Controlled Diffusion of Photoswitchable Receptors by Binding Antielectrostatic Phosphate Oligomers

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Dihydrogen phosphate anions are found to spontaneously associate into anti-electrostatic oligomers via hydrogen bonding interactions at millimolar concentrations in DMSO. Diffusion NMR measurements supported formation of these oligomers, which can be bound by photoswitchable anion receptors to form large bridged assemblies of approximately three times the volume of the unbound receptor. Photoisomerization of the oligomer-bound receptor causes a decrease in diffusion coefficient of up to 16%, corresponding to a 70% increase in effective volume. This new approach to external control of diffusion opens prospects in controlling molecular transport.

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S1. General Experimental

Reagents were purchased from Combi-Blocks or Sigma-Aldrich and used as received. Deuterated NMR solvents were purchased from Cambridge Isotope Laboratories and used as received unless otherwise noted.

NMR experiments were conducted using a Bruker Avance III 500 MHz spectrometer equipped with a TBI (³¹P) probe fitted with standard high-resolution gradient coils capable of generating gradient pulses up to 53.45 G cm⁻¹. Diffusion experiments were prepared and executed through the Bruker **diff5** utility and used the **diffSte** stimulated-echo pulse sequence. Typical parameters for ¹H diffusion experiments were $\Delta = 50$ ms and $\delta = 4$ ms. Typical parameters for ³¹P diffusion experiments were $\Delta = 100$ ms and $\delta = 7$ ms. Gradient pulses were sine-shaped, and gradient lists typically consisted of 12 gradient strengths linearly increasing from 0 – 53.45 g cm⁻¹.

Spectra were automatically phased and baseline-corrected with MestReNova software 12.0, with peak integrals obtained using the built-in data analysis routines and exported as csv data for further processing.

High resolution mass spectra were collected on a Thermo Scientific LTQ-FT-ICR Mass Spectrometer equipped with a nanoESI source and Thermo Xcalibur software used for analysis.

S2. Speciation of acetate and dihydrogen phosphate: literature data

Given the focus placed upon the dihydrogen phosphate anion, it is important to consider potential protonation-deprotonation that may occur under the conditions of wet DMSO. Acid-base properties in non-aqueous solutions are notoriously uncharacterised, but in this case we have access to a carefully-conducted recent study of the acid-base properties of H_2PO_4^- and CH_3CO_2^- in DMSO.^[1] That study also measured an association constant for antielectrostatic H_2PO_4^- dimerization of 180 M^{-1} in DMSO, but did not consider indefinite association to form higher oligomers as is presented here.

It has previously been shown that the deprotonation of H_2PO_4^- in DMSO is highly unfavorable.^[1] As such, we limit our consideration to the possibility of forming of phosphoric acid, H_3PO_4 , by protonation of H_2PO_4^- with water. Literature data for K^{H} , the acid association constant of a species, are given in Table S1.

Table S1. Literature acid-base association constants for phosphate,^[1] acetate,^[1] and water^[2] in DMSO.

$$K^{\text{H}} = \frac{[\text{AH}]}{[\text{H}][\text{A}]}$$

Species	log K^{H} (DMSO)
H_3PO_4 ^[1]	10.8
$\text{H}_3\text{CO}_2\text{H}$ ^[1]	12.8
H_2O ^[2]	28

S2.1 Speciation of phosphate in DMSO

Proton transfer from water to dihydrogen phosphate would occur as:

With the acid-base relationship and using the values in Table S1:

$$\frac{[\text{OH}^-][\text{H}_3\text{PO}_4]}{[\text{H}_2\text{PO}_4^-][\text{H}_2\text{O}]} = \frac{10^{-28}}{10^{-10.8}} = 10^{-17.2}$$

At 0.5% v/v H_2O , $[\text{H}_2\text{O}]_0 = 280 \text{ mM}$. Considering the case for 50 mM $[\text{H}_2\text{PO}_4^-]$ and setting x as the concentration $[\text{OH}^-]$ and $[\text{H}_3\text{PO}_4]$, we can restate this as:

$$\frac{[\text{OH}^-][\text{H}_3\text{PO}_4]}{[\text{H}_2\text{PO}_4^-][\text{H}_2\text{O}]} = \frac{x^2}{([\text{H}_2\text{PO}_4^-]_0 - x)([\text{H}_2\text{O}]_0 - x)} = 10^{-17.2}$$

And then:

$$\begin{aligned} \frac{x^2}{(50 \times 10^{-3} - x)(280 \times 10^{-3} - x)} &= 10^{-17.2} \\ x^2 &= ((50 \times 10^{-3})(280 \times 10^{-3}) - x(280 \times 10^{-3} + 50 \times 10^{-3}) + x^2)10^{-17.2} \\ x &= 9.4 \times 10^{-9} \text{ M}^{-1} \end{aligned}$$

$[\text{H}_3\text{PO}_4]$ is thus expected to be vanishingly small, and is not expected to play any part in supramolecular assembly.

S2.2 Speciation of acetate in DMSO

Following the same steps as above:

$$\frac{[\text{OH}^-][\text{AcOH}]}{[\text{AcO}^-][\text{H}_2\text{O}]} = \frac{10^{-28}}{10^{-12.8}} = 10^{-15.2}$$

And solving for the extent of proton transfer:

$$\frac{x^2}{(50 \times 10^{-3} - x)(280 \times 10^{-3} - x)} = 10^{-15.2}$$

$$x^2 = ((50 \times 10^{-3})(280 \times 10^{-3}) - x(280 \times 10^{-3} + 50 \times 10^{-3}) + x^2)10^{-17.2}$$

$$x = 9.4 \times 10^{-8} \text{ M}^{-1}$$

While the formation of $\text{CH}_3\text{CO}_2\text{H}$ is slightly more favorable than that of H_3PO_4 , it is still expected to only be present at insignificant ($\sim\text{nM}$) concentration.

S2.3 Literature association data^[1]

In a previous study, acetate was proposed to be incapable of forming an antielectrostatic dimer, while a dimerization constant of $K = 180 \text{ M}^{-1}$ was reported for dihydrogen phosphate.^[1] No indefinite association models were considered in that work.

These anions are able to associate with their respective conjugate acid ($K_{1,1}$), with a high ($\sim 10^4 \text{ M}^{-1}$) association constant found for phosphate.

Table S2. Literature association values for dimerization of phosphate and acetate species, with method of determination noted in parentheses.^[1] H_2PO_4 was found to form antielectrostatic dimers with $K = 180 \text{ M}^{-1}$, similar to the 120 M^{-1} isodesmic K_i found here using an indefinite self-association model.

Species	$\log K_{0,2}$ [B ⁻ + B ⁻] → [B ₂] ²⁻	$K_{0,2}$ / M ⁻¹	$\log K_{1,1}$ [HB + B ⁻] → [HB ₂] ⁻	$K_{1,1}$ / M ⁻¹	$\log K_{1,2}$ [HB-B ⁻] + B ⁻ → [HB ₂] ²⁻	$K_{1,2}$ / M ⁻¹
$\text{H}_2\text{PO}_4^{-[1]}$	2.26(6) (ITC)	1.8×10^2	4.23(3) (NMR)	1.7×10^4	2.92 (ITC)	8.3×10^2
$\text{OAc}^{-[1]}$	-	-	2.45(1) (ITC)	2.8×10^2	-	

S3. Synthesis

The *E* and *Z*-stiff-stilbene diamine **S1** was prepared as previously reported.^[3]

S3.1 Preparation of bis-urea hosts *E*-1 and *Z*-1

Compound *E*-S1 (100 mg, 0.38 mmol) was suspended in 50 mL dry CH₂Cl₂, and *p*-tolylisocyanate (101 mg, 95 μ L, 0.76 mmol) was added by syringe. The reaction mixture was stirred at reflux overnight, cooled to rt, and the precipitate was filtered off to obtain crude *E*-1. The crude was washed with CH₂Cl₂ and aq. HCl (1M), suspended in water and sonicated, and the precipitate was collected by filtration to give *E*-1 as a fine beige solid (167 mg, 83%).

¹H NMR (600 MHz, DMSO-*d*₆) δ 8.70 (s, 2H, **H^f**), 8.57 (s, 2H, **H^g**), 7.89 (d, *J* = 1.9 Hz, 2H, **H^e**), 7.35 (d, *J* = 8.1 Hz, 4H, **H^h**), 7.26 – 7.20 (m, 4H, **H^{c,d}**), 7.09 (d, *J* = 8.1 Hz, 4H, **Hⁱ**), 3.11 (m, 4H, **H^a**), 3.04 (m, 4H, **H^b**), 2.25 (s, 6H, **H^j**). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 152.80, 140.12, 138.37, 137.24, 135.08, 130.49, 129.15, 128.48, 124.82, 118.31, 117.58, 114.27, 31.66, 29.82, 20.35. HR-MS found: 529.25972; [C₃₄H₃₂N₄O₂+H]⁺ requires 529.25980 *m/z*.

Figure S1. High resolution mass spectrum of [E-1+H]⁺

Compound **Z-S1** (100 mg, 0.38 mmol) was dissolved in 5 mL of dry CH_2Cl_2 , and *p*-tolylisocyanate (101 mg, 95 μL , 0.76 mmol) was added by syringe. The reaction mixture was stirred at rt overnight and the precipitate was filtered off to obtain crude **Z-1**. The crude was washed with CH_2Cl_2 and aq. HCl (1M), suspended in water and sonicated, and the precipitate was collected by filtration to give **Z-1** as a fine beige solid (152 mg, 75%).

^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 8.59 (s, 2H, H^g), 8.43 (s, 2H, H^f), 8.11 (s, 2H, H^e), 7.42 (dd, $J = 8.2, 1.9$ Hz, 2H, H^d), 7.29 (d, $J = 8.0$ Hz, 4H, H^h), 7.22 (d, $J = 8.2$ Hz, 2H, H^c), 6.88 (d, $J = 8.0$ Hz, 4H, H^i), 2.89 (m, 4H, H^b), 2.77 (m, 4H, H^a), 2.11 (s, 6H, H^j). ^{13}C NMR (151 MHz, $\text{DMSO}-d_6$) δ 152.71, 141.27, 140.05, 137.59, 137.28, 134.86, 130.20, 129.04, 125.10, 118.14, 117.71, 113.62, 34.45, 29.39, 20.22.

S3.2 NMR spectra of *E-1*

Figure S2. ^1H NMR spectrum of *E-1* (600 MHz, $\text{DMSO}-d_6$).

Figure S3. $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of *E*-1 (125 MHz, $\text{DMSO}-d_6$). Low signal-to-noise due to poor solubility.

S3.3 NMR spectra of Z-1

Figure S4. ^1H NMR spectrum of *Z*-1 (600 MHz, $\text{DMSO}-d_6$).

Figure S5. $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of Z-1 (125 MHz, $\text{DMSO}-d_6$).

S4. Viscosity measurements of tetrabutylammonium dihydrogen phosphate solutions

Viscosities and densities were measured at 25 °C using a Lovis 2000M rolling ball viscometer ($\varnothing 1.59$ mm capillary; 1 mm stainless steel ball), with results shown in Table S3.

Table S3. Viscosity measurements of solutions of $[\text{NBu}_4][\text{H}_2\text{PO}_4]$ in $\text{DMSO}-d_6$ with 0.5% v/v added water.

$[\text{NBu}_4][\text{H}_2\text{PO}_4]$ / mM	Density / $\text{g}\cdot\text{cm}^{-3}$	Temperature / °C/	Dyn. Viscosity / $\text{mPa}\cdot\text{s}$	Kin. Viscosity / $\text{mm}^2\cdot\text{s}^{-1}$	Temperature / °C	Error / %
0	1.1833	25.04	2.149	1.816	25.00	0.02
5	1.1833	25.00	2.210	1.868	25.00	0.08
20	1.1828	25.08	2.259	1.910	25.00	0.04
50	1.1820	25.04	2.334	1.974	24.99	0.03
100	1.1805	25.06	2.499	2.117	25.00	0.01
300	1.1752	25.04	3.251	2.767	25.00	0.02

Figure S6. Concentration dependence of the measured viscosity of tetrabutylammonium dihydrogen phosphate solutions in DMSO-*d*₆ with 0.5% added water. Linear fit: $\eta = 2.17 + (3.53 \times 10^{-3})([\text{NBu}_4][\text{H}_2\text{PO}_4])$. For calibrating diffusion measurements, it is convenient to express this in relative form: $\eta/\eta_0 = 1 + 1.63 \times [\text{NBu}_4][\text{H}_2\text{PO}_4] / \text{mM}$

S5. Mathematical modelling of isodesmic self-association

The association of dihydrogen phosphate was fitted to an isodesmic model,^[4] constructed as follows.

S5.1 Isodesmic model for self-association

Consider a monomer A capable of forming an oligomer of arbitrary length, where each association step $A_{n-1} + A \rightarrow A_n$ occurs with the same (isodesmic) association constant K_i :

$$K_i = \frac{[A_n]}{[A][A_{n-1}]} \quad (1)$$

And the total concentration of A is given as:

$$[A]_0 = \sum_{n=1}^{\infty} n[A_n] \quad (2)$$

It is convenient to define $z = [A]K_i$. Then:

$$\begin{aligned} [A_n] &= K_i [A][A_{n-1}] \\ &= z[A_{n-1}] \end{aligned}$$

As $[A_1] = [A]$, by induction:

$$\begin{aligned} [A_n] &= [A]z^{n-1} \\ &= \frac{z^n}{K_i} \end{aligned} \quad (3)$$

We can combine (2) and (3) to build a mass balance equation for $[A]_0$:

$$\begin{aligned} [A]_0 &= \sum_{n=1}^{\infty} n[A_n] \\ &= \frac{1}{K_i} \sum_{n=1}^{\infty} nz^n \\ &= \frac{z}{K_i(z-1)^2} \end{aligned}$$

Allowing us to express z as the root of a quadratic:

$$\begin{aligned} [A]_0 K_i (z-1)^2 &= z \\ [A]_0 K_i z^2 - (2[A]_0 K_i + 1)z + [A]_0 K_i &= 0 \end{aligned}$$

Defining $L = [A]_0 K_i$ and assuming that z is the smallest solution to the quadratic:

$$z = \frac{2L + 1 - \sqrt{4L + 1}}{2L} \quad (4)$$

From z we can obtain the concentration of monomeric (unbound) species $[A] = \frac{z}{K_i}$, and by combining equations (4) and (3) we can calculate the size distribution of oligomers from the starting concentration $[A]_0$ and association constant K_i .

One other relationship may be useful. If we assume that *only the terminal sites* of each oligomer are available for binding by a host (ie each monomeric or oligomeric guest species has two binding sites, regardless of size), then the guest concentration of guest binding sites in solution will be:

$$\begin{aligned} [A_{eff}] &= \sum_{n=1}^{\infty} [A_n] \\ &= \frac{1}{K_i} \sum_{n=1}^{\infty} z^n \\ &= \frac{z}{K_i(1-z)} \end{aligned} \quad (5)$$

S5.2 Isodesmic model for diffusion

The Stokes-Einstein-Sutherland equation for molecular diffusion states that for hard spherical particles^[5]:

$$D = \frac{k_B T}{6\pi\eta R_H} \quad (6)$$

For the sake of simplicity, we can approximate each monomer as a hard sphere of volume V and each n -unit oligomer as a hard sphere of volume nV . This assumption of perfect packing of hard spheres to form a larger hard sphere is clearly unphysical, but the results of this approximation have previously been shown to be quite close to those obtained from more detailed treatments.^[6]

The diffusion coefficient D_n of an n -unit oligomer can then be expressed as

$$D_n = n^{-\frac{1}{3}} D_0 \quad (7)$$

where D_0 is the diffusion coefficient of the monomeric species (*i.e.* at infinite dilution). For system of oligomers in fast exchange on the NMR timescale, the measured diffusion coefficient $\bar{D}$ is a concentration-weighted sum of the diffusion coefficients of all possible oligomers[†]:

$$\bar{D} = \sum_{n=1}^{\infty} x_n D_n \quad (8)$$

Where x_n is the molfraction of the oligomerizing species present in an oligomer of size n , ie $x_n = \frac{n[A_n]}{[A]_0}$

Using the previously obtained expressions (7) and (3) for D_n and $[A_n]$ respectively, we can then say:

$$\bar{D} = \frac{D_0}{[A]_0 K_i} \sum_{n=1}^{\infty} n^{\frac{2}{3}} z^n \quad (9)$$

There is no closed-form analytic solution to this infinite sum, but for numerical modelling and curve-fitting it is convenient to restate it as a polylogarithm. The polylogarithm is a special function defined as

$$\text{Li}_s(z) = \sum_{k=1}^{\infty} \frac{z^k}{k^s}$$

allowing us to express $\bar{D}$ as:

$$\bar{D} = \frac{D_0}{[A]_0 K_i} \text{Li}_{-\frac{2}{3}}([A]K_i) \quad (10)$$

Some results of this model for the concentration-dependent diffusion of isodesmically self-associating species are plotted in Figure S7.

[†] This treatment neglects potential differences in longitudinal relaxation times T_1 across oligomers of different sizes or for the different sites present within an oligomer.

Figure S7. Modelled changes in relative diffusion coefficients D/D_0 under conditions of isodesmic self-association (Equation 10), for five different association constants K_i over the concentration range 1 μ M to 1 M. The 2 – 300 mM concentration range studied here has been shaded in purple. Both plots show the same curves, against a linear (top) and log (bottom) concentration scale.

S5.3 Estimated diffusion of host-guest complexes

Consider a supramolecular complex made up from m host molecules and n guest molecules, of the form $[H_m G_n]$, where the diffusion coefficients of free host and free guest have been obtained as D_H and D_G . Under the same approximation of perfectly packing hard spheres used previously, we can then approximate the diffusion coefficient of the complex as:

$$D(m, n) = (mD_H^{-3} + nD_G^{-3})^{-\frac{1}{3}} \quad (11)$$

As the hosts and guests will not pack perfectly into hard spherical complexes, the diffusion coefficients of complexes estimated by this method can be considered upper bounds for the actual diffusion coefficients.

Assuming fast exchange of species on the NMR timescale, the diffusion coefficient measured for a host species is a population-weighted average of all $H_m G_n$ species present:

$$\overline{D_H} = \frac{1}{[H]_0} \sum_{m=1}^{\infty} \sum_{n=0}^{\infty} m \cdot D(m, n) [H_m G_n] \quad (12)$$

For the case of discrete complexes involving a single host and up to two guests, this can be expanded to

$$\overline{D_H} = \frac{1}{[H]_0} \left([H] D_H + [HG] (D_H^{-3} + D_G^{-3})^{-\frac{1}{3}} + [HG_2] (D_H^{-3} + 2D_G^{-3})^{-\frac{1}{3}} \right) \quad (13)$$

where $[H]$, $[HG]$, and $[HG_2]$ can be calculated using the total concentrations of host and guest and the association constants K_1 and K_2 .^[7] If the guest self-associates using isodesmic kinetics (such as $H_2PO_4^-$), then D_G can be replaced with the concentration-dependent diffusion model given in Equation 10.

S6. Experimental determination of the thermodynamics of H_2PO_4^- self-association

Solutions of tetrabutylammonium dihydrogen phosphate ($[\text{NBu}_4][\text{H}_2\text{PO}_4]$) were prepared in $\text{DMSO-}d_6$ with 0.5% v/v H_2O added (Millipore). 500 μL aliquots were transferred to standard 5 mm NMR tubes (Wilmad) and ^1H and ^{31}P diffusion experiments were obtained and processed as previously described (S1 General Experimental). Diffusion coefficients were then obtained by nonlinear regression of $I = I_0 \exp[-bD]$, where b is the effective attenuation strength of the experiment as calculated by the Bruker **diff5** utility ($b = g^2 \delta^2 \gamma^2 (\Delta - \frac{\delta}{3})$ for square gradient pulses).

Figure S8. Measured diffusion coefficients of tetrabutylammonium and dihydrogen phosphate uncorrected for changes in viscosity. The model for isodesmic diffusion shown in Eq (10) has been regressed unsuccessfully onto measured $D(\text{H}_2\text{PO}_4)$, with obtained parameters $K_i = (2.5 \pm 9) \times 10^3 \text{ M}^{-1}$ and $D_0 = (4.9 \pm 2.7) \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$.

Figure S9. Measured diffusion coefficients of tetrabutylammonium and dihydrogen phosphate corrected for changes in viscosity using the measured viscosities of these solutions (see Table S3; Figure 1 in manuscript). The model for isodesmic diffusion shown in Eq (10) has been regressed onto measured $D(\text{H}_2\text{PO}_4)$, with obtained parameters $K_i = (120 \pm 32) \text{ M}^{-1}$ and $D_0 = (3.39 \pm 0.11) \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$. After correction, no change in $D(\text{NBu}_4)$ is observed (horizontal black line).

Figure S10. Measured diffusion coefficients of tetrabutylammonium and dihydrogen phosphate corrected for changes in viscosity using the change in the measured diffusion coefficient of the DMSO-*d*₆ solvent residual. The model for isodesmic diffusion shown in Eq (10) has been regressed onto measured $D(\text{H}_2\text{PO}_4)$, with obtained parameters $K_i = (333 \pm 110) \text{ M}^{-1}$ and $D_0 = (3.71 \pm 0.16) \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$.

Table S4. Tabulated data for dihydrogen phosphate isodesmic self-association data.

Correction	K_i / M^{-1}	Relative error (K_i)	$D_0 / \text{m}^2 \text{s}^{-1}$	Relative error (D_0)
Uncorrected ^[a]	$(2.5 \pm 9) \times 10^3$	360%	$(4.9 \pm 2.7) \times 10^{-10}$	55%
Indirect correction ^[b]	$(3.33 \pm 1.10) \times 10^2$	33%	$(3.71 \pm 0.16) \times 10^{-10}$	4.3%
Direct correction ^[c]	$(1.20 \pm 0.32) \times 10^2$	27%	$(3.39 \pm 0.11) \times 10^{-10}$	3.2%

[a] Isodesmic fit to diffusion data as-measured; [b] Isodesmic fit to diffusion data corrected for viscosity changes by normalising D for the DMSO solvent residual; [c] Isodesmic fit to diffusion data corrected for viscosity changes using independently measured viscosity data.

Viscosity changes must be corrected for to allow successful fitting of the isodesmic diffusion model with errors smaller than the fitted value themselves (Table S4). Direct corrections using the change in viscosity measured independently by falling-ball viscometry (SI-S4) gave slightly smaller relative errors than an indirect correction approach using the change in measured D for the DMSO-*d*₅ solvent residual peak. These parameters can be used to calculate the size distribution of oligomeric H_2PO_4^- at arbitrary concentrations, as shown in Figure S11.

Figure S11. Visualization of the size distribution of phosphate oligomers using experimentally obtained parameters. Top: distribution of oligomers ($n = 1$ to 30) for solutions of $[A]_0 = 5, 10, 20, 50$, and 300 mM with isodesmic $K_i = 120 \text{ M}^{-1}$ (as experimentally obtained for $[\text{NBu}_4][\text{H}_2\text{PO}_4]$ in $\text{DMSO-}d_6$). Bottom: cumulative distribution functions under the same conditions. For the 50 mM solutions of $[\text{NBu}_4][\text{H}_2\text{PO}_4]$ used repeatedly in this work (red) the median phosphate oligomer contains $n = 4$ units, the 90th percentile contains $n = 10$ units, and the 99th percentile oligomer contains 16 units.

S6.1 Control experiments with tetrabutylammonium acetate

Due to the large quantities of DMSO- d_6 required for falling-ball viscometry, no direct viscosities were measured for solutions of tetrabutylammonium acetate ($[\text{NBu}_4][\text{OAc}]$). Instead, an indirect viscosity calibration was performed from the measured diffusion coefficient of the DMSO- d_6 solvent residual, under the assumption that changes in measured D for the solvent residual would be primarily a result of changing viscosity.

Figure S12. Indirect viscosity calibration for tetrabutylammonium acetate, using the diffusion coefficient of the DMSO solvent residual peak. Linear fit for implied relative change in dynamic viscosity: $\frac{\eta}{\eta_0} = 1 + (0.521 \times 10^{-3})[\text{NBu}_4\text{OAc}]$.

Figure S13. Measured diffusion coefficients of tetrabutylammonium acetate over the concentration range 0 – 300 mM without (left) and with (right) correction for changes in viscosity (see Figure S12). While some acetate self-association appears to occur, the <10% decrease in the measured diffusion coefficient is much smaller than that seen for dihydrogen phosphate.

Table S5. Measured diffusion coefficients for DMSO, tetrabutylammonium, and acetate over a range of tetrabutylammonium acetate concentrations. No correction for viscosity has been applied to these data. ^1H , 500 MHz, $\text{DMSO-}d_6$ with 0.5% added H_2O .

$[\text{NBu}_4][\text{OAc}]$ / mM	$D_{\text{DMSO}}^{[a]}$ / $\text{m}^2 \text{s}^{-1} \times 10^{-10}$	$D(\text{OAc})^{[a]}$ / $\text{m}^2 \text{s}^{-1} \times 10^{-10}$	$D(\text{NBu}_4)^{[a]}$ / $\text{m}^2 \text{s}^{-1} \times 10^{-10}$
2	6.52 ± 0.01	3.96 ± 0.08	2.53 ± 0.01
5	6.51 ± 0.01	3.89 ± 0.05	2.52 ± 0.01
10	6.50 ± 0.02	3.69 ± 0.05	2.52 ± 0.01
20	6.45 ± 0.02	3.59 ± 0.04	2.45 ± 0.01
50	6.28 ± 0.05	3.48 ± 0.08	2.33 ± 0.02
100	6.19 ± 0.02	3.54 ± 0.04	2.38 ± 0.01
200	5.98 ± 0.03	3.33 ± 0.05	2.27 ± 0.01
300	5.60 ± 0.01	3.12 ± 0.03	2.07 ± 0.01

[a] 500 MHz ^1H PGSTE, $\delta = 4$ ms, $\Delta = 50$ ms, $g = 0 - 53.45$ G cm^{-1}

Table S6. Changes in diffusion coefficients for solutions of 2–300 mM tetrabutylammonium dihydrogen phosphate ($[\text{NBu}_4][\text{H}_2\text{PO}_4]$; $D(\text{H}_2\text{PO}_4)$) and tetrabutylammonium acetate (OAc; D_{OAc}), corrected for change in viscosity.

$[\text{NBu}_4][\text{Anion}]$ / mM	$D(\text{H}_2\text{PO}_4)^{[b]}$ / $\text{m}^2 \text{s}^{-1} \times 10^{-10}$	$D(\text{NBu}_4)^{[c]} (\text{H}_2\text{PO}_4)$ / $\text{m}^2 \text{s}^{-1} \times 10^{-10}$	$D(\text{OAc})^{[b]}$ / $\text{m}^2 \text{s}^{-1} \times 10^{-10}$	$D(\text{NBu}_4)^{[b]} (\text{OAc})$ / $\text{m}^2 \text{s}^{-1} \times 10^{-10}$
2	3.17 ± 0.28	2.52 ± 0.01	3.96 ± 0.1	2.53 ± 0.01
5	2.99 ± 0.17	2.52 ± 0.01	3.90 ± 0.08	2.53 ± 0.01
10	2.63 ± 0.08	2.50 ± 0.01	3.70 ± 0.07	2.53 ± 0.01
20	2.51 ± 0.10	2.51 ± 0.02	3.63 ± 0.06	2.48 ± 0.01
50	2.16 ± 0.03	2.50 ± 0.02	3.57 ± 0.1	2.40 ± 0.03
100	2.08 ± 0.02	2.53 ± 0.02	3.72 ± 0.05	2.49 ± 0.01
200	1.80 ± 0.01	2.49 ± 0.01	3.68 ± 0.07	2.52 ± 0.01
300	1.71 ± 0.02	2.48 ± 0.03	3.61 ± 0.05	2.39 ± 0.01

[a] At concentrations below 20 mM the H_2PO_4^- anion (MW = 97) diffuses more rapidly than the $[\text{NBu}_4]^+$ cation (MW = 242), but at concentrations higher than 20 mM the diffusion coefficient of H_2PO_4^- is lower than that of $[\text{NBu}_4]^+$. In contrast, the diffusion coefficient of the acetate anion drops slightly until 50 mM concentration, after which it does not lower any further. Measurements compensated for changes in viscosity using the measured viscosity ($[\text{NBu}_4][\text{H}_2\text{PO}_4]$), or the measured diffusion of residual DMSO ($[\text{NBu}_4][\text{OAc}]$) as an internal standard. $\text{DMSO-}d_6$ with 0.5% added H_2O , 500 MHz ^1H . [b] ^{31}P PGSTE, $\delta = 7$ ms, $\Delta = 100$ ms, $g = 0 - 53.45$ G cm^{-1} . [c] ^1H PGSTE, $\delta = 4$ ms, $\Delta = 50$ ms, $g = 0 - 53.45$ G cm^{-1} .

S7. Host-guest association data

S7.1 Method

Dilution-corrected NMR titrations were performed by adding aliquots of a solution of guest containing 5 mM host to 5 mM solutions of host (in DMSO-*d*₆/0.5% H₂O). The spectra obtained were processed and analysed using MestReNova software to obtain chemical shift data, which were used to obtain association constants using the processing routines given in Script S1 (see section S11). In all cases, the data was fitted to 1:2 H:G binding models^[7] giving K_1 and K_2 , which are defined as:

$$K_1 = \frac{[\text{HG}]}{[\text{H}][\text{G}]}$$

and

$$K_2 = \frac{[\text{HG}_2]}{[\text{HG}][\text{G}]}$$

with cooperativity α defined as

$$\alpha = \frac{K_1}{4K_2}$$

For the *E*-host, the urea binding sites were treated as equal, i.e. a non-cooperative ($\alpha = 1$) model was used. For the *Z*-host, a cooperative model with a floating α was used as two urea groups may be involved in binding one anion.

S7.2 Addition of tetrabutylammonium dihydrogen phosphate to *E*-1

Figure S14. Titration of 50 mM [NBu₄][H₂PO₄] into 5 mM of *E*-1. 400 MHz, DMSO-*d*₆ + 0.5% H₂O.

Figure S15. Data from Figure S14 fitted to a **non-cooperative** 1:2 host:guest association model. $K_1 = 3.6 \times 10^2 \text{ M}^{-1}$, $K_2 = 9.0 \times 10^1 \text{ M}^{-1}$.

Figure S16. Titration of 50 mM $[\text{NBu}_4][\text{H}_2\text{PO}_4]$ into 5 mM of *E*-1. 500 MHz, $\text{DMSO}-d_6 + 0.5\% \text{H}_2\text{O}$.

Figure S17. Data from Figure S16 fitted to a **non-cooperative** 1:2 host:guest association model. $K_1 = 2.3 \times 10^2 \text{ M}^{-1}$, $K_2 = 6.0 \times 10^1 \text{ M}^{-1}$.

S7.3 Addition of tetrabutylammonium dihydrogen phosphate to Z-1 (up to 3 equiv.)

Figure S18. Titration of 50 mM $[\text{NBu}_4][\text{H}_2\text{PO}_4]$ into 5 mM of Z-1, to 9.7 mM guest concentration. 600 MHz, $\text{DMSO}-d_6 + 0.5\% \text{H}_2\text{O}$.

Figure S19. Data from Figure S18 fitted to a 1:2 host:guest association model. $K_1 = 2.1 \times 10^3 \text{ M}^{-1}$, $K_2 = 7.0 \times 10^1 \text{ M}^{-1}$.

Figure S20. Titration of 50 mM $[\text{NBu}_4][\text{H}_2\text{PO}_4]$ into 5 mM of Z-1 to 15.5 mM guest concentration. 500 MHz, $\text{DMSO}-d_6 + 0.5\% \text{H}_2\text{O}$.

Figure S21. Data from Figure S20 fitted to a 1:2 host:guest association model. $K_1 = 3.5 \times 10^3 \text{ M}^{-1}$, $K_2 = 1.1 \times 10^2 \text{ M}^{-1}$.

S7.4 Z-1 dihydrogen phosphate binding fitted to a 1:1 model

Figure S22. Data from Figure S20 over a low concentration range (5 mM Z-1, 0 – 10 mM $\text{NBu}_4\text{H}_2\text{PO}_4$) fitted to a 1:1 host:guest association model. $K_f = 3.8 \pm 0.3 \times 10^3 \text{ M}^{-1}$, within error of the value found for a 2:1 binding model in Figure S21.

S7.5 Addition of tetrabutylammonium dihydrogen phosphate to Z-1 (beyond 3 equiv.)

Figure S23. Titration of 200 mM $[\text{NBu}_4][\text{H}_2\text{PO}_4]$ into 5 mM of Z-1 to 66.7 mM guest concentration. 500 MHz, $\text{DMSO}-d_6 + 0.5\% \text{H}_2\text{O}$. Bottom: the same spectrum, zoomed to focus on the aromatic region and exclude the coalesced urea NH peaks.

Figure S24. Changes in ^1H NMR chemical shifts of the $[\text{HG}]$ and $[\text{HG}_2]$ complexes of Z-1 relative to the free host (ppm, relative to those of free $[\text{H}]$) as obtained from the 1:2 nonlinear fit of the ^1H NMR titration data. The fitted chemical shift changes support the two-step binding fit proposed for the Z isomer in Figure S25. Most notably, the inner aryl proton (circled) is first shielded and then deshielded by the two steps of binding. This is consistent with the expected chemical shift induced by the carbonyl group magnetic anisotropy.

Figure S25. Data shown in Figure S23 fitted to a 1:2 host:guest association model. $K_1 = 13 \times 10^3 \text{ M}^{-1}$, $K_2 = 7 \times 10^1 \text{ M}^{-1}$.

S7.6 Addition of tetrabutylammonium acetate to *E*-1

Figure S26. Titration of 200 mM [NBu₄][OAc] into 5 mM of *E*-1. 500 MHz, DMSO-*d*₆ + 0.5% H₂O.

Figure S27. Data from Figure S26 fitted to a non-cooperative 1:2 host:guest association model. $K_1 = 3.3 \times 10^2 \text{ M}^{-1}$, $K_2 = 8.3 \times 10^1 \text{ M}^{-1}$.

Figure S28. Titration of 200 mM $[\text{NBu}_4][\text{OAc}]$ into 5 mM of *E*-1. 500 MHz, $\text{DMSO}-d_6 + 0.5\% \text{H}_2\text{O}$.

Figure S29. Data from Figure S28 fitted to a non-cooperative 1:2 host:guest association model. $K_1 = 3.5 \times 10^2 \text{ M}^{-1}$, $K_2 = 8.7 \times 10^1 \text{ M}^{-1}$.

S7.7 Addition of tetrabutylammonium acetate to Z-1

Figure S30. Titration of 200 mM [NBu₄][OAc] into 5 mM of Z-1. 500 MHz, DMSO-*d*₆ + 0.5% H₂O.

Figure S31. Data from Figure S30 fitted to a 1:2 host:guest association model. $K_1 = 1.8 \times 10^3 \text{ M}^{-1}$, $K_2 = 7 \text{ M}^{-1}$.

Figure S32. Titration of 200 mM [NBu₄][OAc] into 5 mM of Z-1. 500 MHz, DMSO-*d*₆ + 0.5% H₂O.

Figure S33. Data from Figure S32 fitted to a 1:2 host:guest association model. $K_1 = 1.5 \times 10^3$, M⁻¹, $K_2 = 5$ M⁻¹.

S7.8 Summary of binding data

Calculated association constants for binding of acetate and dihydrogen phosphate to **1** are summarized here and compared to literature values for the previously reported phenyl-substituted stiff stilbene host **S2** (Figure S34).

Figure S34. Anion-binding stiff stilbene hosts characterized in the literature.^[3] These diphenyl bisurea hosts only differ from *p*-tolylurea host **1** in the absence of a methyl group on the terminal aryl rings, and are thus expected to possess similar guest-binding properties.

S7.9 Summary of dihydrogen phosphate binding data

As discussed in detail in S7.11, the standard models of host-guest association are inapplicable here due to the self-associating nature of the H_2PO_4^- anion. One consequence of this is that the estimated errors obtained through nonlinear least-squares regression are nonsensical, as the experimental data is not randomly distributed about the model used. The results obtained for **1** binding H_2PO_4^- are presented here for completeness and for comparison to previous studies, but we again caution that they are obtained using an approach fundamentally inadequate for this associative process.

Table S7. Summary of data for host **1** binding H_2PO_4^- (as $[\text{NBu}_4][\text{H}_2\text{PO}_4]$; DMSO- d_6 + 0.5% H_2O).

Species	E-1 ^[a]	E-1 ^[a]	E-S2 ^[b]	Z-1 ^[a]	Z-1 ^[a]	Z-1 ^[a]	Z-S2 ^[b]
H_2PO_4^- endpoint / mM	18	57		9.8	15.5	67	
K_1 / M^{-1}	$3.6 \pm 3.2 \times 10^2$	$2.3 \pm 3.2 \times 10^2$	1.5×10^2	$2.1 \pm 2.1 \times 10^3$	$3.5 \pm 1.1 \times 10^3$	$1.3 \pm 0.8 \times 10^4$	2.0×10^3
K_2 / M^{-1}	90	60	39	70	110	72	-
α	Forced as 1			0.14 ± 0.33	0.13 ± 0.08	0.02 0.02	-

[a] Measured association constants for compound **1**; [b] Literature association constants for analogous compound **S2**.^[3]

S7.10 Summary of acetate binding data

Table S8. Summary of data for host **1** binding OAc^- (as $[\text{NBu}_4][\text{OAc}]$; DMSO- d_6 + 0.5% H_2O).

	E-1 ^[a]	E-1 ^[a]	E-S2 ^[b]	Z-1 ^[a]	Z-1 ^[a]	Z-S2 ^[b]
OAc^- Endpoint / mM	57	8.6		40	57	
K_1 / M^{-1}	$3.3 \pm 0.35 \times 10^2$	$3.5 \pm 0.9 \times 10^2$	2.1×10^2	$1.8 \pm 0.2 \times 10^3$	$1.5 \pm 0.2 \times 10^3$	1.4×10^3
K_2 / M^{-1}	83	87	52	7	5	-
α	Forced as 1			0.01 ± 0.01	0.01 ± 0.01	

[a] Measured association constants for compound **1**; [b] Literature association constants for analogous compound **S2**.^[3]

S7.11 Host-guest binding models with an aggregating guest

The self-association of dihydrogen phosphate described here can make determination of binding constants challenging. Considering a minimal case of host-guest binding with a 1:1 stoichiometry and no self-association for either host or guest, the concentration of free guest $[G]$ can be found by solving the quadratic:^[7]

$$0 = [G]^2 - [G] \left([G]_0 - [H]_0 - \frac{1}{K_a} \right) - \frac{[G]_0}{K_a} \quad (17)$$

where $[G]_0$ and $[H]_0$ are respectively the total concentration of guest and host, and K_a is the association constant. This quadratic derives from the definition of an association constant and the mass balances for each of host and guest, as shown below:

$$\begin{aligned} K_a &= \frac{[HG]}{[H][G]} \\ [G]_0 &= [G] + [HG] \\ [H]_0 &= [H] + [HG] \end{aligned}$$

If the guest is capable of indefinite self-association, constructing such a model for host-guest binding becomes more challenging. With infinite oligo-guest species present in solution, if the host binding site is capable of binding not only monomeric G but also G_2 dimers, G_3 trimers, G_4 tetramers *etc* there is no longer a single guest but instead many different guest oligomer species that cannot be distinguished. Therefore, there is not a single host-guest association constant, K_a , capable of reflecting the infinite possible host-guest interactions in solution. The mass balance equations for such a system are also complex:

$$\begin{aligned} [G]_0 &= \sum_{n=1}^{\infty} n[G_n] + \sum_{n=1}^{\infty} n[HG_n] \\ [H]_0 &= [H] + \sum_{n=1}^{\infty} [HG_n] \end{aligned}$$

Modelling the titration data to such complicated competitive systems is beyond the scope of this work. Instead, we use the standard equations of host-guest binding (S7.1^[7]) that assume that the guest can only exist in free or host-bound forms and that all host-guest complexes are identical.

It may be possible to measure K_a if the speciation due to one process (i.e. host-guest binding) can be varied while the other process (i.e. the isodesmic self-association) does not change. In practice this requires the two association constants to be sufficiently different that one process can be ignored over a limited concentration range where changes due to the other process can be observed (e.g. measurement of strong association at low concentrations).

S8. Diffusion of static host-guest systems

S8.1 Diffusion measurements with a single host

Figure S35. Measured diffusion coefficients for separate 5 mM solutions of pure *E* or *Z* hosts titrated with $[\text{H}_2\text{PO}_4]$, presented on a single axis for clarity. Diffusion coefficients as-acquired are shown as solid points; diffusion coefficients corrected for changes in viscosity from to increasing $[\text{H}_2\text{PO}_4]$ are shown as empty circles (linear correction from directly measured viscosities of $[\text{NBu}_4][\text{H}_2\text{PO}_4]$ solutions; see Figure S6). Each point represents one diffusion experiment. 500 MHz ^1H , $\Delta = 50$ ms, $\delta = 4$ ms, linear ramp of 12 gradients from $g = 0 - 53.45$ g cm^{-1} .

S8.2 Control experiments with tetrabutylammonium acetate

Figure S36. Comparison of guest-dependent diffusion for separate 5 mM solutions of pure *E* or *Z* hosts in the presence of $[\text{NBu}_4][\text{H}_2\text{PO}_4]$ or $[\text{NBu}_4][\text{OAc}]$, presented on a single axis for clarity. No corrections have been made for changes in viscosity due to the lack of viscometry data for solutions of tetrabutylammonium acetate. Each point represents one diffusion experiment. 500 MHz ^1H , $\Delta = 50$ ms, $\delta = 4$ ms, linear ramp of 12 gradients from $g = 0 - 53.45$ g cm^{-1} .

S8.3 Diffusion measurements for 1:1 *E:Z* mixed systems

Figure S37. Diffusion of hosts **1** in 1 mM mixed solution (1:1 *E:Z*), in the presence of 0 – 70 mM [NBu₄][H₂PO₄]. Diffusion coefficients as-acquired are shown as solid points; diffusion coefficients corrected for changes in viscosity from to increasing [NBu₄][H₂PO₄] are shown as empty circles (linear correction from directly measured viscosities of [NBu₄][H₂PO₄] solutions; see Figure S6). Each point represents one diffusion experiment. 500 MHz ¹H, Δ = 50 ms, δ = 4 ms, linear ramp of 12 gradients from g = 0 – 53.45 g cm⁻¹.

Figure S38. Diffusion of hosts **1** in 5 mM mixed solution (1:1 *E:Z*), in the presence of 0 – 70 mM [NBu₄][H₂PO₄]. Diffusion coefficients as-acquired are shown as solid points; diffusion coefficients corrected for changes in viscosity from to increasing [NBu₄][H₂PO₄] are shown as empty circles (linear correction from directly measured viscosities of [NBu₄][H₂PO₄] solutions; see Figure S6). Each point represents one diffusion experiment. 500 MHz ¹H, Δ = 50 ms, δ = 4 ms, linear ramp of 12 gradients from g = 0 – 53.45 g cm⁻¹.

Figure S39. Diffusion of hosts **1** in 10 mM mixed solution (1:1 E:Z), in the presence of 0 – 70 mM $[\text{NBu}_4][\text{H}_2\text{PO}_4]$. Diffusion coefficients as-acquired are shown as solid points; diffusion coefficients corrected for changes in viscosity from to increasing $[\text{H}_2\text{PO}_4]$ are shown as empty circles (linear correction from directly measured viscosities of $[\text{NBu}_4][\text{H}_2\text{PO}_4]$ solutions; see Figure S6). Each point represents one diffusion experiment. 500 MHz ^1H , $\Delta = 50$ ms, $\delta = 4$ ms, linear ramp of 12 gradients from $g = 0 - 53.45$ g cm^{-1} .

Figure S40. Comparison of measured diffusion coefficients of hosts **1** in 1:1 E:Z mixtures of 1mM, 5 mM, and 10 mM total concentration (as above). All data corrected for changes in viscosity due to the addition of $[\text{NBu}_4][\text{H}_2\text{PO}_4]$ (SI S4). Each point represents one diffusion experiment. 500 MHz ^1H , $\Delta = 50$ ms, $\delta = 4$ ms, linear ramp of 12 gradients from $g = 0 - 53.45$ g cm^{-1} .

S8.4 Viscosity measurements for host-guest systems

Viscosities and densities for 5 mM solutions of host **1** in combination with 50 mM of [NBu₄][H₂PO₄] were measured at 25 °C using a Lovis 2000M rolling ball viscometer (Ø1.59 mm capillary; 1 mm stainless steel ball), with results shown in Table S9. The presence of 50 mM [NBu₄][H₂PO₄] caused a 9.6% increase in dynamic viscosity (η) relative to DMSO-*d*₆ with 0.5% water. Subsequent addition of 5 mM of host **1** to the 50 mM [NBu₄][H₂PO₄] solution as either pure *E*-**1**, pure *Z*-**1**, or 1:1 *E*-**1**:*Z*-**1** caused minor increases in η of 1.44%, 0.51%, or 0.81% respectively with reference to 50 mM [NBu₄][H₂PO₄], indicating that the addition of host **1** has only a minor effect on viscosity.

Table S9. Viscosity measurements of solutions of host and [NBu₄][H₂PO₄] in DMSO-*d*₆ with 0.5% v/v added water.

[NBu ₄][H ₂ PO ₄] / mM	[<i>E</i> - 1] /mM	[<i>Z</i> - 1] /mM	Density / g/cm ³	Temperature / °C	Dyn. Viscosity (η) / mPa·s	Error / %	η/η_0
-	-	-	1.1833	25.04	2.149	0.02	1.000
50	-	-	1.1819	25.06	2.356	0.04	1.096
50	5	-	1.1820	25.05	2.390	0.08	1.112
50	-	5	1.1818	25.06	2.368	0.03	1.102
50	2.5	2.5	1.1820	25.06	2.375	0.02	1.105

While viscosities were not measured directly for 1:1 *E*:*Z* host mixtures at concentrations other than 5 mM, a linear interpolation between $\eta = 2.356$ in the absence of host and $\eta = 2.375$ for the 5 mM mixture was used to estimate $\eta = 2.360$ for 50 mM [H₂PO₄] plus 1 mM 1:1 host and $\eta = 2.394$ for 50 mM [H₂PO₄] plus 10 mM 1:1 host. This interpolated and extrapolated viscosity data was only used for the viscosity-corrected data shown in Table S12.

S8.5 Tabulated diffusion data at 50 mM [NBu₄][H₂PO₄]

Table S10. Changes in diffusion coefficients of pure or 1:1 mixed solutions of *E*-1 and *Z*-1 in the presence of 50 mM [NBu₄][H₂PO₄]. DMSO-*d*₆ with 0.5% added water, 500 MHz. No correction applied for viscosity changes.

[<i>E</i> -1] /mM	[<i>Z</i> -1] /mM	$D(\text{H}_2\text{PO}_4)^{[a]}$ / $10^{-10} \text{ m}^2\text{s}^{-1}$	$D_E^{[b]}$ / $10^{-10} \text{ m}^2\text{s}^{-1}$	$D_Z^{[b]}$ / $10^{-10} \text{ m}^2\text{s}^{-1}$	$D(\text{NBu}_4)^{[b]}$ / $10^{-10} \text{ m}^2\text{s}^{-1}$	$D_{\text{DMSO}}^{[b]}$ / $10^{-10} \text{ m}^2\text{s}^{-1}$
-	-	1.99 ± 0.02	-	-	2.32 ± 0.01	6.32 ± 0.04
0.5	0.5	1.90 ± 0.02	1.15 ± 0.02	1.45 ± 0.01	2.33 ± 0.01	6.35 ± 0.02
2.5	2.5	1.82 ± 0.06	1.10 ± 0.01	1.34 ± 0.01	2.26 ± 0.01	6.28 ± 0.03
5	5	1.69 ± 0.08	1.04 ± 0.01	1.26 ± 0.01	2.14 ± 0.01	6.11 ± 0.03
5	-	1.79 ± 0.04	1.08 ± 0.02	-	2.21 ± 0.01	6.06 ± 0.02
-	5	1.86 ± 0.03	-	1.29 ± 0.01	2.19 ± 0.01	6.02 ± 0.02

[a] ³¹P PGSTE experiment, $\delta = 7$ ms, $\Delta = 100$ ms, $g = 0 - 53.45$ G cm⁻¹. [b] ¹H PGSTE experiment, $\delta = 4$ ms, $\Delta = 50$ ms, $g = 0 - 53.45$ G cm⁻¹.

Table S11. Diffusion data from Table S10, corrected for changes in viscosity using η for 50 mM [NBu₄][H₂PO₄] addition only, ignoring changes in η caused by host.

[<i>E</i> -1] /mM	[<i>Z</i> -1] /mM	η/η_0	$D'(\text{H}_2\text{PO}_4)^{[a]}$ / $10^{-10} \text{ m}^2\text{s}^{-1}$	$D'_E^{[b]}$ / $10^{-10} \text{ m}^2\text{s}^{-1}$	$D'_Z^{[b]}$ / $10^{-10} \text{ m}^2\text{s}^{-1}$	$D'(\text{H}_2\text{PO}_4)^{[b]}$ / $10^{-10} \text{ m}^2\text{s}^{-1}$	$D'_{\text{DMSO}}^{[b]}$ / $10^{-10} \text{ m}^2\text{s}^{-1}$
-	-	1.096	2.18 ± 0.02	-	-	2.54 ± 0.01	6.93 ± 0.04
0.5	0.5	1.096	2.08 ± 0.02	1.26 ± 0.02	1.59 ± 0.01	2.55 ± 0.01	6.96 ± 0.02
2.5	2.5	1.096	2.00 ± 0.07	1.21 ± 0.01	1.47 ± 0.01	2.48 ± 0.01	6.88 ± 0.03
5	5	1.096	1.85 ± 0.09	1.14 ± 0.01	1.38 ± 0.01	2.35 ± 0.01	6.70 ± 0.03
5	-	1.096	1.96 ± 0.04	1.18 ± 0.02	-	2.42 ± 0.01	6.64 ± 0.02
-	5	1.096	2.04 ± 0.03	-	1.41 ± 0.01	2.4 ± 0.01	6.60 ± 0.02

[a] ³¹P PGSTE experiment, $\delta = 7$ ms, $\Delta = 100$ ms, $g = 0 - 53.45$ G cm⁻¹. [b] ¹H PGSTE experiment, $\delta = 4$ ms, $\Delta = 50$ ms, $g = 0 - 53.45$ G cm⁻¹.

Table S12. Diffusion data from Table S10, corrected for changes in viscosity caused by host and guest

[<i>E</i> -1] /mM	[<i>Z</i> -1] /mM	η/η_0	$D'(\text{H}_2\text{PO}_4)^{[a]}$ / $10^{-10} \text{ m}^2\text{s}^{-1}$	$D'_E^{[b]}$ / $10^{-10} \text{ m}^2\text{s}^{-1}$	$D'_Z^{[b]}$ / $10^{-10} \text{ m}^2\text{s}^{-1}$	$D'(\text{H}_2\text{PO}_4)^{[b]}$ / $10^{-10} \text{ m}^2\text{s}^{-1}$	$D'_{\text{DMSO}}^{[b]}$ / $10^{-10} \text{ m}^2\text{s}^{-1}$
-	-	1.096	2.18 ± 0.02	-	-	2.54 ± 0.01	6.93 ± 0.04
0.5	0.5	1.098 ^[c]	2.09 ± 0.02	1.26 ± 0.02	1.59 ± 0.01	2.56 ± 0.01	6.97 ± 0.02
2.5	2.5	1.105	2.01 ± 0.07	1.22 ± 0.01	1.48 ± 0.01	2.50 ± 0.01	6.94 ± 0.03
5	5	1.114 ^[c]	1.88 ± 0.09	1.16 ± 0.01	1.40 ± 0.01	2.38 ± 0.01	6.81 ± 0.03
5	-	1.112	1.99 ± 0.04	1.20 ± 0.02	-	2.46 ± 0.01	6.74 ± 0.02
-	5	1.102	2.05 ± 0.03	-	1.42 ± 0.01	2.41 ± 0.01	6.63 ± 0.02

[a] ³¹P PGSTE experiment, $\delta = 7$ ms, $\Delta = 100$ ms, $g = 0 - 53.45$ G cm⁻¹. [b] ¹H PGSTE experiment, $\delta = 4$ ms, $\Delta = 50$ ms, $g = 0 - 53.45$ G cm⁻¹. [c] Not measured directly but estimated by linear interpolation from other points: see S8.4.

S9. Comparison of modelled and measured guest-dependent diffusion coefficients for pure *E* and *Z* systems

Table S13. Parameters used for modelling concentration-dependent diffusion coefficients of *E*-1 and *Z*-1.

	$D_0 / \text{m}^2 \text{s}^{-1} \times 10^{-10}$	K_1 / M^{-1}	K_2 / M^{-1}	K_3 / M^{-1}
Monomeric H_2PO_4^- (see S6)	3.39	120	-	-
5 mM <i>E</i> -1	1.74	-	360	90
5 mM <i>Z</i> -1	1.87	-	2100	70

Host-guest speciation and diffusion coefficients for pure *E* or *Z* hosts binding up to two H_2PO_4^- oligomers using the parameters given in Table S13 were modelled using Equation 13 (S5.3), and plotted in Figure S41. The modelled dependence of D_E and D_Z on H_2PO_4^- concentration significantly overestimates D at all concentrations studied, particularly for the *E*-1 isomer, indicating that this model does not accurately reflect the structures formed in solution.

Figure S41. Comparison of experimental and modelled $[\text{H}_2\text{PO}_4^-]$ -dependent diffusion coefficients of hosts, using Equation 13 and the parameters shown in Table S13. Top: speciation of *E* (left) and *Z* (right) hosts using a 1:2 host:guest binding model and the association parameters shown in Table S13. Bottom: modelled average diffusion coefficients of hosts binding up to 2 H_2PO_4^- oligomers (see S5.3 for details). This model is clearly insufficient to explain the large decrease in D observed on addition of H_2PO_4^- , particularly for the *E*-1 isomer.

S9.1 Estimated diffusion coefficients of potential supramolecular host-guest complexes

To aid in comparing the sizes of proposed complexes, approximate molecular weights are shown in Table S14. As H_2PO_4^- oligomers have been modelled as forming with a continuous distribution of sizes, no single definitive molecular weight can be given for complexes involving oligomeric H_2PO_4^- . Instead, we provide molecular weights for the calculated median and mean oligomer sizes.

Table S14. Estimated diffusion coefficients for representative supramolecular structures of the form $[\text{H}_m\text{G}_n]$. Estimated diffusion coefficients for complexes (calculated using Eq (11)) are shown in normal text. While a wide distribution of host-guest systems are expected to form in solution, the measured host diffusion coefficients at 5 mM host and 50 mM $[\text{NBu}_4][\text{H}_2\text{PO}_4]$ suggest complexes of approximately 1.5 – 2.5 kDa in size under these conditions.

Complex	Median MW / kDa	Mean MW / kDa	$D(\text{H}_2\text{PO}_4)^{[a]}$ / $10^{-10} \text{ m}^2\text{s}^{-1}$	$D_E^{[b]}$ / $10^{-10} \text{ m}^2\text{s}^{-1}$	$D_Z^{[b]}$ / $10^{-10} \text{ m}^2\text{s}^{-1}$
Free host $[\text{H}_1\text{G}_0]$	0.5		-	1.74 ^[a]	1.87 ^[a]
Oligomeric $\text{H}_2\text{PO}_4^- [\text{G}_n]$ (50 mM $[\text{NBu}_4][\text{H}_2\text{PO}_4]$, $K_i = 120$)	0.4 (4 H_2PO_4^- units)	0.5 (5 H_2PO_4^- units)	2.16 ^[b]		
Monomeric G (H_2PO_4^- , infinite dilution)	0.1		3.39		
Host with monomeric phosphate ($D(\text{H}_2\text{PO}_4) = 3.39$)					
$[\text{H}_1\text{G}_1]$	0.6			1.67	1.78
$[\text{H}_1\text{G}_2]$	0.7			1.61	1.70
Host with phosphate oligomers (50 mM, $D(\text{H}_2\text{PO}_4) = 2.16$)					
$[\text{H}_1\text{G}'_1]$	0.9	1.0		1.51	1.58
$[\text{H}_1\text{G}'_2]$	1.3	1.5		1.37	1.42
$[\text{H}_2\text{G}'_1]$	1.4	1.5		1.22	1.35
$[\text{H}_2\text{G}'_2]$	1.8	2.0		1.20	1.26
$[\text{H}_2\text{G}'_3]$	2.2	2.5		1.14	1.18
$[\text{H}_3\text{G}'_2]$	2.4	2.6		1.09	1.15
$[\text{H}_3\text{G}'_3]$	2.8	3.0		1.05	1.10
$[\text{H}_3\text{G}'_4]$	3.1	3.5		1.01	1.05
5 mM host, 50 mM guest			1.93 ^[b] (E), 2.01 ^[b] (Z)	1.17 ^[a]	1.39 ^[a]

Experimentally obtained values: [a] ^{31}P PGSTE, $\delta = 7$ ms, $\Delta = 100$ ms, $g = 0 - 53.45 \text{ G cm}^{-1}$; [b] ^1H PGSTE experiment, $\delta = 4$ ms, $\Delta = 50$ ms, $g = 0 - 53.45 \text{ G cm}^{-1}$.

S10. Photoswitching studies

S10.1 Time-dependent diffusion with *in situ* irradiation

Photo-diffusion studies were conducted by combining *in situ* light irradiation with time-dependent diffusion experiments previously reported by our group.^[8] It is well known that NMR diffusion measurements are often disrupted by convection, generally caused by the presence of temperature gradients within the sample.^[9] Convection can be challenging at the best of times, but here the use of *in situ* irradiation (and subsequent heating) delivered by fiber to the center of the sample can be almost guaranteed to generate temperature gradients during irradiation. This can be seen in the time-dependent diffusion data presented in Figure S42.

Figure S42. Time-resolved diffusion study while photoswitching under *in situ* irradiation (405 nm LED source). 5 mM host (initially ~90% Z-1) in solution with 50 mM $[\text{NBu}_4][\text{H}_2\text{PO}_4]$ was irradiated with 405 nm light during the shaded periods of time, while diffusion information was continuously acquired using a long list of random gradients as previously reported.^[8] Measured diffusion coefficients can be seen to increase sharply under irradiation, but this is convection driven resulting from temperature gradients caused by uneven sample heating by light irradiation.

S10.2 Monitoring temperatures and suppressing convection

There are many approaches to suppressing convection in diffusion NMR experiments. We chose the crude but effective method of introducing physical obstructions to the sample to disrupt convective flow. We use glass TLC spotting pipettes broken up into 30 – 40 mm irregular lengths to pack the sample and restrict flow. These open-ended capillaries were added to the sample around the 1.5 mm quartz optic fibre and flame-sealed methanol capillary used for thermometry (Figure S43).

To our pleasant surprise, NMR lineshapes were retained and few difficulties were encountered in shimming after packing our samples with glass and quartz (signal intensity was reduced somewhat by the displacement of liquid from the active volume). Representative time-resolved diffusion data are shown in Figure S44.

Figure S43. NMR sample used for photo-diffusion studies with *in situ* irradiation and temperature measurements. In the expansion the optic fiber (quartz, 1.5 mm diameter), flame-sealed methanol capillary (Sigma-Aldrich, 5 μ L capacity glass micropipette), and broken lengths of glass TLC capillaries are highlighted by colored lines.

Figure S44. Time-resolved diffusion study^[8] while photoswitching under *in situ* irradiation (400 nm LED source), with flame-sealed methanol capillary added for *in situ* thermometry and short (30-40 mm) sections of broken glass capillaries added to suppress convection. 5 mM host (initially ~90% Z-1) in solution with 50 mM [NBu₄][H₂PO₄] was irradiated with 405 nm light during the shaded periods of time, while diffusion information was continuously acquired using a long list of random gradients. **Top:** temperature measurements taken from the separation of the methanol CH₃ and OH peaks (top) show an increase in sample temperature of approximately 0.35 K while under irradiation.^[10] The apparent quantization of temperature measurements results from the ~0.5 Hz resolution of the NMR spectra. **Middle:** signal intensity changes of the tolyl peaks of *E*-1 and *Z*-1 during irradiation. **Bottom:** time-dependent diffusion measurements of the same peaks over time. Measured *D* for each species taken from measurements of the 1:1 mixture of photoisomers (SI-S8.5) are shown as dashed horizontal lines for comparison.

S10.3 Demonstration of reversible photoswitching by UV-vis

Figure S45. UV-vis absorption spectrum of *E*-**1** (approx. 25 μ M) in DMSO + 0.5% water. The absorption maximum λ_{max} occurs at 365 nm, identical to that previously reported for analogous host *E*-**S2**.^[9]

Figure S46. Demonstration of reversible photoswitching of compound **1** by UV-vis spectroscopy under irradiation from 385 nm (blue) and 375 nm (violet) LEDs. Solution of pure **1** (approx. 25 μ M) in DMSO + 0.5% water.

The reversibility of photoswitching in DMSO was studied by UV-vis spectroscopy with *in situ* irradiation delivered by 385 nm and 375 nm LEDs. These wavelengths are not ideal for selectively photoswitching this class of compound, but are sufficient to demonstrate the reversibility of photoswitching as shown in Figure S46.

S11. Geometry optimizations by DFT

Input geometries were generated using Avogadro^[11] (UFF optimization). The Gaussian 03 program^[12] was used for further geometry optimization at the DFT B3LYP/6-31G(d,p) level of theory using tight convergence criteria. The DFT optimized geometries were found to have zero imaginary frequencies.

Table S15. Cartesian coordinates of Z-1 (approximate width × height × depth = 15.5 × 10.1 × 13.2 Å).

atom	X	Y	Z
C	-4.4187180000	-2.6292070000	1.3266160000
C	-3.9527390000	-1.5791220000	0.5111400000
C	-5.8729570000	-2.4232700000	1.6806280000
C	-6.3342430000	-1.3578480000	0.6529360000
H	-5.9755270000	-2.0360570000	2.7036870000
H	-6.4586460000	-3.3468050000	1.6303780000
C	-5.0527180000	-0.6315450000	0.2447640000
H	-7.0910580000	-0.6809600000	1.0584210000
H	-6.7811660000	-1.8572210000	-0.2186010000
C	-3.5683980000	-3.6730620000	1.6727960000
C	-2.2574410000	-3.7131230000	1.1939130000
C	-1.8023360000	-2.6928510000	0.3403620000
C	-2.6518610000	-1.6288650000	0.0019210000
H	-3.9213350000	-4.4811900000	2.3083480000
H	-1.5913490000	-4.5217820000	1.4561660000
H	-2.3033540000	-0.8637980000	-0.6848360000
C	-3.5684300000	3.6730450000	-1.6727910000
C	-2.2574770000	3.7131210000	-1.1938980000
H	-1.5913930000	4.5217880000	-1.4561450000
C	-1.8023660000	2.6928510000	-0.3403460000
C	-2.6518800000	1.6288550000	-0.0019140000
N	-0.5165810000	-2.6862610000	-0.2443320000
C	0.5623550000	-3.5111720000	0.0458160000
N	1.6615730000	-3.2297750000	-0.7522750000
C	2.9335330000	-3.8420110000	-0.7334530000
O	0.5451870000	-4.3803600000	0.9072120000
C	3.8773100000	-3.3837580000	-1.6653740000
C	3.3017570000	-4.8591470000	0.1588180000
C	4.5892410000	-5.3906590000	0.0976570000
C	5.1558110000	-3.9296050000	-1.7080860000

C	5.5408340000	-4.9487480000	-0.8284620000
C	6.9185680000	-5.5637080000	-0.8958930000
H	6.9497470000	-6.4025140000	-1.6029560000
H	7.2313860000	-5.9506340000	0.0785830000
H	7.6670300000	-4.8361060000	-1.2250050000
H	2.5852350000	-5.2186980000	0.8826370000
H	4.8566950000	-6.1770440000	0.7994330000
H	3.6100390000	-2.5891770000	-2.3594030000
H	5.8663780000	-3.5520500000	-2.4391240000
H	-0.3719810000	-1.9445230000	-0.9136010000
H	1.5454680000	-2.5424810000	-1.4824010000
C	0.5623220000	3.5111820000	-0.0458070000
N	1.6615350000	3.2298050000	0.7522970000
C	2.9334970000	3.8420380000	0.7334640000
O	0.5451590000	4.3803460000	-0.9072270000
C	3.3017300000	4.8591420000	-0.1588410000
C	3.8772670000	3.3838140000	1.6654070000
C	5.1557690000	3.9296600000	1.7081080000
C	4.5892150000	5.3906520000	-0.0976900000
C	5.5408010000	4.9487710000	0.8284510000
C	6.9185360000	5.5637280000	0.8958700000
H	7.6670010000	4.8361240000	1.2249700000
H	6.9497240000	6.4025310000	1.6029380000
H	7.2313440000	5.9506590000	-0.0786070000
H	2.5852140000	5.2186700000	-0.8826770000
H	3.6099890000	2.5892580000	2.3594610000
H	5.8663300000	3.5521280000	2.4391640000
H	4.8566760000	6.1770120000	-0.7994920000
H	1.5454180000	2.5425490000	1.4824570000
N	-0.5166160000	2.6862790000	0.2443590000
H	-0.3720100000	1.9445390000	0.9136240000
C	-3.9527540000	1.5790980000	-0.5111430000
H	-2.3033680000	0.8637900000	0.6848440000
C	-4.4187400000	2.6291790000	-1.3266200000
H	-3.9213720000	4.4811700000	-2.3083440000
C	-5.8729740000	2.4232240000	-1.6806430000
C	-5.0527240000	0.6315070000	-0.2447770000
C	-6.3342550000	1.3577940000	-0.6529580000
H	-6.7811920000	1.8571600000	0.2185760000
H	-7.0910570000	0.6808970000	-1.0584510000
H	-5.9755300000	2.0360130000	-2.7037050000
H	-6.4586740000	3.3467520000	-1.6303960000

Sum of electronic and zero-point Energies = -1683.883953

Table S16. Cartesian coordinates of *E-1* (approximate width × height × depth = 26.9 × 10.2 × 2.8 Å).

atom	X	Y	Z
C	1.7307360000	2.4455110000	-0.8581430000
C	1.6501510000	1.0686670000	-0.5668060000
C	0.3615640000	3.0232440000	-1.1207740000
C	-0.6039440000	1.9032300000	-0.6565820000
H	0.2273660000	3.2315030000	-2.1906950000
H	0.1930620000	3.9688890000	-0.5944620000
C	0.2418030000	0.6338950000	-0.5797530000
H	-1.4559230000	1.7864390000	-1.3321670000
H	-1.0104210000	2.1500260000	0.3341140000
C	2.9642930000	3.0830780000	-0.8889100000
C	4.1393680000	2.3780140000	-0.6207050000
C	4.0668470000	1.0096400000	-0.3109790000
C	2.8229460000	0.3590410000	-0.2835020000
H	3.0281880000	4.1438660000	-1.1176240000
H	5.1016020000	2.8678410000	-0.6376280000
H	2.7850880000	-0.6897100000	-0.0104510000
C	-2.9642860000	-3.0830350000	-0.8890290000
C	-4.1393650000	-2.3779770000	-0.6208240000
H	-5.1015980000	-2.8678060000	-0.6377670000
C	-4.0668490000	-1.0096120000	-0.3110630000
C	-2.8229490000	-0.3590120000	-0.2835520000
N	5.1973210000	0.2210930000	-0.0055450000
C	6.5296460000	0.6070880000	0.0516980000
N	7.3613870000	-0.4460710000	0.4046280000
C	8.7647880000	-0.4394010000	0.5522650000
O	6.9250110000	1.7412930000	-0.1826970000
C	9.3723340000	-1.6428400000	0.9415430000
C	9.5719080000	0.6871470000	0.3363880000
C	10.9512800000	0.5845210000	0.5096270000
C	10.7510330000	-1.7214050000	1.1083700000
C	11.5736270000	-0.6093010000	0.8925950000
C	13.0737000000	-0.7017300000	1.0410440000
H	13.5028480000	0.2517150000	1.3638680000
H	13.5553750000	-0.9684740000	0.0916330000
H	13.3580790000	-1.4636510000	1.7733150000
H	9.1156970000	1.6215230000	0.0441740000
H	11.5597710000	1.4698500000	0.3409770000
H	8.7595420000	-2.5245240000	1.1197800000

H	11.1925570000	-2.6667620000	1.4137680000
H	4.9830960000	-0.7459300000	0.1878470000
H	6.9311790000	-1.3379360000	0.5996190000
C	-6.5296460000	-0.6070850000	0.0516520000
N	-7.3614020000	0.4460800000	0.4045310000
C	-8.7647970000	0.4393820000	0.5522210000
O	-6.9249950000	-1.7413120000	-0.1826660000
C	-9.5718880000	-0.6872200000	0.3365310000
C	-9.3723670000	1.6428540000	0.9413660000
C	-10.7510600000	1.7213950000	1.1082450000
C	-10.9512570000	-0.5846180000	0.5098200000
C	-11.5736260000	0.6092320000	0.8926570000
C	-13.0736950000	0.7016400000	1.0411630000
H	-13.3580500000	1.4633410000	1.7736740000
H	-13.5028490000	-0.2518970000	1.3637020000
H	-13.5553850000	0.9686890000	0.0918450000
H	-9.1156580000	-1.6216180000	0.0444170000
H	-8.7595970000	2.5245820000	1.1194580000
H	-11.1926020000	2.6667780000	1.4135370000
H	-11.5597260000	-1.4699900000	0.3413160000
H	-6.9312170000	1.3379880000	0.5993750000
N	-5.1973290000	-0.2210700000	-0.0056360000
H	-4.9831070000	0.7459480000	0.1877850000
C	-1.6501490000	-1.0686310000	-0.5668570000
H	-2.7850950000	0.6897320000	-0.0104750000
C	-1.7307300000	-2.4454680000	-0.8582290000
H	-3.0281770000	-4.1438170000	-1.1177700000
C	-0.3615540000	-3.0231940000	-1.1208550000
C	-0.2418010000	-0.6338580000	-0.5797730000
C	0.6039470000	-1.9031910000	-0.6566220000
H	1.0104100000	-2.1500110000	0.3340740000
H	1.4559360000	-1.7863830000	-1.3321920000
H	-0.2273410000	-3.2314270000	-2.1907790000
H	-0.1930590000	-3.9688520000	-0.5945640000

Sum of electronic and zero-point Energies = -1683.887605

Table S17. Cartesian coordinates of H_2PO_4^- (approximate radius = 2.5 Å).

atom	X	Y	Z
P	0.0000000000	0.0000180000	-0.1653310000
O	-1.0101600000	-0.8033300000	0.9083060000
H	-1.8339000000	-0.3034530000	0.8348330000
O	1.0101720000	0.8031570000	0.9084420000
O	-0.8685690000	1.0143440000	-0.8576320000
H	1.8339010000	0.3032610000	0.8349340000
O	0.8685580000	-1.0141790000	-0.8578410000

Sum of electronic and zero-point Energies = -643.562195

S12. Scripts used for data processing

S12.1 Analysis of association data from chemical shifts

Script S1. Python code used for fitting association constants to chemical shift data.

```
import pandas as pd
import numpy as np
import scipy.optimize
import matplotlib.pyplot as plt
from lmfit import minimize, Parameters, report_fit
import itertools
import matplotlib.cm as cm
import seaborn as sns

#Global fit for 2:1 H:G complex data

def Global21(data,figsize=(8,6)):
    '''Fitting function for 2:1 Host:Guest stoichiometry.
    This function calculates association constants from NMR titration data as a Pandas dataframe with
    the following columns
    [Host concentration / Molar] [Guest concentration / Molar] [Peak 1 / ppm] [ Peak 2 / ppm]...
    ...for an arbitry number of peaks. If desired, this can be called from the clipboard using
    pd.read_clipboard(), eg:
    params = Global21(pd.read_clipboard())

    Binding parameters are calculated using a global nonlinear least-squares fit over all provided peaks.
    '''
    def H21(G0,H0,K1,K2):
        '''H21 calculate the concentration of free host, [H], given G0 (total gues concentration), H0
        (total host concentration), K1 (H + G -> [HG] association constant), and K2 (H + [HG] -> [H2G] association
        constant).
        '''
        G0,H0,K1,K2 = np.asarray(G0), np.asarray(H0), np.asarray(K1), np.asarray(K2)
        try:
            length = len(G0)
            coeffs = np.zeros((length,4))
            for i in range(length):
                coeffs[i] = [K1*K2, K1*(2*K2*G0[i] - K2*H0[i] + 1), K1*(G0[i]-H0[i])+1, -H0[i]]
            roots = np.zeros((len(G0),3))
            R = np.zeros(len(G0))
            for i in range(len(G0)):
                roots[i] = np.roots(coeffs[i])
                R[i] = min(roots[i][roots[i] >= 0])
            return R
```

```

except TypeError:
    coeffs = [K1*K2, K1*(2*K2*G0 - K2*H0 + 1), K1*(G0-H0)+1, -H0]
    R = np.roots(coeffs)
    return min(R[R >= 0])

def nmr21(X,K1,K2,delHG,delH2G):
    '''nmr21: calculates chemical shift difference delDel for a given peak from the free host chemical
    shift as a function of X = H0, G0, K1, K2, delHG = chemical shift difference on first association, delH2G
    = chemical shift difference on second association.'''
    H0, G0 = X
    H = H21(G0,H0,K1,K2)
    delDel = (delHG*K1*G0*H + 2*delH2G*G0*K1*K2*H**2)/(H0*(1+K1*H+K1*K2*H**2))
    return delDel

def nmr21_dataset(X,params,i):
    '''Given X = H0, G0 and params = lmfit parameters element for multiple peaks, calculates chemical
    shifts for each peak, for each [G0, H0] pairing'''
    K1 = params['K1_%i' % (i+1)].value
    K2 = params['K2_%i' % (i+1)].value
    delHG = params['delDHG1_%i' % (i+1)].value
    delHG2 = params['delDHG2_%i' % (i+1)].value
    return nmr21(X,K1,K2,delHG,delHG2)

def objective(params, X, data):
    """Calculate total residual for fits to several data sets"""
    dataT = np.array(data.T[2:])
    ndata, nx = dataT.shape
    resid = 0.0*dataT[:]
    # make residual per data set
    for i in range(ndata):
        dataT[i] = dataT[i] - dataT[i][0]
        resid[i, :] = dataT[i, :] - nmr21_dataset(X,params,i)
    # now flatten this to a 1D array, as minimize() needs
    return resid.flatten()

X = data.iloc[:,0],data.iloc[:,1]

dataT = np.array(data.T[2:])
fit_params = Parameters()
for iy, y in enumerate(dataT):
    fit_params.add('K1_%i' % (iy+1), value=1000, min=0.0, max=1e6)
    fit_params.add('K2_%i' % (iy+1), value=100, min=0.0, max=1e4)
    fit_params.add('delDHG1_%i' % (iy+1), value=1.0, min=-4.0, max=4.0)
    fit_params.add('delDHG2_%i' % (iy+1), value=-1.0, min=-4.0, max=4.0)

for iy in range(2,len(dataT)+1):

```

```

fit_params['K1_%i' % iy].expr='K1_1'
fit_params['K2_%i' % iy].expr='K2_1'

result = minimize(objective, fit_params, args=(X, data), nan_policy='omit')
#report_fit(result.params)

palette = itertools.cycle(sns.color_palette())
fig, (ax1, ax2, ax3) = plt.subplots(3,1,sharex=True,figsize=figsize)
plt.rcParams["lines.markeredgewidth"] = 1.5
interp = np.linspace(min(X[1]),max(X[1]),1000)
Xinterp = (interp*0.0+X[0][0]), interp

K1, K2 = result.params['K1_1'].value, result.params['K2_1'].value
Hinterp = H21(Xinterp[1],Xinterp[0],K1,K2)
#Ginterp = (-K1+np.sqrt(K1**2 - 8*K1*K2*(1-Xinterp[0]/Hinterp)))/(4*K1*K2)
Ginterp = Xinterp[1]/(1+K1*Hinterp + K1*K2*Hinterp**2)
HGinterp = K1*Hinterp*Ginterp
H2Ginterp = K2*Hinterp*HGinterp

for i in range(len(dataT)):
    c = next(palette)
    y_fit = nmr21_dataset(X, result.params, i)
    Yinterp = nmr21_dataset(Xinterp, result.params, i)
    ax1.plot(X[1]/X[0], dataT[i, :]-dataT[i,0], 'o', color=c, markerfacecolor='None')
    ax1.plot(Xinterp[1]/Xinterp[0], Yinterp, '-', color=c)
    ax2.plot(X[1]/X[0], dataT[i, :]-dataT[i,0]-y_fit, 'o--', color=c, markerfacecolor='None')
    ax1.set_ylabel('Δδ')
    ax2.set_ylabel('Residual')

    ax2.axhline(y=0,color='k')
    ax3.plot(Xinterp[1]/Xinterp[0], Hinterp/Xinterp[0], label=' [H] ')
    ax3.plot(Xinterp[1]/Xinterp[0], HGinterp/Xinterp[0], label=' [HG] ')
    ax3.plot(Xinterp[1]/Xinterp[0], H2Ginterp/Xinterp[0], label=' [H2G] ')
    ax3.legend(loc='best', frameon=True, framealpha=0.5)
    ax3.set_xlabel('Guest equiv ([G]0 / [H]0)')
    ax3.set_ylabel('Molfraction')

safeVal = lambda x: "ERROR" if result.params[x].value==None else result.params[x].value
safeErr = lambda x: "ERROR" if result.params[x].stderr==None else result.params[x].stderr

ax1.set_title("K1: {:.0f} +/- {:.0f}\n K2: {:.0f} +/- {:.0f}".format(safeVal("K1_1"),
                                                                    safeErr("K1_1"),
                                                                    safeVal("K2_1"),
                                                                    safeErr("K2_1")))

```

```

plt.tight_layout()
fig.align_labels()
plt.show()
return result, fig

```

#Global fit for 1:2 H:G complex data

```
def Global12(data,figsize=(8,6),alpha=None,fmt='o--'):
```

'''data: A pandas dataframe (with header) containining host concentrations in the first column, guest concentrations in the second column, and chemical shifts in subsequent columns.

figsize: Optional. a tuple passed to matplotlib for plotting of association data.

alpha: Optional. Cooperativity used for fitting 1:2 model. Allowed to float if set to None (default). For a non-cooperative 1:2 fit, set alpha=1.

fmt: Formatting string used for residuals of plot.'''

```
def G12(G0,H0,K1,K2):
```

```
    G0,H0,K1,K2 = np.asarray(G0), np.asarray(H0), np.asarray(K1), np.asarray(K2)
```

```
    try:
```

```
        length = len(G0)
```

```
        coeffs = np.zeros((length,4))
```

```
        for i in range(length):
```

```
            coeffs[i] = [K1*K2, K1*(2*K2*H0[i] - K2*G0[i] + 1), K1*(H0[i]-G0[i])+1, -G0[i]]
```

```
        roots = np.zeros((len(G0),3))
```

```
        R = np.zeros(len(G0))
```

```
        for i in range(len(G0)):
```

```
            roots[i] = np.roots(coeffs[i])
```

```
            R[i] = min(roots[i][roots[i] >= 0])
```

```
        return R
```

```
    except TypeError:
```

```
        coeffs = [K1*K2, K1*(2*K2*H0 - K2*G0 + 1), K1*(H0-G0)+1, -G0]
```

```
        R = np.roots(coeffs)
```

```
    return min(R[R >= 0])
```

```
def nmr12(X,K1,K2,delHG,delHG2):
```

```
    H0, G0 = X
```

```
    G = G12(G0,H0,K1,K2)
```

```
    delDel = (delHG*K1*G + delHG2*K1*K2*G**2)/(1+K1*G+K1*K2*G**2)
```

```
    return delDel
```

```
def nmr12_dataset(X,params,i):
```

```
    K1 = params['K1_%i' % (i+1)].value
```

```
    K2 = K1*params['alpha_%i' % (i+1)].value/4
```

```
    delHG = params['delDHG1_%i' % (i+1)].value
```

```
    delHG2 = params['delDHG2_%i' % (i+1)].value
```

```

return nmr12(X,K1,K2,delHG,delHG2)

def objective(params, X, data):
    """ calculate total residual for fits to several data sets"""
    dataT = np.array(data.T[2:])
    ndata, nx = dataT.shape
    resid = 0.0*dataT[:]
    # make residual per data set
    for i in range(ndata):
        dataT[i] = dataT[i] - dataT[i][0]
        resid[i,:] = dataT[i, :] - nmr12_dataset(X,params,i)
    # now flatten this to a 1D array, as minimize() needs
    return resid.flatten()

X = data.iloc[:,0],data.iloc[:,1]

dataT = np.array(data.T[2:])
fit_params = Parameters()
for iy, y in enumerate(dataT):
    fit_params.add('K1_%i' % (iy+1), value=1000, min=0.0, max=1e5)
    if alpha == None:
        fit_params.add('alpha_%i' % (iy+1), value=1, min=0.0, max=10)
    else:
        fit_params.add('alpha_%i' % (iy+1), value=alpha,vary=False)

    fit_params.add('delDHG1_%i' % (iy+1), value=1.0, min=-4.0, max=4.0)
    fit_params.add('delDHG2_%i' % (iy+1), value=-1.0, min=-4.0, max=4.0)

for iy in range(2,len(dataT)+1):
    fit_params['K1_%i' % iy].expr='K1_1'
    fit_params['alpha_%i' % iy].expr='alpha_1'

result = minimize(objective, fit_params, args=(X, data),nan_policy='omit')
#report_fit(result.params)

palette = itertools.cycle(sns.color_palette())
fig, (ax1, ax2, ax3) = plt.subplots(3,1,sharex=True,figsize=figsize)
plt.rcParams["lines.markeredgewidth"] = 1.5
interp = np.linspace(min(X[1]),max(X[1]),1000)
Xinterp = (interp*0.0+X[0][0]), interp

K1 = result.params['K1_1'].value
K2 = K1*result.params['alpha_1'].value/4

Ginterp = G12(Xinterp[1],Xinterp[0],K1,K2)
Hinterp = Xinterp[0]/(1+K1*Ginterp + K1*K2 * Ginterp * Ginterp)

```

```

HGinterp = K1*Ginterp * Hinterp
HG2interp = K2 * HGinterp * Ginterp

for i in range(len(dataT)):
    c = next(palette)
    y_fit = nmr12_dataset(X, result.params, i)
    Yinterp = nmr12_dataset(Xinterp, result.params, i)
    ax1.plot(X[1]/X[0], dataT[i, :]-dataT[i,0], 'o', color=c, markerfacecolor='None')
    ax1.plot(Xinterp[1]/Xinterp[0], Yinterp, '-', color=c)
    ax2.plot(X[1]/X[0], dataT[i, :]-dataT[i,0]-y_fit, fmt, color=c, markerfacecolor='None')
    ax1.set_ylabel('Δδ')
    ax2.set_ylabel('Residual')

    ax2.axhline(y=0, color='k')
    ax3.plot(Xinterp[1]/Xinterp[0], Hinterp/Xinterp[0], label='[H]')
    ax3.plot(Xinterp[1]/Xinterp[0], HGinterp/Xinterp[0], label='[HG]')
    ax3.plot(Xinterp[1]/Xinterp[0], HG2interp/Xinterp[0], label='[HG2]')
    ax3.legend(loc='best', frameon=True, framealpha=0.5)
    ax3.set_xlabel('Guest equiv ([G]0 / [H]0)')
    ax3.set_ylabel('Molfraction')
    safeVal = lambda x: "ERROR" if result.params[x].value==None else result.params[x].value
    safeErr = lambda x: "ERROR" if result.params[x].stderr==None else result.params[x].stderr
    ax1.set_title("K1: {:.0f} +/- {:.0f}\n K2: {:.0f}, alpha: {:.2f} +/-
{:.2f}".format(safeVal("K1_1"), safeErr("K1_1"),

safeVal("K1_1")*safeVal("alpha_1")/4,

safeVal("alpha_1"), safeErr("alpha_1")))

    plt.tight_layout()
    fig.align_labels()
    plt.show()
    return result, fig

#GLOBAL ANALYSIS 1:1 complex
def Global11(data, figsize=(8,6), plotaxis='eq', fmt='o--'):
    import seaborn as sns
    def HG11(G0,H0,K):
        return 0.5*(G0 + H0 + 1/K - np.sqrt((G0-H0-1/K)**2 + 4*G0/K))
    def nmr11(X,K,deldHG):
        H0, G0 = X
        HG = HG11(G0,H0,K)
        out = deldHG*HG/H0
        return out
    def nmr11_dataset(X,params,i):
        K = params['K_%i' % (i+1)].value
        deldHG = params['deldHG_%i' % (i+1)].value

```

```

return nmr11(X,K,deldHG)

def objective(params, X, data):
    """Calculate total residual for fits to several data sets"""
    dataT = np.array(data.T[2:])
    ndata, nx = dataT.shape
    resid = 0.0*dataT[:]
    # make residual per data set
    for i in range(ndata):
        dataT[i] = dataT[i] - dataT[i][0]
        resid[i, :] = dataT[i, :] - nmr11_dataset(X,params,i)
    # now flatten this to a 1D array, as minimize() needs
    return resid.flatten()

X = data.iloc[:,0],data.iloc[:,1]

dataT = np.array(data.T[2:])
fit_params = Parameters()
for iy, y in enumerate(dataT):
    fit_params.add('K_%i' % (iy+1), value=200, min=0.0, max=1e6)
    fit_params.add('deldHG_%i' % (iy+1), value=0.4, min=-4.0, max=4.0)
for iy in range(2,len(dataT)+1):
    fit_params['K_%i' % iy].expr='K_1'

result = minimize(objective, fit_params, args=(X, data),nan_policy='omit')

import matplotlib.cm as cm
import seaborn as sns
palette = itertools.cycle(sns.color_palette())
plt.rcParams["lines.markeredgewidth"] = 1.5
fig, (ax1, ax2, ax3) = plt.subplots(3,1,sharex=True,figsize=figsize)

interp = np.linspace(min(X[1]),max(X[1]),1000)
Xinterp = (interp*0.0+X[0][0]), interp

HGinterp = HG11(Xinterp[1],Xinterp[0],result.params['K_1'].value)
Hinterp = Xinterp[0] - HGinterp

for i in range(len(dataT)):
    c = next(palette)
    y_fit = nmr11_dataset(X, result.params, i)
    Yinterp = nmr11_dataset(Xinterp, result.params, i)
    if plotaxis == 'eq':
        ax1.plot(X[1]/X[0], dataT[i, :]-dataT[i,0], 'o', color=c,markerfacecolor='None')

```

```

ax1.plot(Xinterp[1]/Xinterp[0], Yinterp, '-', color=c)
ax2.plot(X[1]/X[0], dataT[i, :]-dataT[i, 0]-y_fit, fmt, color=c, markerfacecolor='None')
elif plotaxis == 'abs':
    ax1.plot(X[1], dataT[i, :]-dataT[i, 0], 'o', color=c, markerfacecolor='None')
    ax1.plot(Xinterp[1], Yinterp, '-', color=c)
    ax2.plot(X[1], dataT[i, :]-dataT[i, 0]-y_fit, fmt, color=c, markerfacecolor='None')
if plotaxis == 'eq':
    ax3.plot(Xinterp[1]/Xinterp[0], Hinterp/Xinterp[0], label = '[H]')
    ax3.plot(Xinterp[1]/Xinterp[0], HGinterp/Xinterp[0], label = '[HG]')
    ax3.set_xlabel('Guest equiv ([G]0 / [H]0)')
elif plotaxis == 'abs':
    ax3.plot(Xinterp[1], Hinterp/Xinterp[0], label = '[H]')
    ax3.plot(Xinterp[1], HGinterp/Xinterp[0], label = '[HG]')
    ax3.set_xlabel('Guest concentration / M')
ax3.legend(loc='best', frameon=True, framealpha=0.5)
ax1.set_ylabel('Δδ')
ax2.set_ylabel('Residual')

ax2.axhline(y=0, color='k')
ax3.set_ylabel('Molfraction')
safeVal = lambda x: "ERROR" if result.params[x].value==None else result.params[x].value
safeErr = lambda x: "ERROR" if result.params[x].stderr==None else result.params[x].stderr
ax1.set_title("Ka: {} +/- {}".format(safeVal('K_1'), safeErr('K_1')))
plt.tight_layout()
fig.align_labels()
plt.show()
return result, fig

```

S12.2 Processing and analysis of diffusion data

Script S 2. Functions used for analysis and processing of diffusion NMR data. MestReNova was used to obtain integrals of peaks of interest, and

```

'''Module for global fitting of (time-dependent) diffusion NMR data.
This module provides the following functions:
GlobalDiff(data)
    Global nonlinear fitting of the S-T equation for an arbitrary number of gradients and chemical shift
    environments.
    Returns an lmfit parameters object containing fitted I0 intensities for all peaks, as well as a single
    globally fitted D value.
MovingDiff(data, slicelength=10)
    Global moving average fit for diffusion, using GlobalDiff to obtain a D value for each time point.
S
    Takes as input a pandas dataframe with the first column containing B values, and subsequent columns
    containing integrals for the peaks of interest.
    Slicelength sets the number of experiments used for each D(time) point. Slicetime sets the time (in

```


minutes) taken to acquire each gradient slice.

Returns (Dpoints, I0points,Derr,Ierr): four pandas dataframes, each with indices corresponding to time. I0points and Ierr contain a column for each fitted NMR peak, while Dpoint and Der contain a single column with globally fitted diffusion data.

For systems involving multiple chemical species, use SeparateMovingDiffusion to obtain individual (non-globally fitted) diffusion coefficients.

Dpoints, I0points, Derr, and Ierr contain diffusion points, extrapolated intensities, and respective errors for each from fitting.

MovingDiff_csv(fname,slicelength=10,slicetime=2/3)

A wrapper for MogvingDiff to act on a similarly formatted .csv file.

SeparateMovingDiffusion(data,slicelength=10,slicetime=2/3)

Returns (Dpoints, I0points,Derr,Ierr): four pandas dataframes, each with indexes corresponding to time and a column for each peak.

Dpoints, I0points, Derr, and Ierr contain diffusion points, extrapolated intensities, and respective errors for each from fitting.

SeparateMovingDiffusion_csv(fname,slicelength=10,slicetime=2/3)

Generates a pair of pandas dataframes [D,I] containing calculation time-dependent diffusion coefficients and unattenuated integrals.

Acts on a .csv file with the first column containing B-values, and each subsequent column containing the corresponding integrals for a particular chemical shift.

MeOHTemp(dDelta)

Calculates temperature from methanol OH-CH₃ chemical shift separation (in ppm)

MeOHDiff(dDelta)

Calculates expected diffusion coefficient from methanol OH-CH₃ chemical shift separation (in ppm)

'''

def GlobalDiff(data):

'''Function to globally fit a single diffusion coefficient to data from a list of peaks

Input: a pandas dataframe consisting of:

B_0 I0_0 I1_0 ... In_0

B_1 I0_1 I1_1 ... In_1

...

B_m I0_m I1_m ... In_m

where B is the list of B-parameters for all experiments, and each column In_ contains the integrals measured for a single peak.

The function returns a single lmfit Parameters object.

'''

import numpy **as** np

import pandas **as** pd

from lmfit **import** minimize, Parameters, report_fit

def STExp(B,I0,D):

I0,B,D = np.asarray(I0), np.asarray(B), np.asarray(D)

return I0*np.exp(-B*D)

def STExp_dataset(B,params,i):

```

I0 = params['I0_%i' % (i+1)].value
D = params['D_%i' % (i+1)].value
return STExp(B,I0,D)

def objective(params,B,data):
    dataT = np.array(data.T[1:])
    ndata, nx = dataT.shape
    resid = 0.0*dataT[:]
    #Residual per data set:
    for i in range(ndata):
        resid[i,:] = dataT[i,:] - STExp_dataset(B,params,i)
    #Flatten to a 1d array:
    return resid.flatten()

B = data.iloc[:,0]
dataT = np.array(data.T[1:])
fit_params = Parameters()
I0guesses = data.max()[1:]
for iy, y in enumerate(dataT):
    fit_params.add('D_%i' % (iy+1), value = 1e-9, min = 1e-12, max = 1e-8)
    fit_params.add('I0_%i' % (iy+1), value = I0guesses[iy], min = 1, max = 100*I0guesses[iy]) #Give
each I0 parameter a unique guessed I0
for iy in range(2,len(dataT)+1):
    fit_params['D_%i' % iy].expr='D_1'
return minimize(objective,fit_params,args=(B,data))

def MovingDiff(data,slicelength=10,slicetime=2/3):
    '''Fitting for time-dependent diffusion + concentration data.
    Input: pandas dataframe formatted as
    B_0 I0_0 I1_0 ... In_0
    B_1 I0_1 I1_1 ... In_1
    ...
    B_m I0_m I1_m ... In_m

    '''
    import numpy as np
    import pandas as pd
    from tqdm import tqdm_notebook as tqdm
    from lmfit import minimize, Parameters, report_fit

    npoints = data.shape[0]-slicelength
    ipoints = np.arange(0,npoints)
    tpoints = ipoints*slicetime+slicetime*slicelength/2

    cols = data.columns[1:]
    D = pd.DataFrame(index = tpoints,columns = [cols[0]])
    Derr = pd.DataFrame(index = tpoints,columns = [cols[0]])
    I0 = pd.DataFrame(index = tpoints,columns = cols)

```

```

I0err = pd.DataFrame(index = tpoints,columns = cols)

for i in tqdm(range(npoints),desc='Progress:',position=1,leave=False):
    params = GlobalDiff(data.iloc[i:i+slicelength])
    I0slice,I0errslice = [],[]
    for Ival in range(0,len(cols)):
        ParamName = 'I0_{}'.format(Ival+1)
        #I0.insert(params.params[ParamName].value,index=i,col)
        I0slice.append(params.params[ParamName].value)
        I0errslice.append(params.params[ParamName].stderr)
    D.loc[tpoints[i],cols[0]] = params.params['D_1'].value
    Derr.loc[tpoints[i],cols[0]] = params.params['D_1'].stderr
    I0.loc[tpoints[i],cols] = I0slice
    I0err.loc[tpoints[i],cols] = I0errslice
return D,I0,Derr,I0err

def MovingDiff_csv(fname,slicelength=10,slicetime=2/3):
    '''A simple wrapper of MovingDiff() to act on .csv files'''
    import pandas as pd
    return MovingDiff(pd.read_csv(fname),slicelength,slicetime)

def SeparateMovingDiffusion(data,slicelength=10,slicetime=2/3):
    '''Moving average diffusion processing for multiple separate chemical species.
    Acts on a pandas dataframe containing a list of B-values in teh first column, and corresponding peak
    integrals in subsequent columns.
    Returns a pair of pandas dataframes [D,I] containing the calculated diffusion coefficients
    and concentrations for each peak present in the input array. '''
    import pandas as pd
    import numpy as np
    from tqdm import tqdm_notebook as tqdm
    npoints = data.shape[0]-slicelength
    ipoints = np.arange(0,npoints)
    tpoints = ipoints*slicetime+slicetime*slicelength/2
    D = pd.DataFrame(index = tpoints,columns=data.columns[1:])
    I = pd.DataFrame(index = tpoints,columns=data.columns[1:])
    Derr = pd.DataFrame(index = tpoints,columns=data.columns[1:])
    Ierr = pd.DataFrame(index = tpoints,columns=data.columns[1:])

    for peak in tqdm(data.columns[1:],desc='Peak-by-peak progress',position=2):
        td,ti,tderr,tierr =
MovingDiff(pd.concat([data.iloc[:,0],data[peak]],axis=1),slicelength=slicelength,slicetime=slicetime
)

        D[peak] = td
        I[peak] = ti
        Derr[peak] = tderr

```

```

        Ierr[peak] = tierr
    return D, I, Derr, Ierr
def SeparateMovingDiffusion_csv(fname,slicelength=10,slicetime=2/3):
    '''A wrapper of SeparateMovingDiffusion to act on .csv files'''
    import pandas as pd
    return SeparateMovingDiffusion(pd.read_csv(fname),slicelength=slicelength,slicetime=slicetime)

def MeOHTemp(dDelta):
    '''Converts a methanol CH3-OH chemical shift separation (in ppm) to a temperature (in K).
    See J. Magn. Reson. 1982, 46, 319-321'''
    return 409-36.54*dDelta-21.85*dDelta**2
def MeOHDiff(dDelta):
    '''Calculates the expected self-diffusion coefficient of methanol for a given OH-CH3 peak chemical
    shift separation.
    See MacDonald et al, ChemPhysChem 2019, 20, 926-930'''
    import numpy as np
    return 5.124e-7 * np.exp((-1601)/(MeOHTemp(dDelta)))

def MeanGlobularDiffusionD0(A0,K,D0,rho=1,dps=25):
    '''
    Function for modelling the concentration-dependent diffusion coefficient of a supramolecular
    oligomer.
    A0 = species concentration in M (scalar or array)
    K = association constant. If alpha = 1 (isodesmic), K is the association constant for each step of
    oligomerisation.
    D0 = diffusion coefficient of monomer
    rho = cooperativity of system.
        rho = 1: isodesmic model, with each oligomer associating with the same constant.
        rho > 1: dimer (n=2 oligomer) forms more easily (eg electrostatic repulsion), with K(dimer) =
    rho*K
    '''
    #from mpmath import mp, polylog,sqrt,nsum
    from mpmath import fp
    import numpy as np
    #mp.dps = dps #Set mpmath precision. dps = 25 seems to work well.
    import scipy.constants

    #a0k = np.array(A0)*np.array(K)

    try: #First, try to calculate an array of [A] free-monomer concentrations. This should work if A0
    was given as an array
        length = len(A0)
        coeffs = np.zeros((length,4))
        for i in range(length):
            coeffs[i] = [1-rho,
                        2*rho-2-K*A0[i],

```

```

                2*K*A0[i]+1,
                -K*A0[i]]

    roots = np.zeros((length,3))
    R = np.zeros(length)
    for i in range(length):
        roots = np.roots(coeffs[i])
        R[i] = min(roots[roots >= 0])
    A = R/K

except TypeError: #If A0 was given as a single float, not as an array, calculate a single [A]
    coeffs = [1-rho,
              2*rho-2-K*A0,
              2*K*A0+1,
              -K*A0]

    R = np.roots(coeffs)
    A = min(R[R >= 0])/K

try:
    if A == 0:
        sumval = D0 #If there is no monomer present (either A0 = 0 or K = 0, D = D0
    else:
        sumval = D0/(K*A0)*float(A*K + rho*(fp.polylog(-2/3, (K*A))-A*K))
except:
    j = 0
    sumval = []
    while j<len(A):
        if A[j] == 0:
            sumval.append(D0)
        else:
            sumval.append(float(D0/(A0[j]*K)*(A[j]*K + rho*(fp.polylog(-2/3, K*A[j])-A[j]*K))))
        j+=1
    return sumval

```

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Controlled diffusion of photoswitchable receptors by binding antielectrostatic phosphate oligomers

Thomas S. C. MacDonald,^[a] Ben L. Feringa,^[b] William S. Price,^[c] Sander J. Wezenberg,^{*,[b,d]} and Jonathon E. Beves^{*,[a]}

Abstract: Dihydrogen phosphate anions are found to spontaneously associate into anti-electrostatic oligomers *via* hydrogen bonding interactions at millimolar concentrations in DMSO. Diffusion NMR measurements supported formation of these oligomers, which can be bound by photoswitchable anion receptors to form large bridged assemblies of approximately three times the volume of the unbound receptor. Photoisomerization of the oligomer-bound receptor causes a decrease in diffusion coefficient of up to 16%, corresponding to a 70% increase in effective volume. This new approach to external control of diffusion opens prospects in controlling molecular transport.

Active control over molecular transport by synthetic systems is a topic of major contemporary interest.^[1] Recent progress has shown that motors,^[2] enzymes, or other energy consuming nanostructures can effectively drive molecular transport in solution.^[2b, 3] Despite these advances, controlling transport of molecules in solution remains a challenging goal. One way to control transport is by modulating the diffusion rate of species in solution. Various theoretical proposals and experimental data have shown that increasing or decreasing the rate of diffusion can lead to directional transport when coupled with, for example, concentration gradients.^[4] Diffusion rates have been influenced using molecular photoswitches^[5] by altering self-assembled discrete structures^[5c, 5e, 6] or polymers.^[5a, 5e, 6-7] While diffusion NMR^[8] measurements are a known approach for characterizing supramolecular assembly,^[9] the use of switchable assembly to control diffusive transport is relatively unexplored.

In contrast to the chemical intuition that like charges repel, some anions are known to associate through hydrogen-bonds and form polyanionic species.^[10] This antielectrostatic hydrogen bonding (AEHB) is common in the solid state for oxoanions such as HCO_3^- , HSO_4^- , and H_2PO_4^- (Figure 1). While AEHB interactions have been identified in solid-state crystal structures, detection of unchaperoned anion dimers or oligomers in solution is challenging due to limited spectroscopic signatures, weak anion-anion bonds, labile protons and rapidly exchanging bound species. These difficulties can be attenuated by the use of an anion-binding host to template AEHB interactions,^[11] leading to

reports of HSO_4^- dimers^[12] and H_2PO_4^- dimers^[13] and oligomers^[14] in solution. Conductimetric and spectroscopic techniques have been used to establish the formation of AEHB HCO_3^- and H_2PO_4^- dimers in water^[15] as well as in DMSO^[16] and suggested the possibility of higher-order oligomers.^[15c] However, to the best of our knowledge, the unassisted formation of AEHB oxoanion oligomers in solution has yet to be conclusively established.

Figure 1. Antielectrostatic hydrogen-bonding of anions. Anions with a single hydrogen bond donor/acceptor pair (e.g. HSO_4^-) may form dimers^[12a] while anions with multiple donor/acceptors (e.g. H_2PO_4^-) can form oligomers in the solid state.^[14b]

Many small molecular receptors have been developed to selectively bind anions.^[17] Such binding may result in changes in the rate of diffusion of the receptor. If the binding properties could be modified by external stimuli, for example light,^[18] this could represent a means to control the rate of diffusion. Recently, some of us developed the first photoswitchable receptors exhibiting strong dihydrogen phosphate binding.^[19] These receptors were based on molecular motor and stiff-stilbene scaffolds^[20] containing urea anion binding motifs. These hosts could be converted from a weakly guest-binding *E* to a strongly binding *Z* form using near-UV light (Figure 2). We anticipated that the use of this type of photoswitchable anion receptors with AEHB dihydrogen phosphate oligomers would allow changes in diffusion to be controlled by light.

Figure 2. Bis-urea anion binding photoswitch 1. Isomers *E*-1 and *Z*-1 exhibit different binding properties: the binding of *Z*-1 to anions is approximately an order of magnitude stronger than that of *E*-1. The photoisomers can be interconverted by irradiation with near-UV light.

Herein we report quantitative self-association data for the antielectrostatic oligomerization of dihydrogen phosphate in DMSO at millimolar concentrations, and the use of a

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photoswitchable anion receptor to allow reversible binding to control rates of diffusive transport.

Our initial studies of tetrabutylammonium dihydrogen phosphate ($[\text{NBu}_4][\text{H}_2\text{PO}_4]$) solutions in $\text{DMSO}-d_6$ with 0.5% added water^[21] revealed a surprising decrease in the diffusion coefficient of the H_2PO_4^- anion $D(\text{H}_2\text{PO}_4^-)$ at higher concentrations, while the diffusion coefficient of the NBu_4^+ counterion $D(\text{NBu}_4^+)$ remained relatively constant (Figure 3; see SI-S6.1). This behavior cannot be explained by changes in ion pairing or viscosity which must affect the diffusion coefficients of both species equally.

Control experiments conducted with tetrabutylammonium acetate did not show comparable continuing decreases in diffusion coefficients of either NBu_4^+ or acetate over the same concentration range as used for $[\text{NBu}_4][\text{H}_2\text{PO}_4]$ (SI-S6.1), indicating that the formation of oligomers was unique to H_2PO_4^- .

Diffusion coefficients vary approximately proportionally to the inverse cube root of molecular volume, $V^{-1/3}$.^[22] The 50% decrease in $D(\text{H}_2\text{PO}_4^-)$ as the H_2PO_4^- concentration is increased from 2 to 300 mM suggests an *eight-fold increase* in effective volume over this concentration range. We propose this decrease in $D(\text{H}_2\text{PO}_4^-)$ is a result of the formation of AEHB oligomers of H_2PO_4^- in solution.

Figure 3. Diffusion coefficients of tetrabutylammonium dihydrogen phosphate ($[\text{NBu}_4][\text{H}_2\text{PO}_4]$) measured by ^1H (500 MHz) or ^{31}P (202 MHz) NMR over the 2–300 mM concentration range and corrected for changes in viscosity using independent viscosity measurements (see SI-S4). An isodesmic oligomerisation model (red line) was fitted to the measured diffusion coefficients of dihydrogen phosphate giving parameters $K_i = 120 \pm 32 \text{ M}^{-1}$ and $D_0 = 3.39 \pm 0.11 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$. All measurements in $\text{DMSO}-d_6$ with 0.5% added water. Data was processed using scripts given in SI-12.

A simple model for supramolecular oligomerization is the isodesmic model, in which the addition of each monomer to an oligomer occurs with the same association constant K_i (SI-S5.1).^[23] Combining this model with an inverse-cube relationship between D and oligomer size (see SI-S5.2 for derivation and details) gives a model for the concentration dependence of the measured D of a molecular species undergoing reversible oligomerization:

$$\bar{D} = \frac{D_0}{K_i[A]_0} \text{Li}_{-2}(K_i[A]) \quad (1)$$

where $\text{Li}_s(z)$ is the polylogarithm function,^[24] D_0 is the diffusion coefficient of the monomer, K_i is the isodesmic association constant, $[A]_0$ is the total concentration, and $[A]$ is the concentration of the free (monomeric) species which can be obtained from K_i and $[A]_0$. Nonlinear regression of Eq (1) onto the measured diffusion coefficients of H_2PO_4^- gave $K_i = 120 \pm 32 \text{ M}^{-1}$, surprisingly close to the reported dimerization constant of H_2PO_4^- in DMSO (180 M^{-1})^[16]. Our measured K_i corresponds to median complexes comprised of 4 or 10 H_2PO_4^- subunits at 50 or 300 mM concentrations, respectively (Figure 4a, Figure S11), and as far as we know this surprisingly strong process in a polar solvent is the first measurement of indefinite AEHB self-association.

To modify the diffusion rate of a switchable receptor, we turned to the previously developed stiff stilbene^[5c, 5e] bis-urea.^[19c, 19e] The bis-tolyl derivative **1** (Figure 2) was synthesized to take advantage of the convenient methyl ^1H NMR signal for diffusion NMR experiments (see SI-3). The association constants for H_2PO_4^- and OAc^- were measured by NMR titrations and fitted to 1:2 $[\text{HG}_2]$ binding models, with results^[25] comparable to those previously reported for the non-substituted phenyl derivative^[19c] when studied over the same guest concentration range. Binding studies at higher concentrations of H_2PO_4^- , however, resulted in slightly different association constants when fitted to the same binding model (SI-S7.8–7.10) illustrating that competition due to self-association of H_2PO_4^- complicates binding models for association constants.^[26] For example, a recent report of H_2PO_4^- binding^[27] found different binding constants and stoichiometries when measured at different concentrations by UV-vis absorption or NMR spectroscopy, likely due to H_2PO_4^- aggregation.

The photoswitching properties of **1** were studied in $\text{DMSO}-d_6$ with 0.5% added water^[19c] and absorption spectra (Figure S45) are in line with the parent compound.^[19c] The thermal half-life is sufficiently long that no thermal isomerization was observable over the timescales used in the experiments reported here. In the presence of 50 mM $[\text{NBu}_4][\text{H}_2\text{PO}_4]$ the photostationary state under non-optimal irradiation with a 405 nm LED comprised a *E/Z* ratio of 58:42 measured by NMR integration.

The measured diffusion coefficients of *E*-**1** and *Z*-**1** ($D(\text{E-1})$ and $D(\text{Z-1})$) in $\text{DMSO}-d_6$ with 0.5% added water are shown in Table 1. In the absence of H_2PO_4^- , the extended *E*-**1** isomer diffuses slightly more slowly (7%) than the more compact *Z*-**1** (Table 1; entry 1 vs 2). Given the large difference in size between host **1** (MW = 529) and the H_2PO_4^- anion (MW = 97),^[28] we might have anticipated a modest decrease in average host diffusion coefficients at guest concentrations where near-complete complexation occurs based on measured binding constants.^[29] Instead we observe a large decrease in measured D that continues to decrease at concentrations above those predicted for near-complete complexation (e.g. $[\text{H}_2\text{PO}_4^-] = 50 \text{ mM}$, $[\mathbf{1}] = 5 \text{ mM}$: Table 1, entries 4-8; SI-8). After correcting for H_2PO_4^- -induced viscosity changes (see SI-4),^[30] the measured D of pure *E*-**1** or *Z*-**1** in the presence of 50 mM $[\text{NBu}_4][\text{H}_2\text{PO}_4]$ was found to decrease by, respectively, 33% ($D(\text{E-1})$) and 26% ($D(\text{Z-1})$) relative to D without $[\text{NBu}_4][\text{H}_2\text{PO}_4]$ (Table 1; entries 1 vs 4; 2 vs 5; Figure 5 for full $[\text{H}_2\text{PO}_4^-]$ -dependent diffusion data). This suggests greater than 2 or 3-fold increases in effective volumes of the *Z*-**1** or *E*-**1** hosts, respectively.

Figure 4. Proposed supramolecular assemblies present in solution. (a) Anti-electrostatic dihydrogenphosphate (H_2PO_4^-) oligomers, G_n , form from monomeric phosphates. These oligomers are bound by anion-binding **Z-1** (b) and **E-1** (c) bis-urea hosts to form ditopic $[\text{H}(\text{G}_n)]$ or $[\text{H}(\text{G}_n)_2]$ complexes. d) As **E-1** possesses divergent urea binding sites, larger supramolecular structures can form from H_2PO_4^- chains linked by **E-1** hosts.

Table 1. Changes in diffusion coefficients of pure isomers and 1:1 mixed solutions of **E-1** and **Z-1** in the presence of 50 mM $\text{NBu}_4\text{-H}_2\text{PO}_4$.^[a]

Entry	$[\text{H}_2\text{PO}_4^-]$ / mM	$[\text{E-1}]$ / mM	$[\text{Z-1}]$ / mM	$D(\text{H}_2\text{PO}_4^-)$ ^[b] / $10^{-10} \text{ m}^2 \text{ s}^{-1}$	$D(\text{E-1})$ ^[c] / $10^{-10} \text{ m}^2 \text{ s}^{-1}$	$D(\text{Z-1})$ ^[c] / $10^{-10} \text{ m}^2 \text{ s}^{-1}$	$D(\text{NBu}_4)$ ^[c] / $10^{-10} \text{ m}^2 \text{ s}^{-1}$
1	-	5	-	-	1.74 ± 0.03	-	-
2	-	-	5	-	-	1.87 ± 0.01	-
3	50	-	-	2.16 ± 0.03	-	-	2.50 ± 0.02
4	50	5	-	1.93 ± 0.04	1.17 ± 0.03	-	2.39 ± 0.01
5	50	-	5	2.01 ± 0.03	-	1.39 ± 0.01	2.37 ± 0.02
6	50	5	5	1.83 ± 0.08	1.12 ± 0.02	1.36 ± 0.01	2.31 ± 0.01
7	50	2.5	2.5	1.97 ± 0.07	1.19 ± 0.01	1.45 ± 0.03	2.44 ± 0.01
8	50	0.5	0.5	2.05 ± 0.02	1.27 ± 0.03	1.57 ± 0.03	2.52 ± 0.02

[a] $\text{DMSO}-d_6$ with 0.5% added water. [b] 202 MHz ^{31}P PGSTE, $\delta = 7$ ms, $\Delta = 100$ ms, $g = 0 - 53.45 \text{ G cm}^{-1}$. [c] 500 MHz ^1H PGSTE, $\delta = 4$ ms, $\Delta = 50$ ms, $g = 0 - 53.45 \text{ G cm}^{-1}$.

Figure 5. Diffusion measurements of hosts **Z-1** and **E-1** under titration with $[\text{NBu}_4][\text{H}_2\text{PO}_4]$. Measured D for 5 mM solutions of pure **Z-1** or **E-1** ($\circ$), compared with mixed 2.5 mM: 2.5 mM **E-1**:**Z-1** (+).

These substantial decreases in measured D are too large to be explained by the formation of simple ditopic $[\text{HG}_2]$ complexes^[19c] or even $[\text{H}(\text{G}_n)_2]$ complexes, where G_n is an

oligomeric chain of n hydrogen-bonded H_2PO_4^- subunits that forms as in the absence of host^[31] (Figure 4b,c see Figure S11 for modelling).^[19c]

Host **E-1** forms larger structures than host **Z-1** (note that despite **E-1** having a lower measured K_1 for H_2PO_4^- than **Z-1**, there is no free host left in both cases at 50 mM, see Supporting Information S9). This observation is also supported by changes in the measured diffusion of H_2PO_4^- , $D(\text{H}_2\text{PO}_4^-)$, (measured by ^{31}P NMR), which decreases by 11% or 7% in the presence of 5 mM of **E-1** or **Z-1**, respectively (Table 1; entries 3 vs 4, 5). This indicates that H_2PO_4^- is also assembled into larger average structures in the presence of **E-1** than by **Z-1**.

Compared to 5 mM solutions of a single-isomer, $D(\text{E-1})$ and $D(\text{Z-1})$ decrease by just 4% and 2%, respectively, when an additional 5 mM of the other host is present (Table 1; entries 4 vs 6; 5 vs 6), suggesting minimal interactions between the **E**- and **Z**-isomers.

Relative to that of a solution of pure $[\text{NBu}_4][\text{H}_2\text{PO}_4]$, $D(\text{H}_2\text{PO}_4^-)$ decreases on going from 5 mM of **Z-1** (-7%, Table 1, entry 5,) to 5 mM of **E-1** (-11%, entry 4) to 5 mM of each of **Z-1** and 5 mM of **E-1** (-15%, entry 6). $D(\text{H}_2\text{PO}_4^-)$ for a solution of 2.5 mM **E-1** and 2.5 mM **Z-1** (-9%, entry 7) is also the average of that 5 mM solutions of each isomer (entries 4, 5). This also suggests the H_2PO_4^- does not experience anything other than a statistical binding by the hosts, with no evidence of cross-linking between different host isomers.

However, there is evidence suggesting complexes are formed involving multiple host molecules of the same isomer. To test this, titration experiments with $[\text{NBu}_4][\text{H}_2\text{PO}_4]$ were conducted with solutions of 1:1 mixtures of *E*-1:Z-1 at 1, 5, and 10 mM total concentrations (SI-8.3). A small but observable decrease in D for both *E*-1 and Z-1 was found as the total concentration of the host increases from 1 to 5 to 10 mM (e.g. at 50 mM $[\text{NBu}_4][\text{H}_2\text{PO}_4]$: Table 1, entries 6-8; also SI-S8.3). This data supports the formation of structures involving multiple hosts, e.g. structures such as shown in Figure 4d.

Host 1 could also increase the effective size of polyanionic complexes by increasing ion-pairing to the NBu_4^+ cations. This would result in a decrease in $D(\text{NBu}_4)$ with increasing host concentration, but only a 5% decrease in $D(\text{NBu}_4)$ is observed (Table 1, entry 3 vs 4; 3 vs 5). There is also no difference between $D(\text{NBu}_4)$ in the presence of *E*-1 or Z-1, despite *E*-1 forming much larger complexes (Table 1; entries 4 vs 5). These observations suggest the hosts do not significantly change ion pairing between the NBu_4^+ and oligomers of H_2PO_4^- .

From measured diffusion coefficients of free host (5 mM) and oligomeric H_2PO_4^- (50 mM), we estimate the assemblies formed involve 1–2 molecules of 1 with 2–3 chains of oligomeric guest G_n , (where n is the same as that formed at 50 mM $[\text{NBu}_4][\text{H}_2\text{PO}_4]$ in the absence of host, see SI-S9 for discussion of methodology).^[32] From the modelled size distribution of H_2PO_4^- oligomers at 50 mM (Figure S11), this corresponds to complexes incorporating approximately 10 H_2PO_4^- subunits with average molecular weights of 1.5–2.0 kDa. Together, these results indicate that H_2PO_4^- anions not only form aggregates in polar and hydrogen-bond accepting solvents, but that these structures can associate to form larger assemblies with multiple hosts in solution.

As host 1 is a photoswitch, the *E*-1:Z-1 distribution of isomers can be controlled using light (SI-10). By combining *in situ* irradiation within the NMR spectrometer^[33] with recently developed time-resolved diffusion NMR techniques^[34] we simultaneously measured changes in concentration and diffusion coefficients of *E*-1 and Z-1 under 400 nm irradiation (Figure 6).

Photoswitching of organic molecules is expected to result in differences in D , but such changes would typically be minor (e.g. the 7% difference between $D(\text{E-1})$ and $D(\text{Z-1})$ in the absence of anion guest (Table 1, entries 1 and 2). Switchable anion binding might give more control over the effective host D , but complexes with small anions (e.g. Cl^- , OAc^- , NO_3^- , HSO_4^-) might only cause modest changes in host D , as found for control experiments with acetate (8% and 4% decrease, respectively, in $D(\text{E-1})$ and $D(\text{Z-1})$ at 50 mM $[\text{NBu}_4][\text{OAc}]$; SI-S8.2). The use of H_2PO_4^- oligomers allows greater control over D : switching host 1 from Z-1 to *E*-1 causes a “molecular gear change” and a 16% decrease in measured D (Table 1, entries 4 and 5), suggestive of an approximately 70% increase in average molecular volume. This demonstrates the possibility of achieving substantial control over the diffusion rate of small molecules in bulk solution by coupling photocontrol of guest-binding to the ability of H_2PO_4^- to form extended supramolecular structures.^[35] As 1 is a thermally stable (“P-type”) photoswitch, these changes in D will persist in the dark.^[5e]

Figure 6. Photoswitching of Z-1 host in the presence of 50 mM H_2PO_4^- under *in situ* irradiation with 400 nm light (shaded purple areas). Concentrations (top) and diffusion coefficients (bottom) were monitored simultaneously using time-resolved diffusion NMR.^[34a, 34b] Lines and shaded areas on diffusion subplot are respectively values and errors from the Stejskal-Tanner fit. Dashed horizontal lines show measured $D(\text{E-1})$ and $D(\text{Z-1})$ for a 5 mM 1:1 mixture of isomers (Table 1, entry 7). 500 MHz ^1H , $\delta = 4$ ms, $\Delta = 50$ ms, $g = 0 - 53.45$ G cm^{-1} , DMSO- d_6 with 0.5% added water and glass capillaries used to suppress convection.^[36] See Figure S44 for data with *in situ* temperature measurements, and Figure S46 for demonstration of reversibility of switching.^[34b, 37]

In conclusion, we sought to use the switchable anion-binding properties of host 1 to achieve photocontrol of translational diffusion rates. Diffusion NMR allowed characterization of the thermodynamics of the antielectrostatic self-assembly of the bare dihydrogen phosphate anion in solution, a long-suspected^[15a, 15b] but previously uncharacterized phenomenon. We obtained a surprisingly high isodesmic association constant of $K_i = 120 \pm 32 \text{ M}^{-1}$ for H_2PO_4^- self-association, which corresponds to complexes of a median size of 4 (or 10) H_2PO_4^- subunits at concentrations of 50 mM (or 300 mM). Binding studies with H_2PO_4^- in DMSO therefore involve competition between host- H_2PO_4^- and H_2PO_4^- - H_2PO_4^- interactions, which poses problems for fitting titration data (SI-7.11 for further discussion). Because of the limited solubility of our bis-urea receptor, this study was conducted exclusively in wet DMSO. It would be reasonable to assume that in less polar solvents such as DMF, acetonitrile, or dichloromethane self-association of H_2PO_4^- may be more significant.

Combining the unusual anti-electrostatic oligomerization of H_2PO_4^- with a photoswitchable anion-binding receptor allowed light to induce a “gear change” and sharply change the rate of receptor diffusion, equivalent to a 70 % change in effective volume. Can control of diffusion via a spatially selective stimulus (such as light, as demonstrated here) drive directional transport of small molecular species and create concentration gradients? This remains an interesting open question.

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Conflict of interest

The authors declare no conflict of interest.

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- [30] Viscosities of $[NBu_4][H_2PO_4]$ solutions in DMSO- d_6 were measured directly and fitted to a viscosity calibration curve, which was used to compensate for changes in measured D caused by viscosity. See Supporting Information S4 for details.
- [31] Equivalent experiments using $[NBu_4][OAc]$ in place of $[NBu_4][H_2PO_4]$ do not result in similar changes in the measured D of the host, suggesting that the ability of $H_2PO_4^-$ to form extended hydrogen-bound chains is critical for the observed changes in measured D (see Supporting Information SI-8.2).
- [32] This analysis does not rely on the accuracy of the isodesmic binding model and fitted parameters: the proposed $[H_{1-2}(G_n)_{2-3}]$ average structure only requires the experimentally measured effective $D(H_2PO_4^-)$. See Supporting Information S9 for details.
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solution (Table 1; entries 4, 5, 7). The increase is small (2% for $D(E-1)$, 4% for $D(Z-1)$), but a similar trend appears during time-resolved diffusion monitoring of photoswitching hosts (Fig. 6), where switching $Z-1$ into $E-1$ also causes a slight increase in $D(Z-1)$ and decrease in $D(E-1)$.

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Dihydrogen phosphate is complex!

Self-assembled antielectrostatic hydrogen-bonded oligomers of dihydrogen phosphate control the diffusion properties of a photoswitchable anion-binding host in dilute polar solvent.

Thomas S. C. MacDonald, Ben L. Feringa, William S. Price, Sander J. Wezenberg, and Jonathon E. Beves*

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