

# Three-Dimensional Encapsulation of *Saccharomyces Cerevisiae* in Silicate Matrices Creates Distinct Metabolic States as Revealed by Gene Chip Analysis

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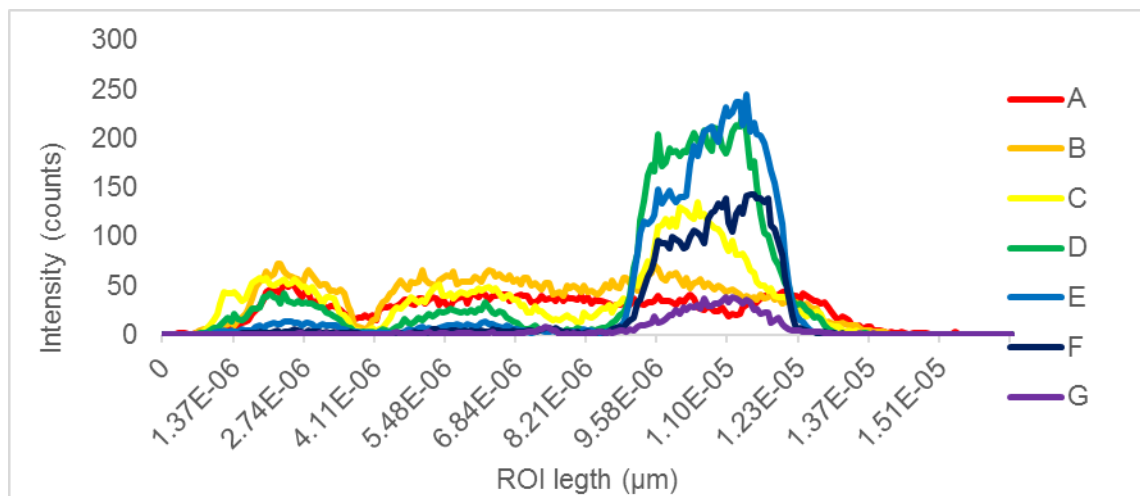
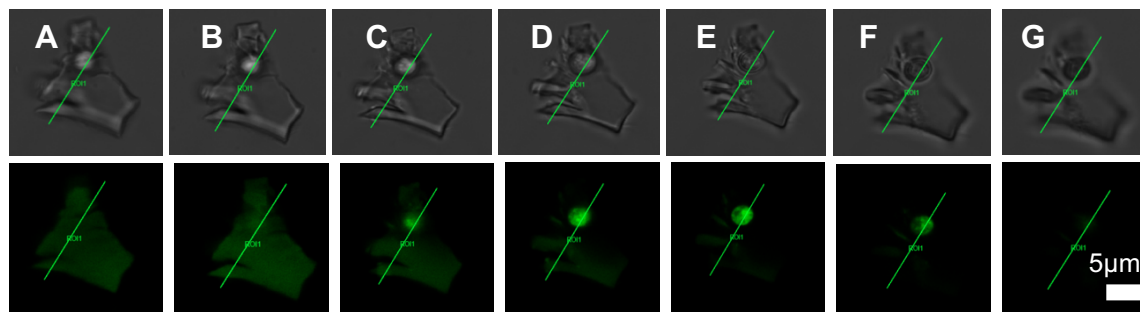
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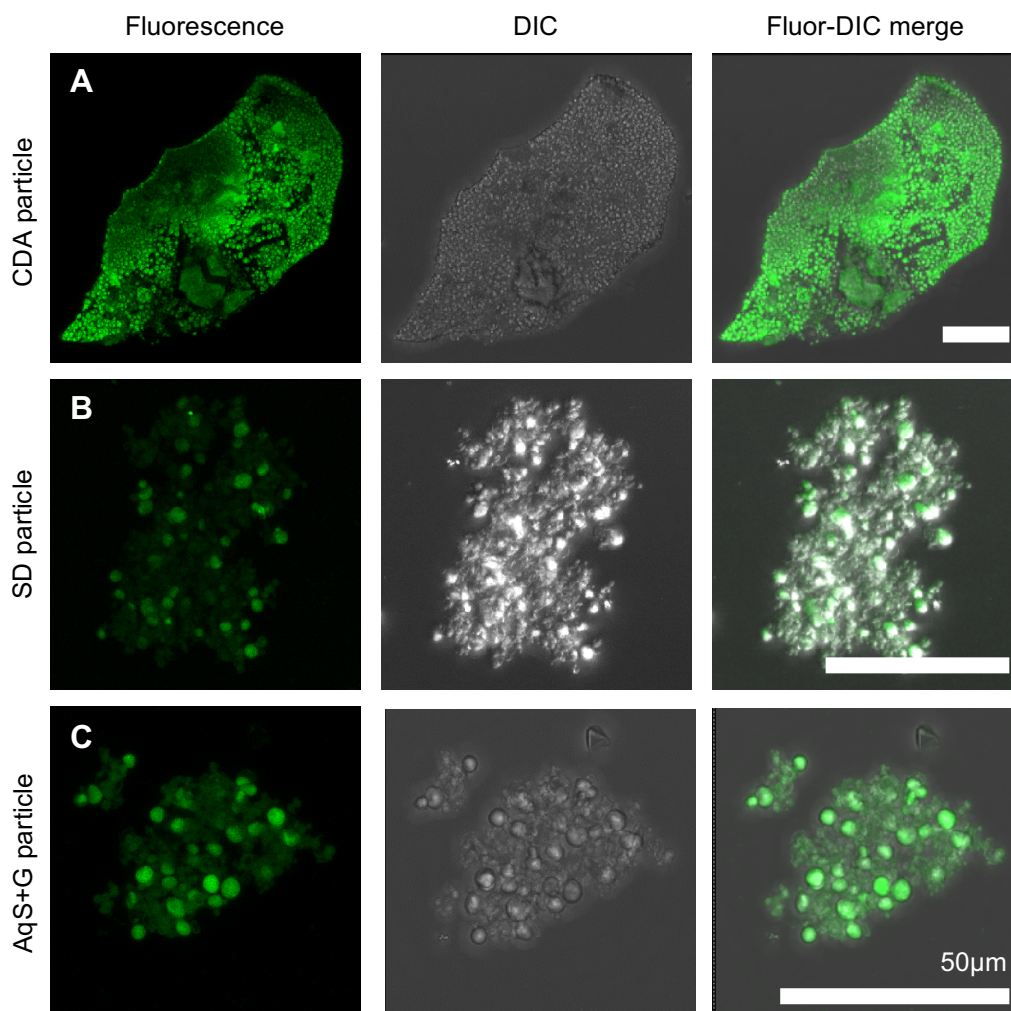
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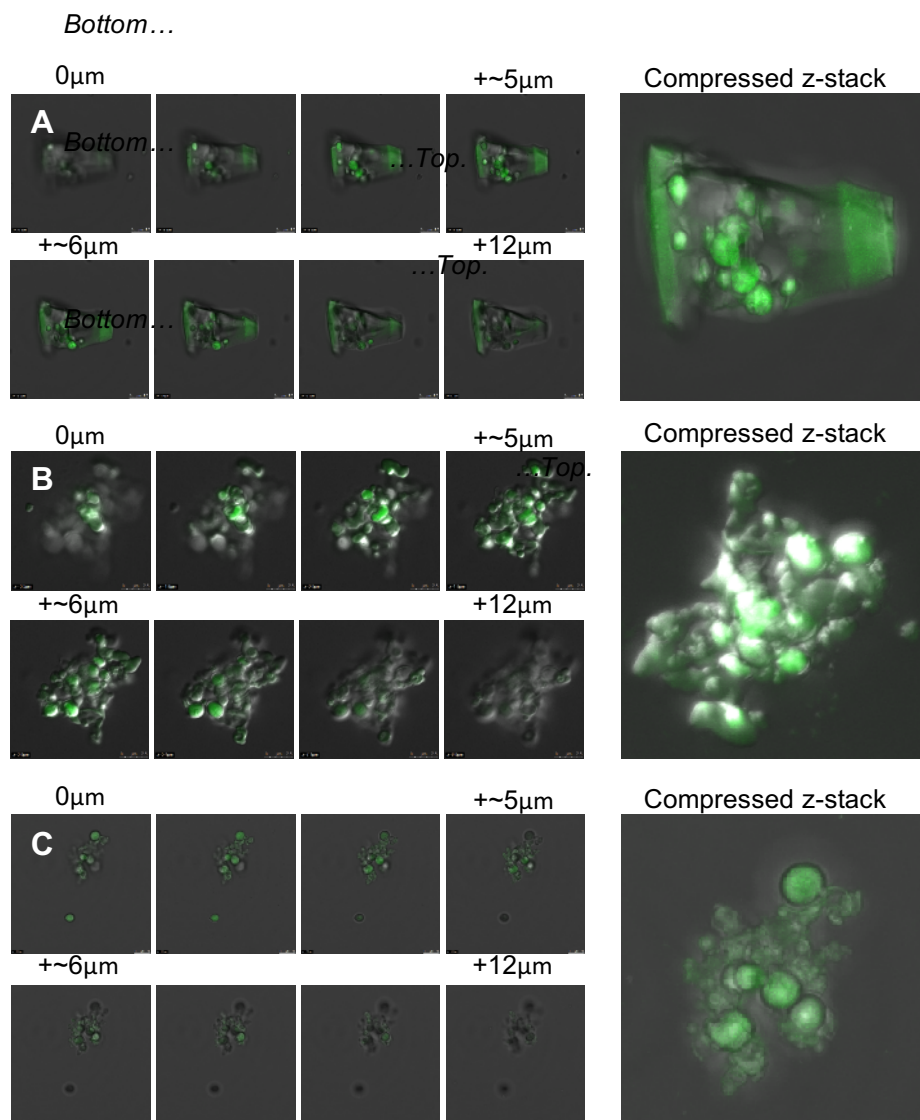
## Supplemental Figures



**Figure S1.** Z-section analysis of confocal images demonstrates complete encapsulation of cells within a CDA matrix. The data set begins at the bottom of a thin sample and traverses through the autofluorescing silica (autofluorescence is about 50 counts in this data) (A-D). The cell “sits” close to the top of the film as can be observed by the cell fluorescence in C and D. (E and F) Cell fluorescence is strong and the matrix autofluorescence begins to subside, suggesting that there is a thin layer of matrix completely surrounding the cell as seen previously.<sup>17</sup> Note the in-focus cell wall in E, which suggests that the center of the cell sits just above the top layer of the bulk Si matrix and the bottom of the cell is “submerged”. (H) Fluorescence intensity plot of the images in A-G.

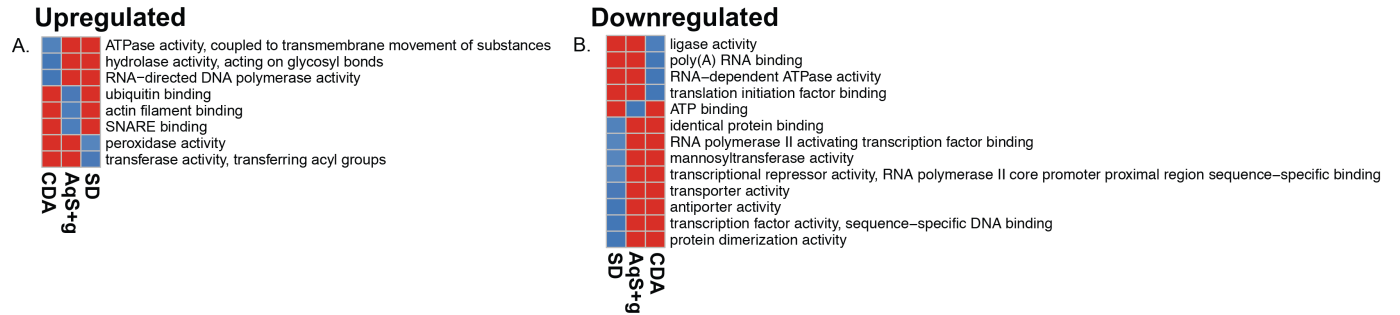


**Figure S2.** Large particles can contain many cells. Depending on the application, samples can be prepared to have few encapsulated cells (*vida infra*) or many encapsulated cells per particle.



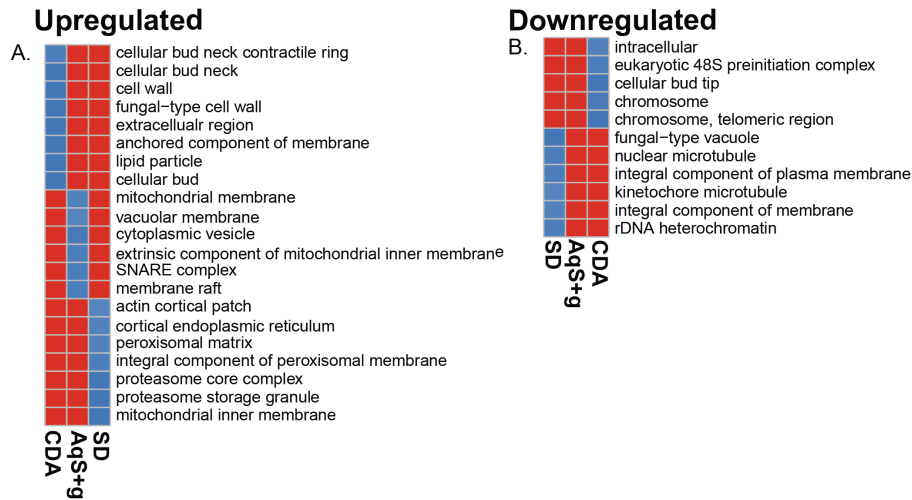
**Figure S3.** Z-stack image gallery of the three particles shown in Figures 2-4 of the manuscript highlights the physical structure of particles as well as cellular distribution. Particles of similar size were chosen for imaging for ease of comparison. In this case, all three particles contain approximately 6-8 cells and the particle depth shown over these eight images (i.e. distance between the first and last image) is approximately  $12 \mu\text{m}$  corresponding to  $\sim 1.5 \mu\text{m}$  per image. Shown are z-stack image galleries and a single, compressed z-stack image for CDA (A), SD (B), and AqS gel (C). Close inspection of the gel sample shows a section of the gel moving with respect to the other, static sections, indicating that the “particle” is likely composed of smaller discrete particles. Note that the compressed z-stack image has been slightly cropped to increase magnification of the particle.

## Molecular Function

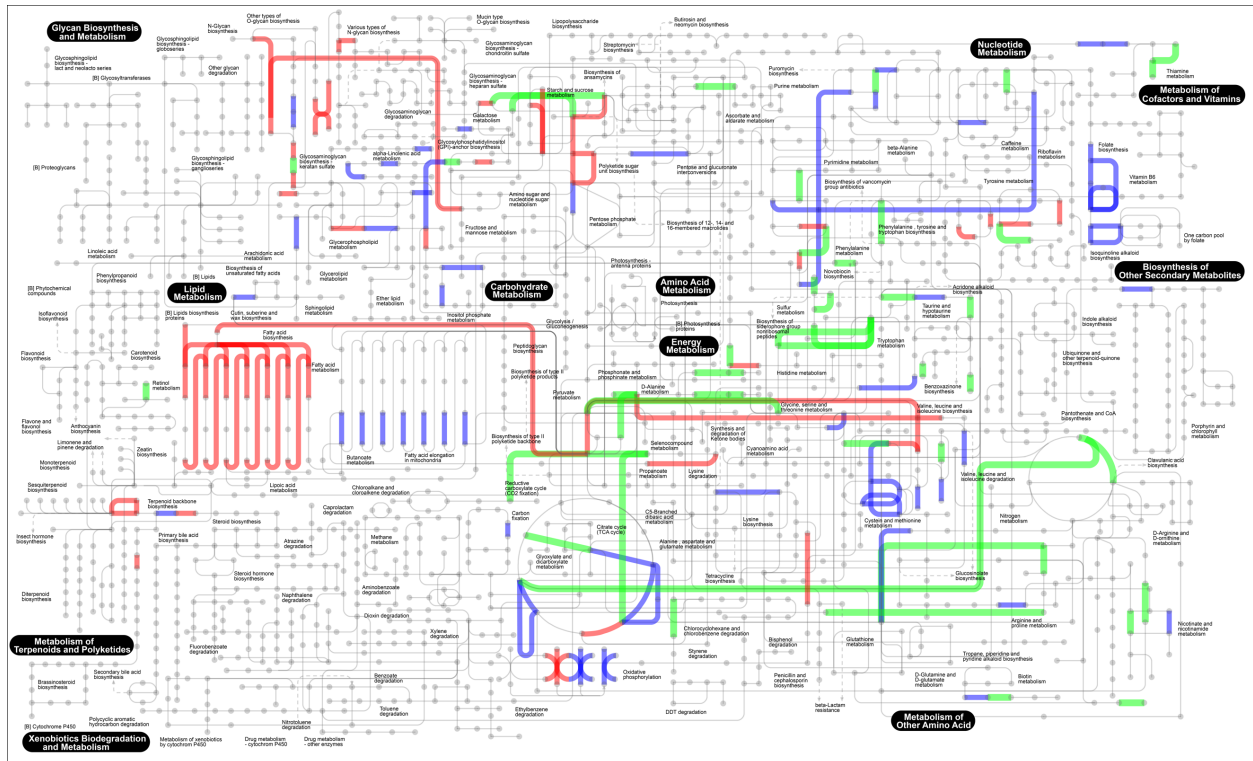


**Figure S4.** Gene Ontology (GO) enrichment was done by The Database for Annotation, Visualization and Integrated Discovery (DAVID). (A) Upregulated molecular functions for *S. cerevisiae* encapsulated via AqS+g, CDA, and SD. (B) Downregulated molecular functions for *S. cerevisiae* encapsulated via AqS+g, CDA, and SD. Red color box show absence of a GO term while blue color box show presence of a GO term in encapsulation method. GO terms with B&H corrected  $p$ -value of 0.05 or less are shown. Individual B&H corrected  $p$ -values are shown in supplemental File 2.

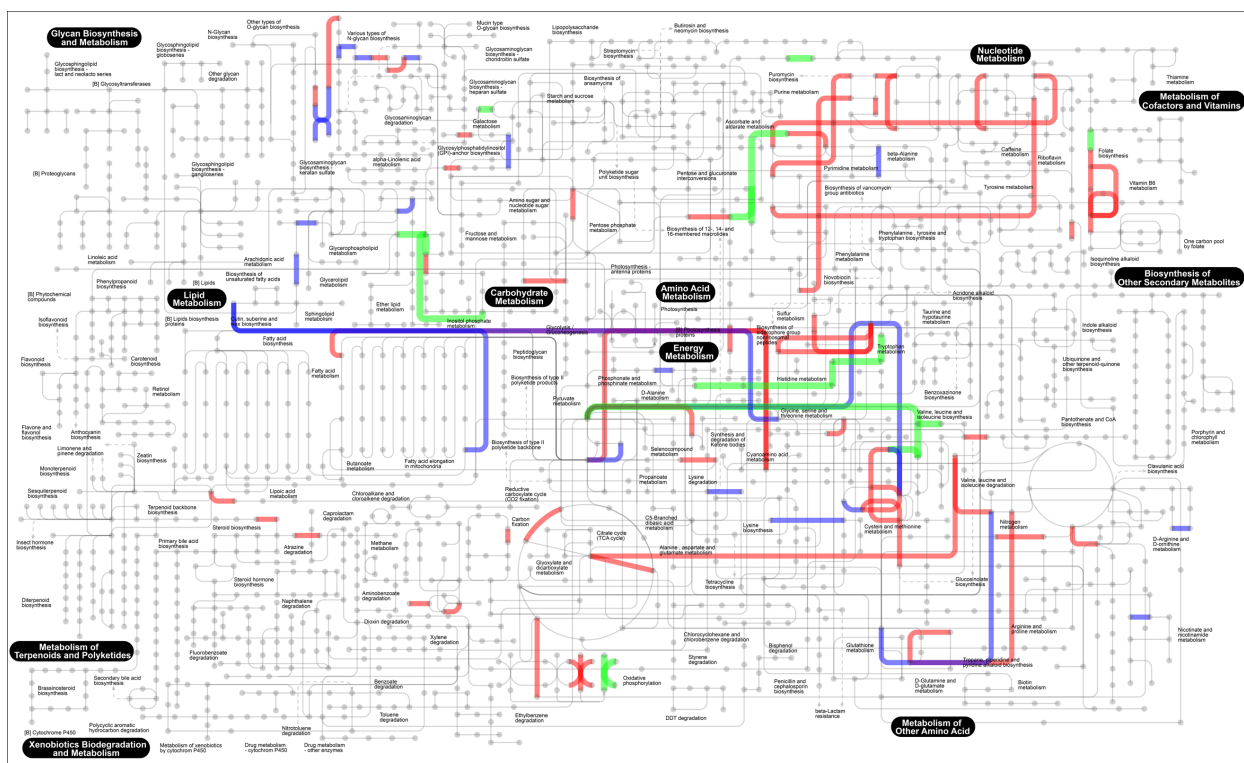
## Cellular Component



**Figure S5.** Gene Ontology (GO) enrichment was done by The Database for Annotation, Visualization and Integrated Discovery (DAVID). (A) Upregulated cellular component for *S. cerevisiae* encapsulated via AqS+g, CDA, and SD. (B) Downregulated cellular component for *S. cerevisiae* encapsulated via AqS+g, CDA, and SD. Red color box show absence of a GO term while blue color box show presence of a GO term in encapsulation method. GO terms with B&H corrected  $p$ -value of 0.05 or less are shown. Individual B&H corrected  $p$ -values are shown in supplemental File 2.

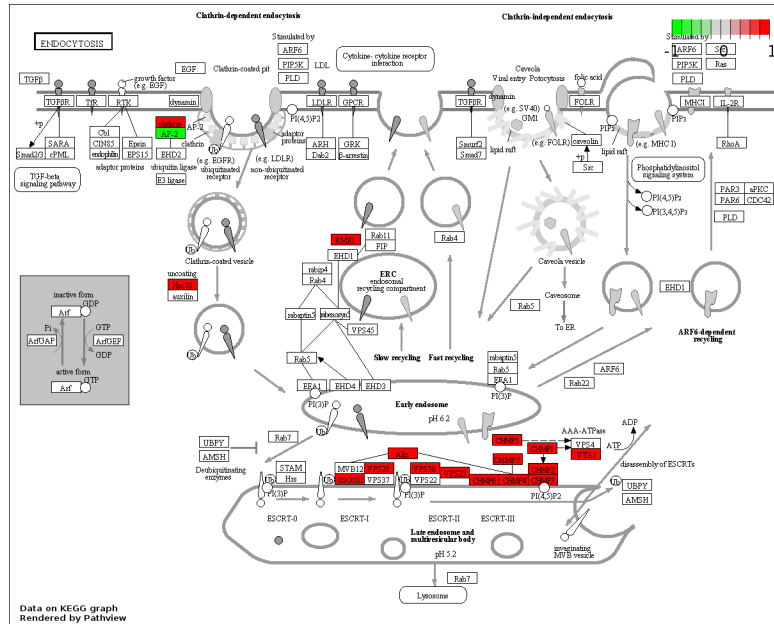


**Figure S6.** Differentially expressed genes from AqS+g, CDA, and SD encapsulated cells were overlaid on global metabolic pathways map of *Saccharomyces cerevisiae* (*S. cerevisiae*). Red lines correspond to upregulated genes in CDA, Green lines corresponds to upregulated genes in AqS+g, Blue lines correspond to upregulated genes in SD. while grey lines show no change. Each node represents a different metabolic compound while edges represent series of chemical reactions.

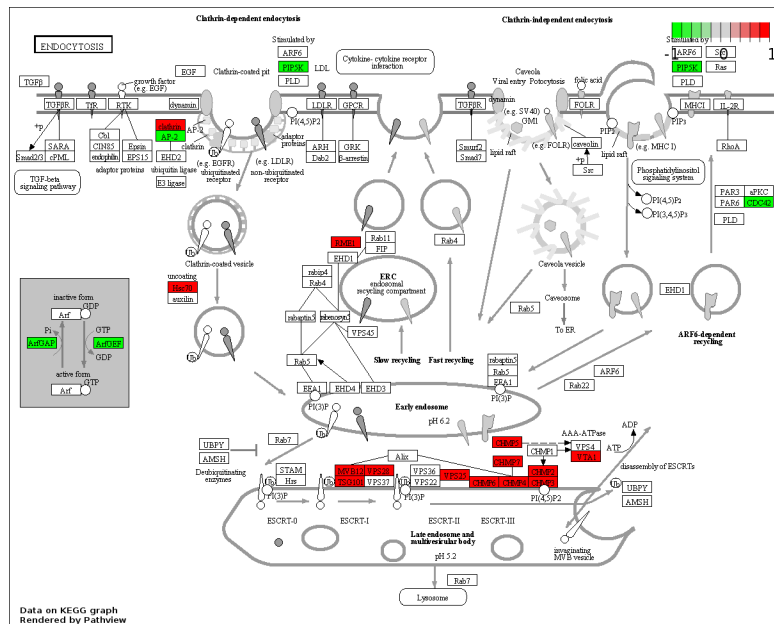


**Figure S7.** Differentially expressed genes from AqS+g, CDA, and SD encapsulated cells were overlaid on global metabolic pathways map of *Saccharomyces cerevisiae* (*S. cerevisiae*). Red lines correspond to downregulated genes in CDA, Green lines corresponds to downregulated genes in AqS+g, Blue lines correspond to downregulated genes in SD. while grey lines show no change. Each node represents a different metabolic compound while edges represent series of chemical reactions.



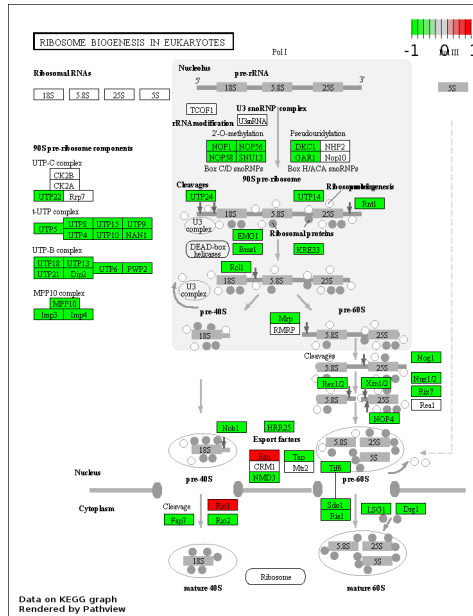


**Figure S8.** Differentially expressed genes from AqS+g encapsulated cells were overlaid on “Endocytosis” of *Saccharomyces cerevisiae* (*S. cerevisiae*). Green Color shows down regulated and Red Color shows upregulated genes.

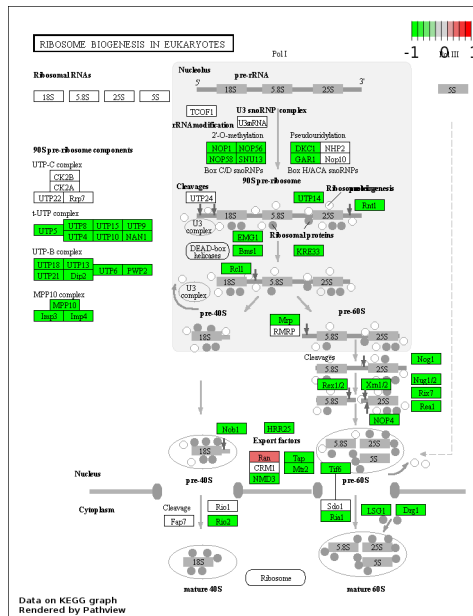


**Figure S9.** Differentially expressed genes from CDA encapsulated cells were overlaid on “Endocytosis” of *Saccharomyces cerevisiae* (*S. cerevisiae*). Green Color shows down regulated and Red Color shows upregulated genes.

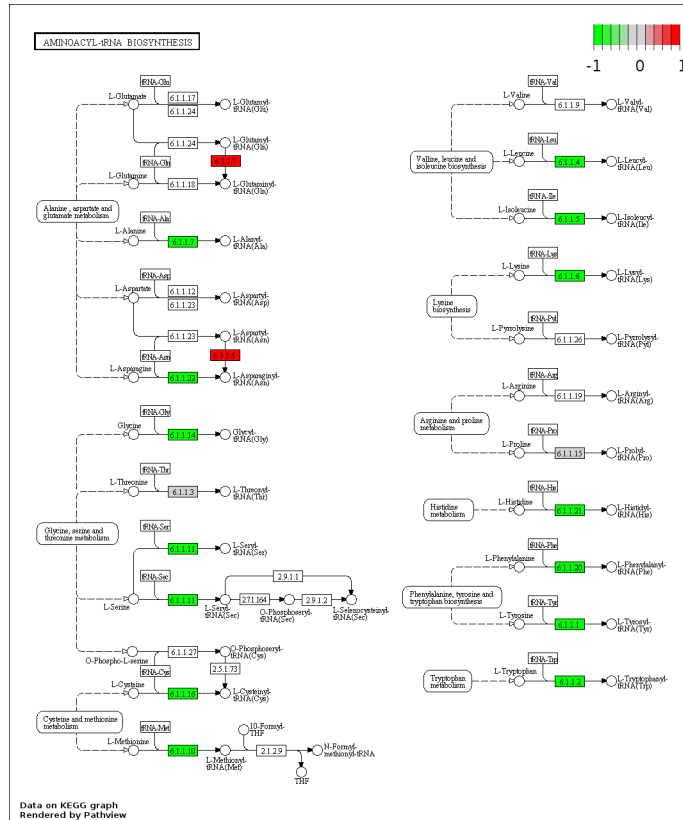




**Figure S12.** Differentially expressed genes from CDA encapsulated cells were overlaid on “Ribosome Biogenesis in Eukaryotes” of *Saccharomyces cerevisiae* (*S. cerevisiae*). Green Color shows down regulated and Red Color shows upregulated genes.



**Figure S13.** Differentially expressed genes from SD encapsulated cells were overlaid on “Ribosome Biogenesis in Eukaryotes” of *Saccharomyces cerevisiae* (*S. cerevisiae*). Green Color shows down regulated and Red Color shows upregulated genes.

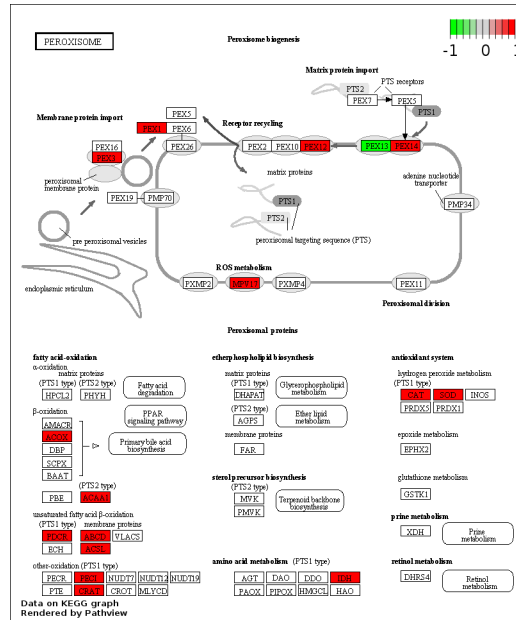


**Figure S14.** Differentially expressed genes from AqS+g encapsulated cells were overlaid on “Aminoacyl tRNA Biogenesis” of *Saccharomyces cerevisiae* (*S. cerevisiae*). Green Color shows down regulated and Red Color shows upregulated genes.

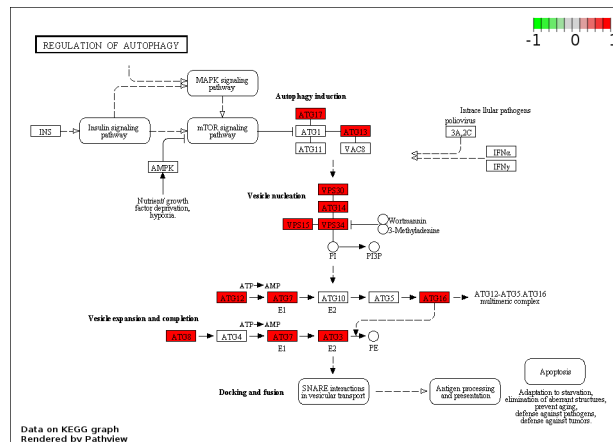








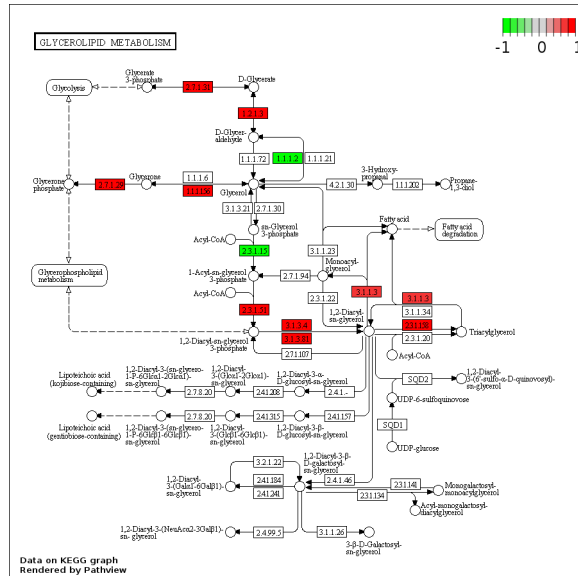
**Figure S19.** Differentially expressed genes from SD encapsulated cells were overlaid on “Peroxisome” of *Saccharomyces cerevisiae* (*S. cerevisiae*). Green Color shows down regulated and Red Color shows upregulated genes.



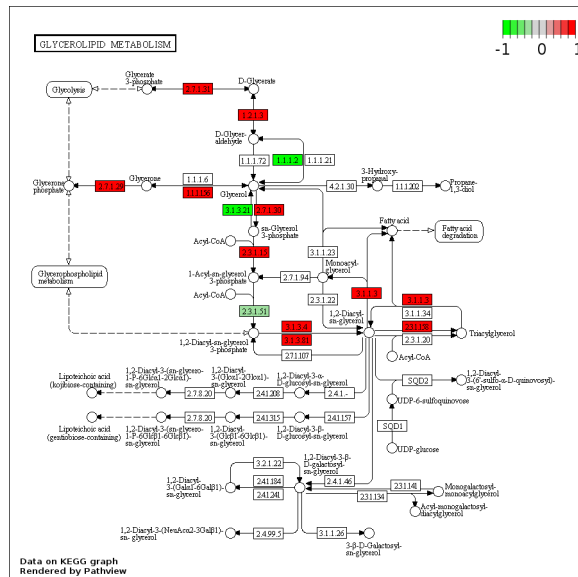
**Figure S20.** Differentially expressed genes from CDA encapsulated cells were overlaid on “Autophagy” of *Saccharomyces cerevisiae* (*S. cerevisiae*). Green Color shows down regulated and Red Color shows upregulated genes.







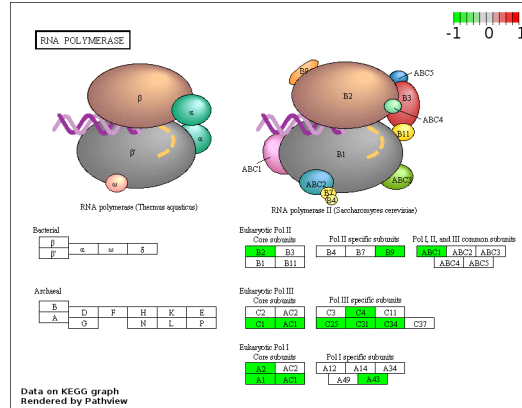
**Figure S23.** Differentially expressed genes from AqS+g encapsulated cells were overlaid on “Glycerolipid Metabolism” of *Saccharomyces cerevisiae* (*S. cerevisiae*). Green Color shows down regulated and Red Color shows upregulated genes.



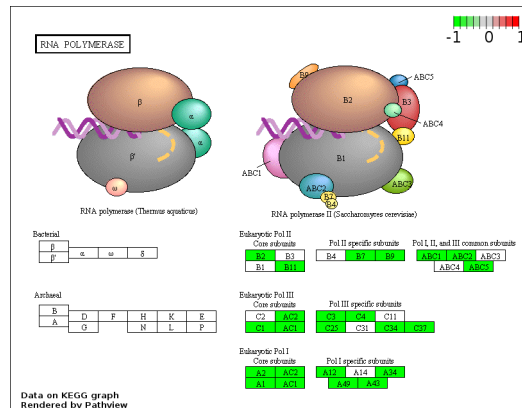
**Figure S24.** Differentially expressed genes from SD encapsulated cells were overlaid on “Glycerolipid Metabolism” of *Saccharomyces cerevisiae* (*S. cerevisiae*). Green Color shows down regulated and Red Color shows upregulated genes.



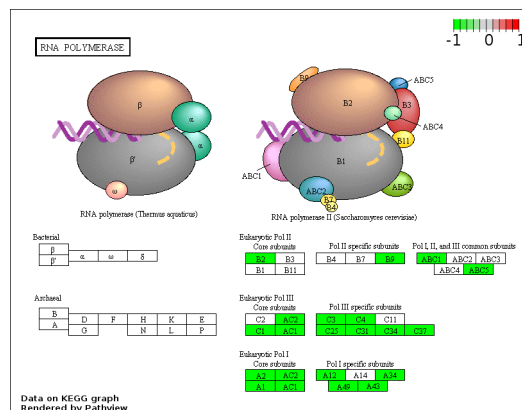




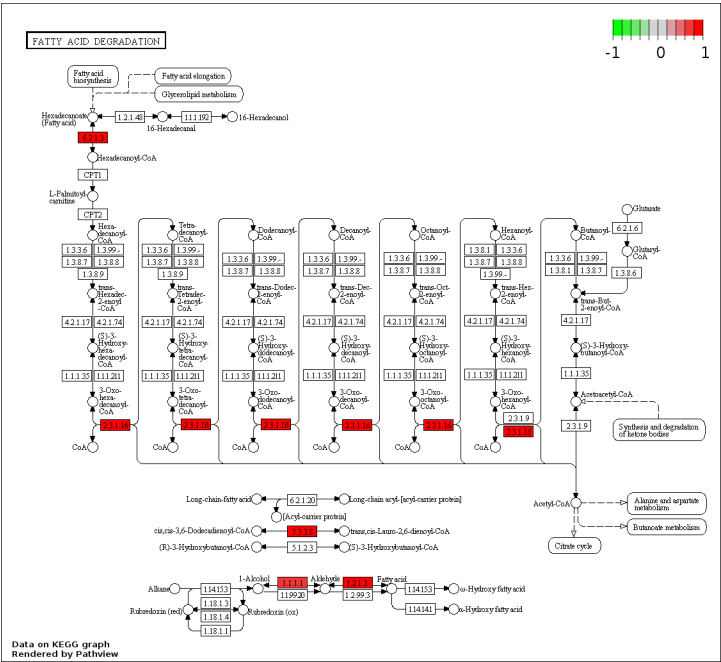
**Figure S29.** Differentially expressed genes from AqS+g encapsulated cells were overlaid on “RNA Polymerase” of *Saccharomyces cerevisiae* (*S. cerevisiae*). Green Color shows down regulated and Red Color shows upregulated genes.



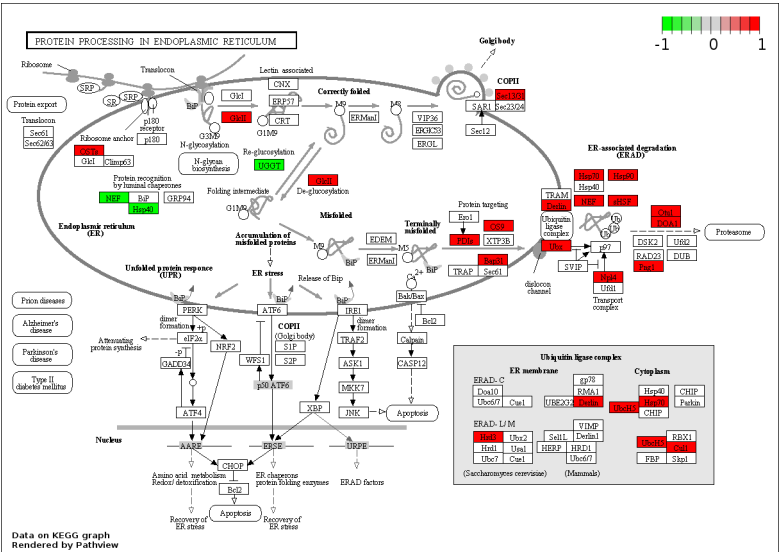
**Figure S30.** Differentially expressed genes from CDA encapsulated cells were overlaid on “RNA Polymerase” of *Saccharomyces cerevisiae* (*S. cerevisiae*). Green Color shows down regulated and Red Color shows upregulated genes.



**Figure S31.** Differentially expressed genes from SD encapsulated cells were overlaid on “RNA Polymerase” of *Saccharomyces cerevisiae* (*S. cerevisiae*). Green Color shows down regulated and Red Color shows upregulated genes.

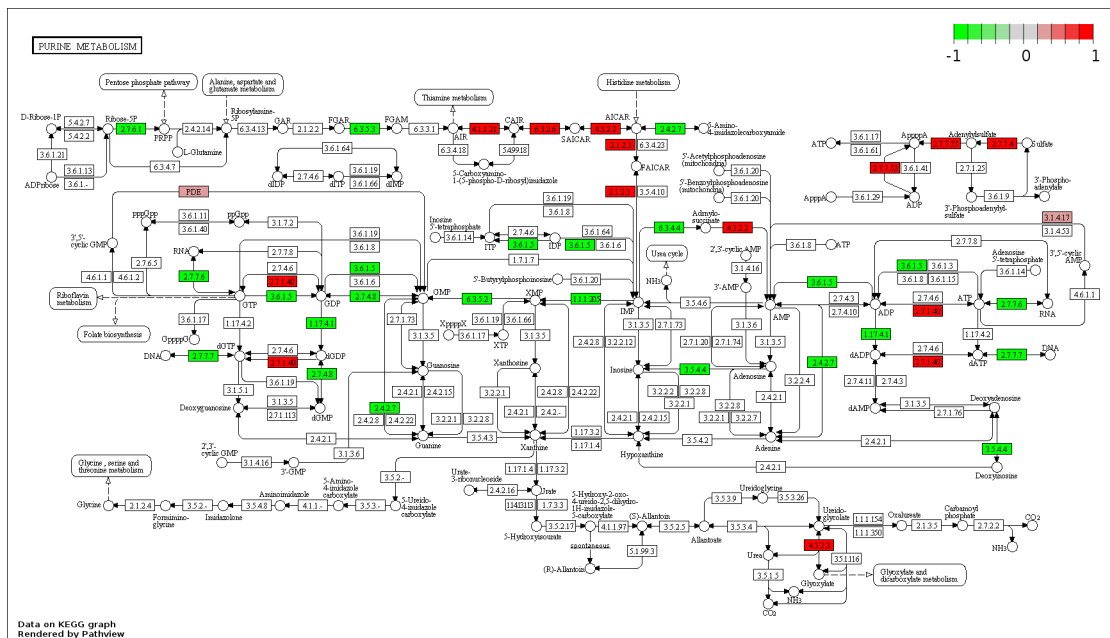


**Figure S32.** Differentially expressed genes from CDA encapsulated cells were overlaid on “Fatty Acid Degradation” of *Saccharomyces cerevisiae* (*S. cerevisiae*). Green Color shows down regulated and Red Color shows upregulated genes.

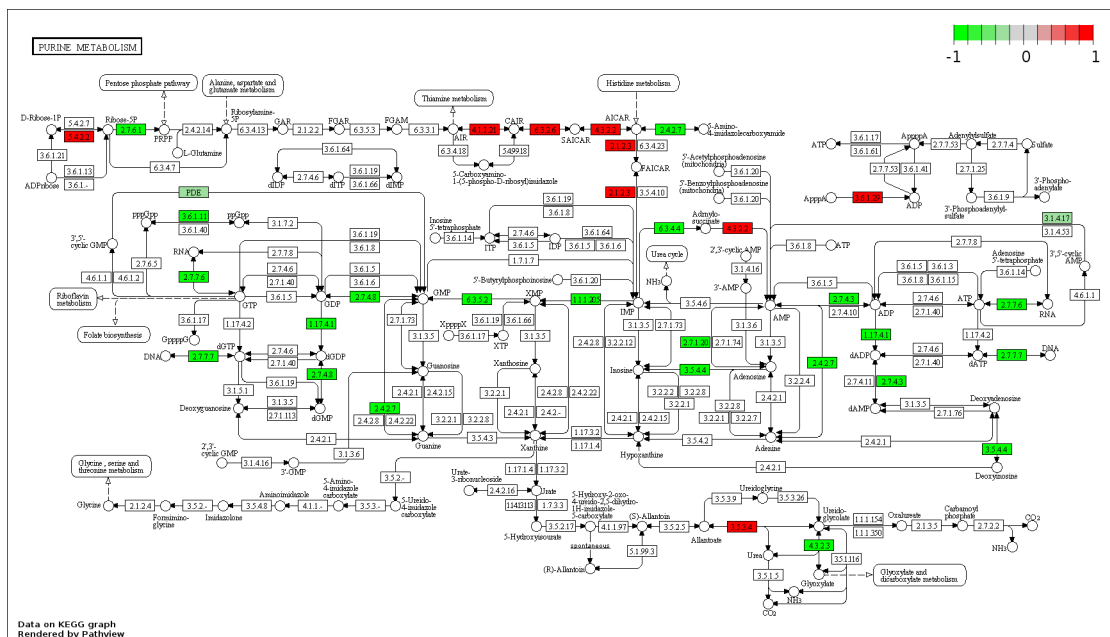


**Figure S33.** Differentially expressed genes from AqS+g encapsulated cells were overlaid on “Protein Processing in Endoplasmic Reticulum” of *Saccharomyces cerevisiae* (*S. cerevisiae*). Green Color shows down regulated and Red Color shows upregulated genes.



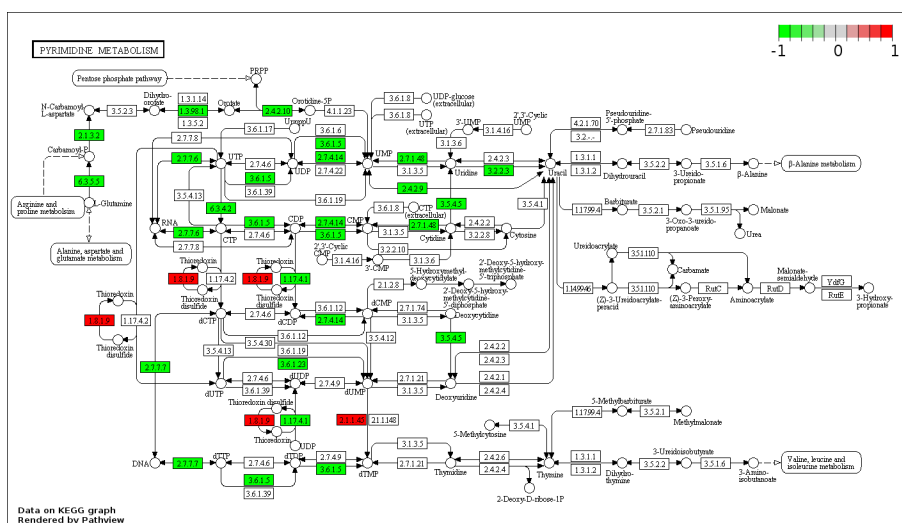
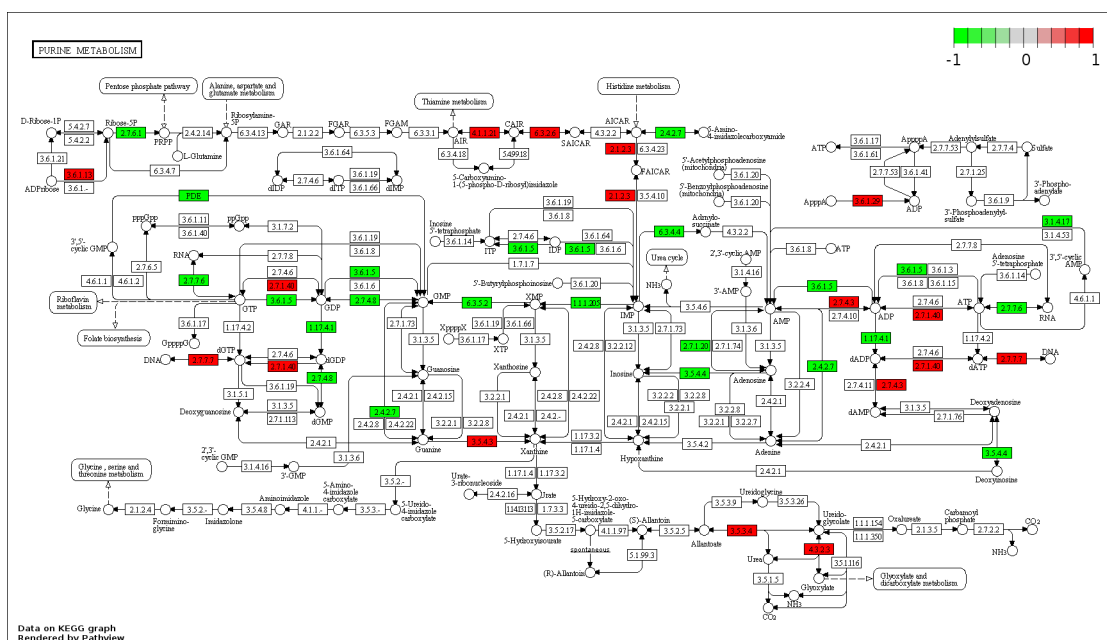


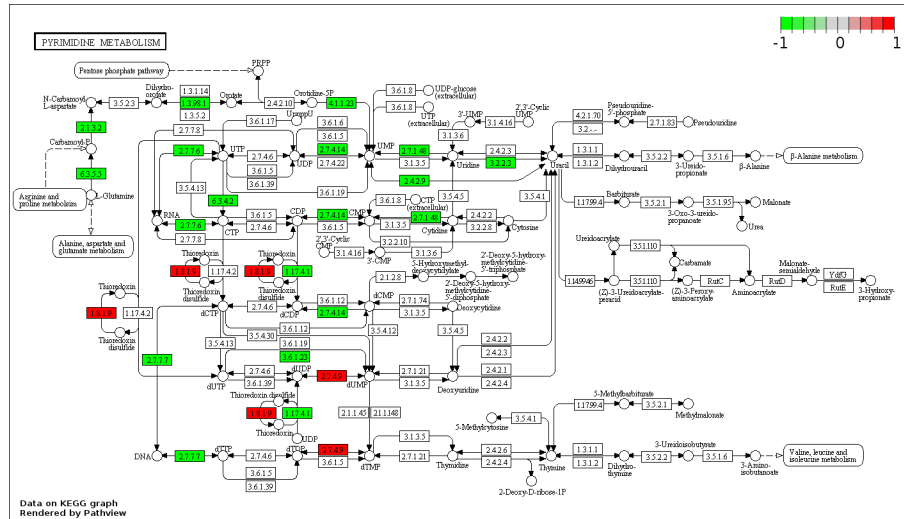
**Figure S36.** Differentially expressed genes from AqS+g encapsulated cells were overlaid on “Purine Metabolism” of *Saccharomyces cerevisiae* (*S. cerevisiae*). Green Color shows down regulated and Red Color shows upregulated genes.



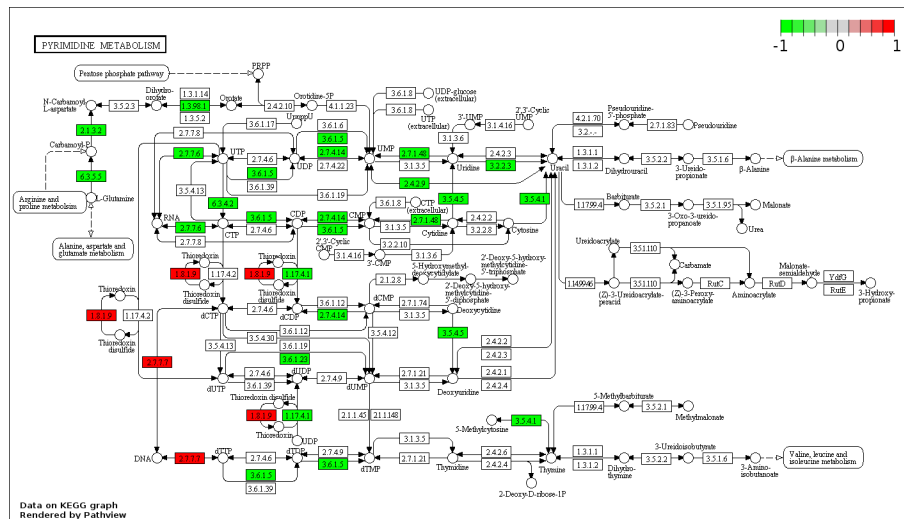
**Figure S37.** Differentially expressed genes from CDA encapsulated cells were overlaid on “Purine Metabolism” of *Saccharomyces cerevisiae* (*S. cerevisiae*). Green Color shows down regulated and Red Color shows upregulated genes.





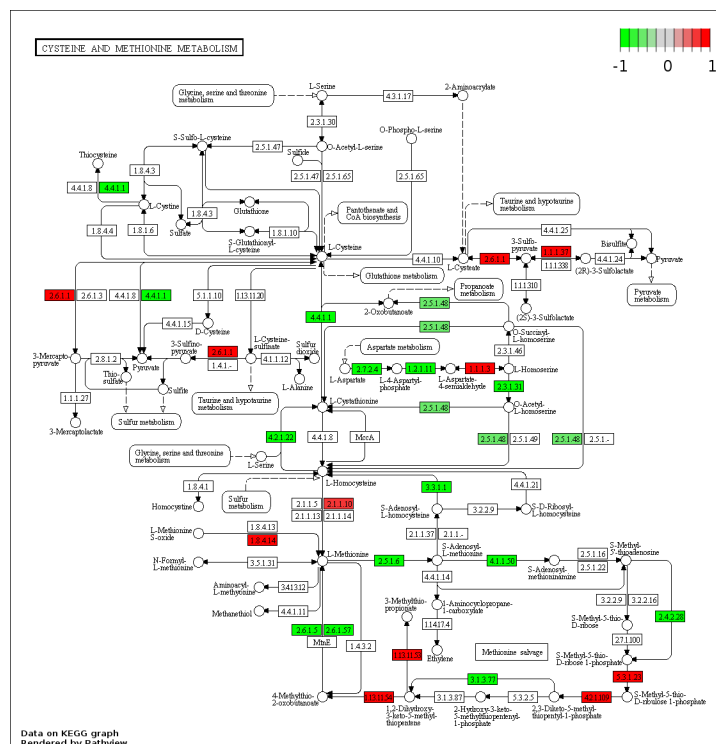


**Figure S40.** Differentially expressed genes from CDA encapsulated cells were overlaid on “Pyrimidine Metabolism” of *Saccharomyces cerevisiae* (*S. cerevisiae*). Green Color shows down regulated and Red Color shows upregulated genes.

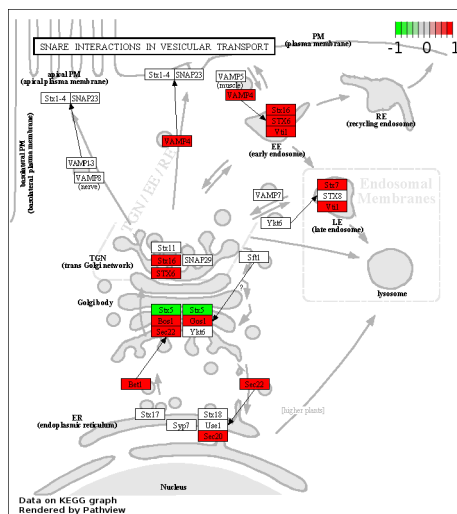


**Figure S41.** Differentially expressed genes from SD encapsulated cells were overlaid on “Pyrimidine Metabolism” of *Saccharomyces cerevisiae* (*S. cerevisiae*). Green Color shows down regulated and Red Color shows upregulated genes.





**Figure S44.** Differentially expressed genes from SD encapsulated cells were overlaid on “Cysteine and Methionine Metabolism” of *Saccharomyces cerevisiae* (*S. cerevisiae*). Green Color shows down regulated and Red Color shows upregulated genes.



**Figure S45.** Differentially expressed genes from SD encapsulated cells were overlaid on “SNARE Interactions in Vesicular Transport” of *Saccharomyces cerevisiae* (*S. cerevisiae*). Green Color shows down regulated and Red Color shows upregulated genes.

