

## Supplementary Information

### Insights into Brain Glycogen Metabolism: The Structure of Human Brain Glycogen Phosphorylase

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Running title: Structure of human brain glycogen phosphorylase

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**SUPPLEMENTARY FIGURE 1. Expression and purification of recombinant bGP.**

Native mass spectrometry analysis of bGP. Phosphorylated bGP (10  $\mu$ M) and non-phosphorylated bGP (10  $\mu$ M) were subjected to native mass spectrometry. The main signal corresponded to the dimeric form of bGP containing the PLP cofactor. The phosphorylated dimer had an additional mass of about 159 Da, corresponding to the additional mass of two phosphate groups per dimer.

**SUPPLEMENTARY FIGURE 2. Electron density of PEG 400 and AMP in the AMP binding site.**

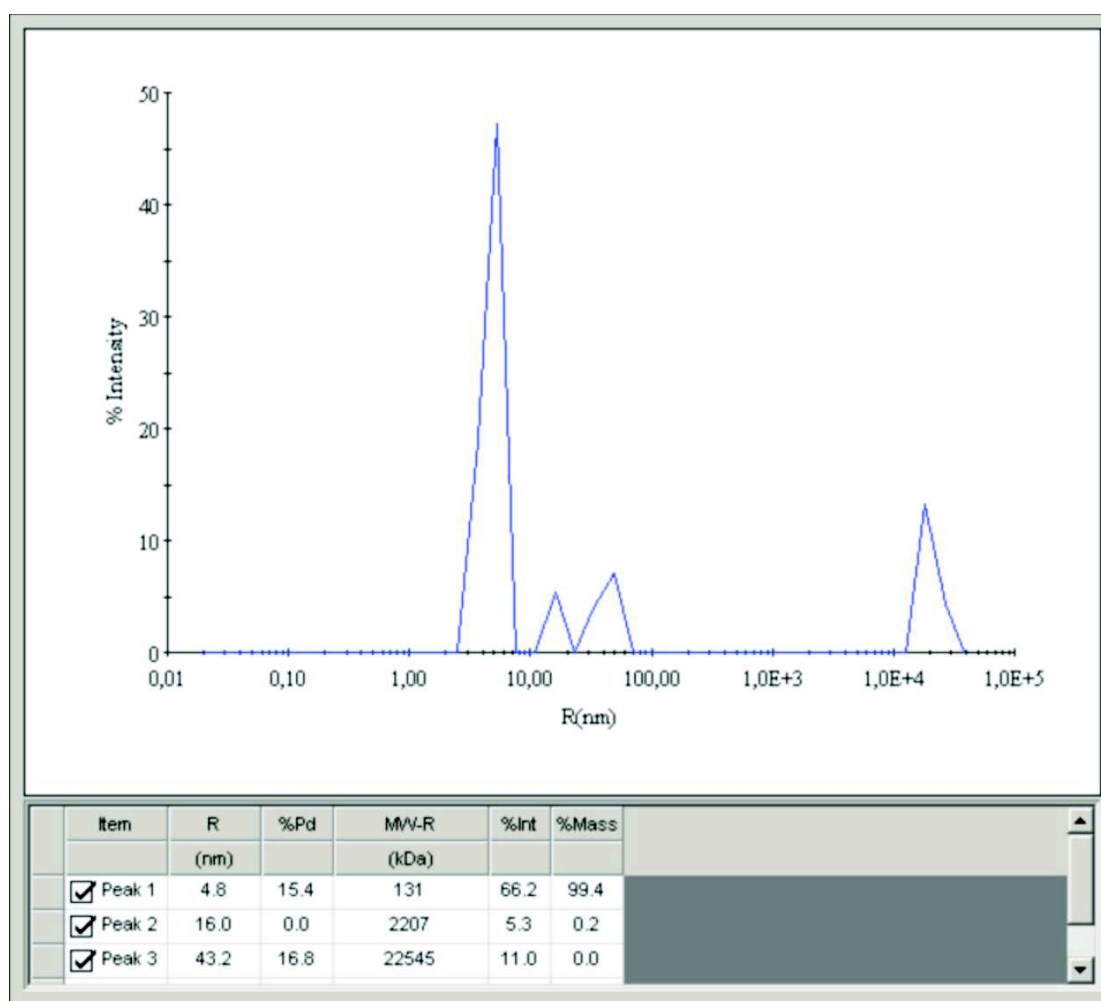
Electron density corresponding to the PEG 400 molecule (upper panel) and the allosteric effector AMP (lower panel) were observed in the AMP-binding site of bGP.

### Supplementary Table 1: Dynamic light scattering (DLS) data

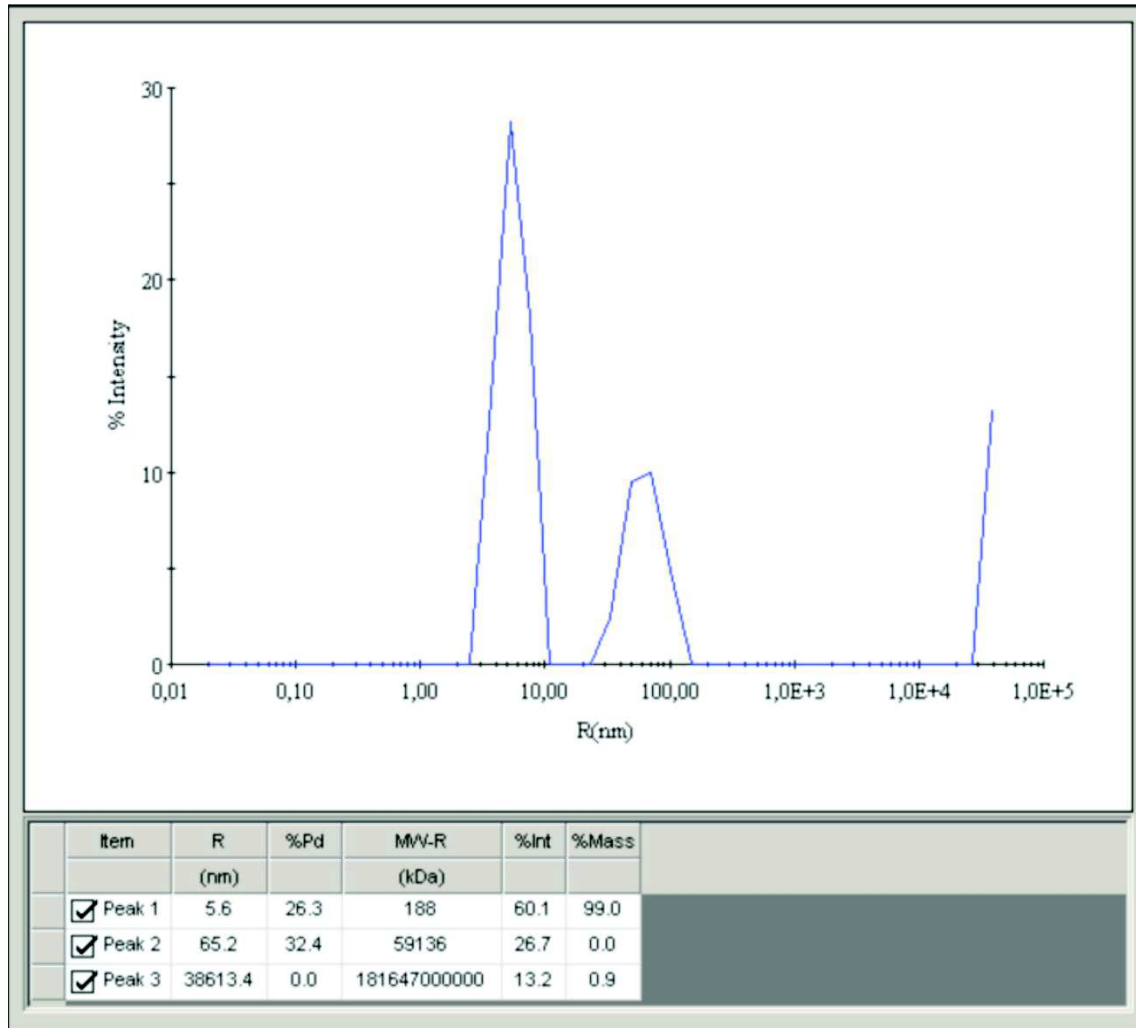
Dynamic light scattering measurements were performed with a DynaPro-MS800 molecular-sizing instrument (Protein solutions, Lakewood, NJ, USA). Protein samples at 3,2mg/ml in 20 mMTris-HCl pH7,0 in the presence or in absence of 5mM AMP were loaded into a 45µl quartz cuvette. The hydrodynamic radius and molecular mass were determined from 50 measurements at 18°C. Data were analyzed using DYNAMICS 6.9.2.11 software. The results are summarized in the table and figures bellow and show that the recombinant bGP protein in the presence or in the absence of are in equilibrium between **monomeric (98 kDa) and dimeric (196 kDa)** form in solution and the presence of AMP does not induce the tetramerization of the protein.

Protein	Hydrodynamic radius(nm)	Apparent Molecular weight estimated by DLS (kDa)	Theoretical molecular weight of monomer (kDa)
bGP	4,8	131	98
bGP + AMP	5,6	188	196

#### DSL in the absence of AMP:



*DLS in the presence of AMP*



**R:** Hydrodynamic radius; **%Pd:** % of polydispersity

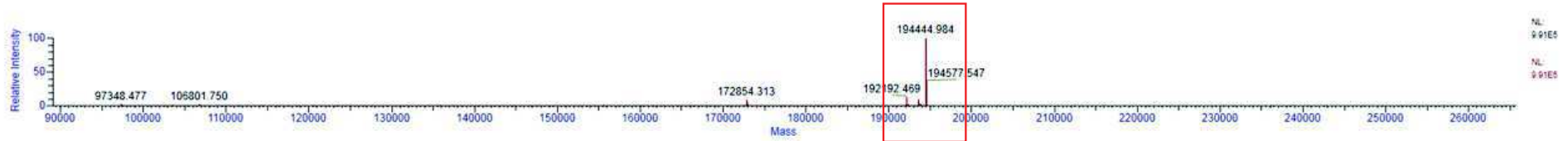
**MW-R:** apparent molecularweight

**% Int :** % of lighthscattered by thismolecularspecies

**%Mass:** is % in terms of population of thismolecularspecies

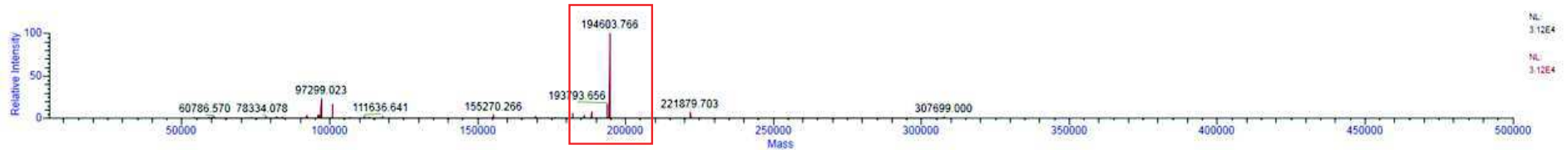
# Supplementary figure 1

## Non phosphorylated bGP



No.	Average Mass	Intensity	Number of Charge States	Mass Std Dev	PPM Std Dev	Delta Mass	Relative Abundance	Fractional Abundance	RT Range	Apex RT	Score
1	194444.984	991,124.62	9	1.29	6.63	0.00	100.0000	55.7571	1.926-2.445	0.8579	37.3887
2	172854.313	85,983.53	4	14.87	86.06	-21,590.67	8.6754	4.8371	1.926-2.445	1.206	3.3760
3	194577.547	360,338.69	7	0.88	4.53	132.56	36.3565	20.2714	1.926-2.445	1.223	26.8028
4	193620.359	93,514.44	7	1.53	7.91	-824.62	9.4352	5.2608	1.926-2.445	1.243	21.2400
5	192192.469	127,770.22	7	1.19	6.21	-2,252.52	12.8914	7.1879	1.926-2.445	4.654	21.1542
6	172974.188	23,461.55	4	15.50	89.60	-21,470.80	2.3672	1.3199	1.926-2.445	1.296	6.2634

## Phosphorylated bGP



No.	Average Mass	Intensity	Number of Charge States	Mass Std Dev	PPM Std Dev	Delta Mass	Relative Abundance	Fractional Abundance	RT Range	Apex RT	Score
1	194603.766	31,163.88	9	15.57	80.02	0.00	100.0000	42.5609	18.62-18.62	0	41.3977
2	100896.625	5,274.53	3	3.35	33.23	-93,707.14	16.9251	7.2035	18.62-18.62	16.62	15.7866
3	97299.023	7,123.86	3	5.40	55.46	-97,304.74	22.8594	9.7292	18.62-18.62	0	15.0293
4	193793.656	5,274.49	5	10.08	52.01	-810.11	16.9250	7.2034	18.62-18.62	18.31	23.9591
5	97067.898	4,106.95	4	2.94	30.32	-97,535.87	13.1786	5.6089	18.62-18.62	0	19.7956
6	307699.000	501.38	7	66.92	217.51	113,095.23	1.6089	0.6847	18.62-18.62	0	28.0075

Supplementary Figure 2

