

SUPPLEMENTARY FIGURES

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NcoI (-2 to 4)                                     NdeI (58-63)
1 M G S S H H H H H S S G L V P R G S H
1 ATGGGCAGCAGCCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGGCAGCCAT

BLA 24↓
21 M H P E T L V K V K D A E D Q L G A R V
61 ATGCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTG
41 G Y I E L D L N S G K I L E S F R P E E
121 GGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTTCGCCCGAAGAA
61 R F P M M S T F K V L L C G A V L S R V
181 CGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTT
81 D A G Q E Q L G R R I H Y S Q N D L V E
241 GACGCCGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAG
101 Y S P V T E K H L T D G M T V R E L C S
301 TACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGT
121 A A I T M S D N T A A N L L L T T I G G
361 GCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGA
141 P K E L T A F L H N M G D H V T R L D R
421 CCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGT

M180↓
161 W E P E L N E A I P N D E R D T T M P A
481 TGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGCA
181 A M A T T L R K L L T G E L L T L A S R
541 GCAATGGCAACAACGTTGCGCAAATTAAGTGGCGAACTACTTACTCTAGCTTCCCGG
201 Q Q L I D W M E A D K V A G P L L R S A
601 CAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCC
221 L P A G W F I A D K S G A G E R G S R G
661 CTTCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGT
241 I I A A L G P D G K P S R I V V I Y T T
721 ATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACG
261 G S Q A T M D E R N R Q I A E I G A S L
781 GGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTG

BLA 286↓
281 I K H W G S G G G G S G G G G S G G G G
841 ATTAAGCATTGGGGATCAggaggcggaggatcaggaggaggtggttctggtggagggtggt

Aβ42 starts↓
301 S G G G G S D A E F R H D S G Y E V H H
901 agtgggggCGGATCCgatgcagaattccgacatgactcaggatatgaagttcatcat
321 Q K L V F F A E D V G S N K G A I I G L
961 caaaaattggtgttctttgcagaagatgtgggttcaacaaaggtgcaatcattggactc

XhoI (1016-1021)
341 M V G G V V I A * * A S * K L * L E
1021 atggtgggCGGtgtgtcatagcgtaatgaGCTAGCtaaAAGCTTtaaCTCGAG
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Figure S1. DNA and protein sequences of BLA-Aβ42. The DNA sequence shown is located on the pET28b plasmid between NcoI and XhoI sites. Residue M180 is indicated with an arrow. BLA protein is 286-residue long, but residues 2-23 of the BLA protein are not included in the construct.

NcoI (-2 to 4) NdeI (58-63)
 1 M G S S H H H H H S S G L V P R G S H
 1 ATGGGCAGCAGCCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGGGCAGCCAT

BLA 24↓
 21 M H P E T L V K V K D A E D Q L G A R V
 61 ATGCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTG
 41 G Y I E L D L N S G K I L E S F R P E E
 121 GGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTTCGCCCCGAAGAA
 61 R F P M M S T F K V L L C G A V L S R V
 181 CGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGT
 81 D A G Q E Q L G R R I H Y S Q N D L V E
 241 GACGCCGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAG
 101 Y S P V T E K H L T D G M T V R E L C S
 301 TACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCGAT
 121 A A I T M S D N T A A N L L L T T I G G
 361 GCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGA
 141 P K E L T A F L H N M G D H V T R L D R
 421 CCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACCTCGCCTTGATCGT

M180↓

161 W E P E L N E A I P N D E R D T T M P A
 481 TGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGCA

BLA 195↓

181 A M A T T L R K L L T G E G S G G G G S
 541 GCAATGGCAACAACGTTGCGCAAATTAACCTGGCGAAGGATCAGGAGGCGGAGGATCA

Aβ42 starts↓

201 G G G G S G G G G S G G G G S D A E F R
 601 GGAGGAGGTGGTTCTGGTGGAGGTGGTAGTGGGGCGGCGGATCCGATGCAGAATTCCGA
 221 H D S G Y E V H H Q K L V F F A E D V G
 661 CATGACTCAGGATATGAAGTTCATCATCAAAAATTGGTGTCTTTGCAGAAGATGTGGGT
 241 S N K G A I I G L M V G G V V I A * * A
 721 TCAAACAAAGGTGCAATCATTTGACTCATGGTGGGCGGTGTGTCATAGCGTAATGAGCT

XhoI (796-801)

261 S * K L * L E
 801 AGCTAAAAGCTTTAACTCGAG

Figure S2. DNA and protein sequences of NBLA-Aβ42. The DNA sequence shown here is located on the pET28b plasmid between NcoI and XhoI sites. NBLA contains residues 24-195 of the BLA protein.

NcoI (-2 to 4) NdeI (58-63)
 1 M G S S H H H H H S S G L V P R G S H
 1 ATGGGCAGCAGCCATCATCATCACAGCAGCGGCCTGGTGCCGCGGGCAGCCAT

BLA 24↓
 21 M H P E T L V K V K D A E D Q L G A R V
 61 ATGCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTG
 41 G Y I E L D L N S G K I L E S F R P E E
 121 GGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTTCGCCCCGAAGAA
 61 R F P M M S T F K V L L C G A V L S R V
 181 CGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGT
 81 D A G Q E Q L G R R I H Y S Q N D L V E
 241 GACGCCGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAG
 101 Y S P V T E K H L T D G M T V R E L C S
 301 TACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCGAT
 121 A A I T M S D N T A A N L L L T T I G G
 361 GCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGA
 141 P K E L T A F L H N M G D H V T R L D R
 421 CCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAAGTTCGCTTGATCGT

M180↓

161 W E P E L N E A I P N D E R D T T M P A
 481 TGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGCA

BLA 195↓ NGR tripeptide

181 A M A T T L R K L L T G E N G R G S G G
 541 GCAATGGCAACAACGTTGCGCAAACTATTAAGTGGCGAAAACGGTTCGTGGATCAGGAGGC

Aβ42 starts↓

201 G G S G G G G S G G G G S G G G G S D A
 601 GGAGGATCAGGAGGAGGTGGTTCTGGTGGAGGTGGTAGTGGGGGCGCGGATCCGATGCA
 221 E F R H D S G Y E V H H Q K L V F F A E
 661 GAATTCGACATGACTCAGGATATGAAGTTCATCATCAAAAATTGGTGTCTTTGCAGAA
 241 D V G S N K G A I I G L M V G G V V I A
 721 GATGTGGGTTCAAACAAAGGTGCAATCATTGGACTCATGGTGGGCGGTGTTGTCATAGCG

XhoI (805 to 810)

261 * * A S * K L * L E
 810 TAATGAGCTAGCTAAAAGCTTTAACTCGAG

Figure S3. DNA and protein sequences of NBLA-Aβ42 containing the NGR tripeptide insertion.
 The DNA sequence shown here is located on the pET28b plasmid between NcoI and XhoI sites. NBLA contains residues 24-195 of the BLA protein. NGR tripeptide is inserted after residue 196 of the BLA protein.

NcoI (-2 to 4)
 BLA 196↓
 1 M G G L L T L A S R Q Q L I D W M E A D
 1 ATGGGCGGACTACTTACTCTAGCTTCCC GGCAACAATTAATAGACTGGATGGAGGCGGAT

21 K V A G P L L R S A L P A G W F I A D K
 61 AAAGTTGCAGGACCACTTCTGCGCTCGGCCCTCCGGCTGGCTGGTTTATTGCTGATAAA

41 S G A G E R G S R G I I A A L G P D G K
 121 TCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAG

61 P S R I V V I Y T T G S Q A T M D E R N
 181 CCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAAT

BLA 286↓

81 R Q I A E I G A S L I K H W G S G G G G
 241 AGACAGATCGCTGAGATAGGTGCCCTCACTGATTAAGCATTGGGGATCAGGAGCGGAGGA

Aβ42 starts↓

101 S G G G G S G G G G S G G G G S D A E F
 301 TCAGGAGGAGGTGGTTCTGGTGGAGGTGGTAGTGGGGGCGGCGGATCCGATGCAGAATTC

121 R H D S G Y E V H H Q K L V F F A E D V
 361 CGACATGACTCAGGATATGAAGTTCATCATCAAAAATTGGTGTCTTTGCAGAAGATGTG

141 G S N K G A I I G L M V G G V V I A * *
 421 GGTTCAAACAAAGGTGCAATCATTGGACTCATGGTGGGCGGTGTTGTCATAGCGTAATGA

HindIII (490 to 495)
 161 A S * K L
 495 GCTAGCTAAAAGCTT

Figure S4. DNA and protein sequences of CBLA-Aβ42. The DNA sequence shown here is located on the pCDFDuet-1 plasmid between NcoI and HindIII sites. CBLA contains residues 196-286 of the BLA protein.

NcoI (-2 to 4) NdeI (58-63)
 1 M G S S H H H H H S S G L V P R G S H
 1 ATGGGCAGCAGCCATCATCATCATCACAGCAGCGGCCCTGGTGCCGCGGGCAGCCAT

BLA 24↓
 21 M H P E T L V K V K D A E D Q L G A R V
 61 ATGCACCCAGAAAACGCTGGTGAAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTG
 41 G Y I E L D L N S G K I L E S F R P E E
 121 GGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTTCGCCCCGAAGAA
 61 R F P M M S T F K V L L C G A V L S R V
 181 CGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGT
 81 D A G Q E Q L G R R I H Y S Q N D L V E
 241 GACGCCGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGTTGAG
 101 Y S P V T E K H L T D G M T V R E L C S
 301 TACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGT
 121 A A I T M S D N T A A N L L L T T I G G
 361 GCTGCCATAACCATGAGTGATAAACTGCGGCCAACTTACTTCTGACAACGATCGGAGGA
 141 P K E L T A F L H N M G D H V T R L D R
 421 CCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGT

M180T↓

161 W E P E L N E A I P N D E R D T T T P A
 481 TGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGACGCCTGCA

BLA 195↓

181 A M A T T L R K L L T G E G S G G G G S
 541 GCAATGGCAACAACGTTGCGCAAATATTAAGTGGCGAAGGATCAGGAGGCGGAGGATCA

BamHI (640-645)

201 G G G G S G G G G S G G G G S D A E H H
 601 GGAGGAGGTGGTTCTGGTGGAGGTGGTAGTGGGGCGGCGGATCCGATGCAGAGCACCAC

221 H H H H *
 661 CACCACCACCTGA

Figure S5. DNA and protein sequences of NBLA-M180T. The DNA sequence shown here is located on the pET28b plasmid.

NcoI (-2 to 4)
 BLA 196↓
 1 M G G L L T L A S R Q Q L I D W M E A D
 1 ATGGGCGGACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGAT

21 K V A G P L L R S A L P A G W F I A D K
 61 AAAGTTGCAGGACCACTTCTGCGCTCGGCCCTCCGGCTGGCTGGTTTATTGCTGATAAA

41 S G A G E R G S R G I I A A L G P D G K
 121 TCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAG

61 P S R I V V I Y T T G S Q A T M D E R N
 181 CCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAAT

BLA 286↓
 81 R Q I A E I G A S L I K H W G S G G G G
 241 AGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGGGATCAGGAGGCGGAGGA

BamHI (343-348)
 101 S G G G G S G G G G S G G G G S E F E L
 301 TCAGGAGGAGGTGGTTCTGGTGGAGGTGGTAGTGGGGGCGGCGGATCCGAATTCGAGCTC

121 G A P A G R Q A C G R I M L K S N R K *
 361 GCGCGCCTGCAGGTCGACAAGCTTGCGGCCGCATAATGCTTAAGTCGAACAGAAAGTAA

Figure S6. DNA and protein sequences of CBLA. The DNA sequence shown here is located on the pCDFDuet-1 plasmid.

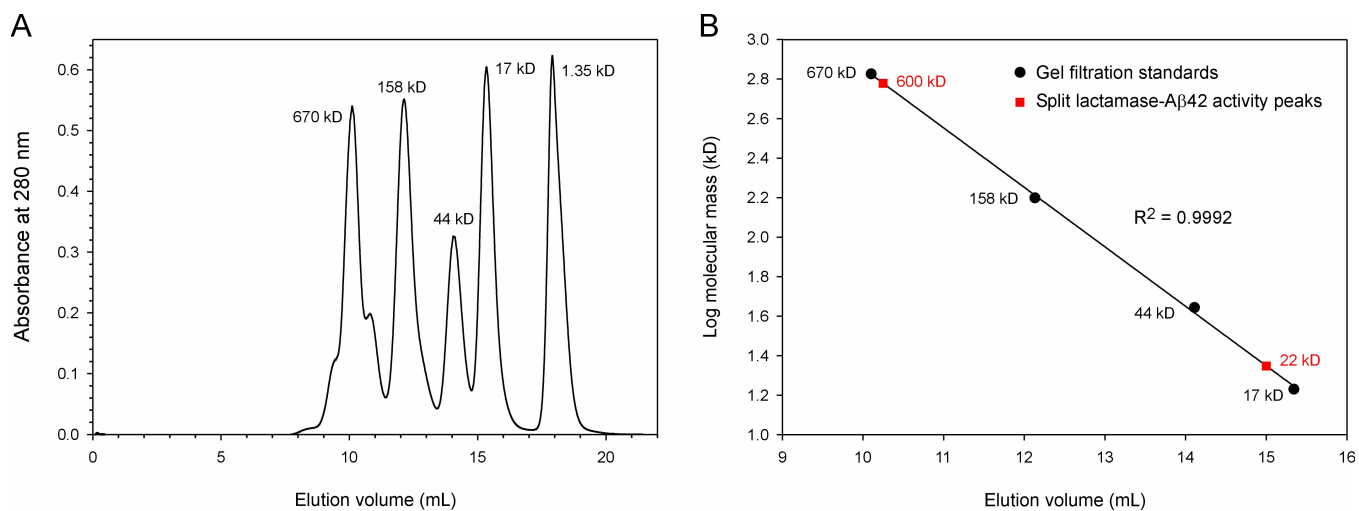


Figure S7. Calibration of the ENrich SEC 650 size exclusion column. (A) Gel filtration standard containing thyroglobulin (670 kD), γ -globulin (158 kD), ovalbumin (44 kD), myoglobin (17 kD), and vitamin B12 (1.35 kD) was run at 1 mL/min in PBS buffer (50 mM phosphate, 140 mM NaCl, pH 7.4). (B) Standard curve using a linear fit to the log molecular mass of the gel filtration standard (excluding vitamin B12) versus elution volume. The molecular mass of the two activity peaks for the split lactamase-A β 42 cell lysate was estimated to be 600 and 22 kD.