Structure	Selenomethionyl derivative of a GID4 fragment
PDB Code	6CCR
Entry clone accession	BC041829.1
Entry clone source	MGC:43491 IMAGE:5268071
SGC clone accession	JMC130-A06
Tag	N-terminal tag: MHHHHHHSSGRENLYFQG
Construct sequence	MHHHHHHSSGRENLYFQG GVATSLLYSGSKFRGHQKSKGNSYDVEVVLQHVDTGNSYL CGYLKIKGLTEEYPTLTTFFEGEIISKKHPFLTRKWDADEDVD RKHWGKFLAFYQYAKSFNSDDFDYEELKNGDYVFMRWKE QFLVPDHTIKDISGASFAGFYYICFQKSAASIEGYYYHRSSEWYQSLNLTHVPEHSAPIYEFR
Vector	pET28-MHL
Expression host	BL21 (DE3) Codon plus RIL (Stratagene)
Growth method	GID4 was expressed in E.coli BL21 (DE3) codon plus RIL in Terrific Broth (TB) in the presence of 50 µg/mL of kanamycin. Cell were grown at 37 ºC to an OD600 of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.2 mM, and incubated overnight at 16 ºC. Cell pellets collected by centrifugation and frozen at -80 ºC. To prepare selenomethionine (SeMet) derivatives, SeMet-GID4 (aa 116-300) was overexpressed in the prepacked M9 SeMet growth media kit (Medicilon) following manufacturer's instructions.
Extraction buffers	**Lysis buffer**: **20 mM Tris-HCl pH 7.5, 400 mM NaCl, 5% glycerol and 2 mM beta-mercaptoethanol**
Extraction procedure	Frozen cell pellet was thawed and suspended in lysis buffer. The cells were lysed by sonication (Virtis408912, Virsonic) on ice: the sonication protocol was 5 sec pulse at half-maximal frequency (5.0), 7 second rest, for 10 minutes total sonication time per pellet. The lysate was centrifuged at 15000rpm for 1h.
Purification buffers	**Wash buffer**: 20 mM Tris pH 7.5, 400 mM NaCl, **5% glycerol** and 25 mM imidazole; **Elution buffer**: 20 mM Tris pH 7.5, 400 mM NaCl, **5% glycerol** and 300 mM imidazole; **Gel filtration buffer**: 20 mM Tris-HCl pH 7.5, 100 mM NaCl and 0.5 mM TCEP
Purification procedure	**The fusion proteins were purified by Ni-NTA agarose column. The His tag was cleaved by His-tagged TEV protease (purified in-house) with an approximate molar ratio of 1 : 20 in the dialysis buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl and 5 mM beta-mercaptoethanol) at 4 °C overnight. The tag and protease were removed by reloading onto the Ni-NTA. The proteins were further purified by Superdex 200 10/300 (GE Healthcare). The gel filtration buffer contains 20 mM Tris-HCl, pH 7.5, 100 mM NaCl and 0.5 mM TCEP**
Protein	**The purified protein was concentrated to 8 mg mL-1** using 15 mL concentrators with a 3,000 molecular weight cut-off (Amicon Ultra-15, UFC900524, Millipore).

stock concentration	
Crystallization	The crystals were grown at 18 °C using sitting drop method by mixing 1 microl protein and 1 microl reservoir solution (10% (*v/v*) 2-propanol, 20% (*v/v*) PEG4000 and 0.1 M Na-HEPES, pH 7.5). The SeMet-labelled proteins were obtained under similar crystallization conditions of wild type.