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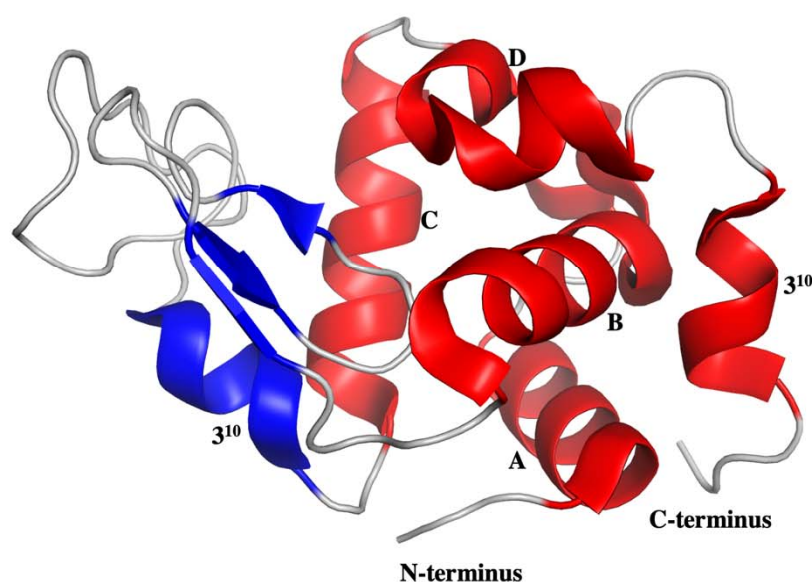
**Supporting information for article:**

**The impact of folding modes and deuteration on the atomic resolution structure of hen egg-white lysozyme**

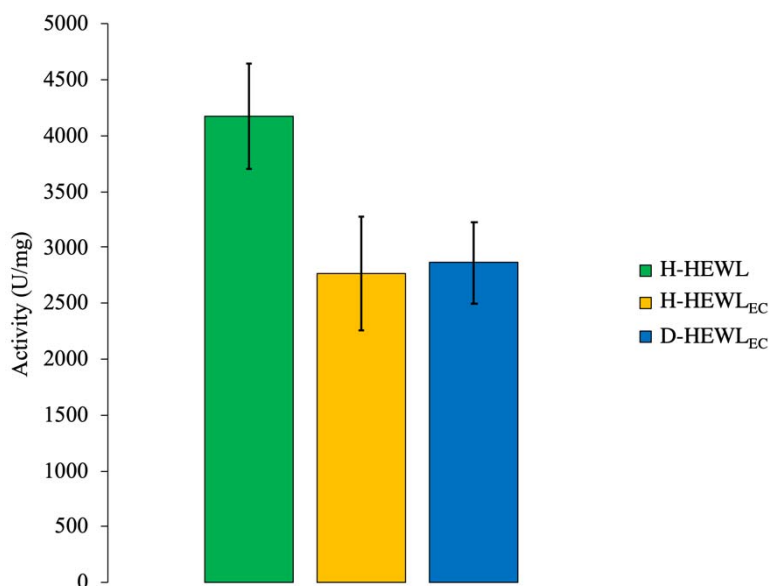
**Joao Ramos, Valerie Laux, Michael Haertlein, V. Trevor Forsyth, Estelle Mossou, Sine Larsen and Annette E. Langkilde**

**Table S1** Melting temperatures and respective standard uncertainties measured by DSF for the three HEWL variants in D<sub>2</sub>O and H<sub>2</sub>O buffer at pH/pD 4.5 and 7.5.

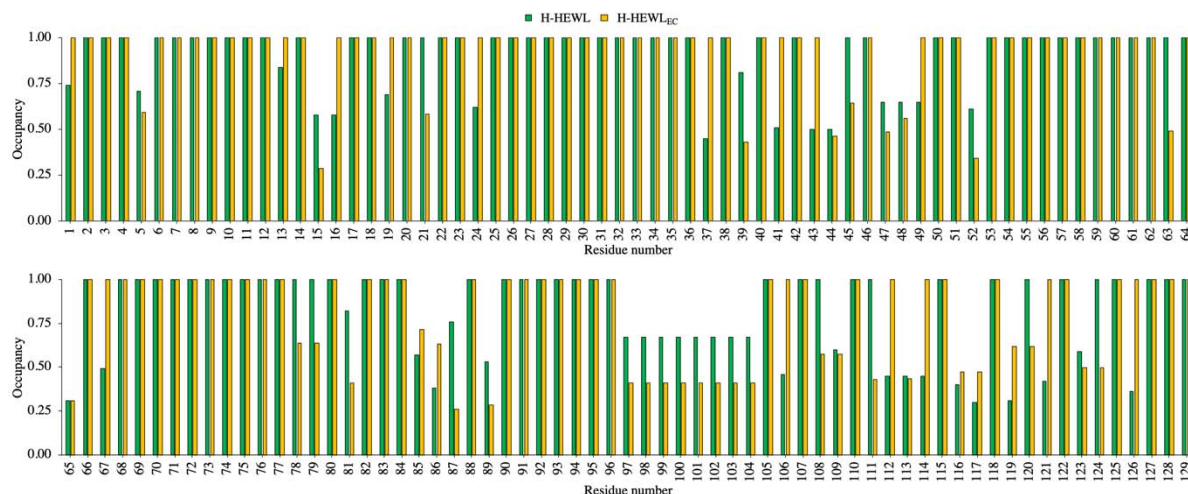
Buffers	T <sub>m</sub> (°C)		
	H-HEWL	H-HEWL <sub>EC</sub>	D-HEWL <sub>EC</sub>
D <sub>2</sub> O sodium acetate pD 4.5	78.5 ± 0.2	73.9 ± 0.1	72.2 ± 0.1
H <sub>2</sub> O sodium acetate pH 4.5	76.4 ± 0.1	71.7 ± 0.1	69.9 ± 0.4
D <sub>2</sub> O sodium phosphate pD 7.5	75.3 ± 0.2	71.4 ± 0.1	70.0 ± 0.2
H <sub>2</sub> O sodium phosphate pH 7.5	72.7 ± 0.3	68.8 ± 0.1	67.3 ± 0.3



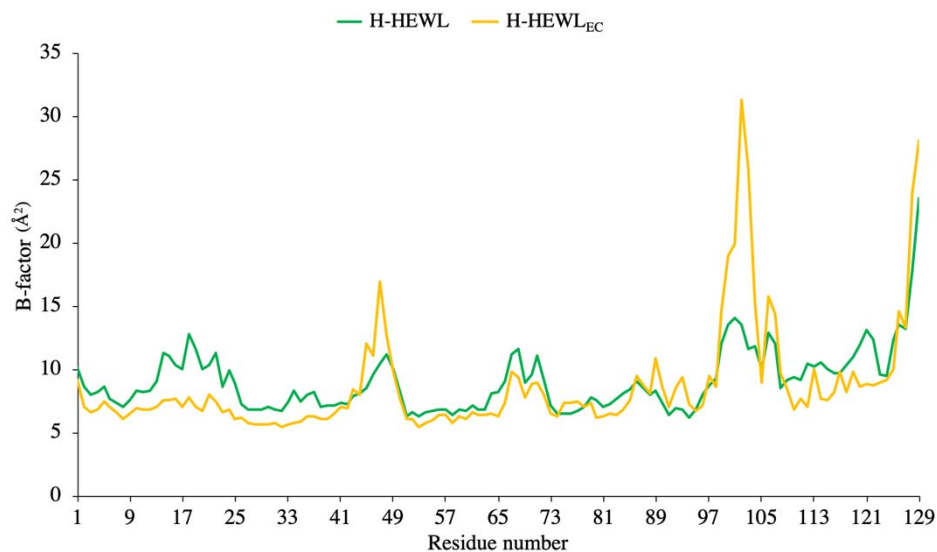
**Figure S1** Cartoon representation of the native H-HEWL structure, highlighting its folding domains. The  $\alpha$ -domain is colored in red and comprises the  $\alpha$ -helices A-D and the Asp119-Arg125  $3^{10}$ -helix. The  $\beta$ -domain is colored in blue, including the triple-stranded antiparallel  $\beta$ -sheet and the Pro79-Ser85  $3^{10}$ -helix. This illustration was produced using PyMOL (version 2.0, Schrödinger).



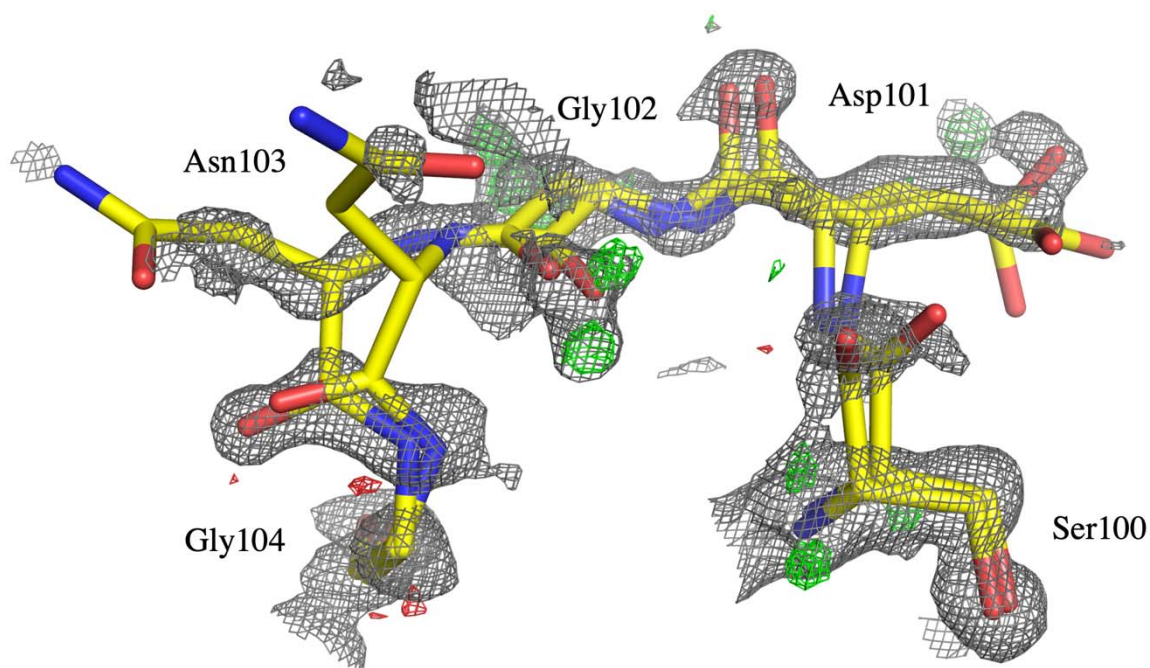
**Figure S2** Measured activity rates of H-HEWL, H-HEWL<sub>EC</sub> and D-HEWL<sub>EC</sub>, at 25° C, in H<sub>2</sub>O buffer solutions at pH 7.5. H-HEWL showed an activity rate of 4172 ± 473 U/mg, while H-HEWL<sub>EC</sub> and D-HEWL<sub>EC</sub> displayed activities rates of 2765 ± 507 U/mg and 2864 ± 365 U/mg, respectively.



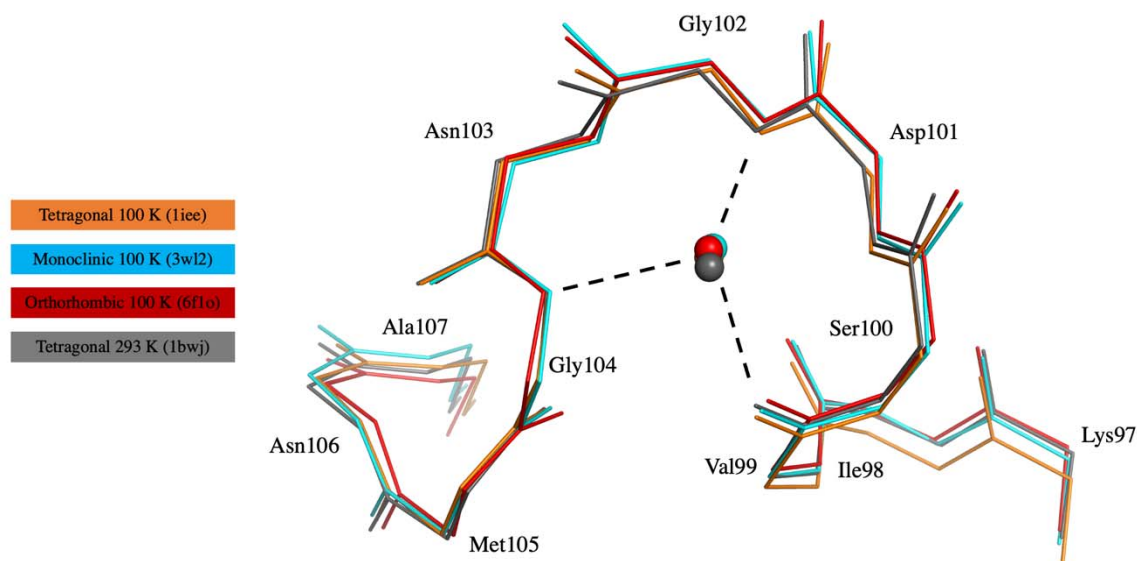
**Figure S3** Refined occupancies of protein residues' conformation A for H-HEWL and H-HEWL<sub>EC</sub>, highlighting their differences in structural disorder.



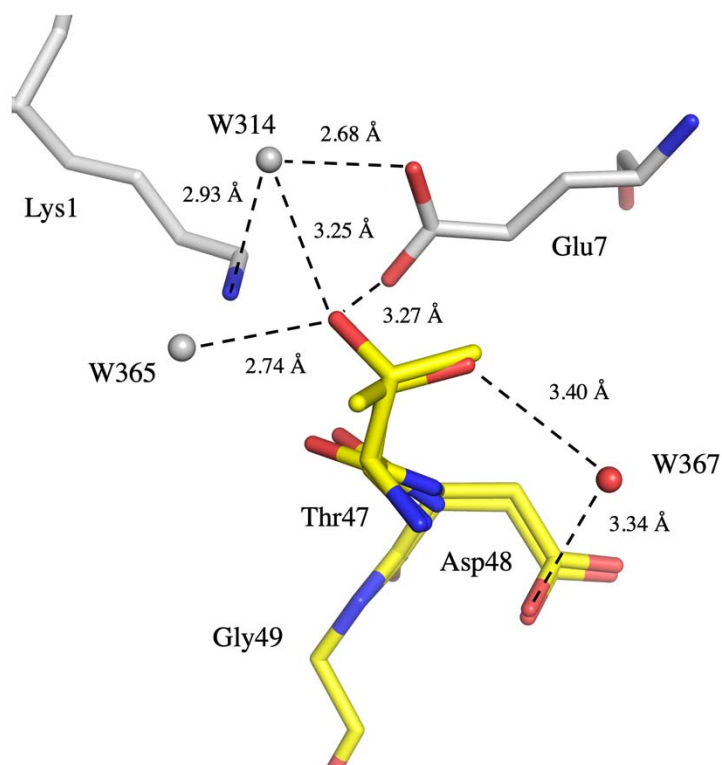
**Figure S4** Mean residue B-factor for the main-chain atoms of H-HEWL and H-HEWL<sub>EC</sub>.



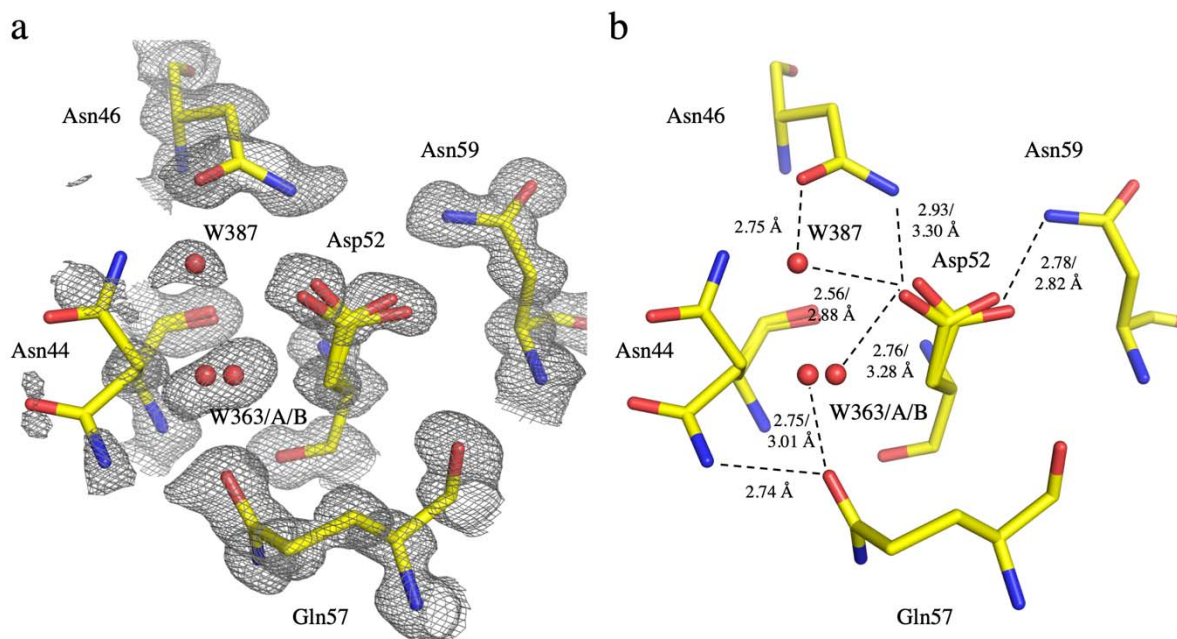
**Figure S5** Backbone disorder of the Lys97-Gly104 loop and complete Asn103 peptide-plane flip in H-HEWL<sub>EC</sub>. The  $2F_o-F_c$  electron density map was contoured at  $1\sigma$ . The positive (green) and negative (red)  $F_o-F_c$  electron density maps were contoured at  $+3\sigma$  and  $-3\sigma$ , respectively. This illustration was produced using PyMOL (version 2.0, Schrödinger).



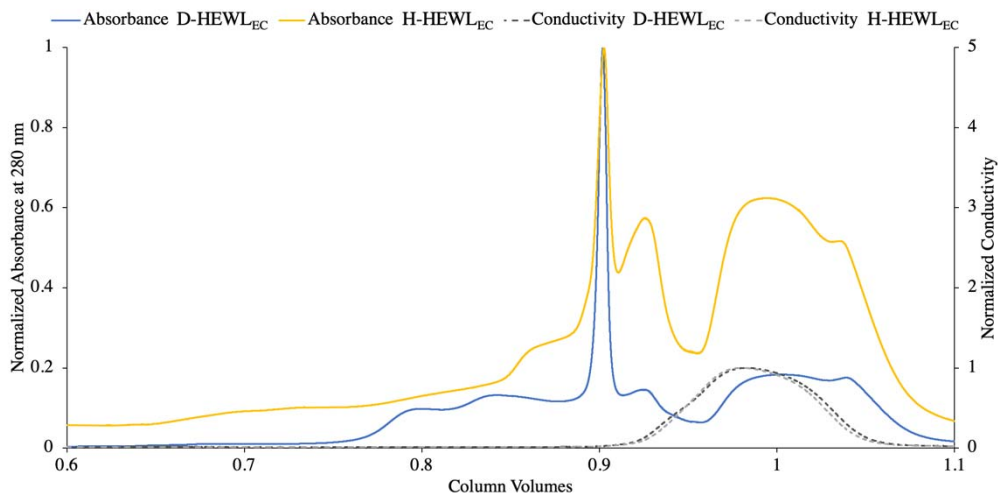
**Figure S6** Localization and respective H-bond interactions of the water molecules in native H-HEWL structures (at 100 K, PDB entries 1ice, 3wl2, 6flo, and room temperature, PDB entry 1bwj) that help stabilize the configuration of the Lys97-Gly104 loop. Water molecules 1010, 312, 316, and 148, of tetragonal 1ice, monoclinic 3wl2, orthorhombic 6flo, and tetragonal 1bwj, respectively, are shown. Dashed lines indicate the H-bonding patterns between these water molecules and Val99 O, Gly102 N(H), and Gly104 N(H). This illustration was produced using PyMOL (version 2.0, Schrödinger).



**Figure S7** Representation of the H-bond interactions of Thr47 conformations A and B via crystal contacts, which promote the backbone disorder in the Thr47-Gly49 region in H-HEWL<sub>EC</sub>. The residues (carbon atoms) and water molecules belonging to adjacent unit cells are colored in grey. This illustration was produced using PyMOL (version 2.0, Schrödinger).



**Figure S8** Alternate conformations of Asn44 and W387 found in the H-HEWL<sub>EC</sub> structure (a), with corresponding active site H-bond interactions (b). The  $2Fo-Fc$  electron density map was contoured at  $1\sigma$ . This illustration was produced using PyMOL (version 2.0, Schrödinger).



**Figure S9** Superposed chromatograms of the refolding gel filtrations of D-HEWL<sub>EC</sub> and H-HEWL<sub>EC</sub>.



<i>Gallus gallus</i>	1	-----KVFGRCELAAAMKRHGLDNYRGYSLGNWVCAAKFESNFNTQATNRNT-DGSTDYGILQINSR	61
<i>Homo sapiens</i>	1	MKALIVLGLVLLSVTVQGKVFERCELARTLKRLGMDGYRGISLANWMLAKWESGYNTRATNYNAGDRSTDYGIFQINSR	80
<i>Mus musculus</i>	1	MKALLTLGLLLSVTAQAKVYNRCELARILKRNGMDGYRGVKLADWVCLAQHESNYNTRATNYNRGDRSTDYGIFQINSR	80
<i>Danio rerio</i>	1	MRLAVVFLCLAWMSSCESKTLGRCDVYKIFKNEGLDGFEGFSIGNYVCTAYWESRFKTHRV--SADTGKDYGIFQINSF	78
<i>Numida meleagris</i>	1	MKSLLILVLCFLPMAALGKVFGRCELAAAMKRHGLDNYRGYSLGNWVCAAKFESNFNSQATNRNT-DGSTDYGVLQINSR	79
<i>Meleagris gallopavo</i>	1	MRSLLILVLCFLPLAALGKVYGRCELAAAMKRLGLDNYRGYSLGNWVCAAKFESNFNTHATNRNT-DGSTDYGILQINSR	79
<i>Gallus gallus</i>	62	WWCNDGRTPGSRNLCNIPCSALLSSDITASVNC AKKIVSDENGMNAWVAWRNRCKGTDVQAWIRGCRL	129
<i>Homo sapiens</i>	81	YWCNDGKTPGAVNACHLSCSALLQDNIADAVACAKRVVDPQGIRAWVAWRNRRCQNRDVRQYVQCGV	148
<i>Mus musculus</i>	81	YWCNDGKTPRSKNACGINCSALLQDDITAAIQCAKRVVDPQGIRAWVAWRTQCQNRDL SQYIRNCGV	148
<i>Danio rerio</i>	79	KWCDDG--TPGGKNLCKVACSDLNDDLKASVGCALIVKM--DGLKSWETWDSYCNCRKMSRWVKGCEQ [ 7 ]	151
<i>Numida meleagris</i>	80	WWCNDGRTPGSRNLCNIPCSALQSSDITATANCAKKIVSDENGMNAWVAWRKHCKGTDVRVWIKGCRL	147
<i>Meleagris gallopavo</i>	80	WWCNDGRTPGSKNLCNIPCSALLSSDITASVNC AKKIASGENGMNAWVAWRNRCKGTDVHAWIRGCRL	147

**Figure S10** Multiple sequence alignment of HEWL (*Gallus gallus*) with other c-type lysozymes (performed in COBALT; [https://www.ncbi.nlm.nih.gov/tools/cobalt/re\\_cobalt.cgi](https://www.ncbi.nlm.nih.gov/tools/cobalt/re_cobalt.cgi)), highlighting high sequence homology and the conservation of residue Asp101.