

## **SUPPLEMENTARY INFORMATION**

### **Structural insights into the unique recognition module between $\alpha$ -synuclein peptide and nanobody**

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Figure S1

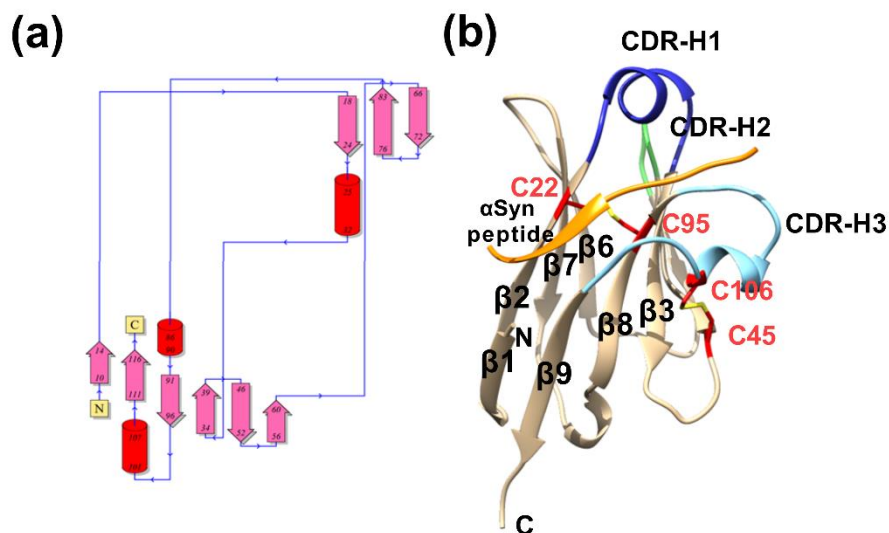
Figure S2

Figure S3

Table S1

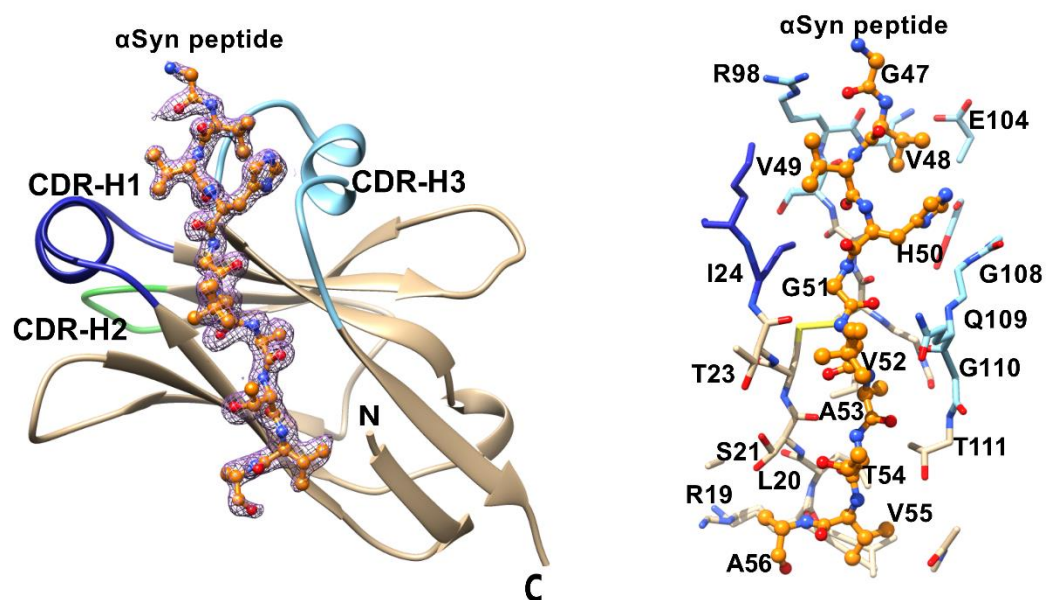
Table S2

**Figure S1.**



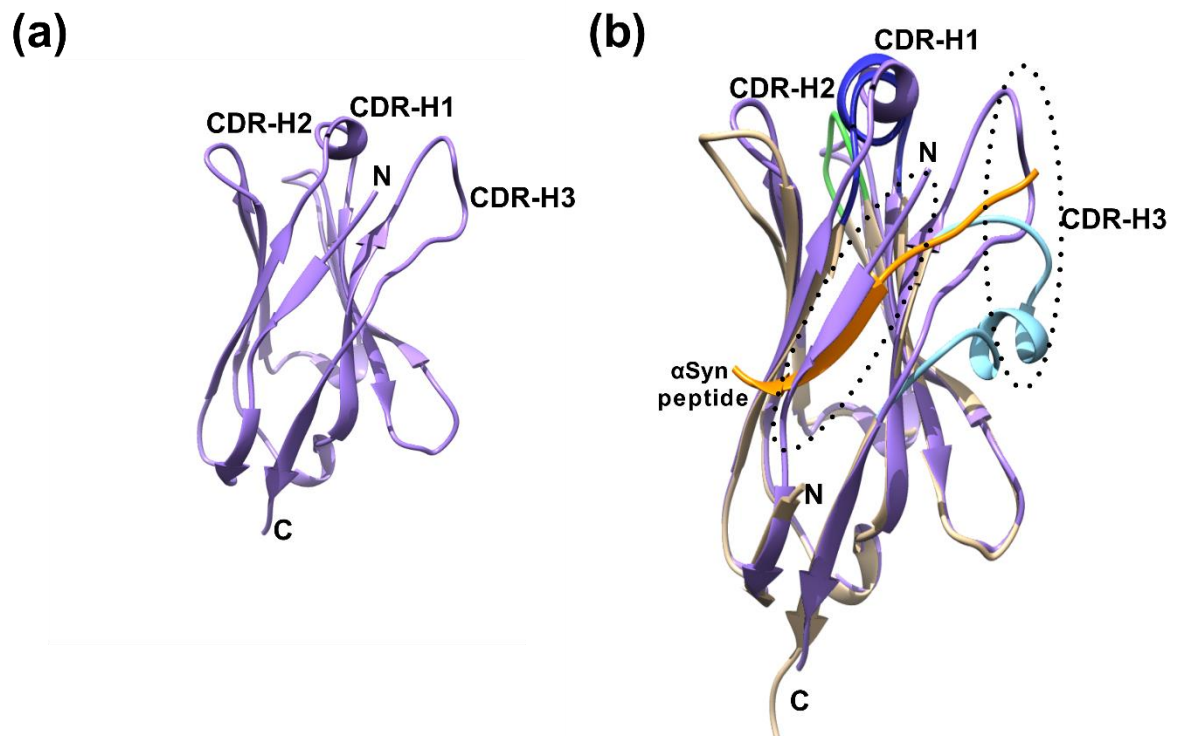
**FIGURE S1. Crystal structure of Nbα-syn01-αSyn peptide complex.** (a) Topology of a typical β-strand arrangement of the immunoglobulin fold of Nbα-syn01 indicating the arrangement of two β-sheets (b) Overall structure of Nbα-syn01-αSyn peptide complex, represented as ribbons, highlighting the 9 β-stands along with the three CDR loops (H1, H2, and H3). The two disulphide bonds (1st between C22 -- C95, 2nd C45 -- C106) are shown in yellow with residue backbone in red. The αSyn peptide is colored orange. CDR-H1, CDR-H2 and CDR-H3 are shown as blue, green and sky blue, respectively.

Figure S2.



**Figure S2: Nbα-syn01- αSyn peptide binding pocket:** 2Fo-Fc electron density map of the αSyn peptide contoured at 1.0 σ. αSyn peptide is shown as orange, Nbα-syn01 in tan. The amino acid residues involved in interaction between αSyn peptide and nanobody are shown as stick.

Figure S3.



**Figure S3: Modelled Nb $\alpha$ -syn01 and comparison to complex structure.** (a) Cartoon representation of homology modelled structure of Nb $\alpha$ -syn01 (apoform), shown as purple. (b) Superposition of structures of Nb $\alpha$ -syn01 in the apo form (purple) and in complex with  $\alpha$ Syn peptide (Nb $\alpha$ -syn01- $\alpha$ Syn complex, tan). The  $\alpha$ Syn peptide is colored orange. The conformational changes in the CDR-H3 region and N-terminal segment are highlighted as dotted circle.

List of peptides designed for epitope mapping of Nb $\alpha$ -syn01.

Peptide ID	Peptide sequence
43-56 (Native)	KTKEGVVHGVATVA
43-56_E46A	KTKE <u>A</u> GVVHGVATVA
43-56_G47A	KTKE <u>A</u> VVHGVATVA
43-56_V48A	KTKEG <u>A</u> VHGVATVA
43-56_V49A	KTKEGV <u>A</u> HGVATVA
43-56_H50A	KTKEGVV <u>A</u> GVATVA
43-56_G51A	KTKEGVVH <u>A</u> VATVA
43-56_V52A	KTKEGVVHG <u>A</u> ATVA

**Table S2**

Secondary structure compositions (%) determined by BeStSel server analysing far-UV CD spectral data.

Proteins/peptide	$\alpha$ -helices	$\beta$ -sheet	Turns	Others
Nb $\alpha$ -syn01	2.8	35.7	11.2	50.3
$\Delta$ NterNb $\alpha$ -syn01	5.0	37.9	12.2	44.9
NbS21A	2.9	35.8	14.1	47.2
$\alpha$ Syn peptide	0.0	45.6	24.3	30.1