nature | methods

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Large-scale evaluation of protein reductive methylation for improving protein crystallization

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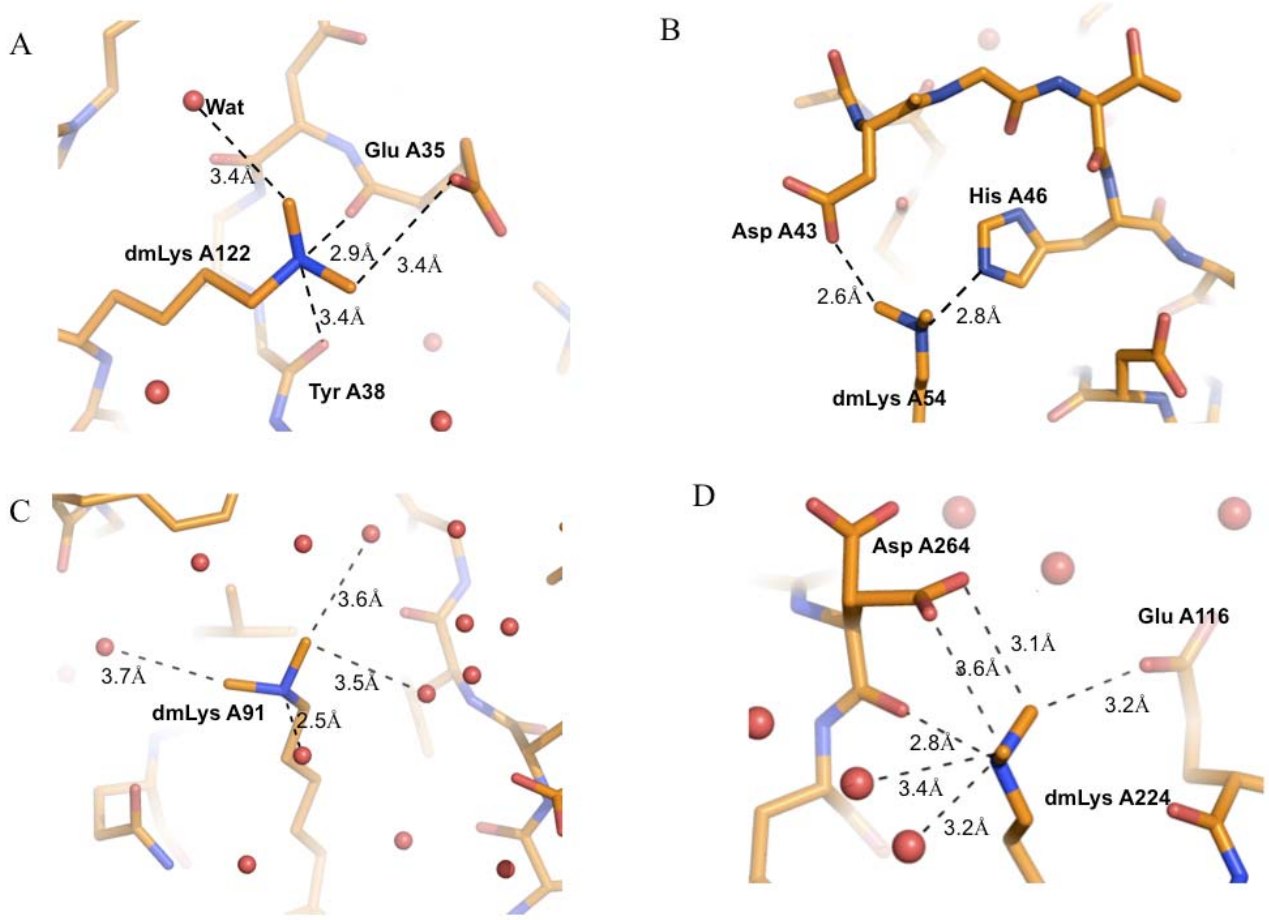
Supplementary Figure 1. Well ordered dmLys are involved in several types of intramolecular interactions with protein side chains, main chain carbonyls and solvent.



A. Interaction with Glu carboxylate and carbonyls - methylated Lys side chain interacting with neighboring residues (2QHQ): NZ of methylated Lys122 is involved in intra-molecular bi-furcated hydrogen bonds to the carbonyl oxygen (O) atoms of Glu35 and Tyr38, and CH1 and CH2 are contacted by OE2 of Glu35 and a water molecule.

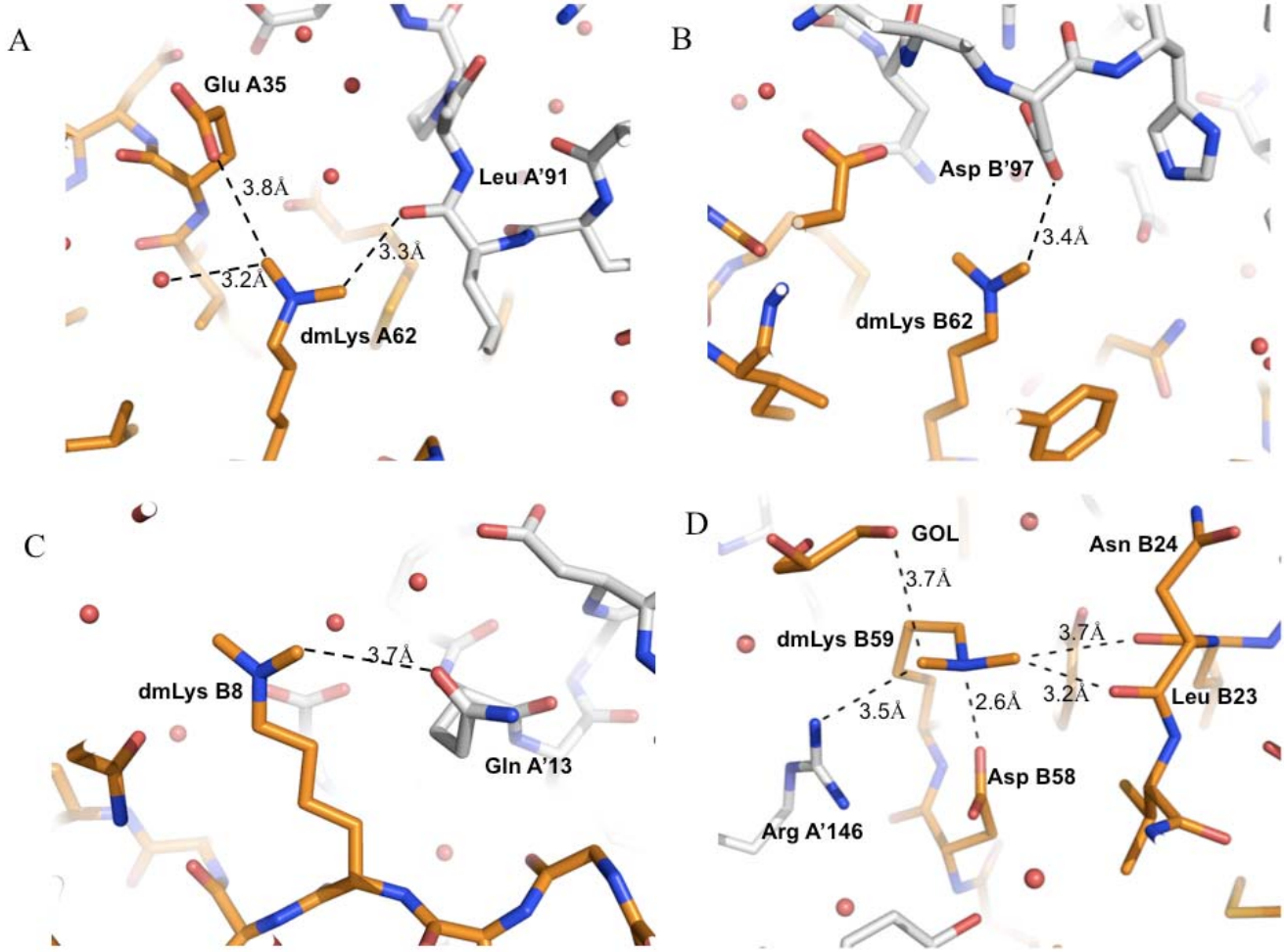
B. Interaction with His nitrogen: in the structure of YtfH (1YYV), a putative transcription regulator containing an HxlR helix-turn-helix DNA binding domain from *Salmonella typhimurium*, dmLysA54 interacts with HisA46 and AspA43.

C. Interaction with water molecules: both polarized carbon atoms and NZ in the dmLys-A91 of the YdhR (1XC3), a fructokinase from *Bacillus subtilis* are making hydrogen bonds with water molecules. Notice that both hydrogen bond distances between polarized carbons and water molecules (3.6 and 3.7 Å) are somewhat longer than that between NZ and a water molecule (2.5 Å).

D. dmLys as a part of a hydrogen bonding network: in the same structure (1XC3), dmLysA224 is in the middle of an extended hydrogen bonding network making multiple contacts with carboxylates (GluA116 and AspA264), a carbonyl (AspA264), and water molecules.

Carbon atoms are in dark yellow, nitrogen atoms in dark blue, and red indicates oxygen atoms. Distances are shown in Å.

Supplementary Figure 2. Examples of intermolecular interaction involved in crystal packing.



A. dmLysA62 in the structure of VPA0580 from *Vibrio parahaemolyticus* (2QHQ) is making crystal packing contact with GluA35 and the main chain carbonyl of LeuA'91 from the symmetry mate of molecule A indicated in the white stick model.

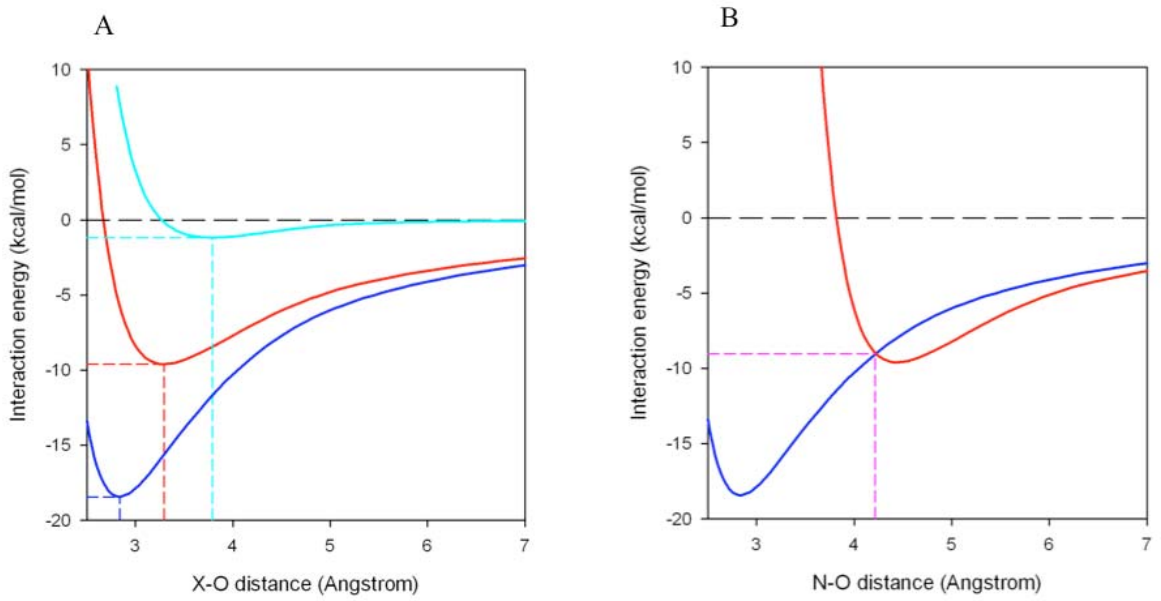
B. dmLysB62 of VPA0580 is making crystal packing contact with AspB'97 from the symmetry mate of molecule B indicated in white stick.

C. dmLysA62 in the structure VPA0580 making crystal packing contact with Gln-A'13 from the symmetry mate of molecule A indicated in white stick.

D. dmLys participating in intra- and intermolecular interactions: dmLys59B in the structure of a transporter associated domain CorC_HlyC from *Haemophilus ducreyi* (2P4P, 1.8 Å) is not only contacting carbonyls of LeuB23 and AsnB24, and carboxlyate AspB58 in the same molecule but also interacting with NZ of ArgA'146 of a symmetry-mate. In white is a stick model of a symmetry mate of molecule A.

Carbon atoms are in dark yellow, nitrogen atoms in dark blue, and red indicates oxygen atoms. Distances are indicated in Å.

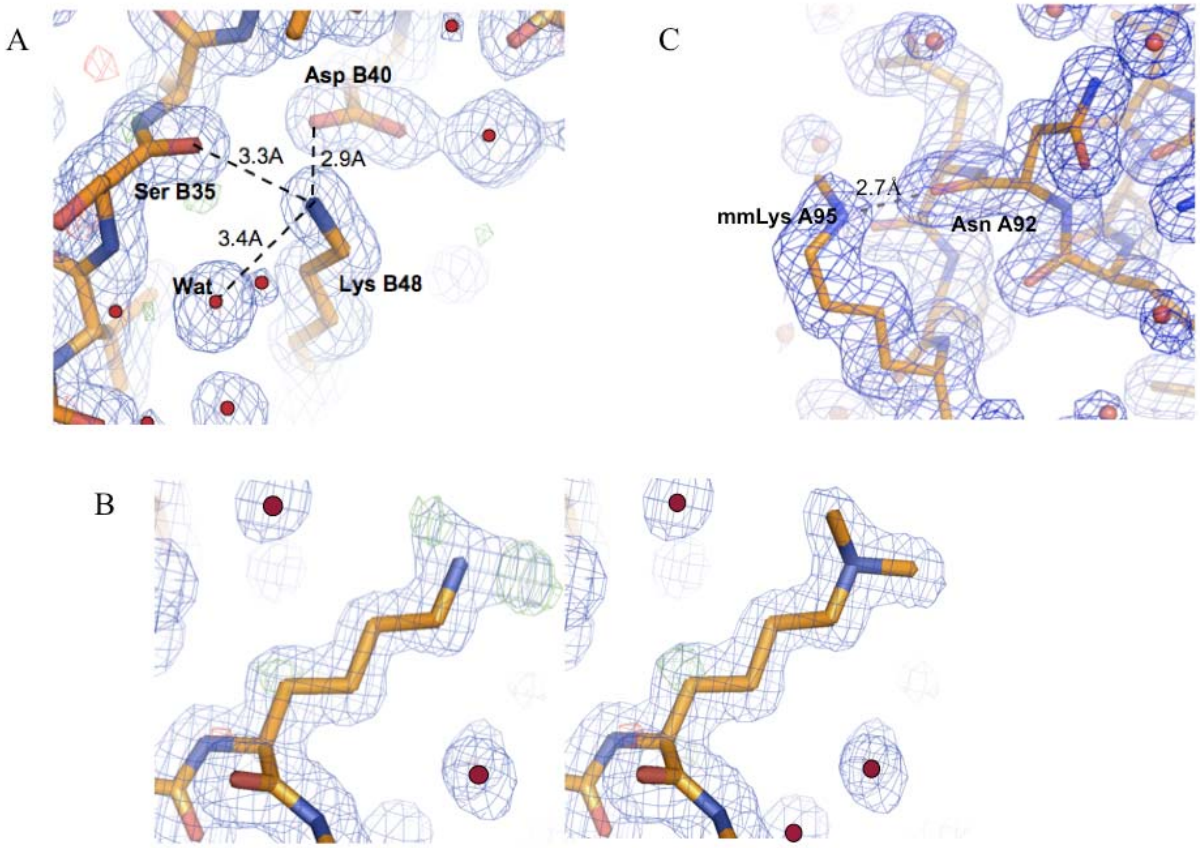
Supplementary Figure 3. The interaction energy profiles for the three bimolecular complexes. (1) Ethylammonium with water, (2) Dimethylethylammonium with water, (3) Dimethylethylamine with water.



A: Interaction energies with respect to the X-O distance, where X is the presumed hydrogen bond donor (N for complex 1 and C for complexes 2 and 3). The profile for complex 1 is shown in blue, complex 2 in red and complex 3 in cyan.

B: Interaction energy profiles for complex 1 (in blue) and complex 2 (in red) with respect to the N-O distance, mimicking the effect of 'inserting' a methyl group between the amine nitrogen atom and the solvent oxygen atom. The energy profiles suggest that at a distance longer than 4.2 Å the interaction of water with dmLys is more favorable than with unmethylated lysine.

Supplementary Figure 4. Unmethylated, monomethylated, and dimethylated lysines with a well defined electron density map.



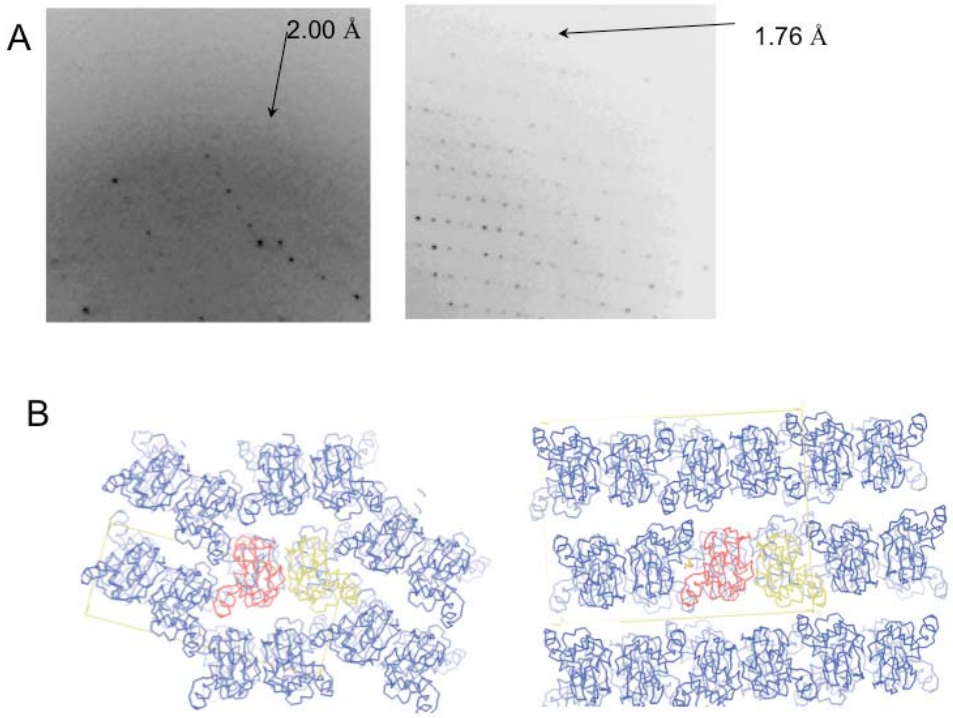
Carbon atoms are in dark yellow, nitrogen atoms in dark blue, and red indicates oxygen atoms. Distances are shown in Å. In navy mesh is a 2FoFc map contoured at 1σ.

A. Electron density map of LysB48 showing no density for two methyl groups suggesting this Lys is not methylated but ordered in the structure of mannose/sorbose specific IIA subunit of phosphotransferase system from *Enterococcus faecalis* (2IAC) determined at 1.45 Å resolution. It is likely that the chemical environment prevented this lysine from being methylated.

B. In the same structure (2IAC), the electron density map around LysA68 for a structure with modeled unmethylated lysines contoured at 1 σ and a difference map contoured at 3 σ showing additional density corresponding to two methyl groups, in the left panel and in the right, electron density map around dmLys-A68 for a structure with modeled dmLys A68 contoured at 1 σ and a difference map contoured at 3 σ showing additional density corresponding to two methyl groups.

C. mmLysA95 in the structure of YdhR (1XC3, 2.7 Å) the carbonyl oxygen of AsnA92 is accepting hydrogen from the NZ of monomethylated lysine mmLysA95 forming a tight contact hindering further methylation of this lysine.

Supplementary Figure 5. Comparison of the diffraction limits and structure of the VP0580 protein from *Vibrio parahaemolyticus* in native and methylated forms.



A. Comparison of the diffraction patterns of the unmethylated (left panel) and the methylated (right panel). The arrow and number indicates the resolution limit for each dataset.

B. Crystal packing of VP0580 protein molecules in the crystal. The unmethylated one is ($C222_1$) on the left and the methylated one ($P2_12_12_1$) is on the right.

Supplementary Table 1. Structures and some properties of methylated proteins used in this study.

PDB ID Methylated/ Native	Identifier APC number	Molecular Weight	Protein pI	Hydropathy	# of Lys in the protein sequence	Diff. Limit Methylated (Å)	Diff. Limit Native (Å)
Δ^a	APC35627	29798	5.01	0.014	6	2.60	3.50
1TWU	APC1848	15588	5.50	-0.363	6	2.00	2.50
1XC3	APC1098	32440	5.18	-0.002	15	1.60	2.20
1YLM	APC1684	16650	4.77	-0.253	10	1.80	
1YQH	APC22703	11795	4.82	-0.192	8	1.30	
1Y9B	APC26917	9683	6.21	0.04	7	2.10	
Δ^b	APC22273	24034	6.63	-0.051	24	3.00	6.00
*2ARK/Δ	APC22280	20444	5.83	-0.116	14	2.40	2.00
1YYV	APC24195	14387	7.97	-0.292	4	2.30	3.20
1XAF	APC27896	26380	6.52	-0.093	11	2.00	3.50
2B20	APC27316	45608	5.76	-0.369	12	2.85	3.20
2IAZ	APC80495	12457	5.19	-0.271	10	2.50	
2OX6	APC83631	18819	5.14	-0.299	11	1.70	
2IAC	APC28805	14548	4.22	0.292	5	1.50	3.30
2FML	APC29501	31463	5.53	-0.516	19	2.30	
*2HO3/2HO5	APC80523	36374	5.77	-0.16	16	2.00	2.60
2P4P	APC86433.2	10074	5.10	-0.511	6	1.80	
2O3F	APC85504	15573	9.70	-0.087	9	1.85	
2HAY	APC29758	25283	7.00	-0.325	18	2.00	
2OZZ	APC28298	25412	4.70	-0.103	7	2.30	
2HKT	APC27974	19210	4.10	-0.022	8	2.50	
2OAI	APC86234.2	10353	3.90	-0.282	1	1.80	
2PLI	APC83979.1	9792	4.70	-0.299	2	1.70	
*2QHQ/2QM2	APC86649	13669	4.10	-0.306	5	1.75	2.10
3BYW	APC90585.2	18440	4.10	-0.187	4	2.35	2.45
*2IDL/Δ	APC80417	12820	4.17	-0.125	2	1.70	2.15

Δ^a, Δ^b - These structures are being prepared for deposition: phenazine biosynthesis-like protein from *B. stearothermophilus*, and Aq_2056 from *A. aeolicus* respectively. * - both native and methylated solved.

Supplementary Table 2. List of proteins used in the reductive methylation experiments. The APC numbers refers to the protein id in the Midwest Center for Structural Genomics database and can be downloaded the following web sites (www.mcsg.anl.gov or http://targetdb.pdb.org/).

APC#	GI #	Fragment	# of Lys residues	Native protein not screened (a reference set)	Native protein screened but no crystal	Native protein crystallized, crystals poor quality	Crystals after methylation	Diffraction quality crystals	PDB id
167	2506917	1-283	12			X			
1004	586860	1-207	20		X				
1010	586874	1-292	26			X			
1013	586885	1-489	38	X			X		
1024	P70976	1-255	20			X	X		
1028	732404	1-414	35						
1036	O31437	1-394	28	X			X		
1047	O34621	1-472	29		X				
1048	O34772	1-453	35		X				
1049	20178041	1-373	28			X	X		
1054	P94400	1-397	23			X			
1058	730830	2-242	12	X					
1060	20178046	1-382	28	X			X		
1063	1175715	1-257	9			X			
1079	P96635	1-352	31	X					
1089	P96684	1-147	8			X	X		
1090	P96693	1-312	13			X			
1098	3914959	1-299	15			X	X	X	1XC3
1108	Q7BVT7	1-104	7			X			
1303	O34682	1-150	13	X					
1341	418462	1-100	11			X			
1343	417830	1-297	17			X	X		
1446	1730918	1-144	7			X			
1448	1730910	1-205	13			X			
1629.1	2635449	284-428	9			X			
1629.2	2635449	271-420	8			X			
1653	O34614	1-194	16		X				
1671	3023263	1-390	22			X	X		
1672	3023262	1-387	24	X			X		
1682	O32118	1-108	10			X			
1684	61680717	1-144	10		X		X	X	1YLM
1685	O32127	1-102	9			X			
1764	P96741	1-286	23	X					
1770	P96720	1-140	23	X			X		
1778	P71037	1-213	18	X					
1779	730100	1-262	17			X			
1781	P70958	1-157	6		X				
1785	1176958	1-184	18		X				
1786	729902	1-195	11			X	X		
1790	1171654	1-184	8		X				
1809	732344	1-416	38			X			
1844	Q45591	1-238	17		X				
1848	586809	1-139	6			X	X	X	1TWU
1932	2633104	1-113	21			X			

1970	**2632933**	**29-166**	**15**			**X**			
22201	**15606645**	**1-176**	**26**			**X**	**X**	**X**	
22228	**15606800**	**1-131**	**14**			**X**			
22273	**15607026**	**1-211**	**24**			**X**	**X**	**X**	**Δ**
22280	**15607053**	**1-185**	**14**			**X**	**X**	**X**	***2ARK/Δ**
22316	**15605894**	**1-141**	**10**		**X**				
22318	**15605896**	**1-222**	**19**		**X**		**X**		
22324	**15605901**	**1-331**	**20**			**X**	**X**	**X**	
22326	**15605917**	**1-133**	**13**		**X**				
22526	**5103929**	**1-149**	**15**		**X**				
22678	**29893867**	**1-97**	**31**			**X**			
22703	**29894173**	**1-106**	**8**			**X**	**X**	**X**	**1YQH**
22737	**29894581**	**1-252**	**11**			**X**			
22812	**29895316**	**1-200**	**19**			**X**	**X**		
22860	**29895590**	**1-125**	**11**		**X**				
22872	**16763422**	**1-572**	**15**			**X**			
22899	**16763553**	**1-327**	**17**		**X**				
22910	**16763608**	**1-241**	**13**			**X**			
23084	**15925984**	**1-97**	**5**			**X**			
23121	**15926233**	**1-227**	**17**			**X**			
23124	**15926255**	**1-121**	**10**			**X**	**X**		
23131	**15926334**	**1-168**	**13**			**X**			
23142	**15926443**	**1-331**	**21**			**X**			
23159	**15926651**	**1-374**	**32**			**X**			
23624	**16766012**	**1-100**	**7**			**X**			
23680	**15928156**	**1-99**	**6**		**X**				
23682	**15928167**	**1-288**	**14**			**X**	**X**		
23890	**16766210**	**1-96**	**8**			**X**			
23892	**16766216**	**1-126**	**3**			**X**			
23896	**16766228**	**1-475**	**22**		**X**				
23906	**16766251**	**1-317**	**21**		**X**				
23911	**16766265**	**1-380**	**12**		**X**				
23930	**16766365**	**1-297**	**10**			**X**			
24116	**16767285**	**1-285**	**9**		**X**				
24132	**16767344**	**1-291**	**15**			**X**			
24195	**16767648**	**1-128**	**4**			**X**	**X**	**X**	**1YYV**
24395	**16764484**	**1-293**	**12**			**X**	**X**	**X**	
24688	**29895377**	**1-140**	**13**			**X**			
24695	**29895450**	**1-419**	**32**			**X**			
24899	**29896750**	**1-168**	**8**		**X**				
24908	**29896822**	**1-181**	**13**			**X**	**X**		
24940	**29897009**	**1-228**	**18**			**X**			
24955	**29897064**	**1-172**	**10**			**X**	**X**	**X**	
25156	**29898379**	**1-190**	**13**			**X**			
25174	**29898487**	**1-260**	**18**			**X**			
25234	**29898852**	**1-344**	**24**			**X**			
25243	**29898909**	**1-196**	**10**			**X**			
25244	**29898914**	**1-203**	**11**			**X**	**X**		
25272	**29893904**	**1-219**	**17**			**X**			
25349	**29894659**	**1-191**	**15**			**X**	**X**	**X**	
25388	**16766124**	**1-176**	**8**			**X**			
25420	**16766410**	**1-239**	**11**			**X**			
25430	**16766519**	**1-378**	**18**			**X**			
25436	**16766556**	**1-154**	**5**			**X**			
25439	**16766570**	**1-167**	**2**			**X**			
25451	**16766653**	**1-209**	**8**			**X**	**X**		
25761	**29894894**	**123**	**6**			**X**			

25811	**29895290**	**1-290**	**19**			**X**			
25824	**29895445**	**1-179**	**21**			**X**			
25896	**29896031**	**1-170**	**8**			**X**			
26071	**29897384**	**1-272**	**23**		**X**				
26161	**29898278**	**1-143**	**10**			**X**			
26175	**29898445**	**1-169**	**7**			**X**			
26366	**9655124**	**1-320**	**17**			**X**			
26562	**9656019**	**1-218**	**6**			**X**			
26720	**9656693**	**1-153**	**8**			**X**			
26721	**9656697**	**1-302**	**16**			**X**			
26745	**9656811**	**1-351**	**18**			**X**	**X**		
26755	**9656843**	**1-147**	**10**			**X**			
26780	**9656989**	**1-256**	**7**			**X**			
26825	**9657253**	**1-188**	**14**			**X**			
26917	**9657717**	**1-90**	**7**			**X**	**X**	**X**	**1Y9B**
26919	**9657722**	**1-92**	**7**			**X**			
26922	**9657729**	**1-155**	**10**			**X**			
27003	**9658060**	**1-126**	**6**			**X**			
27104	**9658473**	**1-656**	**29**			**X**			
27131	**9655086**	**1-139**	**10**			**X**			
27259	**30040098**	**1-485**	**28**			**X**			
27270	**30040126**	**1-269**	**16**			**X**			
27284	**30040174**	**1-349**	**20**			**X**			
27316	**30040286**	**1-400**	**12**			**X**	**X**	**X**	**2B20**
27507	**30040929**	**1-153**	**5**			**X**			
27636	**30041388**	**1-359**	**12**			**X**			
27757	**30041782**	**1-259**	**15**			**X**			
27867	**30042143**	**1-255**	**6**			**X**			
27896	**30042248**	**1-243**	**11**			**X**	**X**	**X**	**1XAF**
27901	**30042267**	**1-227**	**13**			**X**			
27945	**30042393**	**1-109**	**1**			**X**			
27947	**30042396**	**1-454**	**16**			**X**			
27968	**30042474**	**1-400**	**13**			**X**			
27974	**30042499**	**1-169**	**8**		**X**		**X**	**X**	**2HKT**
28000	**30042573**	**1-125**	**3**			**X**			
28012	**30042600**	**1-619**	**31**			**X**			
28013	**30042602**	**1-82**	**2**			**X**	**X**		
28022	**30042635**	**1-193**	**14**			**X**			
28039	**30042689**	**1-338**	**15**			**X**			
28156	**30043091**	**1-413**	**21**			**X**			
28160	**30043099**	**1-236**	**17**			**X**			
28186	**30043187**	**1-494**	**12**			**X**			
28218	**30043292**	**1-240**	**14**			**X**	**X**	**X**	
28290	**30043561**	**1-694**	**37**			**X**			
28291	**30043563**	**1-191**	**8**			**X**			
28298	**30043595**	**1-228**	**7**			**X**	**X**	**X**	**2OZZ**
28331	**30043714**	**1-504**	**31**			**X**			
28344	**30043762**	**1-220**	**13**			**X**			
28356	**30043788**	**1-188**	**13**		**X**				
28359	**30043809**	**1-426**	**15**		**X**				
28365	**30043825**	**1-249**	**4**		**X**				
28379	**30043857**	**1-283**	**16**		**X**				
28381	**30043866**	**1-245**	**16**			**X**			
28386	**30043879**	**1-357**	**13**		**X**				
28394	**30040161**	**1-482**	**28**			**X**			
28492	**29344618**	**1-265**	**13**			**X**			
28560	**24349560**	**1-272**	**13**			**X**	**X**	**X**	

28564	**30041954**	**1-311**	**13**			**X**			
28567	**30043800**	**1-337**	**15**		**X**				
28575	**34397407**	**1-313**	**15**			**X**			
28627	**39984053**	**1-284**	**9**			**X**	**X**	**X**	
28706	**29342205**	**1-226**	**17**			**X**			
28752	**29342415**	**1-159**	**23**			**X**			
28756	**29342421**	**1-182**	**21**			**X**	**X**		
28762	**29342428**	**1-146**	**20**			**X**			
28804	**29342550**	**1-329**	**19**			**X**			
28805	**29342551**	**1-139**	**5**			**X**	**X**	**X**	**2IAC**
28864	**29342741**	**1-314**	**21**			**X**	**X**	**X**	
28878	**29342796**	**1-128**	**3**			**X**			
28883	**29342819**	**1-295**	**17**			**X**	**X**		
29310	**29344107**	**1-96**	**8**			**X**			
29341	**29344208**	**1-599**	**30**			**X**	**X**	**X**	
29367	**29344271**	**1-91**	**9**			**X**			
29391	**29344320**	**1-159**	**8**			**X**	**X**	**X**	
29393	**29344329**	**1-228**	**16**			**X**			
29404	**29344363**	**1-91**	**6**			**X**			
29410	**29344383**	**1-260**	**18**			**X**	**X**	**X**	
29464	**29344524**	**1-335**	**15**			**X**			
29467	**29344532**	**1-387**	**26**			**X**			
29470	**29344539**	**1-230**	**15**		**X**				
29472	**29344544**	**1-275**	**25**			**X**			
29475	**29344554**	**1-126**	**8**			**X**			
29476	**29344562**	**1-180**	**12**		**X**				
29486	**29344605**	**1-257**	**18**			**X**			
29490	**29344615**	**1-281**	**22**			**X**	**X**		
29495	**29344624**	**1-220**	**8**			**X**			
29496	**29344625**	**1-132**	**6**			**X**			
29498	**29344633**	**1-307**	**23**			**X**			
29501	**29344646**	**1-273**	**19**		**X**		**X**	**X**	**2FML**
29502	**29344647**	**1-174**	**7**			**X**			
29504	**29344651**	**1-266**	**28**		**X**				
29505	**29344652**	**1-456**	**40**			**X**			
29516	**29344703**	**1-109**	**6**		**X**				
29520	**29344720**	**1-404**	**17**		**X**				
29528	**29344745**	**1-302**	**17**			**X**			
29535	**29344752**	**1-112**	**9**		**X**				
29566	**29344847**	**1-217**	**10**			**X**			
29608	**29344984**	**1-189**	**14**			**X**			
29685	**13621397**	**1-147**	**13**		**X**				
29695	**13621447**	**1-194**	**17**		**X**				
29700	**13621472**	**1-287**	**21**			**X**			
29706	**13621486**	**1-176**	**11**		**X**				
29717	**13621541**	**1-210**	**11**			**X**			
29722	**13621575**	**1-197**	**16**		**X**				
29740	**13621630**	**1-263**	**21**			**X**			
29742	**13621647**	**1-107**	**9**			**X**			
29747	**13621692**	**1-285**	**23**			**X**			
29749	**13621697**	**1-350**	**24**			**X**			
29758	**13621728**	**1-221**	**18**			**X**	**X**	**X**	**2HAY**
29764	**13621750**	**1-244**	**20**		**X**				
29813	**13621885**	**1-238**	**21**			**X**			
29827	**13621947**	**1-125**	**8**			**X**			
29837	**13621985**	**1-387**	**34**		**X**				
29838	**13621986**	**1-228**	**11**			**X**			

29852	**13622061**	**1-262**	**15**		**X**				
29853	**13622063**	**1-178**	**11**			**X**			
29868	**13622112**	**1-268**	**19**			**X**			
29887	**13622148**	**1-117**	**9**		**X**				
29889	**13622151**	**1-259**	**22**			**X**	**X**		
29893	**13622156**	**1-203**	**17**			**X**	**X**		
29895	**13622164**	**1-415**	**20**			**X**			
29903	**13622186**	**1-451**	**22**			**X**			
29909	**13622202**	**1-141**	**6**			**X**			
35617	**RBSTP0490**	**1-316**	**27**	**X**					
35618	**RBSTP0491**	**1-373**	**14**	**X**					
35619	**RBSTP1439**	**1-379**	**11**			**X**			
35624	**RBSTP1445**	**1-260**	**14**	**X**					
35625	**RBSTP3055**	**1-132**	**13**	**X**					
35626	**RBSTP3059**	**1-395**	**15**			**X**			
35627	**RBSTP2400**	**1-273**	**5**	**X**			**X**	**X**	**Δ**
35646	**126031558**	**1-119**	**5**			**X**			
35682	**RBSTP0886**	**1-333**	**8**		**X**				
35701	**RBSTP3054**	**1-110**	**9**		**X**				
35703	**RBSTP2452**	**1-111**	**9**		**X**				
35732	**134105234**	**1-202**	**12**			**X**			
35872	**RBSTP1905**	**1-352**	**14**			**X**			
36131	**RBSTP1754**	**1-172**	**15**			**X**	**X**	**X**	
36138	**RBSTP2104**	**1-172**	**10**			**X**			
36143	**RBSTP2339**	**1-148**	**5**		**X**				
36150	**RBSTP2382**	**1-154**	**11**			**X**			
80001	**13622564**	**1-170**	**18**			**X**			
80209	**14971708**	**1-445**	**26**		**X**				
80252	**14971897**	**1-255**	**17**		**X**				
80253	**14971903**	**1-129**	**11**		**X**				
80264	**14971938**	**1-261**	**15**			**X**			
80417	**14972581**	**1-114**	**2**			**X**	**X**	**X**	***2IDL/Δ**
80494	**14972854**	**1-282**	**16**			**X**			
80495	**14972858**	**1-112**	**10**			**X**	**X**	**X**	**2B06**
80523	**14972973**	**1-325**	**16**			**X**	**X**	**X**	***2HO3/2HO5**
80559	**14973101**	**1-265**	**12**			**X**			
80587	**14973216**	**1-295**	**14**			**X**			
80592	**14973238**	**1-365**	**23**			**X**			
80594	**14973241**	**1-166**	**14**			**X**			
80844	**34396765**	**1-183**	**10**			**X**			
80873	**34396908**	**1-157**	**7**			**X**			
80877	**34396913**	**1-104**	**12**			**X**			
81263	**29337506**	**1-169**	**15**		**X**				
81383	**29338074**	**1-223**	**18**		**X**				
81522	**29338710**	**1-585**	**33**		**X**				
81523	**29338711**	**1-296**	**17**			**X**			
82694.1	**38200130**	**73-190**	**1**		**X**				
82723	**38200242**	**1-193**	**2**			**X**			
82724.1	**38200243**	**264-407**	**8**			**X**			
82727	**38200258**	**1-511**	**12**			**X**			
82795	**38200519**	**1-145**	**6**		**X**				
82804	**38200552**	**1-322**	**17**			**X**			
82823	**38200636**	**1-147**	**2**			**X**			
83622	**24374351**	**1-83**	**3**			**X**			
83625	**24374472**	**1-262**	**15**			**X**			
83628	**24375155**	**1-105**	**4**			**X**			
83631	**24375337**	**1-166**	**11**			**X**	**X**	**X**	**2OX6**

83633	**24375472**	**1-371**	**15**			**X**			
83640	**24375269**	**1-244**	**17**			**X**			
83734	**7225626**	**1-106**	**8**			**X**			
83766	**7227371**	**1-123**	**8**			**X**			
83772	**7225725**	**1-125**	**12**			**X**			
83979.1	**7225762**	**187-274**	**2**		**X**		**X**	**X**	**2PLI**
84054.1	**7226308**	**226-369**	**10**			**X**			
84081	**7227076**	**1-430**	**21**			**X**			
84110	**7226333**	**1-522**	**24**			**X**			
84720	**14971573**	**8-142**	**8**		**X**				
84723	**14972964**	**5-552**	**38**			**X**			
84725	**14972105**	**1-238**	**24**		**X**				
84740	**14971523**	**87-224**	**9**			**X**			
84747	**14971523**	**87-228**	**10**			**X**			
84773	**14973469**	**346-435**	**7**		**X**				
84780	**14972001**	**149-245**	**8**		**X**				
84791	**14972105**	**87-238**	**11**			**X**			
84809	**14971573**	**19-142**	**8**			**X**			
84824	**28853119**	**19-190**	**4**			**X**			
84913	**28851631**	**1-98**	**8**			**X**			
84956	**28850681**	**1-93**	**7**			**X**			
84986	**28854851**	**1-173**	**13**			**X**			
85006	**28855173**	**110-331**	**12**			**X**			
85246	**9656242**	**2-114**	**10**			**X**	**X**		
85274	**9658162**	**5-175**	**7**			**X**			
85283	**9658034**	**62-319**	**9**			**X**			
85331	**9658162**	**1-175**	**7**			**X**			
85356	**9658034**	**62-331**	**9**			**X**	**X**		
85491	**2632898**	**94-225**	**9**			**X**	**X**		
85504	**2632436**	**1-108**	**9**		**X**		**X**	**X**	**2O3F**
85596	**2632436**	**2-116**	**9**			**X**			
85628.2	**7225808**	**18-164**	**13**			**X**			
85633.3	**18891920**	**350-451**	**12**		**X**				
85637.1	**18893918**	**230-593**	**27**		**X**				
85637.2	**18893918**	**223-583**	**26**		**X**				
85638	**18893184**	**1-349**	**22**			**X**			
85639.1	**18893370**	**1-296**	**17**			**X**			
85684.1	**22534127**	**101-226**	**11**			**X**			
85713	**55737213**	**1-232**	**16**			**X**			
85835.2	**14247285**	**3-142**	**6**			**X**			
85930	**28899021**	**1-266**	**14**		**X**				
85938	**28901389**	**1-251**	**11**			**X**			
85976.2	**28898654**	**3-159**	**11**			**X**			
86055.1	**21647360**	**2-242**	**21**			**X**			
86087.1	**41325261**	**762-1018**	**11**		**X**		**X**		
86097.1	**41326466**	**342-440**	**4**			**X**			
86097.2	**41326466**	**335-433**	**4**			**X**			
86097.3	**41326466**	**342-433**	**4**		**X**				
86112	**20906340**	**1-291**	**16**		**X**				
86134.1	**20906724**	**1-82**	**3**			**X**			
86153.3	**24377441**	**91-222**	**12**		**X**				
86154.2	**24377835**	**127-199**	**3**			**X**			
86156.2	**24377749**	**1-180**	**19**			**X**			
86234.2	**28056530**	**349-439**	**1**			**X**	**X**	**X**	**2OAI/2R8D**
86244.3	**16415076**	**89-341**	**21**			**X**			
86338.1	**27315604**	**116-189**	**7**		**X**				
86344.1	**27316048**	**2-130**	**9**		**X**				

86348.1	**27315659**	**3-144**	**6**			**X**			
86348.2	**27315659**	**3-142**	**6**			**X**			
86368.2	**30138198**	**77-223**	**11**		**X**		**X**		
86389.2	**30138238**	**83-267**	**7**			**X**			
86414.2	**33148123**	**96-220**	**8**			**X**			
86424.1	**33148285**	**500-664**	**19**			**X**			
86428.1	**33148674**	**3-122**	**10**			**X**			
86432.1	**33148154**	**353-440**	**6**			**X**			
86433.2	**33149030**	**347-429**	**6**			**X**	**X**	**X**	**2P4P**
86570.3	**38200659**	**40-341**	**3**			**X**			
86594.1	**22534404**	**3-110**	**6**			**X**			
86649	**28900435**	**1-122**	**5**			**X**	**X**	**X**	***2QHQ/2QM2**
86753.1	**22535124**	**34-317**	**34**			**X**			
86753.2	**22535124**	**34-320**	**35**		**X**				
86774.1	**24377818**	**40-331**	**41**			**X**			
86774.2	**24377818**	**40-333**	**41**		**X**				
86883	**28901276**	**1-100**	**6**			**X**			
86893.1	**29343285**	**36-329**	**24**		**X**				
86893.2	**29343285**	**29-329**	**26**			**X**			
86945.1	**41326430**	**43-330**	**5**		**X**				
87001.1	**55736284**	**43-333**	**28**			**X**			
87001.2	**55736284**	**37-342**	**32**			**X**			
87006.2	**55737184**	**38-333**	**30**			**X**			
87006.3	**55737184**	**29-332**	**30**			**X**			
87683.1	**39985266**	**34-145**	**1**		**X**				
88437	**77461979**	**1-382**	**5**		**X**				
89010	**116490571**	**1-151**	**14**			**X**			
90010.1	**74961517**	**1-100**	**7**		**X**				
90010.2	**74961517**	**1-105**	**7**		**X**				
90010.5	**74961517**	**1-84**	**6**		**X**				
90010.6	**74961517**	**1-88**	**7**		**X**				
90121.1	**34397309**	**371-599**	**20**			**X**			
90214.1	**34397266**	**445-876**	**31**		**X**				
90411.1	**7226307**	**80-674**	**24**			**X**	**X**		
90464.1	**7226683**	**319-658**	**11**			**X**			
90469.1	**7225431**	**377-658**	**11**			**X**	**X**		
90470.1	**7226435**	**289-603**	**15**			**X**			
90585.2	**38199042**	**34-207**	**5**			**X**	**X**	**X**	**3BYW**
90691.1	**38200889**	**155-375**	**5**			**X**	**X**	**X**	
90743.1	**38199203**	**24-443**	**32**		**X**				
91329.1	**28899246**	**32-337**	**14**	**X**					
Total				**16**	**85**	**269**	**72**	**40**	**26**

Δ - These structures are being prepared for deposition. *both native and methylated solved.

Supplementary Methods

Selection of proteins

Initially, 90 random proteins from MCSG targets were selected in three groups; (A) 44 previously screened and crystallized but not good enough to be solved, (B) 30 screened but produced no crystal, (C) 16 new and previously not screened. After six new structures were successfully solved from this set of 90 methylated proteins, reductive methylation has been used routinely at the MCSG. In this Correspondence, the results from altogether 370 applications including the initial 90 targets are discussed.

Protein preparation

All proteins were prepared by following the standard procedure[1] adopted by the MCSG. The open reading frames (ORFs) were cloned in the pMCSG7 vector and over-expressed in *E. coli* BL21 (DE3) - Gold (Stratagene), harboring an extra plasmid encoding three rare tRNAs (AGG and AGA for Arg, ATA for Ile). The pMCSG7 vector bearing a TEV protease cleavage site creates a construct with cleavable His_6-tag fused into the N-terminus of the target protein and adds three additional residues (SerAsnAla) on that end. The cells were grown using seleno-methionine containing enriched M9 medium and conditions known to inhibit methionine biosynthesis[2]. The cells were grown at 37°C to an OD_{600} of ~0.8 and protein expression induced with 1 mM IPTG (Isopropyl β-D-1-thiogalactopyranoside). After induction, the cells were incubated overnight with shaking (180 rpm) at 18°C. The cells were harvested the next morning and re-suspended in 5 volumes of lysis buffer (50 mM HEPES pH 8.0, 500 mM NaCl, 10 mM imidazole, 10 mM β-mercaptoethanol, and 5% v/v glycerol); protease inhibitor (Sigma, P8849) and 1 mg/ml lysozyme were also added. The cells were stored at -20°C. For purification, thawed cells were sonicated; the lysate was clarified by centrifugation at 30,000 x g (RC5C-Plus centrifuge, Sorval) for 60 minutes, followed by filtration through 0.45 μm filter (Pall). The standard purification protocol was followed as described previously[1]. Immobilized metal affinity chromatography (IMAC-I) using a 5-ml HiTrap Chelating HP column charged with Ni^{+2} ions and buffer-exchange chromatography on a HiPrep 26/10 desalting column (GE Healthcare, formerly Amersham Biosciences) were performed using AKTA EXPLORER 3D and AKTA XPRESS (GE Healthcare). His_6-tag was

cleaved using the recombinant TEV protease expressed from the vector pRK508[3]. The TEV protease was added to the target protein in a ratio of 1:50 and the mixture was incubated at 4°C for 72 hours. The proteins were then purified using Ni-NTA agarose (Qiagen) or Ni sepharose 6 fast flow (GE Healthcare) packed in empty PD 10 columns (GE Healthcare). For this project, the volume of protein samples was divided in two; one half was dialyzed and buffer exchanged into crystallization buffer containing 20mM Tris pH 7.5, 250mM NaCl and 2mM DTT, followed by concentration, and the other half was modified as described below.

Reductive methylation protocol

The procedure for reductive methylation used in this study was adapted from the one described by Rypniewski *et al*.[4]. Initially, the experiment was conducted using sodium borohydride as the reducing agent, however, to reduce foaming and subsequent protein denaturation, the protocol was changed to a more gentle treatment with 1 M dimethylamine-borane complex (ABC) as the reducing agent. Typically, 10-20 mg of purified protein at concentrations of 5 – 10 mg/ml prepared in a buffer of 50 mM HEPES pH 8.0, 500 mM NaCl, 5% glycerol and 10 mM β-mercaptoethanol was used. All reagents were prepared fresh the day of experimentation: 1 M dimethylamine–borane complex (ABC) in water, 1 M formaldehyde in water, 0.67 M glycine, 1 M dithiothreitol (DTT) and the reaction buffer (50 mM HEPES pH 8.0, 500 mM NaCl, 5% v/v glycerol and 10 mM β-mercaptoethanol). All solutions were kept at 4°C or on ice as indicated. Protein solutions were concentrated to a volume of 5 ml, at varying protein concentrations, but always below 10 mg/ml. Forty microliters (µl) of 1 M formaldehyde per 1 ml of protein solution was added and mixed gently. This was followed immediately by 20 µl of 1 M ABC per 1 ml of protein solution and again the solution was gently mixed. The solution was incubated at 4°C for 2 hours and the procedure repeated. At the end of incubation, an additional amount of 10 µl of ABC per 1 ml of protein solution was added. The solution was incubated at 4°C overnight. The following day, 1 mg of glycine (final concentration 13.3 mM) (using a 67 mM solution) and DTT (final concentration 5 mM) were added to quench the reaction and the solution was left on ice for 2 hours. The proteins were either buffer exchanged extensively by dialysis or purified by size exclusion chromatography (Superdex 200 26/60, GE healthcare) using buffer conditions

described by Kim *et al.*[1], which not only removed residual reagents from the reaction, but also separated higher molecular weight protein aggregates. In addition, in some cases, analysis of the size exclusion chromatography profile revealed reaction-induced changes in the oligomerization states of the protein. After the size exclusion chromatography step, the proteins were concentrated using Centricon Plus-20 centrifugal concentrators (Millipore) and screened for crystallization conditions using commercial crystallization formulations. Using this procedure the majority of lysines in proteins were methylated as assessed by MS (data not shown). However, both methylated (mLys) and unmethylated lysines were also observed in electron density (Supplementary Fig. 4).

Protein crystallization

Both modified and unmodified samples of each protein were screened for crystallization conditions in sitting drops (Mosquito, TTP Labtech); 0.4 µl of protein was added to 0.4 µl of crystallization solution and equilibrated over 135 µl well solution. Commercial crystallization formulations available from Hampton Research (INDEX), Decode Genetics (Emerald Biostructures) (Wizard I & II), and Qiagen (formerly Nextal Biotechnologies) (PEGSII) were used for the crystal screening. Plates were kept at 4°C and 16°C in Robohotels and imaged with Minstrel III (RIGAKU).

Comparison of native and methylated structures – an observed reduction in isotropic temperature factors

Among 26 protein structures determined after methylation, four protein structures have also been determined and refined in the native state. Comparison of these structures can provide important insights into how methylation affects protein crystallization. One example is described in detail. The native HopJ type III effector protein VPA0580 (pfam08888) from *Vibrio parahaemolyticus* was crystallized in space group $C222_1$ with unit cell dimensions of a = 87.62 Å, b = 90.89 Å, c = 72.44 Å, $\alpha = \beta = \gamma = 90°$ and diffracted to 2.0 Å. However, the structure could not be solved by SAD because of crystal twinning. Moreover, the data suffers from a high mosaicity (near 2.0 degrees) as evident in Supplementary Fig. 5A. After reductive methylation, a different crystal form was obtained ($P2_12_12_1$) with smaller cell of a = 34.80 Å, b = 80.85 Å, c = 87.77 Å, $\alpha = \beta = \gamma =$

90° which diffracted to 1.76 Å. The structure was determined by SAD and refined. Using the same refined model as the search model, the structure of the native VPA0580 was solved by molecular replacement and refined to 2.1 Å. In methylated VPA0580 all lysine residues were found as dmLys by MS, and overall, the native and methylated structures were very similar with a RMSD between Cα atoms of 0.7 Å and both form a similar dimer. However, the dimers were packed differently in the crystal (Supplementary Fig. 5B); the native protein is packed more loosely with a solvent content of 54%, the methylated dimer packed more tightly with a solvent content of 46%. Different regions of each dimer are involved in lattice interactions to form two different symmetric arrangements. The most striking difference, however, is an isotropic average B factor of 40 $Å^2$ vs. 21 $Å^2$ for the native and methylated protein respectively. Interestingly, the average B factor profile over the whole chain is quite similar for both proteins as shown in Fig. 1A. Based on these results we argue that the impact of methylation of lysine residues in a protein is not limited to lysine and neighboring residues but appears to propagate through the entire structure and stabilize the protein.

Computation of hydrogen bonding interaction energies

The interaction energies between the solute molecule (ethylamine and N,N-dimethylethylamine) and the solvent molecule (water) were estimated with a procedure similar to those employed in the development of molecular mechanics force field parameters[5,6]. The geometry of the solute molecules were optimized at the Hartree-Fock 6-31G(d) level of theory and the water molecule was always constrained at the TIP3P[7] geometry. In all three cases, the oxygen atom in the water molecule served as the hydrogen bond acceptor and the only geometrical parameter being optimized in the solute-solvent complex is the distance between the hydrogen bond donor (X) and acceptor (O) (Supplementary Fig. 3). After the energy minima were located, single-point energies were evaluated by gradually changing the X-O distance, thus giving a more detailed description of the interaction energy profile. All calculations were carried out with the GAMESS[8] package on a BlueGene/L supercomputer at Argonne National Laboratory.

Supplementary Material References

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