

## An investigation into structural changes due to deuteration

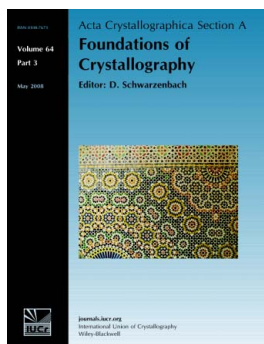
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# An investigation into structural changes due to deuteration

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Perdeuteration of proteins is becoming more commonplace and the assumption is in general that deuteration does not affect protein structure. In this work, the effect of deuteration on structure is examined by data mining, largely of the Cambridge Structural Database but also of the Inorganic Crystal Structure Database, for deuterated and hydrogenated pairs of small-molecule structures analysed by neutron and X-ray crystallography. Differences between these small-molecule structures have been calculated and the results thus far follow the initial assumption. However, functional changes are known, *e.g.* D<sub>2</sub>O is toxic to living systems but H<sub>2</sub>O is not, kinetics change, small pH to pD changes occur, proteins stiffen in D<sub>2</sub>O and ferroelectrics alter their properties.

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## 1. Introduction

There is a major expansion of fully deuterated protein production using microbiological expression for neutron protein crystallography experiments complementary to X-ray protein crystallography (for a review and the latest developments, see Blakeley *et al.*, 2004; Ahmed *et al.*, 2007). The advantages of perdeuterated proteins in neutron protein crystallography have been elaborated by Gamble *et al.* (1994), Shu *et al.* (2000) and Hazemann *et al.* (2005). In essence, the physical crystallography advantage is a more than tenfold reduction of sample background due firstly to a basic elimination of the incoherent scattering by H atoms of the incident neutrons and secondly to a beneficial increase in protein scattering efficiency due to the large number of D atoms which scatter as strongly as the C atoms. Both of these lead to a reduced sample volume requirement and/or improved diffraction resolution or both. The biochemical structural crystallography advantages are an increased visibility of the H atoms (as D atoms) both on the protein and on the bound solvent (see also Habash *et al.*, 2000).

With neutron sources becoming more readily available and with higher flux, and the ability for macromolecules to be fully deuterated by molecular biological expression, it is important to consider the initial assumption that deuteration of a molecule does not affect its structure. It is a well known premise that deuteration does affect kinetics; the reduction in rate of reaction due to replacement of an isotope with a heavier one is known as the kinetic isotope effect (KIE) (Atkins & de Paula, 2002) and can occur as a primary or secondary effect. The primary KIE occurs when the rate-determining step requires the fission of a bond involving the isotope (Ralph *et al.*, 2006) and the secondary KIE occurs when the rate of reaction decreases even though the bond

broken does not involve the isotope (Cheng & Marsh, 2007).

Most of the published work on perdeuteration was performed in the 1960's by Crespi and Berns (Berns, 1963; Hattori *et al.*, 1965). Their experiments, as well as more recent data, indicate that perdeuteration has a destabilizing effect on proteins and concluded that, even though there are observed physicochemical differences (Brockwell, 1996) between hydrogenated and perdeuterated proteins, they are not of an overall structural nature and are, presumably, kinetic. Conversely, biological properties such as ligand binding appear not to be significantly affected (Derrick *et al.*, 1992; Brennan *et al.*, 1994; Brockwell, 1996). Most recently, Olgun (2007) has provided a mini review of biological effects of 'deuteronation', as Olgun refers to it, and a specific study on ATP synthase from the point of view of function with the result that the contribution of deuteration to changing the pK<sub>a</sub> of Asp61 was 0.35. A key investigation into the effects on structure of perdeuteration was performed by Gamble *et al.* (1994) when they determined the structure of perdeuterated *Staphylococcal* nuclease (SNase). The native and perdeuterated forms of SNase behaved similarly throughout the purification procedure and crystallized under the same experimental conditions. X-ray diffraction was used to compare the diffraction characteristics and structure of the native and deuterated protein, firstly by direct comparison of the diffraction data, then by analysis of difference Fourier maps made between the two data sets, and lastly by independent refinement and comparison of the two final structures at 1.9 Å resolution. These comparisons concluded that there are no significant structural differences between native and perdeuterated SNase.

In further addressing this concern as to whether deuteration affects a protein structure, there are a few more recent studies

but, like Gamble *et al.*, most are necessarily at relatively modest diffraction resolution with respect to small-molecule structures. These latter also exist in relatively good quantity compared with protein pairs of H *versus* D structures. Nevertheless, for completeness, we firstly summarize the more recent protein studies since Gamble *et al.* (1994). Cooper *et al.* (1998) compared hydrogenated and deuterated GDP-EFTu at 3.0 Å resolution and concluded that there were no discernible changes. Artero *et al.* (2005) compared the hydrogenated and deuterated structures of  $\gamma$ E-crystallin and reported no significant difference in structure, however, there were changes in *B* factors. Liu *et al.* (2007) compared haloalkane dehalogenase H and D structures at 1.5 Å resolution; they did see a change in the enzyme active site involving a missing water molecule and an associated rotation of a carboxyl group of 20°. Whilst no standard uncertainties were quoted, these changes are significant, *i.e.* based on the overall diffraction precision indices of the two reported structures, the well ordered active-site atoms and the change seen of a well ordered bound water mentioned above. Liu *et al.* concluded that the results of their study underlined the importance of fully characterizing perdeuterated proteins using X-ray crystallography before their use in neutron crystallographic studies of enzyme mechanisms. In another study of two different but closely related enzymes, Kuhn *et al.* (1998) found in their 0.78 Å X-ray structure of a serine protease (*Bacillus lentus* subtilisin; see, for example, their Fig. 3c) that the critical H atom was positioned between His64 and Asp32, in contrast to the findings by neutron protein crystallography of Kossiakoff & Spencer (1980) that it was on the histidine. Kuhn *et al.* (1998) suggested that the different results on the protonation state of the two residues was a consequence of deuterium, which was used in the neutron experiment, having a different zero-point energy and thus being confined to one position; an H atom in contrast, they argued, could be shared. Most recently, Blakeley *et al.* (2008) presented results of combined neutron, X-ray and quantum calculations of the enzyme h-aldose reductase. On the question of structural effects of deuteration, they report that the deuterated X-ray structure determined at 15 K at 0.8 Å resolution showed no overall significant differences with the hydrogenated X-ray structure determined at 100 K. Specifically, the C $\alpha$  r.m.s.d. was overall 0.7 Å, and 0.15 Å in the well ordered active site; they proceeded on that basis both to combine the information from the two structures and to conduct joint X-ray and neutron refinement of the 293 K fully deuterated structure.

Given the above context of applying neutron protein crystallography more widely (Blakeley *et al.*, 2004), this paper examines the possible specific effects of deuteration on hydrogen-bond-geometry differences in more than 50 pairs of the more precise and larger cohort of H and D small-molecule structures than a protein set. With time, it will become possible to analyse for any differences, at a significant level of precision, of a large cohort of ultra-high-resolution protein structures in H and D forms. The latter will require an effort by neutron protein crystallography researchers to characterize their D proteins at the highest resolution possible by X-rays.

**Table 1**

Radiation probe, collection temperature (RT = 283–303 K) and primary citation for all 52 isomorphous compounds studied.

CSD code	H/D	Radiation probe	T (K)	Primary citation
CUHDAK	Hydrogenated	Unknown	RT	Schildkamp <i>et al.</i> (1984)
CUHDAK01	Deuterated	Unknown	RT	Kek <i>et al.</i> (1997)
CYTOSM04	Deuterated	Neutron	82	Weber, Craven <i>et al.</i> (1980)
CYTOSM11	Hydrogenated	X-ray	RT	McClure & Craven (1973)
DINICA11	Hydrogenated	Neutron	15	Cowan <i>et al.</i> (2005)
DINICA13	Deuterated	Neutron	15	Cowan <i>et al.</i> (2005)
DIYJUQ02	Hydrogenated	X-ray	130	Zuniga <i>et al.</i> (1991)
DIYJUQ03	Deuterated	Neutron	RT	Ezpeleta <i>et al.</i> (1996)
FORMAC01	Hydrogenated	X-ray	98	Nahringbauer (1978)
FORMAC02	Deuterated	Neutron	4.5	Albinati <i>et al.</i> (1978)
HAZFAP01	Hydrogenated	X-ray	RT	Reetz <i>et al.</i> (1994)
HAZFAP03	Deuterated	X-ray	RT	Reetz <i>et al.</i> (1994)
HDRZHO02	Hydrogenated	X-ray	RT	Thomas (1973)
HDRZHO03	Deuterated	X-ray	RT	Thomas (1973)
IMDACB02	Deuterated	X-ray	RT	Oskarsson (1976)
IMDACB11	Hydrogenated	Neutron	RT	Oskarsson (1976)
IMZMAL11	Hydrogenated	Neutron	RT	Hsu & Schlemper (1980)
IMZMAL13	Deuterated	Neutron	RT	Hussain <i>et al.</i> (1980)
JUQSOD	Hydrogenated	X-ray	100	Evdokimov <i>et al.</i> (1999)
JUQSOD01	Deuterated	Neutron	20	Evdokimov <i>et al.</i> (2001)
KECYBU06	Hydrogenated	Neutron	RT	Semmingsen <i>et al.</i> (1977)
KECYBU10	Deuterated	Neutron	RT	McMahon <i>et al.</i> (1991)
KFSCCN01	Deuterated	X-ray	RT	Emsley <i>et al.</i> (1981)
KFSCCN10	Hydrogenated	X-ray	RT	Emsley <i>et al.</i> (1982)
KFURDC	Hydrogenated	X-ray	RT	Jaulmes (1978)
KFURDC01	Deuterated	X-ray	RT	Jaulmes (1978)
LABSEM01	Hydrogenated	X-ray	295	Baran <i>et al.</i> (2002)
LABSEM02	Deuterated	X-ray	360	Baran <i>et al.</i> (2002)
LARGPH01	Hydrogenated	X-ray	RT	Saenger & Wagner (1972)
LARGPH07	Deuterated	Neutron	RT	Cheng <i>et al.</i> (1997)
LHOXAL01	Hydrogenated	X-ray	RT	Thomas (1972)
LHOXAL02	Deuterated	X-ray	RT	Thomas (1972)
LIACET02	Hydrogenated	X-ray	RT	Galigné <i>et al.</i> (1970)
LIACET05	Deuterated	Neutron	RT	Kearley <i>et al.</i> (1996)
OXACDH04	Hydrogenated	Neutron	RT	Sabine <i>et al.</i> (1969)
OXACDH28	Deuterated	Neutron	RT	Coppens & Sabine (1969)
POVHEN	Hydrogenated	X-ray	122.4	Madsen & Larsen (1998)
POVHEN01	Deuterated	X-ray	122.4	Madsen <i>et al.</i> (1998)
PUHROZ	Hydrogenated	X-ray	80	Jerzykiewicz <i>et al.</i> (1998)
PUHROZ02	Deuterated	X-ray	80	Jerzykiewicz <i>et al.</i> (1998)
QUICNA02	Hydrogenated	Neutron	100	Takusagawa & Koetzle (1978)
QUICNA11	Deuterated	Neutron	100	Takusagawa & Koetzle (1979)
RUJCI1	Hydrogenated	X-ray	RT	Meervelt <i>et al.</i> (1997)
RUJCI01	Deuterated	X-ray	RT	Meervelt <i>et al.</i> (1997)
SECAZC02	Hydrogenated	Neutron	RT	Roul <i>et al.</i> (1987)
SECAZC03	Deuterated	Neutron	RT	Roul <i>et al.</i> (1988)
URPRBN01	Deuterated	Neutron	116	Weber, Ruble <i>et al.</i> (1980)
URPRBN10	Hydrogenated	X-ray	RT	Colman & Medlin (1970)
WAGMOH01	Hydrogenated	X-ray	276	Ma <i>et al.</i> (2004)
WAGMOH02	Deuterated	X-ray	90	Ma <i>et al.</i> (2004)
WIVDUA01	Deuterated	X-ray	298	Horiuchi <i>et al.</i> (2005)
WIVDUA02	Hydrogenated	X-ray	298	Horiuchi <i>et al.</i> (2005)

Table 2

Space group,  $Z$  number and unit-cell parameters for all isomorphous compounds studied.

The error in the last significant figure is quoted in brackets.

CSD code	H/D	Space group	$Z$	$a$ (Å)	$b$ (Å)	$c$ (Å)	$\alpha$ (°)	$\beta$ (°)	$\gamma$ (°)	Chemical formula
CUHDAK	Hydrogenated	$P2_1/n$	4	9.556 (4)	12.897 (6)	8.128 (3)	90	91.23 (2)	90	$C_5H_{14}As_1N_1O_6$
CUHDAK01	Deuterated	$P2_1/n$	4	9.556 (4)	12.897 (6)	8.128 (3)	90	91.23 (2)	90	$C_5H_{11}D_3As_1N_1O_6$
CYTOSM04	Deuterated	$P2_1/c$	4	7.713 (1)	9.830 (4)	7.505 (4)	90	100.52 (2)	90	$C_4H_2D_3N_3O_2$
CYTOSM11	Hydrogenated	$P2_1/c$	4	7.783 (2)	9.825 (2)	7.668 (2)	90	99.57 (2)	90	$C_4H_7N_3O_2$
DINICA11	Hydrogenated	$P2_1/c$	4	9.7116 (13)	11.1347 (17)	6.4421 (13)	90	108.595 (10)	90	$C_7H_5N_1O_4$
DINICA13	Deuterated	$P2_1/c$	4	9.7196 (13)	11.1396 (17)	6.4540 (13)	90	108.754 (10)	90	$C_7H_3D_2N_1O_4$
DIYJUQ02	Hydrogenated	$Pnma$	4	10.909 (7)	10.029 (3)	10.813 (2)	90	90	90	$C_5H_{15}Ca_1Cl_2N_1O_4$
DIYJUQ03	Deuterated	$Pnma$	4	10.95 (1)	10.15 (1)	10.82 (1)	90	90	90	$C_5D_{15}Ca_1Cl_2N_1O_4$
FORMAC01	Hydrogenated	$Pna2_1$	4	10.241 (1)	3.544 (1)	5.356 (1)	90	90	90	$C_1H_2O_2$
FORMAC02	Deuterated	$Pna2_1$	4	10.2361 (8)	3.5203 (2)	5.3572 (4)	90	90	90	$C_1D_2O_2$
HAZFAP01	Hydrogenated	$P2_1/n$	4	3.800 (1)	11.208 (1)	27.447 (2)	90	92.22 (1)	90	$C_4H_{10}N_2O_4$
HAZFAP03	Deuterated	$P2_1/n$	4	3.793 (1)	11.175 (1)	27.581 (4)	90	91.99 (1)	90	$C_4H_9D_1N_2O_4$
HDRZHO02	Hydrogenated	$P2_1/m\ddagger$	2	3.5792 (3)	13.3228 (16)	5.0965 (3)	90	102.6	90	$C_2H_6N_2O_4$
HDRZHO03	Deuterated	$P2_1\ddagger$	2	3.5858 (5)	13.3085 (16)	5.1001 (5)	90	102.488	90	$C_2D_6N_2O_4$
IMDACB02	Deuterated	$Pmnn$	2	12.8047 (33)	5.8670 (16)	5.0807 (12)	90	90	90	$C_5H_4D_2Br_1N_1O_4$
IMDACB11	Hydrogenated	$Pmnn$	2	12.815 (2)	5.8707 (7)	5.0870 (7)	90	90	90	$C_4H_8Br_1N_1O_4$
IMZMAL11	Hydrogenated	$P2_1/c$	4	10.855 (2)	5.518 (1)	14.616 (4)	90	102.87 (1)	90	$C_7H_5N_2O_4$
IMZMAL13	Deuterated	$P2_1/c$	4	10.858 (1)	5.525 (1)	14.631 (2)	90	102.85 (1)	90	$C_7H_5D_3N_2O_4$
JUQSOD	Hydrogenated	$P2_12_12_1$	4	10.339 (3)	15.495 (2)	4.6265 (10)	90	90	90	$C_6H_{12}O_5$
JUQSOD01	Deuterated	$P2_12_12_1$	4	10.332 (3)	15.446 (2)	4.614 (1)	90	90	90	$C_6H_9D_3O_5$
KECYBU06	Hydrogenated	$P2_1/m$	2	6.143 (2)	5.286 (2)	6.148 (2)	90	89.96	90	$C_4H_2O_4$
KECYBU10	Deuterated	$P2_1/m$	2	6.152 (1)	5.269 (1)	6.165 (1)	90	89.92 (1)	90	$C_4D_2O_4$
KFSCCN01	Deuterated	$Pnam$	4	7.0709 (5)	5.5752 (5)	16.871 (3)	90	90	90	$C_4H_4D_2F_1K_1O_4$
KFSCCN10	Hydrogenated	$Pnam$	4	7.0680 (1)	5.5710 (1)	16.846 (2)	90	90	90	$C_4H_6F_1K_1O_4$
KFURDC	Hydrogenated	$P\bar{1}$	2	8.154 (4)	11.598 (5)	3.7239 (3)	91.75 (3)	100.13 (3)	91.02 (4)	$C_6H_3K_1O_5$
KFURDC01	Deuterated	$P\bar{1}$	2	8.125 (6)	11.583 (5)	3.716 (2)	91.75 (3)	100.24 (4)	91.16 (3)	$C_6H_2D_1K_1O_5$
LABSEM01	Hydrogenated	$P2_1/a$	4	9.793 (1)	8.481 (2)	7.409 (2)	90	100.43 (3)	90	$C_2H_8N_1O_5P_1$
LABSEM02	Deuterated	$P2_1/a$	4	9.824 (1)	8.473 (2)	7.450 (2)	90	100.43 (3)	90	$C_2H_5D_6N_1O_5P_1$
LARGPH01	Hydrogenated	$P2_1$	2	10.898 (3)	7.910 (2)	7.339 (2)	90	97.97 (3)	90	$C_6H_{19}N_4O_7P_1$
LARGPH07	Deuterated	$P2_1$	2	10.843 (3)	7.913 (2)	7.321 (2)	90	97.99 (3)	90	$C_6H_7D_{12}N_4O_7P_1$
LHOXAL01	Hydrogenated	$P1$	1	5.056 (1)	6.140 (1)	3.411 (1)	95.06 (1)	98.93 (1)	78.57 (1)	$C_2H_3Li_1O_5$
LHOXAL02	Deuterated	$P1$	1	5.055 (1)	6.138 (1)	3.410 (1)	95.05 (1)	98.62 (1)	78.57 (1)	$C_2D_3Li_1O_5$
LIACET02	Hydrogenated	$Cmmm$	4‡	6.820 (7)	10.88 (1)	6.620 (7)	90	90	90	$C_2H_7Li_1O_4$
LIACET05	Deuterated	$Cmmm$	2‡	6.82082 (9)	10.88842 (12)	6.59911 (7)	90	90	90	$C_2D_7Li_1O_4$
OXACDH04	Hydrogenated	$P2_1/n$	2	6.119	3.607	12.057	90	106.32	90	$C_2H_6O_6$
OXACDH28	Deuterated	$P2_1/n$	2	6.15	3.605	12.102	90	106.63	90	$C_2D_6O_6$
POVHEN	Hydrogenated	$Pnam$	8	6.4274 (10)	11.826 (2)	17.616 (2)	90	90	90	$C_5H_9N_1O_4$
POVHEN01	Deuterated	$Pnam$	8	6.427 (1)	11.826 (2)	17.616 (2)	90	90	90	$C_5H_5D_4N_1O_4$
PUHROZ	Hydrogenated	$P2_1/c$	4	12.483 (8)	7.551 (5)	16.066 (12)	90	112.15 (7)	90	$C_{14}H_{13}N_3O_6$
PUHROZ02	Deuterated	$P2_1/c$	4	12.488 (7)	7.556 (6)	16.070 (12)	90	112.18(6)	90	$C_{14}H_2D_1N_3O_6$
QUICNA02	Hydrogenated	$P2_1/c$	4	7.415 (5)	12.396 (9)	7.826 (6)	90	117.05 (4)	90	$C_7H_5N_1O_4$
QUICNA11	Deuterated	$P2_1/c$	4	7.422 (2)	12.392 (4)	7.835 (5)	90	117.06 (2)	90	$C_7H_3D_2N_1O_4$
RUJCI	Hydrogenated	$P2_12_12_1$	4	9.547 (1)	10.298 (1)	19.135 (3)	90	90	90	$C_{19}H_{24}N_2O_4$
RUJCI01	Deuterated	$P2_12_12_1$	4	9.641 (3)	10.385 (3)	19.340 (8)	90	90	90	$C_{19}H_{22}D_2N_2O_4$
SECAZC02	Hydrogenated	$P2_12_12_1$	4	7.53 (1)	13.18 (2)	4.67 (1)	90	90	90	$C_1H_6Cl_1N_3O_1$
SECAZC03	Deuterated	$P2_12_12_1$	4	7.55 (1)	13.21 (2)	4.68 (1)	90	90	90	$C_1D_6Cl_1N_3O_1$
URPRBN01	Deuterated	$P2_1/c$	4	8.910 (3)	5.597 (2)	14.525 (6)	90	108.39 (3)	90	$C_4D_6N_4O_4$
URPRBN10	Hydrogenated	$P2_1/c$	4	9.137 (14)	5.623 (3)	15.013 (45)	90	109.13 (3)	90	$C_4H_6N_4O_4$
WAGMOH01	Hydrogenated	$Pca2_1$	4	21.52 (2)	9.645 (7)	6.643 (6)	90	90	90	$C_{10}H_{16}N_4O_6$
WAGMOH02	Deuterated	$Pca2_1$	4	21.483 (3)	9.5933 (14)	6.5701 (10)	90	90	90	$C_{10}H_8D_8N_4O_6$
WIVDUA01	Deuterated	$P2_1/n$	2	12.3997 (9)	3.9144 (3)	17.409 (2)	90	107.882 (6)	90	$C_{18}H_8D_2Br_2N_2O_4$
WIVDUA02	Hydrogenated	$P2_1/n$	2	12.364 (1)	3.9220 (5)	17.395 (2)	90	107.828 (4)	90	$C_{18}H_{10}Br_2N_2O_4$

† Note isomorphous unit cell but change of symmetry in deuterated molecule. ‡ By inspection,  $Z = 2$ , although, for LIACET02, Galigné *et al.* (1970) record  $Z = 4$ .

In any case, this should happen because joint X + N refinement should be the normal option. It remains a limitation of this small-molecule survey that they are not hydrated structures in the same way as proteins are.

Changes in molecular function upon deuteration are known. The effect of deuteration on thermal properties through *structural change* has been documented before and is known as the Ubbelohde effect (Robertson & Ubbelohde, 1939; Katrusiak, 1995). McMahon *et al.* (1998) report the

change in phase-transition temperature due to deuteration for the compound  $KH_2PO_4$  and state how a change in hydrogen-bond geometry is responsible. The structures for  $KH_2PO_4$  and the deuterated  $KD_2PO_4$  can be found in the ICSD (Karlsruhe, 2008). Comparison of the deuterated (ICSD code 201372) and hydrogenated (ICSD code 201370) structures (Nelmes *et al.*, 1982) reveal a change of  $0.73^\circ$  relating to a  $10.3\sigma$  change as previously described by Katrusiak (1995). A survey of isotope effects predominantly on

**Table 3**

Radiation probe, collection temperature (RT = 283–303 K), primary citation, space group, Z number and unit-cell parameters for all three non-isomorphous compounds studied.

CSD code	H/D	Radiation probe	T (K)	Primary citation	Space group	Z	a (Å)	b (Å)	c (Å)	α (°)	β (°)	γ (°)	Chemical formula
AMBONC01	Hydrogenated	Neutron	RT	Schlemper <i>et al.</i> (1971)	<i>P</i> 2 <sub>1</sub> / <i>c</i>	4	12.74 (2)	10.79 (2)	11.81 (2)	90	92.5 (2)	90	C <sub>10</sub> H <sub>25</sub> Cl <sub>1</sub> N <sub>4</sub> Ni <sub>1</sub> O <sub>3</sub>
AMBONC02	Deuterated	Neutron	RT	Hsu <i>et al.</i> (1980)	<i>P</i> 2 <sub>1</sub>	2	11.033 (4)	12.940 (5)	5.862 (2)	90	101.97 (1)	90	C <sub>10</sub> H <sub>18</sub> D <sub>7</sub> Cl <sub>1</sub> N <sub>4</sub> Ni <sub>1</sub> O <sub>3</sub>
GADGUN	Hydrogenated	X-ray	RT	Malarski <i>et al.</i> (1987)	<i>P</i> $\bar{1}$	2	7.408 (6)	8.934 (7)	13.653 (9)	100.15 (6)	118.50 (6)	103.67 (6)	C <sub>12</sub> H <sub>8</sub> Cl <sub>5</sub> N <sub>1</sub> O <sub>1</sub>
GADGUN01	Deuterated	X-ray	RT	Majerz <i>et al.</i> (1990)	<i>C</i> <i>c</i>	4	3.942 (3)	27.73 (3)	13.297 (9)	90	101.81 (6)	90	C <sub>12</sub> H <sub>7</sub> D <sub>1</sub> Cl <sub>5</sub> N <sub>1</sub> O <sub>1</sub>
HAXFER	Hydrogenated	X-ray	RT	Jerzykiewicz <i>et al.</i> (1993)	<i>P</i> $\bar{1}$	2	9.409 (9)	10.813 (7)	12.310 (7)	95.75 (5)	108.17 (7)	91.46 (7)	C <sub>21</sub> H <sub>17</sub> N <sub>5</sub> O <sub>12</sub>
HAXFER01	Deuterated	X-ray	RT	Jerzykiewicz <i>et al.</i> (1993)	<i>P</i> 2 <sub>1</sub> / <i>c</i>	4	11.680 (6)	8.451 (4)	24.382 (9)	90	102.94 (4)	90	C <sub>21</sub> H <sub>15</sub> D <sub>2</sub> N <sub>5</sub> O <sub>12</sub>

bond distances in hydrogen bonds has been made by Ichikawa (2000).

In summary, in order to assess whether or not deuteration affects structure, the CSD (CCDC, 2007) has been data mined for deuterated structures and their hydrogenated counterparts, and their X–O–D and X–N–D bond angles have been analysed in order to determine whether any significant and meaningful change has occurred.

## 2. Data mining

The Cambridge Structural Database was initially data mined for all structures containing either an O–D or an N–D bond; this resulted in roughly 1000 structures being found. From these 1000 structures, the hydrogenated counterpart was searched for, resulting in roughly 100 structures with both hydrogenated and deuterated forms and the Crystallographic Information Files (CIFs) for these 200 compounds were downloaded. The unit-cell parameters of each of the hydrogenated and deuterated forms were compared and compounds with large non-isomorphous differences were kept separate for separate analysis. The hydrogenated forms were checked for the presence of H atoms and if the H atoms were in calculated or real positions. Those in calculated positions were dismissed. This resulted in 26 compounds with both hydrogenated and deuterated forms, with H atoms in non-calculated positions and with isomorphous unit-cell parameters.

The bond angles for each compound were then calculated using the validation and analysis tool *PLATON* (Spek, 2003). The results were filtered for A–O–D/H and A–N–H/D bond angles and the statistical  $\sigma$  level difference was calculated between the deuterated and the hydrogenated bond forms. A standard statistical  $3\sigma$  test was used to identify significant differences between hydrogenated and deuterated structures. The equation used to calculate these differences is

$$\sigma \text{ level} = \frac{|\theta_2 - \theta_1|}{\sqrt{\sigma_{\theta_1}^2 + \sigma_{\theta_2}^2}}. \quad (1)$$

Following this, a secondary cut off of  $\Delta\theta > 5^\circ$  was used in order to identify meaningful differences within this signifi-

cantly screened subgroup. As X-ray structures in chemical crystallography can determine atomic positions to 0.001 Å, the errors can be as low as 0.002 Å and hence bond angles in some cases can be determined to 0.03°. This means that a  $\sigma$  level of  $3\sigma$  can be obtained from a bond-angle difference of  $<1^\circ$ , hence the need for a ‘meaningful’ cut-off.

Table 1 shows the data-mining collection parameters and the primary citation for each of the 52 isomorphous compounds studied and Table 2 shows crystallographic parameters for each of these compounds. Table 3 shows the data-mining collection parameters, primary citation and crystallographic parameters for each of the non-isomorphous compounds studied.

## 3. Results and discussion

At the outset of our study, the effect of deuteration on ferroelectric transition temperature provided an impetus. One of the compounds studied here, WIVDUA (Horiuchi *et al.*, 2005), had been previously investigated for its significant change of 100 K in its ferroelectric phase transition temperature,  $T_c$ , between the hydrogenated and deuterated forms. Horiuchi *et al.*, however, concluded that the change in hydrogen-bond geometry does not fully explain the change in phase-transition temperature for the compound studied in that research, and stated that the mechanism for this compound eludes current knowledge. However, there is no significant change in bond angles, with largest  $\Delta\theta = 9^\circ$  and  $\sigma(\Delta\theta) = 5.8^\circ$ , thus the  $\sigma$  significance level is only  $1.54\sigma$ . It is not discussed further here for this reason.

### 3.1. Isomorphous differences

Table 4 shows nine compounds with a  $\sigma$  level of significance greater than  $3\sigma$  and Table 5 shows four compounds with a  $\sigma$  level greater than  $2\sigma$ . These are discussed in turn.

#### CYTOSM

This compound has bond angles defined with errors as low as 0.03°; hence, with even very small angular changes, large  $\sigma$  levels of significance are produced. The NH<sub>2</sub> group has

**Table 4**  
Bond-angle differences with  $\sigma$  level  $>3$ ; meaningful changes ( $>5^\circ$ ) shown in bold.

CSD code	Bond	$\Delta\theta$ ( $^\circ$ )	$\sigma(\Delta\theta)$ ( $^\circ$ )	$\sigma$ level of significance	Collection temperature
CYTOSM	C(1)—N(1)—H(1)	1.20	0.10	11.49	D (82 K), H (RT)
	C(4)—N(1)—H(1)	0.99	0.10	9.48	
	C(2)—N(3)—H(4)	2.60	0.11	22.80	
	C(2)—N(3)—H(5)	0.49	0.11	4.30	
	H(4)—N(3)—H(5)	2.97	0.15	20.40	
	H(2)—O(1)—H(3)	1.66	0.16	10.20	
FORMAC HDRZHO*	C(1)—O(1)—H(1)	0.85	0.21	4.12	D (4.5 K), H (98) D (RT), H (RT)
	<b>N(2)—N(1)—H(1)</b>	<b>5.70</b>	<b>0.23</b>	<b>24.76</b>	
	N(2)—N(1)—H(1) <i>b</i>	1.06	0.23	4.60	
	H(1)—N(1)—H(1) <i>b</i>	2.29	0.25	9.00	
	N(1)—N(2)—H(2)	4.39	0.21	21.02	
	N(1)—N(2)—H(3)	2.67	0.26	10.42	
	<b>N(1)—N(2)—H(2)<i>b</i></b>	<b>9.15</b>	<b>0.17</b>	<b>53.55</b>	
	H(2)—N(2)—H(3)	3.88	0.19	19.98	
	<b>H(2)—N(2)—H(2)<i>b</i></b>	<b>9.53</b>	<b>0.43</b>	<b>22.31</b>	
	H(3)—N(2)—H(2) <i>b</i>	2.92	0.30	9.65	
	IMDACB*	C(2)—N(1)—H(1)	1.52	0.16	9.27
C(2)—N(1)—H(1) <i>a</i>		1.53	0.16	9.33	
<b>H(1)—N(1)—H(1)<i>a</i></b>		<b>7.10</b>	<b>0.58</b>	<b>12.18</b>	
C(2) <i>a</i> —N(1)—H(1) <i>a</i>		1.53	0.16	9.33	
KECYBU	C(1)—O(1)—H(1)	0.35	0.10	3.54	D (RT), H (RT)
	C(2)—O(2)—H(2)	0.54	0.11	5.08	
LARGPH*	<b>C(2)—N(1)—H(13)</b>	<b>9.60</b>	<b>3.06</b>	<b>3.14</b>	D (RT), H (RT)
	<b>H(6)—N(4)—H(7)</b>	<b>20.60</b>	<b>5.10</b>	<b>4.04</b>	
POVHEN	H(6)—N(1)—H(7)	3.30	0.95	3.48	D (122.4 K), H (122.4 K)
QUICNA	C(1)—N(1)—H(4)	0.83	0.16	5.14	D (100 K), H (100 K)
	C(5)—N(1)—H(4)	0.63	0.17	3.61	
SECAZC*	C(1)—N(1)—H(1)	4.60	0.57	8.13	D (RT), H (RT)
	C(1)—N(1)—H(2)	4.50	0.58	7.72	
	<b>H(1)—N(1)—H(2)</b>	<b>8.30</b>	<b>0.64</b>	<b>12.96</b>	
	N(2)—N(3)—H(4)	4.70	0.64	7.34	
	H(4)—N(3)—H(5)	4.20	0.57	7.42	
	<b>H(4)—N(3)—H(6)</b>	<b>7.60</b>	<b>0.72</b>	<b>10.54</b>	
	<b>H(5)—N(3)—H(6)</b>	<b>6.90</b>	<b>0.78</b>	<b>8.83</b>	

**Table 5**  
Bond angles with  $\sigma$  level  $>2$  and  $<3$ ; meaningful changes ( $>5^\circ$ ) shown in bold.

CSD code	Bond	$\Delta\theta$ ( $^\circ$ )	$\sigma(\Delta\theta)$ ( $^\circ$ )	$\sigma$ level	Collection temperature
IMDACB	C(1)—O(2)—H(3)	1.60	0.58	2.74	D (RT), H (RT)
	H(1)—N(1)—H(3)	2.00	0.92	2.17	
	H(2)—N(1)—H(3)	2.70	0.92	2.93	
LARGPH	<b>H(12)—N(1)—H(14)</b>	<b>10.20</b>	<b>4.08</b>	<b>2.50</b>	D (RT), H (RT)
OXACDH	C(1)—O(1)—H(1)	1.60	0.61	2.63	D (RT), H (RT)
RUJCII*	<b>C(2)—O(1)—H(3)</b>	<b>5.60</b>	<b>2.33</b>	<b>2.40</b>	D (RT), H (RT)
	N(2)—N(3)—H(6)	0.80	0.36	2.22	

changed by  $3^\circ$  resulting in a  $\sigma$  level of 20.4. The movement of the water molecule can presumably be ignored as water of crystallization can change between crystals.

#### FORMAC

This compound has a  $\Delta\theta$  of  $<1^\circ$  and hence suffers from the same circumstance as CYTOSM. This minute change is unlikely to be meaningful.

#### HDRZHO

Although all the  $\sigma$  significance levels are  $>4\sigma$ , the majority of the angular differences are less than  $5^\circ$ . The two N—N—H and the H—N—H bond angles are, however, potentially interesting with  $\Delta\theta$  values of 5.7, 9.15 and  $9.53^\circ$ , respectively.

#### IMDACB

This compound again has very high  $\sigma$  significance levels, all  $>9\sigma$ ; however, all except one of the bond angles has  $\Delta\theta < 5^\circ$ . The bond angle of interest, namely the H—N—H<sub>a</sub> bond, deviates between the hydrogenated and deuterated forms by  $7.1^\circ$ .

#### KECYBU

Both the C—OH bond angles deviate by  $<1^\circ$  and even though they have significant  $\sigma$  significance levels are unlikely to be meaningful.

#### LARGPH

The H—N—H bond angle in this compound has changed by a relatively large  $20.6^\circ$  between hydrogenated and deuterated forms. Even though the bond-angle standard uncertainties are  $5^\circ$ , this still relates to a  $\sigma$  significance level of  $>3\sigma$ . There is also a C—N—H bond angle that varies by  $9.6^\circ$ .

#### POVHEN

The single bond angle with a  $3\sigma$  significant difference only has a  $\Delta\theta$  of  $3^\circ$ , implying that this particular change is most probably meaningless.

#### QUICNA

The  $\Delta\theta$  values for the two C—N—H bond angles are both  $<1^\circ$  and show a  $3\sigma$  significance due to the very small uncertainties on each bond angle. These changes are very unlikely to be meaningful.

#### SECAZC

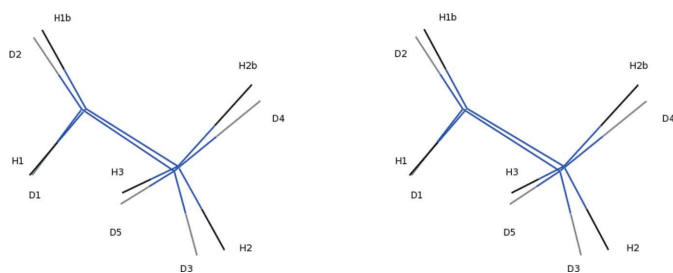
This compound shows deviations greater than  $5^\circ$  on three N—H—N bond angles and all the deviations with  $>3\sigma$  significant differences have  $\Delta\theta > 4^\circ$ . The primary citation for this paper was followed by an erratum (Roul *et al.*, 1989). They detailed how hydrogen-bond geometry had been incorrectly published, however no updated atomic

coordinates were given. This erratum therefore should not affect our analysis as bond angles have been calculated directly from the associated CIF files as opposed to data from within the papers.

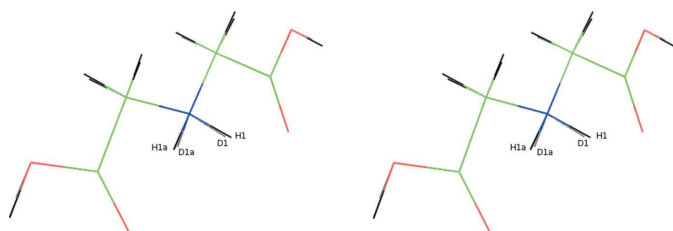
As well as the compounds with a bond-angle difference at  $3\sigma$ , those at  $2\sigma$  were also considered. Table 5 shows the compounds that had  $2\sigma$  differences, as can be seen the first two compounds also appear in the  $3\sigma$  table; however, OXACDH and RUJCII are new additions, OXACDH only shows  $\Delta\theta = 1.6^\circ$  and hence is unlikely to be a meaningful change, RUJCII shows movement of a C—O—H by slightly greater than  $5^\circ$ , which is potentially interesting.

Of the 26 compounds studied, 5 appear to have meaningful and  $3\sigma$  significance changes with  $\Delta\theta > 5^\circ$ . These compounds encompass all four combinations of radiation probe and structure type (X-ray, neutron, hydrogenated, deuterated) and as such show no weakness in the standard uncertainty assessment. For each of these five compounds, the hydrogenated and deuterated forms were superimposed using the CCP4 program *SUPERPOSE* (Collaborative Computational Project, Number 4, 1994), the results are shown below. The labelling of the H and D atoms is arbitrary.

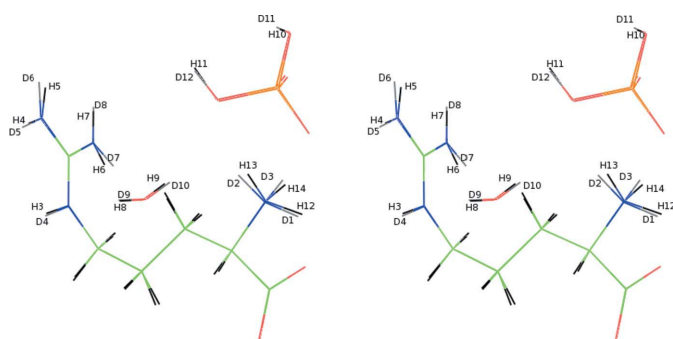
Fig. 1 shows the superimposed HDRZHO structures; the deuterated structure seems to have a slightly distorted tetrahedral geometry about the  $\text{NH}_3^+$  ion in comparison to the hydrogenated structure. The H–N–H bond angle is also slightly smaller for the  $\text{NH}_2$  group in the deuterated case.



**Figure 1**  
Stereoview (cross-eye viewing) of the superimposed hydrazinium hydrogen oxalate (HDRZHO) structures; the change of  $9.53^\circ$  involves H2 N2 H2b, the change of  $9.15^\circ$  involves N1 N2 H2b and the change of  $5.70^\circ$  involves N2 N1 H1.



**Figure 2**  
Stereoview (cross-eye viewing) of the superimposed iminodiacetic acid hydrobromide (IMDACB) structures. The change of  $7.10^\circ$  involves H1 N1 H1a.



**Figure 3**  
Stereoview (cross-eye viewing) of the superimposed L-arginine phosphate monohydrate (LARGPH) structures. The change of  $20.60^\circ$  involves H6 N4 H7 and the change of  $9.60^\circ$  involves C2 N1 H13.

Interestingly, however, the replacement of hydrogen with deuterium has resulted in a distortion away from symmetry with the loss of the mirror plane in the structure due to the lengthening and rotation of one of the N–H bonds about the hydrazinium ion.

Fig. 2 shows the superimposed IMDACB structures; the carboxylic acid C–O–H bond angle is virtually identical for the deuterated and hydrogenated forms, however the protonated amino group does slightly differ between the two.

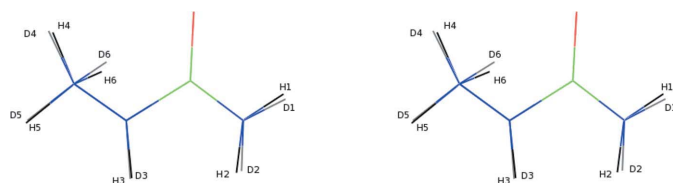
Fig. 3 shows the LARGPH superimposed structures; there are visible deviations of the H/D atoms present on all of the N atoms in the structure. These deviations, although the largest out of all the compounds studied, still only equate to a movement in atomic position of  $0.31 \text{ \AA}$  between atoms D7 and H6.

Fig. 4 shows the SECAZC superimposed structures; the major deviations occur on the terminal  $\text{NH}_3^+$  and  $\text{NH}_2$  groups; however, these deviations although significant are still rather minor, the largest equating to a shift in atomic position by  $0.18 \text{ \AA}$  between the H6 and D6 atoms.

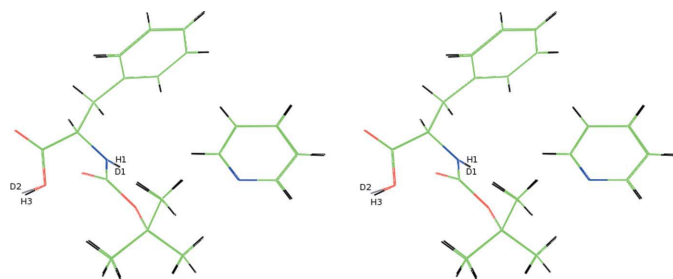
Fig. 5 shows the superimposed RUJCII structures; although only at a  $2\sigma$  difference there is still an angular difference of  $>5^\circ$  at the C–O–H bond. Although greater than  $5^\circ$ , the actual shift in atomic position relates to only  $0.16 \text{ \AA}$  between D2 and H3 which is unlikely to be meaningful.

### 3.2. Non-isomorphous differences

Compounds with non-isomorphous unit cells were also studied. These compounds obviously required structure superimposition. Bond-angle-analysis results are shown in Table 6.



**Figure 4**  
Stereoview (cross-eye viewing) of the superimposed semicarbazide hydrochloride (SECAZC) structures. The change of  $8.30^\circ$  involves H1 N1 H2, the change of  $7.60^\circ$  involves H4 N3 H6 and the change of  $6.90^\circ$  involves H5 N3 H6.



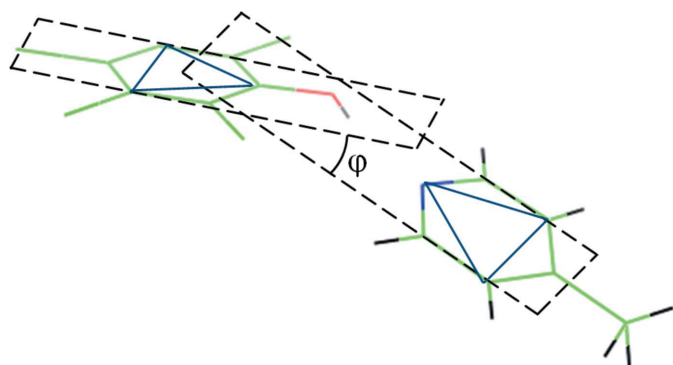
**Figure 5**  
Stereoview (cross-eye viewing) of the superimposed *n*-*tert*-butoxycarbonyl-L-phenylalanine and pyridine complex (RUJCII) structures. The change of  $5.60^\circ$  involves C2 O1 H3.

**Table 6**

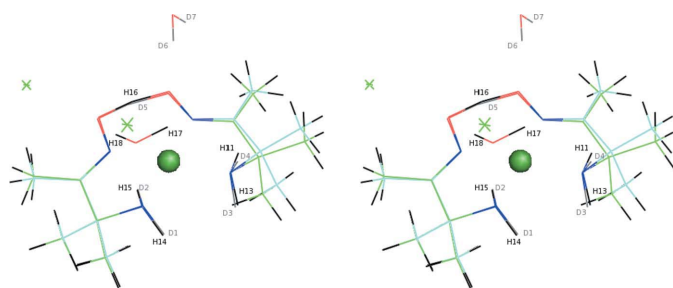
 Bond-angle differences for the non-isomorphous compounds studied; meaningful changes ( $>5^\circ$ ) shown in bold.

CSD code	Bond	$\Delta\theta$ ( $^\circ$ )	$\sigma(\Delta\theta)$ ( $^\circ$ )	$\sigma$ level of significance	
AMBONC	N(1)–O(1)–H(16)	2.00	0.67	2.98	
	Ni(1)–N(3)–H(11)	8.20	0.42	19.33	
	<b>Ni(1)–N(3)–H(13)</b>	<b>10.20</b>	<b>0.42</b>	<b>24.04</b>	
	C(4)–N(3)–H(11)	0.30	0.42	0.71	
	C(4)–N(3)–H(13)	1.20	0.42	2.83	
	H(11)–N(3)–H(13)	2.30	0.57	4.07	
	Ni(1)–N(4)–H(14)	4.80	0.42	11.31	
	Ni(1)–N(4)–H(15)	4.30	0.42	10.14	
	C(5)–N(4)–H(14)	0.50	0.42	1.18	
	C(5)–N(4)–H(15)	0.10	0.50	0.20	
	H(14)–N(4)–H(15)	2.10	0.57	3.71	
	<b>H(17)–O(3)–H(18)</b>	<b>5.40</b>	<b>0.94</b>	<b>5.72</b>	
	GADGUN	<b>C(1)–O(1)–H(1)</b>	<b>7.00</b>	<b>4.24</b>	<b>1.65</b>
	HAXFER	<b>C(21)–O(7)–H(10)</b>	<b>5.00</b>	<b>6.32</b>	<b>0.79</b>
	<b>C(5)–N(1)–H(1)</b>	<b>5.00</b>	<b>4.47</b>	<b>1.12</b>	

The AMBONC structures show a number of significant changes with  $\sigma$  levels of significance as high as 24. The maximum change in  $\Delta\theta$  is  $10.2^\circ$  and is meaningful. Most of these changes are the result of rotation of the H atoms about the  $\text{CH}_3$  methyl groups, however there is also the rotation of the  $\text{C}(\text{CH}_3)_3$  group. The Ni coordination site remains generally unchanged between the two structures.



**Figure 6**  
Example dihedral plane angle for the compound GADGUN.



**Figure 7**  
Stereoview (cross-eye viewing) of the superimposed bis(3-amino-3-methyl-2-butanone oximato)nickel(II) chloride monohydrate (AMBONC) structures. Deuterated structure shown in blue and hydrogenated in green.

**Table 7**

Dihedral plane angle analysis.

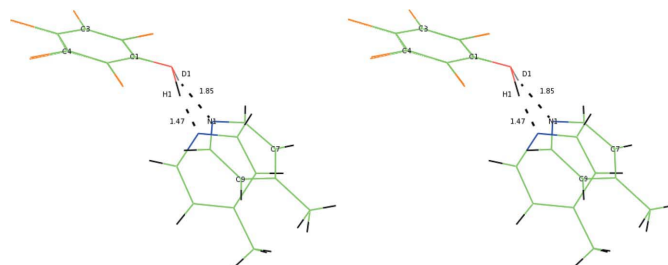
CSD code	Plane 1	Plane 2	Dihedral $\Delta\theta$ ( $^\circ$ )	Dihedral $\sigma(\Delta\theta)$ ( $^\circ$ )	$\sigma$ level of significance
GADGUN	N1 C7 C9	C1 C3 C4	39.60	0.42	93.34
HAXFER	N1 C2 C4	C8 C10 C12	59.60	0.45	133.27
	N1 C2 C4	C16 C18 C20	23.50	0.45	52.55
	C8 C10 C12	C16 C18 C20	42.10	0.45	94.14

Both GADGUN and HAXFER show little in the way of significant changes in bond angle between the hydrogenated and deuterated forms. They do, however, show significant differences in crystal packing (Fig. 6). In order to statistically analyse these differences, dihedral angles were calculated between the molecule centroids along with the associated error. These were calculated by selecting three atoms lying within a plane for each centroid and then using the 'dihedral' option in *PLATON*. From these values, a  $\sigma$  level of significance was calculated using equation (2). See Table 7.

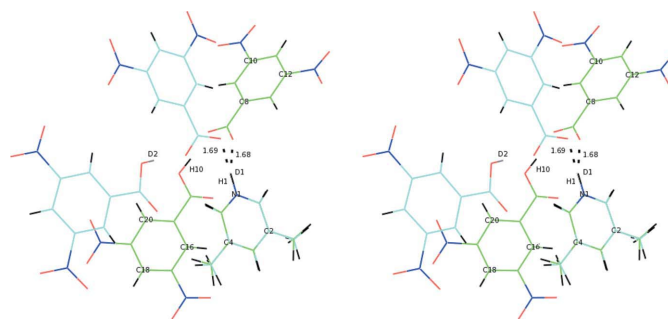
$$\sigma \text{ level} = \frac{|\phi_{\text{H}} - \phi_{\text{D}}|}{\sqrt{\sigma_{\phi_{\text{H}}}^2 + \sigma_{\phi_{\text{D}}}^2}} \quad (2)$$

#### AMBONC

The deuterated and hydrogenated forms of AMBONC (Fig. 7) are very similar despite differences in crystal packing.



**Figure 8**  
Stereoview (cross-eye viewing) of the superimposed 4-methylpyridine with pentachlorophenol (GADGUN) structures. Deuterated structure shown in blue and hydrogenated in green.



**Figure 9**  
Stereoview (cross-eye viewing) of the superimposed 3,5-dimethylpyridine and 3,5-dinitrobenzoic acid complex (HAXFER) structures. The deuterated structure (in blue) lies on a plane, the hydrogenated structure (in green) however does not.



The only major difference between the asymmetric units is the slight shift of one of the CCH<sub>3</sub> groups and the apparent formation of a hydrogen bond to the water molecule.

#### GADGUN

The components of the asymmetric unit are virtually identical in the deuterated and hydrogenated cases. The packing of the components is however significantly different with the dihedral angle between the two centroids differing by 39.6° between the hydrogenated and deuterated forms (Fig. 8).

#### HAXFER

In a similar manner to GADGUN, the components of the asymmetric unit are virtually identical; however, there is once again significant difference in crystal packing (Fig. 9). In the case of the deuterated structure, all three components of the asymmetric unit lie on a plane, in the hydrogenated case they do not. The difference in dihedral angle associated with this change equates to 59.60°.

The large changes in dihedral angles for the GADGUN and HAXFER compounds, although significant are unlikely to be caused solely by deuteration. It is more likely that in these cases a different crystal form has occurred by chance as opposed to by deuteration of the crystal.

### 3.3. Scrutiny of possible structure differences with temperature

The individual structures are sometimes at different temperatures. The question arises to what extent structural changes occur due to that difference rather than a possible deuteration effect. Also, there is the possibility that a deuteration effect is masked by a temperature difference. Within this set of comparison structures, some include hydrogenated or deuterated versions at different temperatures, e.g. LIACET05-D (298 and 1.5 K). The LABSEM structures were collected at 180 and 295 K (H) and 296 and 360 K (D) in order to study the paraelectric and ferroelectric phases. The DINICA structures were studied at 15, 150 and 296 K for both the hydrogenated and deuterated forms and the DIYJUQ03-D compound was studied at both 35 and 293 K. As Tables 4 and 5 show, none of these pairs feature and therefore temperature-induced structural changes can be ruled out as being more than deuteration induced.

In protein crystallography, identical protein structures studied at 100 K and room temperature do show differences in side-chain multiple ordered states (usually two conformers) for approximately 10% of the side chains, sometimes with attached bound waters moving as a result (Deacon *et al.*, 1997). Such temperature-induced differences have been studied in detail by Dunlop *et al.* (2005) of a 'PAK pilin' protein and they conclude that it is advisable to collect both room-temperature and cryo diffraction data for optimum detail (cryo) and characterization of structural changes between cryo and (near-)physiological temperature. Skrzypczak-Jankun *et al.* (2006) performed such comparisons from the point of view of structure-based drug design and conclude that using a (protein) receptor structure determined based on cryogenic data as a target for computational

screening requires flexible docking to enable the expansion of the binding-site cavity and sampling of the alternative conformations of the crucial residues.

With reference to our detailed analyses presented here, we highlight at this point that protein atomic displacement parameters ('*B*' factors) also systematically decrease on cooling. However, since the CSD does not provide such *B*-factor details for small-molecule structures, we have only been able to analyse for possible changes in structure (bond distances and, principally, angles) with temperature and not *B* factors.

## 4. Conclusions

Of the 26 isomorphous compounds studied, four have both significant (>3σ) and meaningful changes with 5 < Δθ < 21°. However, with the shift in atomic position between the relevant H and D atoms being at maximum 0.31 Å, it is unlikely that this level of structural change would cause any anxiety in terms of neutron protein crystallography. Two of the three non-isomorphous compounds did show significant changes in crystal packing and orientation, however it is not possible to deduce whether these changes were caused by deuteration or not, but it is more likely that another crystal form has occurred by chance.

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