Supplementary material

Sugar-coated sensor chip and nanoparticle surfaces for the *in vitro* enzymatic synthesis of starch-like materials

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Table of contents	**Pages**

General experimental

All chemicals were purchased from Sigma Chemical Company Ltd, except where noted. Amyloglucosidase from *Aspergillus niger* was supplied by Fluka, barley β-amylase and porcine pancreatic amylase (PPA) were supplied by Megazyme. All water used was deionised water (≥18MΩ, Millipore) (MQ H_2O)

Cloning of AtPHS2

The PHS2 gene from *Arabidopsis thaliana* was amplified from cDNA, a kind gift of C. Ruzanski by PCR (Pfusion, Finnzymes) using the following primers.

AtPHS2 TOPOF (eurofins MWG): 5'-CACCATGGCAAACGCCAATGGAAAAGC-3'

AtPHS2 R*(Sigma): 5'-TTAGGGAACAGGACAAGCCTC-3'

After separation on a 1% agarose gel in TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA) at 80 V the band corresponding to 2621 bp was extracted from the gel (QIAquick gel extraction kit, Qiagen) and cloned into pET151 using directional TOPO cloning (Invitrogen), forming plasmid pET151-PHS2. This engineered plasmid was transformed into OneShot TOP10 chemically competent *E. coli* (Invitrogen), and transformants were selected on Luria-Bertani (LB) agar using carbenicillin (100 μg ml^{-1}). Plasmids were extracted (QIAprep miniprep kit, Qiagen) and sequences were confirmed by BigDye v3.1 sequencing reactions (Applied Biosystems).

Expression and purification of AtPHS2

E. coli strain Rosetta-2 (DE3) pLysS (Novagen) was transformed with pET151-PHS2 and cultured in LB medium containing carbenicillin (100 μg ml^{-1}). An overnight culture of the cells (8 x 1 ml) was used to inoculate 8 x 1 l cultures of LB which were grown at 37 ^{o}C to OD_{600} = 0.1 and then transferred to 16 ^{o}C for 1 hour. IPTG was then added to a final concentration of 1 mM. After 16 hours the cells were harvested at 7500 g and frozen at -80 ^{o}C until required. When needed, the pellet was thawed and resuspended into 50 ml of lysis buffer (50 mM HEPES, pH 7.5, 100 mM NaCl, a cOmplete, EDTA-free Protease Inhibitor Cocktail Tablet (Roche), 0.02 mg ml^{-1} DNaseI). Cells were lysed using a cell disruptor (one shot mode, 25 kpsi, Constant Systems, UK) and the cell debris removed by centrifugation at 30,000 g for 30 min. Protein was purified at 4 ^{o}C using an ÄKTA Xpress FPLC system (GE Healthcare). The supernatant was passed through a HiTrap Ni-NTA column (5 ml, GE Healthcare), washed with wash buffer (50 mM Tris-HCl, pH 8.0, 0.5 M NaCl) and eluted with elution buffer (50 mM

Tris-HCl, pH 8.0, 0.5 M NaCl, 0.5 M imidazole (BioUltra)). Further purification was performed by gel filtration on a Superdex S75 26/60 column (GE Healthcare) using gel filtration (GF) buffer (50 mM HEPES, pH 7.5, 100 mM NaCl) at a flow rate of 3.2 ml min^{-1}. A total yield of 2.5 mg of purified AtPHS2 per litre of culture was obtained. This material was concentrated to 40 mg ml^{-1} with an Amicon Ultra-15 50 kDa MWCO spin concentrator (Millipore). Protein concentration was estimated by A_{280} using an extinction coefficient of 145190 $M^{-1}cm^{-1}$, calculated from the amino acid composition.[1] Protein quality was analysed by SDS-PAGE and by Dynamic Light Scattering (DynaPro, Wyatt Technologies) (Figure S1).

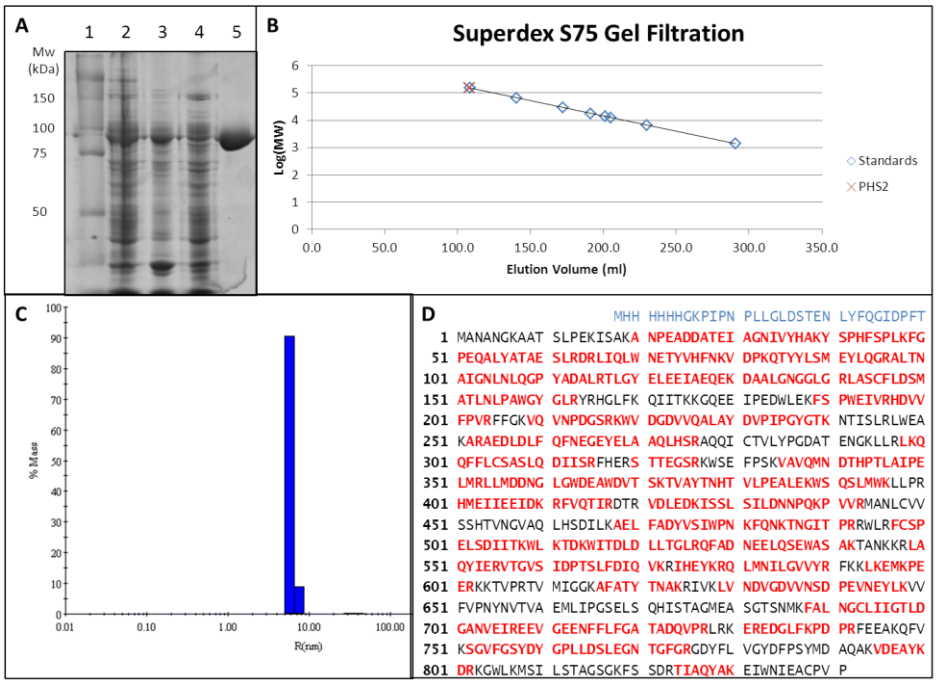

Figure S1. Purification of AtPHS2 by IMAC and GF.

A. SDS-PAGE of protein purification. Lane 1: Kaleidoscope protein standards (Bio-Rad), Lane 2: total cell extract, Lane 3: insoluble cell material, Lane 4: soluble cell lysate, Lane 5; purified protein, showing a single band running just under 100 kDa.

B. Gel filtration shows a single peak eluting around 108 ml, which on a Superdex 75 gives a predicted M_w > 152 kDa when compared to standards shown (vitamin B12 – 1.355 kDa, aprotinin – 6.5 kDa, cytochrome C – 12.4 kDa, ribonuclease A – 13.7 kDa, myoglobin – 17.6 kDa, carbonic anhydrase 29 kDa, albumin 66 kDa and alcohol dehydrogenase – 150 kDa.

C. Dynamic Light Scattering shows that protein is present as monodisperse species with a radius of 6.0 nm, giving a M_w of 221kDa.

D. Sequence of PHS2, with artificial tag in blue, and peptides identified in MALDI-ToF spectrum after tryptic digestion (red).

Enzyme activity assays

All AtPHS2 kinetic analyses were performed in the synthetic direction, measuring release of Pi from Glc-1-P. To determine optimum conditions for the enzyme, assays were performed using 10 mM Glc-1-P, 1 mM maltoheptaose, 8 µg ml^{-1} (80 nM) AtPHS2 (Figure S2). Variation of pH was performed in a mixed buffer (0.1 M Tris, MES, citrate each). Assays were typically performed at 21 ^{o}C. For variable temperature experiments, temperature variation was performed in a thermocycler (GStorm) in MES (20 mM, pH 6.0).

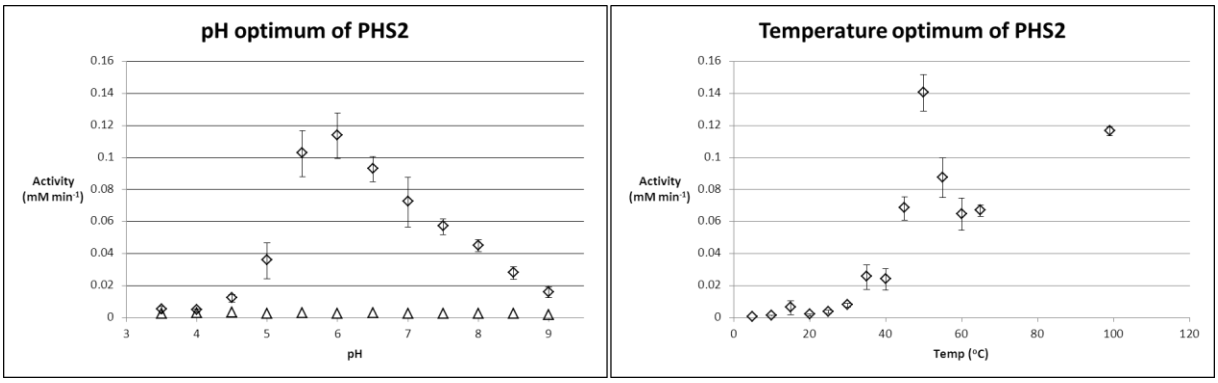

Figure S2. Optimum conditions for the synthetic AtPHS2 reaction.

All reactions measured the release of phosphate from Glc-1-P (10 mM) in the presence of maltopentaose (1 mM). Maximum activity (Δ) is at pH 6.0 (100 mM Tris, MES, citrate) at 37 ^{o}C and at 50 ^{o}C in MES (20 mM, pH 6.0). No release of phosphate is observed in the absence of enzyme (◊) across the pH range tested and the temperature experiments a no-enzyme control was subtracted from the values obtained with the enzyme.

To determine apparent K_m values reactions were conducted in 12.5 µl MES buffer (20 mM, pH 6.0) at 21 ^{o}C using 8 µg ml^{-1} AtPHS2 (Figure S3; Table S1). After 20 min the reactions were terminated by the addition of an equal volume of aqueous ammonium molybdate solution (400 mM), which had been confirmed to stop the reaction completely (Figure S4). The concentration of released Pi was measured colourimetrically using a method modified from De Groeve et al.[2] Briefly, colour solution (75 µl, 0.1 M HCl, 13.6 mM sodium ascorbate) was added to the stopped enzyme reaction (25 µl), containing ammonium molybdate (200 mM), in a microtitre plate. After incubating for 5 min at 21 ^{o}C, stop solution (75 µl, 68 mM sodium citrate, 2 % acetic acid) was added and the A_{620} was measured. Experiments were performed in triplicate in the range 0.1-10 mM Glc-1-P and 0.05-3 mM acceptor glucan. Molybdate inhibition was determined in the range 0.1-200 mM and acarbose inhibition in the range 0.5-500 µM,

under the same conditions as above, using fixed concentrations of Glc-1-P donor (10 mM) and maltoheptaose acceptor (1 mM).

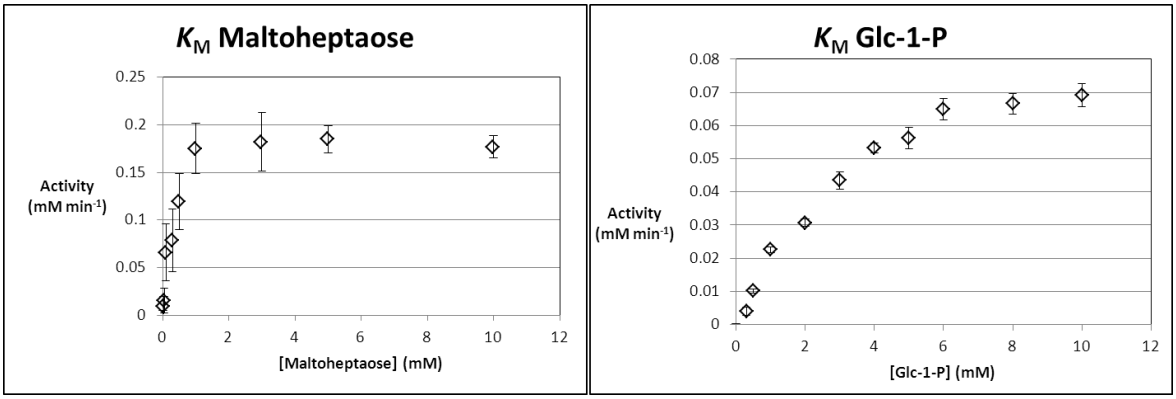

Figure S3. Kinetic analysis of AtPHS2.

Assays were carried out in MES (20 mM, pH 6.0) at 37 ^{o}C. Using maltoheptaose (1 mM) as acceptor the K_m^{app} for Glc-1-P was determined to be 3.9 ± 0.5 mM. K_m^{app} for a standard acceptor (maltoheptaose) was determined to be $K_{m(Glc)7}^{app}$ = 240 ± 50 μM by fixing the concentration of Glc-1-P at 10 mM. Under optimum conditions, specific acitivity = 18 ± 1 μmol min^{-1} mg^{-1} (giving a turnover number of 30 s^{-1}).

Table S1. Acceptor specificity of PHS2.
K_m^{app} decreases as acceptor length increases leading to higher catalytic efficiency, even though the k_{cat} also decreases. NA indicates less than 1% turnover in 20 min.

Acceptor	K_m^{app} (mM)	k_{cat} (min^{-1})	k_{cat}/K_m^{app} (M^{-1}s^{-1})
maltotriose – $(Glc)_3$	NA	NA	NA
maltotetraose – $(Glc)_4$	2.0 ± 0.94	1500 ± 370	12500
maltopentaose – $(Glc)_5$	1.1 ± 0.43	940 ± 170	14200
maltohexaose – $(Glc)_6$	1.0 ± 0.26	1100 ± 120	18300
maltoheptaose – $(Glc)_7$	0.24 ± 0.05	890 ± 62	61700

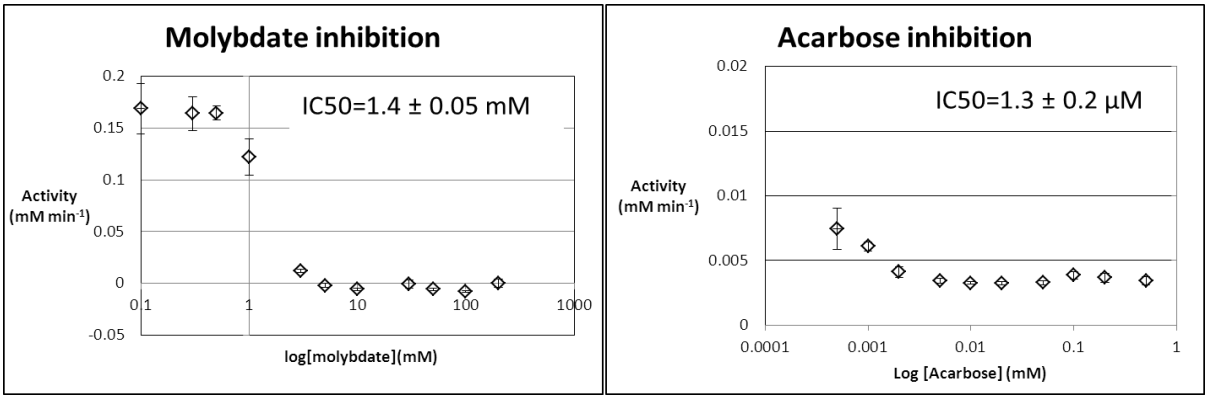

Figure S4. Inhibitor assays with PHS2.

Molybdate, a critical component of the activity assay, was first assessed for its ability to inhibit AtPHS2 by measuring release of Pi from Glc-1-P (10 mM) by AtPHS2 (2.5 µg ml^{-1}) in the presence of $(Glc)_7$ (1 mM) in MES buffer (200 mM, pH 7.0) at 21 ^oC. Acarbose was then assessed as an inhibitor by measuring the release of Pi from Glc-1-P (1 mM) in the presence of $(Glc)_7$ (1 mM) in MES buffer (20 mM, pH 6.0) by AtPHS2 (5 µg ml^{-1}) at 21 ^oC.

APTS labelling of sugars

Sugars were labelled for electrophoresis with 8-aminopyrene-1,3,6-trisulfonic (APTS) according to the PACE method.[3] APTS (0.5 mg, 0.2 M) in aqueous acetic acid (5 µl, 30%) was mixed with $NaBH_3CN$ (0.5 mg, 0.8 M) in THF (5 µl). Maltopentaose (1 mg, 120 mM) was dissolved in the mixture and incubated at 37 ^oC for 18 h. The sample was then loaded onto a 30% 38:2 mono: bis acrylamide Tris-borate (100 mM, pH 8.2) gel and separated by electrophoresis at 400 V in Tris-borate buffer (100 mM, pH 8.2). The carbohydrate band was excised and the labelled sugar was extracted into purified water by grinding, desalted using a PD-10 column (GE Healthcare) and quantified using APTS absorption at 455 nm (17160 M^{-1} cm^{-1}).[4]

Carbohydrate Electrophoresis (CE)

AtPHS2-catalysed glucan synthesis reactions (Figure 1) employed APTS-maltopentaose (2.2 µM), Glc-1-P (10 mM), MES (20 mM, pH 6.0) and AtPHS2 (0.5 µg ml^{-1}) in 100 µl. The reaction was performed at 30 ^oC for 24 h and then placed in boiling water for 5 min to inactivate the enzyme. For phosphorolysis experiments, a 50 µl sample of the synthesis reaction was made up to 100 µl with Pi (100 mM) and fresh AtPHS2 (0.5 µg) was added. For hydrolysis experiments a 50 µl sample was made up to 100 µl

containing amyloglucosidase (5 μg) and incubation at 30 ^{o}C was carried out for 24 h. The reaction was then placed in boiling water for 5 min and all samples were centrifuged for 1 min at 16000 g.

Samples for CE analysis were made up to 50 μl and loaded onto a N-CHO capillary in a PA800 ProteomeLab (Beckman Coulter) by injection at 0.5 psi for 20 s. They were then separated in running buffer (25 mM LiOAc, 0.4% polyethylene oxide, pH 4.75) at 30 kV for 20 or 45 min and detected using LIF (excitation at 488 nm detection at 520 nm).[5]

Atomic Force Microscopy (AFM)

AFM images (Figure 2) were obtained using an MFP-3D BIO microscope (Asylum Research, CA, USA) and was operated in air using AC mode. The cantilevers used were Olympus AC160TS (Olympus, Japan) with a nominal spring constant of ~42 Nm^{-1}, oscillated at a frequency 10% below resonance (typically around 320 kHz). The damping set point for imaging was kept to the minimum value that allowed stable tracking of the sample surface in order to minimise any sample deformation. Images were acquired at a scan rate of 1 Hz.

High sugar density SPR surface

The higher sugar density surface, with a higher FG loading, was made by reacting EDC (1 μl, 0.2 M) and NHS (1 μl, 0.05 M) in DMF with SAM linker (0.25 μmoles, neat) at room temperature for 30 min and adding FG-ADH (1 mg) for 30 min, forming FG-ADH-SAM linker. The resulting solution was then applied to the cleaned gold sensor surface and the thiol was left to immobilise for 2 h before adding SAM spacer (4 μmol) in DMF (10 μl). The SAM was left to assemble overnight before washing and loading the chip into the sensor chip holder and inserting it into the spectrometer.

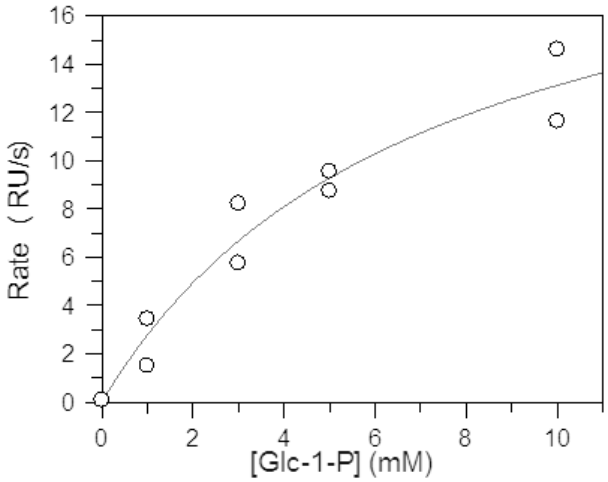

Figure S5. SPR analysis of the extension of the high sugar density surface with PHS2.

The initial SPR response increases with the amount of Glc-1-P donor substrate (0-10 mM) co-injected (100 ul) with the enzyme (5 µg ml^{-1}).

Crystallisation, X-ray structure determination and refinement

Initial protein crystallisation was performed using the sitting drop vapour diffusion method in a 96 well plate format (MRC crystallization plate, Molecular Dimensions). Drops were made by mixing an equal volume (0.3 µl) of filtered (0.1 µm, Millipore) 10 mg ml^{-1} protein solution in GF buffer and well solution ($AmSO_4$ and PEG (Qiagen) and JCSG, Classics and PACT (Molecular Dimensions), 50 µl of which were transferred into wells robotically (Freedom Evo, Tecan), and the drops dispensed robotically (OryxNano, Douglas Instruments). Plates were placed in a CrystalPro (Tritek) crystal hotel at 20 ^oC and monitored over the course of 4 weeks, when hits were selected for further screening in 24-well format using the hanging drop vapour diffusion method.

PHS2 was crystallised at 20 ^oC from 20% (w/v) PEG3350, 100 mM ammonium citrate (pH 8.25), 10% (v/v) glycerol using the hanging drop vapour diffusion method. AtPHS2 (1 µl, 10 mg ml^{-1}) in 25% GF buffer (HEPES 12.5 mM, pH 7.5, NaCl 25 mM) was mixed with 1 µl well solution to give the final drop. Crystals appeared after 2 weeks, at which point 1 mM ligand in 1 µl well solution was added to the drop. After a further week, crystals were flash cooled in liquid nitrogen; no additional cryoprotection was required due to the presence of glycerol in the crystallisation solution.

Crystals were transported to the synchrotron in Unipuck cassettes before being robotically mounted onto the goniostat on either station I02 or I03 at the Diamond Light Source (Oxford, UK) and were maintained at -173 ^{o}C with a Cryojet cryocooler (Oxford Instruments). Diffraction data were recorded using an ADSC Quantum 315 CCD detector. The resultant data were integrated using either MOSFLM[6] or XDS[7] and subsequently scaled by SCALA.[8] Diffraction data were collected from an unsoaked crystal to 1.7 Å resolution in space group $P2_1$ with approximate cell parameters of a = 84.7, b = 117.1, c = 94.2, β = 106.7°. Solvent content estimation gave a value of 46% based on two copies of the monomer in the asymmetric unit. A molecular replacement monomer template was prepared from the structure of rabbit muscle glycogen phosphorylase (GP) (PDB accession code 1GPB) using CHAINSAW[9] guided by an amino acid sequence alignment of GP and PHS2. A molecular replacement search with PHASER[10] using the monomer template was successful in finding two copies of the subunit which, together, yielded a dimer that was consistent with the biological units of both GP and MalP, the *E. coli* homologue. Subsequent rounds of rebuilding with COOT[11] and refinement with REFMAC5[12] yielded a final model with R_{work} and R_{free} values of 0.153 and 0.183, respectively at 1.7 Å resolution. This "apo" structure was used as the starting point for building and refinement of the ligand-bound structures with which it was isomorphous. Data collection and refinement statistics are summarised in Table S2.

Table S2: Summary of AtPHS2 X-ray data and model parameters

Data set	Apo	Acarbose	Maltotriose
Data collection			
Space Group	$P2_1$	$P2_1$	$P2_1$
Cell parameters (Å)	a = 84.7, b = 117.1, c = 94.2, β = 106.7°	a = 84.3, b = 117.1, c = 95.0, β = 106.8°	a = 83.7, b = 116.1, c = 94.3, β = 107.4°
Beamline[a]	I03	I02	I02
Wavelength (Å)	0.971	0.980	0.980
Resolution range[b] (Å)	49.11 - 1.70 (1.79 - 1.70)	66.42 - 2.35 (2.48 - 2.35)	65.81 - 1.90 (2.00 - 1.90)
Unique reflections[b]	190517 (26165)	73427 (10684)	132861 (17494)
Completeness[b] (%)	98.9 (93.5)	99.9 (99.8)	98.2 (88.8)
Redundancy[b]	4.2 (3.2)	3.7 (3.7)	4.0 (3.4)
R_{merge}[b, c]	0.080 (0.433)	0.098 (0.791)	0.064 (0.558)
R_{meas}[b, d]	0.091 (0.523)	0.115 (0.926)	0.073 (0.661)
Mean I/σ(I)[e]	11.8 (2.5)	8.7 (1.6)	12.9 (2.2)
Wilson B value (Å^2)	17.8	45.6	28.1
Refinement			
Reflections: working/free[d]	180900/9587	69673/3670	126128/6643
R_{work}[f]	0.153	0.184	0.196
R_{free}[f]	0.183	0.240	0.241
Ramachandran favoured/ allowed[g] (%)	98.0/99.9	96.4/99.8	97.6/99.9
Ramachandran outliers[g]	2	3	1
rmsd bond distances (Å)	0.016	0.014	0.014
rmsd bond angles (°)	1.46	1.60	1.55
Contents of model			
Protein residues	824 (A chain) 825 (B chain)	824 (A chain) 827 (B chain)	824 (A chain) 824 (B chain)
Pyridoxal phosphate	2	2	2
Ligands[h]	0	2 x ACR; 3 x GLC	2 x M3
Precipitant/cryoprotectant molecules[h]	2 x GOL; 4 x PEG	1 x GOL	1 x GOL; 1 x PEG
Water molecules	1844	344	919
Average atomic displacement parameters (Å^2)			
Main chain atoms	17.2	45.4	31.9
Side chain atoms	18.8	47.3	33.9
Pyridoxal phosphate	15.9	44.1	31.0
Ligands[h]	-	ACR: 72.5; GLC: 77.1	M3: 39.4
Precipitant/cryoprotectant molecules[h]	GOL: 30.8; PEG: 34.6	GOL: 48.3	GOL: 35.0; PEG: 35.0
Water molecules	27.8	39.7	37.5
Overall	19.2	46.4	33.2
PDB accession code	4BQE	4BQF	4BQI

[a] I02, I03 = beamlines at the Diamond Light Source (Oxfordshire, UK).

[b] The figures in brackets indicate the values for outer resolution shell.

[c] $R_{merge} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the ith observation of reflection hkl and $\langle I(hkl)\rangle$ is the weighted average intensity for all observations i of reflection hkl.

[d] $R_{meas} = \sum_{hkl} [N/(N-1)]^{½} \sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where N is the number of observations of reflection hkl.

[e] The data sets were split into "working" and "free" sets comprising 95% and 5% of the data, respectively. The free set was not used for refinement.

[f] The R-factors R_{work} and R_{free} are calculated as follows: $R = \sum(|F_{obs} - F_{calc}|)/\sum|F_{obs}| \times 100$, where F_{obs} and F_{calc} are the observed and calculated structure factor amplitudes, respectively.

[g] As calculated using MOLPROBITY.[13]

[h] Abbreviations: ACR = acarbose; GOL = glycerol; GLC = glucose; PEG = polyethylene glycol; M3 = maltotriose.

Crystal structures of PHS2

PHS2 crystallised in the spacegroup $P2_1$ and the structure was determined to 1.7 Å resolution by molecular replacement using the inactive (B) form of the rabbit muscle GP (PDB entry 1GPB), with which it shares 46% amino acid sequence identity, as the search template. For a summary of AtPHS2 X-ray data and model parameters see Table S2. There are two copies of the subunit per asymmetric unit that together comprise the biologically relevant dimer. The dimer interface of AtPHS2 has about 2600 $Å^2$ of solvent accessible surface buried per monomer, which is comparable in extent to that of the GP dimer. There are many close structural homologues of AtPHS2 in the PDB: 244 entries give a DALI Z score of greater than 45,[14] although these come from just seven non-redundant proteins: mammalian and yeast GP structures and the *E. coli* maltodextrin phosphorylase, MalP. At the subunit level, AtPHS2 most closely resembles the A form mammalian enzymes (e.g. rmsds of 1.17 and 1.18 Å vs. PDB entries 1FA9 and 1GPA, respectively) and the yeast enzyme (rmsd of 1.16 Å vs. PDB entry 1YGP); whilst at the dimer level, AtPHS2 most closely resembles the B form mammalian enzymes (e.g. rmsds of 1.60 and 1.64 Å vs. PDB entries 8GPB and 1GPB, respectively).

Structures of AtPHS2 were obtained in complex with substrates, individually or in combination, as well as the inhibitor acarbose. Upon soaking with oligosaccharides the crystals tended to crack, although this was likely attributable to ligands binding near crystal contacts, rather than to conformational changes. Nevertheless, useable X-ray data were collected for soaks with maltotriose, maltotetraose or β-cyclodextrin (βCD). All three oligosaccharides bound to the surface of the enzyme, although only three glucosyl moieties could be resolved in the electron density for the latter two compounds. Since the maltotriose complex has the highest resolution, it is the only one reported here (Figure S6).

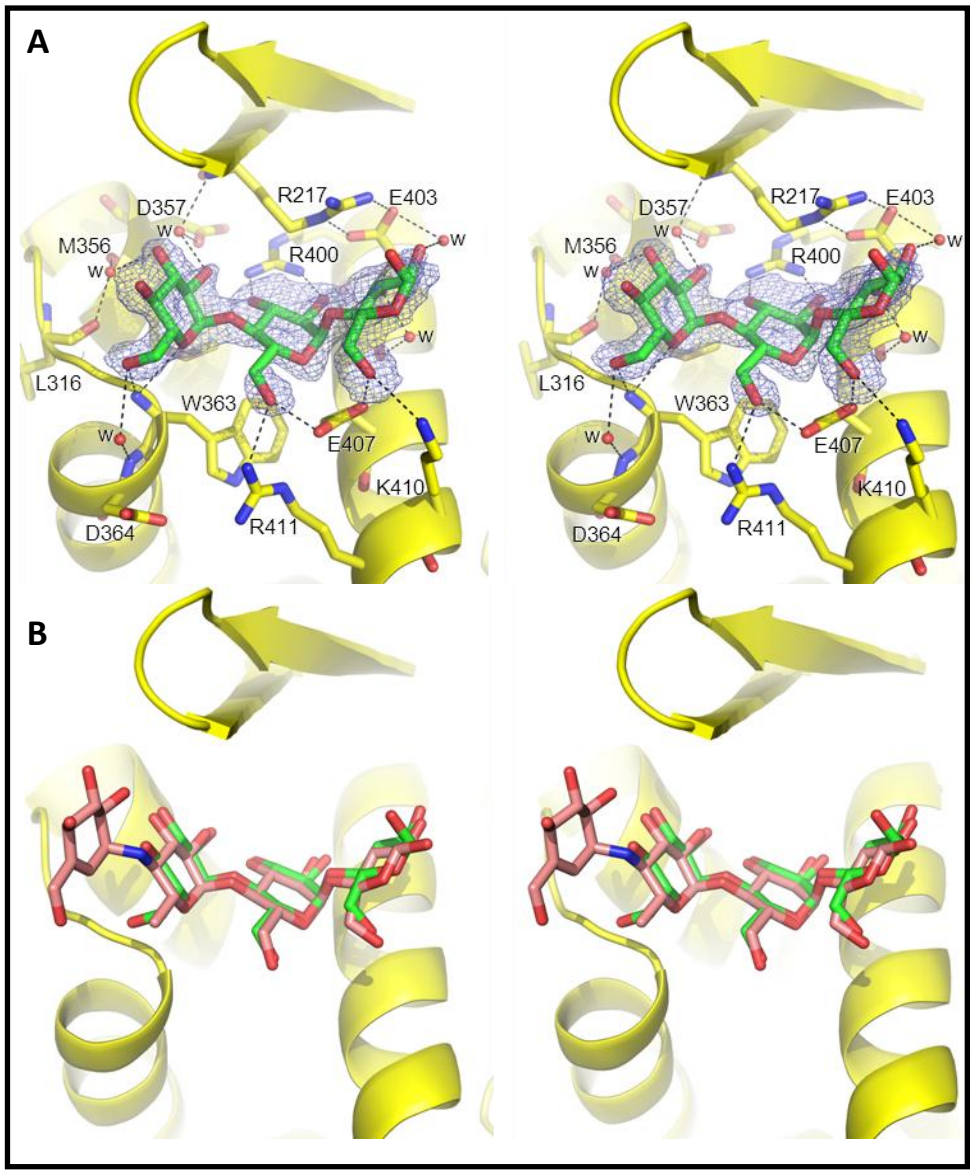

Figure S6. Detail of interactions in the surface binding site of PHS2.
A. Maltotriose (green) binds in the surface site forming 10 H-bonds. Also shown, as a blue mesh, is a difference electron density map calculated from the final model, with maltotriose omitted.
B. Acarbose (pink) also binds in the same mode as maltotriose (green) in the surface site, with the cyclohexitol moiety making no additional contacts.

A structure of AtPHS2 in complex with the inhibitor acarbose was also obtained. This showed acarbose in the same surface site as the maltotriose in the previous structure, making very similar contacts to the protein. In one subunit, the whole of the inhibitor was resolved, whereas only the central two rings could be seen in the other subunit. This was at least in part due to the proximity of a symmetry related subunit, which effectively blocks the site for the cyclohexitol ring. In addition, there was discontinuous residual

density in the active site channel. In one subunit, it was possible to model two separate glucosyl moieties, although it was not clear if they were parts of the same or different acarbose molecules. In the second subunit of PHS2, only the inner site had sufficient density to model a glucosyl moiety. A comparison with the acarbose complex of MalP (PDB entry 2ECP) showed that the ring closest to the PLP (some 9 Å distant) roughly corresponded to the 4-amino-4,6-dideoxy-D-glucopyranose moiety of the acarbose in the MalP complex, whilst the ring further from the PLP (some 14 Å distant) roughly corresponded to the glucosyl moiety at the reducing end of the acarbose in the MalP structure (Figure S7).

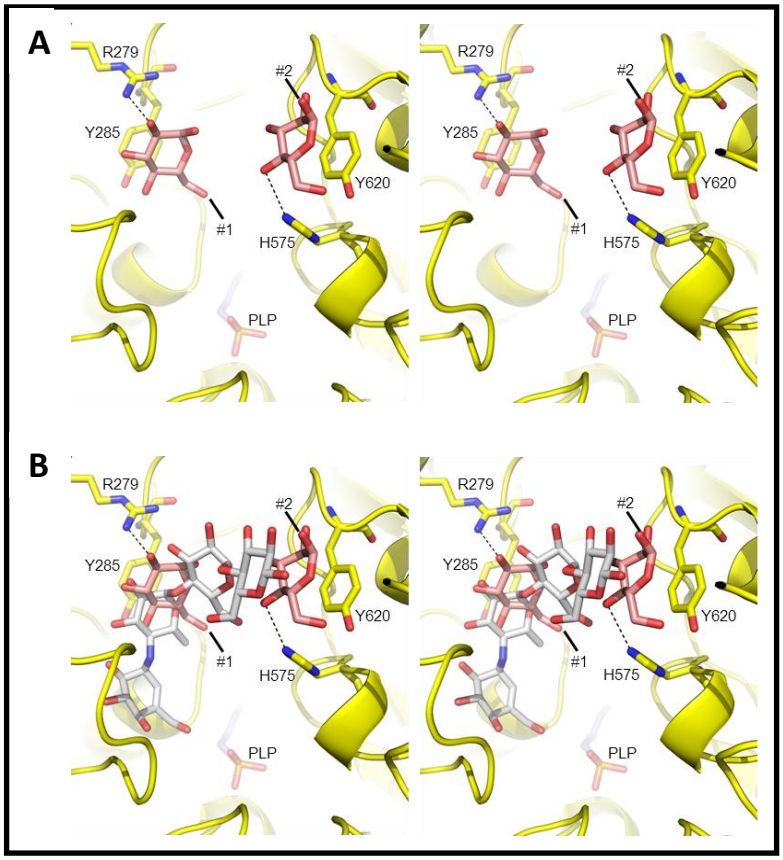

Figure S7. Acarbose bound in active site of AtPHS2
A. When acarbose is bound in the active site only two glucose residues can be modelled (pink).
B. When compared to the acarbose bound to MalP (in white, from PDB entry 2ECP, overlaid onto the AtPHS2), it indicates one of the resolved glucose residues corresponds to the 4-amino-4,6-dideoxy-D-glucopyranose moiety, whilst the other corresponds to the reducing end of the acarbose.

Glycogen-based nanoparticles

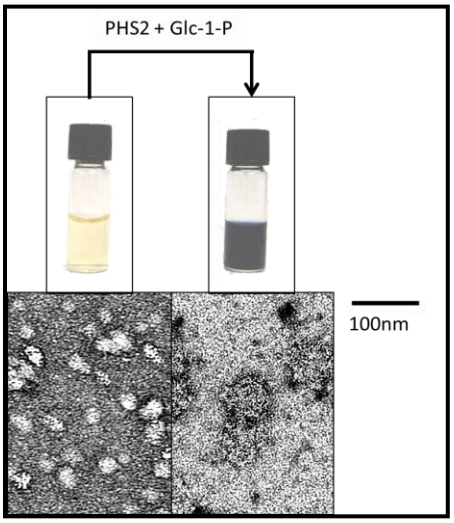

Figure S8 Glycogen-based amylose nanoparticles.

Upon extension of glycogen (1 mg ml^{-1}) by AtPHS2 (10 µg ml^{-1}, 10 mM Glc-1-P) the material stained dark blue with 5 µl of ethanol saturated with iodine and the particle size had increased when imaged using TEM (2% UA stained)

Gold nanoparticles

Citrate stabilised gold nanoparticles (AuNP) of approximately 15 nm were synthesised according to the procedure of Turkevich.[15] Gold (III) chloride ($AuCl_3 \cdot 3H_2O$, 12.5 mg in 100 ml MQ H_2O) was reduced with tri-sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$, 50 mg in 50 ml MQ H_2O), combined with stirring at 60 ^oC and subsequent heating to 85 ^oC for 150 min. The AuNPs were filtered by syringe filtration through a disc filter (0.2 µm, Sartorius Stedim) once they had cooled to room temperature. The particles had a regular round shape and an average diameter of 15 nm according to transmission electron microscopy (TEM) (FEI Tecnai20, at 200 kV, imaged using an AMT digital camera) and a UV absorption maximum (Perkin Elmer Lambda 25 UV/Vis Spectrophotometer) at 524 nm, as expected.[16]

Approximately 1 µmole of FG-ADH-SAM linker, as was made for the high sugar density SPR surface, was added to 10 ml of AuNPs and left to immobilise on the surface for 2 hours. Four µmole of SAM spacer molecule was added and the solution was left to assemble overnight at 4 ^oC to form FG-NPs. The FG-NPs were purified by centrifugation and resuspension and sonication into fresh MQ H_2O six times and stored until ready for use as $5x10^{12}$ particles ml^{-1} in H_2O at 4 ^oC.

The surface was extended by diluting the FG-NPs to $1x10^{12}$ particles ml^{-1} in a solution containing Glc-1-P (10 mM), AtPHS2 (10 µg ml^{-1}) and MES (10 mM, pH 6.5) and incubating at 37 ^{o}C for 60 min. The reaction was stopped by the addition of ProteinaseK (20 µg ml^{-1} in 20 mM MES, pH 7.5) and incubating at 37 ^{o}C for 5 min followed by inhibition of the ProteinaseK by addition of PMSF (1 mM). The reactions were then imaged immediately or stored at 4 ^{o}C for 24 h before depositing on TEM Grids (Pd/Cu with plastic/carbon coating) and negatively stained with 2% uranyl acetate (UA) and imaged using TEM. After 24 h at 4 ^{o}C the particle suspension was diluted two-fold with enzymes in buffer to give final concentrations of PAA (0.3 mg ml^{-1} in 100 mM MES, pH 6.5) or AG (1 mg ml^{-1} in 100 mM MES, pH 4.5) and incubated for 60 min at 37 ^{o}C. The enzymes were inhibited by addition of acarbose (1 mM), a known inhibitor of both enzymes (PPA K_i = 2.1 µM,[17] AG (*Aspergillus*) K_i = 1 pM[18]), and the particles were deposited, stained and imaged immediately afterwards.

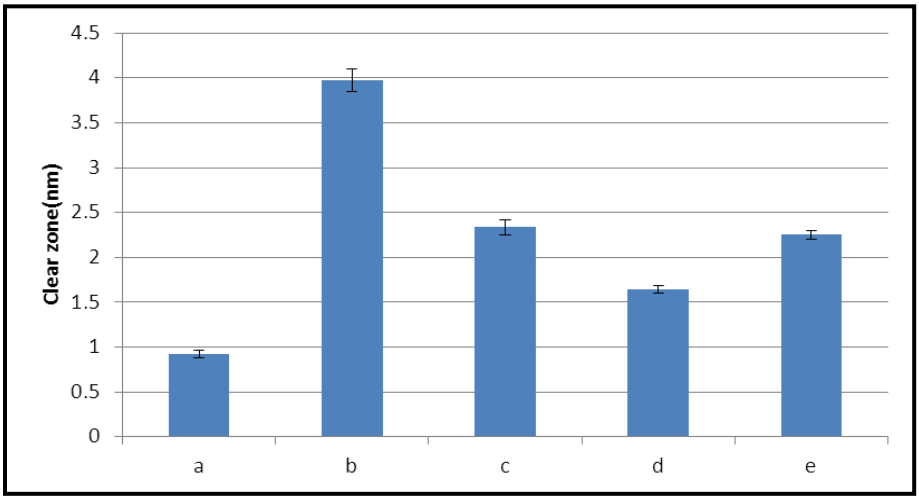

Figure S9. Changes in the glucan layer on FG-nanoparticles.

The glucan layer on the FG-nanoparticles (a) increased after treatment with AtPHS2 (10 µg ml^{-1}, 10 mM Glc-1-P) (b). 24 hours after AtPHS2 was inactivated (c) the thickness of the clear zone around the FG-NPs decreased. After treatment with PAA (0.3 mg ml^{-1}) (d), but not AG (1 mg ml^{-1}) (e), a further decrease was apparent. Clear zones were measured using TEM with negative staining with UA. Means and SE were determined with 31-65 particles for each treatment and all conclusions are at the 5% confidence level (t-test).

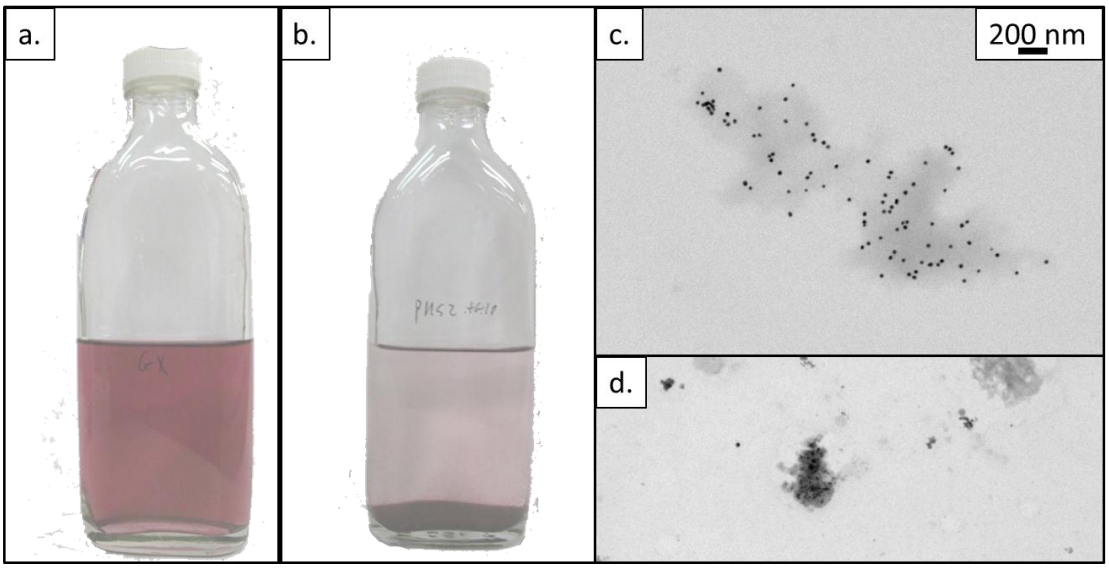

Figure S10. Extended reaction of AtPHS2 on nanoparticles.

a. FG-NPS show characteristic red colour. b. AtPHS2 (5 µg ml^{-1}) and Glc-1-P (10 mM) were added to 75 ml of the FG-NPS for 16 hours at 21 ^{o}C. The nanoparticles precipitated, but did not aggregated, as they remained red in colour. c. Under TEM, extensive glucan synthesised can be, seen stained with UA. d. Positive staining with PATAg shows the material synthesised is carbohydrate.

The periodic acid/thiocarbhydrazide/silver (PATAg) staining protocol was applied in order to specifically stain AuNPs for the carbohydrates.[19] Briefly particles were deposited on the TEM grids that were then inverted onto periodic acid (10 mg ml^{-1}) for 20 min. After washing with MQ H_2O the grids were placed on thiocarbohydrazide (2 mg ml^{-1} in 20% acetic acid) for 2 hours and washed with decreasing aqueous acetic acid (20%, 10%, 5%) and finally MQ H_2O. Grids were then floated on silver proteinate (10 mg ml^{-1}, 8% Ag) for 30 min in the dark and washed with MQ H_2O. Grids were kept in the dark before imaging.

Sequential enzymatic digestion of on-chip-synthesised glucan

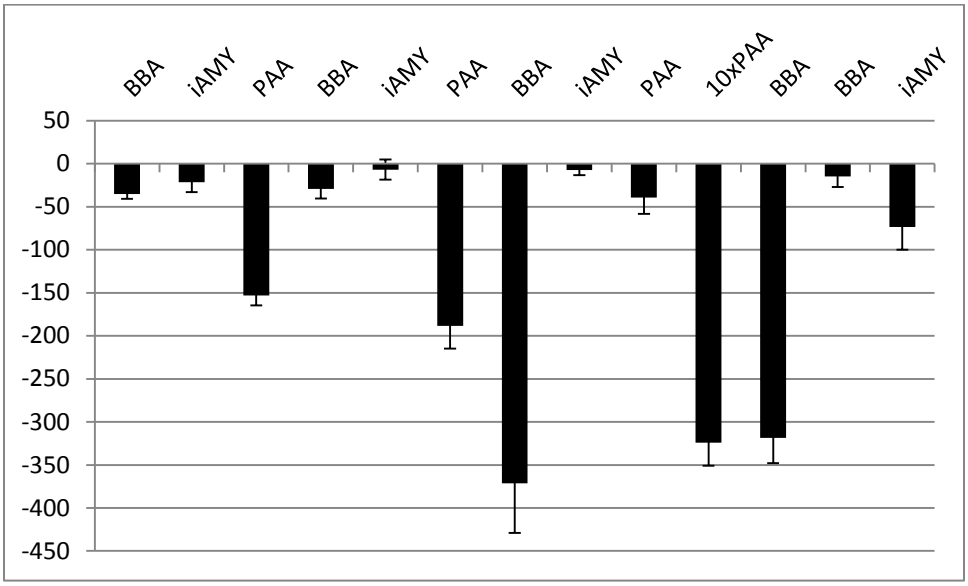

Figure S11 Quantification of the degradation of PHS2 synthesised glucan, as analysed using SPR (Figure 7)
After extending the high sugar density surface with around 4000 RU-worth of material, using PHS2 (5 μg ml^{-1}) and Glc-1-P (10 mM), Sequential injections (100 μl) , in the order shown, of BBA (1 U ml^{-1}), isoamylase (iAMY, 1 U ml^{-1}) and PPA (1 mU ml^{-1}) were used to probe the surface. Standard error bars of three replicates are shown.

References

1. C. N. Pace, F. Vajdos, L. Fee, G. Grimsley and T. Gray, *Protein Sci.*, 1995, **4**, 2411-2423.
2. M. R. M. De Groeve, G. H. Tran, A. Van Hoorebeke, J. Stout, T. Desmet, S. N. Savvides and W. Soetaert, *Anal. Biochem.*, 2010, **401**, 162-167.
3. F. Goubet, P. Jackson, M. J. Deery and P. Dupree, *Anal. Biochem.*, 2002, **300**, 53-68.
4. R. A. Evangelista, M.-S. Liu and F.-T. A. Chen, *Analytical Chem.*, 1995, **67**, 2239-2245.
5. E. Prifti, S. Goetz, S. A. Nepogodiev and R. A. Field, *Carbohydr. Res.*, 2011, **346**, 1617-1621.
6. A. G. Leslie, *Acta Crystallogr. Sect. D*, 2006, **62**, 48-57.
7. W. Kabsch, *Acta Crystallogr. Sect. D*, 2010, **66**, 125-132.
8. P. Evans, *Acta Crystallogr. Sect. D*, 2006, **62**, 72-82.
9. N. Stein, *J. Appl. Crystallogr.*, 2008, **41**, 641-643.
10. A. J. McCoy, R. W. Grosse-Kunstleve, P. D. Adams, M. D. Winn, L. C. Storoni and R. J. Read, *J. Appl. Crystallogr.*, 2007, **40**, 658-674.
11. P. Emsley and K. Cowtan, *Acta Crystallogr. Sect. D*, 2004, **60**, 2126-2132.
12. G. N. Murshudov, A. A. Vagin and E. J. Dodson, *Acta Crystallogr. Sect. D*, 1997, **53**, 240-255.
13. I. W. Davis, A. Leaver-Fay, V. B. Chen, J. N. Block, G. J. Kapral, X. Wang, L. W. Murray, W. B. Arendall, 3rd, J. Snoeyink, J. S. Richardson and D. C. Richardson, *Nucleic Acids Res.*, 2007, **35**, W375-383.
14. L. Holm and C. Sander, *Trends. Biochem. Sci.*, 1995, **20**, 478-480.
15. B. V. Enustun and J. Turkevich, *J. Am. Chem. Soc.*, 1963, **85**, 3317-3328.

16. S. Link and M. A. El-Sayed, *J. Phys. Chem. B*, 1999, **103**, 4212-4217.
17. A. M. Brzozowski, D. M. Lawson, J. P. Turkenburg, H. Bisgaard-Frantzen, A. Svendsen, T. V. Borchert, Z. Dauter, K. S. Wilson and G. J. Davies, *Biochemistry*, 2000, **39**, 9099-9107.
18. J. Sauer, B. W. Sigurskjold, U. Christensen, T. P. Frandsen, E. Mirgorodskaya, M. Harrison, P. Roepstorff and B. Svensson, *Biochim. et Biophys. Acta - Prot. Struc. Mol. Enzymol.*, 2000, **1543**, 275-293.
19. J. P. Thiery, *J. Microsc.-Oxf.*, 1967, **6**, 987-1018.