

Supplementary Material

# ***In-Silico* Analysis of Glucose Oxidase from *Aspergillus niger*: Potential Cysteine Mutation Sites for Enhancing Protein Stability**

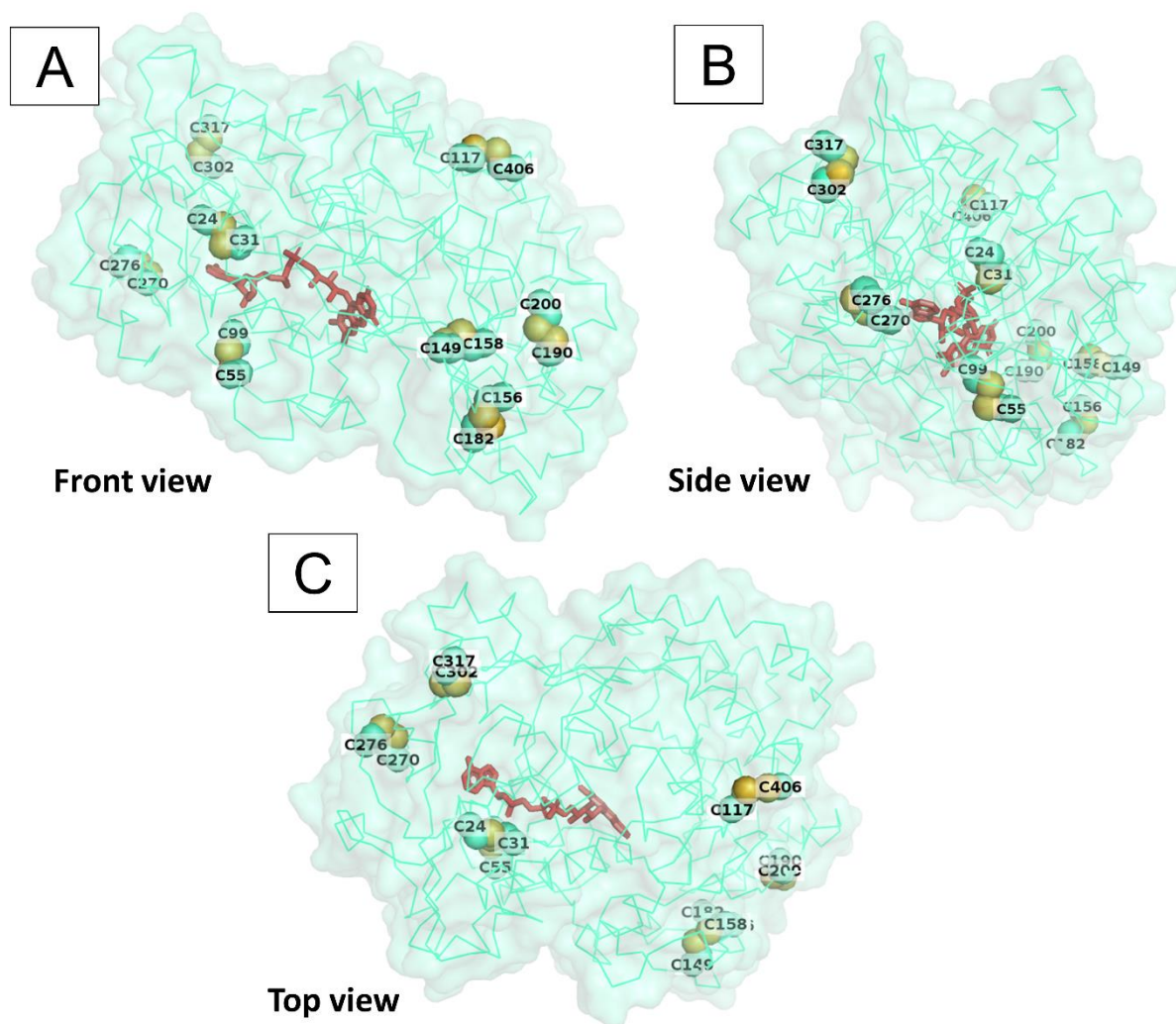
Sirawit Ittisoponpisan<sup>1\*</sup> and Itthipon Jeerapan<sup>2,3,4\*</sup>

1. Center for Genomics and Bioinformatics Research, Division of Biological Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand
2. Center of Excellence for Trace Analysis and Biosensor, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand
3. Division of Physical Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand
4. Center of Excellence for Innovation in Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand

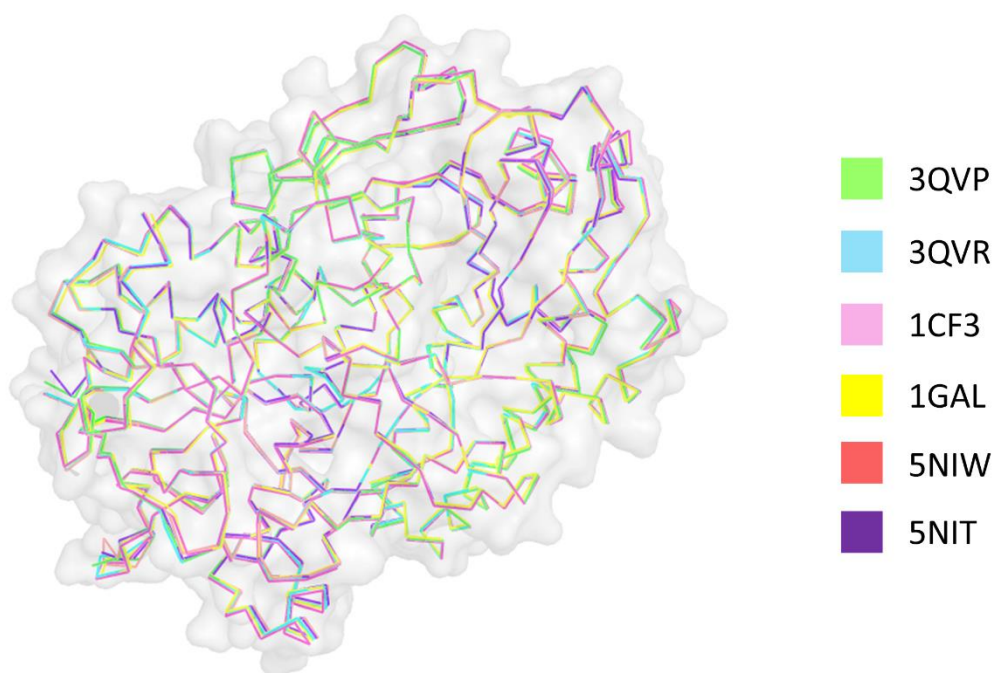
\* Correspondence: sirawit.i@psu.ac.th (S.I.); itthipon.j@psu.ac.th (I.J.)

ORCID of Sirawit Ittisoponpisan: <https://orcid.org/0000-0002-3340-0962>

ORCID of Itthipon Jeerapan: <https://orcid.org/0000-0001-8016-6411>



**Figure S1. Different perspectives of three-dimensional (3D) structures of GOx (Ca trace and surface of the PDB structure 3QVP).** The angles include A) front view, B) side view, and C) top view. Eight possible pairs for cysteine mutations are mapped onto this structure and are represented by spheres with sulfur atoms shown in yellow. Numbers shown in this figure indicate residue number according to the PDB coordinates. FAD (shown in red) can be found located at the pocket of the active site inside the protein.



**Figure S2. Superposition of all PDB coordinates of all GOx used in this study.** The structures are displayed as  $\text{C}\alpha$  traces. To give a general outlook of GOx, the protein surface (based on PDB 3QVP) is shown in light grey.

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3QVP_1|Chain      SNGIEASLLTDPKDVSGRTVDYIIAGGGLTGLTTAARLTENPNISVLVIESGSYESDRGP 60
3QVR_1|Chain      SNGIEASLLTDPKDVSGRTVDYIIAGGGLTGLTTAARLTENPNISVLVIESGSYESDRGP 60
1CF3_1|Chain      SNGIEASLLTDPKDVSGRTVDYIIAGGGLTGLTTAARLTENPNISVLVIESGSYESDRGP 60
1GAL_1|Chain      SNGIEASLLTDPKDVSGRTVDYIIAGGGLTGLTTAARLTENPNISVLVIESGSYESDRGP 60
5NIW_1|Chain      --GIEASLLTDPKDVSGRTVDYIIAGGGLVGLTTAAKL TENPNISVLVIESGSYESDRGP 58
5NIT_1|Chain      --GIEASLLTDPKDVSGRTVDYIIAGGGLVGLTTAARLTENPNISVLVIESGSYESDRGP 58
*****

3QVP_1|Chain      IIEDLNAYGDI FGSSVDHAYETVELATNNQTALIRSGNGLGGSTLVNGGTWTRPHKAQVD 120
3QVR_1|Chain      IIEDLNAYGDI FGSSVDHAYETVELATNNQTALIRSGNGLGGSTLVNGGTWTRPHKAQVD 120
1CF3_1|Chain      IIEDLNAYGDI FGSSVDHAYETVELATNNQTALIRSGNGLGGSTLVNGGTWTRPHKAQVD 120
1GAL_1|Chain      IIEDLNAYGDI FGSSVDHAYETVELATNNQTALIRSGNGLGGSTLVNGGTWTRPHKAQVD 120
5NIW_1|Chain      IIEDLNAYGDI FGSSVDHAYETVELATNNQTALVRSNGNGLGGSTLVNGGTWTRPHKAQVD 118
5NIT_1|Chain      IIEDLNAYGDI FGSSVDHAYETVELATNNQTALVRSNGNGLGGSTLVNGGTWTRPHKAQVD 118
*****

3QVP_1|Chain      SWETVFGNEGWNWDNVAAYS LQAERARAPNAKQIAAGHYFNASCHGVNGTVHAGPRDTGD 180
3QVR_1|Chain      SWETVFGNEGWNWDNVAAYS LQAERARAPNAKQIAAGHYFNASCHGVNGTVHAGPRDTGD 180
1CF3_1|Chain      SWETVFGNEGWNWDNVAAYS LQAERARAPNAKQIAAGHYFNASCHGVNGTVHAGPRDTGD 180
1GAL_1|Chain      SWETVFGNEGWNWDNVAAYS LQAERARAPNAKQIAAGHYFNASCHGVNGTVHAGPRDTGD 180
5NIW_1|Chain      SWETVFGNEGWNWDNVAAYS LQAERARAPNAKQIAAGHYFN T SCHGVNGTVHAGPRDTGD 178
5NIT_1|Chain      SWETVFGNEGWNWDNVAAYS LQAERARAPNAKQIAAGHYFN T SCHGVNGTVHAGPRDTGD 178
*****

3QVP_1|Chain      DYSPIVKALMSAVEDRGVPTKKDFGCGDPHGVSMPFNTLHEDQVRSDAAREWLLPNYQRP 240
3QVR_1|Chain      DYSPIVKALMSAVEDRGVPTKKDFGCGDPHGVSMPFNTLHEDQVRSDAAREWLLPNYQRP 240
1CF3_1|Chain      DYSPIVKALMSAVEDRGVPTKKDFGCGDPHGVSMPFNTLHEDQVRSDAAREWLLPNYQRP 240
1GAL_1|Chain      DYSPIVKALMSAVEDRGVPTKKDFGCGDPHGVSMPFNTLHEDQVRSDAAREWLLPNYQRP 240
5NIW_1|Chain      DYSPIVKALMSAVEDRGVPTKKDFGCGDPHGVSMPFNTLHEDQVRSDAAREWLLPNYQRP 238
5NIT_1|Chain      DYSPIVKALMSAVEDRGVPTKKDFGCGDPHGVSMPFNTLHEDQVRSDAAREWLLPNYQRP 238
*****

3QVP_1|Chain      NLQVL TGQYVGKVL L SQNGTTPRAVGVEFGTHKGNTHNHYAKHEVLLAAGSAVSPTILEY 300
3QVR_1|Chain      NLQVL TGQYVGKVL L SQNGTTPRAVGVEFGTHKGNTHNHYAKHEVLLAAGSAVSPTILEY 300
1CF3_1|Chain      NLQVL TGQYVGKVL L SQNGTTPRAVGVEFGTHKGNTHNHYAKHEVLLAAGSAVSPTILEY 300
1GAL_1|Chain      NLQVL TGQYVGKVL L SQNGTTPRAVGVEFGTHKGNTHNHYAKHEVLLAAGSAVSPTILEY 300
5NIW_1|Chain      NLQVL TGQYVGKVL L SQNGTTPRAVGVEFGTHKGNTHNHYAKHEVLLAAGSAVSPTILEY 298
5NIT_1|Chain      NLQVL TGQYVGKVL L SQNGTTPRAVGVEFGTHKGNTHNHYAKHEVLLAAGSAVSPTILEY 298
*****

3QVP_1|Chain      SGIGMKSILEPLGIDTVVDLPVGLNLQDQTTATVRSRITSAGAGQGQA AWFATFNETFGD 360
3QVR_1|Chain      SGIGMKSILEPLGIDTVVDLPVGLNLQDQTTATVRSRITSAGAGQGQA AWFATFNETFGD 360
1CF3_1|Chain      SGIGMKSILEPLGIDTVVDLPVGLNLQDQTTATVRSRITSAGAGQGQA AWFATFNETFGD 360
1GAL_1|Chain      SGIGMKSILEPLGIDTVVDLPVGLNLQDQTTATVRSRITSAGAGQGQA AWFATFNETFGD 360
5NIW_1|Chain      SGIGMKSILEPLGIDTVVDLPVGLNLQDQTTATVRSRITSAGAGQGQA AWFATFNETFGD 358
5NIT_1|Chain      SGIGMKSILEPLGIDTVVDLPVGLNLQDQTTATVRSRITSAGAGQGQA AWFATFNETFGD 358
*****

3QVP_1|Chain      YSEKAHELLNTKLEQWAE EAVARGGFHNTTALLIQYENYRDWIVNHNVA YSELF LD TAGV 420
3QVR_1|Chain      YSEKAHELLNTKLEQWAE EAVARGGFHNTTALLIQYENYRDWIVNHNVA YSELF LD TAGV 420
1CF3_1|Chain      YSEKAHELLNTKLEQWAE EAVARGGFHNTTALLIQYENYRDWIVNHNVA YSELF LD TAGV 420
1GAL_1|Chain      YSEKAHELLNTKLEQWAE EAVARGGFHNTTALLIQYENYRDWIVNHNVA YSELF LD TAGV 420
5NIW_1|Chain      YSEKAHELLNTKLEQWAE EAVARGGFHNTTALLIQYENYRDWIVNHNVA YSELF LD TAGV 418
5NIT_1|Chain      YSEKAHELLNTKLEQWAE EAVARGGFHNTTALLIQYENYRDWIVNHNVA YSELF LD TAGV 418
*****

3QVP_1|Chain      ASFDVWDL LPFTRGYVHILDKDPYLHFFAYDPQYFLNELDLLGQAAATQLARNISNSGAM 480
3QVR_1|Chain      ASFDVWDL LPFTRGYVHILDKDPYLHFFAYDPQYFLNELDLLGQAAATQLARNISNSGAM 480
1CF3_1|Chain      ASFDVWDL LPFTRGYVHILDKDPYLHFFAYDPQYFLNELDLLGQAAATQLARNISNSGAM 480
1GAL_1|Chain      ASFDVWDL LPFTRGYVHILDKDPYLHFFAYDPQYFLNELDLLGQAAATQLARNISNSGAM 480
5NIW_1|Chain      ASFDVWDL LPFTRGYVHILDKDPYLHFFAYDPQYFLNELDLLGQAAATQLARNISNSGAM 478
5NIT_1|Chain      ASFDVWDL LPFTRGYVHILDKDPYLHFFAYDPQYFLNELDLLGQAAATQLARNISNSGAM 478
*****

3QVP_1|Chain      QTYFAGETIPGDNLAYDADLSAWTEYIPYHFRPNYHGVGTCSMMPKEMGGVVDNAA RVYG 540
3QVR_1|Chain      QTYFAGETIPGDNLAYDADLSAWTEYIPYHFRPNYHGVGTCSMMPKEMGGVVDNAA RVYG 540
1CF3_1|Chain      QTYFAGETIPGDNLAYDADLSAWTEYIPYHFRPNYHGVGTCSMMPKEMGGVVDNAA RVYG 540
1GAL_1|Chain      QTYFAGETIPGDNLAYDADLSAWTEYIPYHFRPNYHGVGTCSMMPKEMGGVVDNAA RVYG 540
5NIW_1|Chain      QTYFAGETIPGDNLAYDADLSAWTEYIPYHFRPNYHGVGTCSMMPKEMGGVVDNAA RVYG 538
5NIT_1|Chain      QTYFAGETIPGDNLAYDADLSAWTEYIPYHFRPNYHGVGTCSMMPKEMGGVVDNAA RVYG 538
*****

3QVP_1|Chain      VQGLRVIDGSIPPTQMSSHVM TVFYAMALKISDAILEDYASMQ 583
3QVR_1|Chain      VQGLRVIDGSIPPTQMSSHVM TVFYAMALKISDAILEDYASMQ 583
1CF3_1|Chain      VQGLRVIDGSIPPTQMSSHVM TVFYAMALKISDAILEDYASMQ 583
1GAL_1|Chain      VQGLRVIDGSIPPTQMSSHVM TVFYAMALKISDAILEDYASMQ 583
5NIW_1|Chain      VQGLRVIDGSIPPTQ VSSHVM TVFYAMALKISDAILEDYASMQ 581
5NIT_1|Chain      VQGLRVIDGSIPPTQ VSSHVM TVFYAMALKISDAILEDYASMQ 581
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**Figure S3. Multiple sequence alignment profile of six GOx structures.** Amino acid variants are highlighted in yellow.