SARS-CoV-2 main protease

M.W. 35.015 kDa

Product Code: BS178678

Introduction

COVID-19, the disease caused by the novel coronavirus SARS-CoV-2 which emerged in December 2019, is an acute health threat to all mankind and has already caused more than 800'000 deaths worldwide. Currently, no effective medication exists to treat this disease.

The main protease (M^{pro}) is a key enzyme of SARS-CoV-2 and is required for viral replication and transcription. M^{pro} activates the viral replicase by digesting replicase polyproteins at 11 conserved cleavage sites. Cleavage occurs at the C-terminal end of glutamine in recognition sequences containing Leu-Gln-(Ser, Ala, Gly) motifs [1]. As SARS-CoV-2 M^{pro} has no closely related homologues in humans, it represents an attractive drug target. In addition, SARS-CoV-2 M^{pro} may be used for diagnostic purposes and for *in vitro* research on viral replication.

SARS-CoV-2 M^{pro} (also referred to as 3C-like protease) is a 33.8 kDa protein forming a homodimer. A crystal structure of SARS-CoV-2 M^{pro} in complex with an inhibitor (PDB 6LU7) was published on the 2nd of February 2020 on protein data bank [2] (Figure 1). Others revealed crystal structures of the unliganded enzyme and of a complex with an α-ketoamide inhibitor [3].

Activity of SARS-CoV-2 M^{pro} can be measured with synthetic fluorogenic peptide substrates. Best activities were obtained with two peptide sequences containing artificial amino acids, Ac-Abu-Tle-Leu-Gln-ACC and Ac-Thz-Tle-Leu-Gln-ACC [1] (ACC: 7-amino-4-carbamoylmethylcoumarin).

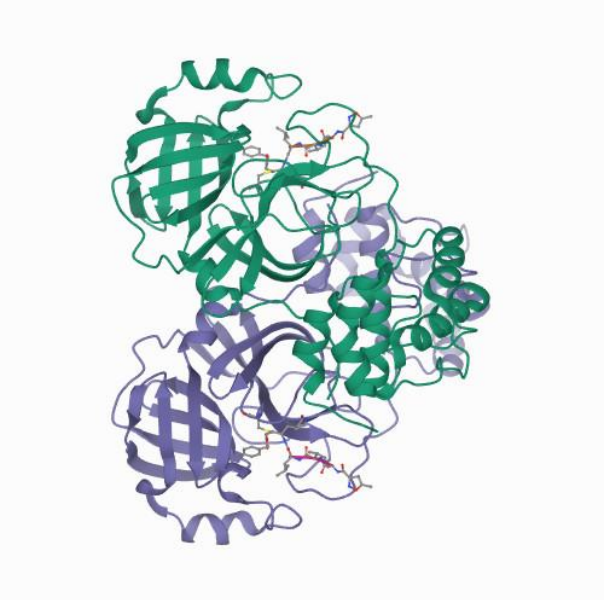

Fig. 1. SARS-CoV-2 main protease (M^{pro}) in complex with inhibitor N3 (PDB 6LU7) [2].

References:

[1] Rut, W., Drag, M. *et al.* (2020). Substrate specificity profiling of SARS-CoV-2 M^{pro} protease provides basis for anti-COVID-19 drug design. bioRxiv preprint doi: https://doi.org/10.1101/2020.03.07.981928.

[2] Jin, Z., Yang, H. *et al.* (2020). Structure of M^{pro} from SARS-CoV-2 and discovery of its inhibitors. *Nature* **582**: 289-293

[3] Zhang, L., Hilgenfeld, R. *et al.* (2020)] Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. *Science* **368**: 409-412.

Application example

SARS-CoV-2 main protease (product code BS178678) was added at the final concentration of 4.7 to 37.5 µg/mL to assay buffer at pH 7.3, containing 0.1 mM of the fluorogenic substrate Ac-Abu-Tle-Leu-Gln-AMC (product code FA178674; AMC: 7-amino-4-methyl coumarin). Assays were performed in

a black 96-well plate in 0.2 mL assay volume. Fluorescence was followed for 10 min with a SpectraMax M5 plate reader (Molecular Devices) at the lowest PMT amplification. Time course of fluorescence (relative fluorescence units, RFU) is shown in Figure 2.

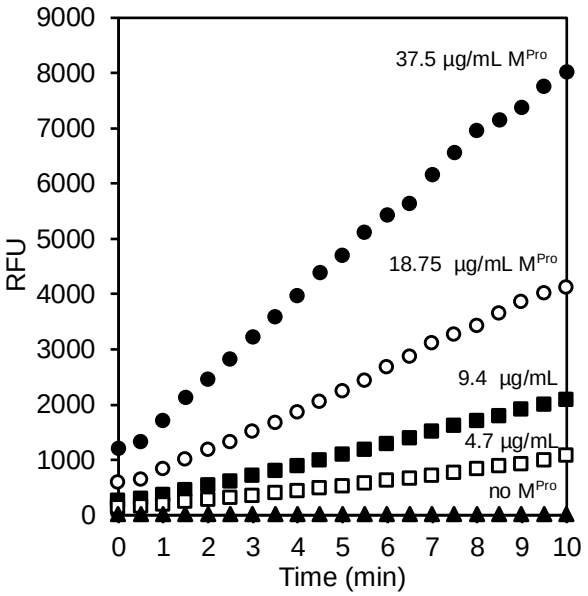

Fig. 2. Time course of fluorescence in assays with fluorogenic substrate Ac-Abu-Tle-Leu-Gln-AMC and different concentrations of SARS-CoV-2 M^{pro}.

Signal-to-background ratio after 10 min (background: RFU without enzyme) in dependency of M^{pro} concentration is shown in Figure 3. Rate of fluorescence increase and endpoint fluorescence intensity exhibited linear correlation to M^{pro} concentration in the assay.

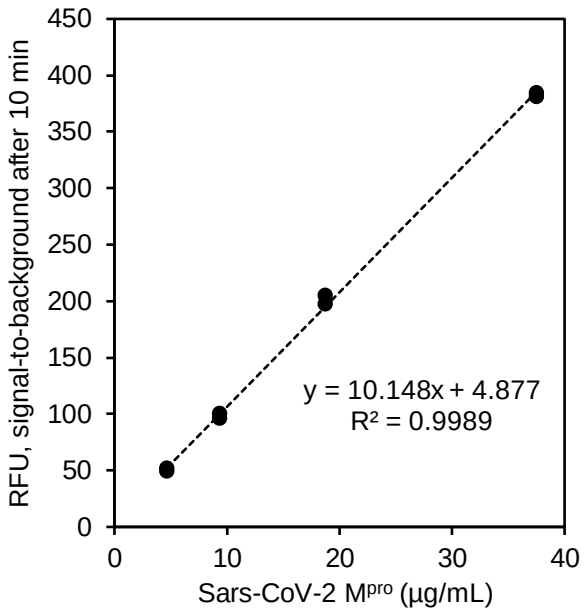

Fig. 3. Signal-to-background ratio (n=2) in dependency of SARS-CoV-2 M^{pro} concentration in assay with fluorogenic substrate Ac-Abu-Tle-Leu-Gln-AMC.

Technical information

SARS-CoV-2 main protease (product code BS178678) has been expressed as a recombinant protein in a pro-form in *Escherichia coli.* The self-cleaved active form has been purified by Ni-affinity chromatography using a C-terminal hexahistidine tag. The C-terminal tag can be removed by the commercially available PreScission protease in order to obtain a fully native protein. The protein sequence is given on page 4.

Storage: Store SARS-CoV-2 main protease (product code BS178678) at -15 °C to -25 °C. Avoid frequent freezing and thawing.

Stock solution: Reconstitute M^{pro} lyophilisate in vial by adding 1 mL 50% v/v glycerol in water. Dissolve solids completely by gentle vortexing. Store 0.1 mL aliquots of stock solution at -15 °C to -25 °C.

Assay buffer:

20 mM TRIS (product code FT15751)

1 mM EDTA (product code FE04031)

150 mM NaCl

adjust pH to 7.3 by adding drop-wise 1 N HCl

then add 1 mM DTT (product code FD02370)

Other assay buffers can also be used, it is recommended to verify the activity of SARS-CoV-2 M^{pro} in alternative buffers in pre-tests with the assay buffer described above as reference.

Enzymatic assay:

Prepare 5 mM stock solution of fluorogenic substrate Ac-Abu-Tle-Leu-Gln-AMC (product code FA178674) in dimethyl sulfoxide and add 20.5 µL per mL to assay buffer (working solution). Add 0.195 mL working solution to wells of a black 96-well plate with black bottom. Pre-warm the plate for 5 to 10 minutes at 37 °C in a plate reader. Add 5 µL SARS-CoV-2 main protease stock solution, mix with pipet and record fluorescence with excitation at 380 nm and emission at 455 nm for 10 min every 30 sec at 37 °C. For higher sensitivity, increase measurement period. M^{pro} stock solution can be pre-diluted in assay buffer if lower activity is desired. Increase in fluorescence is linear for approximately 10 minutes when final concentration of M^{pro} is 0.2 to 40 µg/mL. Rate of fluorescence increase (RLU/min) andendpoint RFU after 10 min should exhibit linear correlation to the final SARS-CoV-2 M^{pro} concentration in the assay.

For quantification of units (µmol fluorigenic substrate transformed per minute) prepare a dilution series of 7-Amino-4-methylcoumarin (AMC, product code FA00826) in assay buffer, ranging from 1.5 to 25 µM. Measure fluorescence of the AMC standards under the same conditions as in enzyme assay. An example for a standard curve is given in Figure 4. Use the slope of the linear regression line of the AMC standard curve for calculation of µM/min values from RFU/min.

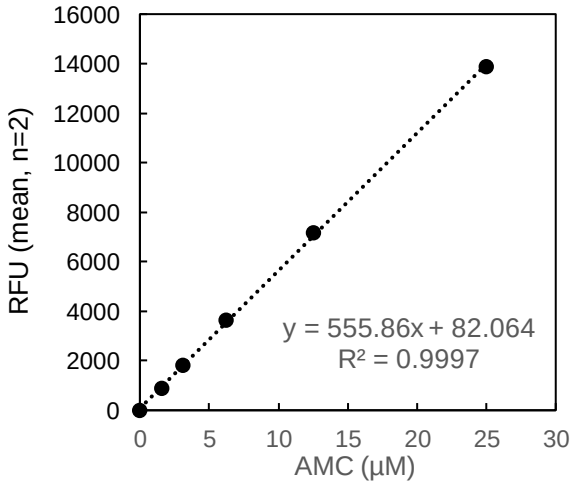

Fig. 4. Standard curve of AMC in assay buffer, measured with the SpectraMax M5 plate reader (black 96-well plate, PMT setting “low”, 0.2 mL assay volume).

Protein parameters

Number of amino acids, including 6H tag: 316

Molecular weight, including 6H tag: 35.015 kDa

Extinction coefficient, including 6H tag (Absorbance of 0.1% = 1 g/l at 280 nm, 1 cm pathlength): 0.961, assuming all pairs of Cys residues form cystines and 0.922, assuming all Cys residues are reduced

Theoretical pI, including 6H tag: 6.20

Number of amino acids without tag: 306

Molecular weight without tag: 33.797 kDa

Theoretical pI without tag: 5.95

Protein sequence of SARS-CoV-2 M^{Pro}, Product Code BS178678

The protein sequence is identical to the published SARS-CoV-2 main protease sequence (PDB 6Y2E, PDB 6LU7A), with the addition of the PreScission cleavage site and the 6xHis tag at C-terminal (boxed). For experiments that require fully native protein, the 6xHis tag can be removed with the commercially available PreScission protease.

```
        10          20          30          40          50          60
SGFRKMAFPS GKVEGCMVQV TCGTTTLNGL WLDDVVYCPR HVICTSEDML NPNYEDLLIR

        70          80          90         100         110         120
KSNHNFLVQA GNVQLRVIGH SMQNCVLKLK VDTANPKTPK YKFVRIQPGQ TFSVLACYNG

       130         140         150         160         170         180
SPSGVYQCAM RPNFTIKGSF LNGSCGSVGF NIDYDCVSFC YMHHMELPTG VHAGTDLEGN

       190         200         210         220         230         240
FYGPFVDRQT AQAAGTDTTI TVNVLAWLYA AVINGDRWFL NRFTTTLNDF NLVAMKYNYE

       250         260         270         280         290         300
PLTQDHVDIL GPLSAQTGIA VLDMCASLKE LLQNGMNGRT ILGSALLEDE FTPFDVVRQC

       310
SGVTFQGPLE HHHHHH
```