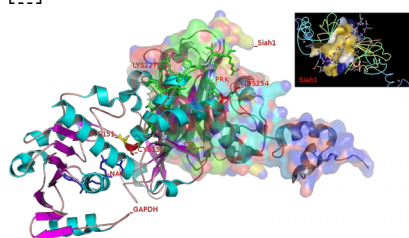


Non-Phosphorylating And Phosphorylating Oxidoreductase Glyceraldehyde-3-Phosphate Dehydrogenase As Part Of A Structure-Based Design In Glycolysis As The Glycolytic Protein G3PD.  
Mark R. Brennehan

Glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) [GAPDH](#)<sup>1</sup>/G3PD, is located in band 12p13.31; related to both [glycolysis](#)<sup>2</sup> and [gluconeogenesis](#)-pathways. G3PD catalyzes reversible oxidative phosphorylation of inorganic phosphate and [nicotinamide](#)<sup>3</sup> adenine dinucleotide ([NAD](#))<sup>4</sup> converting in glycolysis, the glycolytic protein [GAPDH](#)<sup>5</sup> in which adenosine-triphosphate ([ATP](#))<sup>6</sup> is generated when phosphoglycerate kinase ([PGK](#))<sup>7</sup> is produced in the [GAPDH](#)<sup>8</sup>-catalyzed reaction. These intermediate metabolites ([aldolase](#)<sup>9</sup>, triose-[phosphate](#)<sup>10</sup>-isomerase ([TPI](#))<sup>11</sup>) catalyze the Glycolysis reactions, in the sequence of the ten enzyme-catalyzed [Emden](#)<sup>12</sup>-[Meyerhof](#)<sup>13</sup> reactions in the metabolic pathway. Converting phosphoglycerate mutase 1 ([PGM](#))<sup>14</sup> catalyzing the internal steps by [2,3-BPG](#)<sup>15</sup> phosphatase to form by converting D-glyceraldehyde 3-phosphate ([G3P](#))<sup>16</sup> into 1,3-bisphosphoglycerate (1,3-[BPG](#))<sup>17</sup> from its role as 3-Phosphoglyceric acid ([3PG](#))<sup>17a</sup> non-phosphorylating) in glycolysis as the glycolytic protein [GAPDH](#)<sup>18</sup> that catalyzes the first step ([G3P](#)<sup>19</sup> into [1,3-BPG](#)) of the pathway. [Plant](#)<sup>20</sup> cells contain several reactions of [photosynthesis](#)<sup>21</sup> participating in glycolysis and the [Calvin-Benson](#)<sup>22</sup> cycle signaling pathways in plants (cytosolic-[GAPC](#)<sup>23</sup> (*Arabidopsis thaliana*)<sup>24</sup> functions in [plant](#)<sup>25</sup> cells.) its final byproduct is also another Glyceraldehyde-3-P. GAPDH is a [band 3](#)<sup>26</sup> protein that associates with the [cytoplasmic](#)<sup>27</sup> face of human [erythrocyte](#)<sup>28</sup> ([RBC](#))<sup>29</sup> membranes. The cytoplasmic GAPDH exists primarily as a [tetrameric](#)<sup>30</sup> isoform, 4 identical [37 kDa](#)<sup>31</sup> subunits. By subcellular translocation [GAPDH](#)<sup>32</sup> participates in nuclear events [In nuclear membrane the [vesicular](#)<sup>33</sup> tubular cluster [fractions](#)<sup>34</sup> ([VTCs](#))<sup>35</sup> - anterograde transport or [retrograde](#)<sup>36</sup> membrane transport [complexes](#)<sup>37</sup> between the intermediates, these are the [Golgi](#)<sup>38</sup> complex and the endoplasmic reticulum ([ER](#))<sup>39</sup>, in the nucleus, a function is lost in disease\* that exploits this process.], this a change to a [non-cytosolic](#)<sup>40</sup> localization due to the signal transduction pathways (considering [Lm](#)<sup>41</sup>[GAPG L](#).<sup>42</sup> [mexicana](#)<sup>43</sup>-like functions.) involved in [s-nitrosylase](#)<sup>44</sup> activity that mediates, governed by the equilibrium between four cysteine residues ([nitrosylation](#)<sup>45</sup> and denitrosylation [reactions](#))<sup>46</sup>, inhibition of GAPDH nuclear translocation, as a [basis](#)<sup>47</sup> for its [multifunctional](#)<sup>48</sup> activities relating to the extraglycolytic functions of GAPDH. Nuclear [GAPDH](#)<sup>49</sup> promotes glucose metabolism to [sustain](#)<sup>50</sup> cellular [ATP](#)<sup>51</sup> levels, or potentially by inhibiting [targets](#)<sup>52</sup> of [p300](#)<sup>53</sup>/CBP such as [p53](#)<sup>54</sup> dependent phosphorylation. Nitric oxide synthase or neuronal NOS (involved in cellular and human [intracellular](#)<sup>55</sup> nuclei [events](#)<sup>56</sup>, in addition to the cytoplasm) could generate [nitric oxide](#)<sup>57</sup> (NO). GAPDH has [four cysteine](#)<sup>58</sup> residues which are associated with S-[nitrosylation](#)<sup>59</sup>-yielding [NOS](#)<sup>60</sup>-GAPDH which "recruited" its glycolysis [subunit](#)<sup>61</sup> from the [three](#)<sup>63</sup> molecular axes translocation roles (S-[thiolation](#)<sup>64</sup>, S-nitrosylation or [aggregated](#)<sup>65</sup> enzymes (Cys-[152](#)<sup>66</sup> and nearby [156](#)<sup>67</sup> converted into a '[cross-linked](#)<sup>68</sup> soluble' states)), and ([SNO](#)<sup>69</sup>-GAPDH) nitrosylated S-nitrosoglutathione<sup>70</sup> ([GSNO](#))<sup>71</sup> the active site cysteine residue in GAPDH at its [Cys 150](#)<sup>72</sup> residue that binds to Siah1 (seven in absentia homolog 1) acquisition and the translocation of GAPDH into the nucleus, and denitrosylation using a combination of approaches, including [G3P](#)<sup>73</sup>. And NADPH may play a role in (VTC) [vesicle](#)<sup>74</sup> function. The complex would function in the apoptosis [cascade](#)<sup>75</sup> by its molecules translocation, this [may](#)<sup>76</sup> depend on lysine [227](#)<sup>77</sup> in the human [GAPDH](#)<sup>78</sup>-[Siah](#)<sup>79</sup> interaction to another intracellular [position](#)<sup>80</sup> induced by [apoptotic](#)<sup>81</sup> stimuli, augments [p300](#)<sup>82</sup>/CREB binding protein (CBP)-[associated](#)<sup>83</sup> acetylation of nuclear proteins. 'Engineering the cofactor (GAPDH-(Lys) [K160R](#)<sup>84</sup>-K227A) availability [prevents](#)<sup>85</sup> activation of p300/CBP that interferes with GAPDH-Siah1 [binding](#)<sup>86</sup>-prevents the ternary (GAPDH-Siah1) complex associations translocation; that [CGP-3466](#)<sup>87</sup> can [reduce](#) independently with both [cofactors](#)<sup>88</sup>. Dysregulation of protein S-nitrosylation (S-[nitrosocysteine](#)<sup>89</sup> - [247](#)) by lipopolysaccharide (LPS) is associated with [pathological](#)<sup>90</sup> conditions which contributes to disease phenotype, where GAPDH protects ribosomal protein [RP](#)<sup>91</sup>-[L13a](#)<sup>92</sup> from degradation, [L13a](#)<sup>93</sup> and [GAPDH](#)<sup>94</sup> forms a functional

[GAIT](#)<sup>95</sup> complex. One of the functions of GAPDH proteins role in [glycolysis](#)<sup>96</sup> in relation to [DNA](#)<sup>97</sup> synthesis is nuclear accumulation associated by the [NAD](#)<sup>98</sup>(+)-dependent s-[nitrosylation](#)<sup>99</sup> and [denitrosylation](#)<sup>01</sup> reactions both of these isforms are [distinct](#)<sup>02</sup> parallel to the uracil DNA glycosylase ([UDG](#))<sup>03</sup> gene in [mitochondria](#)<sup>04</sup> and in the nucleus is N-terminally processed is the 37-kDa [subunit](#)<sup>05</sup> of the ([GAPDH](#))<sup>06</sup> glyceraldehyde-3-phosphate dehydrogenase protein. This enzyme is an example of [moonlighting](#) protein which is validated and [replaced](#)<sup>07</sup> by alternative reference genes that link (in their nuclear forms) on the [multifunctional](#)<sup>08</sup> properties of the enzyme [GAPDH](#)<sup>09</sup> known as a key enzyme in glycolysis that contributes to a number of diverse cellular functions [unrelated](#)<sup>00</sup> to [glycolysis](#)<sup>001</sup> depending upon its subcellular location. GAPDH is a key enzyme in glycolysis the most commonly used expression is as a [housekeeping](#)<sup>002</sup> gene.

[OBJ]



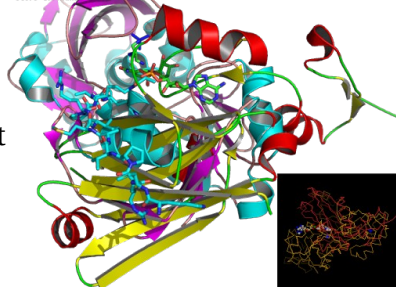
Cytotoxic stimuli [1a.] or Programmed cell death, via nitric oxide generation, lead to the binding of GAPDH from its usual tetrameric form to a dimeric form, to the protein Siah1 [1.] intracellular G-3-P [2.] substrate [3.] protects GAPDH from S-nitrosylation [4.]. The GAPDH-Siah interaction depends on lysine [227](#) [5.], in human GAPDH that interacts with a large groove [6.] of the Siah1 dimer, that connects the GAPDH dimer to PGK in the cytoplasm. The S-nitrosylation [7.,8.]

abolishes catalytic activity and confers upon GAPDH the ability to bind to Siah [9.]. (GAPDH) is physiologically nitrosylated at its Cys 150 residue. GAPDH (SNO-GAPDH) [10.] binds to Siah1 [11.] by forming a protein complex. In the nucleus [12.] GAPDH is acetylated at Lys 160 [13.] and binds to the protein acetyltransferase p300/CBP. Under these conditions siah-1 formed a complex with GAPDH (PDB:4O63) and localized in the nucleus of Müller cells [14.]. GAPDH mutants [15.] that cannot bind Siah1 prevents translocation [16.] to the nucleus to elicit neurotoxicity [17.] and cell apoptosis.

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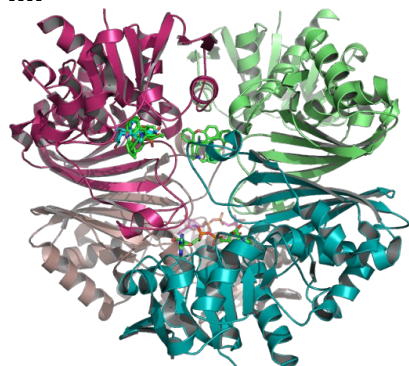
Superimposed 1U8F_Pdb upon 417D_C5dm
Method: Superimpose_CEPROXY
mobile x' = x' -0.667 + y' 0.030 + z' -0.725 + 23.244
mobile y' = y' -0.094 + y' -0.986 + z' -0.138 + 72.096
mobile z' = z' -0.720 + y' -0.158 + z' 0.675 + 89.993
#PFCASCEWGGSLDVAHPHLMHQRKSTTLGQEDVPLATDNLPLGAVDWNWNSQFCGPHPLKQKRYGSHQQ
#FATVQLGSTRKQK RHPATYK LQNHGK RQ LTRWATYRSHGK
IATAIDNSDCLYFDTSIALRKAISGKQINRINHC
#1 PRADNMPYRYSK TQDQSLTSHY
CLAPLAKYVHNSGIDGKLNHMLRATQGTVDGPGSKLWRDGRGALQNIIPASTGAARAVGKIVPELNGKLT
QNA FQVTSQVSYV
Score:1.24 Ems

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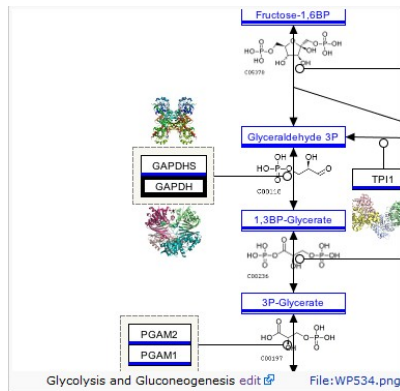
- [1a.] [16492755](#), [8769851](#)<sup>003</sup> [1.][16391220](#), [2.][19542219](#), [22534308](#), [3350006](#)<sup>004</sup>, [19937139](#), [3.][22847419](#), [4.][15951807](#), [5.][20601085](#), [6.][16510976](#), [20392205](#),<sup>005</sup> [7.,8.][22817468](#),<sup>006</sup> [16505364](#),<sup>007</sup> [9.][16633896](#), [10.][16574384](#), [11.][20972425](#), [12.][19607794](#), [13.][18552833](#), [14.][19940145](#), [15.][23027902](#),<sup>008</sup> [16.][24362262](#), [17.][16492755](#).

[OBJ]



Analysis of CGP-3466 Docking (NAD) to Human Placental GAPDH which decreases the synthesis of pro-apoptotic proteins is N-terminally PMID:10677844, processed to which a Rossmann NAD(P) binding fold as seen in figure 1 is a C-terminal domain as seen on this [page](#), PMID:10617673, 26022259, 16510976 ...The structure is also used to build a model of the complex between GAPDH and the E3 ubiquitin ligase Siah1. (Purple Ribbon-1U8F\_Q Figure 1.)

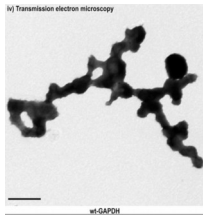
OBJ



In the GAPDH-catalyzed reaction these intermediate metabolites (aldolase, triose-phosphate-isomerase Glycolysis and Glyconeogenesis (TPI)) catalyze the Glycolysis reactions, in the sequence of the ten enzyme-catalyzed Embden-Meyerhof reactions in the metabolic pathway. Converting phosphoglycerate mutase 1 (PGM) catalyzing the internal steps by 2,3-BPG phosphatase to form by converting D-glyceraldehyde 3-phosphate g3p(G3P) into 1,3-bisphosphoglycerate (1,3-BPG) from its role as 3-Phosphoglyceric acid (3PG non-phosphorylating) in glycolysis as the glycolytic protein GAPDH that catalyzes the first step (G3P into 1,3-BPG) of the

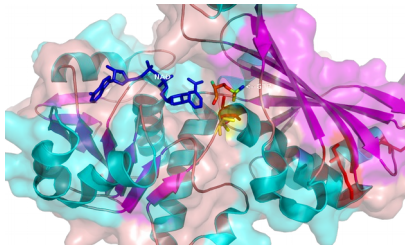
pathway.

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GAPDH homotetramer was studied as represented an assembly of repeating spherical units that harbored a distinct birefringent crystal structure to the optic axis for the p polarization, also (r axis) discernible via transmission electron microscopy. of the relative amount of soluble monomeric GAPDH to G3P in the binding pocket of the NAD(+)-binding site residue located at the active site linked to GAPDH in Figures 5 and 6. PMID:10407144,<sup>009</sup> [25086035](#).

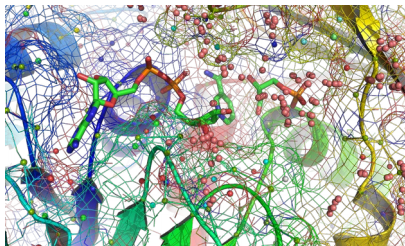
OBJ



Another model building studie indicates that a structure obtained where glyceraldehyde 3-phosphate PDB:3CMC\_Q binds in the P(s) pocket of the natural substrate G3P phosphorylating GAPDH (PDB:1U8F\_Q) at the catalytic cysteine residue site. To define the conditions suitable for affinity for the cosubstrate, the isolation and accumulation of the intermediate metabolites per G3P monomer found in Figure 8 of the equivalent Glc-3-P structure in the binding pocket of the NAD(+)-

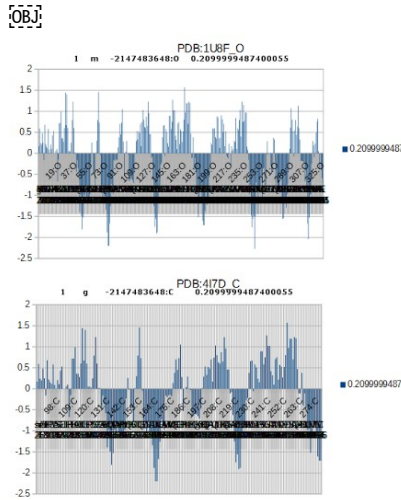
binding site residue located at the active site linked to GAPDH. PMID:[19542219](#), 22534308

OBJ



Correctly known binding sites on ((GAPD/NAD)) structures, polar spheres of the binding catalytic pocket that corresponds to G3P (glyceraldehyde 3-phosphate) aligned to the holographical structure nonbounded spheres (salmon color), these apoenzymes together with the cofactor(s) Cys 151, 152 which corresponds as below the Ps pocket of G3P, on the Green ribbon required for cofactor activity. Together with eliminated crystallographic waters and other possible spheres, these are at least one atom of a amino acid residue in contact with at least one alpha sphere of one binding pocket on the holo protein NAD structure 1U8F\_Q needed to align holo and apo structures included in this data set with G3P (PDB:3CMC\_Q) was tested only on holo structure (NAD), obtained via Pea Green spheres ([link](#)) aligned to 1U8F\_Q ribbons/ligand structure which provide structural recognition insights into the biological 1U8F-Q assembly this includes 29 asymmetric units of its dimeric form, along the

tetrameric 1U8F biological forms axis. PMID:9461340<sup>010</sup>



(Figure 8.) These are the results without the liquid chromatography coupled mass spectrometer, that are known 3D products by two-dimensional sequence analyses with the STRAP alignment tools data sets and which may have any effect on the functions of further analysis involved in more ordered results than this study attempts to show, of the analysis that may be included are identified separated into multiple gradients here in these paired graphs. Therefore in the present work to uncover the exact coincidence of 1U8F\_R and 4I7D\_C, the 3D coordinates of GAPDH (PDB:1U8F\_Q) to the protein Siah1 4I7D were not presenting when subjected to STRAP alignment this apparent discrepancy (Figure 1.) was partially resolved by a (Figure 7) rendering from a more reactive native GAPDH\_R homotetramer model.

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