1 **Dectin-1 mediated DC-SIGN Recruitment to *Candida albicans* Contact**

2 **Sites**

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11 **Abstract**

12 At host-pathogen contact sites with *Candida albicans*, Dectin-1 activates pro-
 13 inflammatory signaling, while DC-SIGN promotes adhesion to the fungal surface. We
 14 observed that Dectin-1 and DC-SIGN collaborate to enhance capture/retention of *C.*
 15 *albicans* under fluid shear culture conditions. Therefore, we devised a cellular model
 16 system wherein we could investigate the interaction between these two receptors during
 17 the earliest stages of host-pathogen interaction. In cells expressing both receptors, DC-
 18 SIGN was quickly recruited to contact sites (103.15% increase) but Dectin-1 did not
 19 similarly accumulate. Once inside the contact site, FRAP studies revealed a strong
 20 reduction in lateral mobility of DC-SIGN (but not Dectin-1), consistent with DC-SIGN
 21 engaging in multivalent adhesive binding interactions with cell wall mannoprotein ligands.
 22 Interestingly, in the absence of Dectin-1 co-expression, DC-SIGN recruitment to the
 23 contact was much poorer—only 35.04%. These data suggested that Dectin-1 promotes the
 24 active recruitment of DC-SIGN to the contact site. We proposed that Dectin-1 signaling

25 activates the RHOA pathway, leading to actomyosin contractility that promotes DC-SIGN
 26 recruitment, perhaps via the formation of a centripetal ActoMyosin Flow (AMF) directed
 27 into the contact site. Indeed, RHOA pathway inhibitors significantly reduced Dectin-1
 28 associated DC-SIGN recruitment to the contact site. We used agent based modeling to
 29 predict DC-SIGN transport kinetics with (“Directed+Brownian”) and without
 30 (“Brownian”) the hypothesized actomyosin flow-mediated transport. The
 31 Directed+Brownian transport model predicted a DC-SIGN contact site recruitment
 32 (108.72%), similar to that we observed experimentally under receptor co-expression.
 33 Brownian diffusive transport alone predicted contact site DC-SIGN recruitment of only
 34 54.02%. However, this value was similar to experimentally observed recruitment in cells
 35 without Dectin-1 or treated with RHOA inhibitor, suggesting that it accurately predicted
 36 DC-SIGN recruitment when a contact site AMF would not be generated. TIRF microscopy
 37 of nascent cell contacts on glucan-coated glass revealed Dectin-1 dependent DC-SIGN and
 38 F-actin (LifeAct) recruitment kinetics to early-stage contact site membranes. DC-SIGN
 39 entry followed F-actin with a temporal lag of 8.35 ± 4.57 seconds, but this correlation was
 40 disrupted by treatment with RHOA inhibitor. Thus, computational and experimental
 41 evidence provides support for the existence of a Dectin-1/RHOA-dependent AMF that
 42 produces a force to drive DC-SIGN recruitment to pathogen contact sites, resulting in
 43 improved pathogen capture and retention by immunocytes. These data suggest that the
 44 rapid collaborative response of Dectin-1 and DC-SIGN in early contact sites might be
 45 important for the efficient acquisition of yeast under flow conditions, such as those that
 46 prevail in circulation or mucocutaneous sites of infection.
 47

1. Introduction

C-type lectin receptors play an important role in recognition of the two major fungal cell wall polysaccharide ligands exposed at the host-pathogen interface. DC-SIGN recognizes abundantly exposed N-mannan in the outer cell wall whereas Dectin-1 recognizes nanoscale exposures of β -(1,3)-glucan. Recognition of β -glucan by Dectin-1 contributes to phagocytosis, oxidative burst, regulation of transcription, production of inflammatory cytokines and chemokines, and initiation of adaptive immunity[1]. DC-SIGN is known to mediate intercellular adhesion, as well as antigen uptake and signaling in dendritic cells (DCs)[2]. We are examining the relationship between Dectin-1 and DC-SIGN to understand, in a simplified model, how an effective host-pathogen contact is built. We focused on the earliest events in fungal contact site biogenesis.

Initial pathogen capture and formation of a stable contact site are the earliest events that must occur for signaling through antifungal receptors to initiate. Our previous work with zymosan particles demonstrated that human monocyte derived dendritic cells (DC) form durable contacts between the DC plasma membrane and extracellular fungal particles, which may be important for the antigen gathering functions of these cells [3]. Rapid formation of adhesive contact site structures is especially important for *C. albicans* capture under conditions involving fluid shear stress, for example by reticuloendothelial macrophages capturing yeast in the bloodstream. Fungal recognition under fluid shear also pertains to phagocytes interacting with *Candida* in the oropharyngeal cavity, a major site of mucocutaneous candidiasis, where the host-pathogen interaction is subject to salivary flow.

70 Various authors have described the accumulation of pattern recognition receptors,
71 such as Dectin-1 and DC-SIGN, at fungal contact sites[4–6]. Immune cells must mobilize
72 receptors to these contact sites for activation, crosstalk and amplification of signaling that
73 directs downstream immune responses. In fact, these contact sites achieve an ordered
74 segregation of molecular components with a peripheral zone enriched in the large
75 transmembrane phosphatase CD45 and a central zone where DC-SIGN and Dectin-1
76 concentrates. Such “phagocytic synapses” can also involve the development of barriers to
77 molecular diffusion that support specialized signaling processes occurring therein[7,8].
78 These findings suggest that PRRs are recruited to fungal contacts in some fashion to
79 support their enrichment at these sites. Active and passive transport processes might
80 conceivably account for observed receptor recruitment, but the molecular mechanisms of
81 innate immunoreceptor recruitment in contact sites with *C. albicans* have not been defined.

82 Previous studies from our group and others have shown the enrichment of DC-
83 SIGN and CD-206 at fungal contact sites[4–6,9]. These studies are typically conducted at
84 longer time scales of hours, which is relevant to processes such as cytokine response and
85 cytotoxic effector responses. However, there is much less information on the dynamics of
86 pattern recognition receptors at fungal contact sites on the time scale of minutes—a time
87 scale that is relevant to the earliest signaling events necessary for innate immune fungal
88 recognition. In the intensely studied immunologic synapse, it is known that
89 immunoreceptors in the T cell/Antigen Presenting Cell (APC) immune synapse are actively
90 transported into the synapse within minutes via their coupling to a centripetal
91 RHOA/Myosin II dependent actomyosin flow (AMF)[10]. Likewise, we previously
92 demonstrated that that Dectin-1 stimulation by glucan activates mechanical contractility

93 signaling via a RHOA/ROCK/Myosin II signaling module within minutes post-stimulation
 94 [11]. Thus, the central hypothesis tested in this study is that Dectin-1 activates a transport
 95 mechanism, through RHOA/ROCK/Myosin II dependent signaling processes, which
 96 facilitates the recruitment of DC-SIGN to the contact site. This would be expected to
 97 improve fungal particle retention by providing higher avidity adhesive interactions with
 98 the fungal cell wall.

99 We used a micropipette-micromanipulation approach to provide very high
 100 spatiotemporal control over host-pathogen contact site formation. We report that Dectin-
 101 1, in collaboration with DC-SIGN, does promote improved capture of *C. albicans* yeast.
 102 This occurs through improved recruitment of DC-SIGN to the contact site in a manner that
 103 is dependent upon Dectin-1 signaling via RHOA, ROCK and myosin II. These findings
 104 provide a high-resolution view of early events in receptor recruitment processes that tailor
 105 the earliest stages of the innate immune anti-fungal response.

106

107 **2. Materials and Methods**

108 *2.1 Cell culture*

109 HEK-293 cells (ATCC, #CRL-1573) were cultured in DMEM containing 10%
 110 FBS, 1% penicillin-streptomycin, 2 mM L-glutamine and 1 mM sodium pyruvate at 37°C,
 111 in a 5% CO₂ environment in an incubator. The identity and mycoplasma-free status of the
 112 cell line was independently confirmed by submission to ATCC Human Cell Line
 113 authentication (STR) and mycoplasma detection (PCR) services.

114

115 *2.2 Transfection*

mApple-Dectin1A-C-10 was a gift from Michael Davidson (Addgene plasmid # 54883; <http://n2t.net/addgene:54883>; RRID:Addgene_54883). pEGFP-DC-SIGN was a generous gift from Ken Jacobson[12]. pUNO1-hDectin-1a was purchased from Invivogen (#punol-hdectin1a). mCardinal-Lifeact-7 was a gift from Michael Davidson (Addgene plasmid #54663). Transfection with plasmid was performed following standard protocols for Fu gene 6 (Promega, #E2691). Cells were selected for stable expression using Geneticin (G418 sulfate) (Thermo Fisher Scientific, 10131035; for pEGFP-DC-SIGN) at 400 µg/ml or Blasticidin (Invivogen #ant-bl-05; for pUNO1-hDectin-1a) at 20 µg/ml for 2 weeks.

124

2.3 Micropipette

1.5 mm outer diameter and 1.12 mm inner diameter borosilicate glass capillaries were purchased from World Precision Instruments (WPI# TW150-4). We optimized fabrication procedures to obtain micropipettes with 2 µm diameter tips. We used a microelectrode puller (WPI #micrPUL-1000) for pulling micropipettes. Our final protocol for pulling micropipette of 2 µm was as follows.

	Heat	Force	Distance	Delay
1	680	200	2.50	55
2	600	170	1.50	60
3	550	150	0.60	35
4	500	100	0.30	20

Table 1. Values of each parameter in WPI microelectrode puller used to pull 2 µm diameter tip micropipettes.

133

134 2.4 Fungal culture

135 *C. albicans* clinical isolate TRL035 was obtained as previously described[13].
 136 Isolate was stored as single-use glycerol stock aliquots -80°C. This stock was transferred
 137 to 5 ml sterile yeast extract-peptone-dextrose (YPD) medium (Becton Dickinson) at
 138 concentration of 1×10^5 cell/ml of YPD and grown for 16 hours at 30°C, with a shaking
 139 speed of 300 rpm. The glycerol stock contained 4×10^7 yeast/ml and was previously
 140 calibrated to provide 3×10^8 /ml yeast cells at the late log phase under the stated growth
 141 conditions.

142

143 2.5 Silicone chambers

144 We used silicone isolators (Grace Bio-labs # 665116 and # 665203) on cover glass
 145 in configurations as shown below (Fig. 1). Whole chamber was sterilized by passing it
 146 through a Bunsen burner blue flame 5 times.

147

Figure 1. Arrangement of silicone chambers for growing HEK-293 cells for contact site studies.

2.6 Contact site studies

HEK-293 cells were transfected with mApple-Dectin1A-C-10 and stable lines were generated, as described above. 2 days before each experiment mApple-Dectin1A-C-10 stable line cells were transiently transfected with pEGFP-DC-SIGN. Next day cells and yeast were seeded into separate compartments of sterilized silicone chambers in a configuration as shown below (Fig. 2).

Figure 2. Separation of HEK-293 cells and yeast within chamber.

Cells were stained with CellMask Deep Red Plasma Membrane stain (CMDR) (Invitrogen, #C10046). Original stock CMDR was freshly diluted 1:100 in culture medium to form a working stock solution. 10 µl working stock CMDR was added per 1 ml of culture medium in the culture vessel. Depending on the condition, along with CMDR, various inhibitors were added during this stage. Blebbistatin (Sigma-Aldrich, #203390) at 12.5 µM, or Y-27632 (Sigma-Aldrich, #Y0503) at 5 µM were added for 1 hour. For RHO inhibitor

169 conditions, cells were treated with C3 transferase (Cytoskeleton Inc., #CT04) 1.5 µg/ml for
170 2 hours. Both CMDR and inhibitor were added to culture dish at the same time. TRL035
171 *C. albicans* were stained with Fluorescent Brightener 28 (Calcofluor White) (Sigma-
172 Aldrich #F3543). 25 µl of 1 mg/ml Calcofluor White was used to stain 1 ml of yeast in
173 PBS pH 7.4 for 15 min. Then yeast were washed three times with PBS and vortexed for 15
174 minutes. *C. albicans* were added to smaller chambers as shown in Figure 2. Glucose
175 oxidase (Sigma-Aldrich # G2133) and catalase (Sigma-Aldrich # C100) were added to the
176 chamber at concentration of 0.5 mg/ml and 40 µg/ml, respectively, during data acquisition
177 to reduce photobleaching.

178 Micropipettes were filled with PBS using MicroFil (WPI #MF28G-5) and
179 syringe. The PBS-filled micropipette was then attached microelectrode holder (WPI #
180 MPH415). The inlet of the microelectrode holder was attached to 1 ml syringe using Luer
181 lock via plastic tubing.

182 For micromanipulation, we used a Sensapex micromanipulator. We attached
183 microelectrode holder to micromanipulator using an electrode handle (WPI #2505). The
184 silicon chamber was then placed on the FV1000 laser-scanning confocal microscope
185 (Olympus, Center Valley, PA) with controlled temperature, 37°C at 5% CO₂. We used a
186 60x super-corrected, 1.40 NA, Plan-Apochromat oil immersion objective for imaging cells.
187 We replaced one microscope eye piece with a Centering Telescope eyepiece (Olympus #
188 U-CT30-2), which allowed a separate focal plane in each eyepiece for ease in positioning
189 the micropipette.

190 We identified a suitable single *C. albicans* yeast for capture and then adjusted the
191 microscope's focal plane to ~20 µm above that yeast. We focused the Centering Telescope

192 eyepiece on the tip of micropipette. The telescope eyepiece was then focused at a plane
 193 closer to the yeast, then we lowered the tip of micropipette via micromanipulator into the
 194 lower focal plane toward the yeast, while being monitored through the telescopic eyepiece.
 195 This process was repeated till we reached the level of fungus. This was done to avoid
 196 breaking of micropipette tip while lowering it. We always lowered telescopic eyepiece
 197 focus first and then adjusted micropipette level. Once at the same level as *C. albicans*, we
 198 applied negative pressure using the syringe to capture a single yeast on the micropipette
 199 tip. Then, we manipulated *C. albicans* to the chamber with HEK-293 cells by moving from
 200 the yeast chamber, over the isolator barrier, and translating into the cell chamber, keeping
 201 the tip submerged at all times. Finally, *C. albicans* was brought near the plasma membrane
 202 of a HEK-293 cell expressing the appropriate receptors, as verified by their fluorescent
 203 protein tags.

204 Confocal fluorescence microscopic observation of contacts sites was conducted
 205 with the following parameters. Calcofluor White (a marker for all yeast) was excited with
 206 a 50 mW, 405 nm diode laser operated at 1% power, and CMDR was excited with a 20
 207 mW, 635 nm diode laser operated at 0.5% power. EGFP–DC-SIGN was excited with a 20
 208 mW, 473 nm diode laser operated at 1% power. mApple-Dectin-1 was excited with a 20
 209 mW 559 nm laser at 1% power. These lines were reflected to the specimen by a
 210 405/473/559/ 635 multi-edge main dichroic element and routed through a confocal pinhole
 211 (110 mm diameter) to secondary dichroic followed by bandpass emission filters in front of
 212 two independent PMT detectors and two independent high-sensitivity GaAsP PMT
 213 detectors (HSD). Specifically, the emission light passed by the main dichroic was directed
 214 to PMT1 (Calcofluor White channel) via reflection from the SDM473 dichroic and passage

215 through a BA430-455 nm bandpass filter. For the CMDR channel, light from SDM473 was
 216 directed to a 640 nm shortpass dichroic and BA575-675 nm bandpass filter. Light from
 217 640 nm shortpass dichroic was directed to SDM560 filter cube to HSD1 (the EGFP-DC-
 218 SIGN channel) via passage through a BA490-540 nm bandpass filter. For mApple-Dectin-
 219 1, light was directed via SDM560 filter cube to HSD2 via passage through a BA575-675
 220 nm. 60x lens with 3x zoom was used to capture images. Further a subregion of interest for
 221 image scanning was selected such that the region was small enough to be scanned at a rate
 222 of 0.400 sec per frame. Overall, pixel size for all images was 7.24 pixels per micron.
 223 Imaging was started and then, with the micromanipulator, contact was made between *C.*
 224 *albicans* and HEK-293 cells. The contact site was imaged for 10 minutes total duration
 225 after contact initiation.

226

227 *2.7 Polystyrene bead control for contact site studies*

228 For making Dextran coated beads, we used 5 μ m streptavidin-coated polystyrene
 229 bead (Spherotech #VP-60-5). We used 1,1'-carbonyldiimidazole in DMSO based system
 230 as described by Tam et al. to conjugate dextran (Sigma-Aldrich # 31388) with beads. Rest
 231 of the procedure for making contact and imaging was exactly same for TRL035 *C.*
 232 *albicans*.

233

234 *2.8 FRAP studies*

235 For FRAP studies, exact same steps as mentioned above for contact site studies
 236 were followed. Then TRL035 contact site was allowed to mature for 10 min. after contact.
 237 Then a rectangular FRAP window was selected so that it included the whole of the contact

238 site. Imaging was started and 5 frames were collected pre-bleach. Then, we photobleached
239 the contact area with 473 nm and 559 laser, 100% power for 500 milliseconds. Imaging
240 was continued for 10 min. to quantify recovery. FRAP analysis was done using
241 easyFRAP[14].

242

243 *2.9 Analysis of contacts site data*

244 For quantifying the contact site, we used the Fiji distribution of ImageJ. We
245 demarcated overlapping pixels between dilated calcofluor channel and CMDR channel.
246 These overlapping pixels denote the contact site. All further calculation of MFI (Mean
247 Fluorescence Intensity) for DC-SIGN, Dectin-1 channel and their normalizations were
248 done from these contact site pixels only. The detailed steps followed were as follows.
249 Calcofluor white (405 channel) was thresholded to make a fungal mask and converted to
250 binary. The binary fungal mask was then dilated 2 times. The fungal mask was divided by
251 255 to make all pixel values 1. This is essential for calculating overlapping areas in next
252 steps. The CMDR (635 channel) was thresholded and converted to binary to create a
253 CMDR mask. The CMDR mask was divided by 255. The fungal mask was multiplied by
254 CMDR mask to mark overlapping pixels as those demarcating the contact site mask, within
255 which each pixel had a numerical value of 1. The remaining non-mask pixels had a
256 numerical value of 0. Overlapping pixels were multiplied with Dectin-1, DC-SIGN and
257 CMDR raw pixel intensities, creating masked Dectin-1, DC-SIGN and CMDR datasets.
258 RawDensity, which is the sum of intensities of all pixels in a dataset, was calculated for
259 each the contact site masked DC-SIGN ,Dectin-1 and CMDR datasets. The same
260 calculation was performed for the contact site mask image, which provides the area (pixels)

261 of the contact site mask. Mean Fluorescence Intensity (MFI) per pixel for DC-SIGN, and
262 Dectin-1, and CMDR were calculated by dividing RawDensity for each of these datasets
263 by the contact site area in pixels. To normalize for variable amount of membrane in a
264 contact site, we divided MFI of DC-SIGN and Dectin-1 by the corresponding contact site
265 CMDR MFI. Finally, to control for possible differential expression/staining of individual
266 HEK-293 cells, we expressed the above normalized receptor MFI signals as a percentage
267 of their value at time 0, on a per cell basis.

268

$$MFI = \left[\frac{\frac{Receptor\ MFI\ at\ time\ x}{CMDR\ MFI\ at\ time\ x}}{\frac{Receptor\ MFI\ at\ time\ 0}{CMDR\ MFI\ at\ time\ 0}} \right] * 100$$

269

270

271 2.10 Yeast capture assay

272 For the yeast capture assay, we used HEK-293 cells stably transfected mApple-
273 Dectin1A-C-10 cells were used or transiently transfected with pEGFP-DC-SIGN. For DC-
274 SIGN only condition parental cells were transiently transfected with pEGFP-DC-SIGN.
275 Overall, the following 4 conditions were used for experiments, 1) EGFP-DC-SIGN +
276 mApple-Dectin-1, 2) EGFP-DC-SIGN, 3) mApple-Dectin-1, 4) Untransfected conditions.
277 Cells were seeded at 2.5×10^4 /dish, overnight in 35 mm Mattek dishes. Then TRL035 *C.*
278 *albicans* stained with calcofluor white were added to dishes at 10×10^4 /dish. Then dishes
279 were kept on rocker shaker (BR200 2d rocker, Southwest Science) for 30 min. Cells were
280 then washed 3 times with PBS and fixed with 4% PFA, and the number of fungi attached
281 to each cell of interest were counted under microscope with imaging condition similar to
282 described in contact site studies. Binding studies with cells expressing both DC-SIGN and
283 Dectin-1 enumerated yeast binding only to cells confirmed to co-express both receptors.

284

285 *2.11 TIRF microscopy*

286 HEK-293 cells were stably transfected with pUNO1-hDectin-1a and mCardinal-
 287 LifeAct-7. 1 days before experiment these cells were transiently transfected with EGFP-
 288 DC-SIGN. Cells were dissociated from dish surfaces using brief exposure to 0.25%
 289 trypsin-EDTA followed by addition of protein rich medium to rapidly quench trypsin
 290 activity and washing into fresh culture medium. Then these cells were put on 35 mm dish
 291 coated with β -glucan. Cells were allowed to settle down on these surfaces in incubator for
 292 30 min. Then cell membrane was observed under Olympus IX83 TIRF/Single Molecule
 293 Localization Microscope. 488 nm and 561 nm lasers were used to excite EGFP-DC-SIGN
 294 and mCardinal-LifeAct respectively. Cell membrane was observed for 5 min. To address
 295 the potential concern that trypsinization would damage transmembrane receptors
 296 sufficiently to render cells non-responsive to fungal ligands, we loaded cells with Fluo-4
 297 calcium dye, trypsinized and settled them on glucan coated glass as above, and observed
 298 calcium flux upon Dectin-1 contact with glucan coated glass. We observed calcium flux
 299 on glucan coated glass but not on glucan free glass surfaces, demonstrating that the cells
 300 remained functional following trypsinization (data not shown)

301

302 *2.12 Coating dishes with β -glucan*

303 The central glass region of Mattek glass bottom 35 mm dishes was coated with β -glucan
 304 to permit TIRF microscopic observations of early contact site membrane dynamics using
 305 a coupling chemistry previously reported by Tam, et al.[6]. The following procedure was
 306 used to produce these surfaces. 200 μ l of 0.01 w/v Poly-l-lysine aqueous solution (Sigma,

P4707) was put on the central portion of 35 mm dishes and adsorption was permitted to occur for 30 min. Excess solution was removed and dishes were washed with DMSO for 3 times. Poly-l-lysine amines were activated by immersion in 0.5M 1,1'-Carbonyldiimidazole (CDI; Sigma, 115533) in DMSO for 1 hour. Dishes were washed with DMSO 3 times. 10 mM Medium Molecular Weight (145 kDa) β -(1,3)-glucan from ImmunoResearch Inc. (Eagan, MN) in DMSO was added to the dishes. The reaction was allowed to incubate overnight, excess solution was removed, and dishes were washed with water 3 times.

315

2.13 Phagocytosis assay

TRL035 *C. albicans* were first stained with Fluorescent Brightener 28 (calcofluor white) (Sigma-Aldrich #F3543). 25 μ l of 1 mg/ml calcofluor white was used to stain 1 ml of 16 hour fungal culture in PBS (Gibco) pH 7.4 for 15 minutes. Then cells were washed 3 times with PBS and stained with 75 μ M CypHer5E NHS ester (GE Healthcare, PA #15401) for 1 hour at 25°C[13]. *C. albicans* were then added to culture dishes with HEK-293 cells transiently transfected with pEGFP-DC-SIGN for 1 hour and observed under a microscope for increased fluorescence in CypHer5E channel as an indicator of fungi which had been phagocytosed.

325

2.14 Agent Based Modeling

An agent-based model of DC-SIGN transport into contact sites was created in the Netlogo modeling environment. Source code and a full description of the parameterization of this model are provided in Supplemental Methods.

3. Results

3.1 Dynamics of Dectin-1 and DC-SIGN recruitment to a contact site for capture of *C. albicans*

We examined the possible role of Dectin-1 and/or DC-SIGN in the capture of fungal particles using a *C. albicans* yeast capture assay. This assay was designed to test the ability of cells expressing these receptors, alone or in combination, to bind and stably capture yeast after a relatively brief exposure to particles under conditions of fluid shear. To unambiguously measure fungal capture attributable to these receptors, we used a HEK293 cell system with no endogenous expression of relevant PRRs, which was then transfected to express one or both receptors. We found that, within 30 min., cells captured an average of 5.1, 1.62, 2.23 and 0.23 *C. albicans* fungal particles per cell under DC-SIGN + Dectin-1, Dectn-1 only, DC-SIGN only and untransfected/parental HEK-293 conditions, respectively (Figure 3a). We found significantly increased capture of fungal particles in the co-expressed condition compared to the condition with either individual receptor expressed alone. This effect was slightly more than additive. Given the normal distribution of yeast capture measured in co-expressing cells, we found that there is only 3.2% probability to find a fungal capture equivalent to the sum of means of yeast capture measured for cells expressing DC-SIGN and Dectin-1 alone. We hypothesized that DC-SIGN and Dectin-1 collaborate to improve capture of *C. albicans* yeast in rapidly developing contact sites with this pathogen.

To look for receptor recruitment dynamics at the contact site, we used micropipette-micromanipulator based system where a single fungal particle was adhered to the tip of the micropipette and advanced into contact with the cell to form a single host-pathogen contact

site at a well-defined location and time (Figure 3b). Fluorescent protein tagged receptors' recruitment to the contact site was followed by live cell confocal fluorescence microscopic observation of the contact site from initiation to 10 minutes post-contact and reported as a relative increase over receptor density at the time of contact site initiation. We additionally reported changes in cumulative receptor intensity (sum of all pixel intensities in contact) and contact site area. First, we measured recruitment of EGFP-DC-SIGN or mApple-Dectin-1A individually expressed in HEK293 cells to contact sites formed as described above. In the DC-SIGN only condition, we found a 35.04% increase in normalized density, 70.76% increase in cumulative receptor intensity and 49.83% increase in the contact site area (Figure 3c). For Dectin-1 only expression, we found no significant increase in normalized Dectin-1 density, cumulative receptor fluorescence and contact site area (Figure 3d). Then, we examined contact sites in HEK-293 cells co-transfected with both EGFP-DC-SIGN and mApple-Dectin-1A (Figure 3e). In dual expressing cells, we found a significant increase in normalized DC-SIGN density of 103.15% compared to pre-contact DC-SIGN density. We did not find a significant increase in Dectin-1 density within 10 minutes post contact in dual expressing cells, similarly to the observation in Dectin-1 only expressing cells. For total amount of receptor in contact sites, we found 154.15% and 45.33% increase in cumulative receptor intensity for DC-SIGN and Dectin-1 respectively. We also found 57.97% increase in contact area in dual expressing cells. With control dextran coated polystyrene beads, we did not find any increase in DC-SIGN or Dectin-1 recruitment (Figure 3f). In summary, we found that DC-SIGN exhibited significantly increased recruitment and accumulation at contact site in the presence of Dectin-1 within

375 the 1st 10 min of *C. albicans* contact. Whereas, Dectin-1 did not show recruitment within
376 the 1st 10 min of *C. albicans* contact.

378

379 **Figure 3.** Dynamics of DC-SIGN and Dectin-1 recruitment to a contact site for capture of
380 *C. albicans*. **(a)** TRL035 *C. albicans* (blue) capture assay by HEK-293 cells transfected
381 with EGFP-DC-SIGN (green) and/ mApple-Dectin-1 (red) (n=61), Dectin-1 only (n=63),
382 DC-SIGN only (n=43) and parental (n=49). Average number of fungal particles captured
383 by a cell under each condition is denoted in the graph (the reported value of “n” denotes
384 the number of total cells pooled from ≥ 3 independent experimental replicates). **(b)**

385 Schematic diagram of micropipette-micromanipulator system. **(c & d)** Dynamics of DC-
 386 SIGN (panel c, n=6) or Dectin-1 (panel d, n=6) density enrichment at the contact site under
 387 individual receptor expression conditions. **(e)** Images showing contact of TRL035 *C.*
 388 *albicans* (blue) with HEK-293 cells transfected with EGFP-DC-SIGN (green) and
 389 mApple-Dectin-1 (red) at time 0 and time 10 min. Graphs show dynamics of normalized
 390 DC-SIGN and Dectin-1 density enrichment, total cumulative receptor intensity and contact
 391 site area under co-expression condition of receptors at TRL035 contact site (n=10). **(f)**
 392 Dynamics of EGFP-DC-SIGN (green) and mApple-Dectin-1 (red) density enrichment
 393 under co-expression of receptors at contact site with dextran coated polystyrene bead (blue)
 394 (n=3). All graphs represent the mean $\pm$ S.D. for the indicated value at each time point).

395

396 *3.2 Actomyosin based active recruitment of DC-SIGN at C. albicans contact site.*

397 We previously reported a novel signaling connection between Dectin-1 activation
 398 and generation of cellular mechanical forces via stimulation of actomyosin contractility
 399 [11]. Actomyosin contractility has previously been found to generate actomyosin flows
 400 (AMFs) that support active transport of plasma membrane proteins (i.e., TCR and BCR
 401 transport at immunological synapses, [10]). Therefore, we next looked for possible
 402 involvement of the Dectin-1 activated actomyosin contractility signaling mechanism in
 403 early active recruitment of DC-SIGN to fungal contact sites. We hypothesized that Dectin-
 404 1 mediated actomyosin contraction leads to an AMF at host-pathogen contact sites, and
 405 that DC-SIGN coupling to this AMF is important for recruitment of DC-SIGN to *C.*
 406 *albicans* contact sites. This is feasible because Dectin-1 signaling gives rise RHOA-
 407 ROCK-Myosin II activation, leading to actomyosin contractility[11]. So, inhibition of this

408 signaling mechanism would be expected to abrogate the development of a hypothesized
 409 AMF and any active receptor recruitment connected to it. We pretreated HEK-293 cells
 410 transfected with DC-SIGN and Dectin-1 with various inhibitors of the actomyosin
 411 contractility signaling axis. We found that cells treated with inhibitors of RHOA, ROCK
 412 and Myosin-II exhibited normalized DC-SIGN density of 41.47%, 62.96% and 44.04%
 413 respectively at 10 minutes post-contact formation (Figure 4a). Thus, there was significantly
 414 less recruitment of DC-SIGN in the presence of each of the 3 inhibitors relative to the
 415 untreated condition. For total cumulative DC-SIGN intensity within the contact sites, we
 416 found an increase of 71.13%, 78.79% and 43.03% with RHOA, ROCK and Myosin-II
 417 inhibitor (Figure 4b). Thus, showing less total accumulation of DC-SIGN in inhibitor
 418 treated contacts as well. Concomitantly, we observed contact site areas increased by
 419 121.77%, 105.43% and 18.36% with RHOA, ROCK and Myosin-II inhibitor, respectively,
 420 at 10 minutes post-contact formation (Figure 4c). Thus, there was an increase in contact
 421 area with RHOA and ROCK inhibitor, but a decrease in contact area with Myosin-II
 422 inhibitor compared to no inhibitor condition.
 423

Figure 4. Role of RHOA-ROCK-Myosin II based system in recruitment of DC-SIGN under co-expression condition of Dectin-1 and DC-SIGN at TRL035 *C. albicans* contact site. **(a)** Normalized receptor density enrichment in fungal pathogen contact sites for DC-SIGN at the end of 10 min. under RHOA (n=11), ROCK (n=9) and Myosin-II (n=9) inhibitory conditions, with comparison to the same in untreated cells. **(b)** Total cumulative DC-SIGN intensity enrichment under the same conditions as panel a. **(c)** Contact site area changes under the same conditions as panel a. All graphs represent the mean $\pm$ S.D. for the indicated value at each time point.

3.3 Diffusivity of DC-SIGN and Dectin-1 within contact site.

Having examined the mechanisms of Dectin-1 dependent DC-SIGN mobilization to contact sites, we finally examined the mobility of these receptors once they

438 arrived at the contact site. We hypothesized that receptor engagement with fungal cell wall
 439 ligands after transport into the contact site would result in reductions in apparent receptor
 440 lateral mobility, especially for DC-SIGN due to its multivalent binding to cell wall
 441 mannoproteins. We used FRAP (Fluorescence Recovery After Photobleaching) on contact
 442 sites after 10 minutes of contact site maturation (Figure 5a). Within contact sites, we found
 443 that DC-SIGN showed 28% mobile fraction with recovery $t_{1/2}$ of 66.19 sec and Dectin-1
 444 showed much larger 79% mobile fraction with recovery $t_{1/2}$ of 42.13 sec (Figure 5b). On
 445 statistical comparison of this contact site FRAP data we found that, relative to Dectin-1
 446 values, DC-SIGN had lower lateral mobility ($p=0.01$) and exhibited a smaller mobile
 447 fraction ($p=0.0003$). For non-contact membrane, we found that DC-SIGN showed 78.82%
 448 mobile fraction with recovery $t_{1/2}$ of 35.13 sec and Dectin-1 showed 79.66% mobile
 449 fraction with recovery $t_{1/2}$ of 14.08 sec (Figure 5c). On statistical comparison of DC-SIGN
 450 within contact sites with non-contact DC-SIGN, we found a significant increase in recovery
 451 $t_{1/2}$ ($p=0.005$) and significant decrease in mobile fraction ($p=0.0002$). For Dectin-1, we
 452 found significant decrease in recovery $t_{1/2}$ compared to non-contact membrane ($p=0.001$)
 453 and no significant difference for mobile fraction ($p=0.39$). These findings indicate that
 454 DC-SIGN is far less mobile than Dectin-1 within contact sites. This conclusion is
 455 consistent with the expectation that highly multivalent DC-SIGN/N-mannan interactions
 456 are likely to result in largely irreversible adhesive interactions of DC-SIGN nanodomains
 457 with the outer cell wall surface of *C. albicans*—the likely physical basis of this receptor’s
 458 importance for rapid and effective capture of yeast. In contrast, Dectin-1 is primarily
 459 monovalent [15] as it enters the contact site, and sites of glucan exposure are quite small
 460 and sparsely distributed [16]. Thus Dectin-1 remains fairly mobile within the contact site,

461 which may increase the efficiency of its search for rare sites of glucan exposure even
462 though $t_{1/2}$ is decreased in the contact as expected from Dectin-1 interactions with available
463 glucans at contact sites.

464 To rule out effects of RHOA inhibitor itself on receptor lateral mobility, we
465 did FRAP studies on cells exposed to RHOA inhibitor (Figure S2). These cells did not have
466 fungal contact sites to facilitate comparison of effects on basal receptor mobility of the
467 receptors. On comparing DC-SIGN lateral mobility in RHOA inhibited cells to untreated
468 controls, we found no significant difference in DC-SIGN recovery $t_{1/2}$ ($p=0.66$) or mobile
469 fraction ($p=0.71$). The same was true for Dectin-1, with no significant difference in $t_{1/2}$
470 ($p=0.53$) or mobile fraction ($p=0.22$). We conclude that RHOA pathway inhibition does
471 not impact the lateral mobility of DC-SIGN or Dectin-1, making such a direct effect an
472 unlikely contributor to the recruitment of these receptors to the contact site.

473

474

475

476 **Figure 5.** Lateral mobility characteristics of DC-SIGN and Dectin-1 at *C. albicans* inside
477 and outside of contact site zones. (a) Example pre/post-FRAP images of TRL035 *C.*
478 *albicans* (blue) contacts with HEK-293 cells transfected with EGFP-DC-SIGN (green) and
479 mApple-Dectin-1 (red). (b) Mean FRAP recovery curves of DC-SIGN (n=7) and Dectin-1
480 (n=7) at contact sites and (c) non-contact membrane DC-SIGN (n=6) and Dectin-1 (n=6)
481 with fluorescence recovery observed for 10 min. All contact sites were allowed to mature
482 for 10 min. after *C. albicans* contact before initiation of FRAP experiments. All graphs
483 represent the mean $\pm$ S.D. for the indicated value at each time point.

484

485 3.4 Evidence for active transport of DC-SIGN into the *C. albicans* contact site

486 In resting cell membranes, the DC-SIGN tetramer self assembles into nanodomains,
 487 which have been described to undergo Brownian diffusion as well as apparent directed
 488 transport in some contexts[17–19]. For instance, DC-SIGN nanodomain tracking studies
 489 have reported that this receptor can undergo rapid, linear transport in the plane of the
 490 plasma membrane, suggesting that some directed mobility of DC-SIGN is
 491 possible[12,18,20,21]. As previously mentioned, directed transport at immunological
 492 contact sites (e.g., T cell or B cell synapses) often involves cytoskeleton/motor protein
 493 dependent centripetal motion of receptors toward the center of the contact site[10]. So, we
 494 proposed that similar mechanism might exist at the fungal contact site. Therefore, we next
 495 used an agent based model to test the hypothesis that a combination of Brownian diffusion
 496 and periods of directed transport into the contact site could explain observed DC-SIGN
 497 recruitment dynamics (“Directed + Brownian transport” model).

498 We created an agent-based computational model in the Netlogo modeling
 499 environment, which was capable of predicting DC-SIGN accumulation rates in contact
 500 sites wherein the DC-SIGN experienced a variable coupling to a centripetally directed
 501 active transport mechanism (i.e., AMF), interspersed with regimes of Brownian diffusion
 502 when uncoupled to the directed transport mechanism. This model was parameterized with
 503 literature reported measurements and systematic parameter studies, as described in
 504 Supplemental Methods. The key parameter in this model determining the degree of active
 505 transport of DC-SIGN was the AMF coupling coefficient, which we systematically varied
 506 to define a range of predicted DC-SIGN contact site recruitment. The Directed+Brownian
 507 transport model was able to predict a DC-SIGN contact site recruitment rate of 108.72%

508 increase over pre-contact density (Figure 6), similar to that observed experimentally in
 509 cells expressing DC-SIGN and Dectin-1 with 103.15% increase over pre-contact density
 510 (Figure 3e). To achieve this density in the model, a coupling coefficient of 40% was
 511 required, indicating the strength of DC-SIGN's attachment to the hypothesized directed
 512 transport mechanism. While there is no direct measurement of this value for DC-SIGN
 513 available at this time, this coupling coefficient is within the physiological range observed
 514 in other systems involving receptor coupling to AMF[10,22]. This result suggests that
 515 partial coupling of DC-SIGN to a centripetally directed active transport mechanism is a
 516 viable model for explaining experimentally observable DC-SIGN recruitment to *C.*
 517 *albicans* contact sites.

518 To determine the level of DC-SIGN recruitment that could be achieved in the
 519 absence of directed motion, we ran simulations at a directed transport coupling coefficient
 520 of zero, leaving only lateral mobility via Brownian diffusion (i.e., "Brownian" model). All
 521 other parameters of the model were kept exactly the same as in the Directed + Brownian
 522 model. We found that the Brownian model predicted contact site DC-SIGN density
 523 increase of 54.02% (Figure 6). This is similar to what we observed in inhibitor conditions
 524 which are supposed to inhibit directed transport of DC-SIGN at contact site (Figure 4).

525

526 **Figure 6.** Agent-based modelling showing role of directed motion in DC-SIGN transport
527 at *C. albicans* contact sites. Top panels are views of DC-SIGN (yellow) distribution relative
528 to the contact site (red) before the start (top left) and after 10 minutes of simulation time
529 for models simulating Brownian diffusion only (top right) or directed transport
530 superimposed upon Brownian diffusion (Directed+Brownian) (top middle). Graphs
531 indicate the percentage increase in DC-SIGN density over pre-contact density within 10
532 minutes of simulation time for Brownian diffusion only model (bottom right) or
533 Directed+Brownian model (bottom left).

534

535 If an AMF forms subjacent to the plasma membrane at nascent fungal contacts, we
536 expected an enrichment of F-actin and DC-SIGN in these regions during the early stages
537 of contact site formation. TIRF microscopy is a useful approach with very high axial and
538 lateral resolution for visualizing protein recruitment dynamics at or very near the plasma

membrane. However, TIRF microscopy requires the membrane region of interest to be immediately adjacent to the cover glass—a condition not met in our previous contact site experimental model. We therefore devised a contact site model amenable to TIRF microscopy wherein HEK-293 cells transfected with EGFP-DC-SIGN, pUNO1-hDectin-1a and mCardinal-LifeAct-7 were dropped onto β -glucan-coated cover glass surfaces. As cells began to interact with this surface, they extended dynamic protrusive structures to explore the glucan-coated glass. We found that DC-SIGN and F-actin (LifeAct) became enriched within cell processes in contact with the glucan-coated surface, and that the kinetics of this enrichment showed a temporal correlation of DC-SIGN and LifeAct fluorescence, but with a lag of DC-SIGN relative to F-actin recruitment. The observed latency of maximum DC-SIGN intensity relative to LifeAct was 8.35 ± 4.57 seconds. This correlation was almost completely abolished by RHOA inhibitor, with DC-SIGN latency of 1.69 ± 4.97 seconds in this condition (Figure S3), which was a statistically significant decrease ($p=0.024$) relative to the non-inhibited condition. Thus giving direct imaging based preliminary evidence for Dectin-1 mediated RHOA dependent actomyosin based transport of DC-SIGN at *C. albicans* contact site.

4. Discussion

At *C. albicans* contacts with immune cells, which have been described as the “phagocytic synapse”, Goodridge et al. showed the accumulation of Dectin-1 within contact sites between myeloid cell types and model fungal particles and fungal pathogen cells wherein regulatory tyrosine phosphatases CD45 and CD148 were excluded from Dectin-1 rich zones of the contact [7]. The evident recruitment of immunoreceptors at these cellular synaptic structures and alterations to their patterns of lateral mobility in the

562 membrane suggest that it is important to understand the molecular mechanisms responsible
563 for their construction. In the case of fungal host-pathogen contacts, many studies have
564 examined receptor distribution at tens of minutes to hours due to the difficulty of achieving
565 precise control over contact site formation that is necessary for examining the earliest
566 stages of host-pathogen interaction. We have overcome that problem in this study by the
567 use of micropipette-micromanipulator based application of fungal particles to cells with
568 high spatiotemporal precision.

569 We looked at the dynamics of DC-SIGN and Dectin-1 recruitment at the ≤ 10 -
570 minute time scale. We propose that events occurring at these earliest stages of contact site
571 formation are important to promote pathogen capture and to stabilize the phagocytic
572 synapse. DC-SIGN showed significant contact site recruitment within the first 10 min.,
573 whereas Dectin-1 did not show recruitment within this period, relative to its initial density.
574 DC-SIGN is optimized for high avidity interactions with fungal pathogens due to its
575 tetramerization via its stalk domain and its organization into multi-tetramer
576 nanodomains[17,18,23]. Improvements in the efficiency of contact site recruitment of DC-
577 SIGN are likely to be important for determining the ability of the phagocytic synapse to
578 retain a fungal pathogen, especially under circumstances where fluid shear forces could
579 destabilize the contact site before internalization of the particle can take place.

580 In the absence of Dectin-1, there was a significant decrease in the recruitment of
581 DC-SIGN. In recently published work, we showed that Dectin-1 stimulation by β -glucan
582 gives rise to RHOA-mediated actomyosin activation for contractile mechanical force
583 generation. Previous research on the immunological synapse has demonstrated the
584 importance of RHO-GTPase mediated actin cytoskeleton organization in adhesion and

early immunological synapse formation[24]. Further, the work of Tsourkas et al. with B cell synapses showed with stochastic simulations that the formation of the synapse occurs only if BCR mobility is enhanced by directed motor-driven transport [25]. Also, Manzo et al. showed the role of lateral mobility of DC-SIGN nanoclusters in enhancing pathogen binding using Monte Carlo simulations [18]. Because Dectin-1 activation appeared to enhance DC-SIGN recruitment, we tested the role of RHOA-mediated actomyosin activation downstream of Dectin-1, leading to active recruitment of DC-SIGN to *C. albicans* contact sites. Using agent-based modeling, we found that a directed transport process for DC-SIGN recruitment was necessary in order for computational predictions of DC-SIGN recruitment kinetics to match experimentally observed rates of DC-SIGN recruitment to host-pathogen contact sites. To further support this finding experimentally, we found that inhibitors of RHOA, ROCK and Myosin II decreased DC-SIGN recruitment to contact sites (Figure 4). The density of contact site DC-SIGN achieved in the presence of these inhibitors was similar to the DC-SIGN density predicted by computational modeling of contact site biogenesis under the assumption that DC-SIGN was only transported by passive diffusion followed by trapping in the contact via high avidity interactions with cell wall N-mannans (Figure 6). This result strongly suggests that RHOA, ROCK and Myosin II are essential components of a Dectin-1 dependent active transport mechanism that enhances DC-SIGN recruitment to the sites of host-pathogen interaction within minutes of pathogen contact.

RHOA and ROCK inhibited contacts exhibited a larger total area than without inhibitor. We expect that the spreading of a cell over a pathogen surface to form a contact site is a net result of protrusive (e.g., RAC1-mediated branched actin in lamellar edges and

608 membrane ruffles) and contractile processes (i.e., RHOA-mediated actomyosin contraction
609 around the contact), both of which are unfolding over the time scale of our studies. The
610 fact that RHOA pathway inhibitors allow greater cell spreading to form larger area contacts
611 is compelling evidence that contractile mechanical forces are being generated during the
612 first ten minutes of host-pathogen interaction with *C. albicans*.

613 We did not find significant enhancement of Dectin-1 density in contact sites relative
614 to non-contact membrane within the first 10 min. of *C. albicans* contact. We think this is
615 because sites of nanoscopic exposures of β -glucan on the *C. albicans* wall surface are
616 limited in spatial extent and sparsely distributed. As described by Ostrowski et al. for
617 phagocytic synapse, upon initial clustering of cognate receptors the ligands on the pathogen
618 surface trigger the signaling pathways that could initiate signaling and phagocytosis[8].
619 However, receptor density enrichment and continuous receptor-ligand interaction is
620 required to complete internalization of phagocytic particle, otherwise phagocytosis will
621 stall[26]. Our results are consistent with a model of *C. albicans* recognition wherein initial
622 Brownian diffusion of Dectin-1 leads to rapid Dectin-1 engagement and RHOA-mediated
623 actomyosin flow formation (Figure 6). Subsequently, DC-SIGN gets coupled to this
624 actomyosin flow, which facilitates its efficient mobilization to the contact site. Soon after
625 being drawn into the contact site, DC-SIGN nanodomains engage in high avidity
626 interactions with the fungal cell wall surface, which is important for strong retention of the
627 fungal particle. The high fraction of pathogen-interacting DC-SIGN in the contact site is
628 evident from our FRAP results where the majority of DC-SIGN is immobile over the time
629 scale of minutes (Figure 5). It is this prolonged pathogen retention that allows Dectin-1,
630 the pro-phagocytic receptor, to engage in the more time-consuming process of searching

631 for and integrating signaling from sparse sites of nanoscale glucan exposure. This is evident
 632 from a large mobile fraction of Dectin-1 in contacts sites at 10 min. At larger time scales,
 633 Dectin-1 may show significant enrichment within phagocytic contacts to amplify its
 634 signaling ultimately giving rise to phagocytosis. This is supported by Tam et al. showing
 635 accumulation of Dectin-1 within phagocytic synapse with glucan coated particles at 30
 636 min. time scale[6]. Also, Strijbis et al. showed importance BTK, VAV1 and F-actin
 637 accumulation within *C. albicans* contact site for efficient phagocytosis of fungus at an hour
 638 scale[5]. Our results on the earliest stages of innate immune fungal recognition provide
 639 some increased insight into host-pathogen contact site evolution, which is evidently a
 640 complex, orchestrated process that involves many receptors being are recruited and
 641 activated across different time scales.

642 Yi et al. showed direct evidence for actin retrograde flow and actomyosin II arc
 643 contractions playing role in driving TCR cluster at T-cell immunologic synapse[10]. We
 644 hypothesize that DC-SIGN can get coupled to a similar actomyosin flow in the phagocytic
 645 synapse, leading to its early recruitment at (Figure 7).

646

647

648 **Figure 7.** Model for early recruitment of DC-SIGN at fungal contact site. Initial Dectin-1
649 activation leads to RHOA mediated actomyosin flow formation. DC-SIGN gets coupled to
650 this AMF and start accumulating within contact site, leading to stable capture of fungal
651 particles. After this early stabilization of contact site, Dectin-1 will get time to look for its
652 rare ligand to amplify its signaling, ultimately leading phagocytosis. (Figure created using
653 Biorender)

654 In contrast to our model, Liu et al. showed constitutive role of microtubule based
655 retrograde transport of DC-SIGN nanoclusters to bring pathogens to the perinuclear region
656 for antigen processing[21]. In this study, DC-SIGN nanoclusters were unladen with

657 pathogen or attached to viral particles. It is possible that microtubule associated transport
 658 is a constitutive retrograde transport process involved in receptor recycling or antigen
 659 acquisition, but the conditions in Liu et al. did not involve AMF generation because Dectin-
 660 1/RHOA axis signaling was absent. Nevertheless, future studies could examine potential
 661 the contribution of microtubule mediated transport of DC-SIGN to nascent fungal contacts.
 662 Cambi et al. discuss the possible role of DC-SIGN in direct phagocytosis of *C. albicans* by
 663 immature dendritic cells [4]. They show enrichment of DC-SIGN within phagosome. This
 664 finding is consistent with DC-SIGN's prominent role in capturing fungal particles, though
 665 it also raises possible pro-phagocytic role of DC-SIGN. However, Rosa et al. showed that
 666 DC-SIGN plays a role in binding of zymosan particles but is uninvolved in coordinating
 667 phagocytic signaling of those particles. Consistent with our findings, Rosa et al. also
 668 highlighted the prominent co-localization of actin and Dectin-1 within zymosan contact
 669 site, as would be expected for Dectin-1 mediated actomyosin reorganization at the contact
 670 site[19].

671 Geijtenbeek et al. and van Gisbergen et al. showed that DC-SIGN plays a role in
 672 intercellular adhesion of DCs with T cells [27,28]. Using an assay of rapid cellular capture
 673 of yeast under fluid shear conditions, we found that when Dectin-1 is co-expressed with
 674 DC-SIGN, cells could capture significantly more fungal particles than when any of these
 675 receptors are expressed individually. Thus, we concluded that the interplay between
 676 Dectin-1 and DC-SIGN is important for optimal fungal capture and retention in early
 677 fungal phagocytic contacts. ALS5 is a fungal amyloid mannoprotein adhesin which
 678 undergoes reorganization into nanodomains under shear. This reorganization of ALS5
 679 under shear exposes binding sites for DC-SIGN, thus making fungal particle sticky for DC-

680 SIGN binding. Thus, it is possible that early, large recruitment of DC-SIGN will improve
681 avidity of interaction with *Candida* through DC-SIGN-ALS5 interactions (or other
682 mannoprotein adhesins) under flow conditions [29], which could be pursued in future
683 research.

684 In conclusion, we showed that Dectin-1 mediated activation of RHOA-ROCK-
685 Myosin II axis plays important role in active recruitment of DC-SIGN to the *C. albicans*
686 contact site. This is important for capture of fungal particles and the formation of stable
687 host-pathogen contact sites.

688

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703 5. Supplementary data

704

705 **Figure S1.** Phagocytosis assay in HEK-293 cells expressing EGFP-DC-SIGN (green) with
 706 TRL035 *C. albicans* (blue, calcofluor, cell wall stain; red, cypher5e, pH sensitive
 707 phagocytosis indicator). There was no uptake of fungal particles in the DC-SIGN only
 708 condition.

709

710

Figure S2. Effect of RHOA inhibitor on DC-SIGN and Dectin-1 mobility in non-contact membranes. (top) DC-SIGN showed 75.72% mobile fraction and recovery $t_{1/2}$ of 35.47 sec. (bottom) Dectin-1 showed 81.77% mobile fraction and recovery $t_{1/2}$ of 14.42 sec

718

719

720 Figure S3. Latency for DC-SIGN compared to F-actin in nascent contact site membranes
 721 for no inhibitor and RHOA inhibitor conditions. Box and whisker plot depicts mean ("x"),
 722 median (horizontal bar within box), 1st and 3rd quartile (top and bottom of colored box) and
 723 minimum and maximum value (whiskers).

724

725

726 **Agent-based modelling: Detailed Methods**

727 For modeling transport of DC-SIGN to fungal contact sites we used Netlogo
728 version 6.1.1. The goal of the simulation was to look at the effects of various modes of
729 lateral transport of DC-SIGN on the kinetics of DC-SIGN recruitment at contact site. By
730 arbitrary convention, we kept world square with the dimension of 5 μm x 5 μm . All other
731 parameters were derived from previously available literature or systematically varied. The
732 essential script for the model is provide below.

733

734 Model Parameterization

735 Parameters which were kept constant:

- 736 • Contact site diameter was set at 1 μm based on a measured value reported
737 previously[9].
- 738 • $1.7 \times 10^{-4}/\text{nm}^2$ mannan binding sites were distributed randomly throughout contact
739 site, based on an estimation of a prominent *C. albicans* mannoprotein previous ly
740 reported [29].
- 741 • 2 DC-SIGN tetramers per domain were randomly distributed at domain density of
742 $1.2 \times 10^{-6}/\text{nm}^2$ throughout world area based on prior optical nanoscopy
743 measurements [17,18,23].
- 744 • Size of DC-SIGN nanodomain in resting membrane was approximated to 75 nm
745 based on prior optical nanoscopy measurements [17].

746

747 Parameters which were varied in model:

748 • For association and dissociation probability, we ran a separate simulation with
749 single DC-SIGN CRD domains with single mannan moieties available for binding
750 in the world. We optimized model for total simulation time, so that quantity of free
751 and bound fraction of receptor and ligand is stabilized at the end of simulation. We
752 could achieve equilibrium binding for all conditions in 1 hour of simulated time.
753 So, we let model run for each combination of association and dissociation
754 probabilities for 1 hour. Then we calculated K_d for each condition using the quantity
755 of unbound ligand, unbound receptor and receptor-ligand concentration. Previous
756 literature has reported experimentally calculated K_d for DC-SIGN and mannan
757 interaction to be 50 μM [30]. Hence, we used association and dissociation
758 probabilities which gave K_d value between 30 to 60 μM for contact site simulation,
759 bracketing this value. K_d values in this range are highlighted as red text in Table 2.
760 Actual values used in simulations for data generation are detailed further below.

	Dissoc_prob				
Assoc_prob	0.01	0.02	0.03	0.04	0.05
0.3	5.22E-05	9.08E-05	1.52E-04	1.82E-04	1.52E-04
0.35	2.18E-05	4.00E-05	1.01E-04	1.11E-04	1.21E-04
0.4	1.83E-05	3.66E-05	1.03E-04	9.28E-05	9.95E-05
0.45	2.39E-05	3.19E-05	8.57E-05	1.03E-04	7.55E-05
0.5	1.40E-05	3.39E-05	6.54E-05	1.11E-04	1.11E-04
0.55	1.79E-05	3.74E-05	6.12E-05	1.37E-04	1.11E-04
0.6	1.52E-05	2.31E-05	5.01E-05	6.03E-05	1.06E-04
0.65	1.09E-05	2.59E-05	4.71E-05	4.87E-05	1.52E-04
0.7	1.71E-05	2.33E-05	4.51E-05	3.69E-05	5.01E-05
Green < 3E-05		Red 3E-05 to 6E-05		Yellow > 6E-05	

761
762 **Table 2.** K_d value obtained after simulation of each condition of association and
763 dissociation probability for 1 hour. Each condition was simulated in triplicate. Red

764 indicate values around experimentally calculated K_d of DC-SIGN-Mannan
765 interaction.

766 • Probability for DC-SIGN carbohydrate recognition domain to couple (coupling
767 coefficient) to actomyosin flow was varied between 0 to 80%. This was from
768 previously available literature for immunologic synapse [10,22].

769

770 *Mathematical formulations*

771 • Step sigma for DC-SIGN is based on diffusion coefficient value of 6.5×10^{-2}
772 $\mu\text{m}^2/\text{sec}$ for DC-SIGN domain reported by Manzo et al.[18] The mean diffusion
773 radius was obtained from

$$774 \quad r^2 = 4Dt \quad (D = 6.5 \times 10^{-2} \mu\text{m}^2/\text{sec}, t = 0.1\text{s time of a clock tick for model})$$

775 Hence, $r = 161 \text{ nm}$.

776 Therefore, we model diffusion as a random normal jump distance with sigma = 161
777 nm. However, Manzo et al. also measured that 10% of trajectories are immobile,
778 so we modeled this as DC-SIGN domain movements with a 90% probability[18].

779 • For directed motion component, actomyosin flow velocity of $0.10 \mu\text{m}/\text{sec}$ was
780 derived from previously reported values for immune synapse[10,31].

781 • With coupling coefficient (x), DC-SIGN domains were modeled to be in directed
782 motion of $0.10 \mu\text{m}/\text{sec}$, fixed heading towards to center of contact site with $x\%$
783 probability each time step.

784 • When not in directed motion, DC-SIGN domains were in random diffusion with
785 random heading and 161 nm diffusion radius in each time step.

786

787 *Running model*

- 788 • To keep number of DC-SIGNs available in world outside constant, we did allow
- 789 wrapping of world. Also, to compensate for DC-SIGN which were bound and
- 790 inside contact site, we replenished the equivalent number of DC-SIGNs to the
- 791 outside world. This was necessary it would have been computationally infeasible
- 792 to model the whole cell's membrane, so we just simulated a small membrane patch.
- 793 This patch (within the model and outside the contact site zone) is assumed to be
- 794 freely connected by diffusion to the rest of the cell, which provides an effectively
- 795 infinite pool of receptor in diffusive equilibrium with the modeled membrane patch.
- 796 So, we assumed that the concentration of receptor outside contact area should
- 797 always approximate the entire cell. Even if receptor is lost to the contact site
- 798 membrane, this would have a negligible impact on the average concentration of
- 799 receptor in membrane outside the simulation area.
- 800 • DC-SIGN immobilization was probabilistic as per association and dissociation
- 801 probability. So, if DC-SIGN is within binding radius of unbound mannan binding
- 802 site then probability that it will bind to mannan binding site was as per association
- 803 probability. Also, bound DC-SIGN dissociated from mannan binding site as per
- 804 dissociation probability.
- 805 • Model itself was run for 10 min. for each condition. This is to keep simulation
- 806 conditions same as actual experimental conditions.

807 808 **Result of contact site simulation**

809 We used only those association and dissociation probabilities which gave K_d around

810 50 μ M as indicated by red in table 3. Each simulation replicate consisted of 3 individual

simulation runs (at a given parameter set) and the single simulation replicate's result was considered as the mean behavior of those individual runs. Table 3 reports the mean of these triplicates' results.

Dissoc prob	0.01	0.02					0.03			0.04		0.05
Assoc proba	0.3	0.35	0.4	0.45	0.5	0.55	0.6	0.65	0.7	0.65	0.7	0.7
Coupling												
0	93.95	9.83	42.29	49.71	54.02	60.59	39.17	72.3	-54.43	-3.52	29.9	11.1
20	173.18	31.74	64.63	72.24	82.46	128.92	45.02	90.57	27.5	18.59	46.96	62.46
40	214.93	45.4	79.51	85.56	108.72	157.02	59.58	94.86	45.59	32.59	49.39	128.92
60	366.76	173.03	170.09	181.73	212.45	260.17	182.88	185.55	125.56	72.48	144.37	270.28
80	646.54	552.1	367.13	221.61	391.75	486.3	238.41	284.37	326.79	158.19	255.02	196.8

Table 3. Final increase in DC-SIGN densities compared to pre-contact densities at the end of 10 min. simulation for each specific condition of association probability, dissociation probability and coupling coefficient. Each condition was simulated in triplicate.

We found that dissociation probability of 0.02 and association probability of 0.5 with coupling of 40% gave increase in DC-SIGN density of 108.72% (highlighted in gray in table 3.3), this is similar to the experimental value of 103.15% obtained with DC-SIGN and Dectin-1 co-expression contact sites (Fig. 3.1e).

This same condition of dissociation probability of 0.02 and association probability of 0.5 with 0 coupling gave increase in DC-SIGN density of 54.02% (highlighted in gray in table 3.3). This is similar to what we obtained with RHOA, ROCK and Myosin-II inhibitors which showed DC-SIGN recruitment of 41.47%, 62.96% and 44.04% respectively (Fig. 3.3).

Overall, in table only dissociation probability of 0.02 and association probability of 0.5 gave a global best fit to what we observed experimentally.

Model Script: Main Simulation

```

832 ;Simulation of DCSIGN domains on a DC interacting with a fungal contact site
833 displaying Als family adhesins.
834 ;The goal of this simulation is to investigate the effects of binding avidity and
835 mode of lateral transport
836 ;on contact site structures formed by DCSIGN and the kinetics of DCSIGN
837 recruitment.
838 ;Aaron Neumann Mar 08 2014
839 ;updated by Rohan Choranghe May 20 2020
840
841 globals
842 [
843     domain_sigma_m
844     step_sigma_m
845     contact_radius_m
846     binding_radius_m
847     domain_x
848     domain_y
849     domain_heading
850     next_heading
851     next_fd
852     domain_counter
853     domain_create_counter
854     domain_number
855     density_setpt
856     DCSIGN_deficit
857     DCSIGN_domain_needed
858     contact_fraction ;the fraction of DCSIGN domains in the contact
859     density_enrichment ;the fold amount of DCSIGN domains in the contact
860     relative to what would be expected by random distribution in the
861         ;contact site's area.
862     radial_shell_domains; counts the number of DCSIGN domains in the outer
863     10% radial shell of the contact.
864     filename_view filename_world
865     coupling_random
866     AMF_m
867     density_enrichment_2
868 ]
869
870 breed [DCSIGNs DCSIGN]
871 DCSIGNs-own [domain_ID bound which_ALS_bound]
872
873 breed [ALSs ALS]
874 ALSs-own [orig_x orig_y ALS_gyration_radius_m bound which_DCSIGN_bound]
875
876 to setup
877 clear-all

```

```

878 reset-ticks
879
880 ;assigns key variables that control domain formation and mobility
881 set domain_sigma_m domain_sigma / patch_size ;size of DCSIGN domains
882 set AMF_m AMF / (patch_size * tick_per_sec) ; how far DCSIGN moves with
883 AFM
884 set step_sigma_m step_sigma / patch_size ;how far DCSIGN domain moves
885 per time tick
886 set domain_number int (domain_density * (world-width * world-height *
887 patch_size ^ 2)) ;how many DCSIGN domains
888 set contact_radius_m contact_radius / patch_size ;size of contact site between
889 DC and yeast
890 set binding_radius_m binding_radius / patch_size ;size of zone within which
891 binding can happen
892
893 set filename_view word (word (word (word "Run#" behaviorspace-run-number )
894 " " ) date-and-time ) ".png"
895 set filename_world word (word (word (word "Run#" behaviorspace-run-number )
896 " " ) date-and-time ) ".csv"
897
898 ;creates domain groups, spreads in gaussian shape of domain, forms links to
899 other members of domain
900 set domain_create_counter 1
901 repeat domain_number
902 [
903   set domain_x random-xcor
904   set domain_y random-ycor
905
906   create-DCSIGNs DCSIGNs_per_domain * DCSIGN_multimerization
907   [
908     set color yellow
909     set bound false
910     set which_ALS_bound -1
911     setxy domain_x domain_y
912     set heading random 360
913     fd random-normal 0.0 domain_sigma_m
914     set domain_ID domain_create_counter
915     ask one-of DCSIGNs with [domain_ID = domain_create_counter]
916     [
917       create-links-with other DCSIGNs with [domain_ID =
918 domain_create_counter]
919     ]
920   ]
921   set domain_create_counter (domain_create_counter + 1)
922 ]
923

```

```

924 ;creates ALS proteins in random distribution and initializes them for gyration
925 mobility
926
927 create-ALSs int (ALS_density * pi * contact_radius ^ 2)
928 [
929     set color red
930     set bound false
931     set which_DCSIGN_bound -1
932     set xcor (random-float (2 * contact_radius_m) - contact_radius_m)
933     set ycor (random-float (2 * contact_radius_m) - contact_radius_m)
934     while [ sqrt(xcor ^ 2 + ycor ^ 2) > contact_radius_m]
935     [
936         set xcor (random-float (2 * contact_radius_m) - contact_radius_m)
937         set ycor (random-float (2 * contact_radius_m) - contact_radius_m)
938     ]
939
940     set orig_x xcor
941     set orig_y ycor
942     set ALS_gyration_radius_m ALS_gyration_radius / patch_size
943 ]
944
945 ;shades the patches in the contact site dark red
946 ask patches with [sqrt((pxcor * patch_size) ^ 2 + (pycor * patch_size) ^ 2) <=
947 contact_radius] [set pcolor 11]
948
949 ;defines the density setpoint that will be used to keep the non-contact area at
950 constant DCSIGN density
951 ;units: DCSIGN polypeptides per um^2 simulation space
952 set density_setpt domain_density * DCSIGNs_per_domain *
953 DCSIGN_multimerization
954
955
956 ;reports the total sim area
957 type "Total simulation area (um^2) = " print (world-width * world-height *
958 patch_size ^ 2) / 1000000
959
960
961 end
962
963 to go
964
965
966 ;determines binding and immobilization of DCSIGNs and ALSs (probabilistic)
967
968 ask DCSIGNs with [bound = false AND count ALSs with [distance myself <=
969 binding_radius_m and bound = false ] > 0 ]

```

```

970     [
971         if (random-float 1 < assoc_prob)
972         [
973             set bound true
974             set color blue
975             set which_ALS_bound [who] of one-of ALSs with [distance myself <=
976 binding_radius_m]
977             ask ALS which_ALS_bound
978             [
979                 set bound true
980                 ;set ALS_gyration_radius_m 0
981                 set color green
982                 set which_DCSIGN_bound [who] of one-of DCSIGNs with [
983 which_ALS_bound = [who] of myself]
984             ]
985         ]
986     ]
987
988
989 ;frees agents from binding interactions and immobilization (probabilistic)
990 ask DCSIGNs with [bound = true]
991 [
992     if random-float 1 < dissoc_prob
993     [
994         set color yellow
995         set bound false
996         ask Als which_ALS_bound
997         [
998             set color red
999             set bound false
1000             ;set ALS_gyration_radius_m ALS_gyration_radius / patch_size
1001             set which_DCSIGN_bound -1
1002         ]
1003         set which_ALS_bound -1
1004     ]
1005 ]
1006
1007 ;causes DCSIGN domains to randomly diffuse in 2D space
1008
1009
1010 set coupling_random random-float 100
1011
1012
1013
1014 set domain_counter 1
1015 repeat domain_create_counter

```



```

1016     ;if coupling_random <= 100
1017
1018   [
1019
1020     set next_heading random 360
1021     set next_fd random-normal 0.0 step_sigma_m
1022     if count DCSIGNALs with [((domain_ID = domain_counter) and (bound = true))]
1023     = 0 and random-float 1 < .1
1024     [
1025       ask DCSIGNALs with [domain_ID = domain_counter]
1026       [ if distancexy 0 0 > contact_radius_m and coupling_random <= 100 [
1027         ask DCSIGNALs with [domain_ID = domain_counter] [set heading
1028         next_heading
1029         fd next_fd ]
1030       ]
1031     ]
1032
1033
1034     ask DCSIGNALs with [domain_ID = domain_counter]
1035     [ if distancexy 0 0 <= contact_radius_m and coupling_random > coupling
1036     [
1037       ask DCSIGNALs with [domain_ID = domain_counter] [set heading
1038       next_heading
1039       fd next_fd ]
1040     ]
1041     ]
1042
1043
1044     ask DCSIGNALs with [domain_ID = domain_counter]
1045     [ if distancexy 0 0 <= contact_radius_m and coupling_random < coupling
1046     [
1047       ask DCSIGNALs with [domain_ID = domain_counter] [ set heading towards
1048       patch 0 0
1049       fd AMF_m ]
1050     ]
1051
1052   ]
1053 ]
1054   set domain_counter (domain_counter + 1)
1055 ]
1056
1057   set contact_fraction (count DCSIGNALs with [distancexy 0 0 <= contact_radius_m]
1058   / count DCSIGNALs)
1059   set density_enrichment (count DCSIGNALs with [distancexy 0 0 <=
1060   contact_radius_m] / (pi * contact_radius ^ 2 * (count DCSIGNALs / (world-width *
1061   world-height * patch_size ^ 2))))

```

```

1062   set density_enrichment_2 (count DCSIGNALs with [distancexy 0 0 <=
1063   contact_radius_m] / (pi * contact_radius ^ 2)) / ((count DCSIGNALs - count
1064   DCSIGNALs with [distancexy 0 0 <= contact_radius_m]) / ((world-width * world-
1065   height * patch_size ^ 2) - (pi * contact_radius ^ 2)))
1066   set radial_shell_domains ((count DCSIGNALs with [distancexy 0 0 <=
1067   contact_radius_m and distancexy 0 0 > (0.9 * contact_radius_m)]) /
1068   (pi * contact_radius ^ 2 * (count DCSIGNALs / (world-width *
1069   world-height * patch_size ^ 2)) - (pi * (0.9 * contact_radius ^ 2) * (count DCSIGNALs
1070   / (world-width * world-height * patch_size ^ 2)))) )
1071
1072   ;create new DCSIGNALs to keep the non-contact area at setpoint as they become
1073   trapped in the contact site
1074
1075   set DCSIGNAL_deficit (int (density_setpt * ((world-width * world-height *
1076   patch_size ^ 2) - (pi * contact_radius ^ 2)) -
1077   (count DCSIGNALs with [distancexy 0 0 > contact_radius_m]) ))
1078
1079   ;DCSIGN_domain_needed tells how many domains need to be created.
1080   ;Nothing should be created in the model until the deficit reaches the size of an
1081   entire domain
1082   set DCSIGNAL_domain_needed int (DCSIGN_deficit / (DCSIGNs_per_domain *
1083   DCSIGNAL_multimerization))
1084
1085   repeat DCSIGNAL_domain_needed
1086   [
1087
1088     set domain_heading random 360
1089
1090     create-DCSIGNs DCSIGNALs_per_domain * DCSIGNAL_multimerization
1091     [
1092       set color yellow
1093       set bound false
1094       set which_ALS_bound -1
1095       set heading domain_heading
1096       fd max-pxcor * 0.9
1097       set heading random 360
1098       fd random-normal 0.0 domain_sigma_m
1099       set domain_ID domain_create_counter
1100       ask one-of DCSIGNALs with [domain_ID = domain_create_counter]
1101       [
1102         create-links-with other DCSIGNALs with [domain_ID =
1103         domain_create_counter]
1104       ]
1105     ]
1106     set domain_create_counter (domain_create_counter + 1)
1107   ]

```

```

1108
1109 tick
1110
1111 end
1112

1113 Model Script: Kd estimation

1114 ;Simulation of single DC-SIGN CRD domains interacting with single mannan
1115 moiety to estimate Kd for interaction.
1116 ;Aaron Neumann. Mar 08 2014
1117 ;edited by Rohan Choranghe April 04 2020.
1118
1119
1120 globals
1121 [
1122     domain_sigma_m
1123     step_sigma_m
1124     binding_radius_m
1125     domain_x
1126     domain_y
1127     domain_heading
1128     multimer_heading
1129     next_heading
1130     next_fd
1131     domain_counter
1132     domain_create_counter
1133     domain_number
1134     density_setpt
1135     DCSIGN_deficit
1136     DCSIGN_domain_needed
1137
1138     patch_volume ;this is the volume for 2D binding calcs assuming a
1139     membrane_separation distance
1140     R L RL Kd ; the apparent dissociation constant at a moment in time and vars
1141     used to calc it. meant to be read out after the model has equilibrated
1142     Kd_list Kd_list_avg
1143     filename_view filename_world
1144 ]
1145
1146 breed [DCSIGNs DCSIGN]
1147 DCSIGNs-own [domain_ID bound which_ALS_bound]
1148
1149 breed [ALSs ALS]
1150 ALSs-own [orig_x orig_y ALS_gyration_radius_m bound which_DCSIGN_bound]
1151

```

```

1152 to setup
1153 clear-all
1154 reset-ticks
1155
1156     set ALS_density 4e-6
1157     set DCSIGN_multimerization 1
1158     set DCSIGNs_per_domain 1
1159     set domain_density 4e-6
1160     set ALS_gyration_radius 50
1161     set domain_sigma 0
1162     set step_sigma 161
1163     set contact_radius 0
1164     set binding_radius 5 ;dont change
1165     set tick_per_sec 10 ;dont change
1166     set patch_size 500 ;dont change
1167     set membrane_separation 25 ;dont change
1168     set Kd_list [0] ;Needed to initialize the Kd_list so that it won't throw an error the
1169 first time.
1170     set Kd_window 100
1171
1172
1173
1174
1175 ;assigns key variables that control domain formation and mobility
1176     set domain_sigma_m domain_sigma / patch_size ;size of DCSIGN domains
1177     set step_sigma_m step_sigma / patch_size ;how far DCSIGN domain moves
1178 per time tick
1179     set domain_number int (domain_density * (world-width * world-height *
1180 patch_size ^ 2)) ;how many DCSIGN domains
1181
1182     set binding_radius_m binding_radius / patch_size ;size of zone within which
1183 binding can happen
1184
1185     set filename_view word (word (word (word "Run#" behaviorspace-run-number )
1186 "_" ) date-and-time ) ".png"
1187     set filename_world word (word (word (word "Run#" behaviorspace-run-number )
1188 "_" ) date-and-time ) ".csv"
1189
1190 ;creates domain groups, spreads in gaussian shape of domain, forms links to
1191 other members of domain
1192 set domain_create_counter 1
1193 repeat domain_number
1194 [
1195     set domain_x random-xcor
1196     set domain_y random-ycor
1197

```

```

1198     create-DCSIGNs DCSIGNs_per_domain * DCSIGN_multimerization
1199     [
1200         set color yellow
1201         set bound false
1202         set which_ALS_bound -1
1203         setxy domain_x domain_y
1204         set heading random 360
1205         fd random-normal 0.0 domain_sigma_m
1206         set domain_ID domain_create_counter
1207     ]
1208     set domain_create_counter (domain_create_counter + 1)
1209 ]
1210
1211 ;creates ALS proteins in random distribution and initializes them for gyration
1212 mobility
1213 ;Following creates the random distribution of ligand for Kd (non-contact) studies.
1214
1215     create-ALSs int (ALS_density * world-width * world-height * patch_size ^ 2)
1216     [
1217         set color red
1218         set bound false
1219         set which_DCSIGN_bound -1
1220         set xcor random-xcor
1221         set ycor random-ycor
1222         set orig_x xcor
1223         set orig_y ycor
1224         set ALS_gyration_radius_m ALS_gyration_radius / patch_size
1225     ]
1226
1227
1228 ;reports the total sim area
1229 type "Total simulation area (um^2) = " print (world-width * world-height *
1230 patch_size ^ 2) / 1000000
1231
1232
1233 end
1234
1235 to go
1236
1237
1238     ask DCSIGNs with [bound = false AND count ALSs with [distance myself <=
1239 binding_radius_m] > 0 ]
1240     [
1241         if (random-float 1 < assoc_prob)
1242         [
1243             set bound true

```

```

1244         set color blue
1245         set which_ALS_bound [who] of one-of ALSs with [distance myself <=
1246 binding_radius_m]
1247         ask ALS which_ALS_bound
1248         [
1249             set bound true
1250             set ALS_gyration_radius_m 0
1251             set color red
1252             set which_DCSIGN_bound [who] of one-of DCSIGNs with [
1253 which_ALS_bound = [who] of myself]
1254         ]
1255     ]
1256 ]
1257
1258
1259 ;frees agents from binding interactions and immobilization (probabilistic)
1260 ask DCSIGNs with [bound = true]
1261 [
1262     if random-float 1 < dissoc_prob
1263     [
1264         set color yellow
1265         set bound false
1266         ask Als which_ALS_bound
1267         [
1268             set color red
1269             set bound false
1270             set ALS_gyration_radius_m ALS_gyration_radius / patch_size
1271             set which_DCSIGN_bound -1
1272         ]
1273         set which_ALS_bound -1
1274     ]
1275 ]
1276
1277
1278
1279 set domain_counter 1
1280 repeat domain_create_counter
1281 [
1282     set next_heading random 360
1283     set next_fd random-normal 0.0 step_sigma_m
1284     if count DCSIGNs with [((domain_ID = domain_counter) and (bound = true))]
1285 = 0 and random-float 1 < .1
1286     [
1287         ask DCSIGNs with [domain_ID = domain_counter]
1288         [
1289             set heading next_heading

```

```

1290         fd next_fd
1291     ]
1292 ]
1293 set domain_counter (domain_counter + 1)
1294 ]
1295
1296 ;causes ALS proteins to gyrate about their fixed position
1297 ask ALSs
1298 [
1299     set xcor orig_x
1300     set ycor orig_y
1301     set heading random 360
1302     fd random-normal 0.0 ALS_gyration_radius_m
1303 ]
1304
1305
1306     set patch_volume ((membrane_separation * patch_size ^ 2) * 1e-9 * 1e-9 * 1e-9
1307 * 1e3);volume of one patch in the model, (1e9)^3 is the conversion from nm^3-
1308 >m^3. 1^3 is the conversion from m^3->L. units=L
1309     set R ((count DCSIGNS with [bound = false] / 6.02214129e23) / (world-width *
1310 world-height * patch_volume));conc (M) of unbound receptor
1311     set L ((count ALSs with [bound = false] / 6.02214129e23) / (world-width * world-
1312 height * patch_volume)); conc (M) of unbound ligand
1313     set RL ((count DCSIGNS with [bound = true] / 6.02214129e23) / (world-width *
1314 world-height * patch_volume)); conc (M) of bound receptor-ligand complex
1315     if RL > 0 [set Kd ((R * L) / RL)]
1316
1317     set Kd_list fput Kd Kd_list
1318     if length Kd_list > Kd_window [set Kd_list remove-item Kd_window Kd_list ]
1319     if ticks > Kd_window
1320     [
1321         set Kd_list_avg mean Kd_list
1322     ]
1323
1324
1325
1326 tick
1327
1328 end
1329
1330

```

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