

Different Strategies Affect Enzyme Packaging into Bacterial Outer Membrane Vesicles

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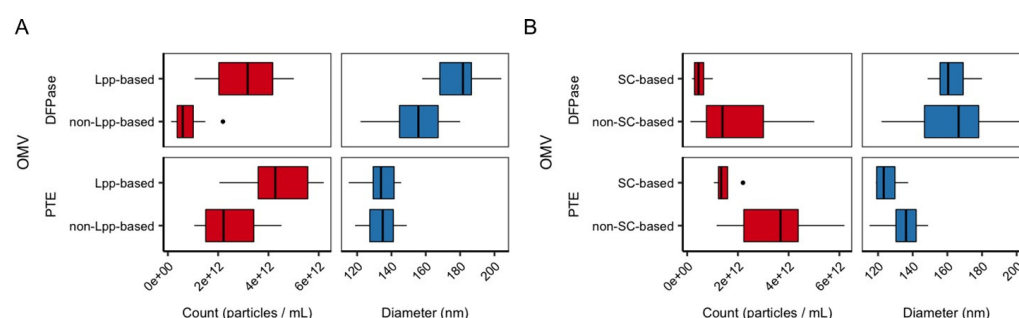


Figure S1. Differences in count and diameter by linker categorization. The count (particles/mL) and diameters (nm) are compared of (A) Lpp'-based versus non-Lpp'-based OMVs and (B) SC-based versus non-SC-based OMVs. Dots represent outliers.

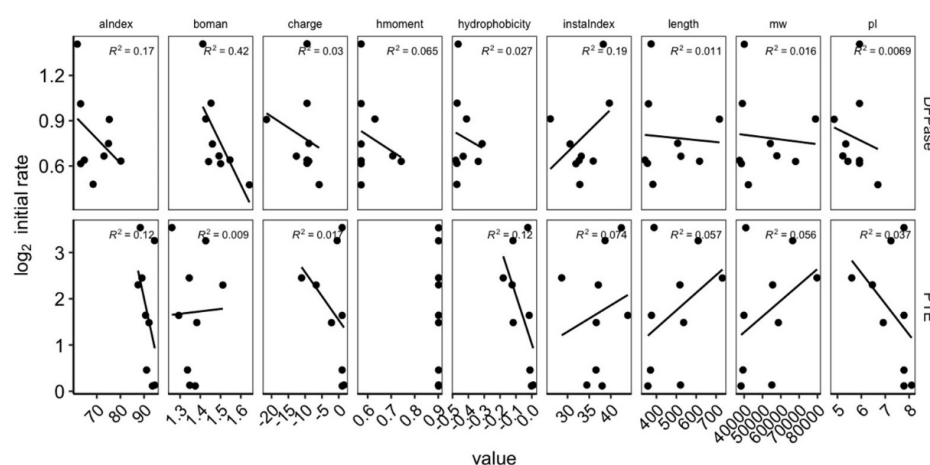


Figure S2. Visual representation of correlations between linker physiochemical characteristics and enzymatic activity (log₂ initial rate). Initial rate taken from the linear portion of the first 20 minutes of the paraoxon degradation reaction. Sequence used for calculating characteristics does not include enzyme.

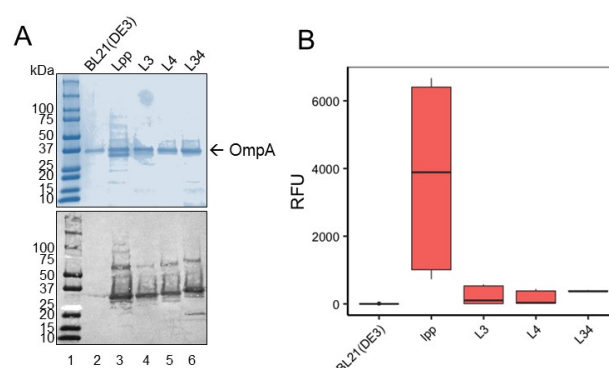


Figure S3. Effect of Lpp'-based linkers on mCherry fluorescence. (A) Coomassie gel of Lpp'-linker mCherry fusions (top) and corresponding immunoblot with anti-mCherry antibody (bottom) showing a major band at ~35 kDa in the total protein gel corresponding to the abundant OmpA, while 6× His-tagged-mCherry fusions are shown at increasing size corresponding to increasing linker length. Lane 1: Molecular weight marker, lane 2: BL21(DE3), lane 3: Lpp'-mCherry, lane 4: L3- mCherry, lane 5: L4- mCherry, lane 6: L34-mCherry. (B) Fluorescence intensity of OMVs from cultures producing Lpp'-based linkers fused to mCherry. Boxplot using data from four biological experiments in triplicate (n = 12).

Table S1. Sequences of anchors/directors and linkers.

Name	Sequence
Lpp'-PTE	<p>TTTGTTTAACTTTAAGAAGGAGATATACCATGAAAGCGACCAAA CTGGTGCTGGGCGCGGTGATTCTGGGCAGCACCTGCTGGCGGG CTGCAGCAGCAACGCGAAAATTGATCAGGGCGGTGGCAGCGG CGGTGGCAGCGGCGGTGGGAGCCGCATTAAACACCGTGCGCGGGCC GATTACCATTAGCGAAGCGGGCTTTACCCTGACCCATGAACATATT GCGGCAGCAGCGGGGCTTTCTGCGCGCGTGGCCGGAATTTTTTGCG AGCCGCAAAGCGCTGGCGGAAAAAGCGGTGCGCGGCCTGCGCCGCG CGCGCGCGGCGGGCGTGCACACCATTTGTGGATGTGAGCACCTTTGAT ATTGGCCGCGATGTGAGCCTGCTGGCGGAAGTGAGCCGCGCGGCGG ATGTGCATATTGTGGCGGCGACAGGCCTGTGGTTTGATCCGCCGCTG AGCATGCGCCTGCGCAGCGTGGAAGAACTGACCCAGTTTTTCTGCG CGAAATTCAGTATGGCATTGAAGATACCGGCATTTCGCGCGGGCATT TTAAAGTGGCGACCAACCGGCAAAGCGACCCCGTTTCAGGAACGGT GCTGAAAGCGGCGGCGCGCGCGAGCCTGGCGACCGGCGTGCCGGTG ACCACCCATACCGCGGCGAGCCAGCGCGATGGCGAACAGCAGGCGG CGATTTTTGAAAGATGATCTGAGCTATCTGACCGCGCTGGCGGCGCG CGGCTATCTGATTGGCCCCGAAGGCCTGAGCCCCGAGCCGCGTGTGCAT TGGCCATAGCGATGATACCGTGGATCATATTCCGCATAGCGCGATTG GCCTGGAAGATAACGCGAGCGCGAGCGCGCTGCTGGGCATTTCGCAG CTGGCAGACCCGCGCGCTGCTGATTAAAGCGCTGATTGATCAGGGCT ATATGAAACAGATTCTGGTGAGCAACGATTGGCTGTTTGGCTTTAGC AGCTATGTGACCAACATTATGGATGTGATGGATCGCGTGAACCCGG ATGGCATGGCGTTTATTCCGCTGCGCGTGATTCCGTTTCTGCGCGAA AAAGGCGTGCCGCGAGAAACCCTGGCGGGCATTACCGTGACCAACC CGGCGCGCTTTCTGAGCCCCGACCTGCGCGCGAGCCACCACCACCA CCACCACTGA</p>
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L4-PTE

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L34-PTE

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BtuF-PTE

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SlyB-PTE

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Lpp'-DFPase

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L3- DFPase

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 TGCACTTCAAGCCGCAGACCAAGACGATTTTGTGACCGAGCATGAG
 AACAATGCAGTTTGGAAATTTGAGTGGCAACGTAACGGTAAGAAAC
 AGTATTGTGAAACCCTGAAGTTTGGTATCTTCCACCACCACCAC
 CACTGA

L4- DFPase

TTTGTTTAACTTTAAGAAGGAGATATACCATGAAAGCGACCAAA
CTGGTGCTGGGCGCGGTGATTCTGGGCAGCACCTGCTGGCGGG
CTGCAGCAGCAACGCGAAAATTGATCAGGCGCGGTGGCAGCGG
CGGTGGCAGCGGCGGTGGGAGCCCGGCATCTCCGGCCCCGCCGG
CTGGTCCAGCACCGCCGGCCCCAACC GCACCGATGGGGGAAATC
CCTGTCATTGAGCCGCTGTTTACCAAAGTCACCGAAGATATCCCGGG
TGCCGAGGGTCCGGTGTTTCGACAAAAACGGTGACTTTTACATCGTTG
CGCCGGCAGTTGAAGTGAATGGCAAGCCTGCTGGTGAGATTCTGCGT
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GCCAACCAACTGTTCTGTGGCAGACATGCGCCTGGGCCTGTTGGTCGT
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GGTCGTCTGATGCAGGGCTGCAATGACTGCGCGTTCGACTACGAGG
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GATCGTTGCCGAGATGCCGACTAAGAAATTGTGGTCGTACGACATCA
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GGGTACGCATGAGGGTGGCGCGGATGGTATGGATTTTGACGAAGAT
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TGGTCCGGATGGTGGCCAGCCGAAGATGCGCATCCGTTGCCCGTTCCG
AGAAGCCGAGCAATCTGCACTTCAAGCCGCAGACCAAGACGATTTT
TGTGACCGAGCATGAGAACAATGCAGTTTGAAAATTTGAGTGCGAA
CGTAACGGTAAGAAACAGTATTGTGAAACCCTGAAGTTTGGTATCTT
CCACCACCACCACCACCTGA

L34- DFPase

TTTGTTTAACTTTAAGAAGGAGATATACCATGAAAGCGACCAAA
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CTGCAGCAGCAACGCGAAAATTGATCAGGCGCGGTGGCAGCGG
CGGTGGCAGCGGCGGTGGGAGCGCCACCGGTCCGGCAAGCGGT
CCGACCAGCGCAGGTCCGCCGGCATCTCCGGCCCCGCCGGCTGG
TCCAGCACCGCCGGCCCCAACC GCACCGATGGGGGAAATCCCTGT
CATTGAGCCGCTGTTTACCAAAGTCACCGAAGATATCCCGGGTGCCG
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ATTGCGGTCCGTCACATGAACGATGGTCGCCCCGTACCAGCTGATCGT
TGCCGAGATGCCGACTAAGAAATTGTGGTCGTACGACATCAAGGGT
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ACCACCACCACCACTGA

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DFPase -SC

ATGGCTAAGTCACTGTTTCAGGGCGCTGGTCGCCCTGTCTTTTCTTG
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 CCGCCAACACTGAACTTGCCCTTGCCGCCGGGATCACGCCGGTTG
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 GAGCAGGTTTCCACCTGGCAGGGGATGAATCTGGAACGCATTGT
 CGCGCTGAAACCCGATCTGGTGATTGCCTGGCGTGAGGTAATG
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 ATGTGGGTCGATGCGACAAGCATTGAACAAATTGCCAATGCGTT
 ACGTCAACTGGCCCCCTGGAGTCCGCAACCAGACAAGGCCGAA
 CAAGCCGCGCAATCCCTGCTGGATCAGTACGCGCAATTGAAAGC
 GCAATATGCTGATAAACCTAAAAACGTGTTTTTCTGCAATTCGG
 CATTAAATCCGCCATTTACCAGTGGAAAAGAGTCGATTGAGAACC
 AGGTACTCGAAGTTTGTGGCGGAGAAAACATCTTTAAAGACAGC

BtuF-DFPase

CGGGTTCCTGGCCGCAAGTTAGCCGCGAACAGGTGTTAGCACG
 CTCGCCACAGGCGATTGTCTATTACAGGCGGACCGGACCAAATTC
 CTAAAATCAAACAATACTGGGGTGAACAGCTCAAAATTCCTCGTT
 ATTCCTCTCACGAGTGACTGGTTTGAACGTGCAAGCCACGTATT
 ATCCTCGCTGCACAACAGCTCTGTAATGCGCTTTCACAGGTAGAT
 AATTCGAGCTCGAACAACAACAATAACAATAACAACAACC
 TCGGGATCGAGGGAAGGATTCACATATGAGCATTGGCACCGGC
 GATATGGGGGAAATCCCTGTCATTGAGCCGCTGTTTACCAAAGTCA
 CGAAGATATCCCGGGTGGCGAGGGTCCGGTGTTCGACAAAAACGGT
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 AGACCAAGACGATTTTTGTGACCGAGCATGAGAAACAATGCAGTTTG
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MBP- DFPase

ATGAAAATAAAAACAGGTGCACGCATCCTCGCATTATCCGCATT
 AACGACGATGATGTTTTCCGCCTCGGCTCTCGCCAAAATCGAAG
 AAGGTAACTGGTAATCTGGATTAACGGCGATAAAGGCTATAAC
 GGTCTCGCTGAAGTCGGTAAGAAATTCGAGAAAGATACCGGAAT
 TAAAGTCACCGTTGAGCATCCGGATAAACTGGAAGAGAAATTC
 CACAGGTTGCGGCAACTGGCGATGGCCCTGACATTATCTTCTGGG
 CACACGACCGCTTTGGTGGCTACGCTCAATCTGGCCTGTTGGCTG
 AAATCACCCCGGACAAAGCGTTCCAGGACAAGCTGTATCCGTTT
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 GATCGCTGTTGAAGCGTTATCGCTGATTTATAACAAAGATCTGCT
 GCCGAACCCGCCAAAAACCTGGGAAGAGATCCCGGCGCTGGAT
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 CGTGGGCGTGATAACGCTGGCGCGAAAGCGGGTCTGACCTTCC
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 CCATCCAAACCGTTTCGTTGGCGTGCTGAGCGCAGGTATTAACGC
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 GGTGAAATCATGCCGAACATCCCCGAGATGTCCGCTTTCTGGTAT
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CGAATGGTATTGCGGTCCGTACATGAACGATGGTCGCCCCGTACCAG
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SLP- DFPaseE

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 TGTGACCGAGCATGAGAACAATGCAGTTTGAAATTTGAGTGGCAA
 CGTAACGGTAAGAAACAGTATTGTGAAACCCTGAAGTTTGGTATCTT
 CGAATTCCTGCAGGTAATTAA

SlyB- DFPase

ATGATTAAACGCGTATTGGTTGTTTCAATGGTAGGTCTGTCTCTTG
 TCGGTTGTGTTAATAACGACACCCTGTCAGGGGATGTTTATACCG
 CTTCTGAAGCGAAACAAGTACAGAATGTCAGCTATGGCACCATC
 GTTAACGTACGTCCGGTACAGATTCAGGGCGGTGATGATTCCAA
 CGTTATCGGTGCAATTGGCGGTGCTGTTCTTGGTGGTTTCTCTGGGG
 AATACTGTTGGTGGCGGAACCGGGCGTTCTCTGGCTACTGCAGC
 AGGCGCTGTTGCAGGTGGCGTAGCTGGTCAGGGCGTACAGAGTG
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 AGCATGAGAACAAATGCAGTTTGGAAATTTGAGTGGCAACGTAACGG
 TAAGAAACAGTATTGTGAAACCCTGAAGTTTGGTATCTTCGAATTC
 CTGCAGGTAATTAA

RBS – Underlined.

Lpp' – Bold

Linker/Fusion partner - Shaded

6x-His – Bold, Italics

PTE/DFPase – Italics, Underlined