Investigating the Binding of Amyloid-beta with the Prion Protein Regulatory Domain

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Background

- It is conventional knowledge that Alzheimer's disease (AD) is caused only by the amyloid beta (Aβ) peptide, but recent studies have found that the cellular prion protein (PrP^C) upregulates the endocytosis of Aβ(1-30) into cells.
- Further investigations by nuclear magnetic resonance (NMR) confirmed interaction of these two proteins in the residue region 94-110 of PrP^C (**Fig. 1**).
  - This region is known to be a docking site for oligomeric Aβ, but monomeric Aβ(1-30) was used in the aforementioned study and in this study.[1]
- X-ray crystallography (XRC) is a biophysical technique that would allow for the modelling of this structure which can then be used to better understand the function of this complex and work to solve biological problems it may be associated with (**Fig. 2**).
  - The dissociation constant (K_D) acquired from isothermal titration calorimetry (ITC) experiments can be used to determine the optimum concentration ratio for complete binding in solution.

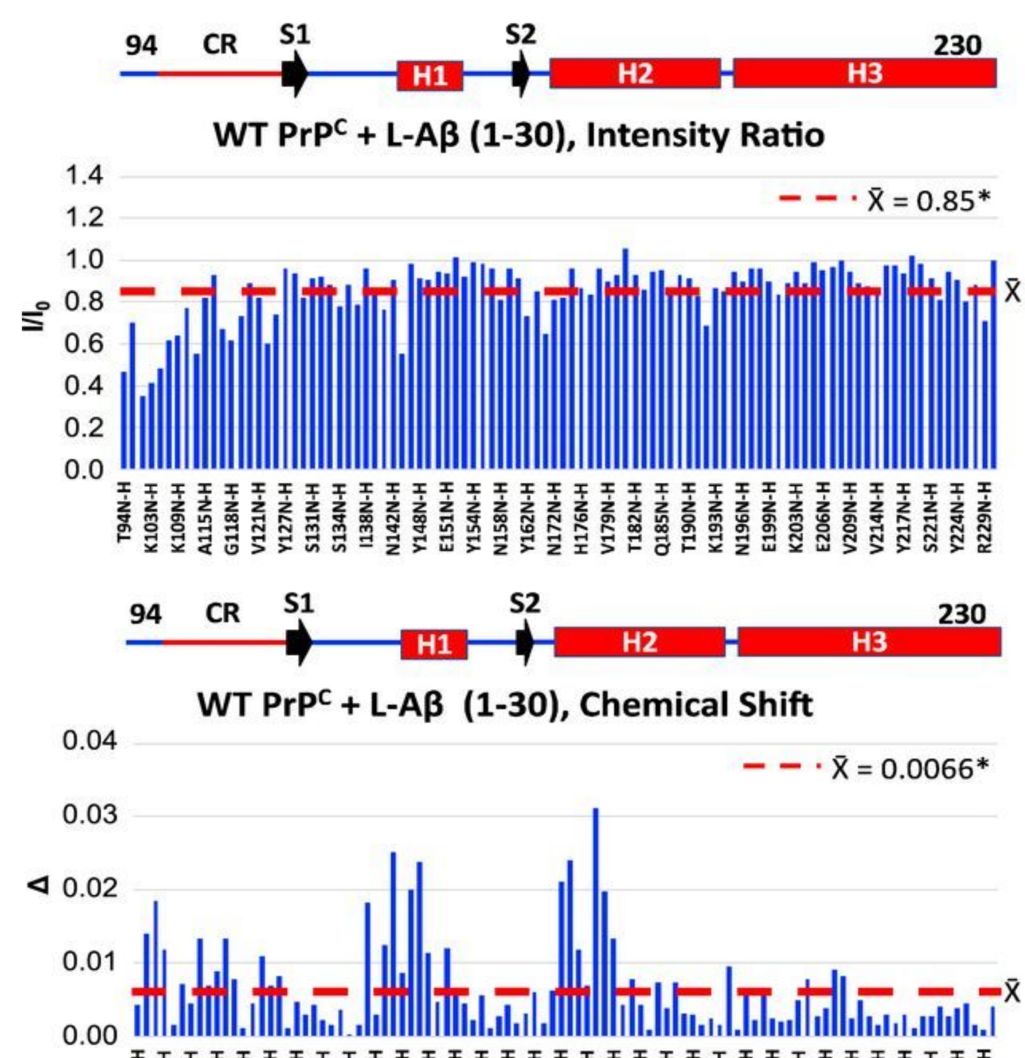

Figure 1. Effects of Aβ (1–30) on the intensity ratios (I/I_0) and chemical shift perturbations (Δ) of PrP^C resonances recorded in a ^1H-^{15}N HSQC NMR. A decrease in the I/I_0 from residue 94 to 110 was observed. Changes in Δ are also seen in this region, indicating moderate affinity for the PrP^c - Aβ (1–30) interaction. Figure originally used in Foley and Roseman et al. (2020) [ref. 1]

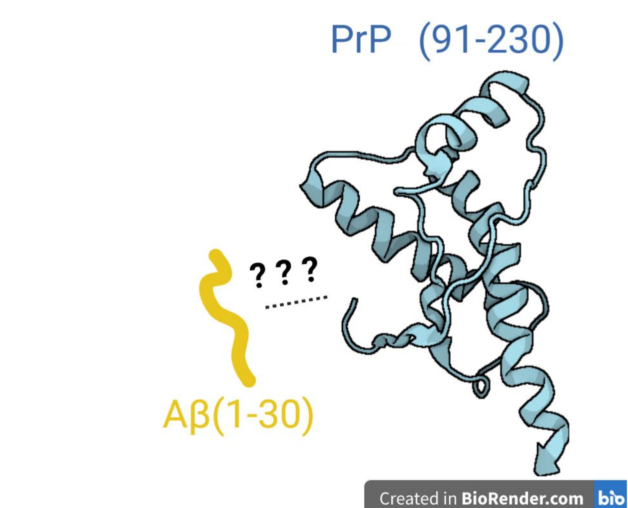

Figure 2. Schematic of the direct, yet poorly understood, interaction between cellular prion protein and amyloid beta.[2]

- The structural details of this interaction are unknown, but the novel implication that PrP^C plays a role in the development of AD motivates our desire to better understand the binary complex.
- This study aims to further characterize the interaction between PrP and Aβ(1-30) through the use of various biophysical methods including XRC and ITC.
- The stabilization of this interaction may be attributed to two salt bridges forming between lysine residues of PrP(91-230) and negatively charged residues of Aβ(1-30) (**Fig. 3**).

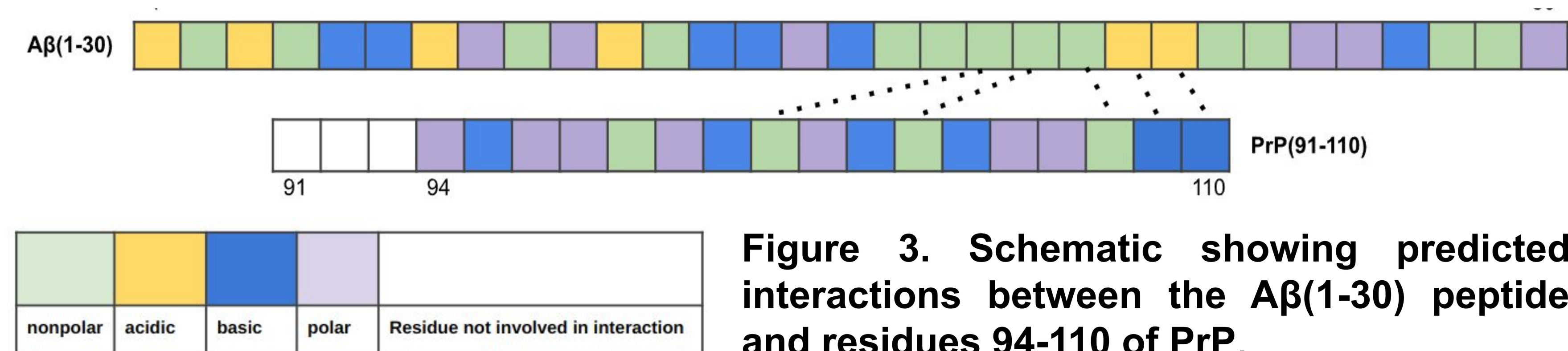

Figure 3. Schematic showing predicted interactions between the Aβ(1-30) peptide and residues 94-110 of PrP.

Methods and Results

Crystallography Methods

- Literature on the crystallization of other PrP homologs was consulted to determine probable buffer conditions for this project.
  - Prepared crystal screens included high-salt conditions at various pHs as well as commercially available screens: MCSG I and II from Anatrace and Hampton Crystal Screen HT.
  - The preparation of some crystal plates included the incubation of PrP(91-230) in $CdCl_2$ based on published crystal structures or $CuCl_2$ as PrP^c is known to coordinate with Cu(II) ions.[3]

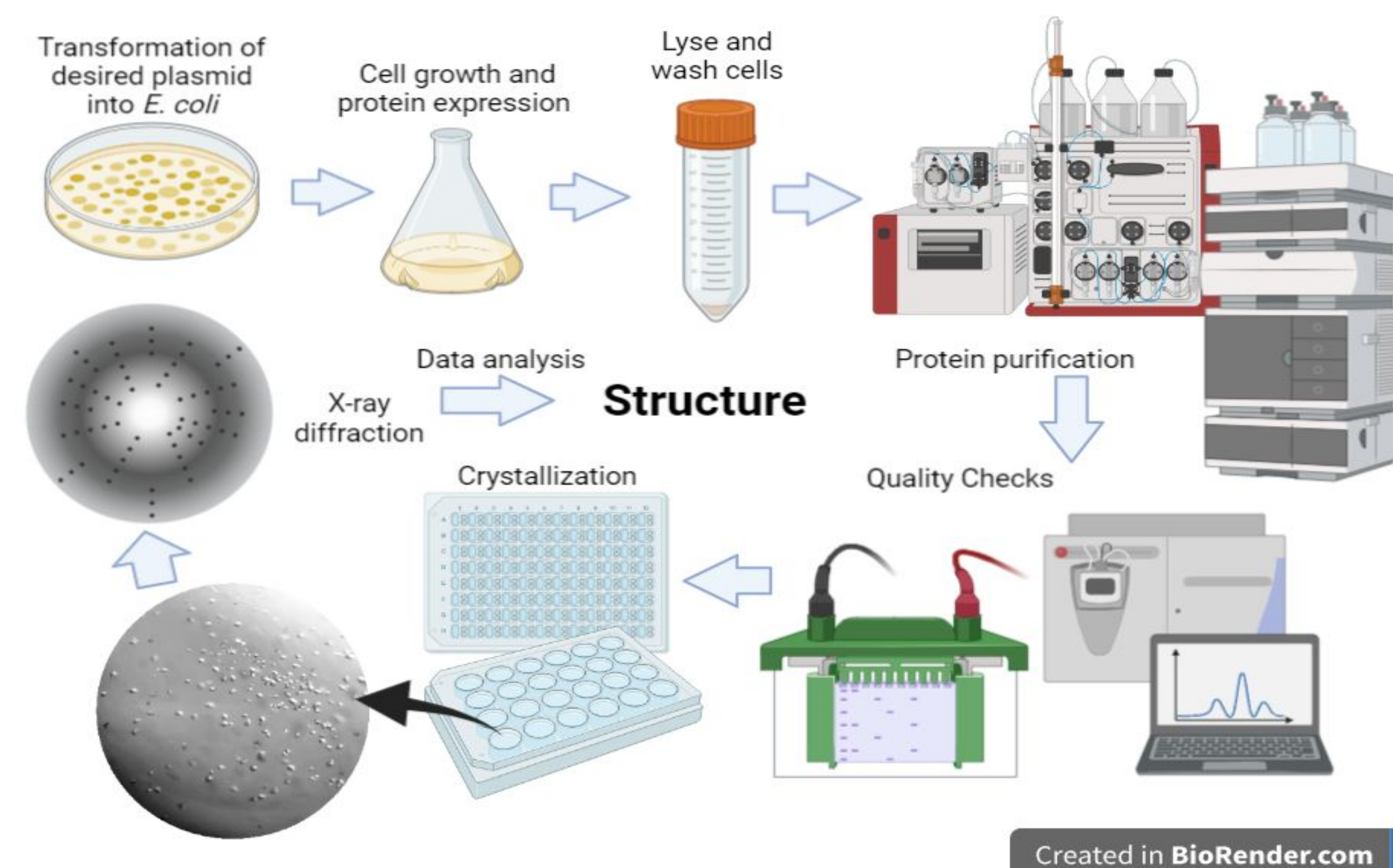

Figure 4. A schematic of the process of protein crystallography. The gene for our protein of interest (PrP^c) is transformed into *E. coli.* and then allowed to grow. Expression of the protein is then induced, but must be freed from inclusion bodies through lyse and wash procedures. This protein is then purified via FPLC and HPLC. Purity and quality of the protein is then checked by MS and gel electrophoresis. After purification the crystallization and optimization of conditions can begin. Once high-quality crystals are formed an X-ray diffraction experiment can be performed. The data from this experiment can then be processed in order to model the structure of the protein complex.

Crystallography Results

- Murine PrP(91-230) did not crystallize under any crystal screening conditions yet.
  - Some conditions yielded oil drops (**Fig. 5**).
- Crystallization did not occur with only mPrP(91-230) in the solution.

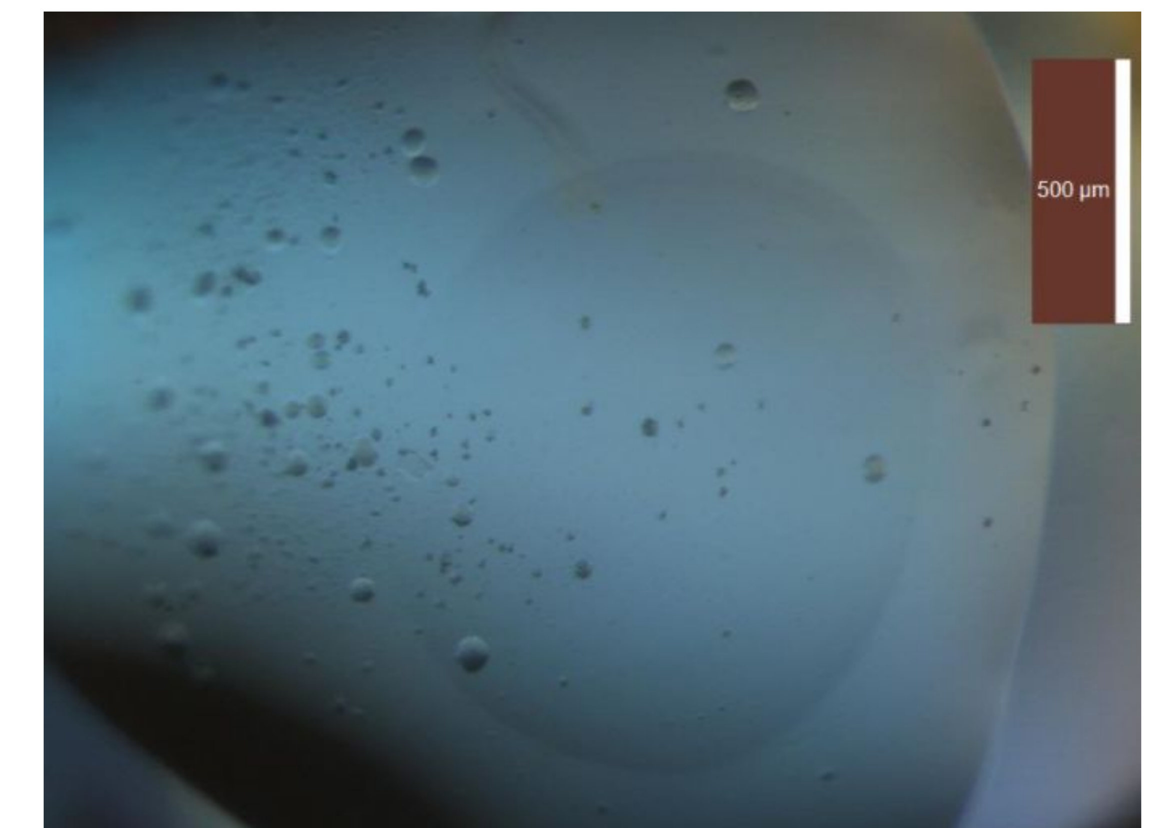

Figure 5. Oil drops of PrP(91-230) formed in a droplet from a sitting-drop tray. The crystal screen used is MCSG II.

ITC Methods and Results

- Lyophilized proteins were resuspended in MilliQ water and were not dialyzed against any buffer due to solubility issues.
- Experiments were performed at 25°C at molar ratios of 1:10 PrP to Aβ(1-30).
  - Aβ(1-30) was titrated into both PrP(91-230) and wildtype PrP.
- Titration of Aβ(1-30) into the ITC cell containing PrP was confirmed by LC/MS (data not shown).
- ITC data showed no significant binding activity between PrP(91-230) and Aβ(1-30); however, full-length PrP and Aβ(1-30) suggest approximately micromolar affinity (**Fig. 6**).

Preliminary ITC Results

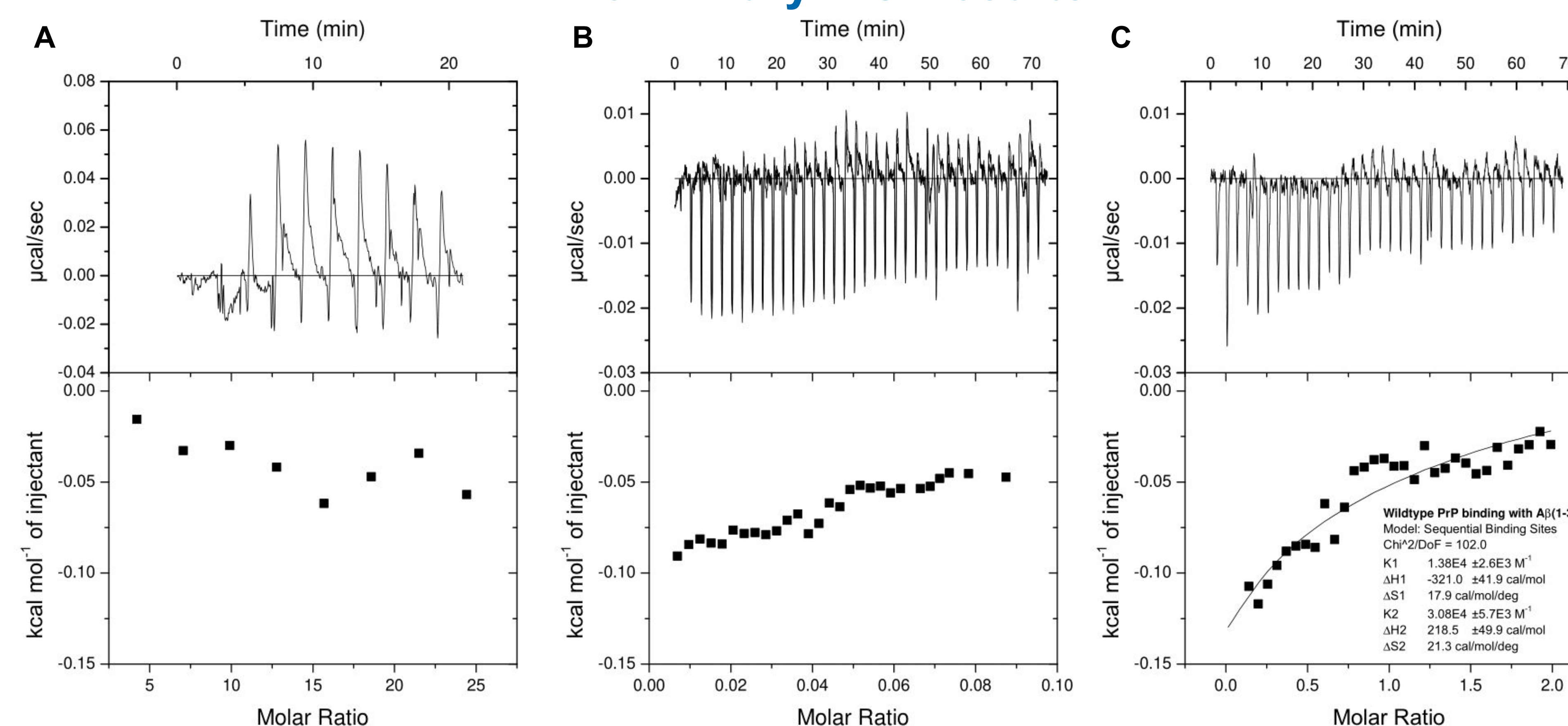

Figure 6. Heat curves (upper) and ΔH graphs (lower) for ITC controls and experiments of Aβ (1-30) with PrP constructs. (A) Control with Aβ(1-30) and MQ water. No binding is seen as there is only one peptide present in the ITC cell. **(B)** Experiment with Aβ(1-30) and PrP(91-230). No significant binding signal is seen. **(C)** Experiment with Aβ(1-30) and full-length PrP WT. Some binding activity can be seen. The ΔH data is fit to a sequential binding model with two sites. K1 and K2 are the association constants; the K_D values are the inverse of the K values reported. These calculations of K_D values are similar to those reported in the literature. Both this data and the literature suggest that Aβ(1-30) has a moderate affinity to bind to PrP (**Fig. 1**).

Future Directions

- The binding seen with full-length PrP may be a result of the presence of two binding regions: 94-110 and 23-27, which is only present in WT PrP.[4]
  - Residues 23-27 of PrP are reported to bind Aβ oligomers.[4]
- ITC results prompted us to consider the possibility of co-crystallizing Aβ(1-30) with full-length PrP instead of PrP(91-230).
- Future experiments may include an investigation of how the presence of copper ions may affect the PrP-Aβ(1-30) interaction, perhaps stabilizing the interaction through coordination.
- Future ITC results may be improved by adjusting the molar ratios of the peptides.
  - A 1:100 PrP WT:Aβ(1-30) ratio may be used if the KD was not previously reached.
  - Or a ratio lower than 1:10 may be investigated as well if PrP WT was saturated too quickly.

References

1. Foley, A. *et al. PNAS.* (2020). 117 (46) 28625-28631.
2. Zahn, R. *et al. PNAS.* (2000). 97 (1) 145-150.
3. Schilling, K. *et al. JMB.* (2020). 432 (16) 4408-4425.
4. Kong, C. *et al. Virol Sin.* (2019). 34 (5) 475–488.

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