Symmetry Controlled, Genetic Presentation of Bio-Active Proteins on the P22 Virus-Like Particle Using an External Decoration Protein

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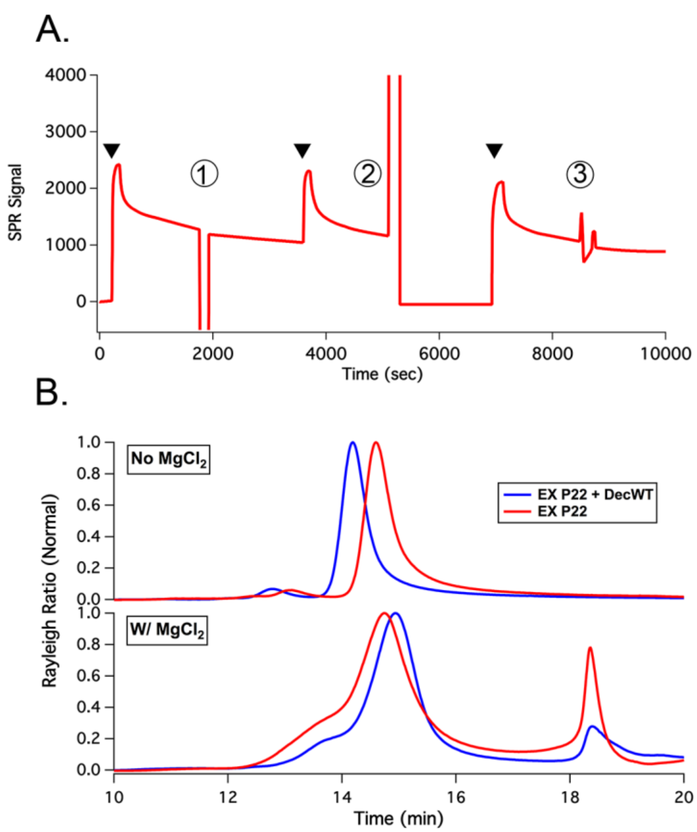
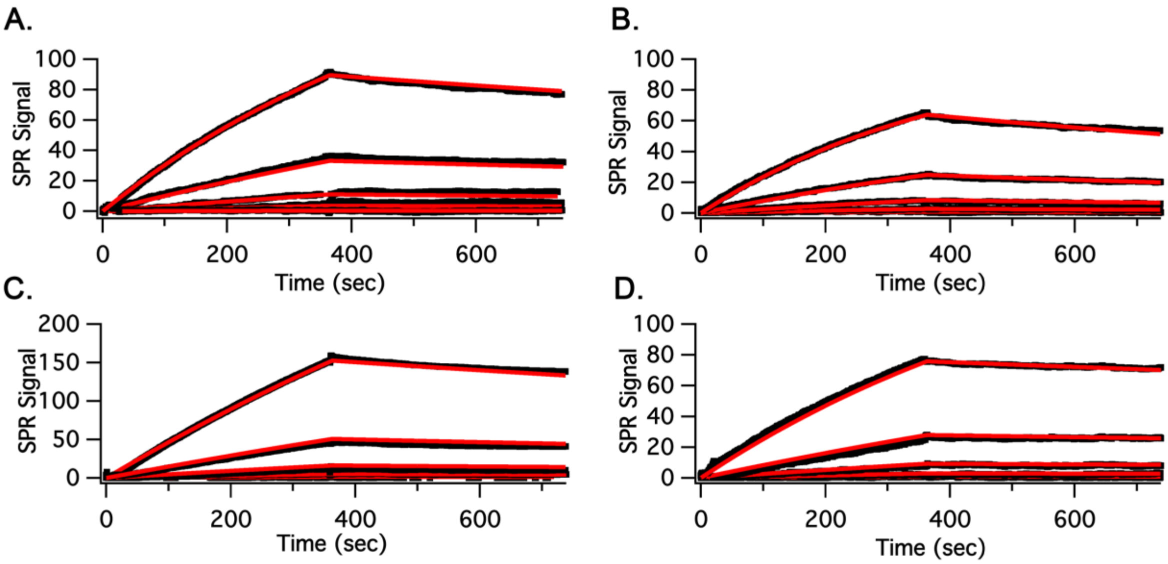
SUPPLEMENTARY INFORMATION



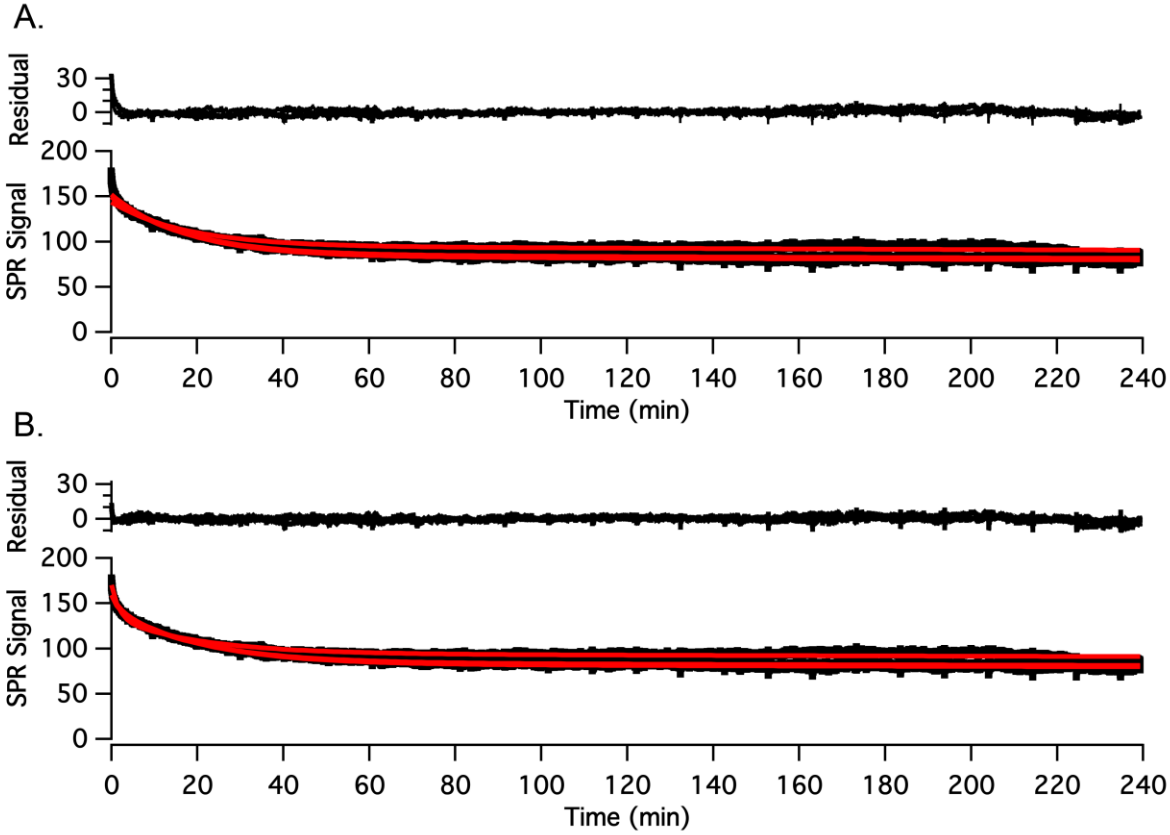
Supplemental Figure 1: High ionic-strength selectively reverses the Dec-P22 interaction but leaves P22 unaffected. A). 4M $MgCl_2$(2) but not 0.35 mM EDTA (1) or 20% acetonitrile (3) regenerated the immobilized P22 surface after injection of 15 μM DecWT. DecWT injection following $MgCl_2$ regeneration reached the same intensity as the injection preceding. DecWT injections are marked by inverted black triangles. B). SEC-MALS of EX-P22 in the presence of DecWT (blue trace) shows a decrease in retention time compared to naked EX-P22. When 4M $MgCl_2$ is present in the sample (but not the running buffer) the retention shift due to Dec is lost but a particle peak is retained.



Supplemental Figure 2: Low-concentration dataset fitting shows only monophasic behavior. DecWT (A), DecΔ11 (B), DecCD40L (C), and DecSelf (D) SPR data from 1-100 nM are fit to a single site model. Parameters (Table) were used as initial inputs for two-site fitting. Each trace shows a sensorgram for a different analyte concentration. All graphs show one representative concentration set. Data traces are shown in black while fits are overlaid in red.

Supplemental Table 1: SPR kinetic binding parameters of Dec constructs and truncations for a one-site binding model. All parameters are the result of global fitting across three full concentration set replicates (1-100 nM Dec). Results were used as initial inputs for subsequent two-site model fitting. Error reflects one standard deviation as reported by the global fit utility in IGOR PRO.

Construct	k_a**($M^{-1}s^{-1}$)**	k_d**(s^{-1})**	K_D**(nM)**
DecWT	13,832 ± 93	3.30 ± 0.03 x 10^{-4}	24.0 ± 0.3
DecΔ11	18,704 ± 82	5.8 ± 0.03 x 10^{-4}	31 ± 0.2
DecCD40L	4,314 ± 55	3.61 ± 0.06 x 10^{-4}	83.4 ± 1.8
DecSelf	13,159 ± 84	2.07 ± 0.03 x 10^{-4}	15.7 ± 0.3



Supplemental Figure 3: Four-hour dissociation demonstrates a DecWT half-life of at least 60 hrs. A. The dissociation traces of 10 μM DecWT injections are shown in triplicate. A two-site dissociation model was fit to the data resulting in k_{d1} of 3.21 ± 0.03 x 10^{-6} sec^{-1} and k_{d2} of 9.55 ± 0.02 x 10^{-4} sec^{-1} B. To account for disagreement of the fit in the early region of the two-site model a third site was introduced which entirely accounted for the discrepancy. Three replicate runs are shown in each plot. Data traces are shown in black while fits are overlaid in red.

Supplemental Table 2: Occupancy of the SPR chip surface relative to DecWT is partially conserved across Dec constructs based on kinetic fit R_{max} ratios. Net R_{max} for each run of each construct was calculated by summing R_{max} for the tight site and the weak site of the two-site model. R_{max} for DecΔ20 was calculated from the single site model. Because the SPR channel was switched between runs net R_{max} ratios were calculated separately for the group of runs from channel 1 and 2. Average ratios are reported and uncertainty reflects one standard deviation. Expected ratios are calculated by dividing the MW of the construct by the MW of DecWT.

Binding Occupancy Relative to DecWT

Construct	Calculated	Expected
DecCD40L	2.61 ± 0.06	2.28
DecSelf	0.94 ± 0.04	1.13
DecΔ11	0.68 ± 0.02	0.89
DecΔ20	0.60 ± 0.02	0.83

Construct Sequences

DecWT

CCATGGGCAGCAGCCATCACCATCATCACCACAGCCTCGTCCCGCGCGGCTCACAGGATCCCATGGCAAACCCAAAC
TTCACGCCATCATGGCCTCTATACAAAGATGCTGACGGTGTATATGTGTCTGCGCTTCCGATTAAAGCTATCAAATA
CGCTAATGACGGAAGTGCAAACGCAGAATTCGACGGCCCGTATGCTGACCAGTACATGTCAGCGCAAACAGTAGCCG
TATTCAAGCCGGAGGTTGGCGGATATCTGTTCCGGAGCCAGTACGGCGAGCTGCTCTATATGAGCAAGACAGCATTT
GAAGCTAACTACACTTCTGCAAGCGGTTCAGTAGCTAATGCAGAGACGGCGGATAAGTTATCTACTGCCCGCACTAT
CACACTAACCGGAGCGGTCACAGGTTCAGCGTCCTTTGATGGTTCGGCTAACGTGACTATCGAAACAACATCAGGAA
GTGAGCTC

DecCD40L

CCATGGGCAGCAGCCATCACCATCATCACCACAGCCAGGATCCCATGGCAAACCCAAACTTCACGCCATCATGGCCT
CTATACAAAGATGCTGACGGTGTATATGTGTCTGCGCTTCCGATTAAAGCTATCAAATACGCTAATGACGGAAGTGC
AAACGCAGAATTCGACGGCCCGTATGCTGACCAGTACATGTCAGCGCAAACAGTAGCCGTATTCAAGCCGGAGGTTG
GCGGATATCTGTTCCGGAGCCAGTACGGCGAGCTGCTCTATATGAGCAAGACAGCATTTGAAGCTAACTACACTTCT
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CACAGGTTCAGCGTCCTTTGATGGTTCGGCTAACGTGACTATCGAAACAACATCAGGAAGTGAGCTCCAAAGAGGTG
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GGCTGAAGCCCAGCAGTGGATCTGAGAGAATCTTACTCAAGGCGGCAAATACCCACAGTTCCTCCCAGCTTTGCGAG
CAGCAGTCTGTTCACTTGGGCGGAGTGTTTGAATTACAAGCTGGTGCTTCTGTGTTTGTCAACGTGACTGAAGCAAG
CCAAGTGATCCACAGAGTTGGCTTCTCATCTTTTGGCTTACTCAAACTCTGAAAGCTT

DecSelf

CCATGGGCAGCAGCCATCACCATCATCACCACAGCCAGGATCCCATGGCAAACCCAAACTTCACGCCATCATGGCCT
CTATACAAAGATGCTGACGGTGTATATGTGTCTGCGCTTCCGATTAAAGCTATCAAATACGCTAATGACGGAAGTGC
AAACGCAGAATTCGACGGCCCGTATGCTGACCAGTACATGTCAGCGCAAACAGTAGCCGTATTCAAGCCGGAGGTTG
GCGGATATCTGTTCCGGAGCCAGTACGGCGAGCTGCTCTATATGAGCAAGACAGCATTTGAAGCTAACTACACTTCT
GCAAGCGGTTCAGTAGCTAATGCAGAGACGGCGGATAAGTTATCTACTGCCCGCACTATCACACTAACCGGAGCGGT
CACAGGTTCAGCGTCCTTTGATGGTTCGGCTAACGTGACTATCGAAACAACATCAGGAAGTGAGCTCGGCAACTATA
CCTGCGAAGTGACCGAACTGACCCGCGAAGGCGAAACCATTATTGAACTGAAATAAAAGCTT

DecΔ11

ATGGGCAGCAGCCATCACCATCATCACCACAGCCAGGATCCCCTATACAAAGATGCTGACGGTGTATATGTGTCTGC
GCTTCCGATTAAAGCTATCAAATACGCTAATGACGGAAGTGCAAACGCAGAATTCGACGGCCCGTATGCTGACCAGT
ACATGTCAGCGCAAACAGTAGCCGTATTCAAGCCGGAGGTTGGCGGATATCTGTTCCGGAGCCAGTACGGCGAGCTG
CTCTATATGAGCAAGACAGCATTTGAAGCTAACTACACTTCTGCAAGCGGTTCAGTAGCTAATGCAGAGACGGCGGA
TAAGTTATCTACTGCCCGCACTATCACACTAACCGGAGCGGTCACAGGTTCAGCGTCCTTTGATGGTTCGGCTAACG
TGACTATCGAAACAACATCAGGAAGTTAA

DecΔ20

ATGGGCAGCAGCCATCACCATCATCACCACAGCCAGGATCCCGTGTCTGCGCTTCCGATTAAAGCTATCAAATACGC
TAATGACGGAAGTGCAAACGCAGAATTCGACGGCCCGTATGCTGACCAGTACATGTCAGCGCAAACAGTAGCCGTAT
TCAAGCCGGAGGTTGGCGGATATCTGTTCCGGAGCCAGTACGGCGAGCTGCTCTATATGAGCAAGACAGCATTTGAA
GCTAACTACACTTCTGCAAGCGGTTCAGTAGCTAATGCAGAGACGGCGGATAAGTTATCTACTGCCCGCACTATCAC

ACTAACCGGAGCGGTCACAGGTTCAGCGTCCTTTGATGGTTCGGCTAACGTGACTATCGAAACAACATCAGGAAGTT
AA

IGOR fitting script

```
if (0<t<t0)

    f(t) = ((Rmax1*A)/((kd1/ka1)+A))*(1-exp(-(ka1*A+kd1)*t))+((Rmax2*A)/((kd2/ka2)+A))*(1-exp(-(ka2*A+kd2)*t))

elseif  (t>t0)

    f(t) = ((Rmax1*A)/((kd1/ka1)+A))*(1-exp(-(ka1*A+kd1)*t0))*exp(-kd1*(t-t0))+((Rmax2*A)/((kd2/ka2)+A))*(1-exp(-(ka2*A+kd2)*t0))*exp(-kd2*(t-t0))

else

    f(t) = 0

endif
```