

Supplementary Information

Delva-Wiley et al.

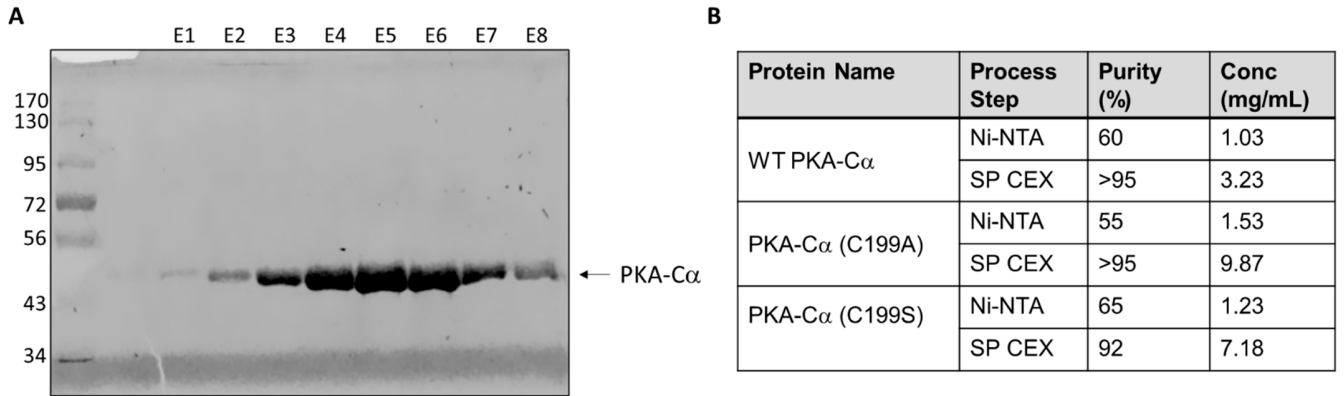


Figure S1. Purification of PKA-C α . **A.** SDS-PAGE gel, stained with colloidal Coomassie-G250, following SP cation exchange (SP CEX) chromatography. The SP product exhibited a migration pattern consistent with His₆-PKA-C α (arrow). **B.** Process purification summary for wild-type (WT) PKA-C α , PKA-C α (C199A), and PKA-C α (199S).

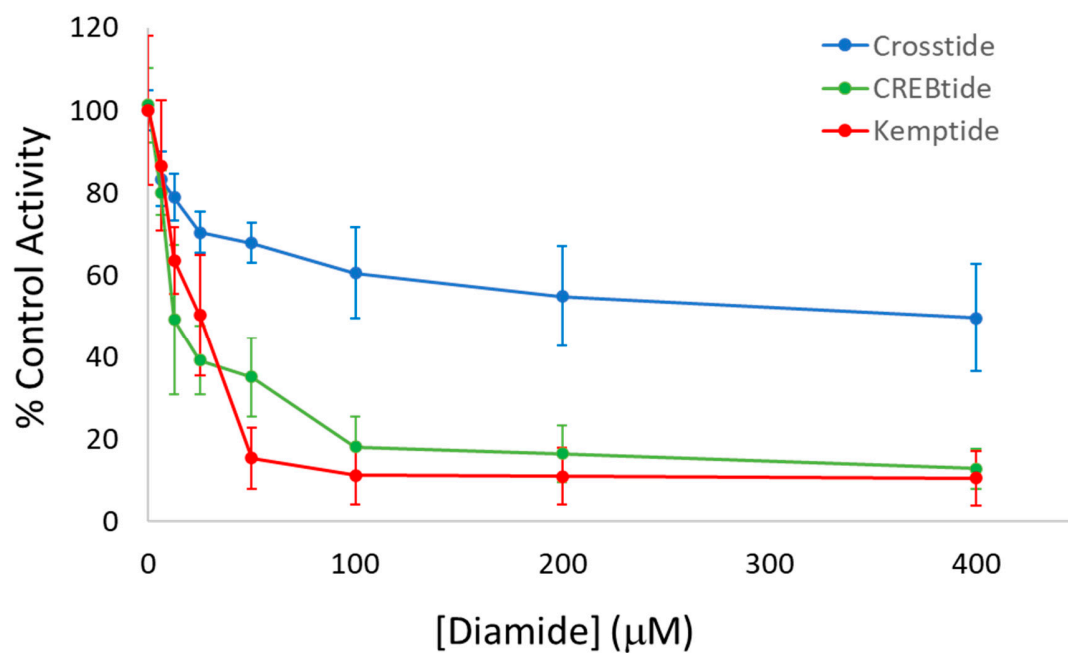


Figure S2. Impact of diamide-mediated oxidation on PKA-C α toward different model substrates following an extended incubation with diamide. Normalized relative activity of PKA-C α toward Kemptide (red), CREBtide (green) and Crosstide (blue) following pre-treatment with the indicated concentrations of diamide for 20 minutes at room temperature.

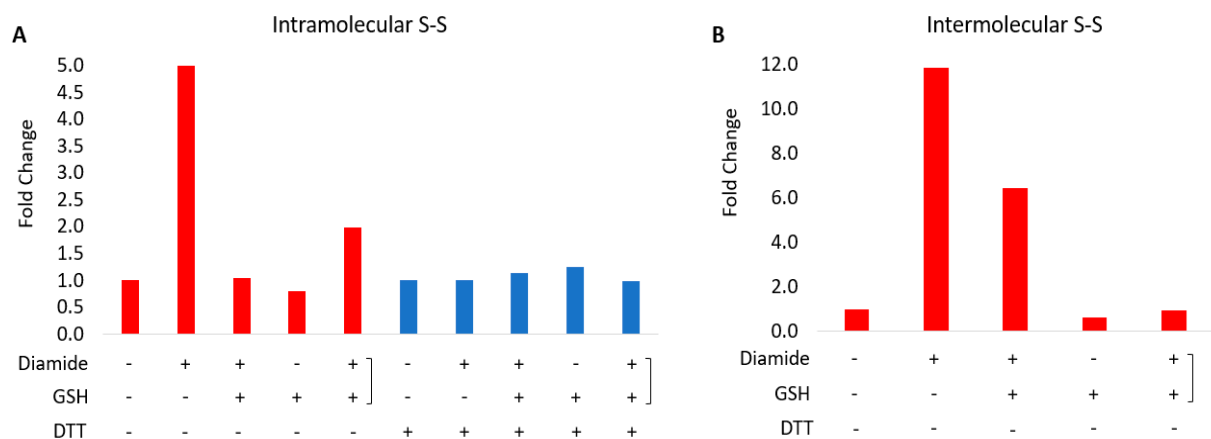


Figure S3. Quantitation of band intensities for intra- and intermolecular disulfide bonded species in non-reducing PAGE following treatment with diamide and/or glutathione. A. Normalized intensities, expressed as fold change relative to the untreated control, of the faster migrating species in Figure 1D (corresponding to PKA-C α that has formed an intramolecular disulfide bond) in the absence (red) and presence (blue) of reducing agent. DTT: dithiothreitol; GSH: reduced glutathione. Brackets in wells 5 and 10 indicate that diamide and GSH were added simultaneously. **B.** Normalized intensities, expressed as fold change relative to the untreated control, of the slower migrating species in Figure 1D (corresponding to PKA-C α that has formed intermolecular disulfide bonds) in the absence of reducing agent. Abbreviations are as in A.

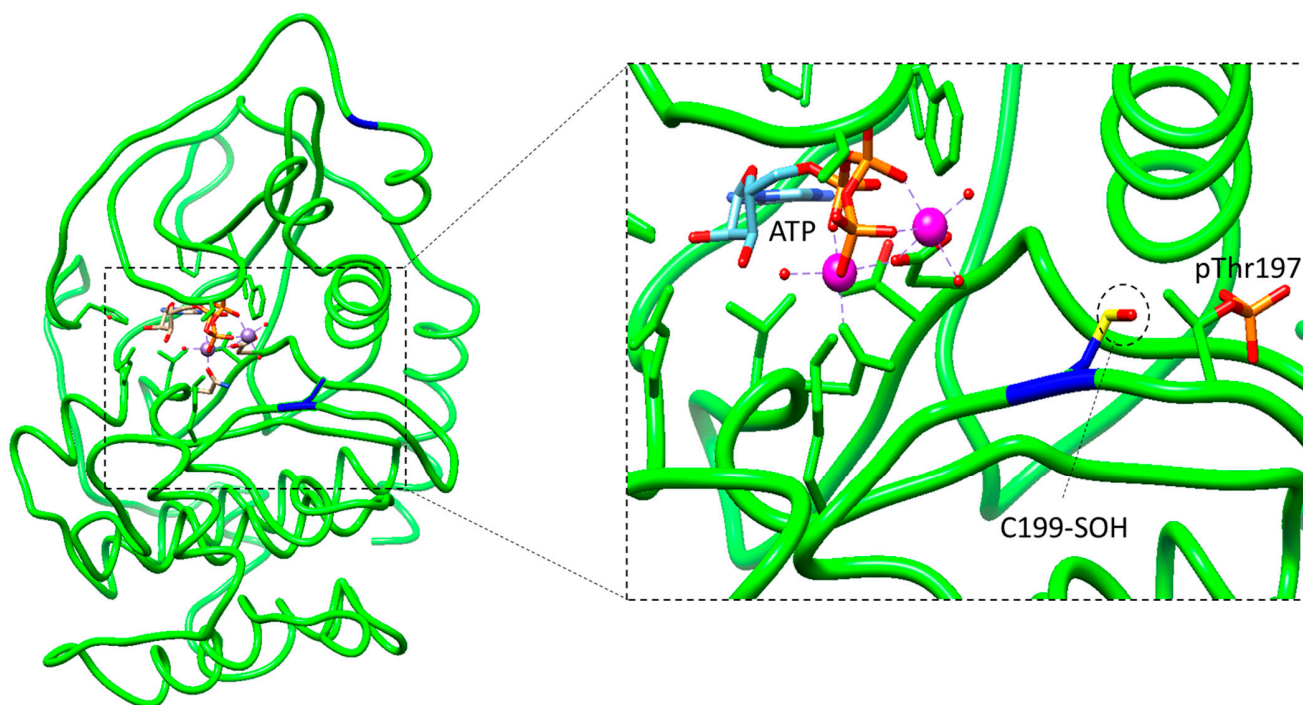


Figure S4. Sulfenylated C199 is well accommodated within PKA-Ca's active site. The sulfhydryl group of C199 in PKA-Ca bound to ATP (PDB: 1ATP) was converted to sulfenic acid in silico using the "build" tool in Chimera-X. The modified structure was then energy minimized by molecular dynamics simulation by UCSF Chimera-X. Sulfenylated C199 (C199-SOH), phospho-T197 (pThr197) and ATP are labeled.

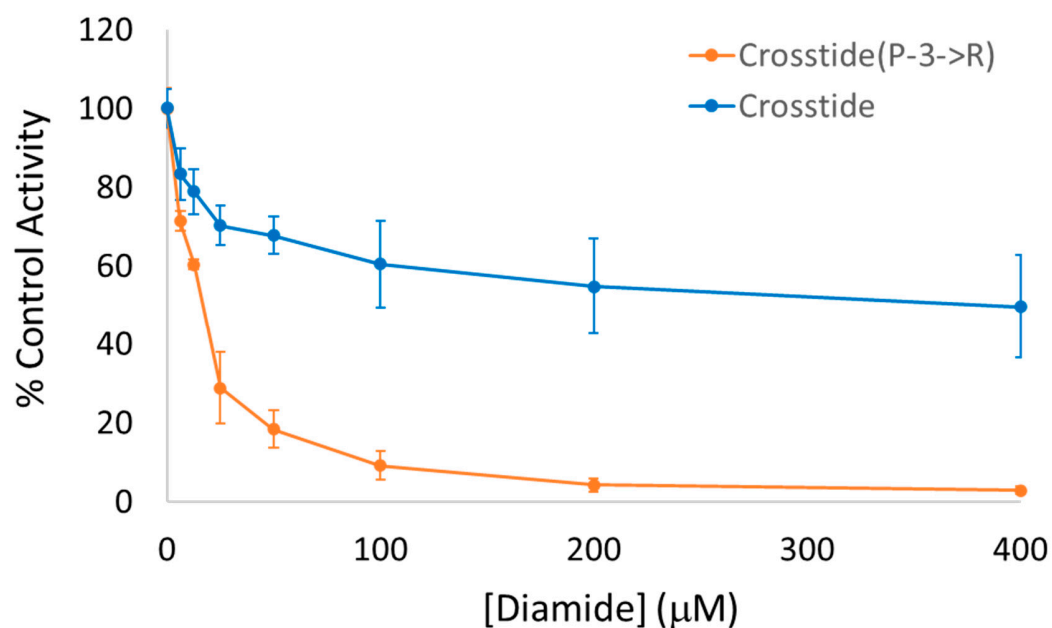


Figure S5. Impact of diamide-mediated oxidation on PKA-C α toward Crosstide and Crosstide(P-3 \rightarrow R). Normalized relative activity of PKA-C α toward Crosstide (blue) and a Crosstide variant in which the Pro residue in the P-3 position has been mutated to Arg (Crosstide(P-3 \rightarrow R)) following pre-treatment with the indicated concentrations of diamide for 10 minutes at room temperature. Error bars represent standard error about the mean of three independent experiments run in duplicate.

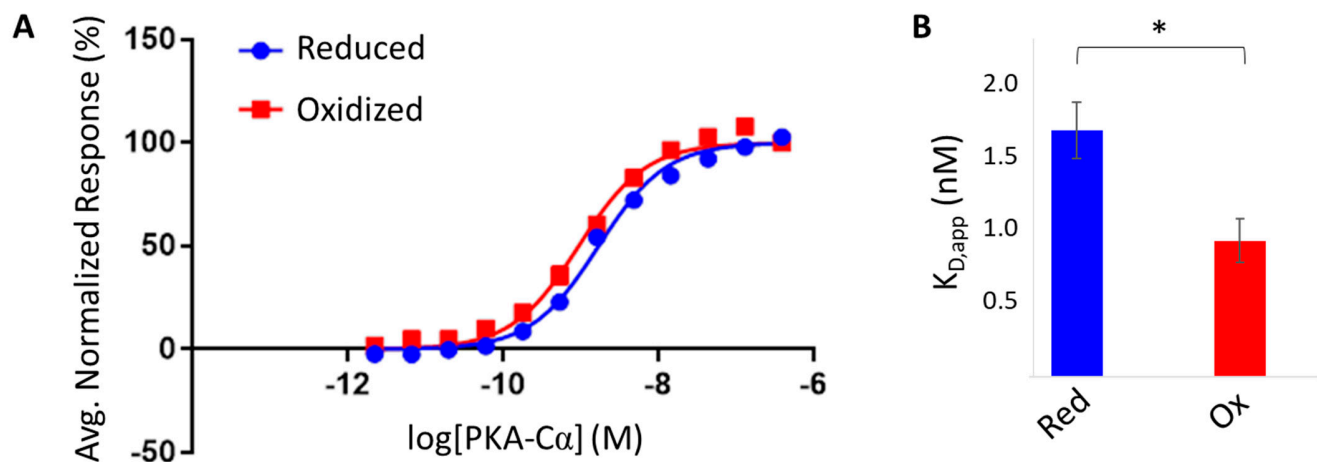


Figure S6. Fluorescence polarization assays to assess the impact of H_2O_2 -dependent oxidation on PKA-C α interactions with FAM-PKI(5-24). **A.** Desalted PKA-C α was treated with either dH_2O (blue circles) or a 20-fold molar excess of H_2O_2 (red squares) for 10 minutes at room temperature before scavenging excess H_2O_2 with catalase. The treated PKA-C α was then incubated with FAM-labeled PKI(5-24) at the indicated concentrations at room temperature for 30 minutes before measuring fluorescence polarization of FAM-PKI(5-24). Error bars represent the standard error about the mean of 3 independent experiments conducted in duplicate. **B.** Average $K_{D,app}$ of PKA-C α for PKI(5-24) following treatment with dH_2O (blue) or 5 μM H_2O_2 alone (red). Error bars represent SE about the mean of at least three independent experiments done in duplicate. *: $p < 0.05$.

PRKACA	GYIQVTDFGFAKRV-----KGRTWTL <u>C</u> GTPEYLAPEIILS	213
PRKACB1	GYIQVTDFGFAKRV-----KGRTWTL <u>C</u> GTPEYLAPEIILS	260
PRKACG	GYLQVTDFGFAKRV-----KGRTWTL <u>C</u> GTPEYLAPEIILS	213
PRKCA	GHIKIADFGMCKEHMM-DG-VTTRTF <u>C</u> GTPDYIAPEIIAY	512
PRKCB1	GHIKIADFGMCKENIW-DG-VTTKTF <u>C</u> GTPDYIAPEIIAY	515
PRKCD	GHIKIADFGMCKENIF-GE-SRASTF <u>C</u> GTPDYIAPEILQG	522
PRKCH	GHIKITDFGMCKENVF-PG-TTTRTF <u>C</u> GTPDYIAPEILLG	529
PRKCI	GHIKLTDFGMCKEGLR-PG-DTTSTF <u>C</u> GTPNYIAPEILRG	427
PRKCQ	GHIKIADFGMCKENML-GD-AKTNTF <u>C</u> GTPNYIAPEILRG	553
PRKCZ1	GHIKLTDFGMCKEGLG-PG-DTTSTF <u>C</u> GTPNYIAPEILRG	425
PRKG1	GYAKLVDFGFAKKIGF---GKKTWTF <u>C</u> GTPEYVAPEIILN	532
PRKAA1	MNAKIADFGLSNM-MS-DG-EFLRTS <u>C</u> GSPNYAAPEVISG	189
PRKAA2	MNAKIADFGLSNM-MS-DG-EFLRTS <u>C</u> GSPNYAAPEVISG	187
RPS6KA1	GHIKLTDFGLSKEAID-HE-KKAYSF <u>C</u> GTVEYMAPEVVNR	236
RPS6KA2A	GHIKITDFGLSKEAID-HD-KRAYSF <u>C</u> GTYEYMAPEVVNR	233
RPS6KA3	GHIKLTDFGLSKESID-HE-KKAYSF <u>C</u> GTVEYMAPEVVNR	242
RPS6KA4A	GHIVLTDFGLSKEFLT-EEKERTFSF <u>C</u> GTYEYMAPEIIRS	211
RPS6KA5A	GHVVLTDFFGLSKEFVA-DETERAYSF <u>C</u> GTYEYMAPDIVRG	227
RPS6KA6	GHIKLTDFGLSKESVD-QE-KKAYSF <u>C</u> GTVEYMAPEVVNR	247
AKT1	GHIKITDFGLCKEGIK-DG-ATMKTF <u>C</u> GTPEYLAPEVLED	323
AKT2	GHIKITDFGLCKEGIS-DG-ATMKTF <u>C</u> GTPEYLAPEVLED	324
AKT3	GHIKITDFGLCKEGIT-DA-ATMKTF <u>C</u> GTPEYLAPEVLED	320
SGK1	GHIVLTDFGLCKENIE-HN-STTSTF <u>C</u> GTPEYLAPEVLHK	271
SGK2A	GHVVLTDFFGLCKEGVE-PE-DTTSTF <u>C</u> GTPEYLAPEVLRK	208
SGK3	GHVVLTDFFGLCKEGIA-IS-DTTTTF <u>C</u> GTPEYLAPEVIRK	335
CAMK1A	SKIMISDFGLSKM-ED-PG-SVLSTA <u>C</u> GTPGYVAPEVLAQ	192
CAMK1D	SKIMISDFGLSKM-EG-KG-DVMSTA <u>C</u> GTPGYVAPEVLAQ	195

Figure S7. Conservation of redox sensitive cysteines among AGC kinase family members. C199 in the P+1 loop of PKA-C α (PRKACA) is conserved among many AGC kinases. The conserved Cys is highlighted in red. Those sites that are known to be oxidized are underlined.