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Crystal structure determination of Cys-S-sulfonated *Hs*GAPDH from protein purified from the supernatant of HEK293F cells.

Summary. *Hs*GAPDH was purified from the supernatant of HEK293F cells treated with 5 μM kifunensine and transfected with the *Ct*HTM1P$_{50-1092}$-pHLsec plasmid for secreted expression of the α-1,2-mannosidase of *Chaetomium thermophilum* HTM1P (*Ct*HTMP1 aka CTHT_0058730, Uniprot G0SCX7, G0SCX7_CHATD). *Hs*GAPDH crystals were grown from the protein sample purified from wash fractions of the IMAC step followed by size exclusion chromatography, and diffraction data were collected on beamline I03@Diamond on 22.07.2019. Structure factor amplitudes collected from samples belonging to four distinct novel *Hs*GAPDH crystal forms labelled P2$_1$-A,B,C,D were processed and scaled to resolutions in the range 1.5-2.4 Å (after anisotropic scaling). Initial structure factor amplitude phases for the highest resolution crystal form P2$_1$-A (PDB ID 6YND) were determined by molecular replacement using the *Hs*GAPDH model in PDB ID 1U8F as a search model. This structure was then used to determine initial molecular replacement structure factor phases for the remaining P2$_1$-B,C,D crystal forms. The catalytic cysteine of the protein in the crystals is partially oxidised to cysteine S-sulfonic acid. The *Hs*GAPDH crystal structures were deposited as PDB IDs 6YND, 6YNDE, 6YNF and 6YNH.

Cloning of the *Ct*HTM1P$_{50-1092}$-pHLsec plasmid

The DNA encoding the *Chaetomium thermophilum* α-1,2-mannosidase *Ct*HTM1P (CTHT_0058730, Uniprot G0SCX7, G0SCX7_CHATD), was purchased from GeneArt/LifeTechnologies, nucleotides 1-3276 (^{1}ATGAGCTGCAGATTTCTGGCCGCTGGCGAT (...)GTGGACGACGGCGACGGCGGAGTTGATGGA^{3276}) encoding *Ct*HTM1P amino-acids 1-1092, with codon optimisation for expression in mammalian cells, inserted after the NdeI site of vector pMK-RQ (kanR), plasmid *Ct*HTM1P-pMK-RQ.

Amplification of the portion of the *Ct*HTM1P gene encoding for residues 50-1092 (in order to avoid the transmembrane domain at the N-terminal of the protein, residues 26-49) for insertion between the AgeI and KpnI sites of the pHLsec vector [1], was effected by polymerase chain reaction (PCR). The PCR mix contained: Q5 High-Fidelity 1X Master Mix (New England Biosciences M0494) 25 μL; 2.5 μl of 10 μM Forward Primer stock to 0.5 μM final concentration; 2.5 μL of 10 μM Forward Primer stock to 0.5 μM final concentration; template *Ct*HTM1P_pMK-RQ DNA 1ng; Nuclease-Free Water to 50 μL. The *Ct*HTM1P$_{50-1092}$ /pHLsec overlapping ends primers were:

*Ct*HTM1P$_{50-1092}$ -pHLsec_FWD (T_m=64.93 °C, T_a=65.9 °C):
gatgggttgcgtagctgaaaccggtCCTCAGTGGAGTGTGATTCC
*Ct*HTM1P$_{50-1092}$ -pHLsec_REV (T_m=67.63°C, T_a=65.9 °C):
gtgatggtggtgcttggtaccTCCATCAACTCCGCCGTC

The PCR amplification was carried out in steps as follows: 1) initial denaturation: T=98°C, t=30"; 2) denaturation: T=98°C, t=10"; 3) annealing: 65.9°C, t=30"; 4) elongation: T=72°C, t=2 minutes (computed from an average

Q5 Hot Start polymerase rate of 1kb/30sec). Then back to step 2), for 33 cycles; 5) final extension: T=72°C, t=2 minutes.

The pHLsec vector was linearised using AgeI at the 5' and KpnI at the 3' end. DNA template used: pHLsec vector (Figure 1A, [1]) encoding a pEGFP fusion of the DNA encoding human Solute carrier family 35 member B1 (SLC35B1 *aka* UGTrel1, Uniprot P78383, S35B1_HUMAN) plasmid pEGFP_SLC35B1-pHLsec, courtesy of Snežana Vasiljević, Department of Biochemistry, Oxford University. 1µg of the template pEGFP_SLC35B1-pHLsec DNA was mixed with 2µL of 10X FastDigest™ green buffer and 0.5µL FastDigest™ enzyme (Thermo Fisher Scientific), adding deionised water up to a total volume of 20µL. The mix was incubated for 1 hour at 37 ºC. Both the PCR amplification mix of the *Ct*HTM1P$_{50\text{-}1092}$ insert with pHLsec AgeI/KpnI ends and the mix of the linearisation of the pEGFP_SLC35B1-pHLsec plasmid cut with AgeI/KpnI were separately run at 100V for 1 hour in an 1% agarose gels with gel electrophoresis in TAE buffer with the SYBR™ Safe DNA Gel Stain (Thermo Fisher Scientific) (see Figures 1B and 1C). The bands corresponding to the amplified *Ct*HTM1P$_{50\text{-}1092}$ insert and the linearised $^{AgeI/KpnI}$pHLsec plasmid (with overlapping ends) were purified from the agarose gels using the GeneJet™ gel extraction kit (Thermo Fisher Scientific).

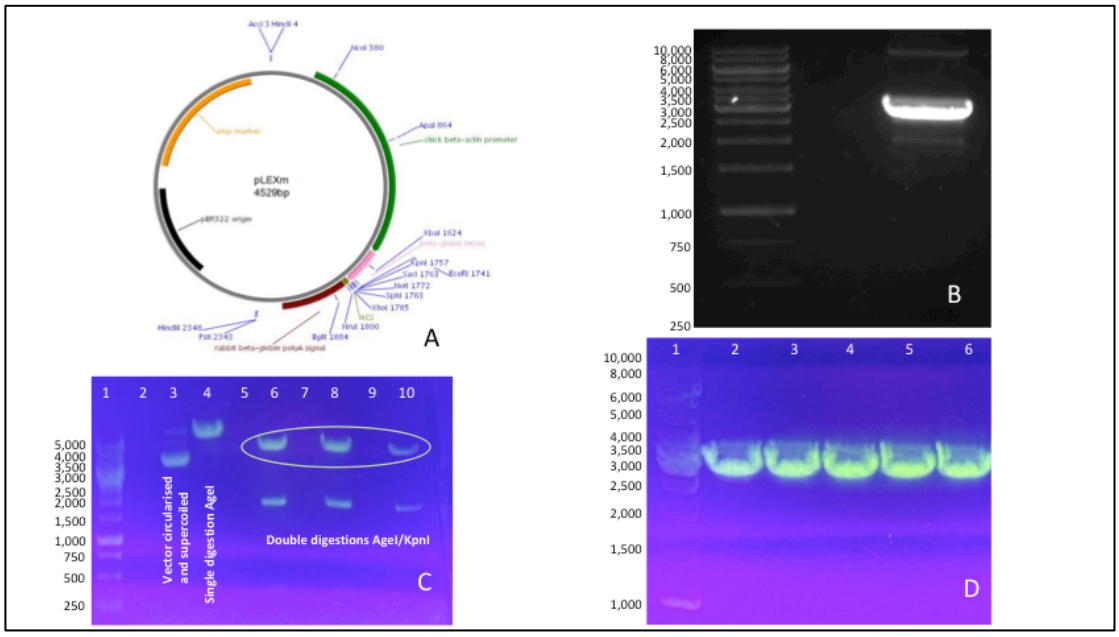

Figure 1. *Ct*HTM1P$_{50\text{-}1092}$-pHLsec cloning. DNA gel electrophoresis experiments were run at 100V for 1 hour in an 1% agarose gel in TAE buffer with SYBR™ Safe DNA Gel Stain (Thermo Fisher Scientific). DNA Ladder: GeneRuler 1kb by Thermo Fisher Scientific. **(A)** pHLsec vector map, from [1]. **(B)** PCR Amplification of the *Ct*HTM1P$_{50\text{-}1092}$ insert from the commercial *Ct*HTM1P-pMK-RQ plasmid. Lane 1: DNA markers; lane 3: amplified *Ct*HTM1P$_{50\text{-}1092}$ DNA with pHLsec AgeI/KpnI overlapping ends (expected: 3129 base pairs). **(C)** Linearisation of the pEGFP_SLC35B1-pHLsec plasmid using AgeI/KpnI. Lane 1: DNA ladder. Lane 3: untreated pEGFP_SLC35B1-pHLsec plasmid (6336 base pairs). Lane 4: digestion of pEGFP_SLC35B1-pHLsec with AgeI; Lanes 6,8,10: digestions of pEGFP_SLC35B1-pHLsec with AgeI and KpnI (the two bands are 4632 and 1704 base pairs, the former band matches the size of the linearised $^{AgeI/KpnI}$pHLsec, enclosed in an ellipse in the figure). **(D)** Colony PCR after transformation of *E. coli* with the *Ct*HTM1P$_{50\text{-}1092}$-pHLsec plasmid (insert: 3129 base pairs).

Lane 1: DNA ladder. Lanes 2-6: PCR amplification of DNA miniprepped from 5 distinct individual colonies.

Ligation independent cloning (*aka* Gibson assembly): the *Ct*HTM1P$_{50-1092}$ DNA insert and the AgeI/KpnI linearised $^{AgeI/KpnI}$pHLsec DNA were mixed in 3:1 molar ratio: 0.06pmol of *Ct*HTM1P$_{50-1092}$ DNA (3129pb x 650/1000 = 122 ng) and 0.02pmol of AgeI/KpnI linearised pHLsec DNA (4632pb x 650/1000 = 60 ng). To this DNA, 10 µL of GIBSON ASSEMBLY MasterMix(2X) (New England Bioscience NEB E2611) were added and the total volume made 20 µL with H_2O. The mix was heated at 50 °C for 1 hour.

A volume of 25µL of frozen glycerol stock of competent DH5α *E.coli* cells (NEB C2987I) were thawed on ice. 1µL of the Gibson assembly mix (as described above) was added to it, mixed, and left on ice for 2 minutes. The cells were then heat-shocked in a water bath at 42°C for 30 seconds and put back on ice for 2 minutes. A volume of 950 µL of Super Optimal broth with Catabolite repression (SOC) media (New England Bioscience) was added and the sample left in a shaking incubator at room temperature for 1 hour. A volume of 100 µL of cells in SOC media were plated on pre-heated LB agar + antibiotic (carbenicillin 100 µg/mL) plates and incubated overnight at 37°C.

Single colonies were picked from the plates and dipped in the same solutions for PCR amplification as described above, using the same primers and PCR protocol utilised the amplification of the *Ct*HTM1P$_{50-1092}$ insert. Figure 1D shows the 1% agarose gel confirming the successful incorporation of the *Ct*HTM1P$_{50-1092}$ insert in all colonies picked. The *Ct*HTM1P$_{50-1092}$-pHLsec plasmid DNA was miniprepped using the the GeneJet Plasmid Miniprep Kit (ThermoFisher Scientific). The sequence was confirmed by DNA sequencing with forward and reverse primers:

pHLsec primer FWD:	5'-CTACAGCTCCTGGGCAACGTG-3'
*Ct*HTM1 FWD:	5'-TGAGACAAGAGGACGACCTG-3'
*Ct*HTM2 FWD:	5'-AGACAGTGGAAGGCGGACT-3'
*Ct*HTM3 FWD:	5'-CCAGGGACGACATCTATCAC-3'
pHLsec primer REV:	5'-CATTGGCCACACCAGCCAC-3'

_Ct_HTM1P$_{50-1092}$-pHLsec plasmid DNA Maxiprep

A single colony of competent DH5α *E.coli* cells transformed with the *Ct*HTM1P$_{50-1092}$-pHLsec plasmid was inoculated in 15 mL of LB with carbenicillin 100 µg/mL, and grown overnight (O/N) at 37°C, then inoculated into 750 mL of the same media in a 2 L baffled flask and grown O/N at 37°C shaking at 220 rpm. The cells were pelleted with a centrifuge spin at 4,000g for 10 mins at 4°C and the pellet resuspended in 12 mL 10 mM Tris/Cl pH 8, 10 mM EDTA. A volume of 4 mL of the same buffer + 50 mg lysozyme was added. The sample was split into 2x 50 mL tubes. The cells were incubated at room temperature (RT) for 5 minutes and then 15 mL of 0.2 M NaOH 1% SDS was added to each tube to complete cell lysis, incubating at RT for 5 mins. A volume of 11 ml of 3 M KAcO pH 4.8 was then added to each tube. The solutions were incubated at RT for 5 mins and then spun at 4,000g for 10

minutes at 4°C to pellet cellular debris. The supernatant (S/N) was filtered through Miracloth (Merck Millipore): two Miracloth squares were cut and each placed on top of a clean 50 mL tube, after which the S/N was poured gently onto the Miracloth square and collected in the tube below. A volume of isopropanol equal to 60% of the solution was then added to precipitate the plasmd DNA. and the solution incubated at RT for 5 minutes. The plasmid DNA was then pelleted by spinning at 4,000g for 10 minutes at 4°C. The two DNA plasmid pellets were resuspended by pipetting in a total of 15 mL of 10 mM Tris/Cl pH 8, 10 mM EDTA. An equal volume of 5 M LiCl (kept at –20°C) was added and incubated on ice for 5 minutes to precipitate contaminating RNA and proteins. The solution was spun at 4,000g for 10 minutes at 4°C, and the pellet discarded. A volume of isopropanol equal to 60% of the solution was then added and incubate at RT for 5 minutes to re-precipitate the purified plasmid DNA. The solution was spun at 4,000g for 10 minutes at 4°C, the S/N discarded and the pellet resuspended by pipetting in 10 mL, 10 mM Tris/Cl pH 8, 1 mM EDTA. To get rid of the remaining RNA, a volume of 50 µL of 10 mg/ml heat-treated RNase A was added, and the solution incubated at RT for 15 minutes. A volume equal to 25% of the sample volume wad added of a solution 30% w/v PEG 6000, 2.5 M NaCl: this re-precipitates the plasmid DNA (the solution went cloudy). The solution was incubated on ice for 30 minutes and then spun at 4,000g for 10 minutes at 4°C. The pellet was re-suspended by pipetting in 10 mL, 10 mM Tris/Cl pH 8, 1 mM EDTA. The PEG was then extracted by addition of 2 mL chloroform and a quick vortex before spinning at 4,000g for 2 minutes at 4°C. The aqueous layer (top) was retained by pipetting and transferred to a clean 50 mL tube. The pure plasmid DNA was then precipitated by addition of a 10% volume of 5 M NaCl (or 3 M NaAcO) and 3 volumes of ethanol. The solution was spun at 4,000g for 10 minutes at 4°C. The DNA plasmid pellet was re-suspended/washed with 20 mL 100% ethanol and spun again at 4,000g for 2 minutes at 4°C. The S/N was discarded and the pellet left to dry overnight in the tube upside down on the bench at RT. The DNA plasmid was re-suspended by pipetting in 2 mL of 10 mM Tris/Cl pH 8, 0.1 mM EDTA (filtered) and left overnight for the plasmid DNA to resuspend fully. The pure *Ct*HTM1P$_{50\text{-}1092}$-pHLsec plasmid DNA was filtered-sterilised with a 0.22 µm filter and stored at -20° C.

_Hs_GAPDH protein expression.

A volume of 450 mL of HEK293F cells (ThermoFisher Scientific) at a concentration of 10^6 cells/mL suspended in GIBCO FreeStyle 293 Media (ThermoFisher Scientific 12338018), supplemented with 5 µM kifunensine (Cayman Chemical Company 109944-15-2), were transfected with the *Ct*HTM1P$_{50\text{-}1092}$-pHLsec plasmid, using the FreeStyle MAX 293 expression system, according to manufacturer instructions (see also [2]). >90 % cell viability was confirmed by trypan blue exclusion. Briefly, 1µg of DNA per mL of culture was used: the DNA plasmid was initially dissolved in 45 mL of Phosphate Buffered Saline (PBS: 0.01 M phosphate buffer pH 7.4, 0.0027 M potassium chloride and 0.137 M sodium chloride; Phosphate Buffered Saline tablets, Sigma Aldrich P4417) and vortexed vigorously for 3 seconds; 1.8 mL of a filter-sterilised solution of 0.5 mg/ml Polyethylenimine (PEI, Sigma

catalogue 408727) was added to the PBS/DNA solution and vortexed vigorously for 3 seconds; the mixture was incubated at room temperature for 20 minutes; the DNA/PEI mixture was added to the cell culture. The cell culture was split into three 500 mL Erlenmeyer flasks with 0.2 µm vent caps (Corning), 150 mL of culture in each flask, and incubated in an orbital shaker incubator at 37 ˚C, shaking at 120 rpm, under a 5% CO_2 atmosphere.

*Hs*GAPDH Protein purification.

Immobilised metal affinity chromatography (IMAC): The HEK293F cells' supernatant was harvested 4 days post-transfection by centrifuging at 4,000 x g for 5 minutes, it was made 1x PBS by adding an appropriate 10xPBS stock solution volume (obtained by dissolving 5 Phosphate Buffered Saline tablets (Sigma Aldrich P4417), and the pH was adjusted to 7.4 by adding a few drops of 2 M NaOH, before bottle-top vacuum filtration through a 0.2 µm filter. The filtered supernatant was then passed onto a 1 mL HisTrap HP Ni IMAC column (GE Healthcare 17-5248-01) equilibrated against binding buffer (50mM sodium phosphate pH 7.5, 100 mM NaCl, 1 mM TCEP (Sigma Aldrich C4706)), using a peristaltic pump at room temperature, at a flow rate of approximately 3 mL/min. The column was washed with 5 column volumes (cV) buffer A, fitted to an ÄKTA purifier FPLC machine in a 4 ˚C cabinet and further washed with 6 mL of elution buffer (20mM HEPES pH 7.5, 500mM imidazole (Honeywell Fluka 56750), 100mM NaCl, 1 mM TCEP).

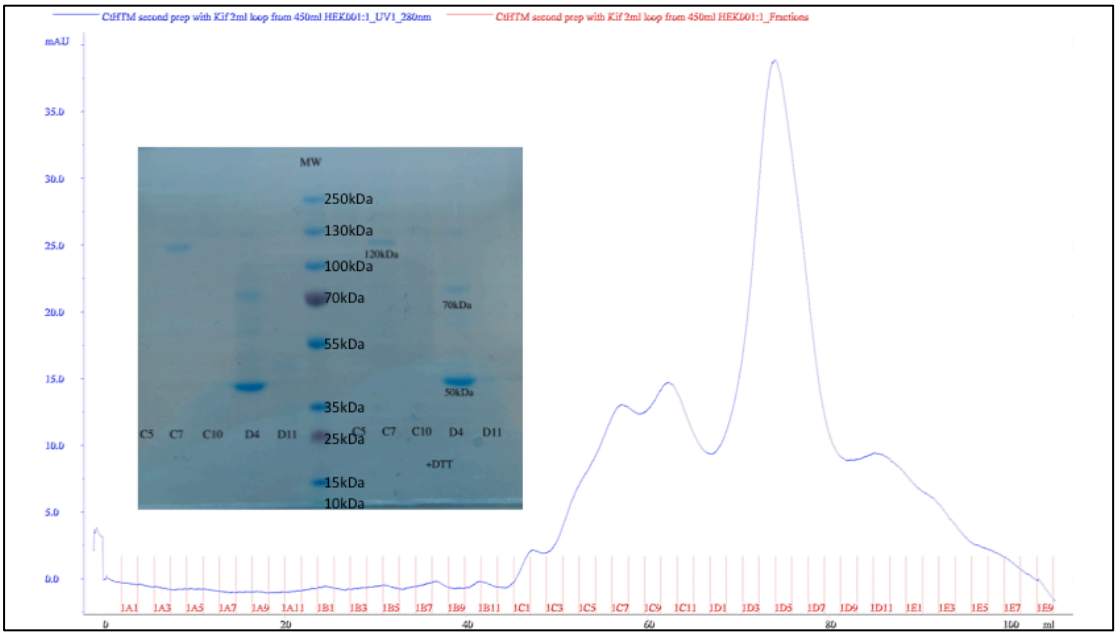

Figure 2. *Hs*GAPDH size exclusion chromatography. The elution profile of the S200 16/60 column on which 2 mL of the concentrated IMAC wash fractions were run. Inset: the SDS-PAGE Bis-Tris 4-12% gel run in MES buffer for 30' at 200 V, stained in Simply Blue™ SafeStain (Thermo Fisher) for 1 h and destained with water. Lanes 1-5: non reducing dye. Lanes 7-11: reducing dye. *Hs*GAPDH is visible as band between the 35 and 55 kDa MW markers in lanes for fraction D4 (lanes 4 and 10).

Size Exclusion Chromatography (SEC): the three wash 1.5 mL fractions were pooled and concentrated down to a volume of 2 mL using a polyethersulfone (PES) membrane, 10 kDa MW cutoff centrifugal ultrafiltration device (Thermo Fisher Scientific 88513). This sample was then filtered through a 0.2 µm filter and applied to a HiLoad Superdex 200 16/60 size exclusion chromatography (SEC) column (GE Healthcare 28-9893-35) equilibrated against SEC buffer: 20 mM NaHEPES pH 7.5 (Sigma H3375), 100 mM NaCl, 1 mM TCEP, running at 0.4 mL/min collecting 1 mL fractions; SDS-PAGE of SEC fractions was used to assess purity (NuPAGE Bis-Tris 4-12% gel (Thermo Fisher) , run at 200 V for 30' in MES buffer, stained in Coomassie Blue for 1 h and destained with water, see Figure 2). The six fractions D2-D7 (Figure 2) were pooled and concentrated as described before to a volume of 70 µL, and protein concentration measured by loading 1.5 µL of sample on a NanoDrop 1000 spectrophotometer (Thermo Scientific). The calculated ε_{280} of *HsGAPDH* = 0.7963 $(mg/mL)^{-1}cm^{-1}$ was then used to estimate the protein concentration: 18.8 mg/mL (A_{280} = 15.0).

Size Exclusion Chromatography with Multi Angle Laser Scattering (SEC-MALS): A volume of 30 µL of the concentrated SEC sample was diluted to 500 µL with filter-sterilised and degassed buffer HEPES 20 mM pH7.5,NaCl 100 mM, TCEP 1mM and injected onto a Superdex S200 10/300 column equilibrated in the same buffer. The mass of the sample was detected on elution with an 18-angle MALS light scattering detector (DawnR HELEOS-R II©) coupled with a differential Refractive Index detector (OptilabR© T-rEX) (Wyatt Technology). Figure 3 illustrates the results of the run. Fractions C3-C4-C5 (the former two corresponding to a tetramer of apparent mass 144 kDa) were pooled and concentrated to a volume of 45µL - the absorbance was OD_{280}=2.46 equivalent to a concentration of 3.0 mg/mL (calculated from ε_{280}=0.7963 $(mg/mL)^{-1}cm^{-1}$). Figure 4 illustrates the full-size gel.

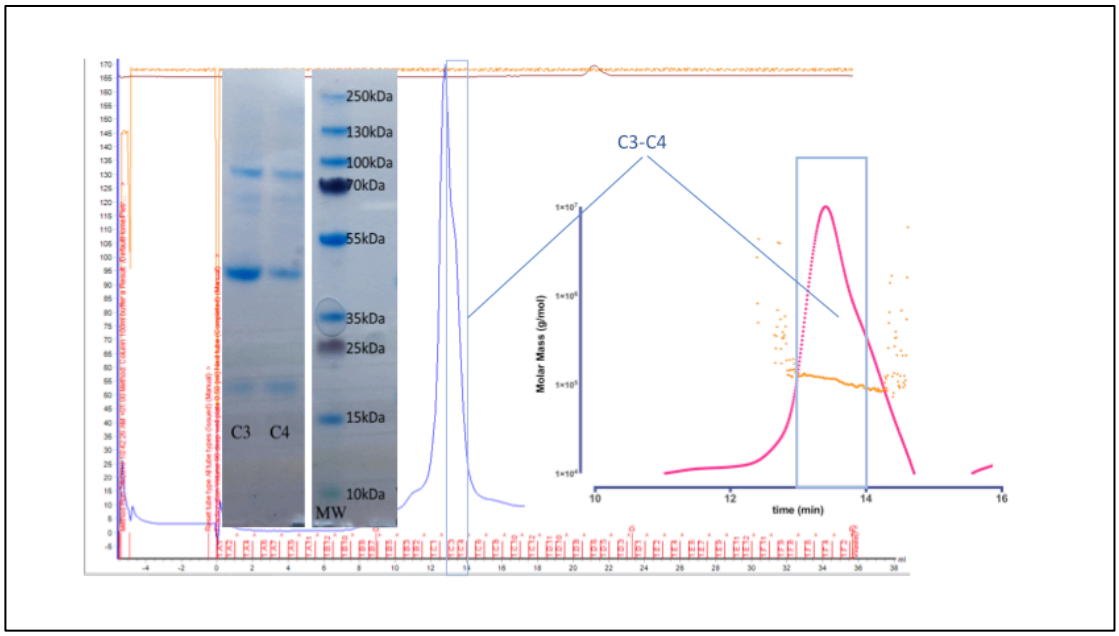

Figure 3. SEC-MALS elution profile of *Hs*GAPDH . Elution profile of the *Hs*GAPDH sample

run on the SEC S200 10/300 column interfaced with the 18-angle MALS light scattering detector (Dawn HELEOS IIR©) coupled with a differential Refractive Index detector (OptilabR©T-rEX) (Wyatt Technology). The fractions C3-C4, eluting between 13 and 14 mL, correspond to a tetramer of apparent mass 144 KDa. Inset: the SDS-PAGE NuPAGE Bis-Tris 4-12% gel (Thermo Fisher), run at 200 V for 30' in MES buffer, stained in Simply Blue™ SafeStain (Thermo Fisher) for 1 h and destained with water.

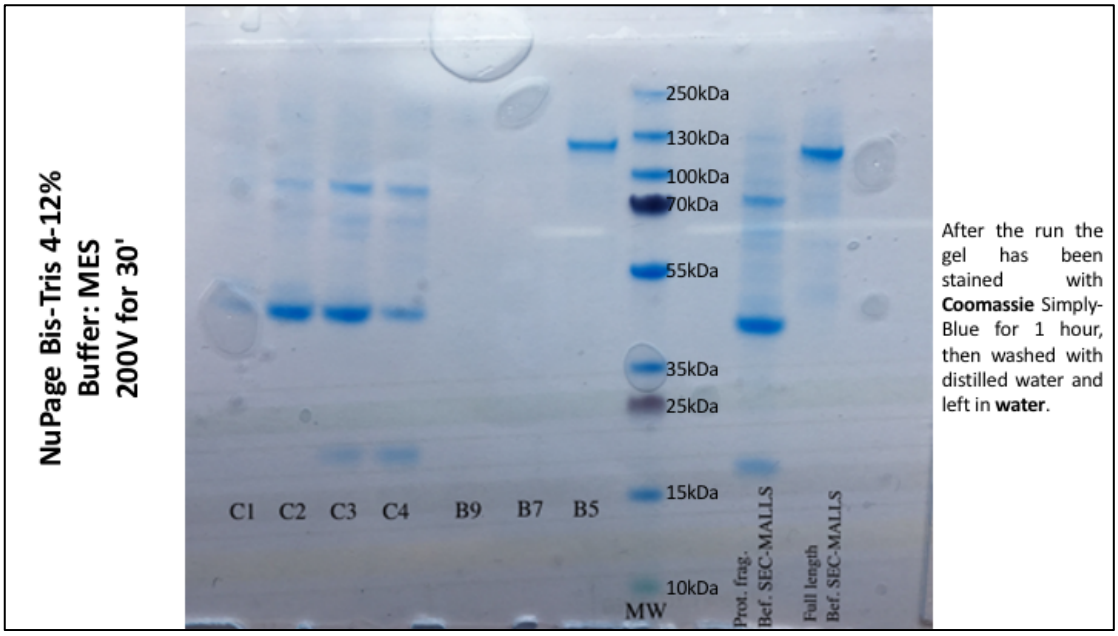

Figure 4. Original full-size version of the gel in Figure 3. Elution fractions of the SEC S200 10/300 column. SDS-PAGE NuPAGE Bis-Tris 4-12% gel (Thermo Fisher), run at 200 V for 30' in MES buffer, stained in Coomassie Simply Blue™ SafeStain (Thermo Fisher) for 1 h and destained with water.

*Hs*GAPDH structure determination

Protein crystallisation: vapour diffusion crystallisation 200 nL sitting drops were set up by mixing *Hs*GAPDH in mother liquor:protein ratioes 1:1 (drop 1) and 1:2 (drop 2) with the 96-conditions MORPHEUS crystallisation screen (Molecular Dimensions, [3, 4]), using a Mosquito crystallisation robot (SPT Labtech) and the drops were left equilibrating at 18 ˚C. **$P2_1$ Forms A and B:** 40 µL of the the protein from the S200 SEC run were used to set up for crystallisation at a concentration of 18.8 mg/mL. **$P2_1$ Form A**: these crystals grew in drop 2 equilibrated against condition H9 of the MORPHEUS crystallisation screen (Molecular dimensions, [3, 4]): 0.1M Amino acids solution (DL-Glutamic acid, DL-Alanine, Glycine, DL-Lysine, DL-Serine); 0.1M Buffer System 3 (Tris (base), bicine pH 8.5); 30% v/v Precipitant Mix 1 (40% v/v PEG 500MME, 20% w/v PEG 20000). **$P2_1$ Form B**: these crystals grew in drop 2 equilibrated against condition F9 of the MORPHEUS crystallisation screen [3, 4]: 0.12M monosaccharides solution (D-Glucose, D-Mannose, D-Galactose, L-Fucose, D-Xylose, N-Acetyl-D-Glucosamine); 0.1M Buffer System 3 (Tris (base), bicine pH 8.5) 30% v/v Precipitant Mix 1 (40% v/v PEG 500MME, 20% w/v PEG 20000). **$P2_1$ Forms C and D:** the protein from the SEC-MALS run was set up for crystallisation at a concentration of 3.0 mg/mL.

Both form C and from D grew in drop 1 equilibrated against the F1 condition of the MORPHEUS crystallisation screen [3, 4]: 0.12M monosaccharides solution (D-Glucose, D-Mannose, D-Galactose, L-Fucose, D-Xylose, N-Acetyl-D-Glucosamine); 0.1M Buffer System 3 (Tris (base), bicine pH 6.5) 30% v/v Precipitant Mix 1 (40% v/v PEG 500MME, 20% w/v PEG 20000).

X-ray data diffraction collection and processing: all crystals were cryo-cooled by plunging them into liquid nitrogen. X-ray diffraction were collected at beamline I03 of the Diamond Light Source in Harwell, England, UK, using wavelength λ=0.97622 Å, and a beam size 80x20 μm. Other data collection parameters are listed in Table 1. Diffraction data were processed with the `autoPROC` suite of programs [5].

Structure determination and refinement: structure determination started from the experimental structure factor amplitudes. **$P2_1$ Form A:** initial phases were computed by molecular replacement, searching for eight copies of the *Hs*GAPDH monomer in PDB ID 1U8F in space group $P2_1$ using the program `CCP4-Molrep` [6]. The eight copies of the *Hs*GAPDH monomer are arranged in two tetramers in the asymmetric unit. Initial automated water addition and positional and individual B-factor refinement were carried out in `autoBUSTER` [7, 8]. Automated non-crystallographic restraints were used throughout [9], including water molecules (assigned to each chain using `CCP4-Sortwater`). At each catalytic Cys152 site, a 0.5:0.5 occupancy ratio mixture of Cys and Cys S-sulfonic acid was initially modelled in Fo-Fc unbiased residual density. At each Cys152 site, occupancies for Cys and Cys S-sulfonic acid were refined and constrained so that they sum up to 1.000±0.005. Crystal form A was deposited in the Protein Data Bank with PDB ID code 6YND. Refinement statistics are reported in Table 2. **$P2_1$ Forms B,C and D:** initial phases were computed by molecular replacement, searching with the $P2_1$ Form A tetramer (PDB ID 6YND) in space group $P2_1$, placing one tetramer per asymmetric unit in $P2_1$ Forms B and D, and two tetramers per asymmetric unit in $P2_1$ Form C, using the program `CCP4-Molrep` [6]. The same refinement protocol and restraints were used as described for $P2_1$ Form A, but for the addition of external secondary structure restraints [9] to the highest resolution $P2_1$ Form A structure. Crystal forms B, C and D were deposited in the Protein Data Bank with PDB ID codes 6YNE, 6YNF and 6YNH, respectively. Refinement statistics are reported in Table 2.

	Form A	Form B	Form C	Form D
PDB ID	6YND	6YNE	6YNF	6YNH
Det. dist., d_{max} (mm, Å)	198.35, 1.5	288.19, 2.0	253.03, 1.8	356.69, 2.4
Photon flux (photons/s)	8.84×10^{11}	8.88×10^{11}	8.85×10^{11}	3.88×10^{12}
Transmission	25%	25%	25%	100%
# Images	3,600	3,600	3,600	3,600
Oscillation range (°)	0.10	0.10	0.10	0.25
Exposure time (s)	0.05	0.05	0.03	0.013
Space Group	$P2_1$	$P2_1$	$P2_1$	$P2_1$
Cell edges: a,b,c (Å)	81.88, 124.45, 141.99	81.79, 124.65, 79.64	87.14, 111.43, 135.94	87.02, 111.30, 69.74
Cell angle β (°)	99.38	117.04	96.02	98.33
Resolution Range (Å)	93.04-1.52 (1.71-1.52)	72.85-1.85 (2.05-1.85)	135.18-2.39 (2.74-2.39)	86.10-2.62 (2.89-2.62)
R_{merge}	0.08 (1.07)	0.226 (1.488)	0.27 (1.28)	0.40 (2.47)
R_{meas}	0.09 (1.16)	0.245 (1.618)	0.29 (1.38)	0.41 (2.54)
Observations	2,029,459 (100,354)	568,580 (26,433)	418,478 (20,849)	496,488 (24,336)
Unique observations	295,270 (14,763)	81,465 (4,074)	59,243 (2,962)	28,207 (1,409)
Average I/σ(I)	11.7 (1.7)	6.5 (1.5)	6.5 (1.6)	9.0 (1.5)
Completeness	69.4 (12.2)	67.6 (12.8)	58.2 (8.7)	71.3 (14.0)
Multiplicity	6.9 (6.8)	7.0 (6.5)	7.1 (7.0)	17.6 (17.3)
$CC_{1/2}$	0.997 (0.608)	0.991 (0.456)	0.986 (0.591)	0.990 (0.464)

Table 1. *HsGAPDH* data processing statistics. In parentheses the values referring to the highest resolution shell.

Table 2. *HsGAPDH* refinement statistics. In parentheses the values referring to the highest resolution shell.

	Form A	**Form B**	**Form C**	**Form D**
PDB ID	6YND	6YNE	6YNF	6YNH
Space Group (Z)	$P2_1$ (16)	$P2_1$ (8)	$P2_1$ (16)	$P2_1$ (8)
Resolution range	140.10-1.52(1.63-1.52)	72.85-1.85 (1.98-1.85)	72.85-1.85 (1.98-1.85)	86.10-2.62 (2.72-2.62)
Reflections working set	280,585 (5,242)	77,398 (1,536)	77,398 (1,536)	25,751 (545)
Reflections free set	14,686 (332)	4,059 (94)	4,059 (94)	1,459 (20)
R,R_{free}	0.182,0.199 (0.209,0.225)	0.190,0.207 (0.218,0.220)	0.190,0.207 (0.218,0.220)	0.176,0.215 (0.231,0.359)
$Rmsd_{bonds}$ (Å)	0.008	0.008	0.009	0.008
$Rmsd_{angles}$ (°)	1.02	1.05	1.04	1.03
Ramachandran favoured	97.4% (2,697/2,768)	97.5% (1,304/1,338)	97.0% (2,586/2,667)	97.5% (1,300/1,333)
Ramachandran allowed	99.7% (2,760/2,768)	99.7% (1,334/1,338)	99.5% (2,653/2,667)	99.7% (1,329/1,333)
Tetramers	(A,C,E,H) and (B,D,F,G)	(B,D,F,G)	(A,C,E,H) and (B,D,F,G)	(B,D,F,G)
Occ. Cys-SO_3H	A:0.13 B:0.15 C:0.09 D:0.18 E:0.13 F:0.15 G:0.15 H:0.08	N/A	A:0.5 B:0.41 C:0.46 D:0.33 E:0.38 F:0.43 G:0.42 H:0.72	B:0.41 D:0.42 F:0.40 G:0.35
Prot.(Wat.) Atoms	20,428 (1,639)	10,108 (569)	20,152 (726)	10,164 (132)
$<B_{prot}>$ ($<B_{wat}>$) ($Å^2$)	27.26 (33.12)	29.40 (36.17)	39.74 (22.93)	45.38 (29.13)

References

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