**SmgGDS-607 regulation of RhoA GTPase prenylation is nucleotide dependent**

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***Supporting Information.***

 Plasmid names, tag order, UniProt ID, backbone plasmid, and selective resistance for expression plasmids used in this study. Final protein sequences from DNA sequencing.

pBJ115 His6-thrombin-linker-human SmgGDS-558 (P52306-2),

 pET28a backbone plasmid, kanamycin resistance

MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSEFELRRQASTMDNLSDTLKKLKITAVDKTEDSLEGCLDCLLQALAQNNTETSEKIQASGILQLFASLLTPQSSCKAKVANIIAEVAKNEFMRIPCVDAGLISPLVQLLNSKDQEVLLQTGRALGNICYDSHSLQAQLINMGVIPTLVKLLGIHCQNAALTEMCLVAFGNLAELESSKEQFASTNIAEELVKLFKKQIEHDKREMIFEVLAPLAENDAIKLQLVEAGLVECLLEIVQQKVDSDKEDDITELKTGSDLMVLLLLGDESMQKLFEGGKGSVFQRVLSWIPSNNHQLQLAGALAIANFARNDANCIHMVDNGIVEKLMDLLDRHVEDGNVTVQHAALSALRNLAIPVINKAKMLSAGVTEAVLKFLKSEMPPVQFKLLGTLRMLIDAQAEAAEQLGKNVKLVERLVEWCEAKDHAGVMGESNRLLSALIRHSKSKDVIKTIVQSGGIKHLVTMATSEHVIMQNEALVALALIAALELGTAEKDLESAKLVQILHRLLADERSAPEIKYNSMVLICALMGSECLHKEVQDLAFLDVVSKLRSHENKSVAQQASLTEQRLTVES

pBJ116 His6-thrombin-linker-human SmgGDS-607 (P52306-1),

 pET28a backbone plasmid, kanamycin resistance

MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSEFELRRQASTMDNLSDTLKKLKITAVDKTEDSLEGCLDCLLQALAQNNTETSEKIQASGILQLFASLLTPQSSCKAKVANIIAEVAKNEFMRIPCVDAGLISPLVQLLNSKDQEVLLQTGRALGNICYDSHEGRSAVDQAGGAQIVIDHLRSLCSITDPANEKLLTVFCGMLMNYSNENDSLQAQLINMGVIPTLVKLLGIHCQNAALTEMCLVAFGNLAELESSKEQFASTNIAEELVKLFKKQIEHDKREMIFEVLAPLAENDAIKLQLVEAGLVECLLEIVQQKVDSDKEDDITELKTGSDLMVLLLLGDESMQKLFEGGKGSVFQRVLSWIPSNNHQLQLAGALAIANFARNDANCIHMVDNGIVEKLMDLLDRHVEDGNVTVQHAALSALRNLAIPVINKAKMLSAGVTEAVLKFLKSEMPPVQFKLLGTLRMLIDAQAEAAEQLGKNVKLVERLVEWCEAKDHAGVMGESNRLLSALIRHSKSKDVIKTIVQSGGIKHLVTMATSEHVIMQNEALVALALIAALELGTAEKDLESAKLVQILHRLLADERSAPEIKYNSMVLICALMGSECLHKEVQDLAFLDVVSKLRSHENKSVAQQASLTEQRLTVES

pBJ138 His6-tev-human RhoA (P61586-1),

 pETM11 backbone plasmid, kanamycin resistance

MKHHHHHHPMSDYDIPTTENLYFQ/GAMAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDTAGQEDYDRLRPLSYPDTDVILMCFSIDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKDLRNDEHTRRELAKMKQEPVKPEEGRDMANRIGAFGYMECSAKTKDGVREVFEMATRAALQARRGKKKSGCLVL

**Figure S1**. Replot of data from Figure 2D fit to either a (*A*) substrate sequestration or (*B*) competitive inhibition model. The indicated concentrations of SmgGDS-607 (µM) were pre-incubated with varying RhoA and then labeled for 4 min by addition of GGTase-I (10 nM) and 2 μM 3H-GGPP. (*A*) Equations 1 and 2 describing Scheme 1 were fit to the data resulting in the following values: *k*cat = 0.05 s-1, *K*M = 1.8 μM, and *K*i = 85 nM. (*B*) For comparison, the same data were fit to a competitive enzyme inhibition model described by equations S1 and S2.

$V=\frac{k\_{cat}[E]\_{total}[S]}{K\_{M}^{App}+[S]}$ (Eq. S1)

$K\_{M}^{App}= K\_{M}\left(1+\frac{[SmgGDS]}{K\_{i}}\right)$ (Eq. S2)



**Figure S2**. Observed rate constants for SmgGDS-607 binding in Figure 4A and B.

**Table S1**. Calculated rates and affinities from Figures 4 and S2 for SmgGDS-607 binding RhoA.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | *k*off (s-1) | *k*on (s-1 M-1) | *K*d calc (nM) | *K*d endpoint (nM) |
| GDP | 0.026 ± 0.005 | 390,000 ± 60,000 | 67 ± 23 | 77 ± 7 |
| GppNp | 0.060 ± 0.005 | 220,000 ± 20,000 | 271 ± 39 | 249 ± 13 |