Electronic Supplementary Material (ESI) for RSC Advances.

Table S1. Amino acid sequences and molecular mass values of chymotrypsin, endoproteinase Glu-C and cyanogen bromide (CB) peptides used to assemble the amino acid sequence of crested porcupine Mb.

Peptide	Sequence position	Experimental molecular mass	Theoretical molecular mass	*Δ* (Da)	Missed cleavage at
chymotrypsin peptides					
C-1	15-29	1494.80	1494.76	0.04	Leu22
C-2	34-43	1185.61	1185.63	0.02	Leu40
C-3	47-55	1100.00	1100.54	0.54	Leu50
C-4	77-89	1390.82	1390.82	0	Leu86
C-5	116-123	929.42	929.45	0.03	
C-6	139-146	950.54	950.50	0.04	
endoproteinase Glu-C peptides					
E-1	7-18	1470.71	1470.81	0.1	
E-2	19-27	869.34	869.37	0.03	
	28-38	1308.72	1308.78	0.06	
E-3	28-41	1651.84	1651.95	0.11	Glu38
E-4	53-59	837.42	837.34	0.14	Glu54
	55-59	593.04	593.27	0.23	
E-5	60-85	2714.49	2714.57	0.08	
E-6	86-105	2244.15	2244.29	0.14	
E-7	110-136	2826.41	2826.44	0.03	
E-8	137-148	1467.74	1467.80	0.06	
CNBr fragments					
CB-1	1-55	6215.31	6215.01	0.30	
CB-2	56-131	8095.04	8095.32	0.28	
	1-131	14338.57	14339.41	0.84	Met55
CB-3	132-153	2499.31	2499.87	0.56	

Table S2. Amino acid sequences and molecular mass values of chymotrypsin and cyanogen bromide (CB) peptides used to assemble the amino acid sequence of reindeer Mb.

Peptide	Sequence position	Experimental molecular mass	Theoretical molecular mass	Δ (Da)	Missed cleavage at
chymotrypsin peptides					
C-1	1-7	763.31	763.32	0.01	Leu2
C-2	10-14	602.10	602.33	0.23	Leu11
C-3	15-29	1508.85	1508.77	0.08	
C-4	30-33	548.14	548.36	0.22	Leu32
C-5	34-40	754.34	754.37	0.03	
C-6	41-46	813.42	813.41	0.01	Phe43
C-7	47-55	1086.63	1086.56	0.07	Phe49
	50-55	708.30	708.32	0.02	
C-8	56-69	1539.92	1539.85	0.07	Phe61
	62-69	896.57	896.53	0.04	
C-9	77-89	1540.95	1540.87	0.08	
C-10	90-103	1621.92	1621.88	0.04	
	116-123	938.49	937.46	0.03	
C-11	116-131	1639.81	1638.74	0.07	Phe123
	124-131	720.32	720.30	0.02	
C-12	139-146	968.46	968.42	0.04	Met142
C-13	147-153	748.43	748.44	0.01	Leu149 and Phe151
CNBr fragments					
CB-1	1-55	6159.35	6159.93	0.58	
CB-2	56-131	8217.73	8217.41	0.32	
	56-142	9524.67	9522.92	0.75	Met131
CB-3	132-142	1275.62	1275.67	0.05	
CB-4	143-153	1181.54	1181.63	0.09	

Fig. S1. Sequence alignment of crested porcupine and reindeer myoglobins. The sequences of both myoglobins are shown with the predicted secondary structure including the eight α-helices. Substitutions are shared by gray. Proximal (position 93) and distal histidine residues (position 64) are reported in red. Amino acid residues involved in the autoxidation mechanism or in the binding for heme are highlighted cyan or green, respectively.

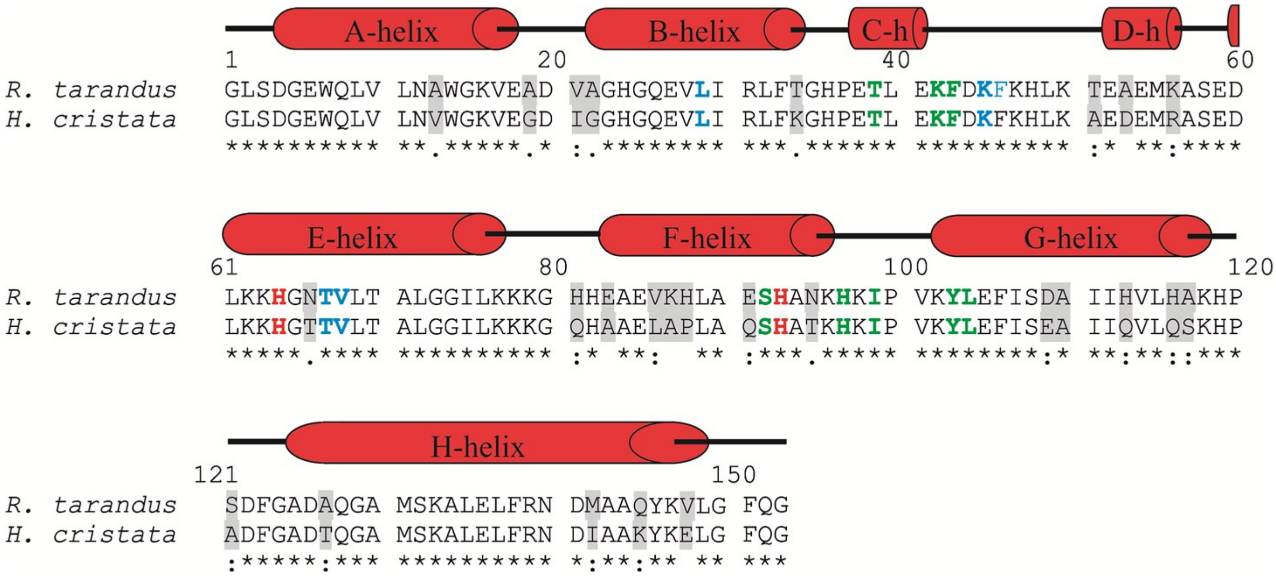


Fig. S2. Ramachandran plot of reindeer Mb (A) and crested porcupine Mb (B).

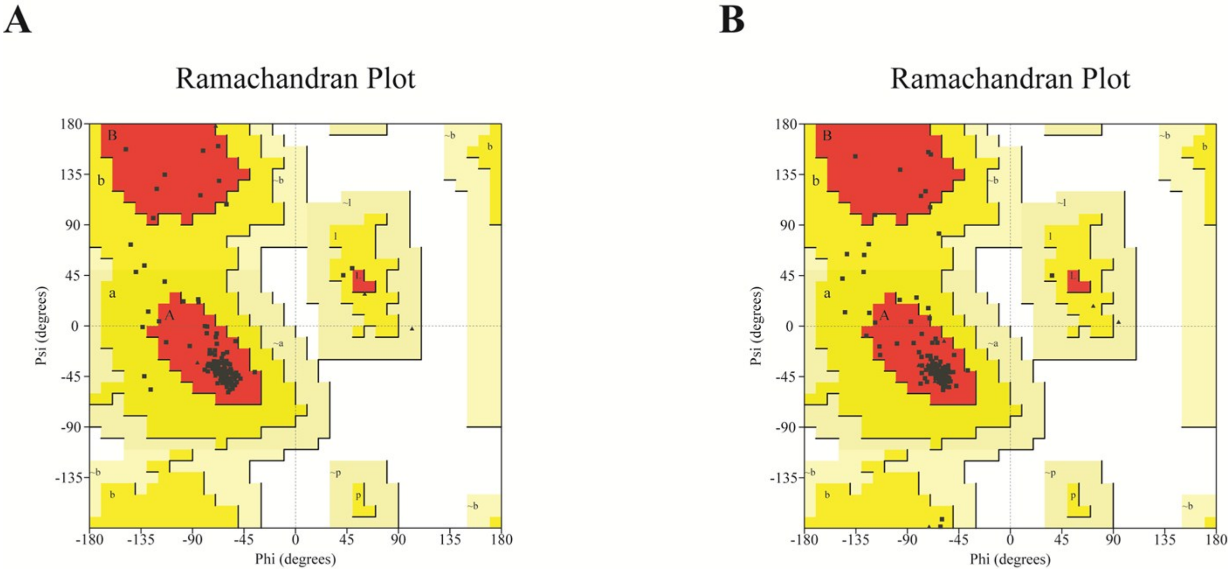
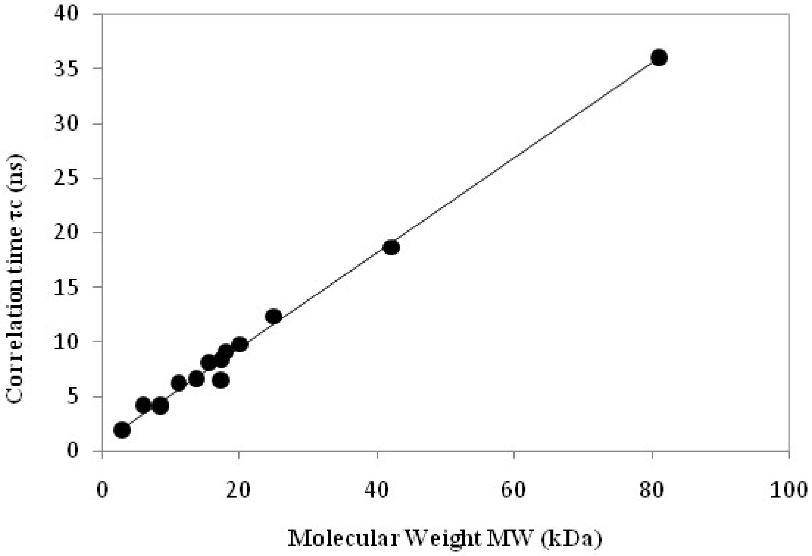


Fig. S3. A, plot of τc versus molecular weight for known monomeric protein. B, table of rotational correlation time and molecular weight used in the plot reported above.

A



B

Molecular Weight MW (kDa)	Correlation time τc (ns)	Reference
2.9	1.9	A.G. Palmer III, M. Rance and P.E. Wright, *J. Am. Chem. Soc*, 1991, 113, 4371-4380
6.0	4.2	N.A. Farrow, O. Zhang, J.D. Forman-Kay and L.E. Kay, *Biochemistry*, 1997, 36, 2390-2402
8.5	4.0	S. F. Lienin , T. Bremi , B. Brutscher , R. Brüschweiler and R. R. Ernst, *J. Am. Chem. Soc*, 1998, 120, 9870-9879
8.5	4.1	D.M. Schneider, M.J. Dellwo and A.J. Wand, *Biochemistry*, 1992, 31, 3645-3652
8.5	4.3	J. Kördel, N.J. Skelton, M. Akke, A. G. Palmer III and W.J. Chazin, *Biochemistry*, 1992, 31, 4856-4866
13.7	6.6	N.A. Farrow, R. Muhandiram, A.U. Singer, S.M. Pascal, C.M. Kay, G. Gish, S.E. Shoelson, T. Pawson, J.D. Forman-Kay and L.E. Kay, *Biochemistry*, 1994, 33, 5984-6003
17.2	6.5	Y. Feng, B.K. Klein and C.A. McWherter, *J. Mol. Biol.*, 1996, 259, 524-541
11.2	6.2	M.J. Stone, W.J. Fairbrother, A.G. Palmer III, J. Reizer, M.H. Jr. Saier and P.E. Wright, *Biochemistry*, 1992, 31, 4394-4406
13.7	6.6	E.L. Kovrigin, R. Cole and J.P. Loria, *Biochemistry*, 2003, 42, 5279-5291
15.6	8.1	J. Lu, D.P. Cistola and E. Li, *J. Mol. Biol.*, 2003, 330, 799-812
17.4	8.3	G.M. Clore, P.C. Driscoll, P.T. Wingfield and A.M. Gronenborn, *Biochemistry*, 1990, 29, 7387-7401
18	9.1	L.E. Kay, D.A. Torchia and A. Bax, *Biochemistry*, 1989, 28, 8972-8979
20	9.8	S.Yao, M.G. Hinds and R.S. Norton, *J. Magn. Reson.*, 1998, 131, 347-350
25	12.3	G.J. Kroon, H. Mo, M.A. Martinez-Yamout, H.J. Dyson and P.E. Wright, *Protein Sci.*, 2003, 12, 1386-1394
42	18.6	V. Tugarinov, R. Muhandiram, A. Ayed and L.E. Kay, *J. Am. Chem. Soc*, 2002, 124, 10025-10035
81	36	V. Tugarinov, R. Muhandiram, A. Ayed and L.E. Kay, *J. Am. Chem. Soc*, 2002, 124, 10025-10035

Fig. S4. Mapping of the residues that affect gas ligand transport (orange) onto the 3D model of reindeer Mb (A-B) and crested porcupine Mb (C-D), respectively.

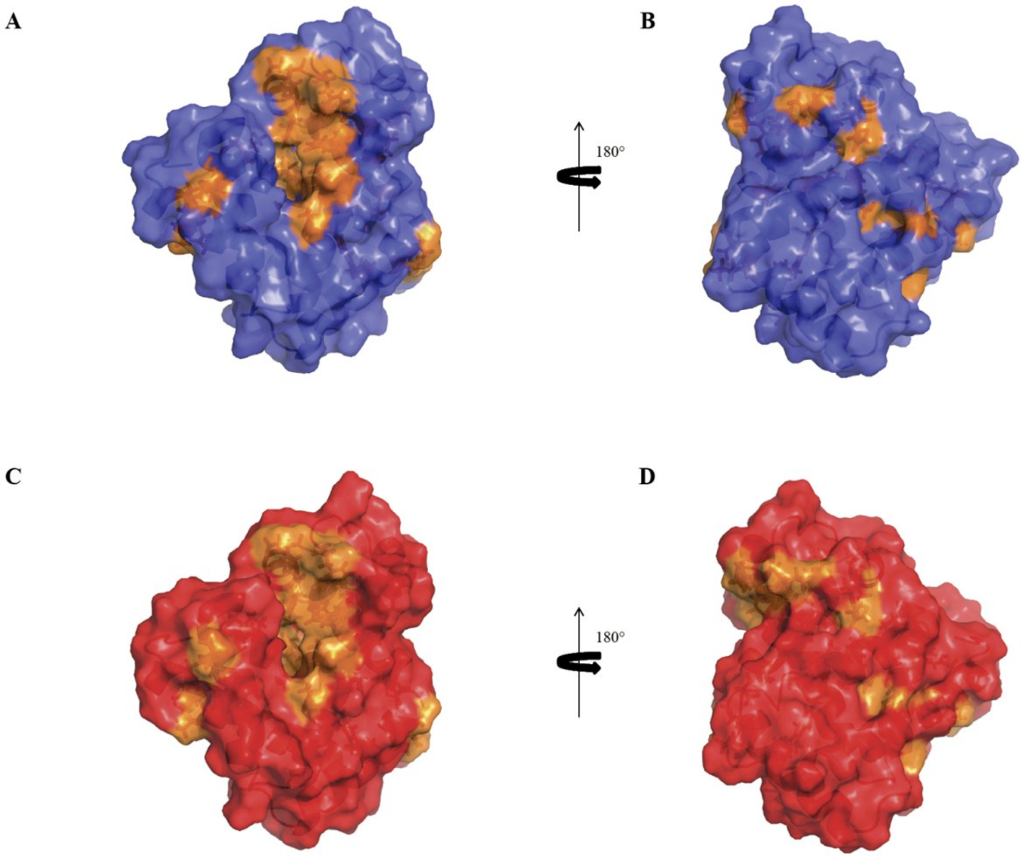
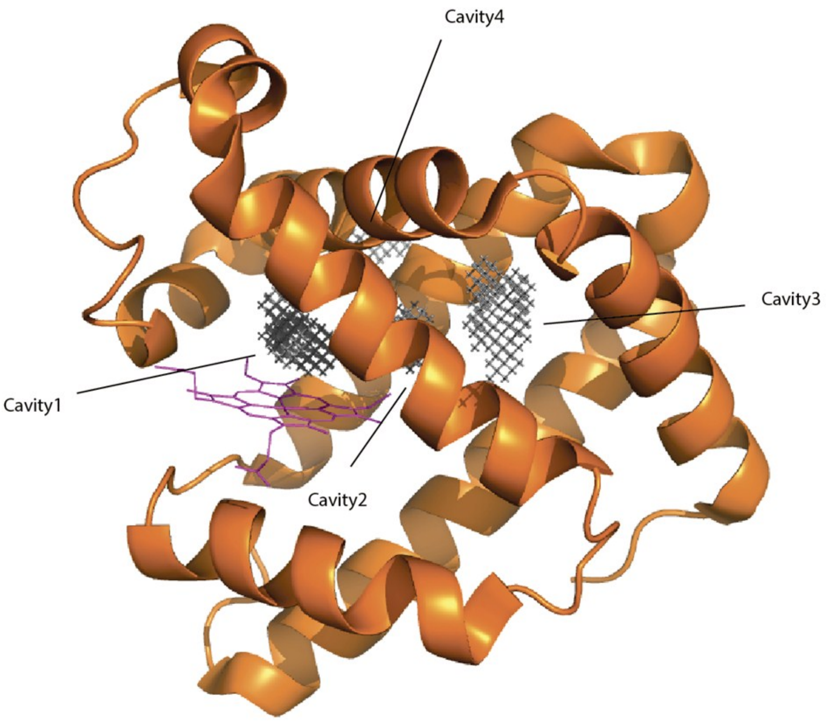


Fig. S5. Mapping of the analyzed cavities onto the x-ray structure of the sperm whale myoglobin (PDB code 1J52). Cavity volume of the studied myoglobins. The heme group is depicted in magenta.

A



B

PDB code	Cavity 1 (Å^3)	Cavity 2 (Å^3)	Cavity 3 (Å^3)	Cavity 4 (Å^3)
1J52	41.90	21.38	32.60	17.71
1LHS	19.00	30.24	41.90	10.80
1WLA	24.84	41.47	32.83	16.42
3RGK	30.24	24.40	44.28	-------
1MYT	23.11	57.90	-------	17.71
1PMB	34.34	37.15	21.38	-------