

Supporting Materials

Sortase-Mediated Ligation as a Modular Approach for the Covalent Attachment of Proteins to the Exterior of the Bacteriophage P22 Virus-like Particle.

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DNA sequences

CP-LPETG

ATGGCTTTGAACGAAGGTCAAATTGTTACTGCGGTAGATGAAATCATCGAAACCATCTCCGCAATC
ACTCCAATGGCGCAGAAAGCCAAGAAATACACCCGCCTGCTGCTTCTATGCAGCGCTCCAGCAATACC
ATCTGGATGCCTGTAGAGCAAGAGTCACCCACTCAGGAGGGCTGGGATTTAACTGATAAAGCGACAGG
GTTACTGGAACCTAACGTCGCGGTAAACATGGGAGAGCCGATAACGACTTCTCCAGTTGCGTGCTGA
TGACTTGGGAGACGAAACTGCGTATCGTCGCCGCATCCAGTCTGCCGCTCGCAAGCTGGCGAACAACGT
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CACTAATACCGCAGACGCCTGGAACCTTGTGGCCGACGCAGAAGAAATCATGTTCTCCCGGAACTTAA
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CTTCGATGATGTCNTGCGCTCTCCGAACTTCTGTGCTGACCAAATCCACCGCAACTGGCATCACTGTA
TCCGGTGCGCAGTCCTTCAAGCCTGTCGCATGGCAACTGGATAACGATGGCAACAAAGTTAACGTTGAT
AACCGTTTTGCTACCGTCACCCTGTCTGCAACTACCGGCATGAAACGCGGCGACAAAATTCGTTTGCTG
GCGTTAAGTTCCTTGGTCAGATGGCTAAGAACGTACTGGCTCAGGATGCGACTTTCTCCGTAGTCCGCGT
TGTTGACGGTACTCATGTTGAAATCACGCCGAAGCCGGTAGCGCTGGATGATGTTTCCCTGTCTCCGGA
GCAGCGTGCCTACGCCAACGTTAACACCTCGCTGGCTGATGCAATGGCAGTGAACATTCTGAACGTTAA
AGACGCTCGCACTAATGTGTTCTGGGCTGACGATGCTATTCGTATCGTGTCTCAGCCGATTCGGGCTAAC
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TACGCAGGGTGATATTTCCACCCTGTCCGGCCTGTGCCGTATTGCGCTGTGGTACGGCGTAAACGCGAC
ACGACCGGAGGCAATCGGTGTTGGCCTGCCTGGTCAGACTGCGAGATCTGCAGGCGGCGGAGGTGCCG
GAGGAGGTACCCTGCCGAAACCGGCGGTAGCTAACTCGAG

*underlined is the flexible peptide spacer used and in yellow highlight is the sortase recognition sequence.

Polyglycine-GFP

ATGGGCGGAGGAGGCAAGGGGGTGAAGGAAGTAATGAAGATCAGTCTGGAGATGGACTGCACTGTTA
ACGGCGACAAATTTAAGATCATTGGGGATGGAACAGGAGAACCTTACGAAGGAACACAGACTTTACAT
CTTACAGAGAAGGAAGGCAAGCCTCTGACGTTTTCTTCGATGTATTGACACCAGCATTTTCAGTATGGAA
ACCGTACATTCACCAATACCCAGGCAATATAACGACTTTTTCAAGCAGACCGTTTTCTGGTGGCGGGTA

TACCTGGGAGCGAAAAATGACTTATGAAGACGGGGGCATAAGTAACGTCCGAAGCGACATCAGTGTGA
AAGGTGACTCTTTCTACTATAAGATTCACTTCACTGGCGAGTTTCCTCCTCATGGTCCAGTGATGCAGAG
GAAGACAGTAAAATGGGAGCCATCCACTGAAGTAATGTATGTTGACGACAAGAGTGACGGTGTGCTGA
AGGGAGATGTCAACATGGCTCTGTTGCTTAAAGATGGCCGCCATTTGAGAGTTGACTTTAACACTTCTTA
CATACCCAAGAAGAAGGTCGAGAATATGCCTGACTACCATTTTATAGACCACCGCATTGAGATTCTGGG
CAACCCAGAAGACAAGCCGGTCAAGCTGTACGAGTGTGCTGTAGCTCGCTATTCTCTGCTGCTGAGAA
GAACAAGGGATCCGGCGGACTGGTGCCGCGCGGCAGCGGACATCACCATCATCACCCTAA

MBP-HAhead (pRK793)

ATGAAAATCGAAGAAGGTAAACTGGTAATCTGGATTAACGGCGATAAAGGCTATAACGGTCTCGCTGA
AGTCGGTAAGAAATTCGAGAAAGATACCGGAATTAAGTCACCGTTGAGCATCCGGATAAACTGGAAG
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GTGGCTACGCTCAATCTGGCCTGTTGGCTGAAATCACCCCGACAAAGCGTTCAGGACAAGCTGTATC
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GCTGATTTATAACAAAGATCTGCTGCCGAACCCGCCAAAAACCTGGGAAGAGATCCCGGCGCTGGATAA
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CAGACACCGATTACTCCATCGCAGAAGCTGCCTTAATAAAGGCGAAACAGCGATGACCATCAACGGCC
CGTGGGCATGGTCCAACATCGACACCAGCAAAGTGAATTATGGTGTAAACGGTACTGCCGACCTTCAAGG
GTCAACCATCCAAACCGTTTCGTTGGCGTGCTGAGCGCAGGTATTAACGCCGCCAGTCCGAACAAAGAGC
TGGCAAAGAGTTCCTCGAAAACCTATCTGCTGACTGATGAAGGTCTGGAAGCGGTTAATAAAGACAAAC
CGCTGGGTGCCGTAGCGCTGAAGTCTTACGAGGAAGAGTTGGCGAAAGATCCACGTATTGCCGCCACC
ATGGAAAACGCCAGAAAGGTGAAATCATGCCGAACATCCCGCAGATGTCCGCTTTCTGGTATGCCGTG
CGTACTGCGGTGATCAACGCCGCCAGCGGTCGTCAGACTGTCGATGAAGCCCTGAAAGACGCGCAGAC
TAATTCGAGCTCGgaaaatctgtactttcaaggtGGCGGCGGTGCAAGCAAGGGCATCGCACCGCTGCAGCTG
GGTAAATGCAATATTGCTGGTTGGCTGCTGGGCAACCCGGAATGTGATCCGCTGCTGCCGGTCCGCAGC
TGGTCTTATATTGTGAAACCCCGAATAGCGAAAACGGTATCTGCTATCCGGGCGATTTCAATTGACTACG
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CGTGGCCGAACCATAATAACCAACGGCGTTACGGCGGCCTGTAGCCACGAAGGTAAAAGCAGCTTTTATC
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TATCAGAATGAAAACGCGTACGTTAGTGTGGTTACCTCCAATTATAACCGTCGCTTCACGCCGAAATTG
CGGAACGTCCGAAGGTCCGCGATCAAGCCGGTTCGTATGAACTATTACTGGACCCTGCTGAAACCGGGC
GACACGATTATCTTTGAAGCGAATGGTAACTGATCGCCCCGATGTACGCGTTCCGCTGTACGCGGGT
TTGGTTCGGGCATTATCACCAGTGGATCCGGCGGACTGGTGCCGCGCGGCAGCGGACATCACCATCATC
ACCACTAA

*lowercase lettering indicates the sequence encoding the TEV protease cleavage site

Protein Sequences

CP-LPETG

MALNEGQIVTLAVDEIIETISAITPMAQKAKKYTPPAASMQRSSNTIWMMPVEQESPTQEGWDLTDKATGLLE
LNVAVNMGEPDNDFFQLRADDLRDETAYRRRIQSAARKLANNVELKVANMAAEMGSLVITSPDAIGTNTA
DAWNFVADAEIIMFSRELNDRDMGTSYFFNPQDYKKAGYDLTKRDIFGRIPEEAYRDGTIQRQVAGFDDVXR
SPKLPVLTKSTATGITVSGAQSFKPVAVQLDNDGKNVVDNRFATVTLSTATTGMKRGDKISFAGVKFLGQM
AKNVLAQDATFSVVRVVDGTHVEITPKPVALDDVLSPEQRAYANVNTSLADAMAVNILNVKDARTNVFW
ADDAIRIVSQPIPANHELFAAGMKTTTSFSIPDVGLNGIFATQGDISTLSGLCRIALWYGVNATRPEAIGVGLPGQ
TARSAGGGGAGGGTLPETGGS

*underlined is the flexible peptide spacer used and in yellow highlight is the sortase recognition sequence.

Poly-glycine-GFP-6xHis

GGGGKGVKEVMKISLEMDCTVNGDKFKIIGDGTGEPYEGTQTLHLTEKEGKPLTFSFDVLTAPAFQYGNRTFTK
YPGNIPDFFKQTVSGGGYTWERKMTYEDGGISNVRSDISVKGDSFYKIHFTGEFPPHGPVMQRKTVKWEK
STEVMYVDDKSDGVLKGDVNMALLLKDGRHLRVDFNTSYIPKKKVENMPDYHFIDHRIEILGNPEDKPKVLY
ECAVARYSLLPEKNKGSGGLVPRGSGHHHHHH

MBP-Poly-glycine-HA-6xHis pRK793

MKIEEGKLVWINGDKGYNGLAEVGGKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAHDHDFGGYA
QSGLLAEITPDKAFQDKLYPFTWDVAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDKELKAKGKSA
LMFNLQEPYFTWPLIAADGGYAFKYENGYDIKDVGVNAGAKAGLTLVDLIKNKHMNADTDYSIAEAAF
NKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEG
LEAVNKDKPLGAVALKSYYEELAKDPRIAATMENAQKGEIMPNIQMSAFWYAVRTAVINAASGRQTVDEA
LKDAQTNSSSENLYFQGGGASKGIAPLQLGKCNIAGWLLGNPECDPLLPVRSWSYIVETPNSENGICYPGDF
IDYEELREQLSSVSSFERFEIFPKESSWPNHNTNGVTAACSHGKSSFYRNLLWLTEKEGSYPKLNKSYVNKKG
KEVLVLWGIHPPNSKEQQNLYQENAYVSVVTSNYNRRFTPEIAERPQVRDQAGRMNYYWTLLKPGDTIIF
EANGNLIAPMYAFALSRGFGSGGIITSGSGGLVPRGSGHHHHHH

*underlined portion is the recognition sequence for TEV protease cleavage after residue Q

Cleaved PolyG-HAhead

GGGGASKGIAPLQLGKCNIAGWLLGNPECDPLLPVRSWSYIVETPNSENGICYPGDFIDYEELREQLSSVSSFE
RFEIFPKESSWPNHNTGVTAACSHGKSSFYRNLLWLTEKEGSYPKLNKSYVNKKGKEVLVLWGIHPPNSKE
QQNLYQENAYVSVVTSNYNRRFTP EIAERPQVRD QAGRMNYYWT LLKPGDTIIF EANGNLIAPM
YAFALSRGFGSGGIITSGSGG LVPRGSGHHH HHH

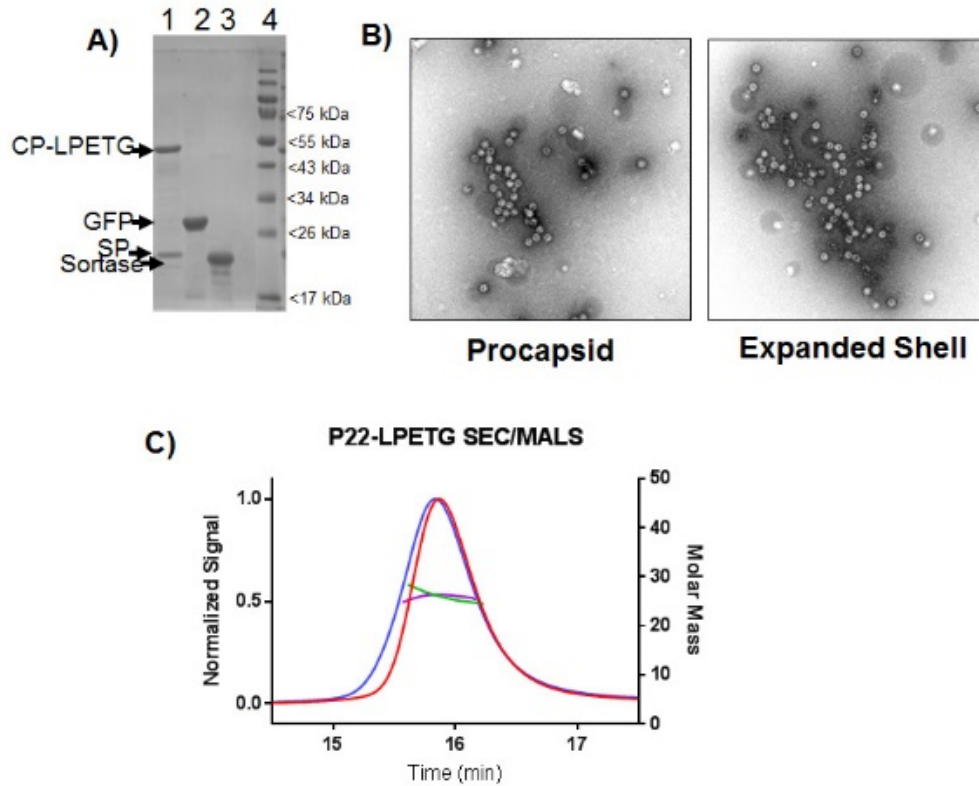


Figure S1. Characterization of purified P22-LPETG VLPs, polyG-GFP, and Sortase. A) SDS-PAGE gel of purified P22-LPETG, polyG-GFP, and Sortase. B) Transmission electron microscopy images of the procapsid and expanded shell P22-LPETG VLPs. C) Size exclusion chromatography elution profiles and multiangle light scattering analysis of PC P22-LPETG (red and green) and EX P22-LPETG (blue and purple).

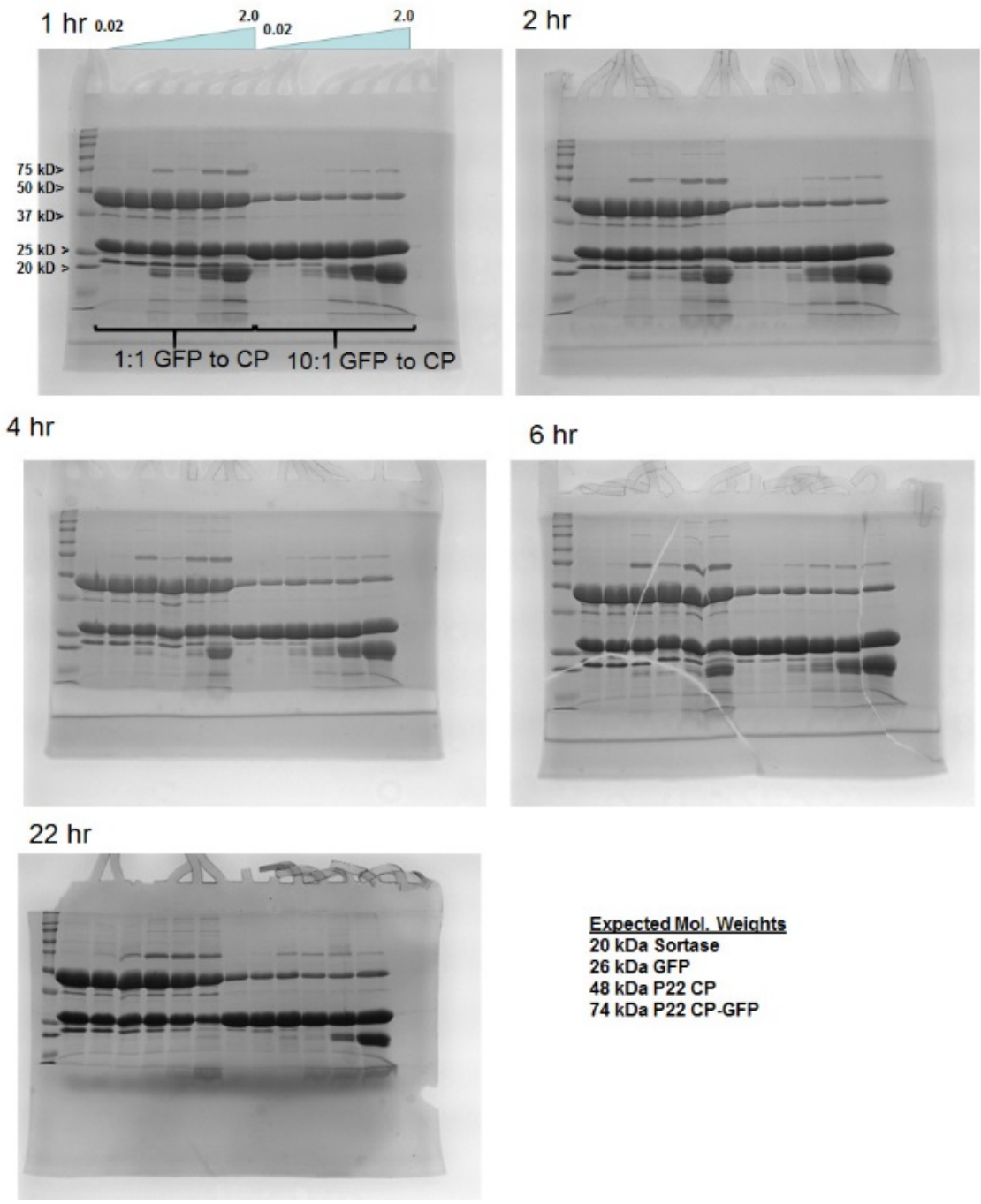


Figure S2. Sortase mediated ligation of polyG-GFP to PC P22-LPETG. SDS-PAGE gels of samples taken at different time points evaluating ligation reactions conditions of different ratios of Sortase to reactants and polyG-GFP to P22 CP-LPETG.

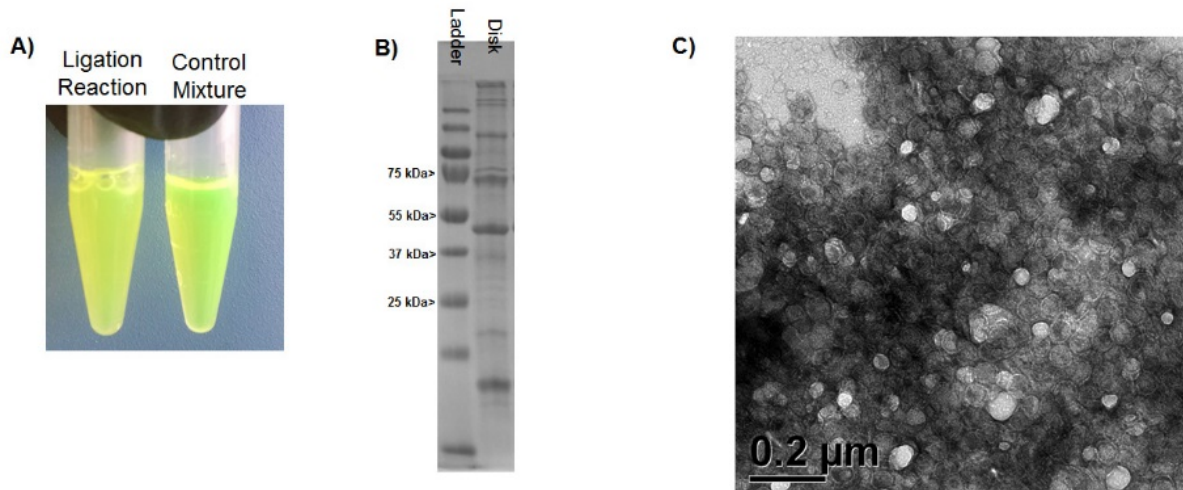


Figure S3. Evaluation of reaction mixtures and products of cesium chloride purification. A) Sortase mediated ligation reaction mixtures showed more increased turbidity in comparison with non-ligated reaction mixtures. B) SDS-PAGE analysis of protein disks produced from cesium chloride gradient purification contains cross-linked P22-GFP. C) TEM image obtained from the protein disk.

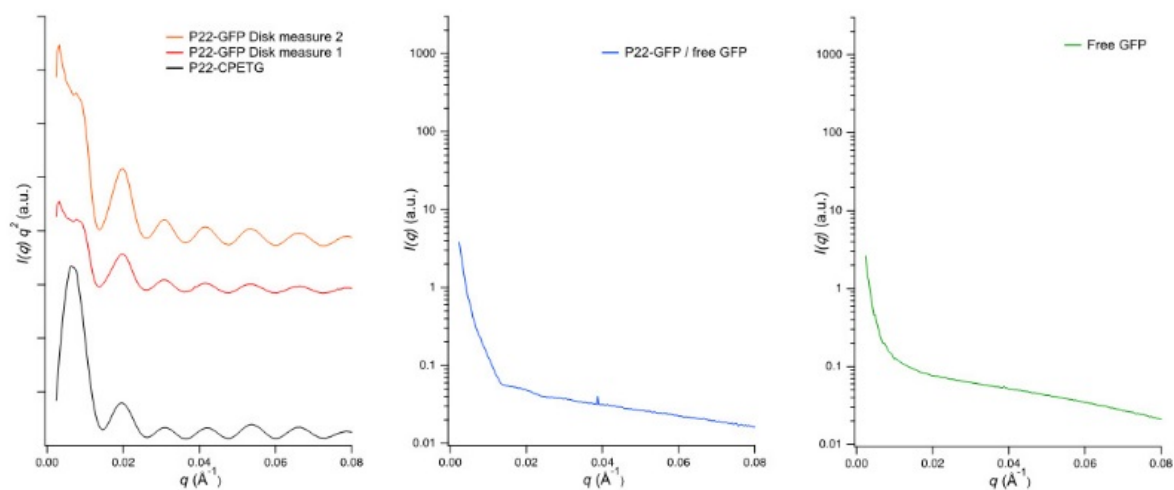


Figure S4. Small angle x-ray scattering of P22-GFP VLP disks and control samples.

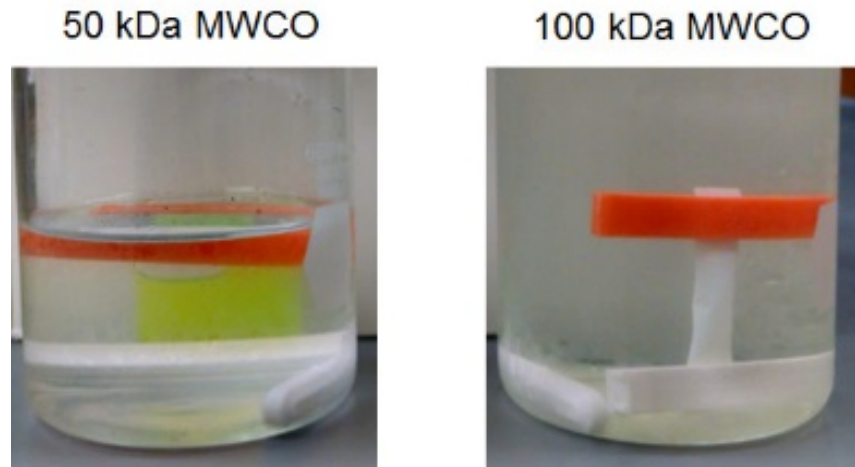


Figure S5. Evaluation of the ability to remove free polyG-GFP by dialysis. 50 kDa molecular weight cutoff (MWCO) dialysis tubing was found to retain most of the free polyG-GFP, whereas 100 kDa MWCO dialysis tubing allow exchange and removal of GFP after two exchanges.

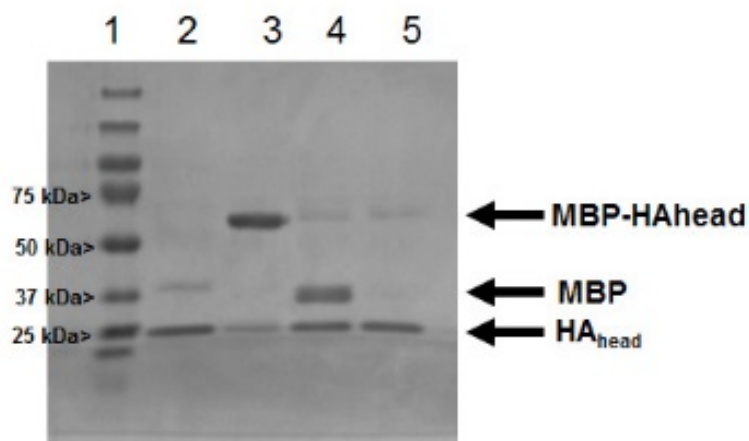


Figure S6. Preparation of polyG-HAhead from the MBP-HAhead fusion protein. SDS-PAGE gel analysis for TEV protease (lane 2), MBP-HAhead (lane 3), MBP-HAhead treated with catalytic amount of TEV (lane 4) and purified polyG-HAhead (lane 5). Expected molecular weights are: MBP-HAhead (68.9 kDa), MBP (43.9 kDa), and HAhead (25 kDa).