Electronic Supplementary Information

Light controlled cell to cell adhesion and chemical communication in minimal synthetic cells

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Protein expression and purification

The plasmids pQE-80L iLID (C530M) and pQE-80L MBP-SspB Nano were gifts from Brian Kuhlman (Addgene # 60408 and # 60409, respectively). pQE-80L iLID (C530M) expresses iLID with an N-terminal His6-tag and pQE-80L MBP-SspB Nano expresses Nano with N-terminal His6-MBP-TEV tag (His6-MBP-TEV-Nano).

Each plasmid was transformed into E. coli BL21 (DE3) and a 10 mL LB medium with 50 µg/mL ampicillin was used to grown the culture overnight at 37 °C at 200 rpm. After that, the cultures were transferred into a 1 L LB medium with 50 µg/mL ampicillin and grown at 37 °C shaking at 200 rpm until the OD_{600} = 0.6−0.8. Expression of the protein was induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and the cultures were grown overnight at 16 °C shaking at 200 rpm. Bacterial culture was spun down centrifuged at 6000 rpm (Beckman Coulter Avanti J-26S XP, JA-10 rotor) for 8 min at 4 °C. The pelleted bacteria were suspended in 20 mL of Buffer A (50 mM Tris, 300 mM NaCl, pH 7.4) with 100 mM PMSF (in methanol) on ice and lysed by sonication on ice. The lysate

was cleared by centrifugation at 12000 rpm (Beckman Coulter Avanti J-26S XP, JA-25.50 rotor) at 4 °C for 30 min. The supernatant was filtrated twice through a 0.45 µm filter (ROTH, KH 55.1). The protein in the supernatant was purified using a N^{i2+}-NTA affinity column. The purified protein was dialyzed against 2 L Buffer A for 24 h and the purified protein was stored at – 80 °C until future use. The purity of proteins was verified on 12% SDS-PAGE gels and the protein concentration was determined by UV-Vis spectroscopy.

GUV preparation and functionalization with proteins

GUVs were formed using the polyvinylalcohol (PVA) - gel assisted method.[1] All lipids were purchased from Avanti Polar Lipids and stock solutions were prepared in chloroform. In general, a thin film was produced on a microscopy glass slide (60 mm x 24 mm, #1.5) using 40 µL of a 5% PVA (MW = 145000 g/mol) and 100 mM sucrose solution in water. The glass slide was incubated for 30 min at 50 °C to dehydrate the PVA layer. After that, 5 µL of lipid solution containing of 10 mg/ml 1-Palmitoyl-2-oleoylphosphatidylcholine (POPC) with 10 mol% 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (POPG), 0.1 mol% 1,2-dioleoyl-sn-glycero-3-{(N-(5-amino-1-carboxypentyl)iminodiacetic acid) succinyl} Ni^{2+}-Salt (DGS-NTA-Ni^{2+}) and 0.1 mol% 1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindodicarbocyanine (DiD) (Thermo Fisher Scientific # D7757) or 0.1 mol% 1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine Perchlorate (DiI) (Thermo Fisher Scientific # D-3911) membrane dye in chloroform was spread onto the PVA film. The glass slide was dried at 30 °C for 1 h to evaporate the chloroform. After this, a chamber was constructed on top of the glass slide with the support of a Teflon frame (ca. 40 mm x 24 mm) and a second glass slide. The lipid layer was hydrated using ca. 1 mL of rehydration buffer (10 mM Tris, 100 mM NaCl, pH 7.4) for 1.5 h at room temperature. Then, the chamber was inverted and GUVs were harvested after 10 min.

The GUVs were functionalized with the appropriate protein using the binding of His-tags on the proteins to the Ni^{2+}-NTA groups on the DGS-NTA-Ni^{2+} lipid. For this, 100 µL of GUVs were incubated with 10 nM protein for 30 min in the dark. Nano functionalized GUVs were kept standing open to air for ca. 1 h at room temperature to osmotically deflate the GUVs.

For the preparation of sender and receiver GUVs the rehydration buffer was incubated with 50mg/ml Chelex 100 resin (Sigma-Aldrich # C7901) on a rotor at 4 °C overnight to eliminate any calcium contamination. The Chelex resin was removed from the buffer by centrifugation at 1500 rpm for 5 min and then the pH of the rehydration buffer was adjusted to 7.4 with HCl.[2]

The sender GUVs were prepared as described above using DiI as a membrane dye but supplementing the rehydration buffer with 2 µM calcium chloride ($CaCl_2$). To remove the excess Ca^{2+} on the outside of the GUVs, the harvested GUVs were allowed to settle down for 3 h at room temperature in LoBind Eppendrof tubes and the top part was carefully removed such that 300 µl of the GUV solution remained. Next, 1.2 ml rehydration buffer were added to the GUVs and the GUVs were allowed to settle for overnight at 4 °C. The next morning, the top 1.2 ml buffer were removed carefully, another added 1 ml of rehydration buffer were added, the GUVs were allowed to settle for 3 h at room temperature and then 0.8 ml of buffer were removed from the top. The buffer removed in the final stage was used as a negative control. This repeated dilution of the external buffer resulted in an external Ca^{2+} concentration of 92 nM for GUVs with an internal Ca^{+2} concentration of 2 µM. Subsequently, the GUVs were functionalized with Nano as described above.

The receiver GUVs were prepared as described using DiD as a membrane dye above but supplementing the rehydration buffer with 500nM Rhod2 dye (Thermo Fisher Scientific #R12220). To remove the excess Rhod2 on the outside of the GUVs, the GUVs were allowed to settle down, the top part of the buffer was removed and the GUVs were diluted with fresh buffer as described above to yield a final external Rhod2 concentration of 50 nM. Subsequently, the GUVs were functionalized with iLID as described above.

Light dependent GUV-GUV adhesion and detachment

The DiD labelled and iLID functionalized GUVs were mixed in equal volume with DiI labelled and Nano functionalized GUVs, which were deflated. To deflate GUVs the functionalized GUV preparation was left standing open to air for ca. 1 h at RT to increase the salt concentration of the buffer and thus osmotically force water out of the GUV. The sample was loaded into a homemade imaging chamber, which was previously coated with 3 wt% bovine serum albumin (BSA) solution to avoid nonspecific adhesion of vesicles to glass. The GUVs were allowed to settle for 15min before imaging them on a Leica SP5 laser scanning confocal microscope. Two GUVs of the opposite type in proximity of each other were chosen and illuminated with a 488 nm laser (Argon laser, 10%) through 63x water objective for 15 min. Images were acquired simultaneously for the whole field of view in the DiI channel (excitation: 557 nm; emission: 576 nm) and DiD channel (excitation: 644 nm; emission: 665 nm). To determine the light dependence of the interaction we observed, the GUVs were imaged in the dark for at least 1 min before illumination with blue light. For reversion experiments, two GUVs in vicinity or with already showing adhesion where illuminated at least 10 min with blue light using the 488 nm laser, before blue light illumination was stopped while continuously imaging in the DiI and DiD channels.

The kinetics of forming GUV-GUV adhesions and their reversion were analyzed using the ImageJ1.8 line tool. The length of the adhesion site was measured as the site where the fluorescence in the DiD and DiI channels overlays and was measured at least every ca. 5s and every ca. 1 s during periods of rapid changes.

Measuring Ca^{2+} transduction from sender to receiver GUVs in bulk

Sender GUVs (loaded with 2 µM Ca^{2+} and functionalized with Nano) and receiver GUVs (loaded with 500 nM Rhod2 and functionalized with iLID) without membrane dyes and washed as detailed above were mixed in 1:1 ratio (V_{total}= 100 µL) in 96-well plates (Greiner 96 Flat Transparent - GRE96ft, incubated with 3% BSA and washed with buffer prior to the experiment). The mixed GUVs were incubated under blue light (471nm, 80 µW/cm^2 - LED light module V10 with TS-110 Controller, CLF Plant Climatics GmbH) or in dark for 1 h with gentle shaking (30 rpm). The Rhod2 fluorescence (Rhod2, excitation: 552 nm, emission: 581nm) of these solutions was measured before and after adding 830 nM ionomycin (Sigma-Aldrich # I0634) over time using a fluorescence plate reader (Tecan). For the control experiment, the buffer above the receiver/sender GUVs in the final washing step was used instead of the GUVs.

Measuring Ca^{2+} transduction from individual sender to receiver GUVs

Sender GUVs (loaded with 2 µM Ca^{2+}, functionalized with Nano, labeled with DiI) and receiver GUVs (loaded with 500 nM Rhod2, functionalized with iLID, labeled with DiD) and washed as detailed above were mixed in equal volumes and loaded into a homemade imaging chamber, which was previously incubated with 3% bovine serum albumin (BSA) solution and washed 3 times with calcium free buffer. The sample was allowed to settle for ca. 20 min before acquiring images on a confocal laser scanning microscope (Leica TCS SP8) equipped with a 63x water objective. Images were acquired in the DiI/Rhod2 channel (538 nm laser, DiI: excitation: 557 nm; emission: 576 nm, Rhod2: excitation: 552 nm; emission: 581nm) and DiD channel (632 nm laser, excitation: 644 nm; emission: 665 nm). The adhesion between sender and receiver GUVs was induced by blue light illumination (488 nm laser) for ca. 15 min. Then, 830 nM ionomycin was added to GUVs either kept in the dark or incubated under blue light and images were acquired in the DiI/Rhod2 and DiD channels after at least 2min. Images were analyzed using Image J 1.52b. The mean Rhod2 fluorescence intensity inside randomly picked receiver GUVs was measured (n=25-30) and corrected for the background.

The data is presented with a mean ± SEM. The statistical significance was determined by unpaired t-test. p values: ns >0.1, *<0.1, **<0.01, ***<0.001. The statistical analysis was done using GraphPad Prism and all figures were prepared using OriginPro9.1.

References

1. A. Weinberger, F. C. Tsai, G. H. Koenderink, T. F. Schmidt, R. Itri, W. Meier, T. Schmatko, A. Schroder and C. Marques, *Biophys J*, 2013, **105**, 154-164.
2. K. Y. Niu, N. C. Noyes and T. W. Abrams, *J Pharmacol Toxicol Methods*, 2012, **65**, 122-125.

Movie 1:

GUV-GUV adhesion under blue light illumination. The deflated Nano functionalized GUV (membrane in green) did not interact with the iLID functionalized GUV (membrane in red) in the dark despite the proximity. Upon turning on the blue light, GUV-GUV adhesions formed between the GUVs within less than 1 min.

Movie 2:

Reversion of the GUV-GUV adhesions in the dark. The deflated Nano functionalized GUV (membrane in green) adhered to the iLID functionalized GUV (membrane in red) over 15 min under blue light. After turning off the blue light, the GUV-GUV adhesion site became smaller over 5 min up to the point that the two GUVs were not interacting anymore.

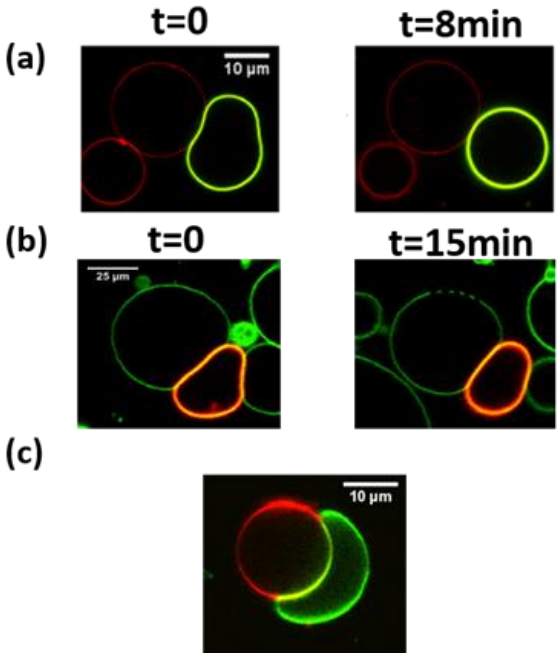

Fig. S1. Reversal of GUV-GUV adhesion in the dark. iLID (shown in red) and Nano (shown in green) functionalized GUVs were first allowed to adhere under blue light before placing them in the dark to observe reversion. a) The GUV-GUV adhesion reversed completely within 8 min and the two GUVs separated from each other. b) The iLID functionalized GUV trapped between two Nano functionalized GUVs still reversed its adhesion at least partially after 15 min in the dark. c) The GUV-GUV interaction was not reversible in cases where the two GUVs strongly interacted as observed over 20 min in the dark.

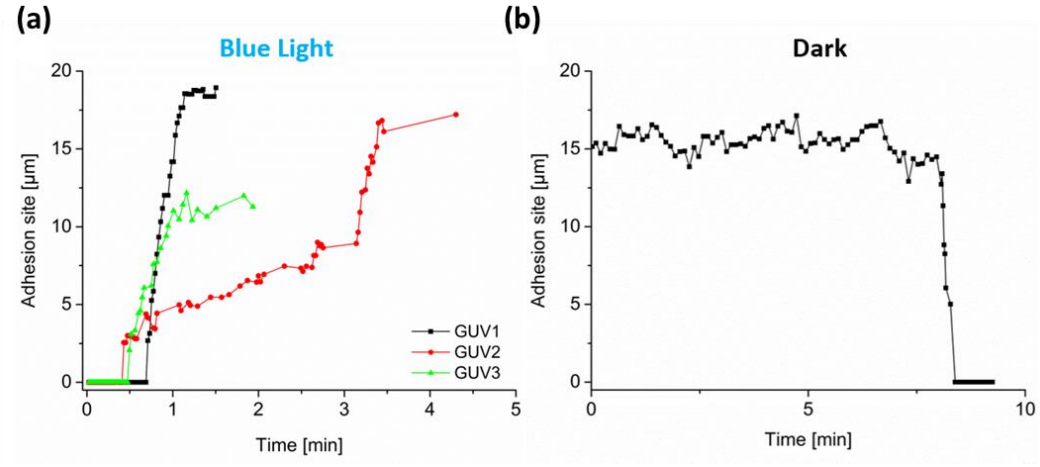

Fig. S2. a) Adhesion kinetics of three different iLID and Nano functionalized GUVs under blue light. After initial contact the adhesion was formed rapidly marked by a steep increase in adhesion site within less than 30 s. The length of the adhesion zone depends on the size of the vesicles and the degree of deflation, which is not controlled in the preparation protocol. b) Reversion kinetics of GUV-GUV adhesions in the dark. The reversion is observed after a few minutes of stopping the blue light illumination.

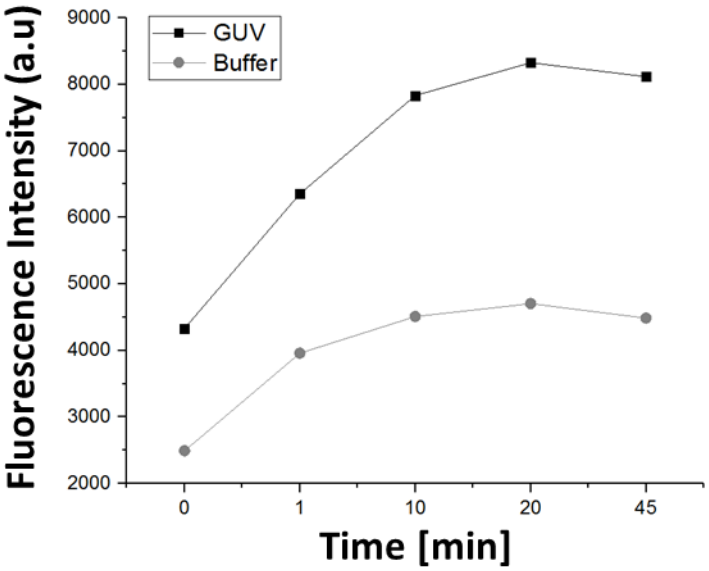

Fig. S3. Fluorescence intensity of receiver GUVs (500nM Rhod2) with ionomycin (830 nM) was quantified over the time upon addition and 2 µM $CaCl_2$ in the bulk solution. The buffer surrounding the receiver GUVs was used as control to demonstrate that the increase in fluorescence is due to the Rhod2 dye inside the receiver GUVs.

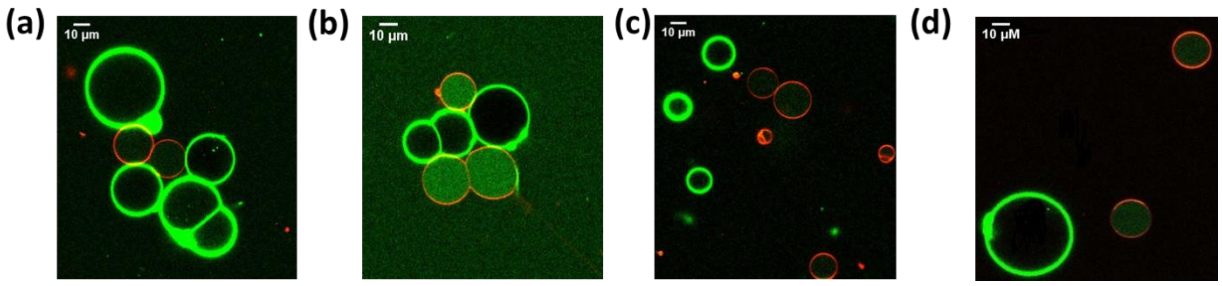

Fig. S4. Blue light dependent formation of multi-GUV clusters and their chemical communication. Confocal microscopy images of sender GUVs (Nano functionalized, loaded with 2 µM Ca^{2+}, DiI membrane dye shown in green) and receiver GUVs (iLID functionalized, loaded with 500 nM Rhod2 shown in green, DiD membrane dye shown in red) under blue light for 1 h a) before and b) after adding ionomycin, and in the dark for 1 h c) before and d) after adding ionomycin. After adding ionomycin a larger increase in Rhod2 fluorescence was observed inside receiver GUVs incubated under blue light (b) than GUVs kept in the dark (d).

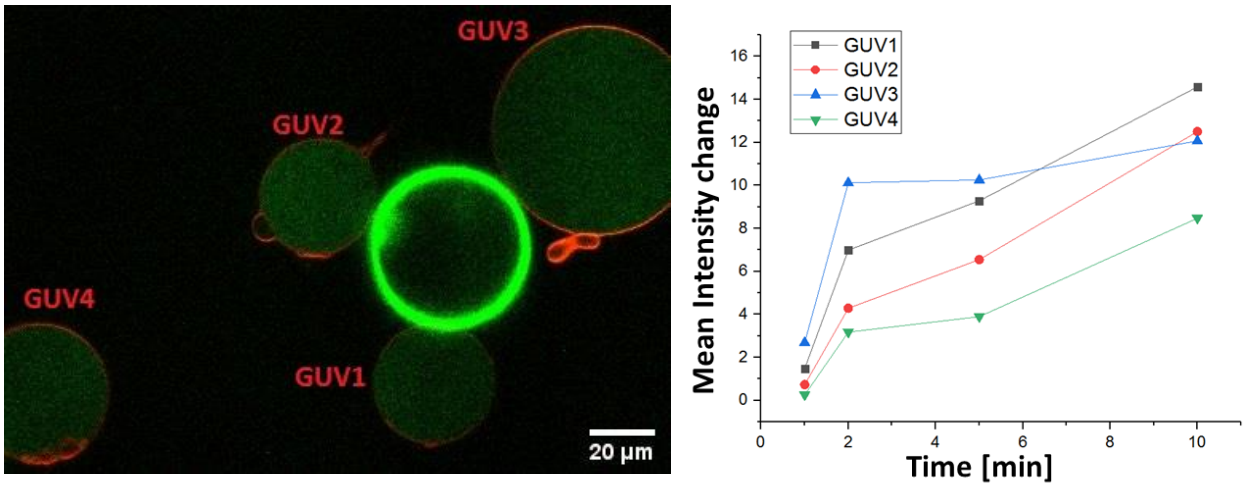

Fig. S5. Confocal microscopy images of sender GUVs (Nano functionalized, loaded with 2 µM Ca^{2+}, DiI membrane dye shown in green) and receiver GUVs (iLID functionalized, loaded with 500 nM Rhod2 shown in green, DiD membrane dye shown in red) under blue light upon addition of ionomycin and quantitation of Rhod2 fluorescence for different receiver GUVs over time in dependence of proximity to the sender GUV. Receiver GUVs in direct contact with the sender GUV (GUV1, GUV2 and GUV3) show faster and higher increase in Rhod2 fluorescence than a GUV which is not in direct contact with the sender GUV (GUV4). The fluorescence intensity before adding the ionophore was subtracted as the background.