Organic & Biomolecular Chemistry

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ARTICLE

A Model β-Sheet Interaction and Thermodynamic Analysis of β-Strand Mimetics

Cite this: DOI: 10.1039/xoxxoooooox

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Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxoooooox

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β-Sheet mediated protein-protein interactions are involved in key signalling pathways in diseases such as cancer. We present small molecule β-strand mimetics and investigate their interactions with a model tripeptide. Using ^{1}H NMR, the thermodynamic parameters for their binding are determined. These give insight into this biologically important interaction.

Introduction

Protein-protein interactions (PPIs) are involved in many cellular processes such as gene expression, proliferation, intracellular communication and apoptosis. Consequently the discovery of small molecule modulators of PPIs has become an important goal in medicinal chemistry.[1]

However, PPIs have proven difficult to target with small molecules due to the challenging nature of their large and flat binding interfaces, which are exposed on protein surfaces.[2-4]

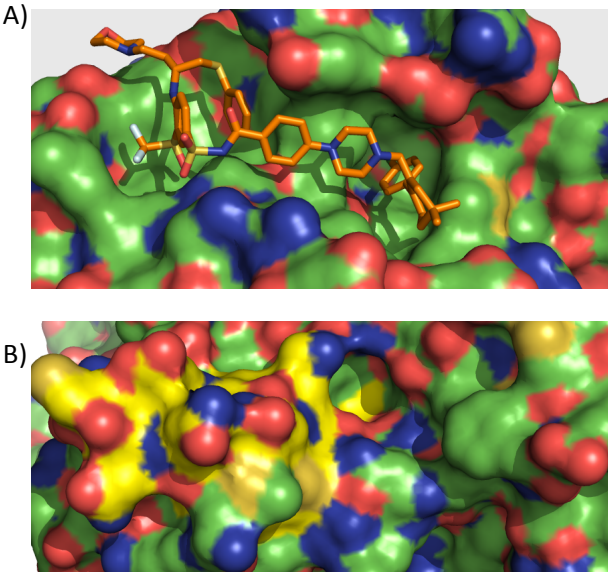


Figure 1: A) Bcl-2 co-crystallised with ABT-263 (PDB code: 4 LVT). B) Rap1A structure. PPI interface is highlighted in yellow. (PDB code: 1GUA)

Despite these challenges inhibitors and tool compounds have been discovered for a number of PPIs.[5-8] For example, inhibitors of the Bcl-2 interactions have been identified using NMR screening technologies to detect fragments that take advantage of shallow lipophilic pockets at the PPI interface of Bcl-2. The biaryl fluoride fragment identified was grown to take advantage of proximal shallow binding pockets and resulted in the potent Bcl-2 family inhibitor ABT-263 (Bcl-xL, Bcl-2 K_i < 1 nM) (**Figure 1A**).[8-10]

A more challenging class of PPIs are those mediated by intermolecular β-sheet interactions, which have been implicated in a number of diseases including HIV, Parkinson's and cancer.[11,12] These β-sheet mediated PPIs generally lack the shallow lipophilic binding pockets of other interactions at the PPI interface (**Figure 1B**) and instead rely upon the formation of intermolecular hydrogen bonds and amino acid side chain interactions to mediate the PPI.[12] Given these highly specific requirements for this class of PPI, a HTS strategy is unlikely to identify a molecule that can adequately mimic these key interactions.

An attractive approach to interfere with β-sheet mediated PPIs is to design synthetic scaffolds that can mimic these key interactions. Many research groups have reported β-sheet/β-strand mimetics, however few of these have progressed beyond model or tool compounds.[11,13-21] To identify novel β-sheet/strand mimetics for interaction with the exposed edge of a β-sheet we have focussed, firstly, on identifying scaffolds that can mimic the hydrogen-bonding array of a β-sheet interaction. In order to measure and optimise the energy of hydrogen bonds a β-sheet/strand mimetic can make with a β-sheet motif we have developed a model system where the thermodynamics of binding can be measured.

Model β-sheet Interaction

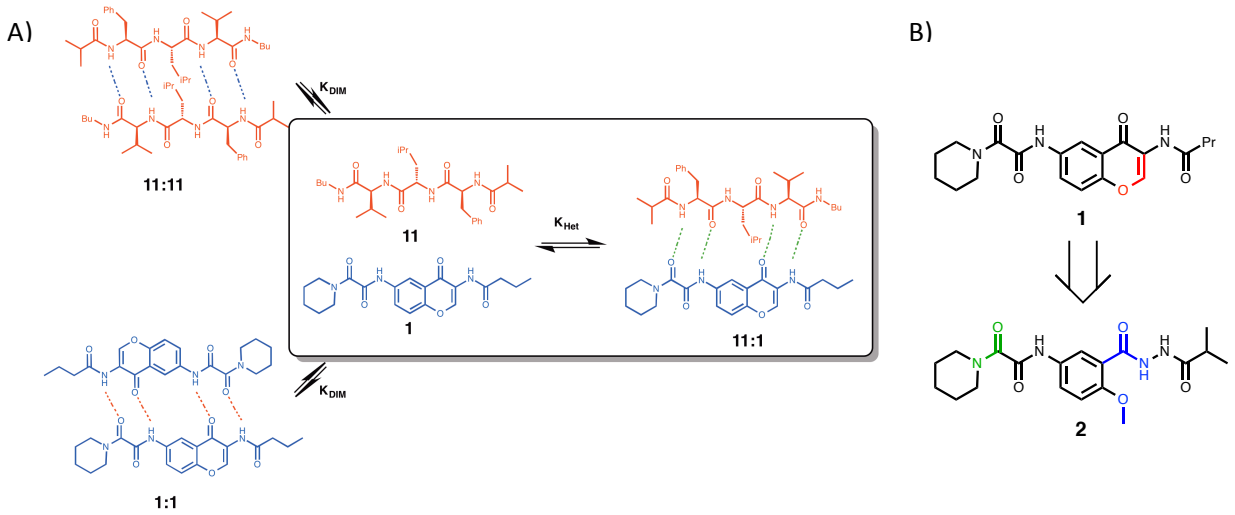


Figure 2: A) Model β-sheet interaction between model β-sheet motif **11** and small molecule β-strand mimetic **1.** B) Chromone β-strand mimetic **1** and β-strand mimetic **2**.

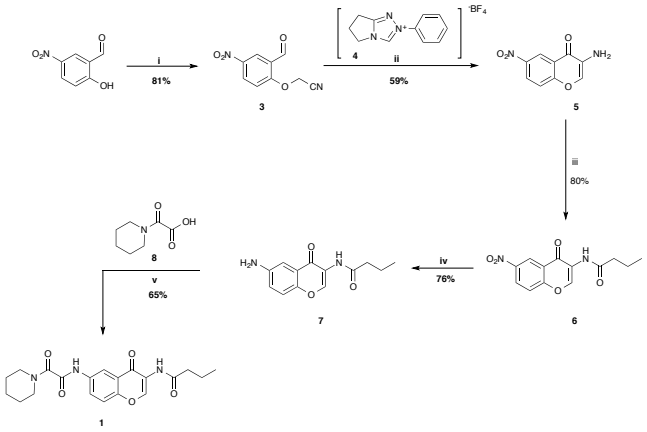
Our model β-sheet interaction involves the binding of β-strand mimetics to a model β-sheet motif (tripeptide **11**) (**Figure 2A**). This interaction was investigated using ^{1}H NMR to monitor chemical shifts of key resonances in **11** to calculate the association constant K_{Het} in deuterated chloroform ($CDCl_3$). The aim of this experiment was to find compounds with a large K_{Het} value, indicating a strong interaction with a model β-sheet motif. Furthermore, this experiment was extended to provide the thermodynamic parameters of binding (ΔH and ΔS) using variable temperature (VT) ^{1}H NMR. Measuring thermodynamic parameters allowed us to understand differences in K_{Het} and provided important information to aid the optimisation of the hydrogen-bonding array of a given scaffold. $CDCl_3$ was chosen as the solvent for these NMR experiments in order to avoid possible complications from solvent interactions.

The starting point for our investigation was chromone **1** (**Figure 2B**) in which the acceptor-donor-acceptor-donor (ADAD) hydrogen-bonding motif is presented *via* a rigid bicyclic aromatic scaffold. In order to place this in context with other β-strand mimetics in the literature we also prepared compound **2** based firmly on the work of Nowick *et al.*[22] The binding of closely-related compounds to a model tripeptide has been described but the thermodynamic parameters have not been reported.[23]

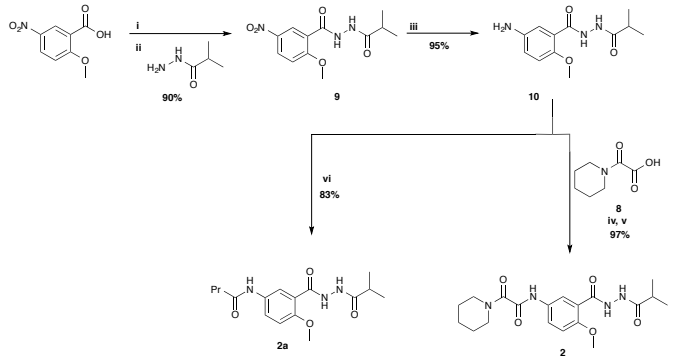
We hypothesised that by using a more rigid bicyclic chromone scaffold we would observe more favourable entropy of binding than the more flexible intramolecularly hydrogen-bonded **2**.

Synthesis of β-Strand Mimetics and Tripeptide

Chromone **1** was synthesised starting from commercially available 2-hydroxy-5-nitrobenzaldehyde (**Scheme 1**). Alkylation with bromoacetonitrile afforded nitrile **3**. A *N*-heterocyclic carbene-catalysed ring closure procedure developed by Vedachalum *et al.* was used to obtain the key 3-amino-6-nitrochromone **5** from nitrile **3**.[24] Acylation of **5** with butyryl chloride, followed by reduction of the nitro moiety



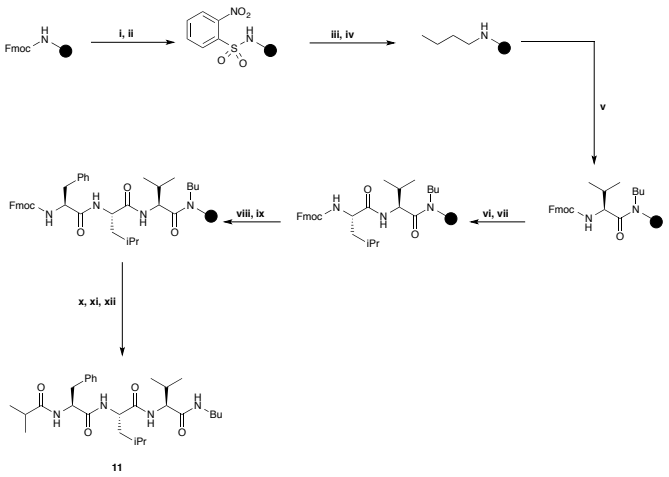
Scheme 1: Synthesis of chromone **1**. Reagents and conditions: i) bromoacetonitrile, K_2CO_3, DMF, rt, 18 h; ii) **4**[25], DBU, DCM, rt, 16 h; iii) Butyryl chloride, DIPEA, DCM, 0 °C to rt, 1 h; iv) Fe, NH_4Cl, EtOH/H_2O (3:1), reflux, 18 h; v) **8**, HATU, DIPEA, DMF, rt, 16 h.



Scheme 2: Synthesis of compound **2**. Reagents and conditions: i) $SOCl_2$, DMF (cat.), 85 °C, 2 h; ii) DIPEA, DCM, 0 °C to rt, 2 h; iii) H_2, Pd/C, THF, 40 psi, 2 h; iv) **8**,

$SOCl_2$, DMF (cat.), 85 °C, 2 h v) DIPEA, DCM, 0 °C to rt, 2 h; vi) Butyric anhydride, pyridine, 0 °C to rt, 2 h.

afforded aniline **7** in good yield. An amide coupling reaction between **7** and carboxylic acid **8** provided **1** in a 65% yield. Compound **8** was synthesised in two steps from piperidine and ethyl chlorooxoacetate (see supplementary information).



Scheme 3: Solid phase synthesis of peptide **11** on PAL-AM resin. Reagents and conditions: i) 20% piperidine in NMP (3 x 15 min); ii) 2-nitrobenzene sulfonyl chloride, collidine, DCM, 3 h; iii) 1-iodobutane, MTBD, DMF, 8 h; iv) mercaptoethanol, DBU, DMF (2 x 3 h); v) Fmoc-Val-OH, HATU, DIPEA, DMF (2 x 2 h); vi) 20% piperidine in NMP (3 x 15 min); vii) Fmoc-Leu-OH, HATU, DIPEA, DMF (2 x 2 h); viii) 20% piperidine in NMP (3 x 15 min); ix) Fmoc-Phe-OH, HATU, DIPEA, DMF (2 x 2 h); x) 20% piperidine in NMP (3 x 15 min); xi) isobutryl chloride, DIPEA, DMF, 2 h; xii) TFA/DCM, 1 h.

The hydrazide compound **2** was synthesised starting from commercially available 2-methoxy-5-nitrobenzoic acid which was first converted to the corresponding benzoyl chloride intermediate in the presence of thionyl chloride (**Scheme 2**). Addition of isobutyrohydrazide to a solution of the benzoyl chloride intermediate in the presence of DIPEA provided compound **9** in an excellent yield.[26] The nitro group was reduced to obtain aniline **10**, which was coupled to the corresponding acid chloride of compound **8** to afford compound **2**.

Compound **2a** was synthesised by addition of butyric anhydride to aniline **10** in the presence of pyridine to give the desired compound in an 83% yield. Tripeptide **11** was synthesised using methodology developed by Barany[22] and Nowick[27] (**Scheme 3**).

Model β-sheet interaction: ^{1}H NMR Homodimer Study

As illustrated in **Figure 2**, compounds **1**, **2** and **11** have the ability to form both homodimer and heterodimer complexes. Homodimer species are observed due to the self-complementary ADAD hydrogen-bonding arrays of these scaffolds. In order to calculate the heterodimer-binding constant of these compounds with tripeptide **11,** the homodimer-binding constants (K_{DIM}) of **1**, **2** and **11** were required.

To determine K_{DIM} for these compounds a ^{1}H NMR homodimer titration was performed. Analysis of the concentration and chemical shift data of key resonances (NH chemical shifts), by HypNMR,[27] afforded homodimer binding constants (K_{DIM}) of 30 M^{-1} (K_d = 30 mM) and 1345 M^{-1} (K_d = 0.7 mM) for **1** and **2** respectively.

A ^{1}H NMR homodimer titration was also performed for tripeptide **11** to give a K_{DIM} of 214 M^{-1} (K_d = 4.6 mM). This value was in accordance with the literature,[22] however we found the error in determining K_{DIM} using the shifts of the NH signals in **11** was large due to their broad nature. As an alternative the concentration dependent shifts of an *alpha* CH proton (leucine) were used and K_{DIM} was determined to be 133 M^{-1} (K_d = 7 mM) (see supplementary section). The error determining K_{DIM} using this shift was much lower than that of the NH shift and as a result the chemical shift of the leucine *alpha* CH signal (**Figure 3B**, H^2) was used throughout to determine both homo- and heterodimer binding constants of **11**. Surprisingly, the K_{DIM} value of **11** was 4-fold larger than the value obtained for **1** (133 M^{-1} vs 32 M^{-1}) but 10-fold less than **2** (133 M^{-1} vs 1345 M^{-1}). To investigate the possible reasons behind the disparity in K_{DIM} a thermodynamic analysis was performed. The thermodynamic parameters, ΔH and ΔS, were determined by obtaining K_{DIM} values for each compound at a range of temperatures by VT ^{1}H NMR and then using the Van't Hoff equation.

Table 1: Summary of Thermodynamic Parameters for Homodimer Complexes of **1**, **2** and **11**

Complex	K_{DIM}^{295K} (M^{-1})	ΔG (kcal mol^{-1})	ΔH (kcal mol^{-1})	$-T\Delta S^{295K}$ (kcal mol^{-1})
1:1	30 ± 0.33	-2.0	-7.8 ± 0.24	5.8 ± 0.25
11:11	133 ± 11.6	-2.8	-10.2 ± 0.29	7.4 ± 0.25
2:2	1345 ± 39	-4.4	-12.6 ± 0.27	8.4 ± 0.30

Values for K, ΔH and -TΔS are the mean ± SEM of three determinations.

The data in **Table 1** show that although **1** pays a lower entropic penalty to form a homodimer, it cannot form hydrogen bonds as effectively as **11** or **2** as illustrated by the lower enthalpic value (ΔH -7.8 vs -10.2 vs -12.6 kcal mol^{-1}). The entropic gain (ΔS 5.8 vs 7.4 vs 8.4 kcal mol^{-1}) of using a rigidified scaffold is in fact cancelled out by a loss in enthalpy. We hypothesised that the increased flexibility of **11** enabled it to adopt a more favourable conformation in order to optimise the angles of the hydrogen bonds it forms with a partner **11** molecule. In contrast, the chromone scaffold cannot optimise its conformation as readily due to its rigid bicylic scaffold. Compound **2** is more rigid than **11** but the intramolecular hydrogen bond maintains a degree of flexibility and allows for a favourable binding conformation. Moreover, the exchange of the hydrazide moiety in **2** for a chromone in **1** is likely to affect the electronics of the hydrogen-bonding array and thus the strength of the hydrogen bonds formed.

It is clear from **Table 1** that there is a critical balance between flexibility (to allow the optimum hydrogen bond formation) and

rigidity (to minimise the entropic penalty) in the formation of homodimer complexes for these scaffolds.

Model β-Sheet Interaction: Heterodimer Studies

Having explored the homodimerisation of **1** and **2**, the heterodimerisation of these compounds with model β-sheet motif **11** was investigated.

The heterodimer binding constant (K_{Het}) was calculated by ^{1}H NMR titration where **11** was held at a constant concentration and the concentration of the small molecule was varied. The data were analysed by HypNMR,[27] using a 1:1 binding model whilst taking the homodimer binding constant of both the small molecule and **11** into account. The heterodimer titrations are summarised in **Figure 3**.

The heterodimer binding constants (K_{Het}) for **1** and **2** were found to be 316 M^{-1} (K_d = 3 mM) and 3568 M^{-1} (K_d = 0.28 mM) respectively (**Table 2**). VT ^{1}H NMR was again used to investigate the thermodynamics of the heterodimer interactions of these compounds with **11**.

The data in **Table 2** show that the difference in K_{Het} is due largely to the variation in the enthalpy of the interactions of **2** and **1** with **11** (-12.5 vs -10.4 kcal mol^{-1}). Although **2** pays a slightly larger entropic penalty (7.6 vs 7.0 kcal mol^{-1}) to bind to **11** than **1**, this is compensated for by the increased enthalpy achieved. We can conclude from this data that **2** can form stronger hydrogen bonds with **11** than **1**. We hypothesise that this is again due to the increased flexibility of **2** with respect to **1**. Due to the importance of geometry in formation of hydrogen bonds,[28,29] it is clear that this increased flexibility is advantageous in achieving the correct orientation to form a hydrogen bond.

Table 2: Summary of Thermodynamic Parameters for **11:1, 11:2** and **11:2a** Heterodimers

Complex	K_{DIM}^{295K} (M^{-1})	ΔG (kcal mol^{-1})	ΔH (kcal mol^{-1})	$-T\Delta S^{295K}$ (kcal mol^{-1})
1:11	**316 ± 11.5**	**-3.4**	**-10.4 ± 0.35**	**7.0 ± 0.38**
2:11[a]	**3568 ± 305**	**-4.9**	**-12.5 ± 0.74**	**7.6 ± 0.93**
2a:11	**1307±132.4**	**-4.2**	**-13.2 ± 0.55**	**9.0 ± 0.55**

Values for K, ΔH and -TΔS are the mean ± SEM of three determinations.
[a]Larger error observed. Limited evidence for multiple heterodimer species

The observation of a strong ROESY correlation between H^2 in **11** and H^4 in **2** gives evidence to support the binding mode proposed in **Figure 3B**.

The increased K_{Het} and ΔH of the **2:11** interaction prompted us

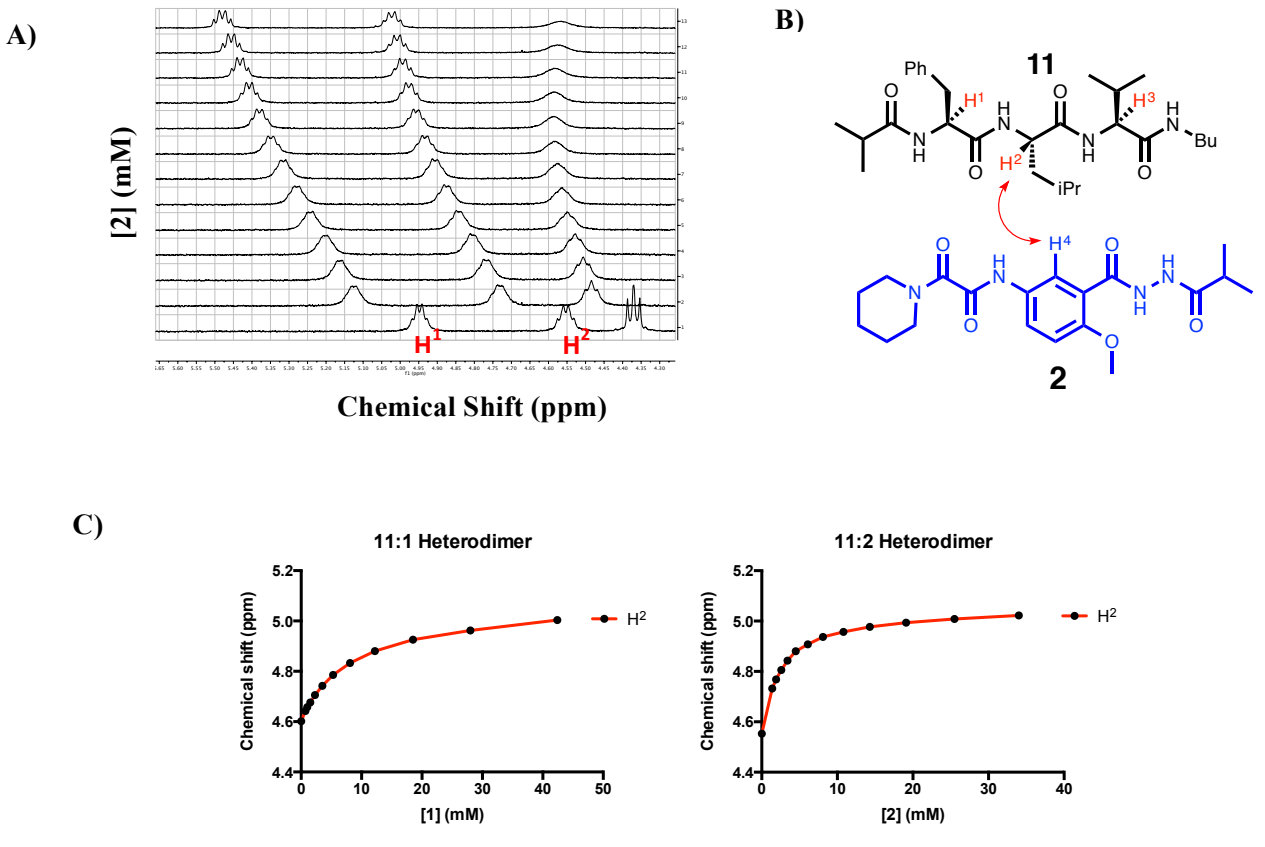


Figure 3: A) Stacked ^{1}H NMR plot of **11:2** heterodimer titration showing αCH shifts of **11** (concentration increasing along y-axis). Key concentration dependent resonances are labelled in red B) Illustration of key intermolecular ROESY correlation (represented by an arrow) observed between H^2 in **11** and H^4 in compound **2** C) Binding curves of **11:1** and **11:2** heterodimer titrations

to further deconstruct the hydrogen-bonding array of compound **2**. To this end we synthesised compound **2a** (**Scheme 2**), where one hydrogen bond acceptor (highlighted in green in **Figure 2**) was removed to present a DAD hydrogen-bonding array.

The heterodimer-binding constant of **2a** with **11** was measured and found to be 1307 M^{-1} (K_d = 0.77 mM). From the data in **Table 2** it is clear that compound **2a** has a lower heterodimer-binding constant with **11** than **2** but has an increased enthalpic value (-13.2 vs -12.5 kcal mol^{-1}). This suggests that although **2a**, has one hydrogen bond acceptor fewer than compound **2,** it is forming stronger hydrogen bonds with **11**. The clear inference from the data is that the contributions of the individual hydrogen bonds are not equal.

The comparison of **2** and **2a** serves to highlight the importance of measuring thermodynamic parameters of this interaction, as compound **2a** may have been discarded based solely on K_{Het}, but is in fact superior to compound **2** in terms of the stronger hydrogen bonds it can form with **11**.

In conclusion, we have studied a series of model β-sheet interactions that have allowed us to assess small molecule β-strand mimetics and their thermodynamics of binding to a model β-sheet motif. Using this model system we have found that the known β-strand scaffold **2**, is superior to a chromone **1** in its ability to bind to a peptide motif. The increased flexibility and the hydrazide moiety of **2** facilitates the formation of stronger intermolecular hydrogen bonds. This shows there is a balance to be found between the pre-organisation of a scaffold into a favourable binding conformation, with that of the flexibility of the scaffold. Furthermore by investigating the thermodynamic parameters of binding, our model β-sheet interaction provides a tool to optimise the hydrogen-bonding array of a given scaffold, as illustrated by the comparison of compounds **2** and **2a**.

Using this approach we aim to find novel β-strand mimetics with optimal hydrogen bonding arrays for interaction with relevant PPIs.

Acknowledgements

We would like to thank The Institute of Cancer Research for PhD Studentship funding and acknowledge funding from Cancer Research UK [grant number: C309/A11566]. We thank Dr. Maggie Liu and Dr. Harry Parkes for help with NMR experiments and Meirion Richards for help with peptide purification.

Notes and references

[a] Cancer Research UK Cancer Therapeutics Unit, Institute of Cancer Research, Haddow Laboratories, 15 Cotswold Road, Sutton, London, SM2 5NG

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

(1) J. A. Wells and C. L. McClendon, *Nature,* 2007, **450**, 1001.
(2) S. Jones and J. M. Thornton, *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 13.
(3) L. L. Conte, C. J. Chothia and J. Janin, *J. Mol. Biol.*, 1999, **285**, 2177.
(4) A. L. Hopkins and C. R. Groom *Nat. Rev. Drug Discov.,* 2002, **1**, 727.
(5) K. Ding, Y. Lu, Z. Nikolovska-Coleska, S. Qui, Y. Ding, W. Gao, J. Stuckey, K. Krajewski, P. P. Roller, Y. Tomita, D. A. Parish, J. R. Deschamps and S. Wang, *J. Am. Chem. Soc.,* 2005, **127**, 10130.
(6) L. T. Vassilev, B. T. Vu, B. Graves, D. Carvajal, F. Podlaski, Z. Filopovic, N. Kong, U. Kammlott, C. Lukacs, C. Klein, N. Fotouhi and E. A. Liu, *Science,* 2004, **303**, 844.
(7) D. L. Buckley, I. V . Molle, P. C. Gariess, H. S. Tae, J. Michel, D. J. Noblin, C. M. Crews, A. Ciulli and W. L. Jorgensen, *J. Am. Chem. Soc* 2012, **134**, 4465.
(8) C. M. Park, M. Bruncko, J. Adickes, J. Bauch, H. Ding, A. Kunzer, K. C. Marsh, P. Nimmer, A. R. Shoemaker, X. Song, S. K. Tahir, C. Tse, X. Wang, M. D. Wendt, X. Yang, H. Zhang, S. W. Fesik, S. H. Rosenberg and S. W. Elmore, *J. Med. Chem.*, 2008, **51**, 6902.
(9) T. Oltersdorf, S. W. Elmore, A. R. Shoemaker, R. C. Armstrong, D. J. Augeri, A. B. Belli, M. Bruncko, T. L. Deckwerth, J. Dinges, P. J. Hajduk, M. K. Joseph, Shinichi Kitada, S. J. Korsmeyer, A. R. Kunzer, A. Letai, C. Li, M. J. Mitten, D. G. Nettesheim, S. Ng, P. M. Nimmer, J. M. O'Connor, A. Oleksijew, A. M. Petros, J. C. Reed, W. Shen, S. K. Tahir, C. B. Thompson, K. J. Tomaselli, B. Wang, M. D. Wendt, H. Zhang, S. W. Fesik and S. H. Rosenberg, *Nature*, 2005, **435**, 677.
(10) A. M. Petros, J. Dinges, D. J. Augeri, S. A. Baumeister, D. A. Betebenner, M. G. Bures, S. W. Elmore, P. J. Hajduk, M. K. Joseph, S. K. Landis, D. G. Nettesheim, S. H. Rosenberg, W. Shen, S. Thomas, X. Wang, I. Zanze, H. Zhang, and S. W. Fesik, *J. Med. Chem.*, 2006, **49**, 656.
(11) W. A. Loughlin, J. D. A. Tyndall, M. P. Glenn, Timothy A. Hill and D. P. Fairlie, *Chem. Rev.*, 2010, **110**, 32.
(12) N. Nassar, G. Horn, C. Herrmann, C. Block, R. Janknecht and A. Wittinghofer, *Nat. Struct. Biol.*, 1996, **3**, 723.
(13) H. Diaz, J. R. Espina and J. W. Kelly, *J. Am. Chem. Soc.*, 1991, **144**, 8316.
(14) J. S. Nowick, *Acc. Chem. Res.*, 1999, **32**, 287.
(15) S. T. Phillips, M. Rezac, U. Abel, M. Kossenjans, and P. A. Bartlett, *J. Am. Chem. Soc.*, 2002, **124**, 58.
(16) P. A. Bartlett and M. C. Hammond, *J. Org. Chem.*, 2007, **72**, 3104.
(17) A. G. Jamieson, D. Russel and A. D. Hamilton, *Chem. Commun.*, 2012, **48**, 3709.
(18) C. L. Sutherell, S. Thompson, R. T. W. Scott and Andrew D. Hamilton, *Chem. Commun.*, 2012, **48**, 9834.
(19) H. Lingard, J. T. Han, A. L. Thompson, I. K. H. Leung, R. T. W. Scott, S. Thompson and A. D. Hamilton, *Angew. Chem. Int. Edit.*, 2014, **53**, 3650.
(20) A. B. Smith, A.K Charnley and R. Hirschmann, *Acc. Chem. Res.*, 2011, **44**, 180.

(21) E. A. German, J. E. Ross, P. C. Knipe, M. F. Don, S. Thompson and A. D. Hamilton, *Angew. Chem. Int. Edit.*, 2015, 54, 2649.
(22) J. S. Nowick, M.D. Chung, K. Maitra, S. Mairita, K. D. Stigers and Y. Sun, *J. Am. Chem. Soc.*, 2000, **122**, 7654.
(23) J. S. Nowick, J. H. Tsa and D. B. Quoc-Chuong, *J. Am. Chem. Soc.*, 1999, **121**, 8409.
(24) S. Vedachalam, J. Zeng, B. K. Gorityala, M. Antonio and X.Liu, *Org. Lett.*, 2010, **12**, 352.
(25) J. E. Thomson, C. D. Campbell, C. Concellon, N. Duguet, K. Rix, A. M. Z. Slawin, and A. D. Smith, *J. Org. Chem.*, 2008, **73,** 2784.
(26) J. S. Nowick, J. H. Tsai, Q. C. D. Bui and S.Maitra, *J. Am. Chem. Soc.,* 1999, **121**, 8409.
(27) C. Frassineti, S. Ghelli, P. Gans, A. Sabatini, M. S. Moruzzi, and A. Vacca, *Anal. Biochem.*, 1995, **231**, 374.
(28) M. C. Etter, *Acc. Chem. Res.*, 1990, **23**, 120.
(29) R. Taylor and O. Kennard, *Acc. Chem. Res.*, 1984, **17**, 320.