Combining *in-situ proteolysis and microseed matrix screening to promote crystallization of PrP^{C}-nanobody complexes*

Romany N. N. Abskharon[1], Sameh Soror[1*], Els Pardon[1], Hassan El Hassan[1], Giuseppe Legname[2], Jan Steyaert[1] and Alexandre Wohlkonig[1]

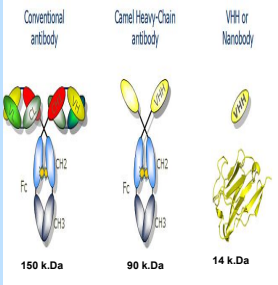
1- VIB Department Structural Biology, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussel, Belgium
2- Scuola Internazionale Superiore di Studi Avanzati - International School for Advanced Studies (SISSA-ISAS), Trieste, Italy, Institute for Neurodegenerative Diseases
*Contact author ssoror@vub.ac.be.

ABSTRACT

Prion proteins (PrPs) are difficult to crystallize, probably due to their inherent flexibility. Several PrPs structures have been solved by nuclear magnetic resonance (NMR) technique; however, only three structures were solved by X-ray crystallography. Here we combined *in-situ* proteolysis[1] with automated microseed matrix[2] screening (MMS) to crystallize two different PrP(C)/ nanobody (Nb) complexes. Nanobodies are single-domain antibodies derived from heavy-chain-only antibodies of camelids. Initial crystallization screening of mouse prion(23-230) in complex with a nanobody (Nb_PrP_01) did not produce any crystals. However, *In-situ* proteolysis gave poor diffracting thin needle-crystals. We used these microcrystals as nucleants for automated MMS. Good-quality crystals were obtained from mouse PrP(89-230)/Nb_PrP_01 which diffracted to 2.1 Å resolution using synchrotron radiation. Human PrP (90-231)/Nb_PrP_01 crystals diffracted to 1.5 Å resolution. This combined strategy benefits from the power of the MMS technique without suffering from the drawbacks of the *in-situ* proteolysis. It proved to be a successful strategy to crystallize PrP/ nanobodies complexes.

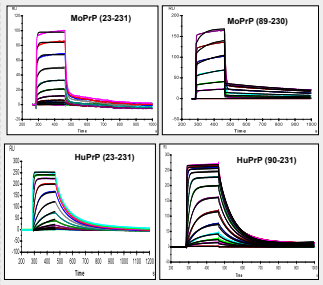
INTRODUCTION

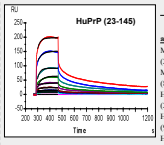
conversion of the cellular monomeric prion protein PrP^{C} into the pathogenic isoform PrP^{Sc}. The transition mechanism is still unknown, however it has been shown that PrP^{C}, exhibits an α-helical fold as the infectious isoform (PrP^{Sc}), is formed of β-sheets. Camelidae antibodies are a class of immunoglobulin that lacks the light chains. It is named heavy-chain antibody and also referred to as nanobodies (Nb). They are composed of a single domain and posses all features of conventional antibodies making them one of the smallest functional immunoglobulin. Due to the absence of light chains, they are referred to as variable domain of heavy chain antibody or VHH. Recently, nanobodies have been used to help in crystallizing difficult protein[3], making them a promising tool to crystallize prion proteins



RESULTS

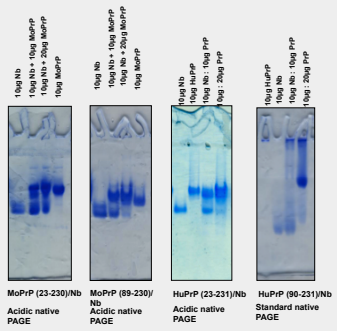
Measuring Nb_PrP_01/PrPs proteins interaction using Surface plasmon resonance (SPR)





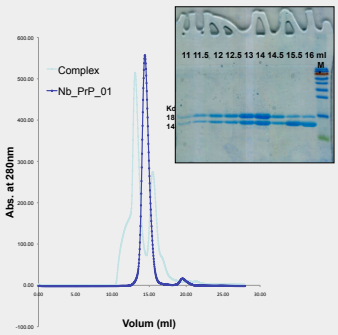
antigen	KD (M)	K_{on} (1/Ms)	K_{off} (1/s)	Immunization
MoPrP (23-231)	4,05E-08	3,18E+05	0,0129	MoPrP(23-231)
MoPrP (89-230)	5,03E-08	2,32E+04	1,17E-03	MoPrP(23-231)
HuPrP (23-231)	1,17e-8	3,93e5	4,6e-3	MoPrP(23-231)
HuPrP (90-231)	9,42E-09	1,32E+06	0,0124	MoPrP(23-231)
HuPrP (23-145)	7,72e-6	1,35e3	0,0105	MoPrP(23-231)

Detection of nanobody/prion protein complexes using native-PAGE electrophoresis



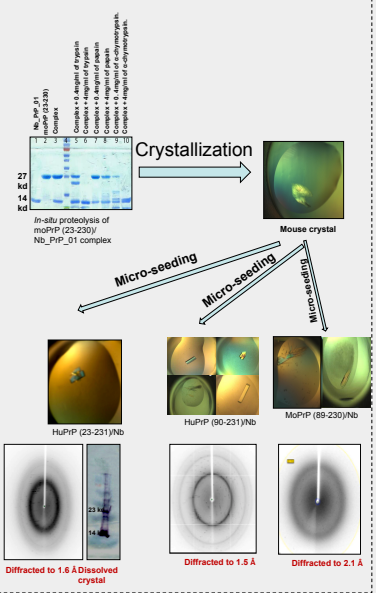
Native-PAGE is an accurate technique that allows studying and quantifying the complex formation between proteins.

Purification of Nb/HuPrP (1:1) complex by gel filtration

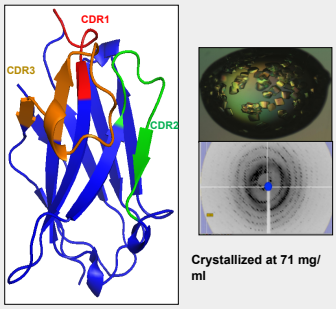


Size-exclusion chromatography of HuPrP/ Nb_PrP_01 complex on Superdex 75 HR 10/30

Crystallization PrP /Nb_PrP_01 complexes



Crystal structure of Nb_PrP_01

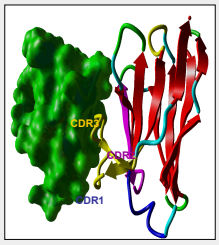


Crystallized at 71 mg/ ml

We obtained high resolution crystal structure of Nb_PrP_01 (1.2 Å)[4].

Crystal structure of HuPrP /Nb_PrP_01 complex

Resolution 1.5 Å

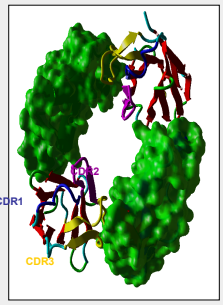


Recombinant HuPrP $^{90-231}$ was crystallized in complex with Nb_PrP_01, crystal structure shows the presence of one molecule of Nb_PrP_01 and one molecule of prion in the asymmetric unit.

High resolution crystal structure shows interaction of CDR2 and CDR3 of Nb_PrP_01 with huPrP(90-231).

Crystal structure of MoPrP / Nb_PrP_01 complex

Resolution 2.1 Å



We obtained successfully the first crystal structure of recombinant MoPrP$^{89-230}$ in a complex with Nb_PrP_01. The crystal structure shows that two molecules of Nb_PrP_01 and two molecules of prion are found in the asymmetric unit.

CONCLUSIONS

1.Our results highlight how we combined different crystallization techniques: *in-situ* limited proteolysis and MMS, to produce well-ordered crystals that diffract to high resolution.

2.Our protocol details how using limited proteolysis of a complex between two proteins can produce poor-quality crystals, which can be used as nucleants for homologous and heterogonous seeding in a high-throughput microseed matrix screening to obtain crystals of better diffraction quality.

3.Nb_PrP_01 aided in producing good quality crystals (2.1Å) of mouse PrP^{C} (89-230), which has never been crystallized before and high resolution crystal structure of human PrP^{C} (90-231) (1.5Å) [5].

REFERENCES

1. Ireton,G.C. and Stoddard,B.L. (2004) Acta. Crystallogr. D Biol. Crystallogr., 60, 601–605.
2. Wernimont,A. and Edwards,A. (2009). PLoS One, 4, e5094.
3. Rasmussen G.F.S, Choi H. J, Fung J. J, Pardon E, Casarosa P, Chae S.P, DeVree B.T, Rosenbaum M. D, Thian S. F, Kobilka S.T, Schnapp A, Konetzki I, Sunahara K. R, Gellman H. S, Pautsch A, Steyaert J, Weis I. W & Kobilka K. B. Nature 469,175–180 (2011).
4. Abskharon R.N., Soror S.H., Pardon E., El Hassan H., Legname G., Steyaert J. and Wohlkonig A. (2010). *Acta Crystallogr Sect F Struct Biol CrystCommun*. 66, 1644-1446.
5. Abskharon R.N., Soror S.H., Pardon E., El Hassan H., Legname G., Steyaert J. and Wohlkonig A. (2011). Protein Engineering, Design, and Selection, 24(9): 737-41 .