Structure-based analysis of the guanine nucleotide exchange factor SmgGDS reveals armadillo-repeat motifs and key regions for activity and GTPase binding

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Small GTPases are molecular switches that have critical biological roles and are controlled by GTPase-activating proteins and guanine nucleotide exchange factors (GEFs). The Smg GDP dissociation stimulator (SmgGDS) protein functions as a GEF for the RhoA and RhoC small GTPases. SmgGDS has various regulatory roles, including small GTPase trafficking and localization and as a molecular chaperone, and interacts with many small GTPases possessing polybasic regions. Two SmgGDS splice variants, SmgGDS-558 and SmgGDS-607, differ in GEF activity and binding affinity for RhoA depending on the lipidation state, but the reasons for these differences are unclear. Here we determined the crystal structure of SmgGDS-558, revealing a fold containing tandem copies of armadillo repeats not present in other GEFs. We also observed that SmgGDS harbors distinct positively and negatively charged regions, both of which play critical roles in binding to RhoA and GEF activity. This is the first report demonstrating a relationship between the molecular function and atomic structure of SmgGDS. Our findings indicate that the two SmgGDS isoforms differ in GTPase binding and GEF activity, depending on the lipidation state, thus providing useful information about the cellular functions of SmgGDS in cells.

Small GTPases are molecular switches that have critical biological roles by switching between an active GTP-binding form and an inactive GDP-binding form. Small GTPases usually have low hydrolysis activity and are controlled by GTPase-activating proteins and guanine nucleotide exchange factors (GEFs). In addition to the regulation of GTP/GDP forms, the trafficking and localization of small GTPases are controlled by their post-translational modification.

SmgGDS was originally identified as a GEF for many types of small GTPases, including Rho and Rac family members (1, 2). In particular, SmgGDS interacts with small GTPases possessing a polybasic region (PBR) at the C-terminal region, such as RhoA, Rac1, Rap1A, K-Ras4B, and Di-Ras2 (3–5). SmgGDS has various roles in the regulation of small GTPases, including those in trafficking, localization, and molecular chaperone functions, in addition to its general role as a GEF. SmgGDS has two main splicing variants (6); SmgGDS-558 consists of 558 amino acid residues and SmgGDS-607 is composed of 607 amino acid residues (Fig. 1A). These isoforms are thought to have different physiological roles because SmgGDS-558 prefers prenylated RhoA, whereas SmgGDS-607 prefers nonprenylated RhoA (6–8).

SmgGDS levels are elevated in some cancers, such as non-small cell lung carcinoma (9), prostate cancer (10), breast cancer (11), and pancreatic cancer (11). Patients with high SmgGDS expression in tumors have worse clinical outcomes in breast cancer; furthermore, the knockdown of SmgGDS-558, but not SmgGDS-607, in breast cancer cells decreases proliferation, in vivo tumor growth, and RhoA activity (11). SmgGDS also controls the localization of Rac1 (12, 13). Recent studies have revealed that SmgGDS is up-regulated by statin, resulting in the nuclear transport and degradation of Rac1 (14). Additionally, SmgGDS is a crucial mediator of the inhibitory effects of statins on cardiac hypertrophy (15, 16).

Recently, SmgGDS was re-evaluated as a GEF specific for RhoA and RhoC (17). In humans, there are 69 typical RhoGEFs in the Dbl family and 11 atypical RhoGEFs known as DOCK family proteins. GEF mechanisms for proteins in both families have been well-characterized by biochemical and structural studies (18, 19). Dbl and DOCK family proteins contain the Dbl homology (DH) domain associated with a PH domain and DOCK homology region 2 (DHR2), respectively, as a catalytic domain. Interestingly, SmgGDS is thought to contain armadillo-repeat motifs (ARMs), suggesting a novel GEF mechanism distinct from those of GEFs harboring the DH domain or DHR2 domain.

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In this study, we determined the crystal structure and solution structure of SmgGDS-558. Our structural and biochemical analyses revealed that SmgGDS recognizes RhoA at distinct sites, and GEF activity differs substantially depending on the isoform of SmgGDS and lipidation state of RhoA. It is possible that this recognition mechanism exists in other small GTPases. These results provide useful information regarding the unique cellular functions of SmgGDS.

**Results**

**Overall crystal structure of hSmgGDS-558**

We determined the crystal structure of truncated human SmgGDS-558 lacking 60 N-terminal residues, hereafter referred to as SmgGDS-558(61–558), at 2.1 Å resolution (Fig. 1B and supplemental Table S1). SmgGDS-558(61–558) consists of 10 completely folded ARMs (ARM D–M) with a partially unfolded ARM at its N-terminal region (aa 79–87, part of ARM B). In the N-terminal region, 18 residues (aa 61–78) were disordered, and side chains for 66 C-terminal amino acid residues were not assigned owing to the poor electron density. As the truncated 70 N-terminal residues are predicted to fold into the ARM repeat structure, full-length SmgGDS-558 would consist of 12 ARMs. In addition, SmgGDS-607 is predicted to have an additional ARM between ARM B and ARM D (Fig. 1A); therefore, full-length hSmgGDS-607 would comprise 13 ARMs. SmgGDS-558 folds into a superhelical structure distinct from known GEF structures such as those of Dbs, Sos1, and Dock9 (supplemental Fig. S1). A structural comparison of SmgGDS-558(61–558) against the Protein Data Bank (PDB), using DALI (20), showed that SmgGDS shares structural homology with functionally unrelated proteins containing ARMs such as OR497 (PDB ID: 4RV1), importin subunit α-1A (PDB ID: 4BQK), and β-catenin (PDB ID: 1TH1) (supplemental Fig. S2). However, in each of these cases, the root-mean-square deviation was above 3 Å and the structural similarity was limited, suggesting that the proteins were not biologically related.

**Solution structures of SmgGDS and its RhoA complex**

For a more detailed analysis of the structure of SmgGDS and the SmgGDS-RhoA complex, we performed SEC-MALS (size-exclusion chromatography multi-angle light scattering) and SEC-SAXS (size-exclusion chromatography small-angle X-ray scattering) analyses. Based on SEC-MALS, the SmgGDS-558–RhoA complex was 74 kDa, indicating that the complex is formed with a 1:1 stoichiometry, considering their theoretical molecular weights (61 and 22 kDa, respectively) (supplemental Fig. S3). Based on SEC-SAXS, the structure of the apoform of full-length SmgGDS-558 in solution was elongated and well fit to the crystal structure of SmgGDS-558(61–558). The maximum dimension (Dmax) of the RhoA-bound form is shorter than that of the unbound form. To assess the binding mode of RhoA, the solution structures of the SmgGDS-558–RhoA and SmgGDS-607–RhoA complexes were determined (Fig. 1C and supplemental Fig. S4). It was difficult to correctly determine the orientation and docking mode due to the low resolution, but both structures showed additional dummy atom models at the concave surface, which is appropriate to accommodate RhoA.

**SmgGDS isoforms exhibit differences in GEF activity and binding affinity for RhoA depending on the lipidation state of RhoA**

We first examined the effect of lipidation on the GEF activity of SmgGDS because the C-terminal CAAX motif of RhoA was prenylated as a post-transcriptional modification. RhoA is generally modified by a geranylgeranyl group at the CAAX motif, but geranylgeranylated RhoA is easily precipitated (21). Instead, we employed farnesylated RhoA in this work. Wild-type SmgGDS-607 strongly promoted the release of BODIPY-GDP from non-farnesylated RhoA, whereas SmgGDS-558 did not show significant activity (Fig. 2 and supplemental Fig. S5 and supplemental Table S3). Conversely, wild-type SmgGDS-558 promoted GEF activity against farnesylated RhoA, demonstrating that each SmgGDS isoform exhibits different GEF activity depending on the C-terminal lipidation state of RhoA (Fig. 2 and supplemental Fig. S6). SmgGDS-607 against farnesylated RhoA exhibited decreased activity, to a level similar to that of SmgGDS-558. It should be noted that SmgGDS-558(61–558), evaluated in the structural study, showed GEF activity similar to that of SmgGDS-558 (supplemental Fig. S6).

Next, we examined whether the binding affinities of both isoforms depend on the lipidation state of RhoA. To quantitatively evaluate the binding affinity of both isoforms, we measured the binding kinetics of SmgGDS for RhoA, using surface plasmon resonance (SPR). SmgGDS tightly binds to RhoA.
under Mg$^{2+}$-free conditions (17); accordingly, we performed SPR using an EDTA-containing solution. SmgGDS-558 exhibited a higher affinity for farnesylated RhoA ($K_D = 2.3$ nM) than non-farnesylated RhoA ($K_D = 51.3$ nM). In contrast, SmgGDS-607 exhibited a lower affinity for farnesylated RhoA ($K_D = 3.1$ nM) than non-farnesylated RhoA ($K_D = 0.8$ nM) (Table 1 and supplemental Fig. S7). These results showed a positive correlation between the $K_D$ value and the GEF activity. For farnesylated RhoA, the binding affinity of SmgGDS-558 was almost the same as that of SmgGDS-607.

**SmgGDS-558 has characteristic surface electrostatic potential**

An electrostatic potential map of SmgGDS-558 (61–558) demonstrated that SmgGDS had a characteristic negatively charged region (negative region) formed by ARM B–F and a small positively charged region (positive region) formed by ARM H and I at its concave surface (Fig. 3A). A recent in silico docking study and mutational analysis suggest that acidic residues (Asp-239, Glu-242, Glu-246, Glu-253, and Asp-255) of SmgGDS-607 are responsible for Rap1 PBR binding (22). Mutations at Glu-213, Asp-239, Glu-242, Glu-246, Glu-253, and Asp-255 of SmgGDS-607 also decrease its GEF activity toward RhoA (17). Mapping these residues of SmgGDS-558 to the crystal structure indicated that they are located in or near the negative region (Fig. 3B), suggesting the importance of the negative region for the recognition of the small GTPase PBR.

Asn-338 of SmgGDS-558 (Asn-387 of SmgGDS-607) is a critical residue for binding to small GTPases (4, 17). For example, an N338A mutant of SmgGDS-558 exhibits an inability to interact with Di-Ras2 (4). Mutations of conserved Asn-342, Arg-345, His-379, Ser-383, and Lys-395 in SmgGDS-607 on the concave surface, in addition to N387A, decrease GEF activity toward RhoA (17). These residues were located near the positive region, suggesting that the positive region is also important for the recognition of small GTPases (Fig. 3B).

**Positive region of SmgGDS is responsible for GEF activity and RhoA binding**

Considering the SAXS analysis suggesting that RhoA binds to the concave surface of SmgGDS as well as the reduced GEF activity associated with mutations in the positive region, this region is a strong candidate for RhoA binding. To assess the functional importance of residues constituting the positive region, we performed a GDP dissociation assay using SmgGDS-558 mutants (H330A, R337A, N338A, K372A, R378A, and M379A in the positive region) and SmgGDS-607 mutants (H379A, R386A, N387A, K421A, R427A, and M428A in the positive region) (Fig. 2). Five mutants (H379A, R386A, N387A, K421A, R427A, and M428A in the positive region) significantly decreased dissociation rates compared with the wild type, or a complete lack of dissociation. Of note, Asn-387 in SmgGDS-607 (Asn-387 in SmgGDS-558) is a critical residue for GEF activity, consistent with previous results (17). In contrast, a mutation of a methionine (M379A) resulted in a slight reduction in GEF activity. Regardless of whether RhoA was farnesylated or not and whether SmgGDS-558 or SmgGDS-607 was

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**Table 1**

| GEF protein   | $k_{on}$  | $k_{off}$ | $K_D$  |
|---------------|-----------|-----------|--------|
| SmgGDS-558    |           |           |        |
| Non-farnesylated | $1.9 \times 10^4$ | $9.9 \times 10^{-4}$ | 51.3   |
| Farnesylated  | $7.2 \times 10^4$ | $1.7 \times 10^{-4}$ | 2.3    |
| SmgGDS-607    |           |           |        |
| Non-farnesylated | $2.6 \times 10^5$ | $2.2 \times 10^{-4}$ | 0.8    |
| Farnesylated  | $1.3 \times 10^5$ | $3.9 \times 10^{-4}$ | 3.1    |

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Figure 2. Guanine nucleotide dissociation assay of RhoA. Relative guanine nucleotide dissociation rates of farnesylated or non-farnesylated RhoA are shown in a bar chart ($n = 3$). ***, $p < 0.001$. The dissociation rates were normalized by the control rate constant, i.e. the dissociation rate in the absence of SmgGDS. Absolute values derived from kinetics analyses are shown in supplemental Table S3.
used, mutants of both isoforms both showed decreased GEF activity.

We evaluated the stability of the protein complexes between nonprenylated RhoA and the positive-region mutants of SmgGDS-558 and SmgGDS-607 by SEC (Fig. 4). Among the mutants of SmgGDS-558 with reduced GEF activity, H330A, R337A, N338A, and K372A showed dissociation, suggesting that the stability of the protein complex was reduced. In particular, the N338A mutant of SmgGDS-558 exhibited remarkable dissociation. These SEC analyses revealed that this positive region is a RhoA-binding site; interactions at this site are critical for forming a stable RhoA–SmgGDS-558 complex and for GEF activity.

In contrast, none of the mutants of SmgGDS-607 exhibited dissociation, suggesting that they retained sufficient stability of RhoA–SmgGDS-607 complex, although their GEF activities were apparently reduced (Fig. 4). In SmgGDS-607, both WT and the N387A mutant formed a stable complex with RhoA, independent of farnesylation. For SmgGDS-558, the N338A mutant formed a complex with farnesylated RhoA but not with non-farnesylated RhoA (Fig. 4 and supplemental Fig. S8). These results suggested that SmgGDS had other binding sites outside of the positive region.

The negative region is a PBR-CAAX-binding site

Considering that the SmgGDS-558 structure exhibits the characteristic negative region at the N-terminal region (ARM B and D–F) and prefers PBR-containing small GTPases, we hypothesized that the negative region accommodates PBR and CAAX (PBR-CAA(X)) in the C-terminal region of RhoA. To examine this hypothesis, we measured the binding affinity between SmgGDS and the RhoA PBR-CAA peptide by isothermal titration calorimetry (ITC). The heat released or absorbed upon the SmgGDS-558 and peptide interaction was too small to determine the dissociation constant, whereas SmgGDS-607 showed strong binding to the RhoA PBR-CAA peptide (K_D = 104 nM) (Fig. 5A and supplemental Table S4). Next, to confirm that the negative region of SmgGDS-607 was a PBR-binding site, we performed a competitive binding assay by SEC. When we added a 50-fold excess of the C-terminal peptide of RhoA,181ARRGKKKSGCLVL193 (numbers denote the amino residue numbers for the first and last residues), to the RhoA/SmgGDS-607 (N387A) complex solution, complex dissociation was observed (Fig. 5B), strongly suggesting that the negative region of SmgGDS is a PBR-CAA motif–binding site.

Discussion

This is the first study to describe the crystal structure of SmgGDS-558, which harbors a unique fold consisting of tandem ARMs. The structure of SmgGDS was distinct from those of other GEFs. Proteins harboring ARMs have a variety of functions, such as signaling, protein transport, molecular chaperon-
ing, and cell adhesion (23). Of the ARM proteins, p120 catenin (catenin/H9254-1) binds to RhoA and inhibits its activity (23), but the ARMs of p120 catenin do not bind to RhoA directly; rather, the inserted loop is thought to interact with RhoA (24). DOCK harboring the ARM domain functions as a RhoGEF, but the role of the ARM domain is not known (25).

The crystal structure demonstrated that SmgGDS-558 has a characteristic positive region on its concave surface and a negative region. Our structural and biochemical analyses revealed that the positive region is responsible for RhoA binding and GEF activity. In this study, we determined that SmgGDS recognizes the C-terminal PBR-CAA\textsubscript{X} motif of RhoA in the negative region.

Our in vitro SEC binding assay and SPR analysis results were highly consistent with those of previous in vivo studies, showing that SmgGDS-558 prefers prenylated RhoA to nonprenylated RhoA, whereas SmgGDS-607 shows the opposite preference (6–8). Furthermore, our GEF assay showed that SmgGDS-558 acts as a GEF only for prenylated (farnesylated) RhoA. In contrast, the splice variant SmgGDS-607 has strong GEF activity for prenylated (non-farnesylated) RhoA and decreased activity for prenylated RhoA. SmgGDS differs with respect to GEF activity and binding affinity depending on the lipidation state of the substrate. This result supports those of a previous report (10) and suggests that SmgGDS-558 mainly functions as a GEF for more mature substrates.

Taking the results together, we proposed a binding model of SmgGDS and RhoA (Fig. 6). The positive region provides a primary interface for the core region of RhoA. SmgGDS-558 has a negative region, but it is insufficient for the formation of a stable complex with the PBR-CAA\textsubscript{X} motif of RhoA. This isoform, showing a preference for prenylated RhoA, harbors a prenyl group–binding site. In fact, a hydrophobic groove is located near the negative region (supplemental Fig. S9). By the insertion of ARM C in SmgGDS-607, the negative region is organized to bind to the C-terminal PBR-CAA\textsubscript{X} motif of RhoA, and the prenyl group–binding site is disrupted. Further structural analyses and crystal structure determination of the RhoA-SmgGDS complex will be required to clarify substrate binding and GEF activity mechanisms. Crystallization trials of the complex are in progress. Our results, indicating differences in binding and GEF activity between SmgGDS isoforms depending on lipidation state, shed light on the multifunctional roles of SmgGDS in cells.

**Experimental procedures**

**Preparation of recombinant SmgGDS, RhoA, and FTase**

The following cDNA were subcloned into pGEX6P-1 (GE Healthcare) vector: \textit{h}SmgGDS-558 (UniProt ID: P52306-2, aa 1–558 or 61–558) and \textit{h}SmgGDS-607 (UniProt ID: P52306-1, aa 1–607). A point mutation of \textit{h}SmgGDS was generated by PCR-based site-directed mutagenesis. \textit{Escherichia coli} (BL21(DE3) RIPL) was used as a host cell. Protein expression was induced with 0.1 mM isopropyl-\textbeta-D-1-thiogalactopyranoside when the A\textsubscript{600} was 0.4–0.8, and then the cells were cultured at 18 °C overnight. To produce selenomethionine (SeMet)-labeled protein, transformed \textit{E. coli} (BL21(DE3) RIPL) was cultured in the minimum medium (7 g of Na\textsubscript{2}HPO\textsubscript{4}, 3 g of KH\textsubscript{2}PO\textsubscript{4}, 0.5 g of NaCl, 4 g of glucose, 20 mg of thiamine, 20 mg of biotin, 20 mg of adenosine, 20 mg of guanosine, 20 mg of cytidine, 20 mg of thymidine, 0.5 mg of FeCl\textsubscript{3}, 120 mg of MgSO\textsubscript{4}, 6.3 mg of MnCl\textsubscript{2}, 1 g of NH\textsubscript{4}Cl, and 11 mg of CaCl\textsubscript{2} were added to 1 liter of MilliQ). When the A\textsubscript{600} was 0.3, 100 mg of Lys, 100 mg of Phe, 100 mg of Thr, 50 mg of Ile, 50 mg of Leu, 50 mg of Val, and 60 mg of SeMet were added per 1 liter of medium. Protein expression was induced with 0.25 mM isopro-
Crystal structure of SmgGDS

pyl β-D-1-thiogalactopyranoside when the $A_{600}$ was 0.7, and then the cells were cultured at 18°C overnight. Following cell lysis and purification, the steps were almost the same between the wild type and the SeMet-labeled protein. The harvested cells were sonicated in buffer A (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 1 mM DTT) with 1 mM PMSF. GST-fused SmgGDS was loaded on glutathione-Sepharose 4B (GE Healthcare) and washed with buffer A for the wild type and with buffer B (20 mM Tris-HCl (pH 7.5), 500 mM NaCl, and 1 mM DTT) for SeMet-labeled protein. GST tag was removed with on-column tag cleavage using PreScission protease. The supernatant solution containing the tag-removed SmgGDS was collected, and further purification was performed with HiTrap Q (GE Healthcare) with buffer C (20 mM Tris-HCl (pH 7.5) and 1 mM DTT) and buffer D (20 mM Tris-HCl (pH 7.5), 1 mM DTT, and 1 mM NaCl) and with Superdex 200 prep grade (GE Healthcare) with buffer A.

The following cDNA were subcloned into the pET-44a(+) vector (Novagen): hRhoA (UniProt ID: P61586-1, aa 1–193) harbored His6 and PreScission protease recognition sequences at the N terminus. hRhoA$^{189\text{A}}$ was generated by PCR-based site-directed mutagenesis. The host cell and protein expression procedure were the same as described above. The harvested cells were sonicated with buffer E (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 5 mM MgCl2, and 1 mM DTT) containing 1 mM PMSF. His tag−fused RhoA was purified with nickel-nitrioltriacetic acid resin (Qiagen) or complete His-tag purification resin (Roche Life Science). After the first affinity chromatography purification, the proteins were purified with HisTrap (GE Healthcare) His-tag affinity chromatography, and then the His tag was removed with PreScission protease. Finally, the proteins were purified with Superdex 200 prep grade (GE Healthcare) size-exclusion chromatography with buffer E. Cys190 farnesylated RhoA$^{189\text{A}}$ was prepared as described previously (21).

FTase was expressed by dual expression following a procedure similar to that described previously (26). The α-subunit, ribosomal binding site, and β-subunit coding sequences were subcloned tandemly into the pGEX6P-1 vector. GST-fused α-subunit and non-tagged β-subunits were co-expressed. E.coli (BL21(DE3)) was used as the host cell. The protein expression procedure was the same as described above. The harvested cells were sonicated in buffer F (5 mM sodium phosphate (pH 7.2), 75 mM NaCl, 5 mM DTT, and 1 mM PMSF). The expressed FTase, comprising the GST-fused α-subunit and β-subunit, was purified with glutathione-Sepharose 4B (GE Healthcare), and GST tag was removed with PreScission protease. Further purification was performed with HiTrap Q (GE Healthcare) using buffers C and D, both containing 10 μM Zn(OAc)$_2$.

Crystallization of hSmgGDS-558

Crystallization was performed using the sitting-drop vapor-diffusion method. For crystallization, N-terminally truncated SmgGDS-558(61–558) was used. Purified SmgGDS-558 solution was concentrated up to 5 mg/ml. The same volume of protein and a reservoir solution containing 100 mM MgCl$_2$, 100 mM HEPES (pH 7.3–8.1), and 8–20% (w/v) PEG 3350 were mixed at 10°C. Needle crystals were found in 1–3 days and grown for 1–2 weeks. SeMet-labeled proteins were crystallized under the same conditions as the native ones.

Data collection and structure determination of hSmgGDS-558

The X-ray diffraction data set of hSmgGDS-558 crystal was collected on beamline BL44XU at SPring-8 (Hyogo, Japan), and the data set of SeMet-labeled SmgGDS-558 crystal was collected on beamline BL17A at the Photon Factory (Tsukuba, Japan). 30% (w/v) glycerol-containing reservoir solution was used as a cryoprotectant. The collected data of hSmgGDS-558 and SeMet-labeled hSmgGDS-558 were integrated, merged, and scaled using the HKL2000 (27) and XDS programs (28), respectively. Phase determination was performed by using the SAD method with the program SHARP/autoSHARP (29). All 16 selenium sites could be identified. Initial model building was performed with the program Buccaneer (30, 31). Molecular replacement was performed with the program MolRep (32). Manual model building was performed with the Coot program (33). The model structure was refined with the program Refmac5 (34).

SEC-SAXS/MALS

SEC-SAXS and SEC-MALS data for hSmgGDS-558, hSmgGDS-558−hRhoA complex, hSmgGDS-607, and hSmgGDS-607−hRhoA complex were collected on beamline BL10C at the Photon Factory. SEC was performed using a Superdex 200 Increase column (GE Healthcare) and buffer containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 5 mM EDTA, and 1 mM DTT solution as the mobile phase. First, SEC-MALS was performed to determine the molecular weight of each sample and to evaluate the retention volume and peak dilution rate during SEC analysis. In SEC-SAXS analysis, the flow rate was changed from 0.5 to 0.05 ml/min when protein elution was started. The elution profile was evaluated using a UV-visible spectrometer installed at the irradiation position. Protein concentration was calculated using the value of $A_{280}$. Background data were collected at a position before sample was eluted. Ten images were collected, and the average data were used as background data. Scattering intensities were measured at 293 K on a PILATUS3 2M detector with a sample-to-detector distance of 2.0 m. Over 200 images were collected in one measurement, and all scattering data processing and radius of gyration ($R_g$) calculations were performed using the program package SÁnger (36). Around the elution top peak, the apparent concentration-dependent particle interaction could not be detected. The measurement details of the collected data are summarized in supplemental Table S2.

Solution structure determination by SEC-SAXS

For solution structure determination by SEC-SAXS, only one scattering image of the elution top peak was used. $R_g$ was derived by the Guinier approximation using the program PRI-MUS (37, 38), and the pair distance distribution functions (P(r))
function) were determined using the program GNOM (39). To evaluate measurement condition, the agreement of the $R_g$ values calculated by the Guinier approximation and $P(r)$ function was confirmed. The $P(r)$ functions also were used to determine the maximum dimension ($D_{max}$) of the macromolecules and to estimate their shape. The experimental scattering curves, Guinier plots, and $P(r)$ functions are shown in supplementary Fig. S11. The $P(r)$ function was used to calculate a dummy atom model using the program DAMMIN (40). After 10 times calculation of DAMMIN, 10 independent dummy atom models were generated. Ten or 9 models were selected and averaged using the program DAMAVER (41), and then a second model calculation using DAMMIN was performed with a damast model derived from DAMAVER as starting model. Superpositions of the crystal structure onto SAXS envelopes of $h$SmgGDS-558 and $h$SmgGDS-607 were performed using the program SUPCOMB (42). Superpositions of the crystal structure onto SAXS envelopes of $h$SmgGDS-558/hrRhoA and $h$SmgGDS-607/hrRhoA were performed manually. The experimental SAXS curves of SmgGDS-558 and SmgGDS-607 were fit to the theoretical scattering curves calculated from the X-ray crystal structure with χ values of 2.25 and 2.72, respectively, using the program CRYSOL (43). Detailed structural parameters are summarized in supplemental Table S2.

Preparation of BODIPY-loaded RhoA or farnesylated RhoAL193A

Recombinantly processed RhoA or farnesylated RhoAL193A (10 μM final concentration) was incubated with 100 μM final concentration of BODIPY® FL GDP (Invitrogen, G22360) in buffer F (20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 10 mM EDTA, and 1 mM DTT) at 4 °C in the dark overnight. The reaction solution was purified by a Sephadex G-25 gel filtration column (GE Healthcare) in buffer G (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 5 mM EDTA, and 0.005% Tween 20) at 25 °C. At a flow rate of 30 μl/min, RhoA (wild type or farnesylated) was injected for 240 s and dissociated for 600 s as an analyte. In a single cycle, a five-point concentration series of non-farnesylated RhoA (10, 50, 100, 200, and 400 nM for SmgGDS-558 and 0.4, 2, 10, 50, and 100 nM for SmgGDS-607) and farnesylated RhoA (2, 10, 50, and 200 nM for SmgGDS-558 and 0.4, 2, 10, 50, and 100 nM for SmgGDS-607) was sequentially injected without regeneration. Under a 1:1 binding model, the association rate constants ($k_a$), dissociation rate constants ($k_d$), and dissociation constants ($K_D$) were determined by evaluating the single-cycle kinetic analysis.

Isothermal titration calorimetry

The ITC experiments were carried out at 25 °C in a buffer condition of 20 mM Hepes (pH 7.5) and 150 mM NaCl using MicroCal ITC200 (GE Healthcare). 10 μM SmgGDS was titrated by 100 μM RhoA PBR-CAA peptide (ARRGKKSGCLVL). The titration sequence included a single 0.4-μl injection followed by 18 injections of 2 μl each.
Crystal structure of SmgGDS

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