MS08 Serial crystallography, obtaining structures from many crystals

MS8-02

Structural insights into mechanism of glycine reuptake inhibition

A. Shahsavar [1], P. Stohler [2], G. Bourenkov [3], I. Zimmermann [4], M. Siegrist [2], W. Guba [2], E. Pinard [2], S. Sinning [5], M. Seeger [6], T. Schneider [3], R. Dawson [4], P. Nissen [5]

[1]University of Copenhagen (Denmark), [2]Roche (Switzerland), [3]EMBL Hamburg (Germany), [4]Linkster Therapeutics (Switzerland), [5]Aarhus University (Denmark), [6]University of Zurich (Switzerland)

Abstract

Glycine transporter GlyT1 is the main regulator of neuronal excitation and inhibition mediated by neurotransmitter glycine in the brain. Prolonging glycinergic signalling through selective inhibition of GlyT1 has been pursued extensively over the past two decades as a key strategy for the treatment of a broad range of neurological/psychiatric disorders including schizophrenia. GlyT1 inhibitors achieve antipsychotic and pro-cognitive effects against many symptoms of schizophrenia, however a successful drug candidate has to come. To elucidate structure-based mechanism for inhibition in GlyT1, we have investigated its complex with a benzoylpiperazine chemotype inhibitor. We have combined a variety of tools including protein engineering to introduce stabilising mutations and grafting stable protein scaffolds as fusion constructs, exploring various expression systems, as well as generating stabilising inhibition state-specific sybodies to enable structure determination. Lipidic cubic phase crystallisation yielded microcrystals (< 10 μm) of GlyT1 in complex with the small-molecule inhibitor and the sybody. Diffraction data were collected from hundreds of microcrystals using serial helical line scans as available on the EMBL P14 beamline at the PETRA III storage ring (DESY, Hamburg). Merging the oscillation patterns yielded a complete dataset at 3.4 Å resolution. The GlyT1 structure reveals the selective inhibitor-bound state, adopting an inward-open conformation. The data unveil a dual nature of non-competitive inhibitors of functional transport exhibiting also competitive binding to the substrate binding site of glycine. Catching the transporter in a clinically relevant conformation helps re-evaluate the efforts for the development of efficacious GlyT1 inhibitors and provides insight in finding new strategies to target glycine reuptake transporter.