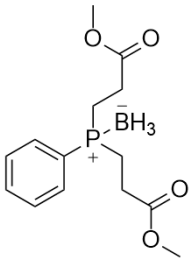
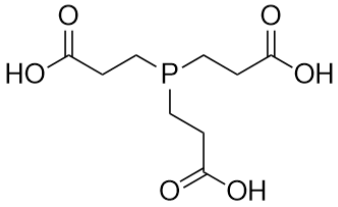
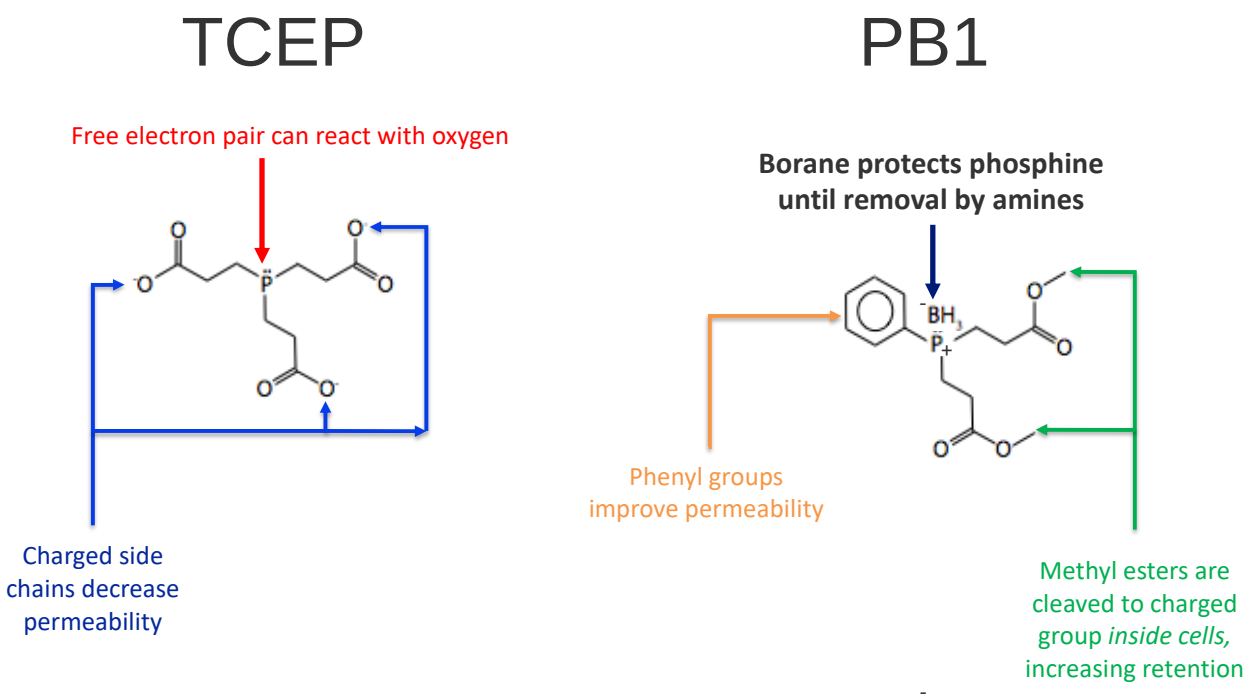
chemical properties of PB1 and TCEP are listed in **Supplementary Table S1**.

Supplementary Table S1. Comparison of PB1 and TCEP based on molecular characteristics calculated in Molecular Operating Environment software.

Feature	PB1	TCEP
Hydrogen bond acceptor atoms	2	6
Hydrogen bond donor atoms	0	6
Heavy atoms	20	16
Rotatable bonds	7	9
Aromatic bonds	6	0
Bond count	42	30
Dipole	0.63	0.56
SlogP	1.87	0.89
Molar refractivity (SMR)	8.51	5.83
Topological polar surface area (TPSA)	52.6	125.5
Van der Waals surface area (VSA)	342.4	267.4
Molecular weight	296.1	250.2
Structure		

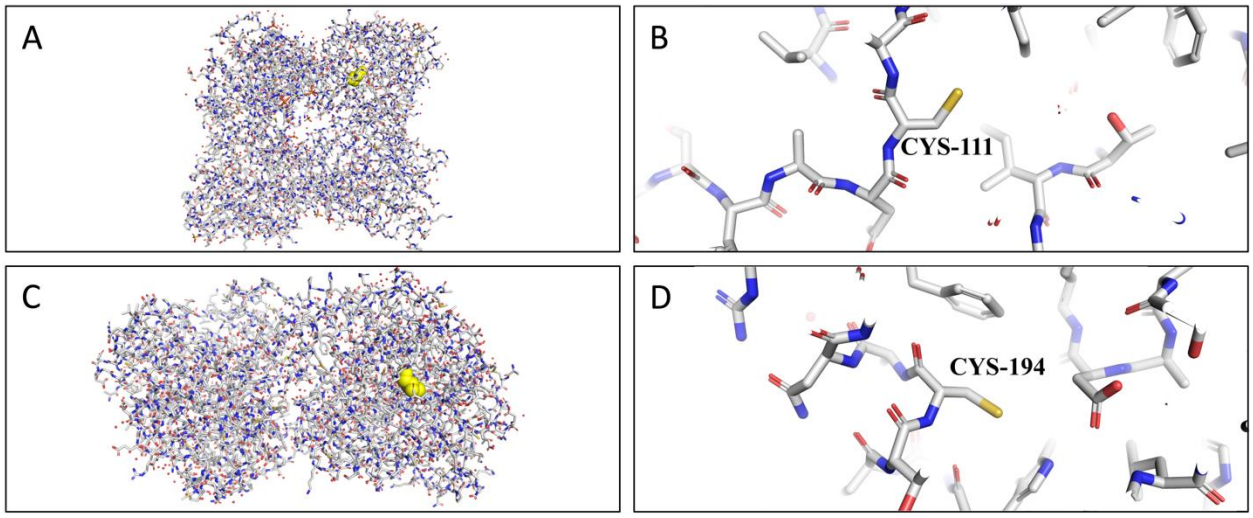


Supplementary Figure S1. Summary of major changes between prototype phosphine TCEP and phosphine-borane complex PB1

Proteins that underwent significant disulfide reduction or sulfhydryl modification with PB1

Elongation factor 1-alpha 1. PSM [K].NMITGTSQADcAVLIVAAGVGEFEAGISK.[N] showed a ratio of PB1/control of 0.49, with the PSM from positions 101-129 labeled at position 111 **(Supplementary Figure S2AB)**. Cys111 is located near the inside of the protein, isolated from other cysteines.

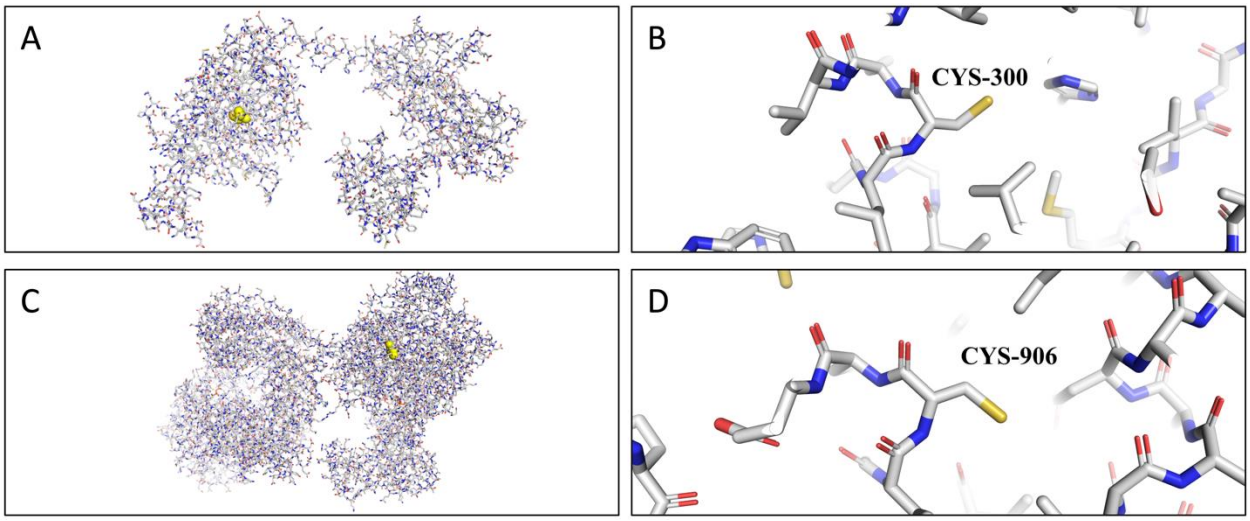
Elongation factor 1-gamma. PSM [K].NMITGTSQADcAVLIVAAGVGEFEAGISK.[N] had a PB1/control ratio of 1.7. The PSM from position 190-201 was labeled at Cys194 **(Supplementary Figure S2CD)**. Cys194 is isolated from other cysteines and near the surface of the protein.



Supplementary Figure S2. Computational images of labeled elongation factors. (A) Overall protein structure of elongation factor 1 alpha 1 from Oryctolagus cuniculus, with a sequence identity of 92% with Mus musculus(6RA9). (B) Cys111 of elongation factor 1 alpha. (C) Human elongation factor 1 gamma overall protein structure (5JPO). (D) Cys194 of elongation factor 1 alpha.

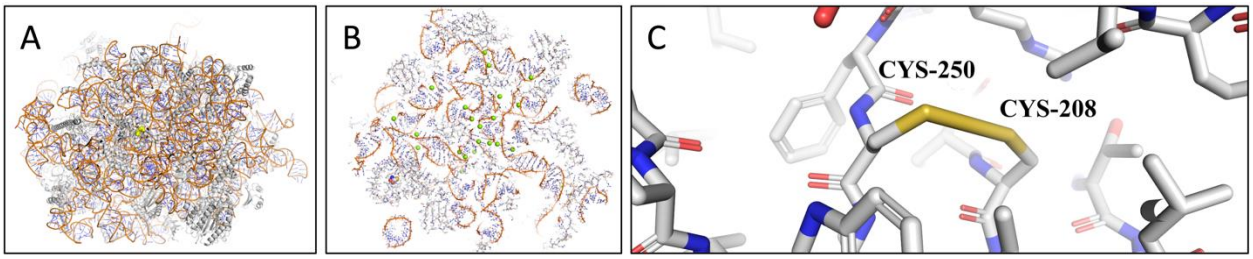
Ubiquitin carboxyl-terminal hydrolase 7 (USP 7) PSM [K].SFGWETLDSFMQHDVQELcR.[V] showed a ratio of PB1/control of 0.341. The PSM from positions 283-302 was labeled once at position 300, which is within a USP domain from 215-522 **(Supplementary Figure S3AB)**. The PDB 5FWI shows that Cys300 is isolated, with no neighboring cysteines with which to form intramolecular disulfide bonds.

Ubiquitin-like modifier activating enzyme 1 (UBA1). PSM [K].IIPAIATTTAAVVGLVcLELYK.[V] showed a PB1/control ratio of 1.87. The PSM from position 890-911 was labeled at Cys906 **(Supplementary Figure S3CD)**. The PDB 6DC6 of the human ubiquitin activating enzyme E1 shows Cys906 to be isolated and not exposed to solvent.



Supplementary Figure S3. Computational images of labeled ubiquitin-related proteins. (A) Overall protein structure of catalytic domain of ubiquitin carboxyl-terminal hydrolase 7 (5FWI). (B) Cys300 of Ubiquitin carboxyl-terminal hydrolase 7. (C) Human ubiquitin-like modifier activating enzyme 1 overall protein structure (6DC6). (D) Cys906 of ubiquitin-like modifier activating enzyme 1.

60S ribosomal protein. Two subunits of 60S ribosomal protein were labeled. PSM [R].FcIWTESAFR.[K] of parent protein 60S ribosomal protein L4 showed a ratio of PB1/control of 3.01. The PSM from positions 249-258 was labeled at position 250 **(Supplementary Figure S4)**. In addition, PSM [K].FGIIcMEDLIHEIYTVGK.[R] of 60S ribosomal protein L7 showed a ratio of PB1/control of 4.48. The PSM from positions 204-221 was labeled at position 208. Although two different proteins were labeled, they are connected by a disulfide bond extending from Cys208 on L7 to Cys250 on L4. This intermolecular disulfide is visualized in the PDB 6SWA of mouse ribosome 60S protein bound to Ebp1.



Supplementary Figure S4. Computational images of labeled 60S ribosomal subunit proteins. (A) Overall 60S ribosomal protein structure (6SWA). (B) Local protein environment surrounding disulfide between L4 and L7. (C) Disulfide between Cys250 of L4 protein and Cys208 of L7 protein.

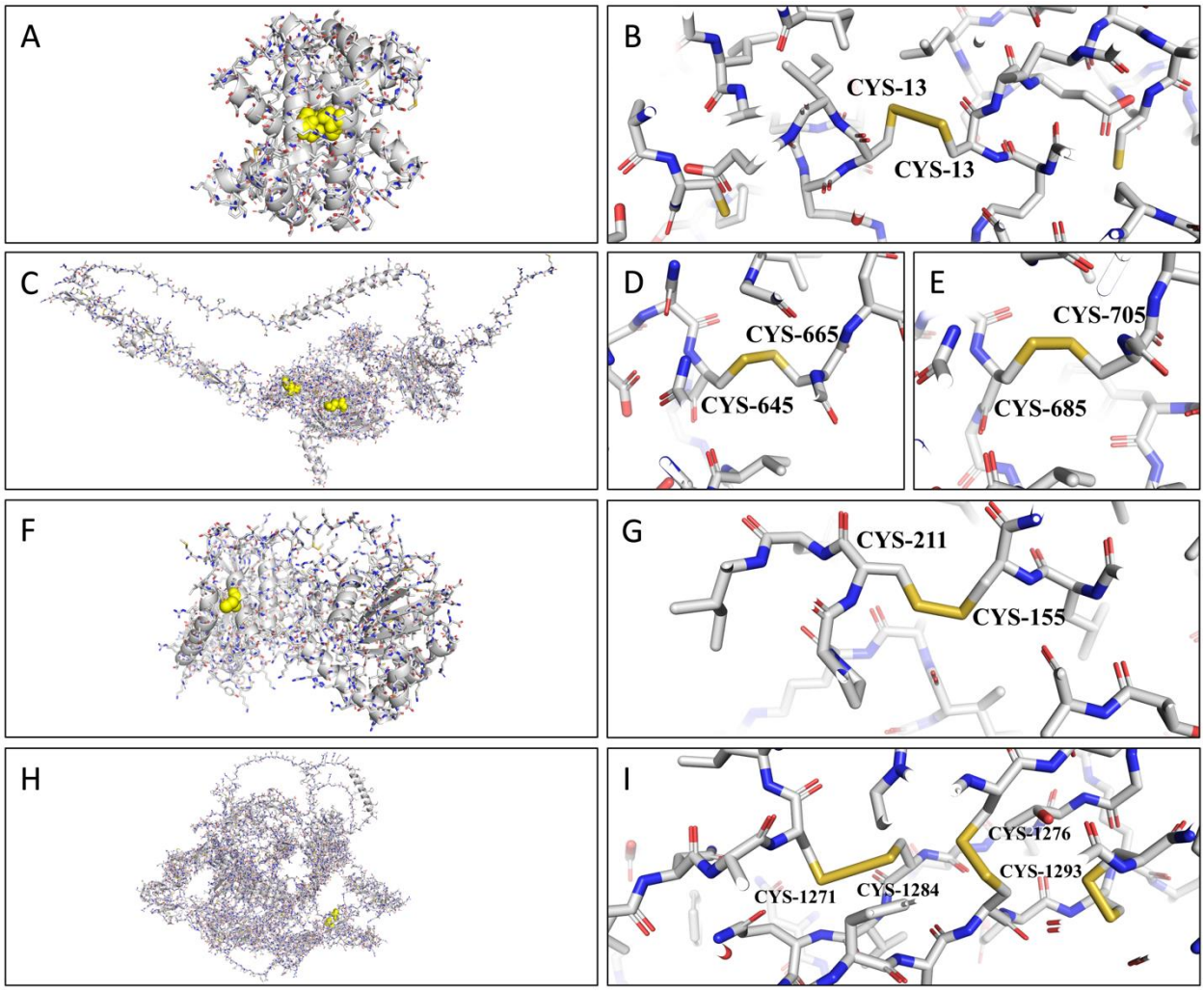
S100-A11. PSM [R].cIESLIAVFQK.[Y] showed a PB1/control ratio of 1.3. The PSM was labeled at Cys13, forming a homodimeric disulfide between two instances of the protein **(Supplementary Figure S5AB)**.

Thrombospondin-4. PSM [K].DGIGDEcDDDDDNDGIPDLVPPGPDNcR.[L] showed around a 3-fold decrease in the cells exposed to PB1 compared to the control with a ratio of 0.329. The PSM from 659-686 was labeled at positions 665 and 685 **(Supplementary Figure S5CD)**. This stretch of the protein contains a TSP type-3

repeat from 654-693. Similarly to Notch1, the PSM was labeled on two cysteines, both of which form disulfide bonds but not with each other. The two disulfide bonds in question connect cysteine residues 645-665 and 685-705.

DNA dC->dU-editing enzyme APOBEC-3. PSM [R].LYNVQDPETQQNLcR.[L] showed a ratio of PB1/control of 2.55. The PSM from positions 142-156 was labeled at position 155 **(Supplementary Figure S5FG)**. This is within a CMP/dCMP-type deaminase domain from 49-165. This protein is involved in deoxycytidine deaminase activity. Structural data show cysteine 155 participates in an intramolecular disulfide bond with cysteine 211.

Neurogenic locus notch homolog 1 (Notch1). PSM [R].CEGDVNEcLSNPcDPR.[G] showed a drastically reduced abundance in the cells exposed to PB1 compared to the control with a ratio of PB1/control of 0.067. The PSM from 1264-1279 was labeled twice, on positions 1271 and 1276 **(Supplementary Figure S5HI)**. The labeled cysteines participate in two separate disulfide interactions, the first extending from Cys1271 to Cys1284 and the second from Cys1276 to Cys1293.



Supplementary Figure S5. Computational images of labeled disulfide forming cysteines. (A) Human S100 calcium-binding protein A11 overall structure (2LUC). (B) Cys13 to Cys13 disulfide linking S100 calcium-binding protein A11 homodimer. (C) AlphaFold-predicted Mus musculus thrombospondin-4 overall protein structure. (D) Cys645 to Cys665 disulfide of thrombospondin-4. (E) Cys685 to Cys705 disulfide of thrombospondin-4. (F) AlphaFold-predicted Mus musculus structure for DNA dC->dU-editing enzyme APOBEC-3. (G) Cys155 to Cys211 intramolecular disulfide in DNA dC->dU-editing enzyme APOBEC-3. (H) AlphaFold-predicted Mus musculus structure of Notch1. (I) Disulfides between Cys1271-Cys1284 and Cys1276-Cys1293 for Notch1.

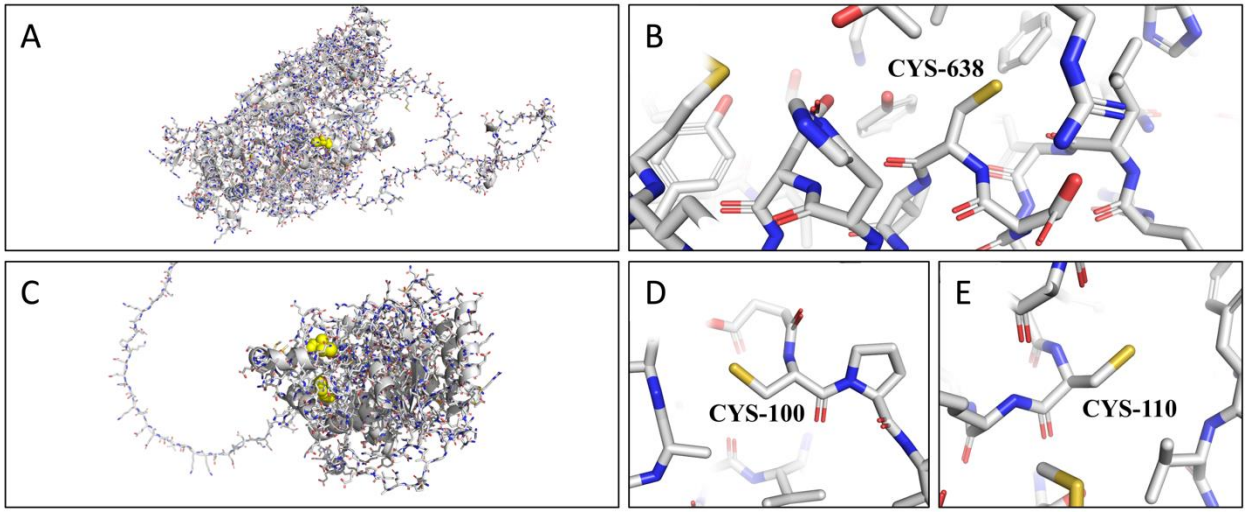
116 kDa U5 small nuclear ribonucleoprotein component. PSM

[K].VEESGEHVILGTGELYLDcVMHDLR.[K] showed a ratio of PB1/control of 2.61. The

PSM from positions 620-644 was labeled at position 638 **(Supplementary Figure**

S6AB). Structural data of the ribonuclear protein show Cys639 to be isolated and near the surface of the protein.

Histone deacetylase 1. PSM [R].FNVGEDcPVFDGLFEFcQLSTGGSVASAVK.[L] showed a ratio of PB1/control of 0.34. The PSM from positions 94-123 was labeled at 100 and 110 **(Supplementary Figure S6CDE)**. This protein has been implicated in the Notch-HLH activation pathway. The structure shows that although cysteines 100 and 110 were both labeled, they do not appear to participate in an intramolecular disulfide bond due to their physical separation from other cysteines.



Supplementary Figure S6. Computational images of additional labeled proteins (A) AlphaFoldpredicted Mus musculus 116 kDa U5 small nuclear ribonucleoprotein overall structure. (B) Cys638 of 116 kDa U5 small nuclear ribonucleoprotein. (C) AlphaFold-predicted Mus musculus overall protein structure of histone deacetylase 1. (D) Labeled cysteine 100 of histone deacetylase 1. (E) Labelled cysteine 110 of histone deacetylase 1.