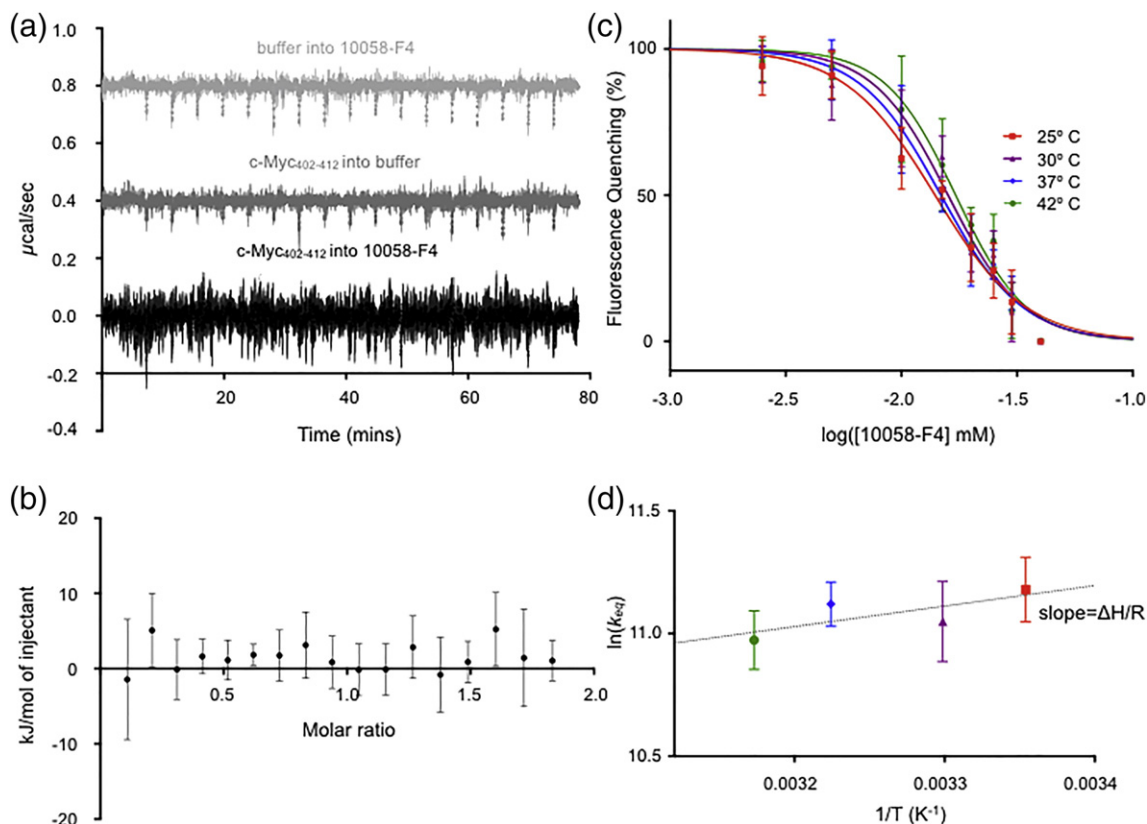
## Thermodynamic characterization

As c-Myc<sub>402–412</sub> is disordered, we asked whether the binding mechanism of this monomeric peptide to 10058-F4 could be characterized in terms of equilibrium thermodynamics. To ensure that we were indeed working with the monomeric peptide, dynamic light scattering measurements were taken of the sample prior to any other measurements (Fig. S1).

Given the absence of well-defined binding pockets within this short, disordered peptide, the identification of a thermodynamic signature of binding is fundamental for understanding the mechanism of this interaction. Therefore, we performed isothermal titration calorimetry (ITC) on this system (Fig. 1a), which is one of the most conventional and well-established methods for

direct, label-free measurements of enthalpic changes [13,17]. Using this method, we detected heats of dilution indicative of low enthalpic contributions to the binding at 25 °C (Fig. 1b). As similar results were obtained by repeating the experiments at 15 °C, we were prompted to more closely consider entropic contributions.

To unambiguously elucidate the contributions of enthalpy and entropy in this interaction, we performed a van't Hoff analysis (Fig. 1d) using fluorescence titration experiments (Fig. S2) at different temperatures (Fig. 1c), taking advantage of the intrinsic fluorescence of a tyrosine residue within the peptide sequence, and found consistent results with the ITC experiments. At room temperature, we observed an affinity of 14  $\mu\text{M}$  with a 95% confidence interval (95% CI 12.2, 15.9  $\mu\text{M}$ ), consistent with the affinity previously reported of  $13 \pm 1 \mu\text{M}$  [10]. This analysis showed that the binding free energy ( $-27.6 \pm -8.5 \text{ kJ/mol}$  at 25 °C) predominantly comprised entropic contributions ( $-20.7 \pm -4.2 \text{ kJ/mol}$ ), while enthalpic contributions were also observed ( $-7.0 \pm -4.3 \text{ kJ/mol}$ ). We thus concluded that the binding of 10058-F4 to the disordered c-Myc peptide is associated with an increase of entropy of the system.



**Fig. 1.** Thermodynamic characterization of the interaction between the disordered 11-residue c-Myc peptide, Ac-YILSVQAEK-NH<sub>2</sub> (c-Myc<sub>402–412</sub>) and 10058-F4. (a) ITC binding isotherms in which c-Myc<sub>402–412</sub> (590  $\mu\text{M}$ ) was titrated into the compound solution 10058-F4 (30  $\mu\text{M}$ ) with corresponding heats of dilution. (b) Integrated peaks from ITC, accounting for heats of dilution. (c) Intrinsic fluorescence titrations performed at various temperatures. Error bars represent the SD from three independent experiments. (d) Van't Hoff analysis of the data shown in panel c.

## Metadynamic metainference simulations

To gain mechanistic insight into the specificity of this entropic binding, we determined a structural ensemble representing the bound (holo) state of c-Myc<sub>402–412</sub> and 10058-F4. To achieve this result, we incorporated NMR measurements into molecular dynamic simulations by performing metadynamic metainference simulations [24,25] which combine a physical model of the system with NMR data [26,37]. Specifically, we used previously published backbone chemical shifts data (apo: 11 <sup>13</sup>C $\alpha$  chemical shifts, 7 <sup>13</sup>C $\beta$  chemical shifts, 11 <sup>1</sup>H $\alpha$  chemical shifts, and 11 <sup>1</sup>H backbone amide chemical shifts; holo: 11 <sup>13</sup>C $\alpha$  chemical shifts, 4 <sup>13</sup>C $\beta$  chemical shifts, and 11 <sup>1</sup>H $\alpha$  chemical shifts) [10]. We performed metadynamic metainference simulations [24,25,36] with GRO-MACS [34] equipped with PLUMED [35].

We parameterized a classical force field based on quantum mechanical calculations for the 10058-F4 molecule (see Methods), as we found that the Generalized Amber Force Field [27] tends to overestimate the potential energy barrier for dihedral rotation between the two rings of 10058-F4 (Fig. S3, see Methods). To ensure convergence, simulations of c-Myc<sub>402–412</sub> in the presence and absence of 10058-F4 were performed in duplicate for at least 5  $\mu$ s each, at which point the estimated free energies remained constant (Fig. S4) and the populations of conformational clusters were consistent between runs (Fig. S5). As a validation of the accuracy of the simulations, we first computed the number of expected nuclear overhauser effect (NOE) crosspeaks between the small molecule and each residue within the peptide sequence (see Supplementary Information) and found good agreement with the corresponding experimental measurements (Fig. S6) [10]. We then computed the distribution of distances between terminal C $\alpha$  atoms throughout the simulation and found close agreement with dynamic light scattering measurements of the c-Myc<sub>402–412</sub> peptide (Fig. S7). The ability to predict multiple independent observables suggests that the metadynamic metainference simulations rather accurately represent the conformational ensemble of c-Myc<sub>402–412</sub>.

The results of the metadynamic metainference simulations suggest that this binding is diffuse, or “fuzzy” [28], as c-Myc<sub>402–412</sub> remains disordered and 10058-F4 is delocalized across several residues of the peptide, consistent with previous simulations of this interaction and those of other small molecules identified to bind c-Myc (Fig. 2) [22,29].

## Sequence specificity

To probe the sequence specificity of this entropic interaction, we directly compared the simulations described above with additional metadynamics

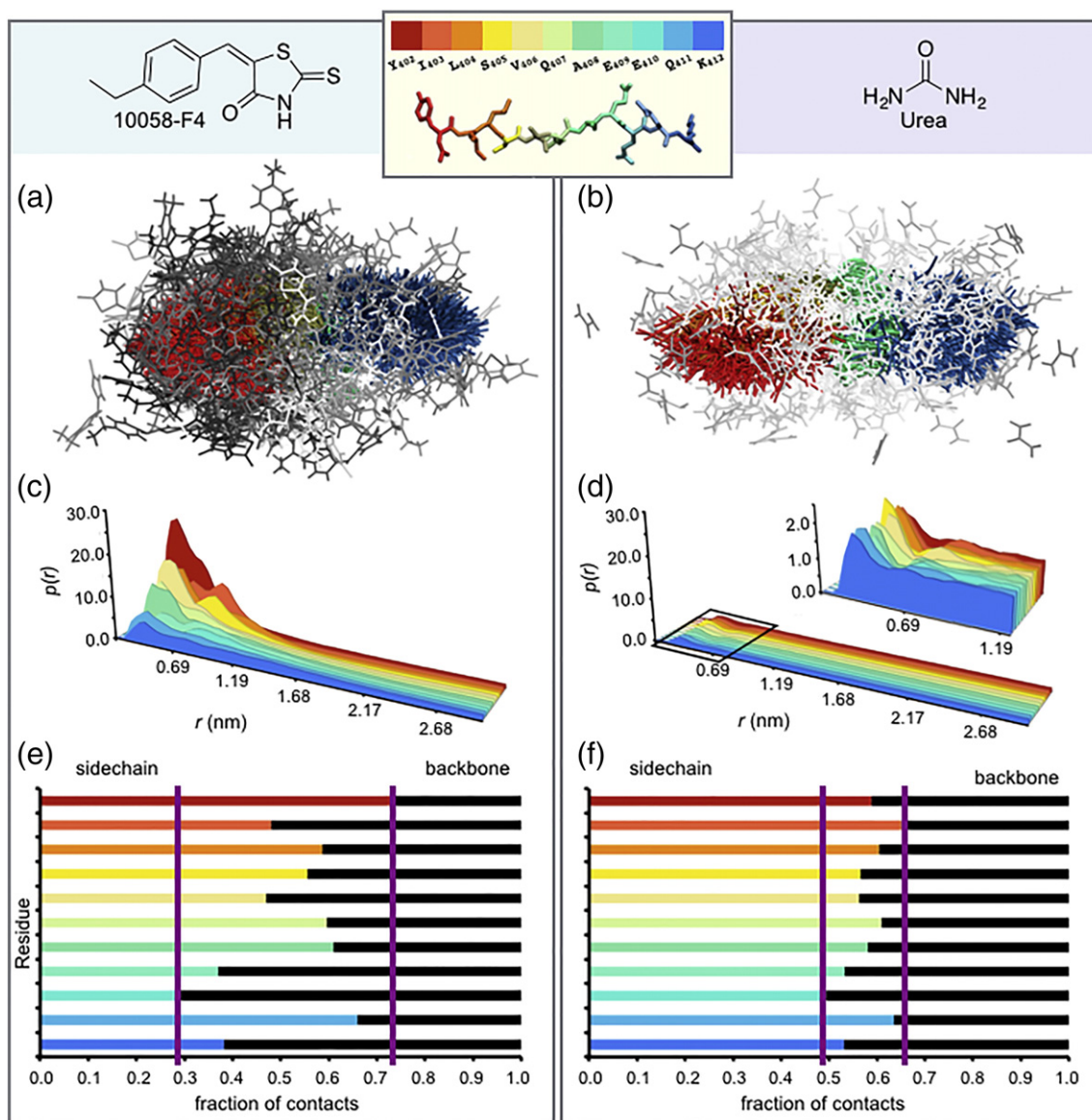
simulations of c-Myc<sub>402–412</sub> in the presence of urea, which is known to interact promiscuously with proteins (Fig. 2). Comparison of the results of these two sets of simulations suggests that while both 10058-F4 and urea bind to c-Myc<sub>402–412</sub> in a delocalized manner, 10058-F4 exhibits notable sequence specificity, which results in a preference for the hydrophobic N-terminus of the peptide (Fig. 2a, b) and large differences in residue-dependent side chain *versus* backbone contacts in bound conformations (Fig. 2c). In contrast, urea interacts with each residue with a more homogenous manner (Fig. 2b, d), while the preference of side chain *versus* backbone contacts is largely independent of residue type (Fig. 2f).

To quantify sequence specificity, we defined a quantitative, residue-specific measure of specificity,  $\tilde{k}_i$  (see Supplementary Information). This value is not a measure of affinity to each single amino acid alone within the sequence, but rather of measure of specificity within the context of its neighboring residues. Large differences in the value of  $\tilde{k}_i$  between residues in the case of 10058-F4 ( $\sigma=54\%$ ) characterize the degree of sequence specificity of this interaction (Fig. 3a). To establish a reference value for this measure of specificity, we calculated it in the case of urea, finding a much smaller value ( $\sigma=9\%$ ; Fig. 3a, inset).

In addition to this distance-based measure of specificity, we also measured sequence specificity using a Voronoi volume approach (see Supplementary Information), which quantifies the probability of a given residue being involved in binding in terms of the number of small-molecule atoms with which it exclusively interacts (10058-F4: Fig. 3b, urea: Fig. S8). For example, residue Y<sub>402</sub> interacts with 10058-F4 in 30.8% of the bound conformations, involving side chains in the majority of cases (87.5%). The three residues with the highest probabilities of interacting with 10058-F4 are Y<sub>402</sub>, Q<sub>407</sub>, and L<sub>404</sub>. Interestingly, Q<sub>407</sub> and L<sub>404</sub> are the two point mutations that were previously reported to impair the binding [10], thus providing further validation to our simulations. While the two measures of sequence specificity proposed here yield complementary information, they both demonstrated that the binding is diffuse along the c-Myc<sub>402–412</sub> sequence, with preferential binding to specific residues.

## Mutagenesis studies to validate the simulations

Our analysis identified Q<sub>407</sub> and L<sub>404</sub> as important residues for binding. These point mutations along with V<sub>406</sub>A and E<sub>409</sub>V were previously reported to impair binding of the basic-helix-loop-helix-leucine-zipper (residues 351–439), which encompasses c-Myc<sub>402–412</sub>, to 10058-F4 [10]. As such, we performed fluorescence quenching experiments of the same mutants within c-Myc<sub>402–412</sub> and observed



**Fig. 2.** Characterization of sequence specificity in the binding of 10058-F4 to c-Myc<sub>402–412</sub>. A structural ensemble that illustrates the mechanism of “specific-diffuse binding” for 10058-F4 (a) and urea (b). The statistical weights of different bound conformations are illustrated by varying opacity. Radial distribution function  $p(r)$  between 10058-F4 (c) or urea (d) and each residue of c-Myc<sub>402–412</sub>. The inset on panel d shows the same plot on a different scale. Fractions of side chain *versus* backbone contacts for bound conformations ( $r < 0.75$  nm) for each residue in the case of 10058-F4 (e) and urea (f). The purple bars are placed at the minimum and maximum values of the fraction of contacts across all residues.

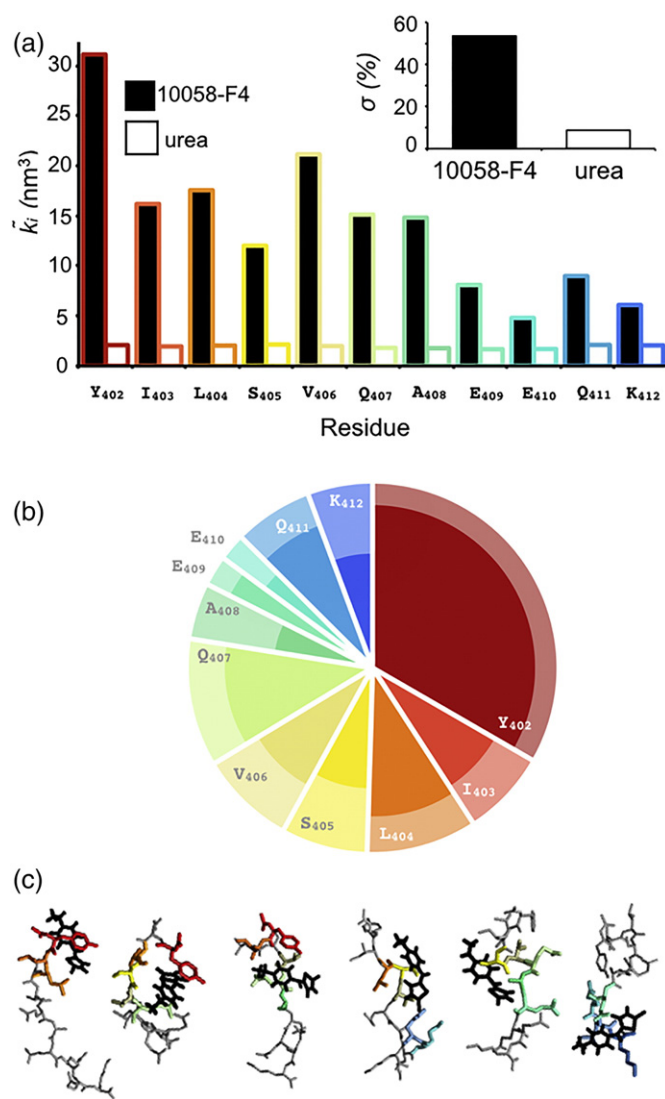
that the binding of 10058-F4 is modestly weakened by these mutations (L<sub>404</sub>P = 15.8  $\mu$ M, 95% CI [14.3, 17.4  $\mu$ M]; Q<sub>407</sub>K = 16.7  $\mu$ M, 95% CI [16.3, 17.2  $\mu$ M]; and V<sub>406</sub>A-E<sub>409</sub>V = 20.7  $\mu$ M, 95% CI [20.0, 21.4  $\mu$ M]; Fig. S9). These results suggest a lower cooperativity in the binding of 10058-F4 with the c-Myc<sub>402–412</sub> variants, which may reflect a less diffuse binding. These experiments not only validate our simulations but also the use of the 11-residue model to represent the full basic-helix-loop-helix-leucine-zipper region,

as our ranking of these affinities is consistent with the previously reported changes in fluorescence polarization of the full zipper region [10].

### Analysis of the nature of 10058-F4/c-Myc<sub>402–412</sub> interaction

We investigated the physico-chemical origin of this “specific-diffuse” mechanism by characterizing



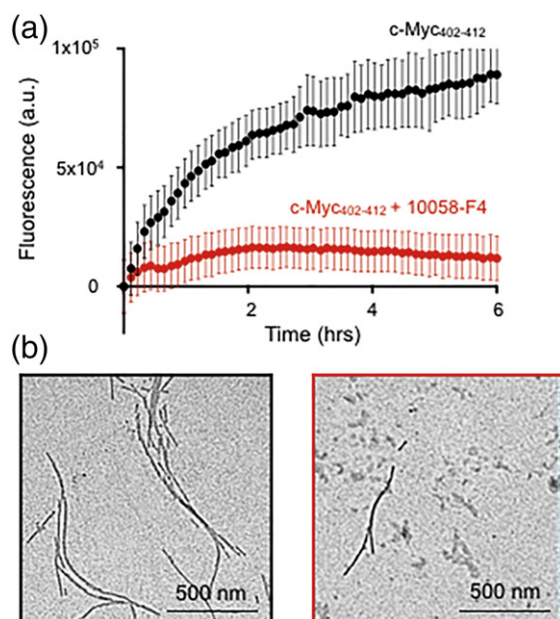


**Fig. 3.** Quantification of the sequence specificity in the binding of 10058-F4 to c-Myc<sub>402–412</sub>. (a) A measure of specificity ( $\tilde{k}_i$ ) for each residue  $i$  with 10058-F4 (black) and urea (white). The inset shows the relative SD ( $\sigma$ ) of  $\tilde{k}_i$  computed over all the residues for 10058-F4 and urea. (b) Voronoi analysis showing the probability of a given residue interacting with 10058-F4. Results are weighted based on the number of atoms within 10058-F4 with which each residue interacts. Solid colors indicate fraction of side chain interactions, whereas faded colors indicate backbone interactions. (c) Structures illustrate the interactions underlying this specificity, including  $\pi$ - $\pi$  stacking between the phenyl rings of 10058-F4 and the terminal tyrosine, hydrophobic interactions, hydrogen bonding and electrostatic interactions, among others.

entropic and enthalpic contributions to the binding. We first quantified the change in conformational entropy of c-Myc<sub>402–412</sub> upon binding to the small molecule (see Supplementary Information). Since we could not observe a significant change in the conformational entropy of the peptide, our results suggest that the main source of entropy observed experimentally is likely due to the release of water molecules. We then analyzed possible enthalpic contributions to the binding (see Supplementary Information). While strong electrostatic interactions were observed for the charged C-terminal region, we did not observe such interactions to the N-terminus, suggesting that the predominant binding to this region is driven by hydrophobicity (Fig. S10).

### Modulation of the behavior of c-Myc<sub>402–412</sub> upon binding 10058-F4

While it has been shown that 10058-F4 is effective in inhibiting the cancer-promoting dimerization of c-Myc to its partner Max [9,23], we asked whether or not this sequence-specific, delocalized, entropic binding could be a general strategy to modulate protein behavior. Using protein aggregation as a model of protein behavior [30], we tested the efficacy of 15  $\mu$ M 10058-F4 on the aggregation of 50  $\mu$ M of the c-Myc<sub>402–412</sub> peptide, monitored by 8-Anilino-naphthalene-1-sulfonic acid fluorescence. We observed that the presence of the compound slows down aggregation and changes the morphology of c-Myc aggregates



**Fig. 4.** The small molecule 10058-F4 inhibits the aggregation of the c-Myc peptide. (a) Kinetic profile of c-Myc<sub>402–412</sub> (50 μM) aggregation in the presence (red) and absence (black) of 15 μM 10058-F4 monitored by ANS fluorescence. Dashed lines represent standard error. (b) Structural characterization of aggregates using transmission electron microscopy. The coloring schemes are the same as in panel a.

(Fig. 4). These results provide initial evidence that targeting the monomeric states of disordered proteins via entropic binding may lead to the development of therapeutic strategies to modify the behavior of these proteins, including those involved in cancer [9,31] and in neurodegeneration [30,32].

## Concluding remarks

The high prevalence of disordered proteins in human disease makes them ideal drug targets, yet a poor understanding of their binding mechanisms with small molecules has largely prevented the development of small molecules capable of altering the disease-promoting behavior of these proteins. Specifically, drug development efforts targeting disordered proteins are hindered by concerns about specificity, as many of the small molecules identified to bind disordered regions have general promiscuity toward other proteins. Despite evidence that its binding is localized to the 11-residue region within the c-Myc protein [10], 10058-F4, like many of the other c-Myc binders, contains a benzylidene rhodanine scaffold, which binds a wide range of diverse biomolecules [33].

However, since 10058-F4 does exhibit sequence specificity toward this peptide, a quantitative, structural understanding of this property may offer novel insights for the development of molecules capable of binding disordered proteins into viable therapeutics. In this study, we have shown that a small molecule can bind to a disordered peptide via an entropically driven process, which is diffuse and yet shows sequence specificity. To achieve this result, we exploited metainference metadynamics, an integrative technique that combines experimental data with the power of simulations to generate a quantitative, all-atom structural understanding of this specificity [26].

On the basis of these results, we anticipate that more small molecules will be discovered that are capable of modulating the disease-promoting behavior of disordered proteins and that detailed analyses of their mechanisms of interaction will facilitate the understanding of the factors that can be exploited to increase their specificity.

## Acknowledgments

We thank Dr. Aleksandr Sahakyan for assistance, and Dr. Johnny Habchi and Dr. Alessandro Barducci for useful discussions. G.T.H. is supported by the Churchill Scholarship and the Gates Cambridge Trust Scholarship.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.jmb.2017.07.016>.

Received 20 February 2017;  
Received in revised form 16 July 2017;  
Accepted 20 July 2017  
Available online 22 July 2017

### Keywords:

disordered proteins;  
small molecules;  
entropy;  
drug binding;  
specificity

### Abbreviations used:

ITC, isothermal titration calorimetry; NMR, nuclear magnetic resonance spectroscopy; 95% CI, 95% confidence interval.

## References

- [1] P. Tompa, Intrinsically disordered proteins: a 10-year recap, *Trends Biochem. Sci.* 37 (2012) 509–516.
- [2] C.J. Oldfield, A.K. Dunker, Intrinsically disordered proteins and intrinsically disordered protein regions, *Annu. Rev. Biochem.* 83 (2014) 553–584.
- [3] J. Habchi, P. Tompa, S. Longhi, V.N. Uversky, Introducing protein intrinsic disorder, *Chem. Rev.* 114 (2014) 6561–6588.
- [4] P.E. Wright, H.J. Dyson, Intrinsically disordered proteins in cellular signalling and regulation, *Nat. Rev. Mol. Cell Biol.* 16 (2015) 18–29.
- [5] V.N. Uversky, C.J. Oldfield, A.K. Dunker, Intrinsically disordered proteins in human diseases: introducing the D2 concept, *Annu. Rev. Biophys.* 37 (2008) 215–246.
- [6] M.M. Babu, R. van der Lee, N.S. de Groot, J. Gsponer, Intrinsically disordered proteins: regulation and disease, *Curr. Opin. Struct. Biol.* 21 (2011) 432–440.
- [7] A. Bhowmick, D.H. Brookes, S.R. Yost, H.J. Dyson, J.D. Forman-Kay, D. Gunter, M. Head-Gordon, G.L. Hura, V.S. Pande, D.E. Wemmer, P.E. Wright, T. Head-Gordon, Finding our way in the dark proteome, *J. Am. Chem. Soc.* 138 (2016) 9730–9742.
- [8] P. Sormanni, D. Piovesan, G.T. Heller, M. Bonomi, P. Kukic, C. Camilloni, M. Fuxreiter, Z. Dosztanyi, R.V. Pappu, M.M. Babu, S. Longhi, P. Tompa, A.K. Dunker, V.N. Uversky, S.C.E. Tosatto, M. Vendruscolo, Simultaneous quantification of protein order and disorder, *Nat. Chem. Biol.* 13 (2017) 339–342.
- [9] S.J. Metallo, Intrinsically disordered proteins are potential drug targets, *Curr. Opin. Chem. Biol.* 14 (2010) 481–488.
- [10] A.V. Follis, D.I. Hammoudeh, H. Wang, E.V. Prochownik, S.J. Metallo, Structural rationale for the coupled binding and unfolding of the c-Myc oncoprotein by small molecules, *Chem. Biol.* 15 (2008) 1149–1155.
- [11] A.K. Dunker, V.N. Uversky, Drugs for “protein clouds”: targeting intrinsically disordered transcription factors, *Curr. Opin. Pharmacol.* 10 (2010) 782–788.
- [12] G.T. Heller, P. Sormanni, M. Vendruscolo, Targeting disordered proteins with small molecules using entropy, *Trends Biochem. Sci.* 40 (2015) 491–496.
- [13] A. Bronowska, Thermodynamics of ligand–protein interactions: implications for molecular design, *Thermodynamics—Interaction Studies—Solids, Liquids and Gases 2011*, pp. 1–49.
- [14] G. Klebe, Applying thermodynamic profiling in lead finding and optimization, *Nat. Rev. Drug Discov.* 14 (2015) 95–110.
- [15] T. Flock, R.J. Weatheritt, N.S. Latysheva, M.M. Babu, Controlling entropy to tune the functions of intrinsically disordered regions, *Curr. Opin. Struct. Biol.* 26 (2014) 62–72.
- [16] V. Csizmek, A.V. Follis, R.W. Kriwacki, J.D. Forman-Kay, Dynamic protein interaction networks and new structural paradigms in signaling, *Chem. Rev.* 116 (2016) 6424–6462.
- [17] G.T. Heller, F.A. Aprile, M. Vendruscolo, Methods of probing the interactions between small molecules and disordered proteins, *Cell. Mol. Life Sci.* 74 (2017) 3225–3243.
- [18] L.I. Iconaru, D. Ban, K. Bharatham, A. Ramanathan, W. Zhang, A.A. Shelat, J. Zuo, R.W. Kriwacki, Discovery of small molecules that inhibit the disordered protein, p27Kip1, *Sci Rep* 5 (2015) 15686.
- [19] G. Toth, S.J. Gardai, W. Zago, C.W. Bertoncini, N. Cremades, S.L. Roy, M.A. Tambe, J.C. Rochet, C. Galvagnion, G. Skibinski, S. Finkbeiner, M. Bova, K. Regnstrom, S.S. Chiou, J. Johnston, K. Callaway, J.P. Anderson, M.F. Jobling, A.K. Buell, T.A. Yednock, T.P.J. Knowles, M. Vendruscolo, J. Christodoulou, C.M. Dobson, D. Schenk, L. McConlogue, Targeting the intrinsically disordered structural ensemble of  $\alpha$ -synuclein by small molecules as a potential therapeutic strategy for Parkinson's disease, *PLoS One* 9 (2014), e87133.
- [20] K. Ono, L. Li, Y. Takamura, Y. Yoshiike, L. Zhu, F. Han, X. Mao, T. Ikeda, J. Takasaki, H. Nishijo, Phenolic compounds prevent amyloid  $\beta$ -protein oligomerization and synaptic dysfunction by site-specific binding, *J. Biol. Chem.* 287 (2012) 14631–14643.
- [21] D.I. Hammoudeh, A.V. Follis, E.V. Prochownik, S.J. Metallo, Multiple independent binding sites for small-molecule inhibitors on the oncoprotein c-Myc, *J. Am. Chem. Soc.* 131 (2009) 7390–7401.
- [22] J. Michel, R. Cuchillo, The impact of small molecule binding on the energy landscape of the intrinsically disordered protein C-Myc, *PLoS One* 7 (2012).
- [23] A.V. Follis, D.I. Hammoudeh, A.T. Daab, S.J. Metallo, Small-molecule perturbation of competing interactions between c-Myc and Max, *Bioorg. Med. Chem. Lett.* 19 (2009) 807–810.
- [24] M. Bonomi, C. Camilloni, M. Vendruscolo, Metadynamic meta-inference: enhanced sampling of the meta-inference ensemble using metadynamics, *Sci Rep* 6 (2016) 31232.
- [25] M. Bonomi, C. Camilloni, A. Cavalli, M. Vendruscolo, Meta-inference: a Bayesian inference method for heterogeneous systems, *Sci. Adv.* 2 (2016), e1501177.
- [26] M. Bonomi, G.T. Heller, C. Camilloni, M. Vendruscolo, Principles of protein structural ensemble determination, *Curr. Opin. Struct. Biol.* 42 (2017) 106–116.
- [27] J. Wang, R.M. Wolf, J.W. Caldwell, P.A. Kollman, D.A. Case, Development and testing of a general Amber force field, *J. Comput. Chem.* 25 (2004) 1157–1174.
- [28] M. Fuxreiter, P. Tompa, Fuzzy complexes: a more stochastic view of protein function, *Adv. Exp. Med. Biol.* 725 (2012) 1–14, [http://dx.doi.org/10.1007/978-1-4614-0659-4\\_1](http://dx.doi.org/10.1007/978-1-4614-0659-4_1).
- [29] F. Jin, C. Yu, L. Lai, Z. Liu, Ligand clouds around protein clouds: a scenario of ligand binding with intrinsically disordered proteins, *PLoS Comput. Biol.* 9 (2013).
- [30] C.M. Dobson, Protein folding and misfolding, *Nature* 426 (2003) 884–890.
- [31] K.-C. Jeong, K.-O. Ahn, C.-H. Yang, Small-molecule inhibitors of c-Myc transcriptional factor suppress proliferation and induce apoptosis of promyelocytic leukemia cell via cell cycle arrest, *Mol. Biosyst.* 6 (2010) 1503–1509.
- [32] T.P.J. Knowles, M. Vendruscolo, C.M. Dobson, The amyloid state and its association with protein misfolding diseases, *Nat. Rev. Mol. Cell Biol.* 15 (2014) 384–396.
- [33] J.B. Baell, G.A. Holloway, New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays, *J. Med. Chem.* 53 (2010) 2719–2740.
- [34] S. Pronk, S. Páll, R. Schulz, P. Larsson, P. Bjelkmar, R. Apostolov, M.R. Shirts, J.C. Smith, P.M. Kasson, D. Van Der Spoel, B. Hess, E. Lindahl, GROMACS 4.5: a high-throughput and highly parallel open source molecular simulation toolkit, *Bioinformatics* 29 (2013) 845–854.

- 
- [35] G.A. Tribello, M. Bonomi, D. Branduardi, C. Camilloni, G. Bussi, PLUMED 2: new feathers for an old bird, *Comput. Phys. Commun.* 185 (2014) 604–613.
- [36] J. Pfandtner, M. Bonomi, Efficient sampling of high-dimensional free-energy landscapes with parallel bias metadynamics, *J. Chem. Theory Comput.* 11 (2015) 5062–5067.
- [37] P. Robustelli, K. Kohlhoff, A. Cavalli, M. Vendruscolo, Using NMR chemical shifts as structural restraints in molecular dynamics simulations of proteins, *Structure* 18 (2010) 923–933.