Conformational switching of a foldamer in a multicomponent system by ph-filtered selection between competing noncovalent interactions
Brioche, Julien; Pike, Sarah; Tshepelevitsh, Sofja; Leito, Ivo; Morris, Gareth; Webb, Simon; Clayden, Jonathan

DOI: 10.1021/jacs.5b03284
License: Creative Commons: Attribution (CC BY)

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (Harvard):
Brioche, J, Pike, S, Tshepelevitsh, S, Leito, I, Morris, G, Webb, S & Clayden, J 2015, 'Conformational switching of a foldamer in a multicomponent system by ph-filtered selection between competing noncovalent interactions', Journal of the American Chemical Society, vol. 137, no. 20, pp. 6680-6691. https://doi.org/10.1021/jacs.5b03284

Link to publication on Research at Birmingham portal

General rights
Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

• Users may freely distribute the URL that is used to identify this publication.
• Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
• Users may use extracts from the document in line with the concept of ‘fair dealing’ under the Copyright, Designs and Patents Act 1988 (?).
• Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy
While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

Download date: 09. Nov. 2020
Conformational Switching of a Foldamer in a Multicomponent System by pH-Filtered Selection between Competing Noncovalent Interactions

Julien Briouche,† Sarah J. Pike,† Sofja Tshepelevitsh,‡ Ivo Leito,† Gareth A. Morris,† Simon J. Webb,§,† and Jonathan Clayden*,†

†School of Chemistry, University of Manchester, Oxford Road, Manchester M13 9PL, United Kingdom
‡Institute of Chemistry, University of Tartu, Ravila 14a, Tartu 50411, Estonia
§Manchester Institute of Biotechnology, University of Manchester, 131 Princess Street, Manchester M1 7DN, United Kingdom

ABSTRACT: Biomolecular systems are able to respond to their chemical environment through reversible, selective, noncovalent intermolecular interactions. Typically, these interactions induce conformational changes that initiate a signaling cascade, allowing the regulation of biochemical pathways. In this work, we describe an artificial molecular system that mimics this ability to translate selective noncovalent interactions into reversible conformational changes. An achiral but helical foldamer carrying a basic binding site interacts selectively with the most acidic member of a suite of chiral ligands. As a consequence of this noncovalent interaction, a global absolute screw sense preference, detectable by 13C NMR, is induced in the foldamer. Addition of base, or acid, to the mixture of ligands competitively modulates their interaction with the binding site, and reversibly switches the foldamer chain between its left and right-handed conformations. As a result, the foldamer–ligand mixture behaves as a biomimetic chemical system with emergent properties, functioning as a “proton-counting” molecular device capable of providing a tunable, pH-dependent conformational response to its environment.

INTRODUCTION

A defining difference between biological and chemical systems lies in biology’s ability to store, process, and amplify information in the midst of immense chemical complexity.1–4 The remarkable selectivity displayed by biomolecules in their binding of other biomolecules, ligands, or metabolites allows the simultaneous independent but interactive control of numerous chemical signaling pathways. As a result, multiple biochemical processes may be controlled, all taking place within the same physical phase.5 Communication events in biological systems typically couple selective molecular recognition to some form of conformational response,6–9 allowing modulation of function in a peptide, protein, or nucleic acid. Classic examples10 include G-protein coupled receptors, which modify their conformation in response to the binding of an extracellular ligand, and the allosteric protein hemoglobin,11,12 which adjusts its conformation on binding of oxygen, and phosphorylases.13 Other proteins exhibit pH-dependent conformational switching.14,15 Signaling pathways result when further biochemical events are initiated as a consequence of these conformational changes—the release of GDP into a cell, further cooperative binding of oxygen, or phosphorylation of active hydroxyl groups.

This relay of information through reversible conformational changes may be mimicked16–20 by artificial, conformationally defined extended molecular structures (foldamers21–23) that adjust their global conformational preference as a result of the reversible covalent binding of a ligand. Three-dimensional structural information about the ligand is thus transmitted from a binding site in the artificial receptor to a remote reporter group.24 However, in a real biological system, every binding site is continually buffeted by a menagerie of potential ligands, among which it must recognize and bind a suitable partner, leading to a corresponding selective conformational response.

We now report a receptor mimic that incorporates a basic binding site, whose conformational preference is reversibly modulated by selective noncovalent interactions. When several alternative acidic ligands are presented simultaneously to the receptor, its response is dictated by the ligands’ pKa values and pH-dependent binding ability. The receptor’s tunable selective response to the ligand is communicated conformationally to a remote site in the molecule, where the resulting global conformational preference is revealed by NMR. Through a characteristic combination of noncovalent ion-pairing and...
hydrogen-bonding interactions, each potential ligand induces a quantitatively different, spectroscopically quantifiable, conformational preference in the receptor mimic. Cycling between conformational outputs is made possible by the selective activation or silencing of ligands by varying the pH (and hence protonation state) of the system. The multicomponent mixture of ligands plus the receptor thus constitutes a chemical system with emergent properties, functioning as a device capable of counting protons and providing a tunable, conformationally encoded output.

## RESULTS AND DISCUSSION

### Identifying a Versatile Binding Site.

Conformational change induced by noncovalent binding of a ligand is a well established feature of supramolecular systems. In the context of extended dynamically switchable helical foldamer structures, Inai and co-workers showed that a noncovalent interaction between an enantiopure N-protected α-amino acid and the free amino terminus of an achiral but helical polyamide is capable of eliciting a circular dichroism (CD) response from the foldamer, indicating the induction of some degree of screw-sense preference in the helical structure. The perturbation of the equilibrium between the left- and right-handed conformers arises from a combination of localized ion pairing and hydrogen bonding interactions in a 1:1 complex between the carboxylic acid and the foldamer. Nonetheless, excess ligand produced complexes with higher stoichiometry that interfered with the conformational responses. Building on Inai’s work, we aimed first to quantify the screw-sense preference induced in a conformationally labile foldamer as a result of noncovalent hydrogen bonding/ion pairing interactions, and second to identify a more versatile basic binding site that would be able to maintain selective and strong 1:1 binding interactions even in the presence of a mixture of different competing ligands.

Because of their well established ability to form conformationally uniform, hydrogen-bonded 3-helical structures in a range of solvents, we used foldamers consisting of oligomers of 2-aminoisobutyric acid (Aib) residues. Helical Aib oligomers are achiral, and therefore necessarily conformationally racemic, but may be induced to adopt a globally preferred screw sense (left-handed M or right-handed P) by a covalently attached terminal chiral residue.

A small library of potential binding sites B0−7 were ligated to the N-terminus of 4−9 Aib residue oligomers to form achiral helical foldamers (F0−7) (for synthetic details, see the Supporting Information, SI). Several chiral acids (HA1−6) or anions (A7−, A8−) with a range of gross structural features and pKₐ values were chosen as potential chiral ligands (Figure 1). To allow us to quantify the global conformational change in any of F0−7 induced by interaction with any of HA1−6, a ¹³C NMR reporter of helical screw-sense preference was incorporated into the foldamers F0−7 at a position remote from the binding site. The C-terminal Aib residue was labeled with ¹³C at both enantiotopic methyl groups. At ambient temperature under normal conditions of rapid screw sense inversion, the anisochronicity (Δδ) of the two diastereotropic ¹³CH₃ signals of the NMR probe is proportional to the imbalance between the population of M and P conformers of the foldamer F (the helical excess, h.e.).

The anisochronicity Δδ was typically measured by recording ¹³C NMR spectra at 296 K in CDCl₃ of mixtures of HA and F at concentration of [F] = 10 mM (sufficiently low to avoid foldamer aggregation) and in a ratio HA:F = 1.2:1. The values of Δδ are reported in Table 1 as anisochronicity (in ppb) and as a screw-sense preference (helical excess, h.e.) calculated from Δδ as described in the SI.

### Quantifying the Effect of Ligands.

Initial experiments employed primary amine binding sites B1 and B2 (Table 1, entries 1, 2). In the case of free Aib-terminated F1, carboxylic acid HA1 and N-trifyl phosphoramidate HA6 failed to induce a screw-sense preference in the achiral foldamer (Δδ = 0). However, phosphoric acids HA2−HA5 induced a conformational preference in F1 having a maximum value of 70% h.e. for HA5 (entry 1). With F2, which contains a β-alanine binding site, all of HA1−HA6 induced at least some conformational preference in F2 (entry 2), with a maximum value of 63% h.e. for HA4. The parent, nonbasic azido-substituted foldamer (entry 0) displayed almost no conformational induction with HA1, HA4, or HA6, showing that nonspecific interactions of HA with the Aib oligomer were insignificant.

Inai had shown that N-terminal β-alanine-bearing foldamers participate with N-Boc protected amino acids in stable 1:1 interactions which retain their conformational preference in the presence of moderate excesses of the amino acid. However, we found that this was not the case for the β-alanine-capped foldamer−phosphoric acid pair HA4−F2: in this case, both the ratio HA4:F2 and the concentration [F2] had a significant effect on the conformational preference of the helical foldamer.
Anisochronicity (Δδ) measured in F2 increased with the amount of HA4 up to a maximum value corresponding to ca. 70% h.e. at 1:1 HA4:F2 but then decreased in the presence of excess HA4 dropping to 56% h.e. with 2.5 equiv. HA4 (SI Figure S34). Conformational control in the HA4↔F2 mixture was also concentration-dependent, increasing in a linear manner up to a concentration [F2] = 2.5 mM and then dropping (SI Figure S48).

Phosphoric acids HA2−5 and N-triflyl phosphoramides HA6 are evidently capable of inducing relatively powerful conformational preferences in helical foldamers, but higher order complexes that diminish the h.e. are evidently possible. These competing interactions may arise from multiple hydrogen bonds to the NH3+ group in the protonated binding site B2H+, disrupting the stoichiometric acid–base interaction HA4↔F2.

This information prompted us to investigate alternative basic binding sites, and especially N-terminal pyridyl substituents B3−6 (Figure 1): such motifs can accept or (when protonated) donate only one hydrogen bond. The Δδ values induced by acids HA1−6 and anions A7− and A8− were measured in CDCl3 (plus 28% MeOH for A7− and A8−) using the protocol described above (Table 1, entries 3−8).

In the case of F3, the phosphoric acids (HA2, HA4, HA5) and phosphoramid HA6 induced a weak conformational preference (entry 3). Moving to the more flexible but more basic N-terminal 2- and 3-pyridinylacetyl motifs B4 and B6 led to higher levels of conformational induction from all three groups of acid ligands. More specifically, HA4, HA1, and HA6 resulted in three distinct, decreasing chemical shift separations in N-terminal 2-pyridylacetyl foldamer F4 (entry 4). A similar trend, with similar values for the induced helical excess, was observed with the longer oligomer F4′ (entry 5). By contrast, conformational preferences in the N-terminal 3-pyridyl foldamer F6 were reduced, except with HA6 (entry 6). As a control experiment, HA4, HA1, and HA6 were added to nonbasic foldamer F7 (entry 9). Zero or very low induced screw-sense preferences were measured, confirming that any control arising from the chiral ligands occurs almost entirely from interactions at the N-terminal binding site.

Mixing chiral anions A7− and A8− with the methylated foldamers F5Me+ and F6Me+ induced some detectable conformational preferences, even in the presence of methanol, showing that ion pairing alone may be sufficient to transfer chiral information from the ligand to the foldamer, but the level of control was low (entries 7, 8). (The conformational

| entry | foldamer F | n | binding site B | HA1a | HA2 | HA3 | HA4 | HA5 | HA6 | A7f | A8f |
|-------|------------|---|---------------|------|-----|-----|------|-----|-----|-----|-----|
| 0     | F0         | 0 | B0            | 0    | −   | −   | 58   | −   | 40  | −   | −   |
| 1     | F1         | 0 | B1            | 0    | 525 | 88  | 500  | 1236| 0   | −   | −   |
| 2     | F2         | 1 | B2            | 124  | 105 | 131 | 1127 | 171 | 55  | −   | −   |
| 3     | F3         | 1 | B3            | 0    | 99  | 0   | 166  | 102 | 37  | −   | −   |
| 4     | F4         | 1 | B4            | 838  | 88  | 306 | 1035 | 234 | 169 | −   | −   |
| 5     | F4′        | 5 | B4            | 783  | −   | −   | 1068 | −   | 73  | −   | −   |
| 6     | F6         | 1 | B6            | 33   | 22  | 127 | 492  | 237 | 169 | −   | −   |
| 7     | F5Me+      | 1 | B5Me+         | −    | −   | −   | −    | −   | −   | −   | −   |
| 8     | F6Me−      | 1 | B6Me−         | −    | −   | −   | −    | −   | −   | −   | −   |
| 9     | F7         | 1 | B7            | 0    | −   | −   | 26   | −   | 0   | −   | −   |

Entries shaded in grey indicate the greatest levels of non-covalent conformational induction, and these combinations were used further in later experiments. a Measured using 1.5 equiv HA. b Using a 19F NMR-based probe. c 8 equiv. A−. d 10 equiv. A−. e 4 equiv. A−. f In the presence of 28 vol % methanol; empty table cell, value not measured.

---

Anisochronicity (Δδ) measured in F2 increased with the amount of HA4 up to a maximum value corresponding to ca. 70% h.e. at 1:1 HA4:F2 but then decreased in the presence of excess HA4 dropping to 56% h.e. with 2.5 equiv. HA4 (SI Figure S34). Conformational control in the HA4↔F2 mixture was also concentration-dependent, increasing in a linear manner up to a concentration [F2] = 2.5 mM and then dropping (SI Figure S48).

Phosphoric acids HA2−5 and N-triflyl phosphoramides HA6 are evidently capable of inducing relatively powerful conformational preferences in helical foldamers, but higher order complexes that diminish the h.e. are evidently possible. These competing interactions may arise from multiple hydrogen bonds to the NH3+ group in the protonated binding site B2H+, disrupting the stoichiometric acid–base interaction HA4↔F2. This information prompted us to investigate alternative basic binding sites, and especially N-terminal pyridyl substituents B3−6 (Figure 1): such motifs can accept or (when protonated) donate only one hydrogen bond.

Foldamers F3−6 were constructed containing the pyridine-carboxamide and pyridylacetamide binding sites B3−6, along with the two methylated pyridinium sites B5Me+ and B6Me+ that can ion-pair but not hydrogen bond. The Δδ values induced by acids HA1−6 and anions A7− and A8− were measured in CDCl3 (plus 28% MeOH for A7− and A8−) using the protocol described above (Table 1, entries 3−8).

In the case of F3, the phosphoric acids (HA2, HA4, HA5) and phosphoramid HA6 induced a weak conformational preference (entry 3). Moving to the more flexible but more basic N-terminal 2- and 3-pyridinylacetyl motifs B4 and B6 led to higher levels of conformational induction from all three groups of acid ligands. More specifically, HA4, HA1, and HA6 resulted in three distinct, decreasing chemical shift separations in N-terminal 2-pyridylacetyl foldamer F4 (entry 4). A similar trend, with similar values for the induced helical excess, was observed with the longer oligomer F4′ (entry 5). By contrast, conformational preferences in the N-terminal 3-pyridyl foldamer F6 were reduced, except with HA6 (entry 6). As a control experiment, HA4, HA1, and HA6 were added to nonbasic foldamer F7 (entry 9). Zero or very low induced screw-sense preferences were measured, confirming that any control arising from the chiral ligands occurs almost entirely from interactions at the N-terminal binding site.

Mixing chiral anions A7− and A8− with the methylated foldamers F5Me+ and F6Me+ induced some detectable conformational preferences, even in the presence of methanol, showing that ion pairing alone may be sufficient to transfer chiral information from the ligand to the foldamer, but the level of control was low (entries 7, 8). (The conformational
preferences in these cationic foldamers were quantified using a different set of $^{19}$F-containing NMR reporters: see the SI for details.69)

**Nature of the Ligand-Binding Site Interaction.** Having identified the 2-pyridylacetamide motif B4 as a strong candidate in the search for a versatile and effective binding site for the development of a multicomponent signaling system, we next studied the stability of the ligand-foldamer pairs HA1↔F4, HA4↔F4, and HA6↔F4 with respect to excess ligand and concentration. Varying the ratio HA1:F4 (Figure 2) gave a maximum induced helical excess of 55% for a ratio HA1:F4 >1:1. A similar trend was observed for HA4↔F4 with a maximum value around 59% h.e. for a ratio >3:1. A similar trend was observed for HA4 maximum induced helical excess of 55% for a ratio >1:1. In the case of HA6↔F4, the maximum conformational induction (around 6% h.e.) was obtained with a ratio HA6:F4 = 1.2:1. In this case only, the $\Delta \delta$ value dropped in the presence of an excess of HA6, falling to 0 in the presence of 2.7 equiv of the ligand.

The change of h.e. upon binding of HA1 and HA4 to F4 was fitted using a 1:1 binding model (see the SI). For HA1, a good fit to the data was found for a binding constant of $K = (1 \pm 0.3) \times 10^4$ M$^{-1}$, while for HA4 the binding constant was found to be $>10^5$ M$^{-1}$. This large difference in binding affinity (by a factor of $>10^2$) was critical in allowing the development of complex systems capable of conformational switching. For HA6, the variation of h.e. on binding was fitted using a 2:1 binding model, which gave a good fit to the data with $K = 10^4$ M$^{-1}$ and $K' = 10^6$ M$^{-1}$ (see the SI).

Conformational induction in the HA4↔F4 pair was remarkably concentration-independent: the induced helical excess was constant for [F4] ranging from 10 mM to 0.1 mM (ratio HA:F fixed at 1:2:1, Figure 3). In HA6↔F4, the conformational preference was likewise almost constant down to 0.1 mM. In the less strongly bound pair HA1↔F4, h.e. varied little between 5 and 10 mM, but fell markedly at lower concentrations. These results also give a qualitative indication of the strength of binding in the HA↔F4 pairs, with HA6↔F4 ≥ HA4↔F4 > HA1↔F4. The conformational effect of all three ligands was much weaker in the presence of a protic solvent: for example, addition of 2% MeOH to the solution in CDCl$_3$ induced a significant drop in the value of $\Delta \delta$ for the HA4↔F4 interaction (SI Figure S56).

The nature of the interaction between the ligands and the binding site of F4$^{62,70–72}$ was examined by following the change in $^{13}$C and $^1$H NMR spectra as HA1, HA4, or HA6 were titrated into a solution of F4 in CDCl$_3$ at 296 K (SI Figures S35–37, S39–41, and S43–45). Addition of either HA1, HA4, or HA6 led to gradual migration of $^1$H NMR signals of the pyridine binding site to new positions, with the change in chemical shift being much more significant for HA4 or HA6 than for HA1. During the titration with HA1, none of the four proton signals from the pyridyl ring migrated by more than 0.13 ppm, though two of the NH protons of the foldamer chain exhibited a downfield shift. By contrast, during the titrations with HA4 and HA6, the protons in the 4- and 5-positions of the pyridine ring migrated downfield by 0.5–0.6 and 0.25 ppm respectively, while the proton in the 6-position migrated upfield by 0.6–0.7 ppm. In addition, the migration of the peaks to new positions is complete after the addition of 1.0 equiv for HA4, while with HA6, the addition of more than one equivalent of the acid leads to further changes in the $^1$H NMR spectrum that could be explained by protonation of other, less basic sites within F4 by this extremely strong acid. Finally, similar experiments with HCl led to downfield shifts (of 0.1–0.7 ppm) for all pyridyl protons (SI Figure S57).

In nonpolar solvents, neutral bases such as amines and pyridines are markedly less basic than anionic species, such as carboxylates, due to poor stabilization of charged species.73 Acid–base interactions in chloroform are likely to start from hydrogen bonding, which under certain conditions can evolve into proton transfer from acid to base and, infrequently, dissociation of the resulting hydrogen-bonded ion pair. The
extent of proton transfer in a hydrogen-bonded complex is dictated mainly by the difference of basicities of the acid anion and base in the given medium. The titration results suggest that the interaction of F4 with the stronger acids HA4 and HA6 leads to extensive proton transfer from acid to the pyridine binding site and formation of a strong ionic hydrogen bond (a tightly hydrogen-bonded ion pair). HA1, by contrast, is insufficiently acidic to allow proton transfer to the pyridine site, yet still forms a hydrogen-bonded complex with F4 that is additionally stabilized by interaction with two of the NH protons at the N terminus of the foldamer chain. Estimates of the relative pK values of HA1, HA4, and HA6 in 1,2-dichloroethane (DCE) are shown in Table 2 along with the reported so-called “ion-pair” pK values for AcOH and HCl in DCE (all pK values are relative to 2,4,6-trinitrophenol). 1,2-Dichloroethane was used as a model for chloroform due to scarcity of reported acidity data in the latter, and the similarity between the properties of these two solvents was confirmed using COSMO-RS calculations. The pK estimates were calculated using linear regressions between pK values in DCE and acetonitrile (HA4, HA6), and between pK values in DCE and acid dissociation energies by COSMO-RS (HA1) (for further details, see SI). Pyridine is known to be remarkably less tightly hydrogen-bonded ion pair.

| acid   | base  | relative pK (DCE) |
|--------|-------|-------------------|
| AcOH   | AcO⁻  | 15.5⁷⁻     |
| HA1    | A⁻    | 12⁷⁻       |
| HA4    | A⁻⁺   | 3       |
| HCl    | Cl⁻   | −0.4⁶⁻    |
| HA6    | A⁻⁺   | −5.2⁷⁻    |

“Calculated value from ref 73. ²Estimated using COSMO-RS calculations. ³Estimated using experimental pK values in acetonitrile. ⁴Experimental value from ref 75.

Screw-sense inversion of Aib oligomers occurs on a submillisecond time scale at room temperature. In other words, room temperature ¹³C (ref 67) and ¹H (refs S8, S22) NMR spectra lie in the fast exchange regime with respect to screw sense inversion. The peak shapes in the ¹H NMR spectra resulting from titrations of F4 with HA1 or HA6 remain constant and more or less sharp throughout the experiment (SI Figures S35, S36, and S43–44). This result is consistent with rapid exchange (on the NMR time scale) of F4 not only between screw-sense conformers, but also between bound and unbound states when a substoichiometric quantity of either ligand is present. By contrast, addition of substoichiometric amounts of HA4 to F4 gives rise to exchange broadening in the ¹H NMR spectrum, with peaks sharpening again as more of the ligand is added (SI Figures S39 and S40). This result suggests slower exchange of F4 between bound and unbound states, with the coalescence temperature for this exchange process lying close to ambient temperature.

Related behavior was evident in the ¹³C NMR spectra (SI Figures S37, S41, and S45). With HA1, fast exchange between bound and unbound F4 led to the separation between the peaks arising from the two ¹³C labels increasing successively with additional quantities of HA1 to reach a maximum of 959 ppb, corresponding to 54% h.e. Evidence for fast exchange between bound and unbound states was further provided by variable temperature ¹³C and ¹H NMR experiments of a mixture of F4 and 0.5 equiv HA1. The ¹³C NMR spectrum of this mixture showed just one pair of sharp signals above 293 K that start to undergo decoalescence on lowering the temperature to 235 K (SI Figure S65). With HA6, similar incremental increases in peak separation were seen, but when more than one equivalent of acid was added, the Δδ value dropped to 0 ppb and the ¹³C label signal migrated to a new position, presumably due to protonation of the peptide chain.

Behavior in the ¹³C NMR spectrum during titration of F4 with HA4 was different, showing broadened signals characteristic of spectra in the intermediate exchange regime even at

Table 2. Estimated and Reported pK Values of Acids in 1,2-Dichloroethane Relative to 2,4,6-Trinitrophenol (see SI for Details)
room temperature. Variable temperature $^{13}$C and $^1$H NMR experiment of a mixture of 0.5 equiv HA4 with F4 (SI Figure S66) were consistent with a mixture of bound (a pair of signals in the $^{13}$C NMR) and unbound (a single signal) states that are exchange-broadened at all temperatures between 235 and 313 K and that undergo coalescence at around 270 K. At 235 K, the singlet arising from the unbound state is just above coalescence, behavior consistent with the slowing of screw sense inversion to a time scale slightly faster than that of ligand binding.

Line shape simulations of the $^{13}$C NMR spectra obtained during the titration of F4 with HA4 and of the VT $^{13}$C NMR spectra obtained from the mixture F4 with 0.5 equiv HA4 (SI Figure S68) lend further support to our interpretation of these results in terms of exchange between bound and unbound states, and between left- and right-handed screw-sense, on a time scale of $10^{-5} - 10^{-6}$ s at 295 K (see the SI).

**Competition between Ligands: A Three-Component System.** At this stage of the study, it was clear that the 2-pyridylacetyl motif B4 was capable of sustaining stable 1:1 interactions through hydrogen bonding and/or ion pairing with a range of chiral acids, with [F] from 10 to 7 mM or less and HA1·F, HA4·F, and HA6·F ratios from 1:1 to 1:5:1. We now needed to set up ligand exchange experiments between competing foldamer-ligand pairs HA4 ↔ F vs HA4 ↔ F. In order to establish which of the alternative pairs predominated, we chose systems in which competing ligands would each induce an opposite absolute screw sense in the foldamer. Absolute screw-sense preference in HA4 ↔ F pairs was determined using labeled foldamer F4* in which the C-terminal (R)-Ab*OMe residue is asymmetrically enriched in $^{13}$C, with 75% $^{13}$C in the pro-R Me group and 25% in the pro-S.63 As a result, the major $^{13}$C NMR signal appears downfield of the minor signal when the residue finds itself terminating a P-helix and upfield of the minor signal in an M-helix, allowing $^{13}$C NMR to report on both the relative and absolute sense of conformational induction in the foldamer.17,24,82 Preliminary experiments with F4*, mixing with either (S)·HA1, (S)·HA4 or (S)·HA6, showed that (S)·HA1 and (S)·HA4 induced a right-handed (P) screw sense while (S)·HA6 induced (more weakly) a left-handed (M) screw sense (Figure 5a-c).

Now the scene was set for a competition experiment70 between two ligands. (S)·HA1 (1.5 equiv) was added to a solution of F4* (1.0 equiv) in CDCl3 to induce P screw sense (Figure 6a,b) with the anisochronicity +904 ppb characteristic of the ca. 50% h.e. induced in the HA1↔F4 pair (cf. Table 1, entry 4). On addition of the stronger acid (R)·HA4 (1.5 equiv) to the mixture (Figure 6c), the major signal in the $^{13}$C NMR spectrum moved upfield of the minor, indicating a switch in the screw sense preference of F4* from P to M.72,84 The anisochronicity of the signals also increased in magnitude to $-945$ ppb, suggesting almost exclusive formation of a paired (R)·HA4↔F4* ligand-foldamer complex (Table 1, entry 4). Evidently, HA4 can completely displace HA1 from the pyridyl binding site, a result that is most readily understood as a consequence of the tighter pairing between the foldamer F4* and the stronger acid HA4.82 In the absence of detailed knowledge about the extent of proton transfer in the acid–base pairs in this and subsequent studies, foldamer F4* is represented by a neutral pyridine ring irrespective of its probable protonation state: it may be assumed that this pyridine pairs with the most acidic species available, by a mechanism that we leave undefined diagrammatically.

We reasoned that the interaction with, and hence the influence of, the carboxylic acid HA1 would be restored by the addition of a base stronger than F4* in CDCl3. Addition of ammonia (1.5 equiv) indeed induced a screw sense inversion back from M to P (Figure 6d). Presumably, the allocation of the proton available from HA4 to NH determines an ion pair NH$_4^+$A4* that allows the neutral, acidic HA1 and the neutral, basic F4* to reform a screw-sense inducing HA1↔F4* interaction. The reduced anisochronicity of +423 ppb does however suggest some interference in this hydrogen-bonded interaction from the other acidic and basic species in solution. Given that the relative dominance of competing ligands HA1 or HA4 may evidently be decided by the availability of protons, we reasoned that screw sense in F4* should be switchable simply by addition either of base (to favor the P helical pairing HA1↔F4*) or of acid (to favor the M helical pairing HA4↔F4*). Adding HCl (1.5 equiv) to the previous mixture of HA1, HA4, F4* and NH$_3$ led to a switch in screw sense from P back to M (Figure 6e) as the HA4↔F4* interaction is restored by the additional proton now made available. This change was accompanied by a white precipitate, attributed to the formation of NH$_4$Cl. The protonation was reversible, and adding again NH$_3$ (1.5 equiv) switched back on the HA1↔F4* interaction and inverted again F4*’s screw sense from P to M (Figure 6f).

After each switching cycle, the anisochronicity of ca. $-950$ ppb induced in F4* by the stronger acid HA4 was resilient in the presence of the other species in the mixture, while that induced by HA1 continued to decline, despite the apparent removal of NH$_4$Cl from solution by precipitation.

Having demonstrated reversible switching between the two states M (in HA4↔F4*) and P (in HA1↔F4*), we added another 1.5 equiv of NH$_3$. As a result, F4* entered a third conformational state in which it recorded no screw-sense preference (Figure 6g). Presumably the stronger base NH$_3$ now displaces the weaker F4* from the HA1↔F4* pair, leaving F4* unable to interact with HA1. This conformationally racemic ±
The resting state could be “reactivated” with HCl: adding two portions (3.0 equiv) to the previous solution reprotonated the excess ammonia and reinstated the HA4 ↔ F4* pair, restoring a powerful (Δδ = −984 ppb) M screw-sense preference (Figure 6h).

Overall then, the three-component chemical system comprising F4*, HA1 and HA4 may be switched at will between three alternative conformational states (in this case ± → P → M → P → M → P → ± → M) by successive additions and subtractions of protons, forcing the pH-dependent selective exchange of ligands at the pyridylacetyl binding site.

Switching in Four-Component Systems. Encouraged by the responsiveness of this three-component system, we investigated the potential for acidity-driven conformational switching in a four-component system composed of F4*, (R)-HA1, (S)-HA4, and (S)-HA6. Starting with a solution of F4* (1.0 equiv) in CDCl₃ (Figure 7a) we added (R)-HA1 (1.5 equiv), inducing an M screw sense in F4* (Figure 7b), followed by (R)-HA4 (1.5 equiv), inverting the screw-sense of F4* from M to P (Figure 7c). Now, addition of (S)-N-trityl phosphoramidate HA6 (1.5 equiv) to this mixture induces a second helical inversion from P back to M (Figure 7d), with a
value of $\Delta \delta = -112$ ppm. We presume that HA6 takes control of the conformation of the foldamer F4* by protonating the HA4↔F4* pairing of the weaker acid HA4 and inducing a conformational preference characteristic of the new (probably largely ion-paired\(^{62}\)) HA6↔F4* interaction (cf. Table 1, entry 4).

Next, 4.5 equiv of ammonia was added in 1.5 equiv portions to the four-component system. The screw sense of F4* switched from M to P (Figure 7e) as the first 1.5 equiv was added. Portions of the \(^{13}\)C NMR spectra of the mixtures containing the labeled signals of F4* are shown, with anisochronicty $\Delta \delta$ reported as the difference in chemical shift between the major and minor labeled signals of F4*, $\delta_{\text{maj}} - \delta_{\text{min}}$, measured in ppb. Protonated species available for interaction with the F4* binding site (represented by the pyridine in the colored rectangle) are indicated by blue/green (for chiral species) or gray (for achiral species) disks, and acids HA are stacked in order of $pK_a$. The number of protons available is represented by the number of discs, building up from the bottom of the stack. Proposed conformation-inducing interactions with F4* (whether these are hydrogen-bonded or ion-paired is left undefined) are coded by matched colors: blue indicates induction of a P screw-sense; green indicates induction of an M screw-sense; red indicates no screw-sense induction. The most significant interaction is assumed to be between F4* and the top (typically the most acidic) protonated species in each multiply protonated stack. 7(g) is an exception: A1 is probably protonated rather than NH3, but the lack of screw sense preference in F4* suggests interaction preferentially with an NH4+ ion.

Figure 7. Conformational switching of foldamer F4* with three competing chiral ligands. [F4*] = 10 mM, CDCl3, 296 K; all subsequent additions are of 1.5 equiv relative to F4*. Portions of the \(^{13}\)C NMR spectra of the mixtures containing the labeled signals of F4* are shown, with anisochronicty $\Delta \delta$ reported as the difference in chemical shift between the major and minor labeled signals of F4*, $\delta_{\text{maj}} - \delta_{\text{min}}$, measured in ppb. Protonated species available for interaction with the F4* binding site (represented by the pyridine in the colored rectangle) are indicated by blue/green (for chiral species) or gray (for achiral species) disks, and acids HA are stacked in order of $pK_a$. The number of protons available is represented by the number of discs, building up from the bottom of the stack. Proposed conformation-inducing interactions with F4* (whether these are hydrogen-bonded or ion-paired is left undefined) are coded by matched colors: blue indicates induction of a P screw-sense; green indicates induction of an M screw-sense; red indicates no screw-sense induction. The most significant interaction is assumed to be between F4* and the top (typically the most acidic) protonated species in each multiply protonated stack. 7(g) is an exception: A1 is probably protonated rather than NH3, but the lack of screw sense preference in F4* suggests interaction preferentially with an NH4+ ion.
added, then back to M with the second 1.5 equiv (Figure 7f), and then to the \( \pm \) state with the third 1.5 equiv (Figure 7g).

Neutralizing the 4.5 equiv ammonia with 4.5 equiv HCl took the system back to the P state (Figure 7h) corresponding to the HA4 interaction, with magnitude of conformational control similar to that observed originally (cf. Figure 7d,h).

The cyclic switching of screw sense with successive additions of ammonia can be accounted for by ammonia first disrupting the most acidic triflamide pairing HA6\(\leftrightarrow\)F4\(^*\) (Figure 7e) then the phosphoric acid–pyridine pairing HA4\(\leftrightarrow\)F4\(^*\) (Figure 7f) and finally the least acidic carboxylic acid–pyridine pairing HA1\(\leftrightarrow\)F4\(^*\) (Figure 7g). In other words, the three additions of ammonia each provide a favorable, basic destination for the three protons initially supplied to the system by the three acids, leaving the foldamer F4\(^*\) to choose a partner from the acid species that remain after each addition. In Figure 7e the selective interaction of F4\(^*\) with HA4 rather than HA1 may be driven principally by the relative acidity of HA4, while in Figure 7f an additional factor in the choice of HA1 over the probably more acidic NH4\(^+\) may be the involvement of NH4\(^+\) in stronger ion pairing interactions with A6\(^-\) and A4\(^-\). As ammonium counterions build up in solution, the level of conformational induction is reduced, presumably because they compete as (achiral, screw-sense neutral) acid ligands for F4\(^*\). The final addition of NH3 (Figure 7g) offers even the weakest acid HA1 a more basic partner than F4\(^*\), so F4\(^*\) is left unpartnered in an achiral environment. Final addition of 4.5 equiv HCl precipitated the added base from solution as ammonium chloride, and completed the cycle of switching of F4\(^*\) from \( \pm \) \(\rightarrow\) M \(\rightarrow\) P \(\rightarrow\) M \(\rightarrow\) P \(\rightarrow\) M \(\rightarrow\) \(\pm\) and restores fully the degree and sense of conformational control supplied by the triflamide HA6.

Aiming to avoid the deteriorating conformational control that appears to result from the accumulation of hydrogen-bond

Figure 8. Conformational switching of foldamer F4\(^*\) with three competing chiral ligands in a single phase. PS = proton sponge. [F4\(^*\)] = 10 mM, CDCl\(_3\), 296 K; all subsequent additions are of 1.5 equiv relative to F4\(^*\). Portions of the \(^{13}\)C NMR spectra of the mixtures containing the labeled signals of F4\(^*\) are shown, with anisochronicity \( \Delta \delta \) reported as the difference in chemical shift between the major and minor labeled signals of F4\(^*\), \( \delta^{\text{maj}} - \delta^{\text{min}} \), measured in ppb. protonated species available for interaction with the F4\(^*\) binding site (represented by the pyridine in the colored rectangle) are indicated by blue/green (for chiral species) or gray (for achiral species) disks, and acids HA are stacked in order of pK\(_a\). The number of protons available is represented by the number of discs, building up from the bottom of the stack. Proposed conformation-inducing interactions with F4\(^*\) (whether these are hydrogen-bonded or ion-paired is left undefined) are coded by matched colors: blue indicates induction of a P screw-sense; green indicates induction of an M screw-sense; and red indicates no screw-sense induction. The most significant interaction is assumed to be between F4\(^*\) and the top (typically the most acidic) protonated species in each multiply protonated stack.
donating ammonium ions, we repeated the acid–base switching experiment with proton sponge86 (PS, 1,8-bis(dimethylamino)-naphthalene), selected as an alternative base to NH3 that will sequester the accepted proton with an internal hydrogen bond. The results are shown in Figure 8, where stages α–d match the switching process of Figure 7a–d. Addition of PS (1.5 equiv) to the four component system F4*+HA1+HA4+HA6 of Figure 8d induced a conformational switch from M to P (Figure 8e) as observed with ammonia (Figure 7e), but with much greater presence of PSH+ (Figure 8e), compared with +190 ppb in the absence of cations (Figure 8c) and only +190 ppb in the presence of NH4+ (Figure 7e). The second addition of PS (1.5 equiv) switched screw-sense from P to M (Figure 8f) but this time with only marginally greater conformational control than with NH3 (Figure 7f). Unlike with NH3, the third addition of PS (1.5 equiv) did not result in the system switching to the resting ± state: an induced M screw sense was still observed (Figure 8g). Working back up the acidity scale, a first addition of HCl (1.5 equiv) had no influence on the system, which remained M (Figure 8h), and a second addition of HCl (1.5 equiv) induced a switch from M to P with good recovery of the conformational control typical of HA4→F4*. However, a third addition of HCl (1.5 equiv) did not result in the switch from P to M as seen with ammonia as base (Figure 7h)—instead F4* remained in the P screw-sense, but with a reduced magnitude of conformational control extent (Figure 8i).

The inability of PS to disrupt the HA1→F4* interaction (Figure 8g) suggests that while PS and NH3 are both insufficiently basic to deprotonate carboxylic acid HA1, NH3 hydrogen bonds strongly to HA1, disrupting its interaction with F4* (Figure 7g). In contrast, steric hindrance at the basic site in PS may prevent strong hydrogen-bonding to HA1, leaving the HA1→F4* interaction intact.

The lack of recovery of M screw sense on acidification to the final F4*+HA1+HA4+HA6+(3 × PS)+(3 × HCl) mixture (Figure 8i) seems likely to arise from the contrasting behavior of NH4Cl (which precipitates from chloroform) and PS-HCl (which remains in solution). In this final mixture, the system has six protons to distribute between seven bases, so the mixture presumably contains 3 × PSH+, HF4+, HA1, HA4, A6, and three Cl− ions. Before the final acidification step, the conformation-controlling interaction is that between the strongest base F4* and the strongest acid HA4 (Figure 8i). We expected addition of HCl to protonate A6*, hence disrupting HA4→F4* and allowing HA6→F4* to form, an opportunity which it evidently nonetheless does not take. This observation can be rationalized by assuming that the weak M preference of the HA6→F4* ion pair (seen in Table 1 entry 4, Figure 7d and Figure 8d) is sensitive to disruption by the high concentration (4.5 equiv relative to F4*) of other ionic material in solution. More strongly hydrogen-bonded, rather than principally ion-paired, interactions seem less susceptible to interference by the presence of dissolved salts (cf. Figure 8c, e.g.; Figure 8f,h).

### CONCLUSIONS

Selecting interactions among the many possible within a multicomponent chemical mixture leads a peptidomimetic foldamer to adopt a specific conformational preference, which may be quantified by 13C NMR. Alternative permutations of mutual interactions among the components of the system may be activated by controlling the protonation state of the system. In response to changes in acidity, the foldamer chooses, from a suite of ligands of graded basicity, a partner whose binding is identifiable by the specific, ligand-dependent conformational preference it induces in the foldamer. Competing ligands are simultaneously rendered ineffective by stronger silencing interactions with alternative acids or bases, but each may nonetheless be restored to activity by adding or subtracting protons. By choosing chiral ligands of appropriate configuration, pH changes can be used to switch the foldamer reversibly between left- and right-handed conformations84,88 with conformational preferences characteristic of the ligands employed. The chemical system thus behaves as a simple proton-counting device.25,26,89–91 It can also be viewed as an acidity-sensitive conformational indicator,92–103 whose analogue spectroscopic output (measurable by the relative positions of two peaks in the 13C NMR spectrum) is dictated by a conformational preference that is itself a function of the number of protons available in the mixture. Future work will seek to develop more complex synthetic networks of conformationally responsive interacting molecules.

### ASSOCIATED CONTENT

#### Supporting Information

Characterization data for all new compounds. Full details of titration and NMR experiments. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b03284.

### AUTHOR INFORMATION

#### Corresponding Author

*clayden@man.ac.uk

#### Notes

The authors declare no competing financial interest.

### ACKNOWLEDGMENTS

This work was funded by the ERC (AdG ROCOCO) and the BBSRC (Grant I007962). The work of S.T. and I.L. was supported by institutional research funding I007962. The work of S.T. and I.L. was supported by institutional research funding I007962. The work of S.T. and I.L. was supported by institutional research funding I007962. The work of S.T. and I.L. was supported by institutional research funding I007962. The work of S.T. and I.L. was supported by institutional research funding I007962.

### REFERENCES

1. Lehn, J.-M. Chem.—Eur. J. 2000, 6, 2097–2102.
2. Sarma, R. J.; Nitschke, J. R. Angew. Chem., Int. Ed. 2008, 47, 377–380.
3. Thomas, S. W.; Chiuchi, R. C.; LaFratta, C. N.; Webb, M. R.; Lee, A.; Wiley, B. J.; Zakin, M. R.; Walt, D. R.; Whitesides, G. M. Proc. Natl. Acad. Sci. U. S. A. 2009, 106, 9147–9150.
4. Safont-Sempere, M. M.; Fernández, G.; Wüthrich, F. Chem. Rev. 2011, 111, 5784–5814.
5. Milroy, L.-G.; Grossmann, T. N.; Hennig, S.; Brunsveld, L.; Ottmann, C. Chem. Rev. 2014, 114, 4695–4748.
6. Grauer, A.; König, B. Eur. J. Org. Chem. 2009, 2009, 5099–5111.
7. Ha, J.-H.; Loh, S. N. Chem.—Eur. J. 2012, 18, 7984–7999.
8. Smith, C. A.; Ban, D.; Pratihar, S.; Giller, K.; Schwegle, C.; de Groot, B. L.; Becker, S.; Griesinger, C.; Lee, D. Angew. Chem., Int. Ed. 2015, 54, 207–210.
9. Nevola, L.; Giral, E. Chem. Commun. 2015, 51, 3302–3315.
10. Perutz, M. F. Q. Rev. Biophys. 1989, 22, 139–236.
11. Shibayama, N.; Sugiyama, K.; Tame, J. R. H.; Park, S.-Y. J. Am. Chem. Soc. 2014, 136, 5097–5105.
(12) Yuan, Y.; Tam, M. F.; Simplanceau, V.; Ho, C. Chem. Rev. 2015, 115, 1702–1724.
(13) Perutz, M. F. Nature (London) 1988, 336, 202–203.
(14) Dudek, T.; Lim, C. Sci. Rep. 2015, 5, 7864.
(15) Davis, R. B., Jr.; Lecomte, J. T. J. Proteins 2005, 63, 336–348.
(16) Krauss, R.; Koert, U. Synlett 2003, 598–608.
(17) Solà, J.; Fletcher, S. P.; Castellanos, A.; Clayden, J. Angew. Chem., Int. Ed. 2010, 49, 6836–6839.
(18) Clayden, J.; Lund, A.; Vallverdu, L. S.; Hellwell, M. Nature (London) 2004, 431, 966–971.
(19) Clayden, J.; Vassiliou, N. Org. Biomol. Chem. 2006, 4, 2667–2678.
(20) Clayden, J. Chem. Soc. Rev. 2009, 38, 817–829.
(21) Gellman, S. H. Acc. Chem. Res. 1998, 31, 173–180.
(22) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. Chem. Rev. 2001, 101, 3893–4012.
(23) Hecht, S.; Hu, I. Foldamers: Structure, Properties and Applications; Wiley-VCH: Weinheim, 2007.
(24) Brown, R. A.; Diemer, V.; Webb, S. J.; Clayden, J. Nat. Chem. 2013, 5, 853–860.
(25) Shigeno, M.; Kushida, Y.; Kobayashi, Y.; Yamaguchi, M. Chem.—Eur. J. 2014, 20, 12759–12762.
(26) Pischel, U. Angew. Chem., Int. Ed. Engl. 2007, 46, 4026–4040.
(27) Knipe, P. C.; Thompson, S.; Hamilton, A. D. Chem. Sci. 2015, 6, 1630–1639.
(28) Knipe, P. C.; Lingard, H.; Jones, I. M.; Thompson, S.; Hamilton, A. D. Org. Biomol. Chem. 2014, 12, 7937–7941.
(29) Mendez-Arroyo, J.; Barroso-Flores, J.; Lifschitz, A. M.; Sarjeant, A. A.; Stern, C. L.; Mirkin, C. A. J. Am. Chem. Soc. 2014, 136, 10340–10348.
(30) Sairenji, Y.; Akine, S.; Nabeshima, T. Tetrahedron Lett. 2014, 55, 1987–1990.
(31) Sui, J.-M.; Naidu, V. R.; Liu, X.; Lah, M. S.; Jeong, K.-S. J. Am. Chem. Soc. 2011, 133, 13938–13941.
(32) Jones, I. M.; Hamilton, A. D. Angew. Chem., Int. Ed. 2011, 50, 4597–4600.
(33) Ulrich, S.; Lehn, J.-M. J. Am. Chem. Soc. 2009, 131, 5546–5559.
(34) Clayden, J.; Vallverdu, L.; Clayton, J.; Hellwell, M. Chem. Commun. 2008, 561–563.
(35) Meudtner, R. M.; Hecht, S. Angew. Chem., Int. Ed. 2008, 47, 4926–4930.
(36) Nishimura, T.; Maeda, K.; Yashima, E. Chirality 2004, 16, S12–S22.
(37) Tamoto, R.; Daugey, N.; Buffeteau, T.; Kauffmann, B.; Takafuji, A.; Sairenji, S.; Akine, S.; Nabeshima, T. Tetrahedron Lett. 2014, 55, 1987–1990.
(38) Ousaka, N.; Takeyama, Y.; Iida, H.; Yashima, E. Chem.—Eur. J. 2014, 20, 15981–15990.
(39) Ousaka, N.; Takeyama, Y.; Iida, H.; Yashima, E. Eur. J. Org. Chem. 2015, 2015, 3963–3972.
(40) Ousaka, N.; Takeyama, Y.; Iida, H.; Yashima, E. J. Am. Chem. Soc. 2009, 131, 8534–8536.
(41) Ousaka, N.; Takeyama, Y.; Iida, H.; Yashima, E. J. Am. Chem. Soc. 2009, 131, 8533–8534.
(42) Ousaka, N.; Takeyama, Y.; Iida, H.; Yashima, E. J. Am. Chem. Soc. 2009, 131, 8533–8534.
(43) Ousaka, N.; Takeyama, Y.; Iida, H.; Yashima, E. J. Am. Chem. Soc. 2009, 131, 8533–8534.
(44) Ousaka, N.; Takeyama, Y.; Iida, H.; Yashima, E. J. Am. Chem. Soc. 2009, 131, 8533–8534.
(45) Ousaka, N.; Takeyama, Y.; Iida, H.; Yashima, E. J. Am. Chem. Soc. 2009, 131, 8533–8534.
(46) Ousaka, N.; Takeyama, Y.; Iida, H.; Yashima, E. J. Am. Chem. Soc. 2009, 131, 8533–8534.
(47) Ousaka, N.; Takeyama, Y.; Iida, H.; Yashima, E. J. Am. Chem. Soc. 2009, 131, 8533–8534.
(48) Ousaka, N.; Takeyama, Y.; Iida, H.; Yashima, E. J. Am. Chem. Soc. 2009, 131, 8533–8534.
(49) Ousaka, N.; Takeyama, Y.; Iida, H.; Yashima, E. J. Am. Chem. Soc. 2009, 131, 8533–8534.
(50) Ousaka, N.; Takeyama, Y.; Iida, H.; Yashima, E. J. Am. Chem. Soc. 2009, 131, 8533–8534.
(51) Ousaka, N.; Takeyama, Y.; Iida, H.; Yashima, E. J. Am. Chem. Soc. 2009, 131, 8533–8534.
absolute chemical potential of the proton in the solution and expresses the proton donicity of the overall solution, taking into account any acidic species in the solution (including of course protonated solvent molecules, if they exist).

(81) Kubasik, M.; Kotz, J.; Szabo, C.; Furlong, T.; Stace, J. Biopolymers 2005, 78, 87–95.

(82) Brown, R. A.; Marcelli, T.; De Poli, M.; Solà, J.; Clayden, J. Angew. Chem., Int. Ed. 2012, 51, 1395–1399.

(83) Fletcher, S. P.; Solà, J.; Holt, D.; Brown, R. A.; Clayden, J. Beilstein J. Org. Chem. 2011, 7, 1304–1309.

(84) Sairenji, S.; Akine, S.; Nabeshima, T. Chem. Lett. 2014, 43, 1107–1109.

(85) Cullen, W.; Turega, S.; Hunter, C. A.; Ward, M. D. Chem. Sci. 2015, 6, 625–631.

(86) Alder, R. W.; Bowman, P. S.; Steele, W. Chem. Commun. 1968, 723–724.

(87) Alder, R. W.; Bryce, M. R.; Goode, N. C. J. Chem. Soc., Perkin Trans. 2 1982, 477–483.

(88) Akine, S.; Sairenji, S.; Taniguchi, T.; Nabeshima, T. J. Am. Chem. Soc. 2013, 135, 12948–12951.

(89) Andréasson, J.; Pischel, U. Chem. Soc. Rev. 2015, 44, 1053–1069.

(90) de Silva, A. P.; Vance, T. P.; West, M. E. S.; Wright, G. D. Org. Biomol. Chem. 2008, 6, 2468–2480.

(91) de Silva, A. P.; McClanaghan, N. D. Chem.—Eur. J. 2004, 10, 574–586.

(92) Ahn, H.; Hong, J.; Kim, S. Y.; Choi, I.; Park, M. J. ACS Appl. Mater. Interfaces 2015, 7, 704–712.

(93) Siebler, C.; Erdmann, R. S.; Wennemers, H. Angew. Chem., Int. Ed. 2014, 53, 10340–10344.

(94) Knipe, P. C.; Jones, I. M.; Thompson, S.; Hamilton, A. D. Org. Biomol. Chem. 2014, 12, 9384–9388.

(95) Jones, I. M.; Lingard, H.; Hamilton, A. D. Angew. Chem., Int. Ed. 2011, 50, 12569–12571.

(96) Su, X.; Aprahamian, I. Org. Lett. 2011, 13, 30–33.

(97) Cho, J.-I.; Tanaka, M.; Sato, S.; Kinbara, K.; Aida, T. J. Am. Chem. Soc. 2010, 132, 13176–13178.

(98) Landgo, S. M.; Aprahamian, I. J. Am. Chem. Soc. 2009, 131, 18269–18271.

(99) Okamoto, I.; Nabet, M.; Minami, T.; Nakashima, A.; Morita, N.; Takeya, T.; Masu, H.; Azumaya, I.; Tamura, O. Tetrahedron Lett. 2007, 48, 573–577.

(100) Kanamori, D.; Okamura, T.-A.; Yamamoto, H.; Ueyama, N. Angew. Chem., Int. Ed. 2005, 44, 969–972.

(101) Dolain, C.; Maurizot, V.; Huc, I. Angew. Chem., Int. Ed. 2003, 42, 2738–2740.

(102) Kolomietz, E.; Beer, V.; Odriozola, I.; Stadler, A.-M.; Kyritsakas, N.; Lehnh, J.-M. Chem. Commun. 2003, 2868–2869.

(103) Mutter, M.; Gassmann, R.; Buttka, U.; Altmann, K. H. Angew. Chem., Int. Ed. 1991, 30, 1514–1516.