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A universal measure of chaotropicity and kosmotropicity

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Summary

Diverse parameters, including chaotropicity, can limit the function of cellular systems and thereby determine the extent of Earth's biosphere. Whereas parameters such as temperature, hydrophobicity, pressure, pH, Hofmeister effects, and water activity can be quantified via standard scales of measurement, the chao-/kosmotropic activities of environmentally ubiquitous substances have no widely accepted, universal scale. We developed an assay to determine and quantify chao-/kosmotropicity for 97 chemically diverse substances that can be universally applied to all solutes. This scale is numerically continuous for the solutes assayed (from +361 kJ kg^{-1} mol^{-1} for chaotropes to –659 kJ kg^{-1} mol^{-1} for kosmotropes) but there are key points that delineate (i) chaotropic from kosmotropic substances (i.e. chaotropes ≥ +4; kosmotropes ≤ –4 kJ kg^{-1} mol^{-1}); and (ii) chaotropic solutes that are readily water-soluble (log *P* < 1.9) from hydrophobic substances that exert their chaotropic activity, *by proxy*, from within the hydrophobic domains of macromolecular systems (log *P* > 1.9). Examples of chao-/kosmotropicity values are, for chaotropes: phenol +143, $CaCl_2$ +92.2, $MgCl_2$ +54.0, butanol +37.4, guanidine hydrochloride +31.9, urea +16.6, glycerol [> 6.5 M] +6.34, ethanol +5.93, fructose +4.56; for kosmotropes: proline –5.76, sucrose –6.92, dimethylsulphoxide (DMSO) –9.72, mannitol –6.69, trehalose –10.6, NaCl –11.0, glycine –14.2, ammonium sulfate –66.9, polyethylene glycol- (PEG-)1000 –126; and for relatively neutral solutes: methanol, +3.12, ethylene glycol +1.66, glucose +1.19, glycerol [< 5 M] +1.06, maltose –1.43 (kJ kg^{-1} mol^{-1}). The data obtained correlate with solute interactions with, and structure–function changes in, enzymes and membranes. We discuss the implications for diverse fields including microbial ecology, biotechnology and astrobiology.

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Introduction

Cells are essentially aqueous systems with an architecture that is based on water : biomacromolecule structures, the integrity and interactions of which depend on temperature, pH, physicochemical activities of solutes, and other factors (Fig. S1; Brown, 1990; Daniel *et al.*, 2004; Chaplin, 2006). Dissolved substances can determine the operational windows for macromolecular, and therefore cellular, systems and do so via any of a number of distinct stress parameters (water activity, hydrophobicity, ionic strength etc.; Scott, 1957; Brown, 1990; Sikkema *et al.*, 1995; Hallsworth and Nomura, 1999; Lo Nostro *et al.*, 2005; Bhaganna *et al.*, 2010; Leong *et al.*, 2011; Somero, 2012). An increasing body of evidence, mostly from studies of microbial species, indicates that the chaotropic activity of dissolved substances can control cellular systems and processes at multiple levels (often via genome-mediated changes), impact the competitive abilities and community composition of microbes, determine the limits of cellular function and survival, and define the extent of the functional biosphere (see Fig. S1) in specific locations on Earth (Sikkema *et al.*, 1995; Hallsworth, 1998; Hallsworth *et al.*, 2003a; 2007; Duda *et al.*, 2004; 2005; Lo Nostro *et al.*, 2005; 2006; Kashangura *et al.*, 2006; McCammick *et al.*, 2009; Williams and Hallsworth, 2009; Bhaganna *et al.*, 2010; Chin *et al.*, 2010; Ezeji *et al.*, 2010; Zangi, 2010).

Chaotropicity and kosmotropicity are defined according to the effects of dissolved substances (see Fig. S1) on macromolecular systems rather than colligative properties of pure solutions (see Hamaguchi and Geiduschek, 1962; Collins, 1997; McCammick *et al.*, 2009; Ezeji *et al.*, 2010)[1]

[1]Whereas chaotropicity was originally used to describe the impact of ions on nucleic-acid structure without any implied mechanism (Hamaguchi and Geiduschek, 1962), a recent convention has arisen to define chaotropes as water-structure breakers that may modify the behaviour of liquid water even though this assumption remains controversial (see Dixit *et al.*, 2002). The use of entropic measurements of binary liquid mixtures (e.g. Frank and Evans, 1945) to

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and in this way (like the Hofmeister series that ranks ions according to ability to solubilize or precipitate proteins; Hofmeister, 1888) they differ qualitatively from temperature and absolute parameters such as water activity, and pH. Chemically diverse substances can exert chaotropic activity via distinct mechanisms (see Sachs and Woolf, 2003; Hallsworth *et al*., 2007; Bhaganna *et al*., 2010; Chin *et al*., 2010; Zangi, 2010), and whereas some recent studies suggest that substances located within the hydrophobic domains of macromolecular systems can exert chaotropic activity *by proxy* (see Fig. S1; Sachs and Woolf, 2003; McCammick *et al*., 2009; Bhaganna *et al*., 2010; Zangi, 2010), there has been no assay available to determine which cellular stressors can behave in this way.

The consequences of chao- and kosmotropicity for the structure and activities of macromolecular systems have been well characterized *in vitro* whereas the degree to which they facilitate and/or limit the activities of cellular macromolecules *in vivo* remains relatively obscure (e.g. Duda *et al*., 2005; Chin *et al*., 2010). Nevertheless it has been established that chaotropicity-mediated stresses elicit specific stress responses in microbial cells (Hallsworth *et al*., 2003a; Bhaganna *et al*., 2010). Chaotropicity has been shown to not only limit life processes but can render potential habitats sterile including alcoholic fermentations and environments containing chaotropic salts (Hallsworth, 1998; Hallsworth *et al*., 2007; Bodaker *et al*., 2010). The chao- and kosmotropic activities of both cellular metabolites and environmentally ubiquitous substances can determine the biotic windows for metabolic activity and growth of phylogenetically diverse microorganisms (Hallsworth *et al*., 2003b; Williams and Hallsworth, 2009; Bhaganna *et al*., 2010; Chin *et al*., 2010). Indeed it has been suggested that these solute activities drive microbial evolution, have given rise to a novel class of extremophile (*chaophiles*; Hallsworth *et al*., 2007; Williams and Hallsworth, 2009), and can play a key role – via their antimicrobial activities – in the ecology of weed species that recurrently dominate microbial habitats (e.g. the chaotropicity-mediated antimicrobial activity of ethanol produced by *Saccharomyces cerevisiae*; Hallsworth, 1998; J. A. Cray, A. N. W. Bell, P. Bhaganna, A. Y. Mswaka, D. J. Timson, and J. E. Hallsworth, unpublished). The chao- and kosmotropic activities of solutes are utilized in molecular biology and protein-purification protocols (Harris and Angal, 1989; Sambrook *et al*., 1989) as well as a wide range of biotechnological processes (e.g. Dennison and Lovrient, 1997; Gaur *et al*., 2007; Sen *et al*., 2011), and have contributed to policy documents to determine the special regions on Mars where physicochemical conditions would be permissive for cellular activity (http://planetaryprotection.nasa.gov/documents; Kminek *et al*., 2010). Studies to elucidate the diverse mechanisms by which chaotropic (and other types of) solute modify water : macromolecule interactions are ongoing (Sachs and Woolf, 2003; McCammick *et al*., 2009; Bhaganna *et al*., 2010; Zangi, 2010), and there have been a number of insightful studies on the interaction of proteins with ions in relation to the chao- or kosmotropicty of the latter (e.g. Zhao, 2006; Zhao and Song, 2007; Yeh *et al*., 2010). Remarkably, however, there is currently no standard method for the quantification of chaotropic and kosmotropic activity based on a homogenous aqueous system (such as water : agar gels) that has predictive value for diverse macromolecular structures, can be used for different solute types (including ions, organic solutes, surfactants, and polysaccharides), and provides one continuous ranking/scale for both chaotropes and kosmotropes.

The current study was carried out to: (i) develop a simple assay to quantify the chao- and kosmotropic activities that substances exert towards macromolecular systems; (ii) classify and rank solutes according to these activities (and therefore predict the relative potency of biologically relevant substances as potential stressors and/or protectants of cellular systems); and (iii) determine the chao- and kosmotropic activities of environmentally and biologically relevant substances. We quantified the impacts of solutes on gelation temperature of a hydrophilic polymer, agar, as a model system to enable the spectrophotometric quantification of the chaotropicity and kosmotropicity for a range of 97 chemically diverse substances in order to produce a classification of compounds, and a universal scale for the quantification of chao-/kosmotropicity.

Results and discussion

Spectrophotometric determination of chao-/kosmotropicity

In order to establish whether a spectrophotometric protocol could be used to determine solute-induced modifications of agar gel-point temperature, a wavelength scan of liquid (48°C) and solidified agar (38°C) were carried out (Fig. 1A). The solidification of dissolved agar occurs when large numbers of hydrogen (and other non-covalent) bonds form between the polymer chains which, collectively, induce gel formation. A purified agar was used at a concentration of 1.5% w/v (see *Experimental procedures*). For both visible and ultraviolet wavelengths there were marked differences between the liquid and solid agar spectra, as high as 1.5 absorbance units between 230 and 280 nm (Fig. 1A). Furthermore, the spectra for solid agar remained consistent even when the tempera-

predict chao-/kosmotropicity is based on this assumption; we believe that in order to quantify chao-/kosmotropic activity it is preferable to assay the solute-induced changes in water : macromolecule interactions that are the basis of this activity.

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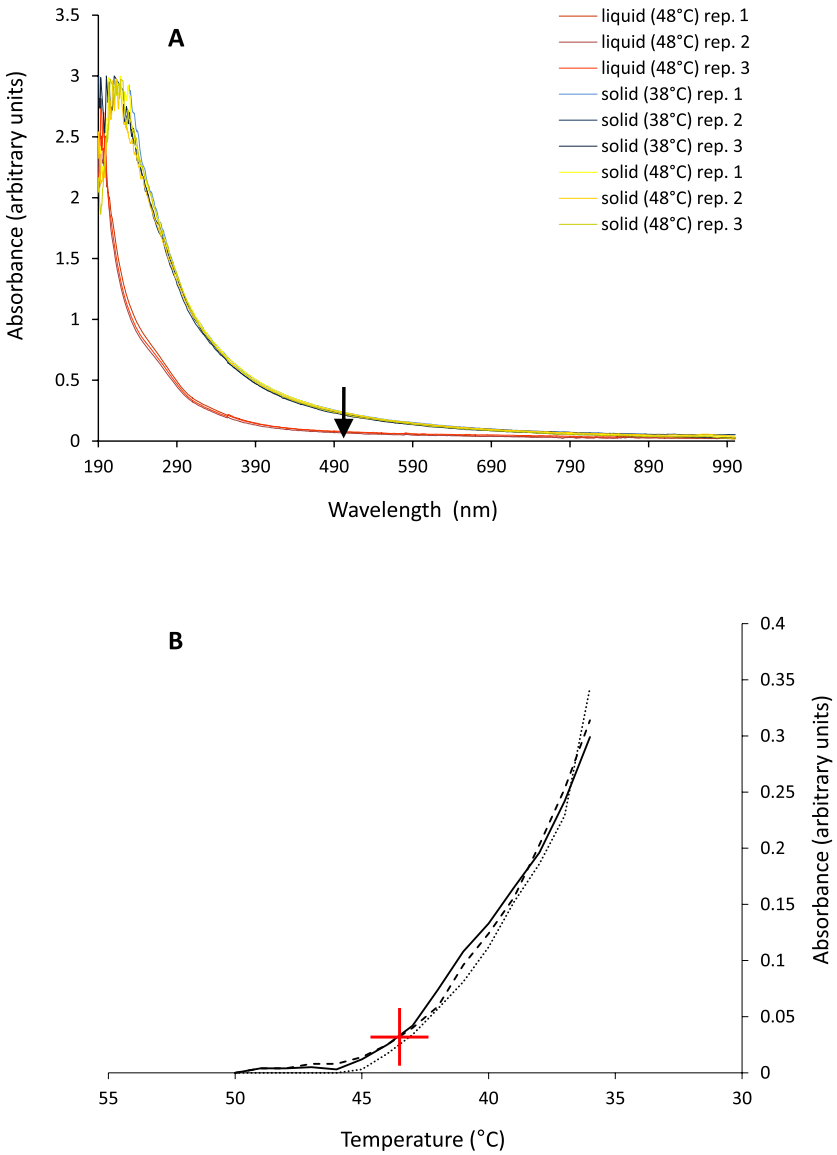


Fig. 1. Absorption spectra of liquid (48°C) and solid agar (38°C or 48°C; A); the black arrow indicates the wavelength selected to use for solute-activity assays. Absorption spectra (at 500 nm) of agar solutions over temperature, during the gelation process as solutions were cooled (B); the red cross indicates the point at which the agar had solidified (i.e. gelled agar units > 4 mm diameter ≡ 0.03 absorbance units) according to manual assessment techniques (see Hallsworth *et al.*, 2003a). The agar concentration in each case was 1.5% w/v; see also *Experimental procedures*.

ture was elevated from 38 to 48°C, indicating that these absorbance readings were not temperature-sensitive (Fig. 1A). A visible wavelength of 500 nm was selected for solute-activity assays (see Fig. 1A) to avoid interference with readings from aromatic solutes that absorb in the ultraviolet range, and to keep absorbance readings below 0.5 in order to maintain accuracy. Whereas the absorbance spectrum of agar solutions prior to, and immediately after, the gelation process shows a consistent increase (Fig. 1B) the gelation of agar as determined visually (see Hallsworth *et al.*, 2003a) is immediate and occurs at an absorbance value of 0.03 units (Fig. 1B). We compared the spectrophotometric determination of agar gelation at 0.03 absorbance (500 nm) with the manual assessment developed previously (Hallsworth *et al.*, 2003a) and found consistency to ± 0.25°C. Hence an absorbance value of 0.03 was used as the basis for spectrophotometric solute-activity assays (see below).

For solute-activity assays, 97 substances (see Table 1) were each incorporated into agar solutions as described previously (Hallsworth *et al.*, 2003a; Bhaganna *et al.*, 2010). Stock solutions of agar were heated to 95°C to ensure dissolution, allowed to cool and then solutes were added over a range of concentrations (see *Experimental procedures*). The concentration at which there was a 1°C change in agar gel-point temperature was recorded (Table 1) and used to calculate the molar chaotropic activity (≡ gel-point decrease) or molar kosmotropic activity of each solution (≡ gel-point increase). The chao- or kosmotropic activity values were calculated using the heat capacity of water- and agar-solutions (Cornillon and Andrieu, 1995) and are therefore expressed as kJ kg^{-1}.

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Table 1. Chao- and kosmotropic activities of stressors, metabolites and other biologically relevant substances quantified using an agar model system.

Compound (log $P_{octanol-water}$)	M_r	Concentration (mM) at which there is a 1°C increase (+) or decrease (–) in agar gel-point[a]	Chaotropic (+) or kosmotropic activity (–) (kJ kg^{-1} mole added compound^{-1})[a,b]
Hydrophobic substances			
1,2,3 trichlorobenzene (4.04)	181.45	n/a	none
ethyl octanoate (3.90)	172.27	n/a	none
gamma-hexachlorocyclohexane (3.7)	290.83	n/a	none
n-hexane (3.5)	86.18	n/a	none
pentane (3.39)	72.15	n/a	none
β-cyclocitral (3.10)	152.23	n/a	none
octanol (2.9)	130.23	n/a	none
1-heptanol (2.62)	116.2	n/a	none
2,5-dichlorophenol (2.5)	163.0	n/a	none
toluene (2.5)	92.14	n/a	none
isoamyl acetate (2.26)	130.19	n/a	none
benzene (2.1)	78.11	n/a	none
hexanol (1.94)	102.17	n/a	none
benzoic acid (1.9)	122.12	n/a	none
Chaotropic solutes			
Tween® 80[c]	1 300	11.5 (–)	+361
vanillin	182.15	23.9 (–)	+174
phenol	94.11	29.0 (–)	+143
Triton X-100[d]	646.87	30.8 (–)	+135
Tween® 20[c]	227.54	32.6 (–)	+127
$HgCl_2$	271.52	33.3 (–)	+125
guanidine thiocyanate	118.16	34.2 (–)	+121
o-cresol	108.13	43.0 (–)	+96.5
$CaCl_2$[e]	110.98	45.0 (–)	+92.2
benzyl alcohol	108.13	46.0 (–)	+90.2
sodium benzoate	144.11	48.0 (–)	+86.5
KI	166.01	48.1 (–)	+86.3
$FeCl_3$	162.20	60.3 (–)	+69.2
KSCN	97.18	61.5 (–)	+67.5
NaSCN	81.07	65.9 (–)	+63.0
ethyl acetate	88.11	67.6 (–)	+61.4
$MgCl_2$	95.23	76.9 (–)	+54.0
cetyltrimethylammonium bromide	364.46	91.4 (–)	+45.4
butanol	74.12	111 (–)	+37.4
acetone	58.08	112 (–)	+37.0
$FeCl_2$	126.75	123 (–)	+33.3
guanidine hydrochloride	95.53	130 (–)	+31.9
ammonium nitrate (NH_4NO_3)	80.04	132 (–)	+31.4
LiCl	42.40	204 (–)	+20.3
SDS	288.38	208 (–)	+20.0
urea	60.06	250 (–)	+16.6
propanol	60.10	253 (–)	+16.4
NaBr	102.91	324 (–)	+12.8
LiBr	86.84	410 (–)	+10.1
ammonium chloride (NH_4Cl)	53.49	418 (–)	+50.5
xylose	150.13	529 (–)	+7.84
glycerol (6.5–10 M)	92.09	655 (–)	+6.34
formamide	45.05	667 (–)	+6.22
ethanol	46.07	700 (–)	+5.93
fructose	180.16	910 (–)	+4.56
Relatively neutral solutes			
methanol	32.04	1330 (–)	+3.12
ethylene glycol	62.07	2500 (–)	+1.66
glucose	180.16	3500 (–)	+1.19
glycerol (< 5 M)	92.08	3920 (–)	+1.06
maltose	360.32	2910 (+)	–1.43
erythritol	122.12	2030 (+)	–2.04
Kosmotropic solutes and polysaccharides			
proline	115.1	720 (+)	–5.76
xylitol	152.1	620 (+)	–6.69
mannitol	182.17	620 (+)	–6.69

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Table 1. *cont.*

Compound (log $P_{octanol-water}$)	M_r	Concentration (mM) at which there is a 1°C increase (+) or decrease (–) in agar gel-point[a]	Chaotropic (+) or kosmotropic activity (–) (kJ kg^{-1} mole added compound^{-1})[a,b]
sorbitol	182.17	610 (+)	–6.80
sucrose	342.30	600 (+)	–6.92
dimethylsulphoxide (DMSO)	78.13	427 (+)	–9.72
trehalose	342.31	390 (+)	–10.6
NaCl	58.44	376 (+)	–11.0
KCl	74.55	367 (+)	–11.3
KH_2PO_4	136.09	300 (+)	–13.8
glycine	75.07	293 (+)	–14.2
polyethylene glycol (PEG) 200	200	277 (+)	–15.0
ectoine	142.16	250 (+)	–16.6
betaine	117.15	163 (+)	–25.5
trimethylamine oxide (TMAO)	75.11	160 (+)	–25.9
PEG 300	300	133 (+)	–31.2
Na_2SO_4	142.04	82.1 (+)	–50.5
PEG 400	400	78.2 (+)	–53.2
$MgSO_4$	120.37	64.3 (+)	–64.5
ammonium sulfate ($(NH_4)_2SO_4$)	132.14	62.4 (+)	–66.9
$FeSO_4$	151.91	61.0 (+)	–68.0
K_2SO_4	174.26	61.1 (+)	–67.8
PEG 600	600	56.2 (+)	–74.0
Li_2SO_4	109.94	53.0 (+)	–78.3
EDTA disodium salt	354.24	46.5 (+)	–90.2
sodium citrate	276.1	42.5 (+)	–97.6
polyvinylpyrrolidone (PVP) 10 000	10 000	33.3 (+)	–125
PEG 900	900	33.1 (+)	–126
PEG 1 000	1 000	33.2 (+)	–126
dextran 10 000	10 000	30.3 (+)	–138
PVP 40 000	40 000	15.0 (+)	–277
PEG 3 350	3 350	9.72 (+)	–429
PEG 6 000	6 000	6.31 (+)	–659

a. Based on the heat capacity (see *Experimental procedures*). All measurements were made in triplicate and values were consistent to ± 0.3°C. n/a, not applicable.
b. Care was taken to use solute concentrations below the level at which agar precipitation occurs otherwise quantitatively inaccurate values are obtained such as those that led to misleading conclusions that the kosmotropic substances $MgSO_4$ and $LiSO_4$ can exert chaotropic activity (Crisler *et al*., 2012). For substances with a solubility limit of < 1 M (e.g. phenol) and those that precipitate agar at solute concentrations of < 1 M the listed value was derived by extrapolation. None = insufficiently soluble in water to exert chaotropic activity as a solute.
c. Polyoxyethylene sorbitan monooleate.
d. $C_{14}H_{22}O(C_2H_4O)_n$.
e. $CaCl_2$ was assayed using gelatine (see *Experimental procedures*) and gel-point values transformed to be in line with those of the agar system.

Plots of solute concentration versus agar gel-point are typically linear (Hallsworth *et al*., 2003a; Bhaganna *et al*., 2010), and chaotropicity correlates with log *P* (with two exceptions – salts that dissociate and surfactants such as Tween® 80, Triton™ X-100 and Tween® 20, cetyltrimethylammonium bromide, and sodium dodecyl sulfate – that have hydrophobic regions; see Table 1; Bhaganna *et al*., 2010). Nevertheless chaotropic activity cannot be predicted from solution chemistry for all substances because chaotropicity is ultimately an empirical measure on water : macromolecule interactions; this relates, in part, to whether the solute enters the aqueous phase of a hydrated macromolecular system (Arakawa and Timasheff, 1984). Furthermore, there are multiple mechanisms by which solutes can exert chaotropic activity (Sachs and Woolf, 2003; Bhaganna *et al*., 2010). The interaction of hydrophobic substances, including antimicrobials such as ethyl octanoate and β-cyclocitral and oil-derived substances (e.g. benzene and toluene), with macromolecular systems can be quantified using log *P* and/or dispersal or micellization of SDS or other lipids (Wu *et al*., 1998; Bhaganna *et al*., 2010).

Glycerol and fructose are unique among compatible solutes in their chaotropic, rather than neutral or kosmotropic, nature (Table 1). This is consistent with studies of their respective physical chemistries and interactions with macromolecular and cellular systems (Back *et al*., 1979; Brown, 1990; Lelong *et al*., 2009; McCammick *et al*., 2009; Williams and Hallsworth, 2009; Chin *et al*., 2010; Rivero *et al*., 2010). The chao-/kosmotropicity of salts shows some correlations with the Hofmeister series (Hofmeister, 1888; Hallsworth *et al*., 2007), but the activities of

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chaotropic salts may induce cellular stress primarily via fluidization of the plasma membrane. Salts such as $MgCl_2$, $CaCl_2$ and KI and guanidine-hydrochloride impair the functional activities of macromolecular systems (Arakawa and Timasheff, 1984; and are utilized for this purpose in biochemical protocols; Hallsworth *et al*., 2003b; Bodaker *et al*., 2010), induce chaotrope-mediated water stress in microbial cells at sublethal concentrations (Hallsworth *et al*., 2003a; 2007), ultimately denature but preserve macromolecules (Hallsworth *et al*., 2007), and can kill and mummify cellular systems (Duda *et al*., 2005), and at sufficient concentrations prevent all life processes (e.g. in bitterns, the Dead Sea, the deep-sea Discovery Basin, Don Juan Pond; Hallsworth *et al*., 2007; Bodaker *et al*., 2010) via their chaotropic activities.

The data obtained (Table 1) were consistent with the known denaturant properties/solute activities of chaotropic substances (e.g. urea, phenol, $MgCl_2$, guanidine thiocyanate; see Hamaguchi and Geiduschek, 1962; Duda *et al*., 2004; Hallsworth *et al*., 2007) and the structuring/stabilizing activities of kosmotropic substances (e.g. NaCl, betaine, ammonium sulfate, sucrose, polyethylene glycol; Rudolph *et al*., 1986; Washabaugh and Collins, 1986; Daffonchio *et al*., 2006) and their interactions with macromolecular systems (see Hofmeister, 1888; Scott, 1957; Harris and Angal, 1989; Sambrook *et al*., 1989; Hallsworth *et al*., 2003a; 2007; Miyawaki and Tatsuno, 2011; Nazari-Robati *et al*., 2012). For example thermodynamically induced unfolding of RNase by sucrose, urea, glycerol, guanidine hydrochloride and a range of aliphatic alcohols correlates with the chao-/kosmotropicity values in Table 1 ($R^2 = 0.94$; see Fig. 2A); the disruption of plasma membrane integrity by alcohols correlates with their chaotropic activity ($R^2 = 0.91$; Fig. 2B); and the stabilization of a thermally sensitive enzyme (chondroitinase) by co-solvents correlates with the kosmotropic activity of the latter ($R^2 = 0.81$; Fig. 2C; Table 1). For the salts $FeCl_3$, $MgCl_2$, $FeCl_2$, LiCl, NaBr, LiBr, NH_4Cl, NaCl, KCl, and $(NH_4)_2SO_4$ standard molar entropy of aqueous solutions (Lide, 1998) correlates well with their chao- and kosmotropic activities ($R^2 = 0.86$; Table 1). Remarkably, the chao- and kosmotropic activities of organic, inorganic, ionic and non-ionic solutes can also correlate with the solute concentrations that inhibit microbial growth rates (Scott, 1957; Hallsworth *et al*., 2003b; Lo Nostro *et al*., 2006; Williams and Hallsworth, 2009; Bhaganna *et al*., 2010).

A universal scale of chaotropicity and kosmotropicity

It has been long established that chaotropicity and kosmotropicty have potent impacts on macromolecular systems (see Nelson and Schubert, 1928; Hamaguchi and Geiduschek, 1962; Washabaugh and Collins, 1986; Collins, 1997; Crowe *et al*., 1998). Furthermore we now know that chaotropicity, like water activity, hydrophobicity, temperature and pH, can define the biotic windows for both cellular and ecological systems (see above). Units and scales of measurement for other stress parameters have been established for some time: temperature in 1742 (Celsius, 1742), hydrophobicity in 1872 (Berthelot and Jungfleisch, 1872), ions in 1888 (Hofmeister, 1888), pH in 1909 (Sörensen, 1909), and water activity in 1957 (Scott, 1957). In contrast, chaotropicity may be the only parameter able to limit life processes which has not been quantified via a scale. The spectrophotometric quantification of chao-/kosmotropic activities (Table 1) resulted in a scale on which the macromolecular-stabilizing/disordering activities of diverse substances have now been ranked and compared on a qualitative (hydrophobic, chaotropic, neutral, or kosmotropic) as well as a quantitative basis. This ranking is universally applicable to diverse types of macromolecular and cellular system, diverse habitats and environmental locations. Furthermore it can be used to quantify the net effect of complex and/or unknown solute-mixtures (see Hallsworth *et al*., 2007; Williams and Hallsworth, 2009).

Whereas the chao-/kosmotropic-activity scale is numerically continuous for the solutes assayed (ranging from +361 to –659 kJ kg^{-1} mol^{-1}; Table 1) there are key points on the scale that delineate (i) chaotropic from kosmotropic substances (i.e. chaotropes $\geq +4$; kosmotropes ≤ -4 kJ kg^{-1} mol^{-1}); and (ii) chaotropic solutes that are readily water-soluble from hydrophobic substances that exert chaotropic activity, *by proxy*, from within the hydrophobic domains of macromolecular systems (Table 1). These hydrophobic substances with a log $P > 1.9$ are therefore unable to act as (water-soluble) solute stressors (see also Bhaganna *et al*., 2010). Whereas the majority of chaotropic solutes have a M_r between 42 and 182, the majority of the kosmotropic solutes assayed have a M_r of > 182 (Table 1). For ionic compounds, that dissociate in water, the chaotropic- and kosmotropic-activity values refer to the net effect of the ionic solution on the macromolecular system (see Hallsworth *et al*., 2007; Williams and Hallsworth, 2009).

Many microbial habitats and natural environments on Earth are chaotropic in nature; for example those dominated by low-M_r aromatic compounds such as benzene and phenol; aliphatic alcohols; urea; glycerol; specific sugars such as fructose; and chaotropic salts such as LiCl, $MgCl_2$, and $CaCl_2$. Other microbial habitats are kosmotropic in nature (see Fig. S1), especially those dominated by substances such as sucrose; trehalose; hydrophilic polymers (e.g. those found in extracellular polymeric substances), NaCl, and sulfate salts. The microbial and enzyme systems used for industrial applications are typically exposed to, and can be inhibited by,

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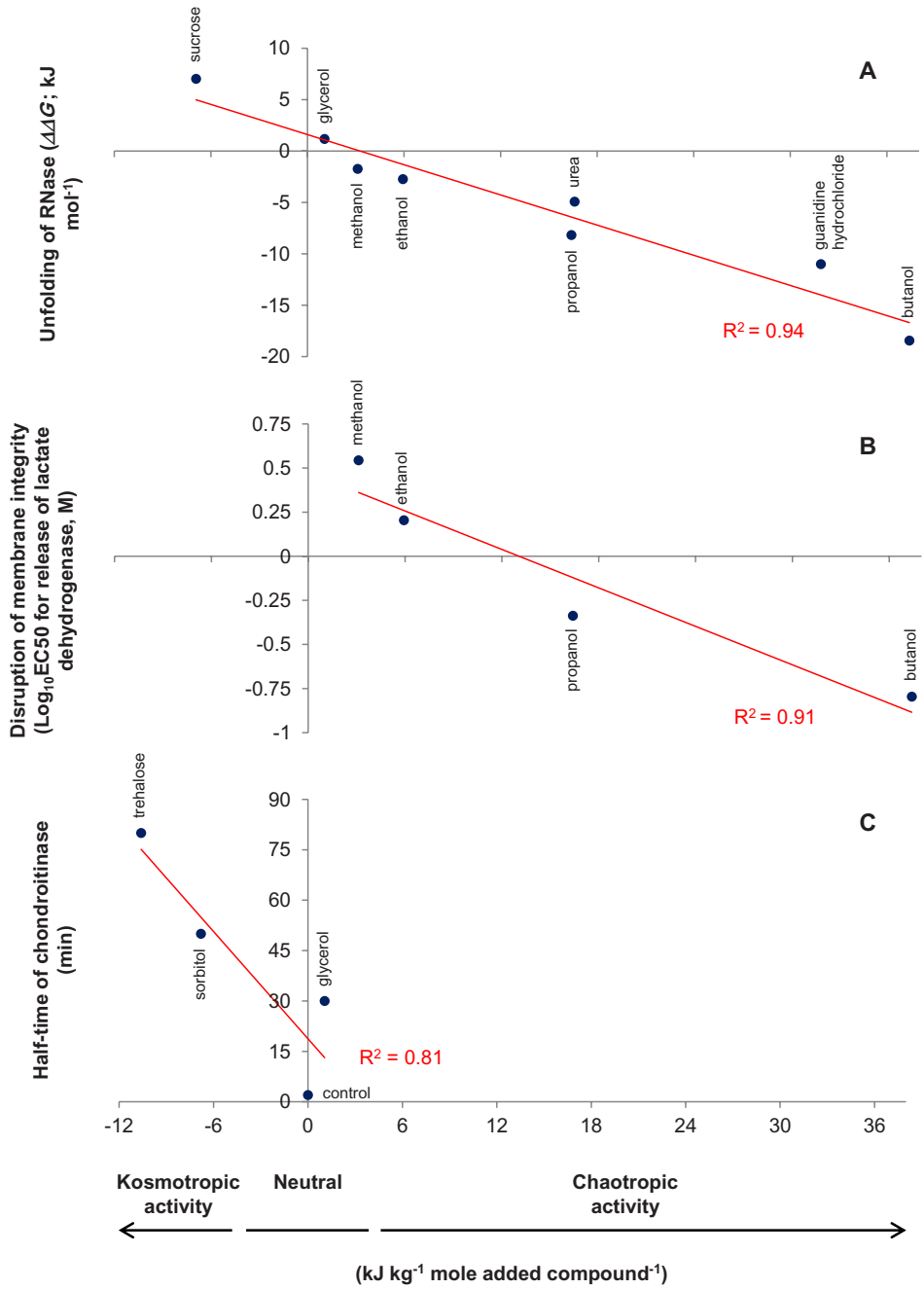


Fig. 2. Linear regression between the effects of solutes on the (A) unfolding of RNase (ΔΔG, where stabilizing effects result in a positive value) and their chao-/kosmotropic activity where positive values indicate chaotropicity and negative ones kosmotropicity; (B) disruption of plasma membrane integrity by alcohols and their chaotropic activity and, (C) rate of unfolding of chondroitinase (at 25°C) in the presence of cosolvents and the kosmotropic activity of the latter. Data on chao-/kosmotropic activity are from this article (Table 1), data on solute effects on RNase from Miyawaki and Tatsuno (2011) and were converted from molal to molar values via empirical determines of solution volume changes, data on membrane disruption from McKarns and colleagues (1997), and those on chondroitinase from Nazari-Robati and colleagues (2012). See also *Experimental procedures*.

the solute activities of substances in the growth medium or reaction mixture (including microbial substrates and products) and recent analyses of extraterrestrial environments that may have the potential to support life have identified a significant number of strongly chaotropic and kosmotropic substances (e.g. Squyres *et al.*, 2004; Tielens, 2008; Hecht *et al.*, 2009). All cellular systems are intimately associated with solutes of biotic as well as abiotic origin, all of which have either chaotropic or kosmotropic activity and can potentially induce collateral

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damage (Hallsworth *et al*., 2003a; Duda *et al*., 2004; Bhaganna *et al*., 2010; Chin *et al*., 2010). Depending on the biological context, solute activities such as chaotropicity may be stressful (Hallsworth *et al*., 1998; 2003a,b; Duda *et al*., 2004; 2005; Bhaganna *et al*., 2010) or protective; for example kosmotropicity can offset chaotrope stress, and chaotropes can counteract low-temperature stress in microbial cells (Hallsworth, 1998; Hallsworth *et al*., 2007; Chin *et al*., 2010). The scale has a predictive value for the understanding of how solute activities impact on living systems, and raises questions such as: (i) can empirically derived measurements of chao- and kosmotropicity be used to construct a thermodynamic scale; (ii) to what extent are chao-/kosmotropic activities additive or synergistic; (iii) do cells have mechanisms for sensing and regulating chao- and kosmotropicity (as they do for pH, ionic concentrations etc; see Korem *et al*., 2010); (iv) does the kosmotropic activity of intra- and extracellular polysaccharides confer compatible solute-type protective abilities; (v) can quantification of these solute activities enable optimization of current, and development of novel, biotechnologies; and (vi) to what degree do the net chao-/kosmotropic activities of solutes interact with temperature (Chin *et al*., 2010; Kellogg *et al*., 2011) to determine the boundaries of Earth's biosphere or those that may exist on other planetary bodies such as Mars?

Experimental procedures

Spectrophotometric determination of agar gelation

Extra-pure reagent-grade agar, gel strength 600–700 g cm^{-2} (Nacalai Tesque, Kyoto, Japan), at a concentration of 1.5% w/v (made up in 18 MΩ-cm deionized reverse osmosis water; gel point = 43.5 ± 0.3°C) was used to determine molar chao- or kosmotropicity values for added solutes (see Hallsworth *et al*., 2003a). A Cecil E2501 spectrophotometer fitted with a thermoelectrically controlled heating block (Milton Technical Centre, Cambridge, England) was used to determine the wavelength and absorbance values at which to assay compounds (see Fig. 1; *Results and discussion*). Prior to each experiment, agar (1.5% w/v) was dissolved by heating to 95°C in a water bath and maintaining this temperature for 1 h. The agar was then transferred to a water bath at the desired experimental temperature (i.e. approximately 5–10°C above the anticipated gel-point) for 30 min. The thermoelectrical controller of the spectrophotometer was used to regulate the heating block to the required experimental temperature and the agar or agar + solute mixture (2 ml) was placed into a 3 ml cuvette (path length: 1 cm). Each compound was assayed up to saturation, or otherwise up to the concentration that induced precipitation of the agar.

The initial wavelength scan was carried out from 190 nm to 1000 nm (see Fig. 1A) at a rate of 5 nm s^{-1}. For all other readings the temperature of the heating block was set at 5–10°C above the anticipated gel-point and then progressively decreased by 1°C per 5 min until gelation occurred. For volatile compounds, mineral oil was used to seal the surface of the solution in the cuvette. The chao-/kosmotropic activity was calculated as the deviation of gelation temperature from that of the control.

Gelatine (300-Bloom) was obtained from Sigma, USA and used to test the gel point of $CaCl_2$ that has specific interactions with agar. The gelatine was dissolved at 45°C and the gel-point values obtained were then transformed in line with those derived from agar solutions (see Table 1; Hallsworth *et al*., 2003a). The effect of pH on gel-point of agar and gelatine was assessed previously and did not vary between the values of 2 and 11 (Hallsworth *et al*., 2003a). All measurements were made in triplicate and values were consistent to ± 0.3°C. The data obtained were used to calculate the chaotropic activity of each substance (in kJ kg^{-1} mole added $compound^{-1}$) and the values were calculated based on the knowledge that the heat capacity for a 1.5% (w/v) agar gel is 4.15 kJ kg^{-1} $°C^{-1}$ (slightly less than the heat capacity of water at 25°C which is approximately 4.20 kJ kg^{-1} $°C^{-1}$; see Cornillon and Andrieu, 1995). The gelation assay can be performed over a range of agar concentrations in order to determine or confirm chao-/kosmotorpicity values for a range of temperatures.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Definitions of scientific concepts pertinent to the biology of chao- and kosmotropic substances.

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