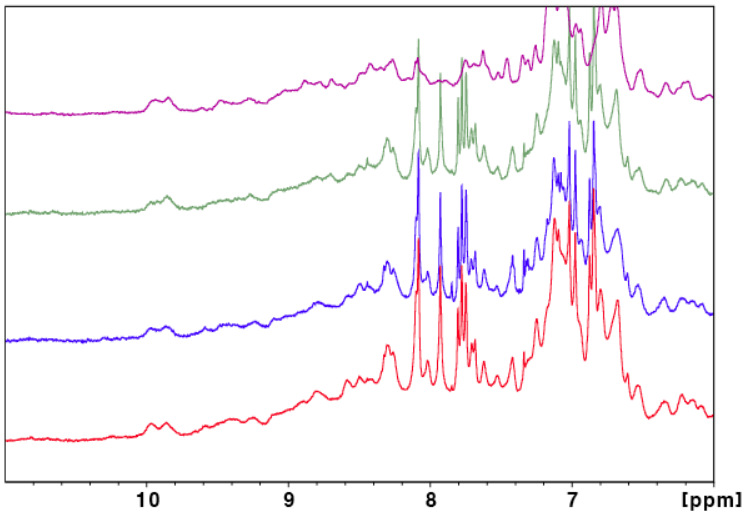
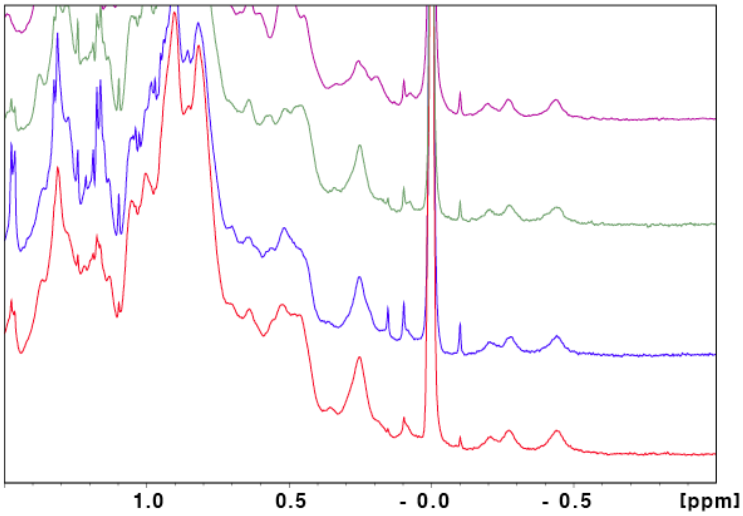
Supplemental material

Supplemental Figure S1. Overlay of regions of the ^{1}H 1D spectra of Pulmozyme (magenta), wild type DNase I (green), DNase I N7A mutant (blue) and DNase I N7S mutant (red). Characteristic peaks for protein resonances are nearly the same in all spectra in both downfield (panel A) and upfield (panel B) regions. The patterns of peaks upfield of 0 ppm are indicative of a structured correctly folded protein and these and the signals at 10 ppm downfield are essentially identical in all proteins. The sharp signals around 7 and 8 ppm in panel A stem from the histidine tag present in wild type, N7S and N7A DNase I proteins, but absent in Pulmozyme.

A



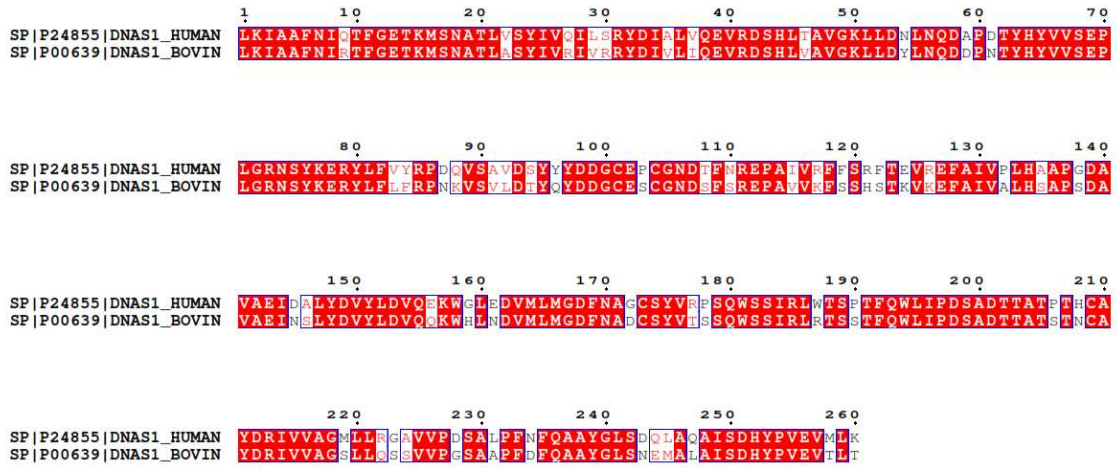
B

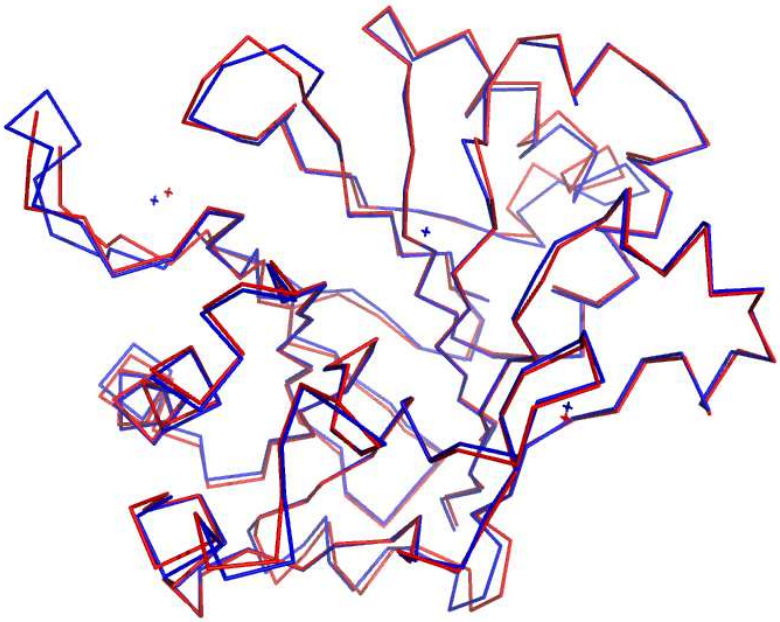


Supplemental Table S2. Conservation of active site residues in the DNase I-like superfamily used in pairwise structural overlays (seven or eight C_α pairs) with rhDNase I. Residues in shaded boxes were not used in pairwise overlay. All indicated residues are identical to those in DNase I, except for the His134 which is a Tyr in APE / Exo III. Residue numbering used refers to numbering used in the accession codes for structures of the respective proteins.

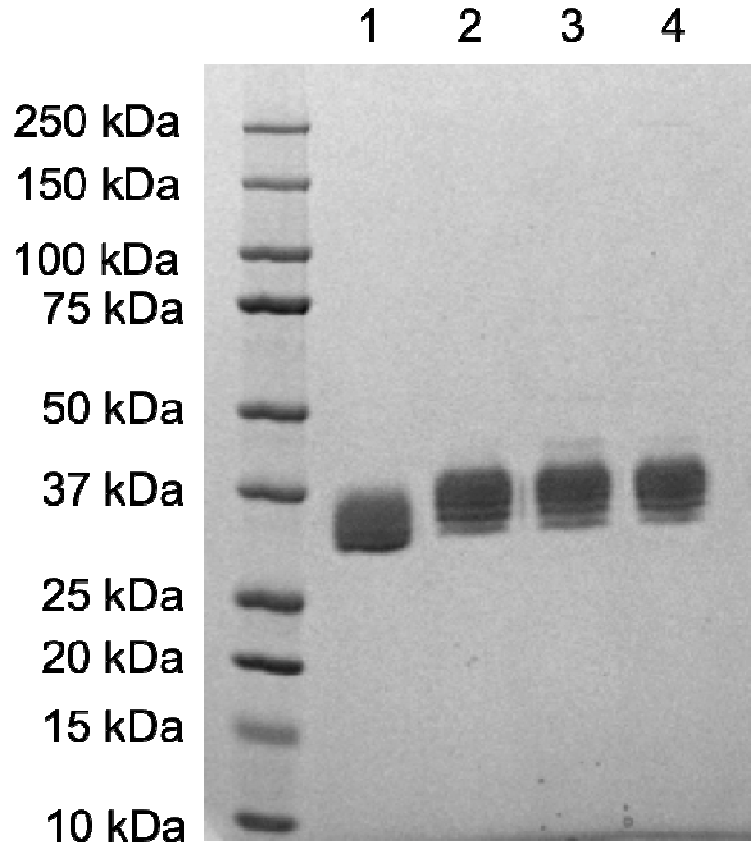
	PDB ID	Asn	Glu	His	Asp	Asn	Asp	Asp	His	rmsd C_α
rhDNase I human	4AWN	7	39	134	168	170	212	251	252	0
bDNase I bovine	1DNK	7	39	134	168	170	212	251	252	0.183
SMase *B. cereus*	2DDT	16	53	151	195	197	253	295	296	0.493
SMase *L. ivanovii*	1ZWX	51	88	185	229	231	282	324	325	0.543
CNOT6L human	3NGN	195	240	360	410	412	489	528	529	0.406
APE1 human	1DE9	68	96	*Tyr171*	210	212	283	308	309	0.498
APE *L. major*	2J63	136	167	*Tyr276*	335	337	411	436	437	0.511
Exo III *E coli*	1AKO	7	34	*Tyr109*	151	153	229	258	259	0.408
Exo III *N. meningitidis*	2CJ4	7	34	*Tyr105*	146	148	217	246	247	0.366
Exo III *A. fulgidus*	2VOA	8	35	*Tyr105*	146	148	218	247	248	0.554
IPP5 *S. pombe*	1I9Z	566	597	699	740	742	816	838	839	0.654

Supplemental Figure S3. Sequence alignment and structural superposition of human DNase I and bovine DNase I. The sequence alignment was produced using T-Coffee (Notredame, C., Higgins, D.G., Heringa, J. (2000). T-Coffee: A novel method for fast and accurate multiple sequence alignment. *J Mol Biol.* **302** 205-17) and ESPRIPT (Gouet, P., Courcelle, E., Stuart, D.I. and Metoz, F. (1999). ESPript: multiple sequence alignments in PostScript. *Bioinformatics*. **15** 305-8). C_α tracing of the structural superposition of rhDNase I (PDB ID: 4AWN ; blue) and bDNase I (PDB ID: 3DNI ; red) using PyMOL (The PyMOL Molecular Graphics System, Version 1.4.1 Schrödinger, LLC.). Cations are shown as small crosses.

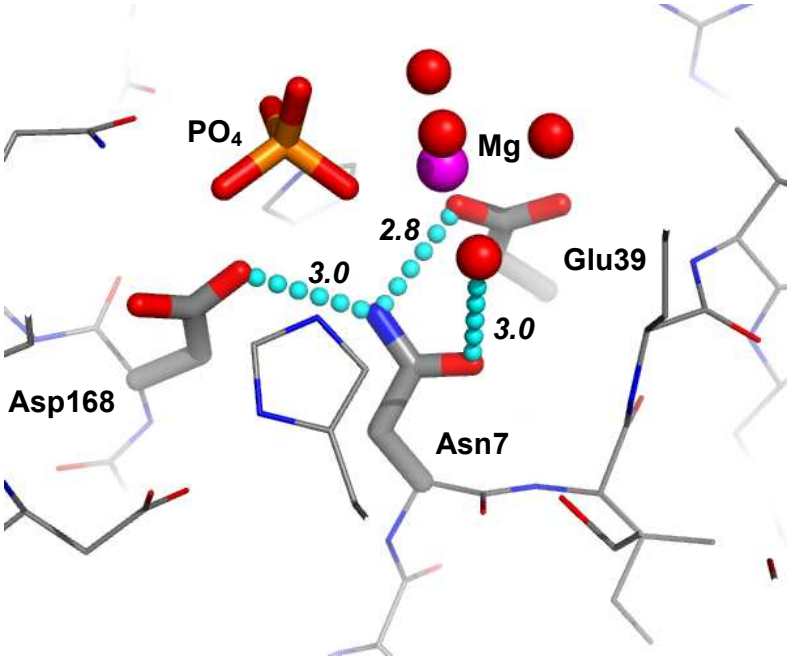




Supplemental Figure S4. SDS-PAGE of purified proteins used for activity assays. Lanes 1-4 contain 2 µg each of Pulmozyme (dornase alfa), wildtype rhDNase I, rhDNase I Asn7Ala or rhDNase I Asn7Ser, respectively. Proteins were stained with Coomassie Blue. The MW of Pulmozyme is slightly lower since it does not contain the His_9 tag. All proteins are glycosylated and show a typical multiband pattern.



Supplemental Figure S5. Interactions of Asn7 in the active site of rhDNase I. H-bonds of Asn7 with Asp168, Glu39 and a water atom (red sphere) from the coordination sphere of the Mg^{2+} ion (magenta sphere) comprising their length in Å are indicated. The phosphate ion could either be the monoprotonated (HPO_4^{-2}) or diprotonated ($H_2PO_4^-$) state.



Supplemental Table S6. Sequence alignment of key residues in the active site of mature mammalian DNase I and human DNase I-like proteins. Positions in human DNase I that are involved in metal ion binding either directly or via water are labeled with "M". Nonconserved residues are in shaded boxes.

		M		M						M	
Position	Uniprot ID	7	9	39	78	134	168	170	212	251	252
human DNase I	**P24855**	N	Q	E	E	H	D	N	D	D	H
bovine DNase I	**P00639**	N	R	E	E	H	D	N	D	D	H
murine DNase I	**P49183**	N	R	E	E	H	D	N	D	D	H
rat DNase I	**P21704**	N	R	E	E	H	D	N	D	D	H
porcine DNase I	**P11936**	N	R	E	E	H	D	N	D	D	H
ovine DNase I	**P11937**	N	R	E	E	H	D	N	D	D	H
rabbit DNase I	**O18998**	N	R	E	E	H	D	N	D	D	H
horse DNase I	**Q4AEE3**	N	R	E	E	H	D	N	D	D	H
dog DNase I	**Q767J3**	N	R	E	E	H	D	N	D	D	H
chicken DNase I	**Q9YGI5**	N	R	E	E	H	D	N	D	D	H
human DNAS1L1[a)]	**P49184**	N	Q	E	E	H	D	N	D	D	H
human DNAS1L2[b)]	**Q92874**	N	Q	E	E	H	D	N	D	D	H
human DNAS1L3[c)]	**Q13609**	N	R	E	E	H	D	N	D	D	H

[a)]DNAS1L1 is also known as DNase X or XIB.
[b)]DNAS1L2 is also known as DHP-1.
[c)]DNAS1L3 is also known as LS-DNase, DNase-gamma, DHP-2 or nhDNase.

Supplemental Table S7. Selection of members of the DNase I-like superfamily with available structures in the absence or presence of bound ligands. Comments in parenthesis refer to the comparative structural analysis with the present rhDNase I structure.

Protein Name	**PDB ID**	**Ligand in active site**	**Reference**
DNase I (bovine)	2DNJ	DNA octamer (cleaved missing phosphate)	(*1*)
DNase I (bovine)	3DNI	none	(*2*)
DNase I (bovine)	1DNK	DNA octamer (in phosphate site)	(*3*)
DNase I (bovine)	1ATN	none; complex with actin	(*4*)
APE1 human	1DE9	1 Mn^{2+} ion (good fit); DNA (cleaved)	(*5*)
APE1 human	1DE8	DNA	(*5*)
APE1 human	1E9N	2 Pb^{2+} ions	(*6*)
APE1 human	1HD7	Pb^{2+} ion (displaced)	(*6*)
APE1 human	1DEW	DNA (cleaved)	(*5*)
APE1 human	2ISI	1 Mg^{2+} ion (displaced), 1 PO_4^{3-} ion (distal)	[unpublished]
APE1 human	1BIX	1 Sm^{3+} ion	(*7*)
APE *Neisseria meningitidis*	2JC5/2CJ4	1 Mg^{2+} ion (displaced 1.8 Å)	(*8*)
APE *Leishmania major*	2J63	none	(*9*)
SMase *Bacillus cereus*	2DDS	2 Co^{2+} ions	(*10*)
SMase *Bacillus cereus*	2DDR	1 Ca^{2+} ion	(*10*)
SMase *Bacillus cereus*	2DDT	1 Mg^{2+} ion (perfect fit)	(*10*)
SMase *Bacillus cereus*	2UYR	1 Mg^{2+} ion (perfect fit); MES (distal)	[unpublished]
CNOT6L nuclease human	3NGQ	2 Mg^{2+} ions (1 perfect fit, 1 at 4 Å site IVa)	(*11*)
CNOT6L nuclease human	3NGO	2 Mg^{2+} ions (1 perfect fit, 1 at 4 Å site IVa); DNA	(*11*)
CNOT6L nuclease human	3NGN	AMP (in phosphate site)	(*11*)

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