Supporting Information

Reduction of Nanoparticle Load in Cells by Mitosis but not Exocytosis

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Figure S1 NP stability in cCCM. A) The 20 nm Au@PVP (left) and the 46 nm Au@PVP NPs (right) were incubated for 24 hours in cCCM at 37 °C. Immediately after the incubation started (red) and 24 hours later (black) their UV-Vis extinction spectra were collected. No red shift is observed for either of the Au NPs indicating that the aggregation in cCCM is negligible and the PVP surface coating is sufficient to stabilize the NPs. B) SiO$_2$ NPs were incubated in cRPMI (250 µg mL$^{-1}$) for 24 hours and further visualized using CryoTEM. Single particles were clearly visible and little to no aggregation was observed. Scale bar= 250 nm
Figure S2 Cytotoxicity assays show no reduction of cell viability at 20 μg mL⁻¹. Neither of the 3 different NPs (SiO₂, 20 nm Au@PVP and 46 nm Au@PVP) used showed significant alterations in cell membrane permeability (LDH, upper panel) nor cell metabolism (Resazurin, lower panel) for any cell type used after 24 hours of exposure and 48 hours of post-exposure. The NP treated cells were compared to the untreated negative control and the 0.2 % Triton X-100 treated positive control.
Figure S3 NP-Lysosome co-localization. Live cell imaging of the different cells previously incubated for 24 hours with the SiO$_2$ NPs (red) and 15 min with cCCM supplemented with a lysosomal stain (green), co-localization appears in yellow. Co-localization coefficient is annotated in the top right corner. Scale bar = 20 µm.
Figure S4 Average spot size increases in DF microscopy with increasing post-exposure time. The DF micrographs were binarized using a threshold (same threshold for same conditions) and further analyzed for their spot sizes using particle tracker from FIJI. The box plots show medians (line in the box) and 5-95 percentiles (whiskers) of the analysis performed for J774A.1 (A), MDM (B) and HeLa (C). Significant increases of sizes were observed for both particle types in J774A.1 and for the 20 nm Au@PVP particles in the case of MDMs. No significant increase was detected for any NPs in HeLa cells. Significance was calculated using student’s t-test.
Figure S5 Stability of fluorescent particles over time in the lysosomal environment. 0.5 mg mL\(^{-1}\) of the fluorescently labelled SiO\(_2\) NPs were incubated in either artificial lysosomal fluid (ALF) or PBS. The relative fluorescence was measured after 1, 2, 3 and 30 days using a plate reader. No significant differences in the fluorescence over time can be seen when comparing ALF and the PBS control. Within the first 48 hours in ALF the fluorescence remained at 97 % of the initial fluorescent signal and at 90 % after 30 days. Dotted lines: 99 % confidence band.
Figure S6 Release of β-Hexosaminidase by MDMs upon stimulation with ionomycin.

MDMs were exposed to 20 µM ionomycin for 6 hours. After the exposure the supernatant was collected and the total amount of released enzyme was quantified based on its enzymatic activity and compared to the lysed cells. Upon stimulation, approximately 30 % of the total amount of the enzyme was released in the supernatant in contrast to the untreated control cells that released < 1 % of the enzyme into the supernatant.
Figure S7 Flow cytometry shows little reduction of the MFI in proliferation inhibited cells. In contrast to normal growth conditions (Figure 4A) the MFI remains on a high level in proliferation inhibited J774A.1 (red) and HeLa (green) cells. The small amount of reduction in the MFI can be explained by cells which were still proliferating or the release of NPs in microvesicles induced by the stress conditions during starvation. Data is shown as the mean ± standard deviation of 3 individual experiments. Lower panel shows J774A.1 cells grown for 24, 48 and 72 hours in the reduced serum conditions.
# Simulation characteristics

- **cell_numberStart** = 1000  #number of mother cells seeded
- **experiments** = 25  #number of experiment repetitions
- **hours** = 48  #total length (in hours) of each experiment
- **Pfactor** = 1/0.516  #fluorescence reduction factor due to mitosis
- **timestep** = 1  #time increment (in hours)
- **doublingTime** = 26.9  #Doubling time (in hours), see below

# Defining the limits of the simulation of each cell's cycle inherent speed (some cells are faster, some are slower), based on a Gaussian around the doubling time (-2 to +2 hour).

- **variation_min** = -2  #Standard deviation of the cell cycle’s speed
- **variation_max** = 18  #Standard deviation of the cell cycle’s speed

# Create a matrix where the final values will be stored

- **total_timepoints** = hours * timestep  #the number of points simulated
- **MeanMatrix** <- matrix(0, nrow= total_timepoints, ncol = experiments)
- **headersize** = 4  #create space for metadata
- **total_cells_MFI** <- c(0)  #declare variable for MFI of the culture
- **mitotic_Event** <- c(0)  #declare variable for the mitotic events

# Run the experiment

# E xperiment = 1000 cells, for each timepoint and each cell

for (experiment in rep(1:experiments,1)) {  #LOOP 1: number of experiments loop
    #create the experiment matrix containing the cell cycles
    result <- matrix(0, nrow = cell_numberStart, ncol = total_timepoints + headersize)
    #create the experiment matrix containing the MFI
    MFI_tail <- matrix(0, nrow = cell_numberStart, ncol = total_timepoints)
    MFI_start <- rep(100, cell_numberStart)
    MFI <- cbind(MFI_start, MFI_tail)
    #define header info and seed
    #assign incremental numbers for each cell (=column 1)
    result[,1] <- rep(1:cell_numberStart, 1)
    # load the entire start array with generation 0 (=column 2)
    result[,2] <- rep(0, cell_numberStart)
    # allow variation in the mitosis point (some cells are slower, some are faster)
    result[,3] <- runif(cell_numberStart, variation_min, variation_max)
    #simulate a random position along the cell cycle
    result[,4] <- runif(cell_numberStart, 1, doublingTime)

    for (timesetting in rep(1:total_timepoints, 1)) {  #LOOP 2: Timepoints
        #loop through all timepoints and update the results matrix
        result[,timesetting+headersize] <- result[,timesetting+headersize-1] + timestep
        #increase the last timepoint with the timestep
        MFI[,timesetting+1] <- MFI[,timesetting]
        for (cell in rep(1:nrow(result), 1)) {  #LOOP 3: cell
            #loop through each cell
            #Check if a mitosis event occurs (i.e. cell is older than doubling time obtained experimentally):
            if(result[cell,timesetting+headersize] > result[cell,3] + doublingTime){  #if YES:
                #reset the cell’s age to 0(reset cell cycle)
                result[cell,timesetting+headersize] <- 0
                #add a new row (=cell) to the cell cycle matrix. This is
daughter cell 2. The mother cell becomes Daughter cell 1.

```r
row2add <- c(nrow(result)+1, result[1,2] + 1, runif(1, -1, 1), rep(0, total_timepoints+1))
result <-rbind(result,row2add)
```

# adjust the MFI of the mother cell based on the 51.6% factor obtained experimentally
MFI[cell,timesetting+1] <- MFI[cell,timesetting]/Ffactor

```r
row2add <- c(rep(0, timesetting),
            MFI[cell,timesetting]/Ffactor, rep(0,ncol(MFI)-timesetting-1))
MFI <- rbind(MFI, row2add)
```

# Update the mitotic events counter with one
```
mitotic_Event = mitotic_Event + 1
```

# The MFI matrix now contains the fluorescence of each cell at each
timepoint. Now, run through the MFI matrix to sum (and average) the
fluorescence values at each time point to reach the MFI of the culture at
each time point.

```r
for(timepoint in rep(1:total_timepoints, 1)) {
  Sumvalue = 0
  for (cell in rep(1:sum(MFI[,timepoint]!=0 ), 1)) {
    Sumvalue = Sumvalue + MFI[cell, timepoint]
  }
  MeanMatrix[timepoint, experiment] = MeanMatrix[timepoint, experiment] + Sumvalue / sum(MFI[,timepoint]!=0 )
}
```

# print/export the results for this experiment
readout <- cat("Experiment ", experiment, " finished (" , cell_numberStart , ", nrow(MFI), " cells) 
")

```r
#Update the total MFI of all cells
total_cells_MFI = total_cells_MFI + nrow(MFI)
```

#Calculate the Mean/Median/standard deviation fluorescence intensity from the
arrays
```r
sd <- apply(MeanMatrix, 1, sd)
mean <- apply(MeanMatrix, 1, mean)
median <-apply(MeanMatrix, 1, median)
```

#Output the data as a plot of the MFI over all timepoints and summarize the
simulation
```r
plot(mean)
```
readout <- cat("Started with ", experiments * cell_numberStart , ", mother cells and ended with ", total_cells_MFI, " daughter cells after ", mitotic_Event, " mitotic events")