

A Strong ^{13}C Chemical Shift Signature Provides the Coordination Mode of Histidines in Zinc-Binding Proteins

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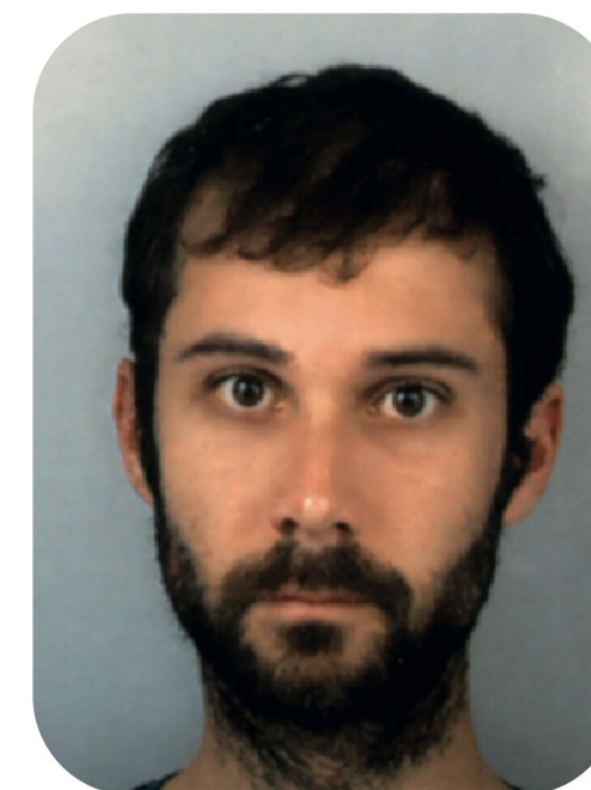
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Abstract

Zinc is the second most abundant metal ion incorporated in proteins, and is in many cases a crucial component of protein three-dimensional structures. Zinc ions are frequently coordinated by cysteine and histidine residues. Whereas cysteines bind to zinc via their unique S^γ atom, histidines can coordinate zinc with two different coordination modes, either $\text{N}^{\delta 1}$ or $\text{N}^{\epsilon 2}$ is coordinating the zinc ion. The determination of this coordination mode is crucial for the accurate structure determination of a histidine-containing zinc-binding site by NMR. NMR chemical shifts contain a vast amount of information on local electronic and structural environments and surprisingly their utilization for the determination of the coordination mode of zinc-ligated histidines has been limited so far to ^{15}N nuclei. In the present report, we observed that the $^{13}\text{C}^{\delta 2}$ chemical shift is sensitive to the histidine coordination mode and that the chemical shift difference $\delta\{^{13}\text{C}^{\epsilon 1}\} - \delta\{^{13}\text{C}^{\delta 2}\}$ provides a reference independent marker of this coordination mode. The present approach allows the direct determination of the coordination mode of zinc-ligated histidines even with non-isotopically enriched protein samples and without any prior structural information.

1. $\text{N}^{\delta 1}$ vs. $\text{N}^{\epsilon 2}$ coordination mode

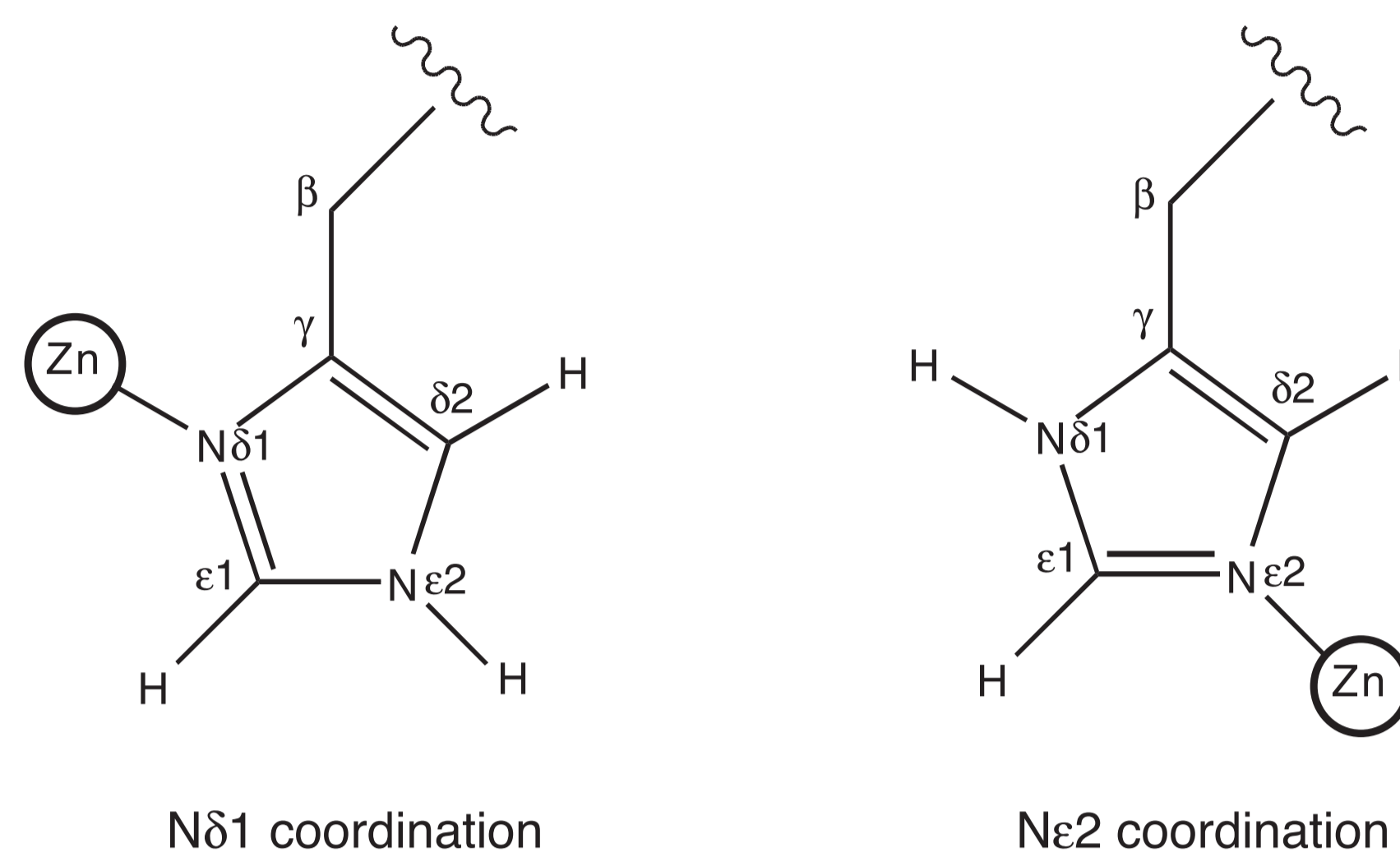


Fig. 1 Two different coordination modes of histidines

Contrary to cysteine ligands, for which the atom that directly contacts the zinc ion is unique (Cys S^γ), histidine ligands bind to zinc ions with two different coordination modes involving either one or the other of the two endocyclic nitrogens $\text{N}^{\delta 1}$ or $\text{N}^{\epsilon 2}$ (Figure 1). It is important to notice that these coordination modes are intrinsically associated with the tautomeric form of the histidine ring, where a $\text{N}^{\delta 1}$ coordination mode is uniquely compatible with a protonation on $\text{N}^{\epsilon 2}$ and vice versa. Since the two endocyclic nitrogens are 2.2 Å apart, the correct determination of the histidine coordination mode is of primary importance for the accurate structure determination by NMR of a histidine-containing zinc-binding site.

2. Currently used methods for coordination mode determination

Method 1: Preliminary structure calculation assuming one or the other histidine coordination modes. Further NOE violation analysis.

Drawbacks: Indirect method. Can be influenced by homologous structures. NOE violation analysis may not be sufficient to unambiguously determine histidine coordination modes.

Method 2: Determination of the histidine tautomeric state using ^{15}N chemical shifts measured via long-range ^{15}N - ^1H correlation.

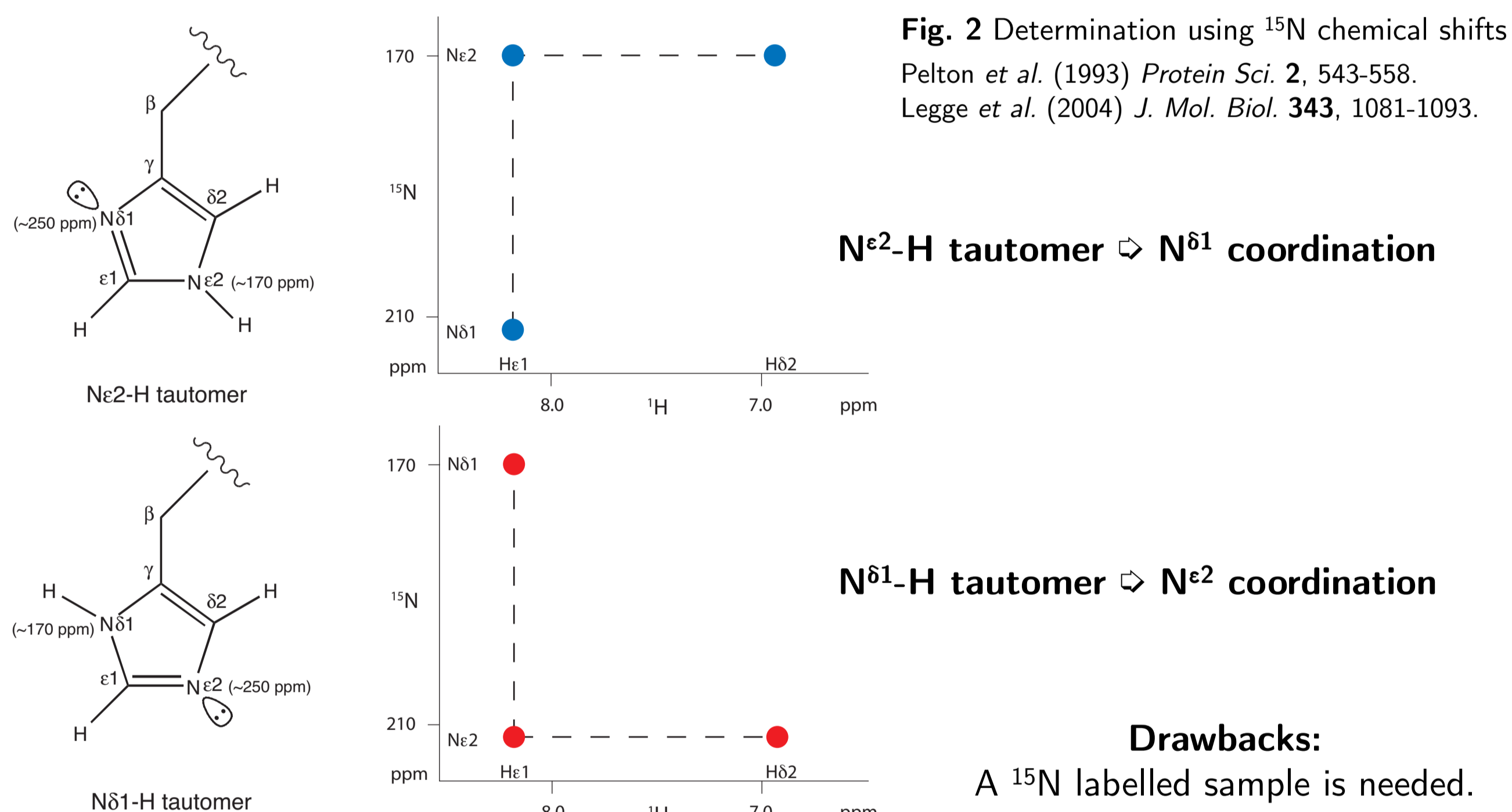


Fig. 2 Determination using ^{15}N chemical shifts Pelton *et al.* (1993) *Protein Sci.* 2, 543-558. Legge *et al.* (2004) *J. Mol. Biol.* 343, 1081-1093.

$\text{N}^{\epsilon 2}\text{-H}$ tautomer \rightarrow $\text{N}^{\delta 1}$ coordination

$\text{N}^{\delta 1}\text{-H}$ tautomer \rightarrow $\text{N}^{\epsilon 2}$ coordination

Drawbacks:
A ^{15}N labelled sample is needed.

3. Initial questions and genesis of the project

In the structure determination process of a protein domain with an unexpected zinc-binding site (Barraud *et al.* 2011), we noticed the presence of a downfield shifted $\text{C}^{\delta 2}$ of a histidine side chain ($\text{C}^{\delta 2} = 126.1$ ppm). This is rather unusual in proteins where most histidine $\text{C}^{\delta 2}$ have chemical shifts around 120 ppm. We wondered whether this downfield shifted $\text{C}^{\delta 2}$ was a characteristic of zinc-coordinated histidines. On one hand, downfield shifted $\text{C}^{\delta 2}$ in histidines are sometimes presented as a characteristic of zinc-coordinated histidines. But on the other hand, we also noticed numerous examples where zinc coordination was not associated with a particularly downfield shifted $\text{C}^{\delta 2}$, showing that confusion exists in the literature about this point.

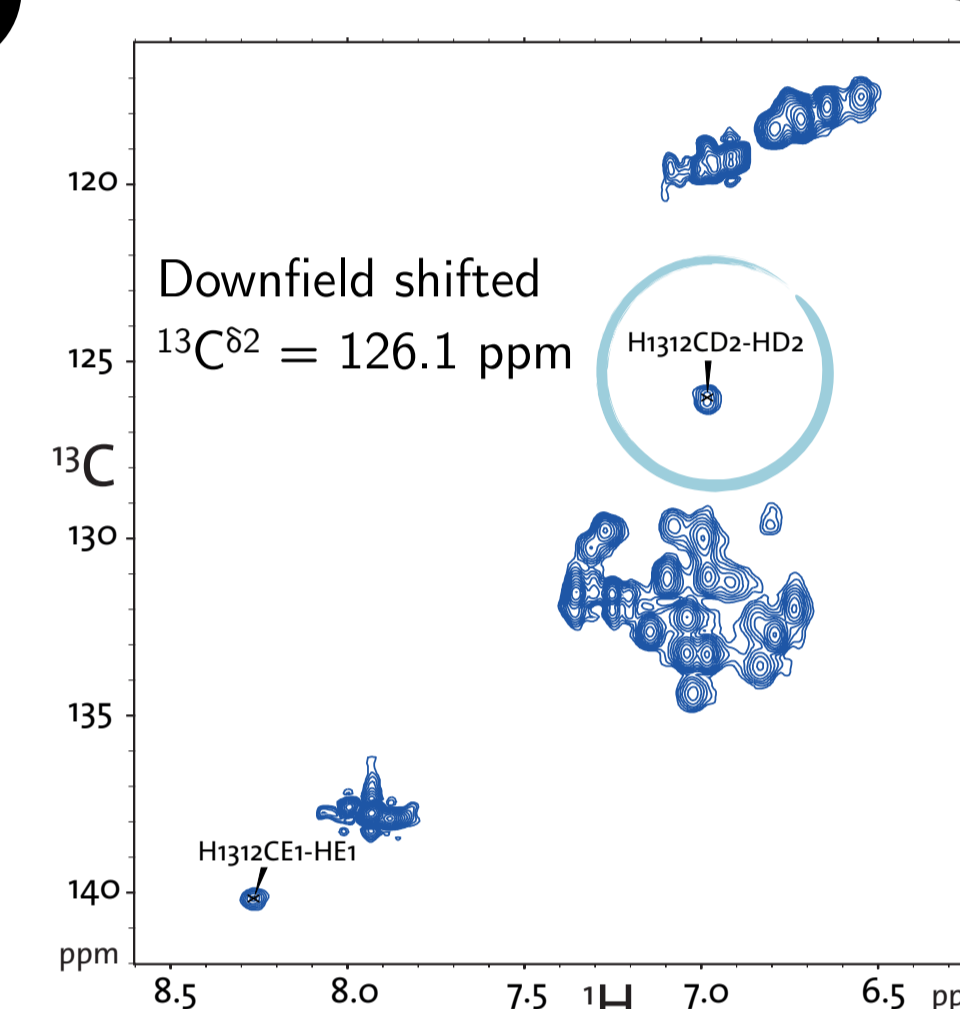


Fig. 3 Aromatic ^{13}C - ^1H HSQC

4. Chemical shift of $\text{C}^{\delta 2}$ aromatic carbon is a sensitive probe of histidine coordination mode

In order to understand the exact correlation between zinc-coordination, tautomeric form and downfield shifted $\text{C}^{\delta 2}$ in histidines, we decided to perform a systematic survey of the chemical shifts of aromatic carbons in zinc-coordinated histidines. For this, we identified zinc-coordinated histidines in all entries of the BMRB and extracted their ^{13}C aromatic carbon chemical shifts, namely $\text{C}^{\delta 2}$ and $\text{C}^{\epsilon 1}$.

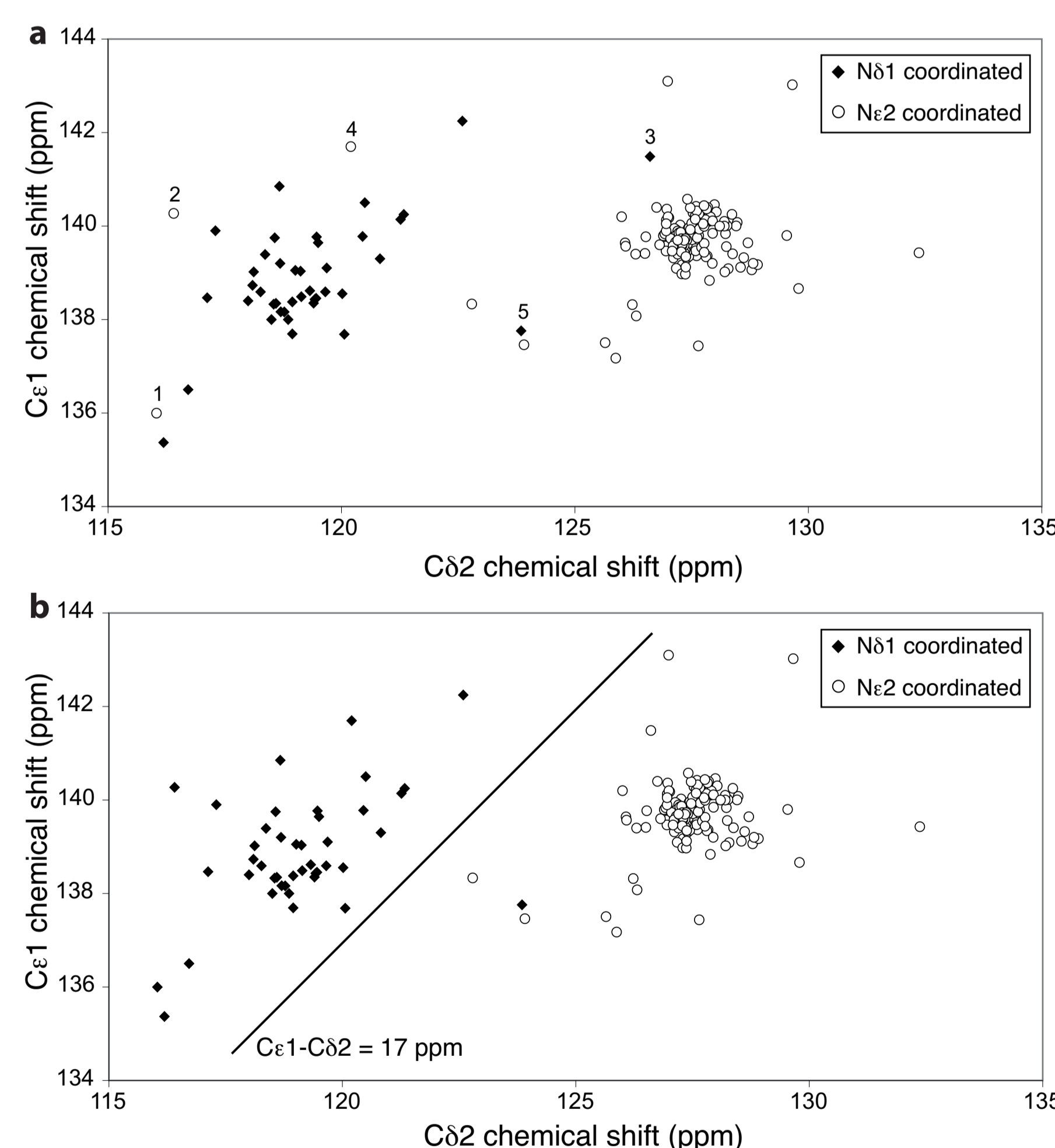


Fig. 4 Two dimensional plots of $^{13}\text{C}^{\delta 2}$ and $^{13}\text{C}^{\epsilon 1}$ chemical shifts of zinc-coordinated histidines as a function of the coordination mode.

a Initial database prior to any manual corrections.
b Final corrected database showing a clear distinction between the two coordination modes of histidines. The solid line with its corresponding equation ($\text{C}^{\epsilon 1} - \text{C}^{\delta 2} = 17$ ppm) delineate the two clusters of the $\text{N}^{\delta 1}$ and $\text{N}^{\epsilon 2}$ coordination modes.

	$\text{N}^{\delta 1}$ coordination		$\text{N}^{\epsilon 2}$ coordination	
	$^{13}\text{C}^{\delta 2}$	$^{13}\text{C}^{\epsilon 1}$	$^{13}\text{C}^{\delta 2}$	$^{13}\text{C}^{\epsilon 1}$
Number of shifts	43	43	223	223
Average δ (ppm)	119.1	138.9	127.4	139.7
Min value δ_{min} (ppm)	116.0	135.4	122.8	137.2
Max value δ_{max} (ppm)	123.8	142.2	132.4	143.1
Standard dev. σ_{δ} (ppm)	1.54	1.30	0.75	0.59

Table 1 Summary of the statistical values of the $^{13}\text{C}^{\delta 2}$ and $^{13}\text{C}^{\epsilon 1}$ chemical shifts for 266 histidines coordinating zinc via $\text{N}^{\delta 1}$ or $\text{N}^{\epsilon 2}$.

The two-dimensional landscape on Figure 4 shows that $^{13}\text{C}^{\delta 2}$ chemical shift in zinc-coordinated histidines constitutes an extremely potent probe for the determination of the coordination mode of histidines. However, the chemical shift is a relative measure that depends strongly on correct calibration to a standard. Incorrect chemical shift referencing could then potentially lead to a wrongly predicted coordination mode of histidines if the $^{13}\text{C}^{\delta 2}$ chemical shift were used exclusively. To circumvent this problem, we sought to establish a simple reference-independent parameter which will constitute a more robust probe for the determination of the coordination mode of histidines.

5. The chemical shift difference $\text{C}^{\epsilon 1}\text{-C}^{\delta 2}$ is a reference independent marker of the coordination mode of histidines

As the $^{13}\text{C}^{\epsilon 1}$ chemical shift is rather insensitive to the coordination mode of histidines (Figure 4; Table 1), the chemical shift difference $\text{C}^{\epsilon 1} - \text{C}^{\delta 2}$ retains all the information content of the $^{13}\text{C}^{\delta 2}$ chemical shift regarding the coordination mode of histidines, but in addition constitutes a reference independent marker of this coordination mode. The complete histogram showing the distribution of this chemical shift difference $\Delta_{\delta\epsilon}$ is presented on Figure 5. In case of an $\text{N}^{\epsilon 2}$ coordination mode, $\Delta_{\delta\epsilon}$ is distributed around an average value of 12.32 ± 0.82 ppm whereas for an $\text{N}^{\delta 1}$ coordination mode, $\Delta_{\delta\epsilon}$ is found around 19.77 ± 1.49 ppm.

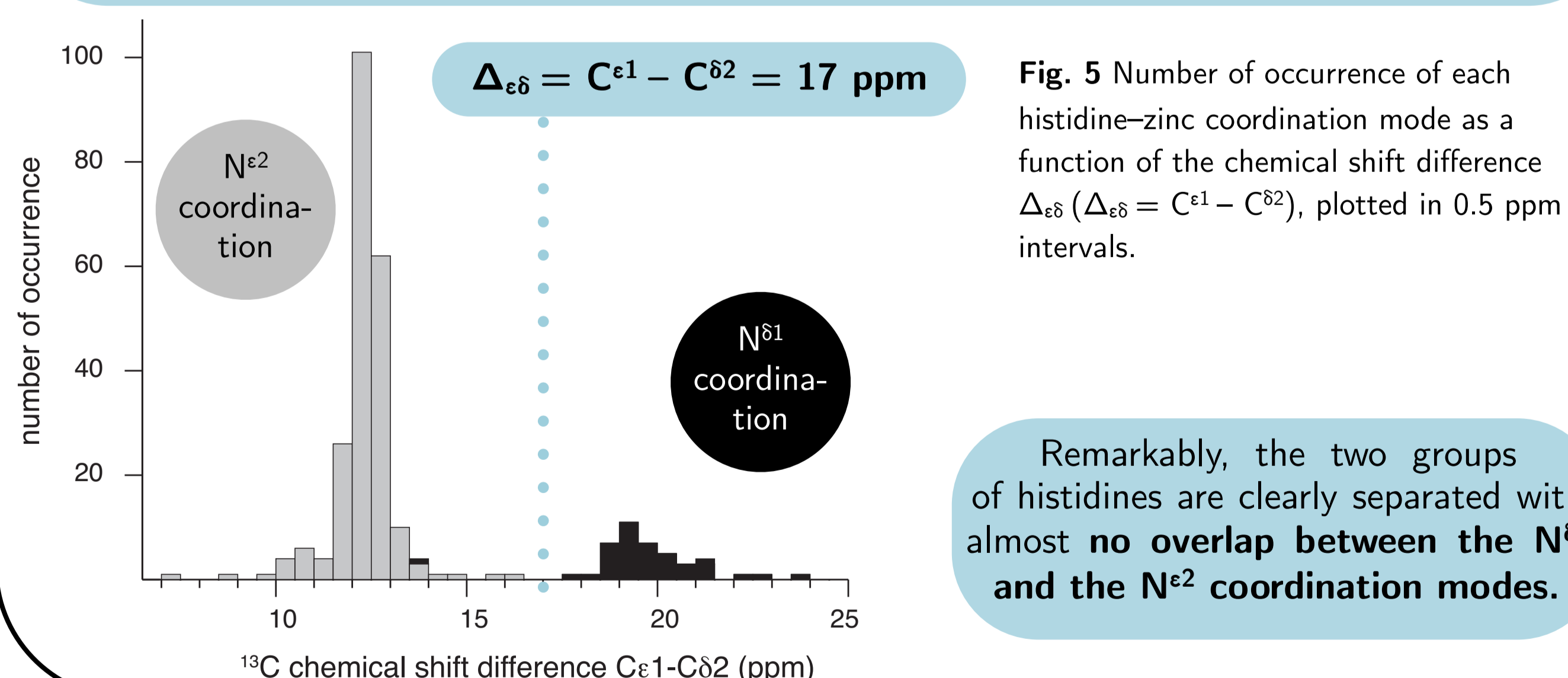


Fig. 5 Number of occurrence of each histidine-zinc coordination mode as a function of the chemical shift difference $\Delta_{\delta\epsilon}$ ($\Delta_{\delta\epsilon} = \text{C}^{\epsilon 1} - \text{C}^{\delta 2}$), plotted in 0.5 ppm intervals.

Remarkably, the two groups of histidines are clearly separated with almost no overlap between the $\text{N}^{\delta 1}$ and the $\text{N}^{\epsilon 2}$ coordination modes.

Advantages of the method and conclusion

We showed that ^{13}C aromatic carbon chemical shifts can be used to distinguish between the $\text{N}^{\delta 1}$ and the $\text{N}^{\epsilon 2}$ coordination mode of histidines in zinc-binding proteins. This provides an alternative method to the currently used approaches for the determination of the coordination mode of histidines. The method presented here is perfectly applicable to unlabelled protein samples. Furthermore, the coordination mode can be determined with a direct measurement and not with an indirect method making use of preliminary structure calculations. It also does not rely on previously published structures, therefore avoiding two approaches that could strongly bias the structure determination of new classes of zinc-binding domains. Moreover, with the present method, the information on the histidine coordination mode is available directly after chemical shift assignment prior to any structure calculation. This could for example allow the early identification of an unusual zinc-binding site with an unconventional histidine coordination mode. Moreover, the discrimination revealed in this study could be used by structural and chemical shift databases as internal check for the determination of histidine coordination modes. Finally, the present study with a comprehensive survey of the ^{13}C aromatic chemical shifts of histidines should definitely help clarify the relations between zinc-coordination, tautomeric form and downfield shifted $\text{C}^{\delta 2}$ in histidines.

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