Supporting Information

Zasloff et al. 10.1073/pnas.1108558108

SI Materials and Methods

Computer Simulation (Laboratory of E.L.). All modeling was performed by means of molecular dynamics simulations using the LAMMPS package (1). To increase computational efficiency, the lipid molecules, squalamine molecules, Rac1 proteins, and their counterions were coarse-grained and represented by spherical beads or assemblies of beads that interacted through generic short-range potentials. In addition, all Coulombic forces were fully accounted for by Ewald summation.

Coarse graining. The system was set up to simulate the interaction between squalamine molecules and Rac1 proteins with the outer surface of a lipid bilayer membrane. This surface was modeled by 400 beads of uniform size of 8.5 Å, which were confined to the x–y plane. Using the small-angle X-ray scattering setup, we used a membrane in which 20% of the beads carried a charge of $-1e$. Coarse-grained models of the Rac1 and squalamine molecules were obtained using the VMD Shape-Based Coarse-Graining (SBCG) tool (2, 3). To accurately and efficiently reproduce the shape of a molecule, the SBCG tool generally uses a distribution of bead sizes. However, because the distance of closest approach between two beads determines the electrostatic binding, we opted for beads of uniform size, equal in diameter to the 8.5-Å beads representing the lipid head groups. This uniformity was achieved by adjusting the number of coarse-grained beads so that the average bead size was as close to 8.5 Å as possible, and then, all beads were set to that uniform size. For squalamine, this uniformity required three beads, with a separation of 9.6 Å between the head and body beads and a separation of 12.4 Å between the body and tail beads. The charge on each bead was assigned manually based on the coarse-grained model (i.e., $-1e$ for the acidic head, 0 for the hydrophobic body, and $+3e$ for the basic tail). For the coarse graining of the Rac1 protein, we isolated the Rac1 molecule from the Protein Data Bank file 2RMK (4) and then separated the protein into a tail domain and a body domain. The polybasic tail was coarse-grained with the same 8.5-Å building block as the squalamine and the lipid head groups to ensure that the electrostatic interaction strength between the tail and the membrane was modeled in a manner consistent with the squalamine–membrane attraction. The total charge on the tail was +5, which was divided into partial charges of +0.38, +1.53, +2.09, +1.09, +0.86, −0.04, and −0.91, respectively. For the weakly charged body of Rac1, steric interactions dominate, and therefore, a larger bead size (11.6 Å) could be used to increase computational efficiency while maintaining an accurate representation of the excluded volume. For both the body and the tail, the VMD SBCG tool was used to obtain the number of beads, their relative positions, equilibrium bond lengths between beads, and individual charges on each molecule.

Bead interactions. The interaction between bonded beads was modeled by a harmonic potential (Eq. **S1**):

$$U_{\text{bond}} = 200\varepsilon(r - r_0)^2, \qquad \textbf{[S1]}$$

where r_0 was the equilibrium bond length and ε was the Lennard–Jones (LJ) unit of energy. For squalamine, a bond angle potential was introduced to model its stiffness (Eq. **S2**):

$$U_{\text{angle}} = 4\varepsilon\,(\theta - \theta_0)^2, \qquad \textbf{[S2]}$$

with $\theta_0 = 180°$ of the equilibrium angle between the two bonds. All nonbonded beads interacted through a short-range, purely repulsive-shifted and truncated LJ potential that was truncated at the LJ energy minimum $r_{\text{cut}} = 2^{1/6}\,\sigma$, where σ is the LJ unit of length that is shifted by ε so that both energy and force vanished at r_{cut}. The cutoff r_{cut} was chosen as the effective diameter of the particle and equated to the bead diameter chosen in the coarse-graining procedure. Electrostatic energies and forces were computed using Ewald summation with a relative accuracy of 10^{-4}. Using information in the work by Stevens and Kremer (5) and customary lengths in coarse-grained simulations of electrostatic complexation phenomena, the Bjerrum length was set to 3σ. The solvent was modeled as a homogeneous dielectric medium, with Brownian effects represented by a Langevin thermostat with damping time 10τ, where τ is the LJ unit of time (Eq. **S3**):

$$\tau = \sqrt{\frac{m\sigma^2}{\varepsilon}}, \qquad \textbf{[S3]}$$

with m as the LJ unit of mass. The equations of motion were integrated using the velocity Verlet algorithm. The temperature was controlled by the Langevin thermostat as well, and it was set to $T = 1.2\varepsilon/k_B$, where k_B is Boltzmann's constant.

Simulation setup. The total number of squalamine molecules and Rac1 proteins was determined from the ratio of the total net (positive) charge on all molecules of either species to the total negative charge on the membrane surface. The net charge on one squalamine molecule is $+2e$, the net charge on one Rac1 protein is $+4e$, and a charge ratio of 1.5 with respect to the membrane charge was imposed. Accordingly, 60 squalamine molecules and 30 Rac1 proteins were placed in the simulation box. In addition, 80 positive and 120 negative monovalent counterions were present in the system to maintain charge neutrality. No additional salt was added. An orthorhombic simulation box was used with a linear size of 172 Å (corresponding to 20 lipid bead diameters) and periodic boundary conditions in the x and y directions. The height of the cell was 344 Å (40 lipid bead diameters), with repulsive LJ walls at the top and the bottom of the simulation box. These walls interacted with the same purely repulsive-shifted and truncated LJ potential as the beads. The molecular dynamics time step was set to 0.01τ, and a typical simulation run took 10 million steps, corresponding to $1 \times 10^5\tau$.

X-Ray Diffraction Measurements (Laboratory of G.C.L.W.). 1,2-Dioleoyl-sn-Glycero-3-[Phospho-L-Serine] (sodium salt), 1,2-Dioleoyl-sn-Glycero-3-Phosphocholine, and 1,2-Dioleoyl-sn-Glycero-3-Phosphoethanolamine, all purchased from Avanti Polar Lipids, were used without additional preparation. Lipid solutions in chloroform were dried under N_2 and desiccated under vacuum overnight. Dried lipids were rehydrated with buffer containing 50 mM Tris (pH 7.4) and 100 mM NaCl to a final concentration of 20 mg/mL, incubated at 37 °C overnight, sonicated, and extruded through a 0.2-μm Nucleopore (Whatman) filter to form small unilamellar vesicles. WT human Rac1 protein, purchased from Cytoskeleton, was reconstituted to 5 mg/mL in the following buffer: 50 mM Tris (pH 7.5), 0.5 mM $MgCl_2$, 50 mM NaCl, 0.5% (wt/vol) sucrose, 0.1% (wt/vol) dextran. Squalamine was dissolved in buffer containing 50 mM Tris (pH 7.4) and 100 mM NaCl to 1 mg/mL. Protein solutions were incubated with small unilamellar vesicles at specific protein to lipid molar ratios and sealed in quartz capillaries. X-ray diffraction was performed at the small-angle X-ray scattering spectrometer at the California Nanosystems Institute, University of California at Los Angeles, using CuKα radiation (λ = 1.54 Å) X-rays. The scattered in-

tensity was collected using a MAR Research Image Plate Detector (pixel size = 150 μm). Additional experimental details can be found in the work by Yang et al. (6).

Dengue Virus (Laboratory of I.M.). Viral infection of a line of human dermal microvascular endothelial cells that were grown on uncoated glass coverslips followed a published protocol (7). Den V2 was obtained from the Institute of Diagnostic and Epidemiological Reference in Mexico City. The strain was isolated from a patient who developed dengue fever. Monolayers of human dermal microvascular endothelial cells were pretreated with squalamine (10–100 μg/mL) for 2 h at 37 °C in MCDB-131 Medium (GIBCO); fresh medium without squalamine was added before viral exposure. Virus (at a multiplicity of infection of 3.0) remained in contact with cells for 30 min at 4 °C followed by 90 min at 37 °C. The medium was then replaced with fresh medium lacking virus, and cells were maintained at 37 °C for 48 h. Cells were then fixed and processed for immunohistochemical detection of viral E protein or for F-actin labeling with rhodamine–phalloidin. Viral E protein expression was used to monitor the percentage of infection (7). In the absence of drug, about 38% of cells were infected. Ten randomly chosen fields were counted in control coverslips and those coverslips treated with squalamine.

Hepatitis Viruses (Laboratory of J.M.T.). Monolayer cultures of primary human hepatocytes were infected with human hepatitis B virus (HBV) or human hepatitis δ-virus (HDV) using a modification of procedures as described in the work by Taylor and Han (8). Cells were exposed to HBV in the presence of 5% polyethylene glycol in serum-free hepatocyte growth medium (HGM; Cellzdirect) for 16 h and then replaced with virus-free medium. In one series of experiments, squalamine was present 1 h before addition of virus and remained in the medium for 16 h. In a second series, squalamine was added at 24 h after viral infection and remained in the medium for 16 h. Medium-containing virus and squalamine was replaced with fresh medium (drug- and virus-free), and both treated and untreated cells were maintained for 12 d. Total RNA was extracted, with concentrations as indicated, and assayed per unit of RNA by real-time PCR for HBV sequences as described (8). The experimental points were performed in at least triplicate.

Infections with HDV were performed and assayed in a manner very similar to HBV, except that HDV exposure was limited to 3 h and was performed in the absence of polyethylene glycol (8). The period of infection was reduced to 7 d.

We could not directly evaluate the virucidal activity of squalamine against HBV because of the dilute concentration of virus available to us, which would require additional dilution of the pretreated sample before assay. Because HDV uses the same viral envelope as HBV, we evaluated the virucidal activity against HDV by incubating the HDV inoculum with squalamine (20 μg/mL) in HGM or an equivalent volume of drug-free HGM for 3 h at 37 °C. The samples (run in triplicate) were diluted with HGM containing 5% PEG, and primary hepatocytes were exposed overnight followed by replacement with fresh medium. Viral replication was assayed as above at 7 d.

Yellow Fever Virus (Laboratory of J.J.). ***Animals.*** Female Golden Syrian hamsters (*Mesocricetus auratus*) with an average weight of 110 g were used after a quarantine period of greater than 48 h. Experiments were conducted in the biosafety level 3 animal suite at the Utah State University Laboratory Animal Research Center. All personnel continue to receive special training on bloodborne pathogen handling by this university's Environmental Health and Safety Office. Standard operating procedures for biosafety level 3 were used.

Virus. Jimenez, a hamster-adapted yellow fever virus strain, was obtained as a gift from Robert B. Tesh (Galveston, TX). The virus was inoculated into five adult female hamsters. The livers of the infected hamsters were removed 3 d postinjection and homogenized in a 2× volume of sterile PBS. This liver homogenate had a titer of $10^{6.0}$ 50% cell cultures infectious doses/mL ($CCID_{50}$) and served as the stock for subsequent studies.

Virus inoculations, treatment, and postexposure monitoring. Hamsters were randomly assigned to groups, with 10 included in each group and 20 placebo-treated controls. A 10^{-4} dilution ($10^{2.0}$ $CCID_{50}$/mL) of the virus was prepared in minimal essential media. Hamsters were injected i.p. with 0.1 mL diluted virus (10 $CCID_{50}$/animal). Squalamine was administrated s.c. at a total daily dose of either 15 or 30 mg/kg as indicated one time per day. To evaluate protection, dosing began at 24 h before introduction of virus and ended on day 6; in the case of the treatment experiment, dosing began on either day 1 or 2 after infection and continued until days 8 or 9, respectively. Ribavirin was administered i.p. at total daily doses of 3.2, 10, or 32 mg/kg per d administered two times daily beginning 24 h before introduction of virus and ending on day 6 (protection experiment) or at a total daily dose of 75 mg/kg administered i.p. two times daily (37.5 mg/kg). Hamsters were observed daily for mortality for a total of 21 d, and body weight was recorded on 0, 3, and 6 d postinfection. Serum alanine amino transferase was measured as previously described (9).

Statistical analysis. Survival data were analyzed using the Wilcoxon log-rank survival analysis. and all other statistical analyses were done using one-way ANOVA followed by Bonferroni's multiple comparison test (Prism 5; GraphPad Software, Inc). Values of $P \leq 0.05$ were considered significant.

Eastern Equine Encephalitis Virus (Laboratory of A.P.A. and S.C.W.). ***Animals.*** Sixteen 6-wk-old Golden Syrian hamsters were purchased from Charles River Laboratories and housed in the animal biosafety level 3 facilities at the University of Texas Medical Branch (UTMB). All experimental animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee at UTMB.

Virus. The North American eastern equine encephalitis virus strain FL93-939 was provided by the UTMB World Reference Center for Emerging Viruses and Arboviruses and was originally isolated from a 1993 Florida pool of *Culiseta melanura* mosquitoes. An infectious cDNA clone of this strain was subsequently developed by RT-PCR amplification and cloning as previously described (10). Virus was subsequently rescued from the infectious cDNA clone, and virus stocks were titered by plaque assay using Vero (monkey kidney) cells as previously described (11).

Virus inoculations, treatment, and postexposure monitoring. Sixteen animals received an s.c. inoculation of 3.0 $\log_{10}$ pfu strain FL93-393 in a 0.1-mL volume; 24 h before infection and on days 0–6 after infection, one cohort of 10 animals was treated with squalamine (10 mg/kg s.c.), and another cohort of 6 animals was treated s.c. with D5W placebo. After infection, all animals were monitored daily for clinical signs of disease, including anorexia, lethargy, abnormal neurologic signs, or mortality. At −1 to 14 d postinfection, body weight was recorded, and at 1–4 d postinfection, blood samples were collected from the retroorbitus for virus titration. Virus titrations were performed on the sera by plaque assay using Vero cells as previously described (11). The limit of detection for the assay was 100 pfu/mL.

Statistical analysis. Survival data were analyzed using the log-rank test, and for viremia data, a statistical comparison was performed using a one-way ANOVA followed by Bonferroni's multiple comparison test (GraphPad). Values of $P \leq 0.05$ were considered significant.

Murine Cytomegalovirus (Laboratory of A.C.). ***Animals.*** Male BALB/c mice (H-2^d haplotype) were purchased from Harlan Laboratories and housed in the animal facility at East Virginia Medical

School in sterile microisolator cages with sterile food, water, and bedding. All animal procedures were approved by the Animal Care and Use Committee of Eastern Virginia Medical School and adhered to the guidelines established by the US Animal Welfare Act.

Virus. The Smith strain of murine cytomegalovirus (VR-194) was used and prepared for inoculation through passage in 3-wk-old male BALB/c weanling mice as previously described (12).

Virus inoculations, treatment, and postexposure monitoring. In all experiments, adult mice (6 wk of age) were infected by i.p. injection with 1×10^3 pfu virulent salivary gland passaged murine cytomegalovirus. Squalamine was administered either i.p. or s.c. at a dose of 10 mg/kg beginning 1 d before virus inoculation. Controls received an equivalent volume of vehicle (D5W) through the i.p. route. Each cohort was represented by 18 animals. Groups of mice ($n = 6$) were killed at days 3, 7, and 14 d postinfection, and livers, spleens, lungs, and salivary glands were harvested. These tissues were then processed for viral titers as described (12). The limit of detection was 10 pfu/mL.

Statistical analysis. For viral titers, statistical comparisons were performed using a one-way ANOVA (GraphPad). Values of $P \leq 0.05$ were considered significant.

Videos of Rac1 and Squalamine near a Model Membrane. ***Description of video. rac1_only.mp4.*** This video (Movie S1) shows 68 Rac1 proteins (accompanied by 180 positive and 272 negative monovalent counterions) in thermal equilibrium. The purple Rac1 tails associate with the blue membrane beads representing charged lipids. For Rac1 in solution, the tails are frequently seen to latch onto nearby Rac1 proteins; this finding is because of negatively charged domains on those proteins.

The run time of this video is 679τ (see description of the time unit τ below), with one frame per time unit.

squalamine_only.mp4. This video (Movie S2) shows 135 squalamine molecules (accompanied by 180 positive and 270 negative monovalent counterions) in thermal equilibrium. The red (positive) sides of squalamine near the membrane associate with blue (negatively charged) lipids.

The run time of this video is 679τ, with one frame per time unit.

squalamine_rac1_mixture.mp4. This video (Movie S3) shows 68 squalamine molecules and 135 Rac1 proteins (accompanied by 180 positive and 542 negative monovalent counterions) in thermal equilibrium. The Rac1 proteins can now be observed to be considerably more dispersed through the bulk and less associated with the membrane than in the absence of squalamine (Movie S1).

The run time of this video is 679τ, with one frame per time unit.

rac1_displacement.mp4. The components in this system (Movie S4) are identical to the components in Movie S3, but now, the initial state is created from an equilibrium configuration of 68 Rac1 proteins associated with the membrane (Movie S1), to which 135 squalamine molecules have been added in the top one-half of the simulation cell. Movie S4 displays how the system reaches thermal equilibrium (i.e., the state depicted in Movie S3). During this process, squalamine diffuses to the membrane and displaces part of the Rac1 proteins. This process is particularly noticeable in the last one-third of Movie S4, where the number of Rac1 proteins near the membrane has decreased significantly.

The run time of this video is 2,716τ (four times longer than Movies S1–S3), with one frame per four time units.

Color coding. The lipid membrane is represented by 900 blue and yellow beads of uniform size of 8.5 Å. Yellow beads are neutral, and blue beads carry a charge of $-1e$. Charged beads represent 20% (180 of 900) of all lipids.

Each *Rac1* molecule is represented by two domains: the body is composed of cyan-colored beads of size 11.6 Å, and the tail is composed of purple-colored beads of size 8.5 Å. The body of Rac1 carries a net charge of $-1e$, and the tail carries a net charge of $+5e$.

Each squalamine molecule is represented by two pink beads and one red bead of uniform size of 8.5 Å. The red bead carries a charge of $+3e$, and the pink bead on the opposite end carries a charge of $-1e$.

Ions are represented by green and orange beads, with orange ions carrying a positive unit charge and green ions carrying a negative unit charge. The counterions are simulated using size 8.5 Å, but for visual clarity, they are represented at one-half their size in Movies S1–S4.

Technical information regarding the simulations. Movies S1–S4 were created from coarse-grained molecular dynamics simulations in which all excluded volume and electrostatic interactions were fully taken into account (model as described in *SI Materials and Methods*). The simulation cell has a width and depth of 255 Å and a height of 340 Å, and it is periodically replicated in the x and y directions. All simulations used a molecular dynamics time step of 0.01τ, with τ defined in Eq. **S3**.

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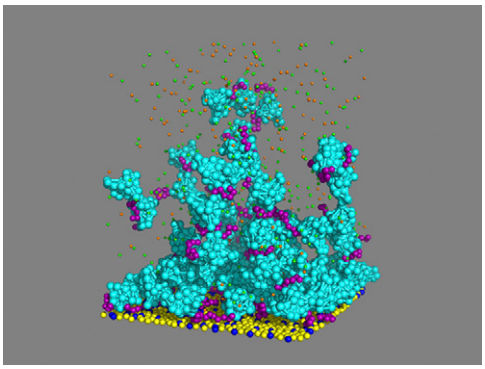
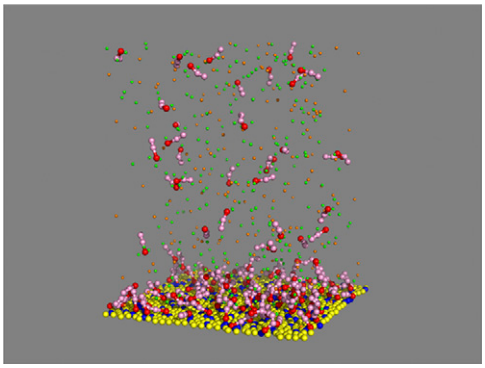


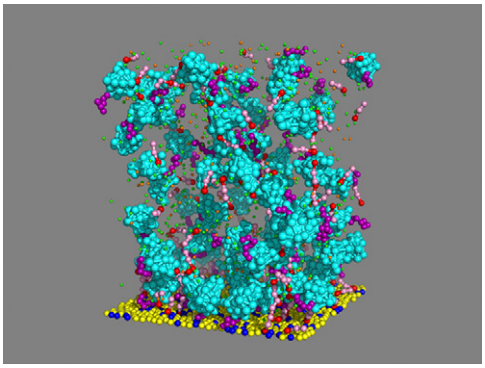
Movie S1. Rac1 only. Details of movie in *SI Materials and Methods*.

Movie S1



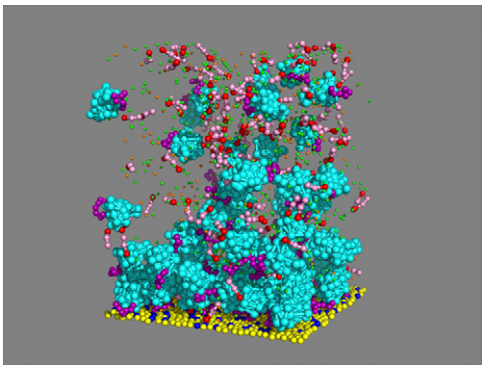
Movie S2. Squalamine only. Details of movie in *SI Materials and Methods*.

Movie S2



Movie S3. Squalamine Rac1 mixture. Details of movie in *SI Materials and Methods*.

Movie S3



Movie S4. Rac1 displacement by squalamine. Details of movie in *SI Materials and Methods*.

Movie S4