Supporting Information

Bacterial Aggregation Triggered by Fibril Forming Tryptophan-Rich Sequences: Effects of Peptide Side Chain and Membrane Phospholipids

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Figure S1. Changes of the bacterial growth turbidity upon incubation with the 14 days old HHC nanofibrils. The experiments were performed once in triplicate.

Figures S2–S9. Representative TEM and FE-SEM micrographs of control *S. aureus* (ATCC 12600) and the cells exposed to HHC-10 and ^{4Har}HHC-10 at ½ MIC and 2 MIC, as well as the bacteria treated with the peptide nanofibrils. The bacterial aggregates are marked by orange arrows. Yellow arrows highlight nanostructure of different morphologies including but not limited to peptide aggregates formed over the course of bacterial aggregation. Membrane disruption (deformation) and degradation of cell wall septum are, respectively, presented by green and cyan arrows. The colorful square insets were magnified in the images outlined by the same color.

Figures S10–S17. Representative TEM and FE-SEM micrographs of control *E. coli* (ATCC 10536) and cells exposed to HHC-10 and ^{4Har}HHC-10 at ½ MIC and 2 MIC, as well as the bacteria treated with the peptide nanofibrils. Orange arrows highlight the aggregates of bacteria. Yellow arrows point to nanostructure of different morphologies including but not limited to peptide aggregates formed over the course of the cell aggregation. Pink arrows mark the inside cell protein aggregates that likely result from the interruption of bacterial proteostasis. Membrane buds (red), blebs (blue), thinning (cyan), and disruption and separation of bacterial outer and cytoplasmic membranes (green) are presented by the noted colors. The colorful square insets were magnified in the images outlined by the same color.

Figure S18. Fluorescence emission spectra for the membrane (SUVs) binding of HHC peptides.

Figure S19. CD spectra of ^{4Har}HHC-10 upon interaction with the SUVs. The peptide concentration was 200 μM at $c_P/c_L = 0.04$, where the optical artifacts due to the liposomal particulates were minimal. The peptide and lipid were incubated at 37 °C for 30 min prior to the measurement.

Figure S20. FRET emission spectra for the interaction of POPE/POPG [3/1 (mol/mol)] (A) single/double labelled LUVs and (B) single labelled SUVs with HHC-10 and ^{4Har}HHC-10 (λ_{ex} = 460 nm). The spectra were registered for the fluorescence emission of NBD at 480–650 nm and Rhod at 580–650 nm.

Figures S21–S29. FE-SEM and TEM of ultrastructure images of anionic SUVs alone and in the presence of HHC peptides (c_P/c_L = 0.08). Red arrows highlight the exemplar free (and/or control) SUVs of different phospholipid composition. Yellow arrows point to the aggregates of lipid vesicles (SUVs) in complexation with the HHC peptides. The micrographs show different morphologies and aggregate nanostructures of POPC/POPG liposomes in comparison with those made of POPE/POPG [3/1 (mol/mol)].

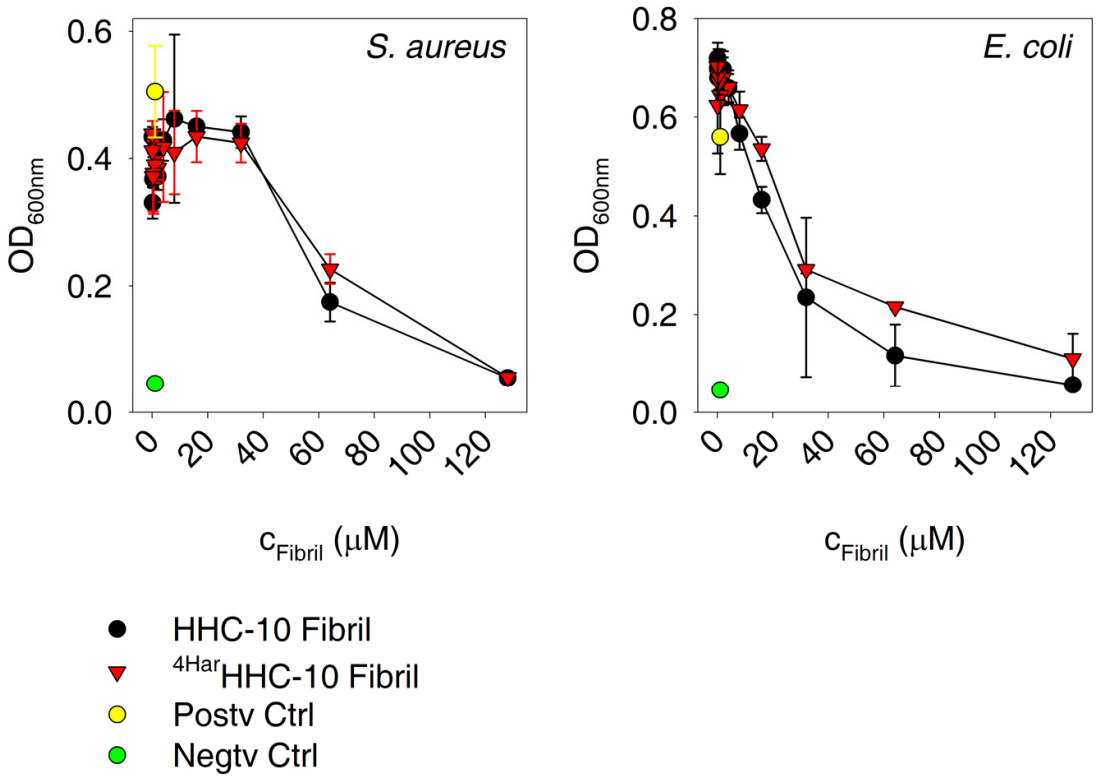
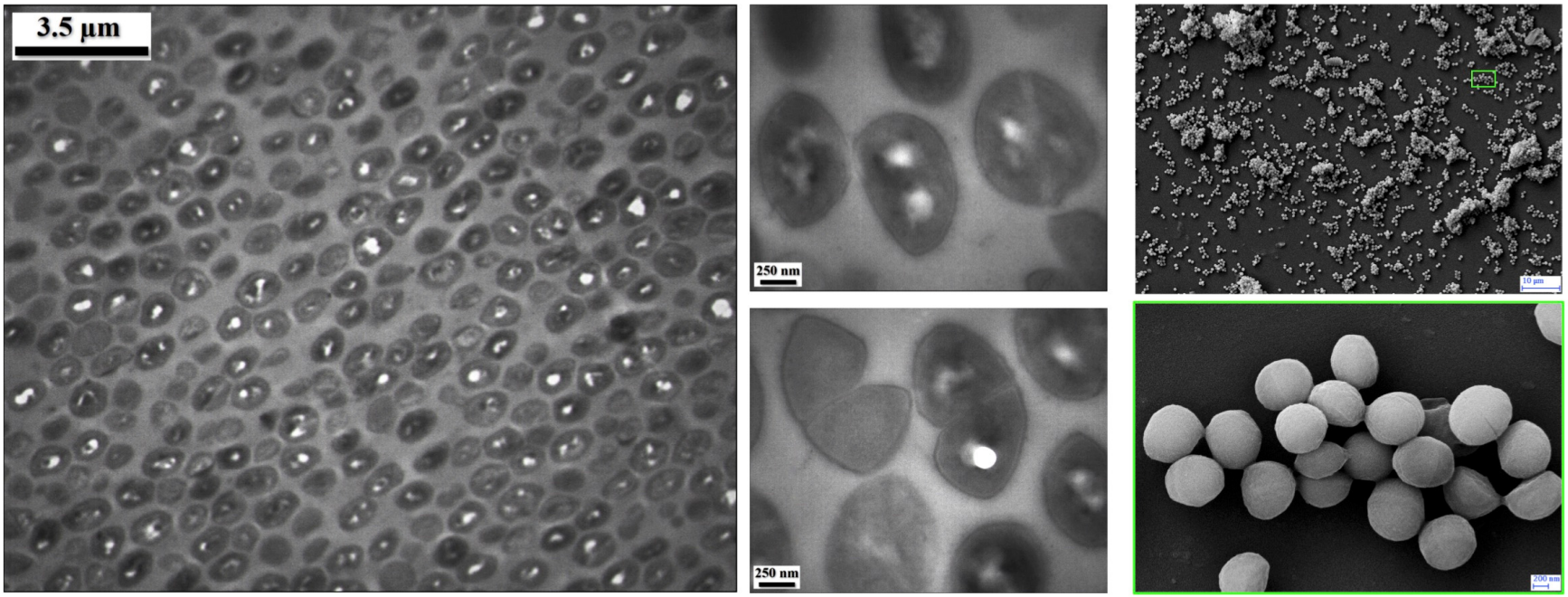
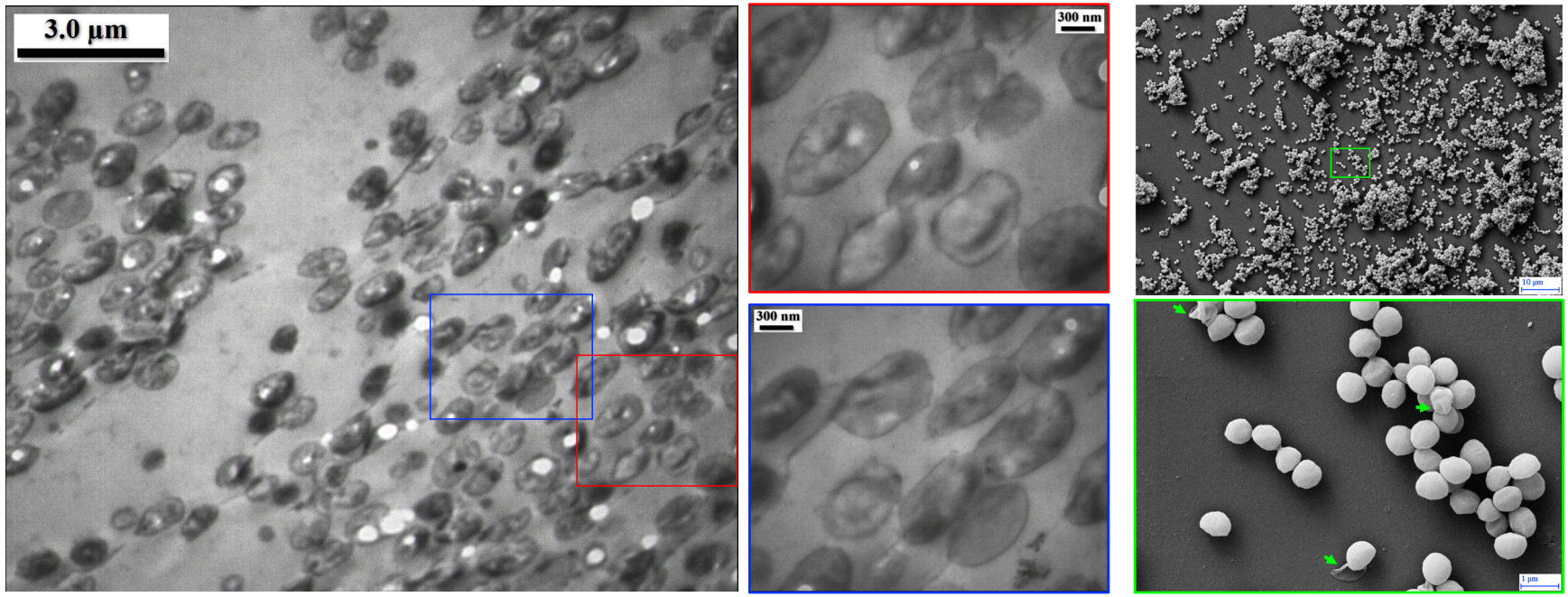

S. aureus
E. coli
OD600nm
cFibril (μM)
HHC-10 Fibril
4HarHHC-10 Fibril
Postv Ctrl
Negtv Ctrl

Figure S1



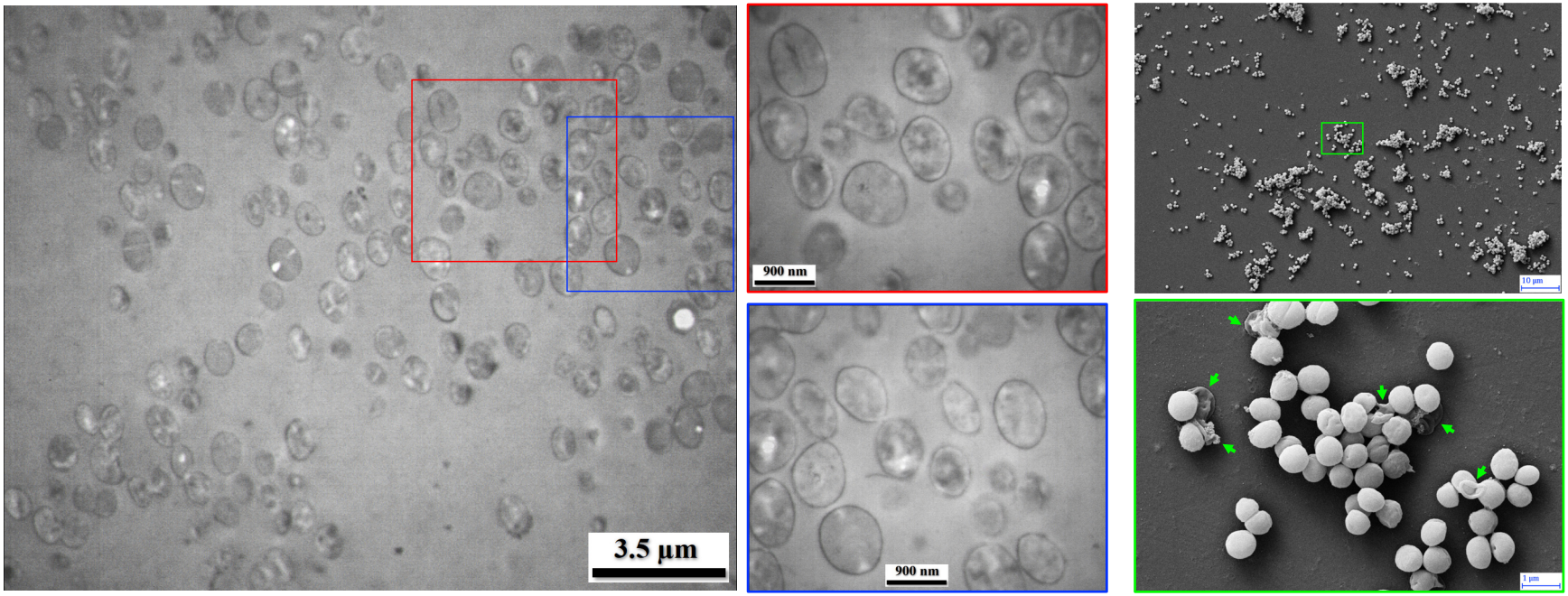
S. aureus Control

Figure S2



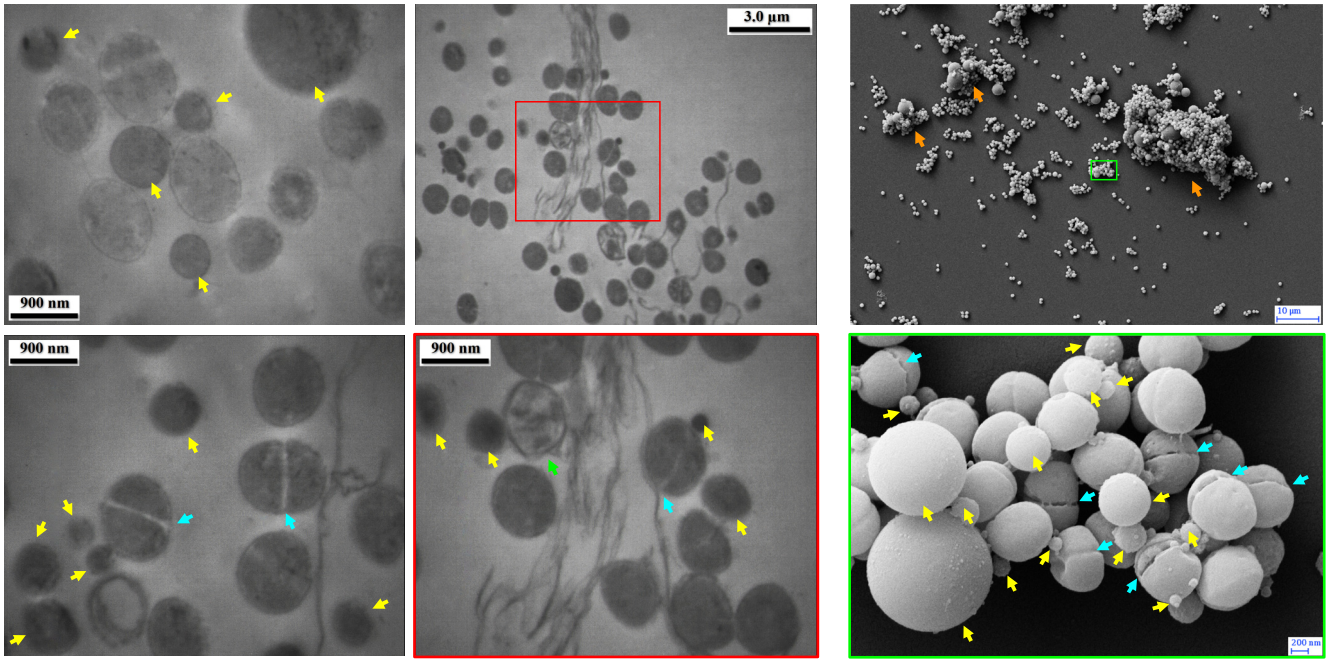
HHC-10 @ ½ MIC

Figure S3



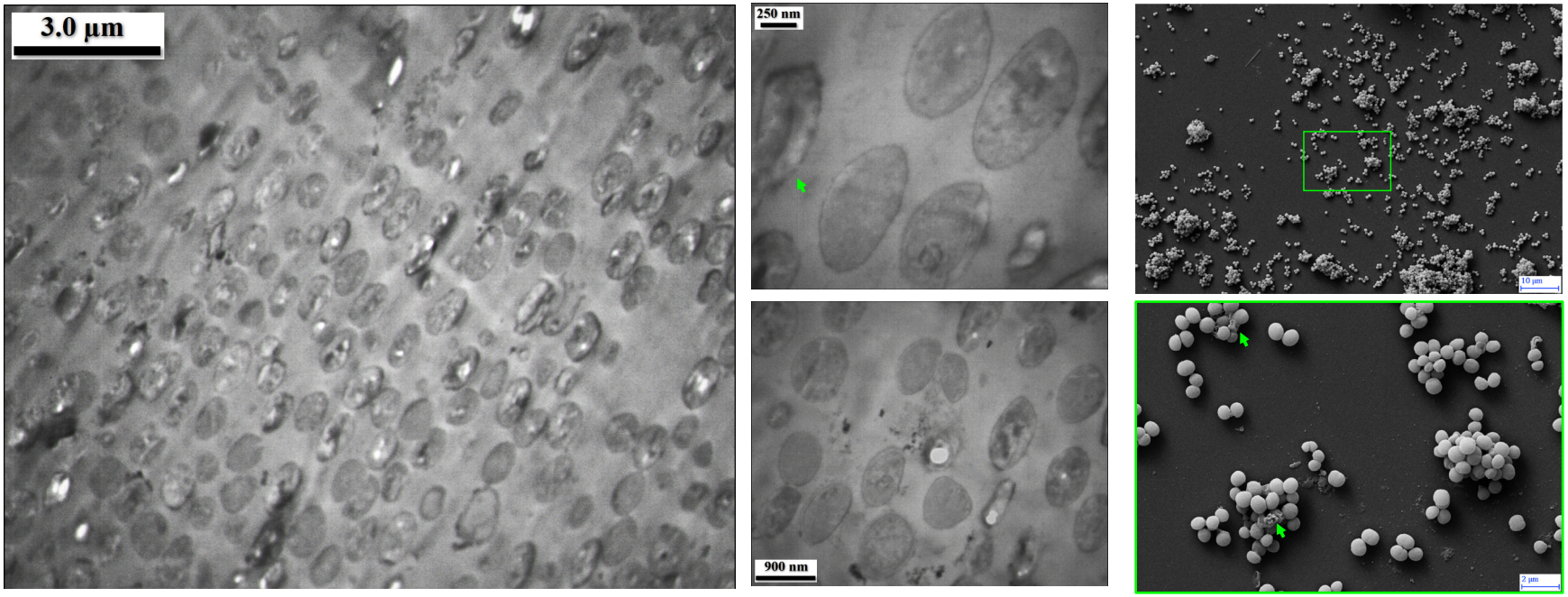
HHC-10 @ 2 MIC

Figure S4



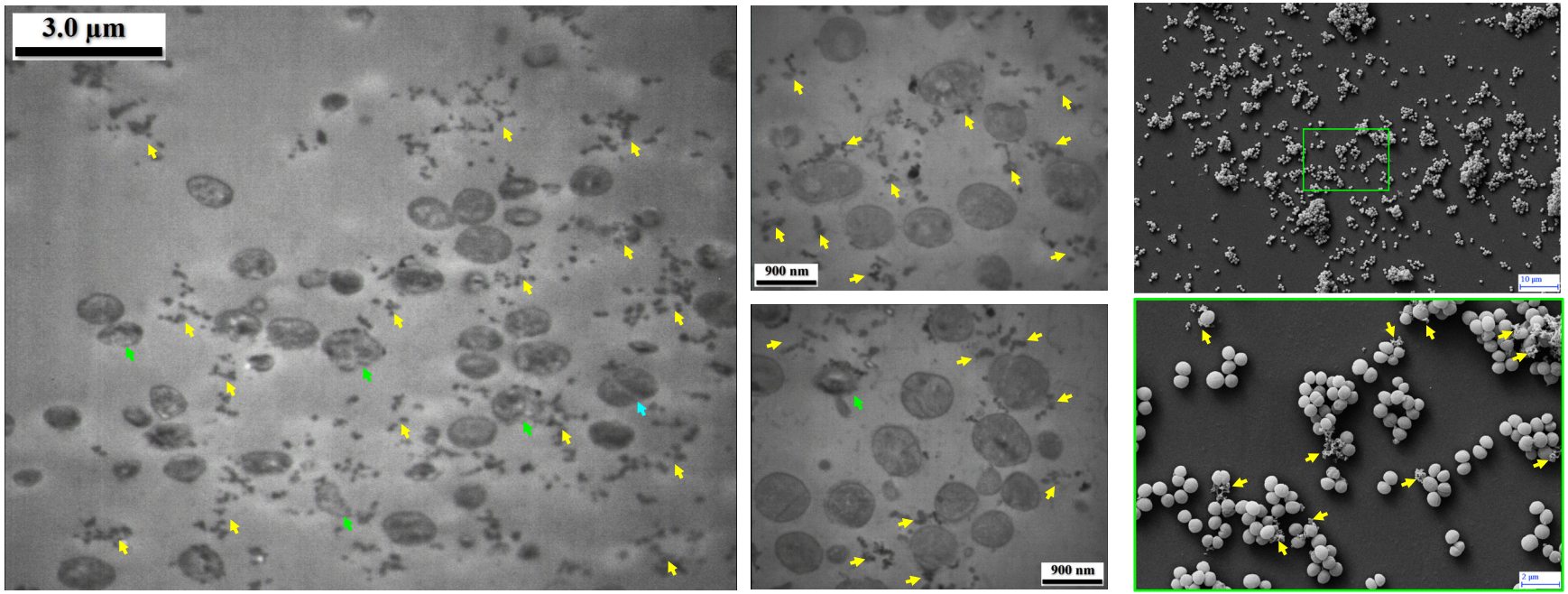
HHC-10 @ 128 ∝M

Figure S5



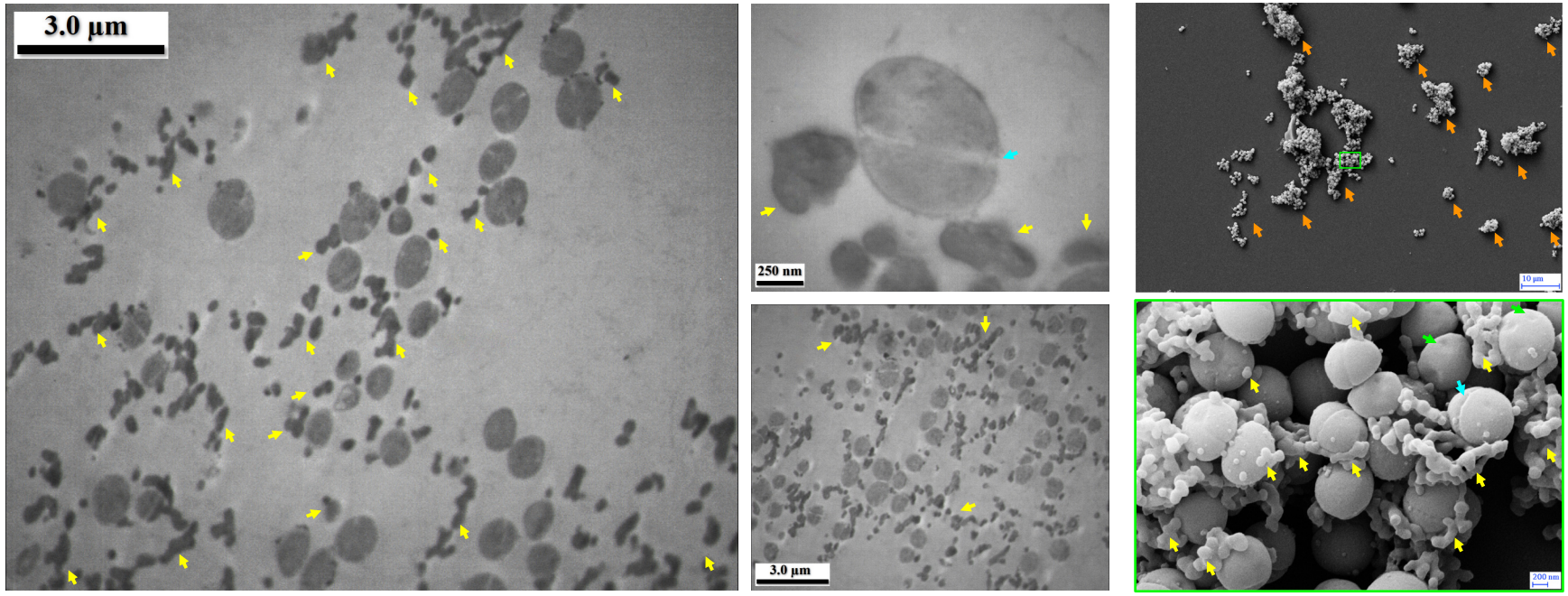
^{4Har}HHC-10 @ ½ MIC

Figure S6



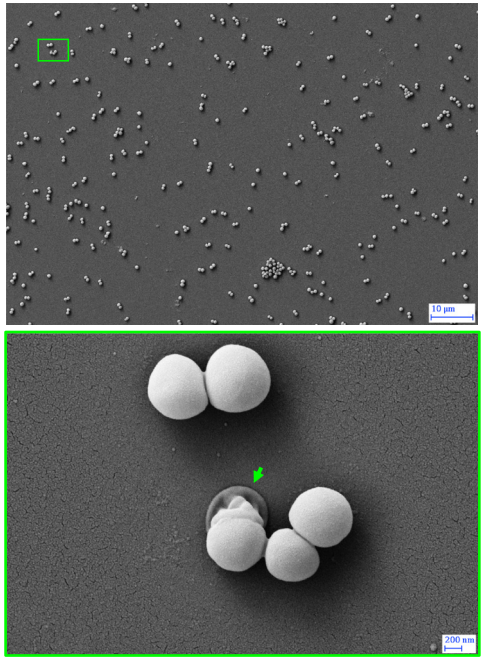
^{4Har}HHC-10 @ 2 MIC

Figure S7

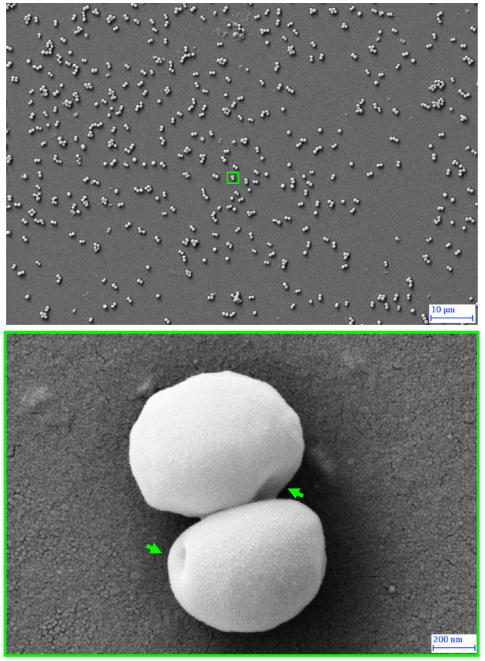


^{4Har}HHC-10 @ 128 ∝M

Figure S8

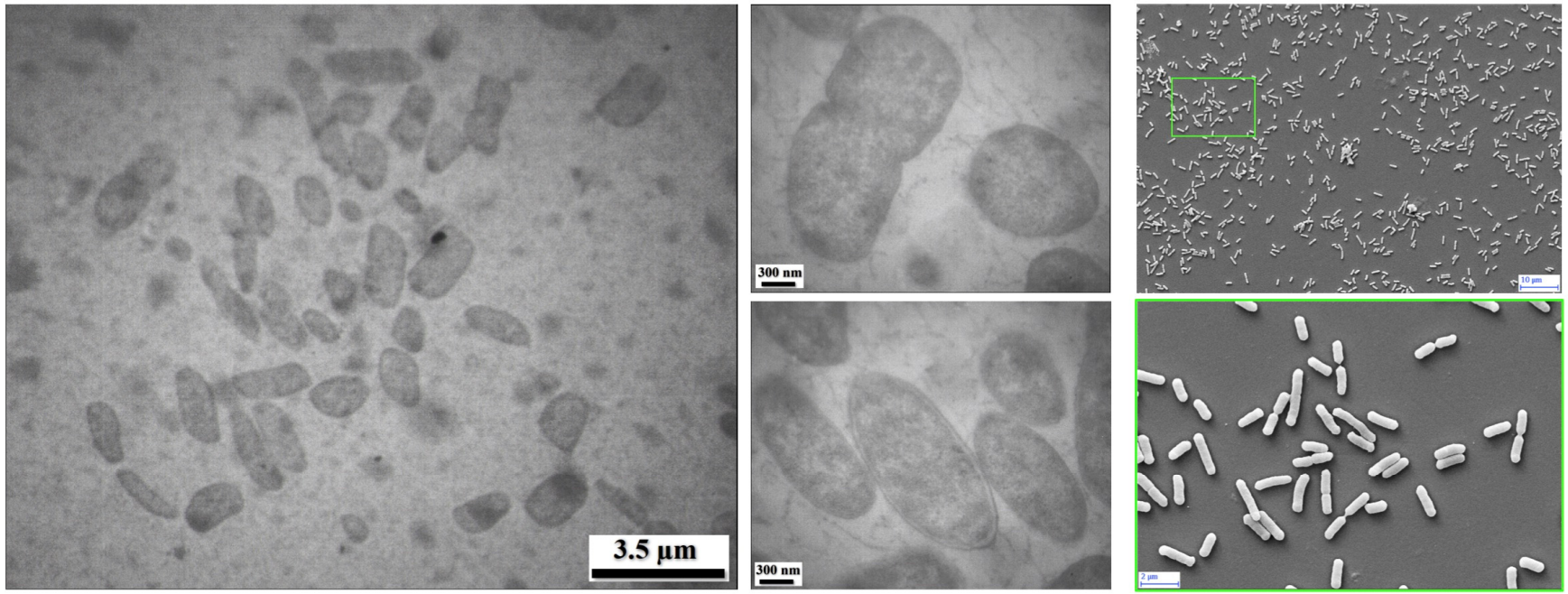


HHC-10 nanofibers



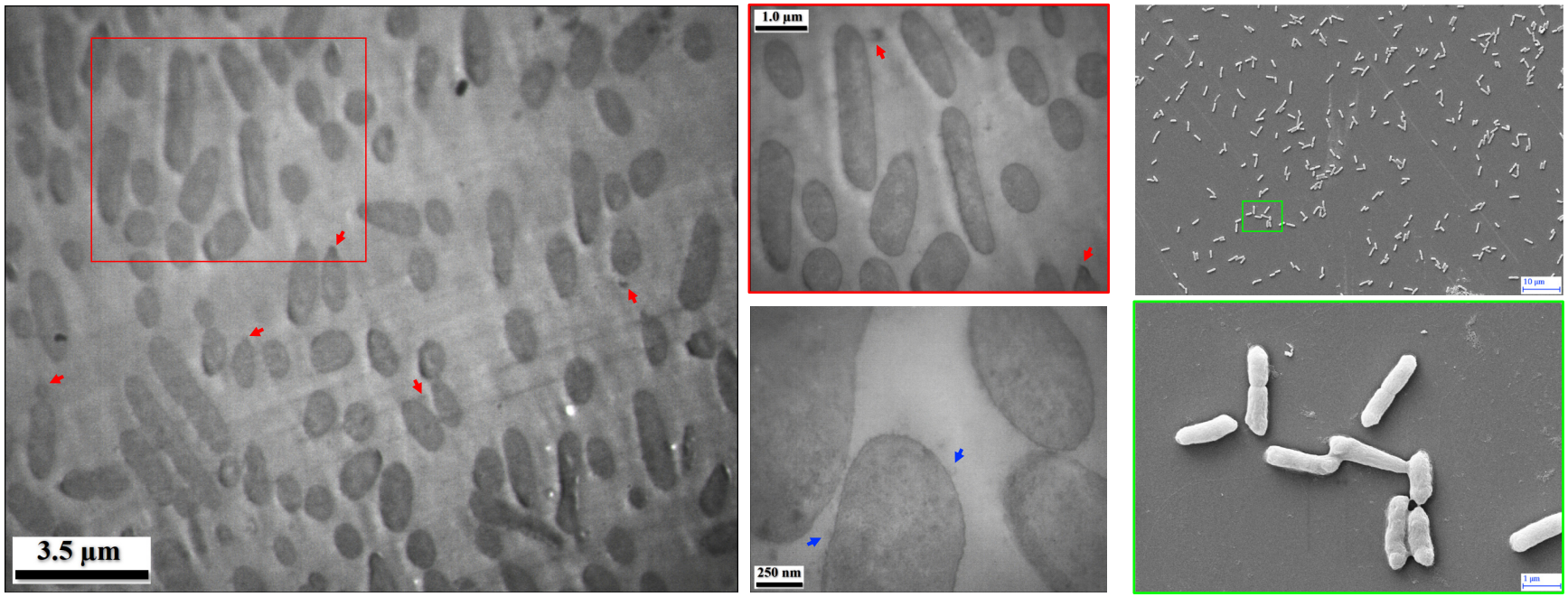
^{4Har}HHC-10 nanofibers

Figure S9



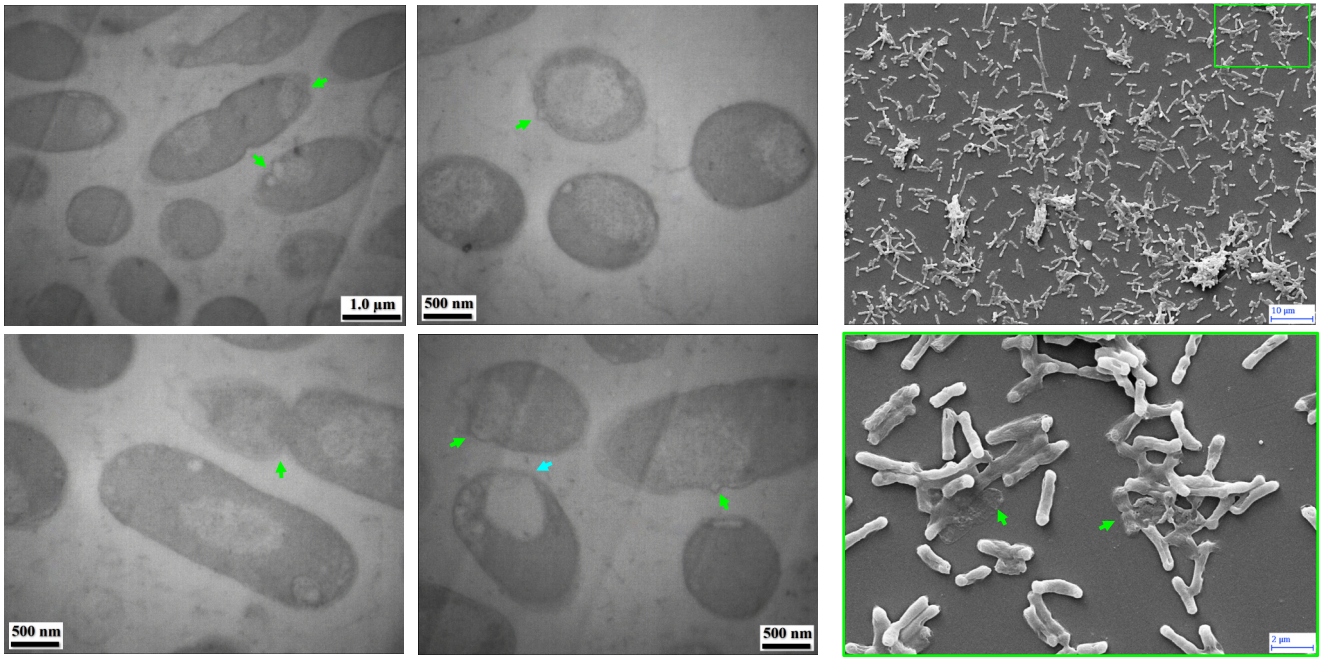
E. coli Control

Figure S10



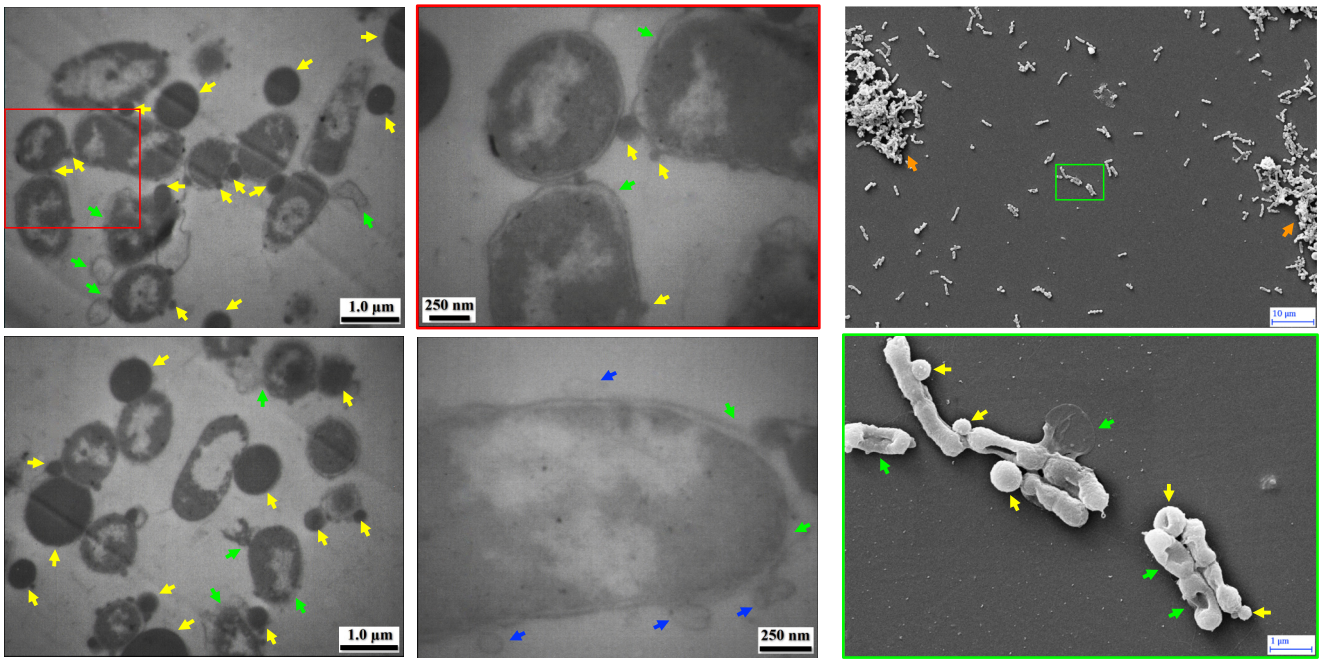
HHC-10 @ ½ MIC

Figure S11



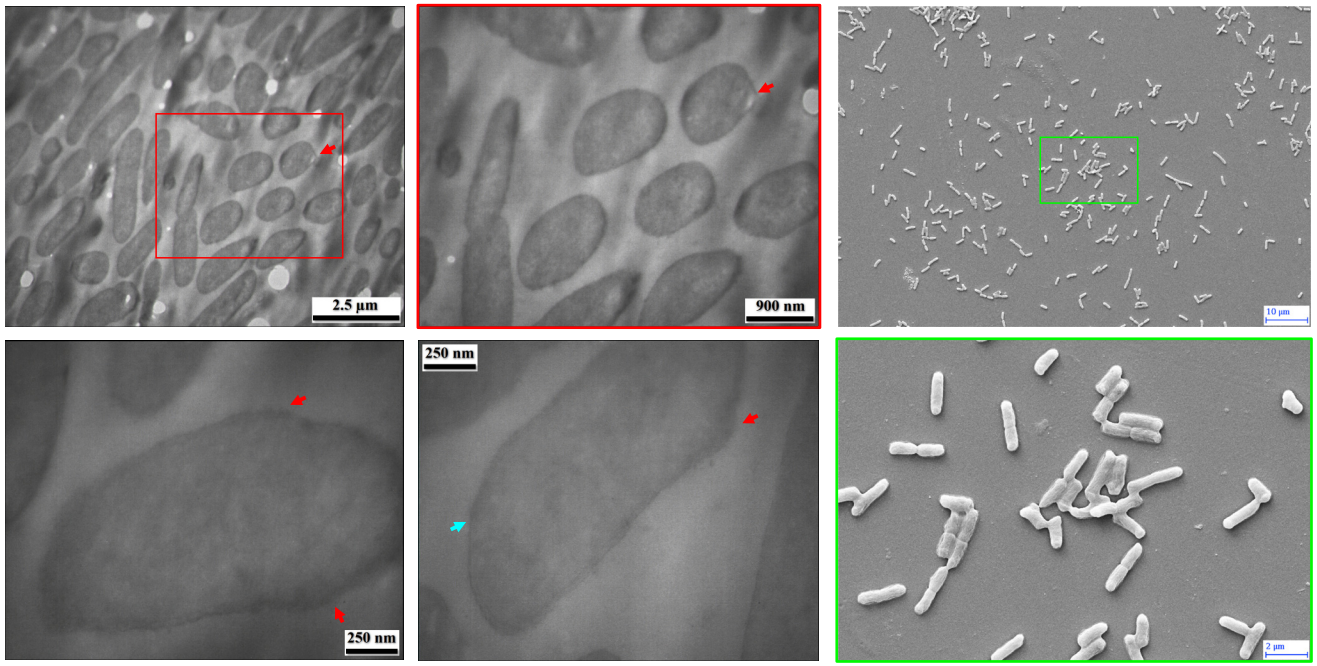
HHC-10 @ 2 MIC

Figure S12



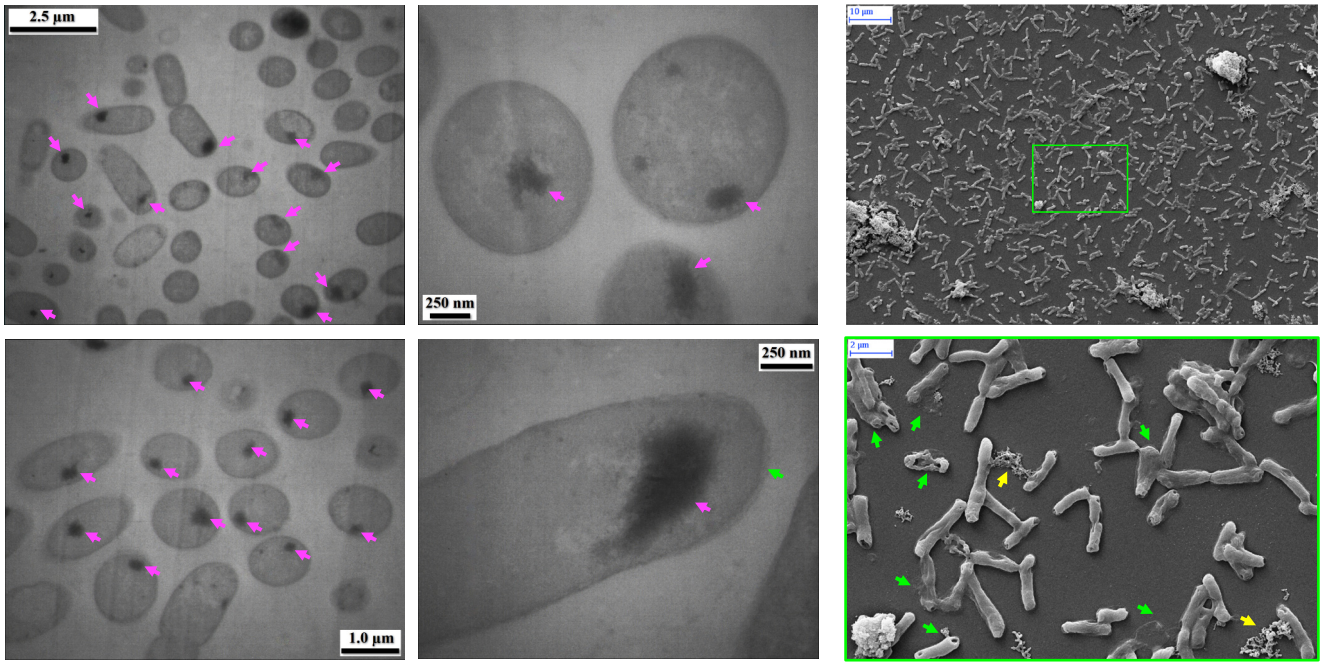
HHC-10 @ 128 ∝M

Figure S13



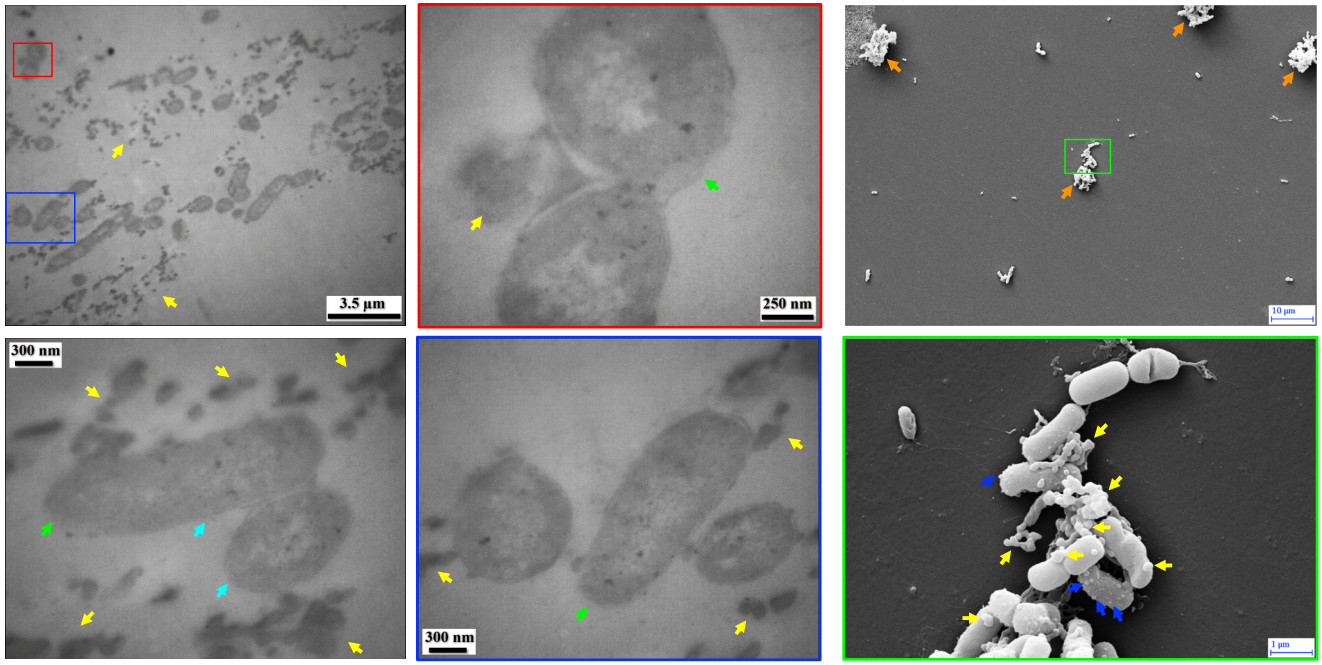
^{4Har}HHC-10 @ ½ MIC

Figure S14



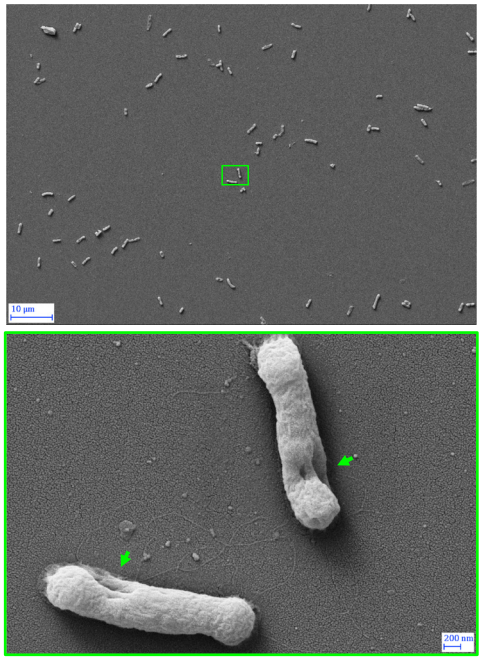
^{4Har}HHC-10 @ 2 MIC

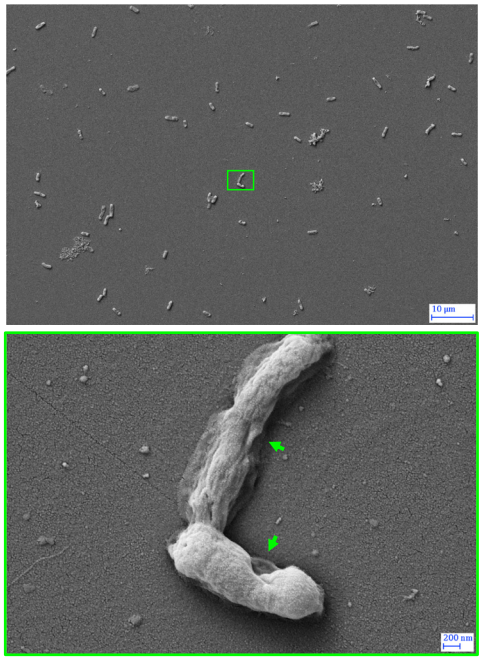
Figure S15



^{4Har}HHC-10 @ 128 ∝M

Figure S16





HHC-10 nanofibers ^{4Har}HHC-10 nanofibers

Figure S17

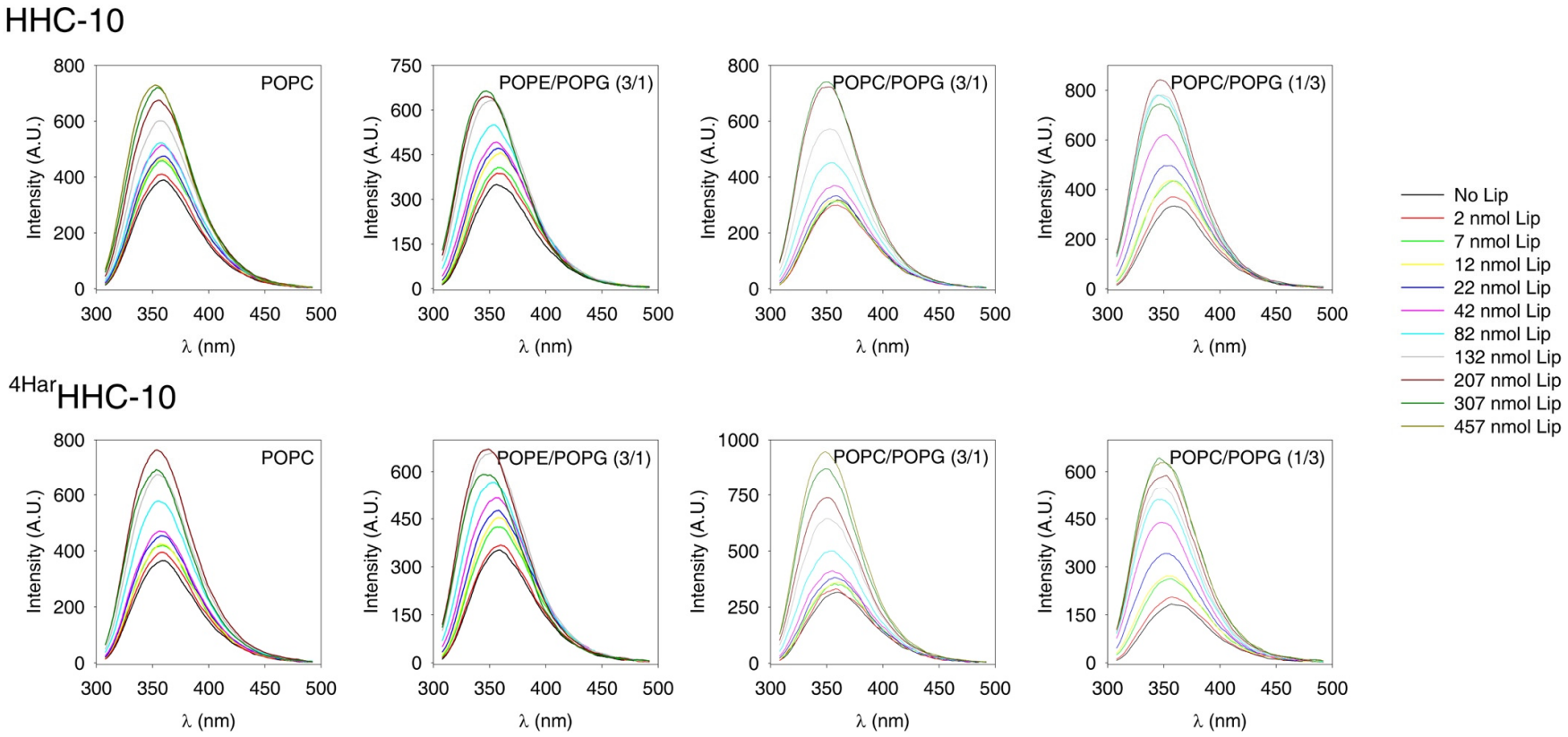

HHC-10
POPC
POPE/POPG (3/1)
POPC/POPG (3/1)
POPC/POPG (1/3)
4HarHHC-10
POPC
POPE/POPG (3/1)
POPC/POPG (3/1)
POPC/POPG (1/3)
Intensity (A.U.)
λ (nm)
No Lip
2 nmol Lip
7 nmol Lip
12 nmol Lip
22 nmol Lip
42 nmol Lip
82 nmol Lip
132 nmol Lip
207 nmol Lip
307 nmol Lip
457 nmol Lip

Figure S18

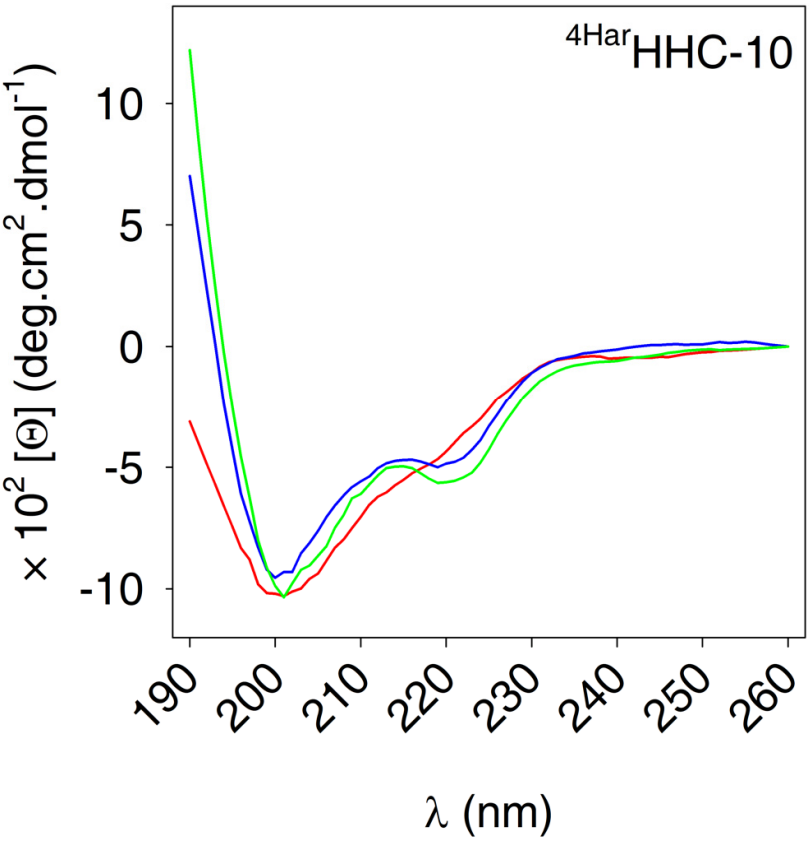

4HarHHC-10
10
5
0
-5
-10
× 10² [Θ] (deg.cm².dmol⁻¹)
190
200
210
220
230
240
250
260
λ (nm)

Figure S19

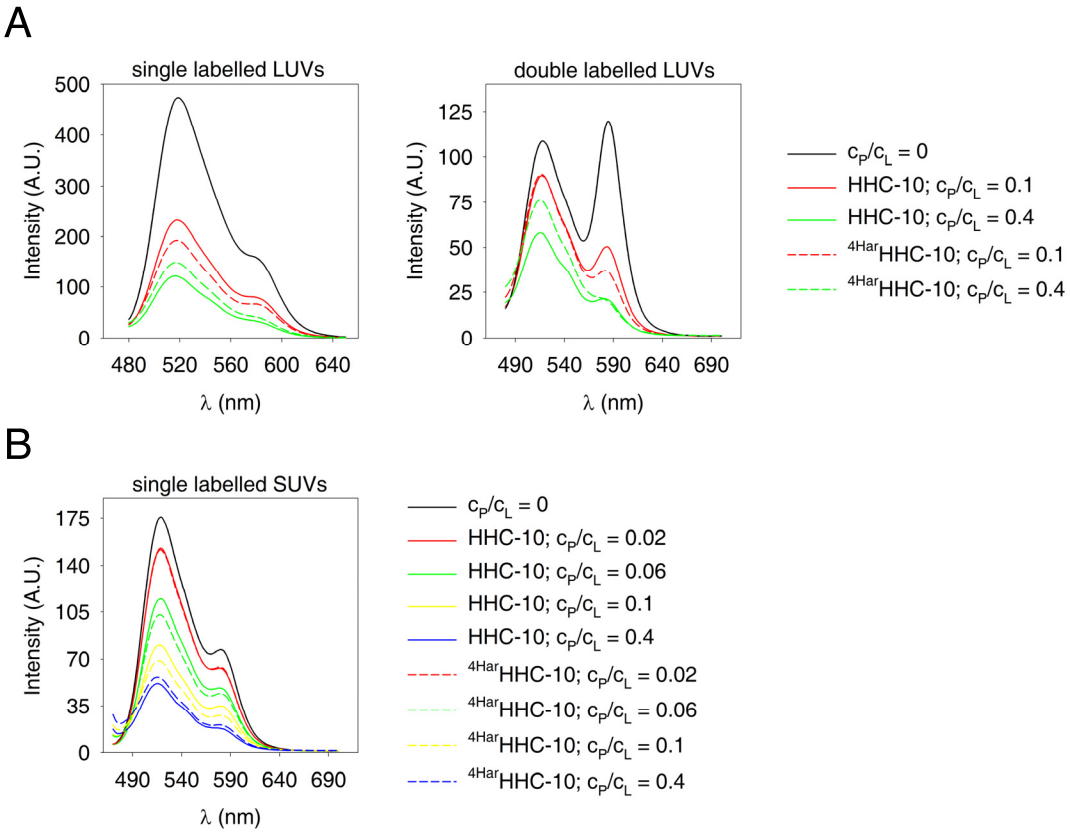
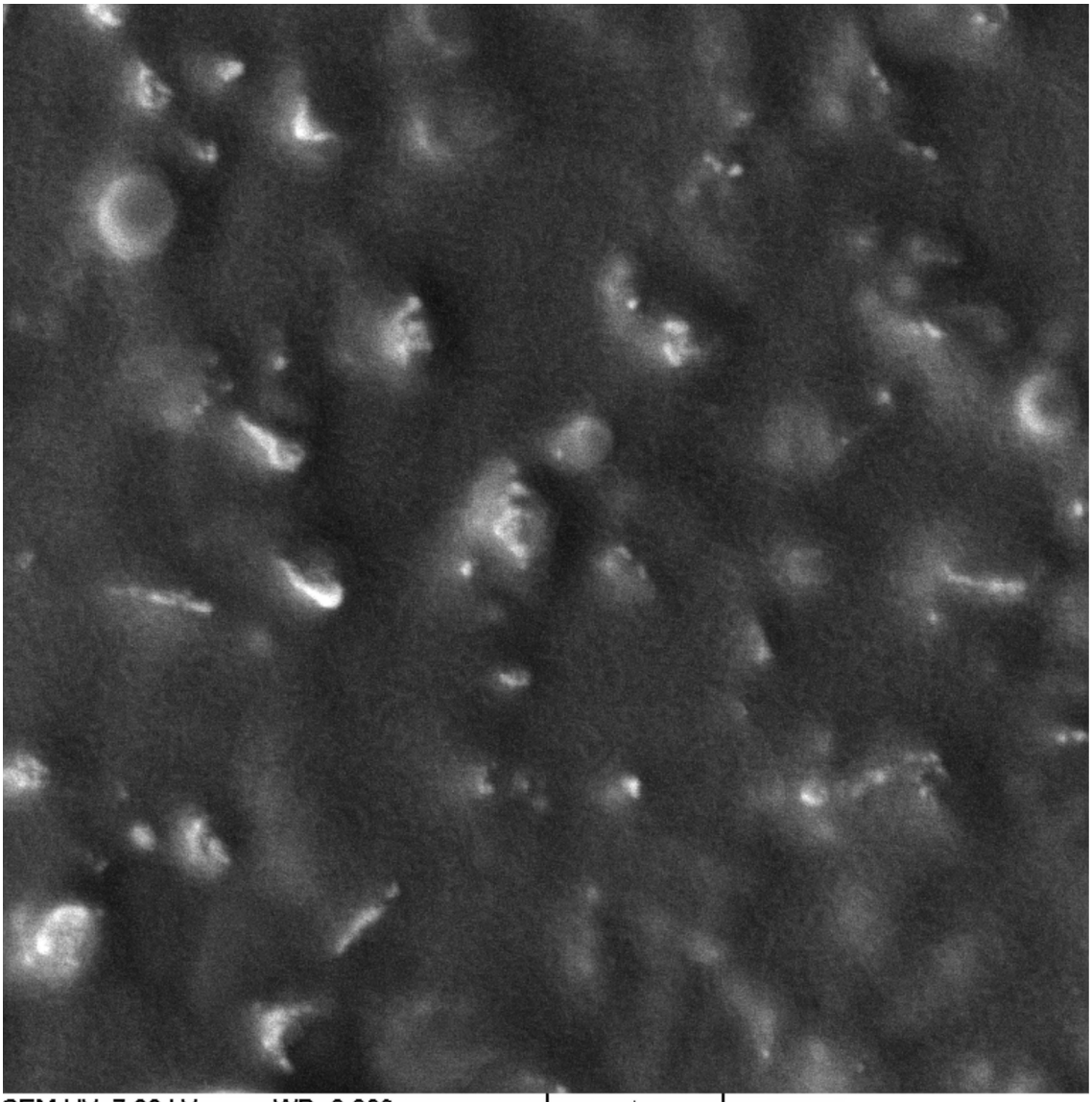
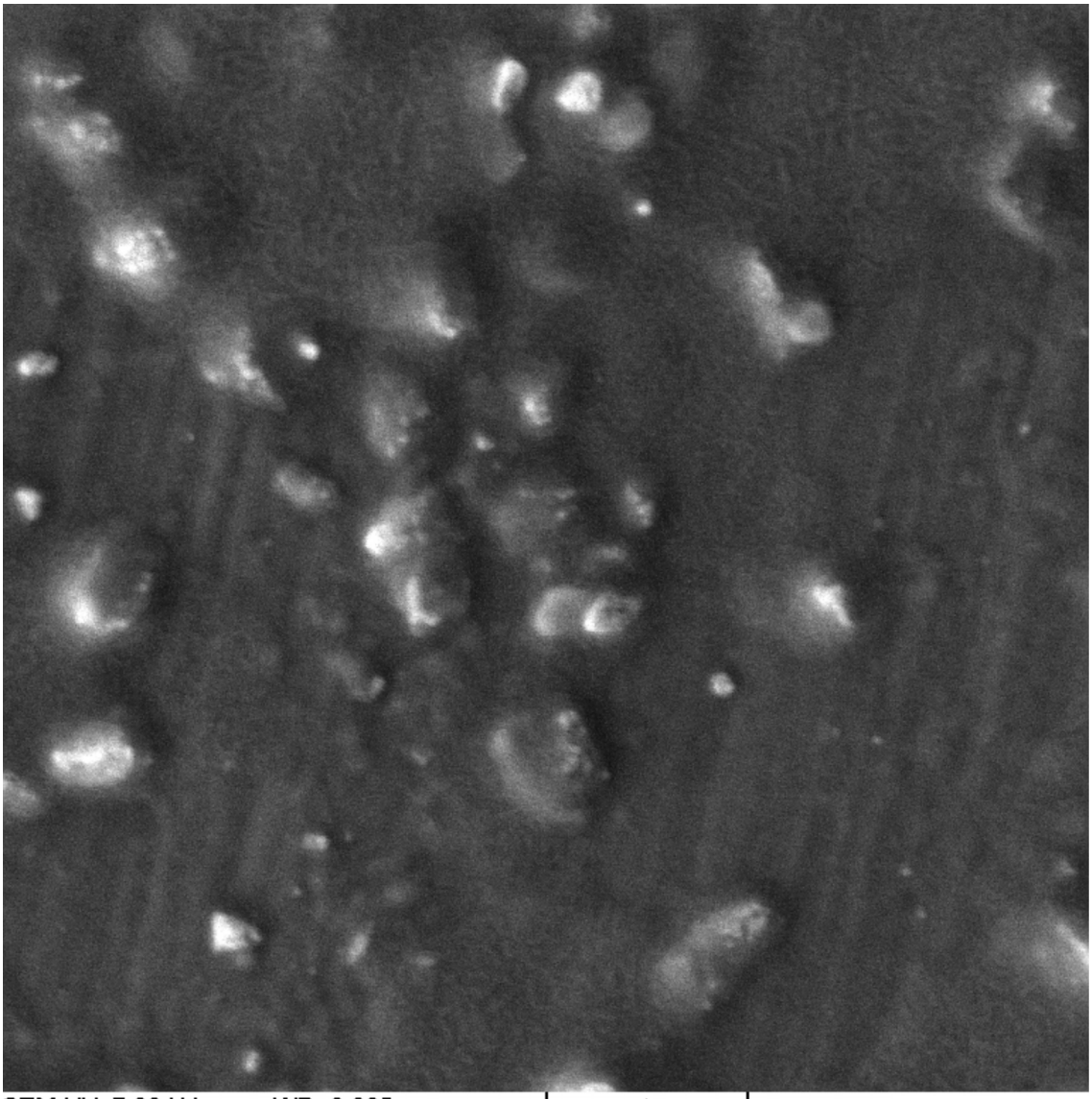

A
single labelled LUVs
double labelled LUVs
Intensity (A.U.)
λ (nm)
c_P/c_L = 0
HHC-10; c_P/c_L = 0.1
HHC-10; c_P/c_L = 0.4
^{4Har}HHC-10; c_P/c_L = 0.1
^{4Har}HHC-10; c_P/c_L = 0.4
B
single labelled SUVs
c_P/c_L = 0
HHC-10; c_P/c_L = 0.02
HHC-10; c_P/c_L = 0.06
HHC-10; c_P/c_L = 0.1
HHC-10; c_P/c_L = 0.4
^{4Har}HHC-10; c_P/c_L = 0.02
^{4Har}HHC-10; c_P/c_L = 0.06
^{4Har}HHC-10; c_P/c_L = 0.1
^{4Har}HHC-10; c_P/c_L = 0.4

Figure S20



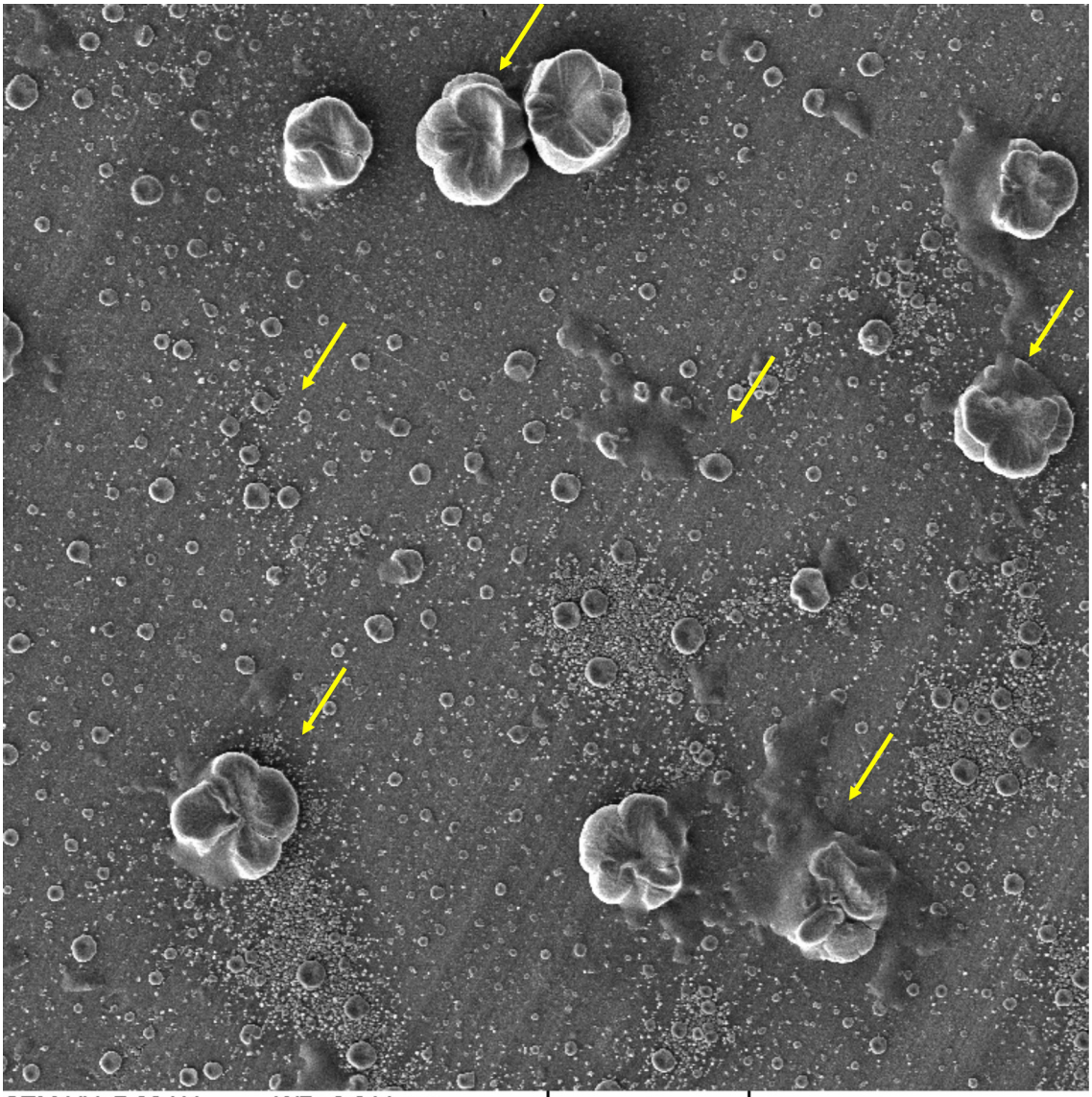
POPE/POPG [3/1 (mol/mol)]

Figure S21



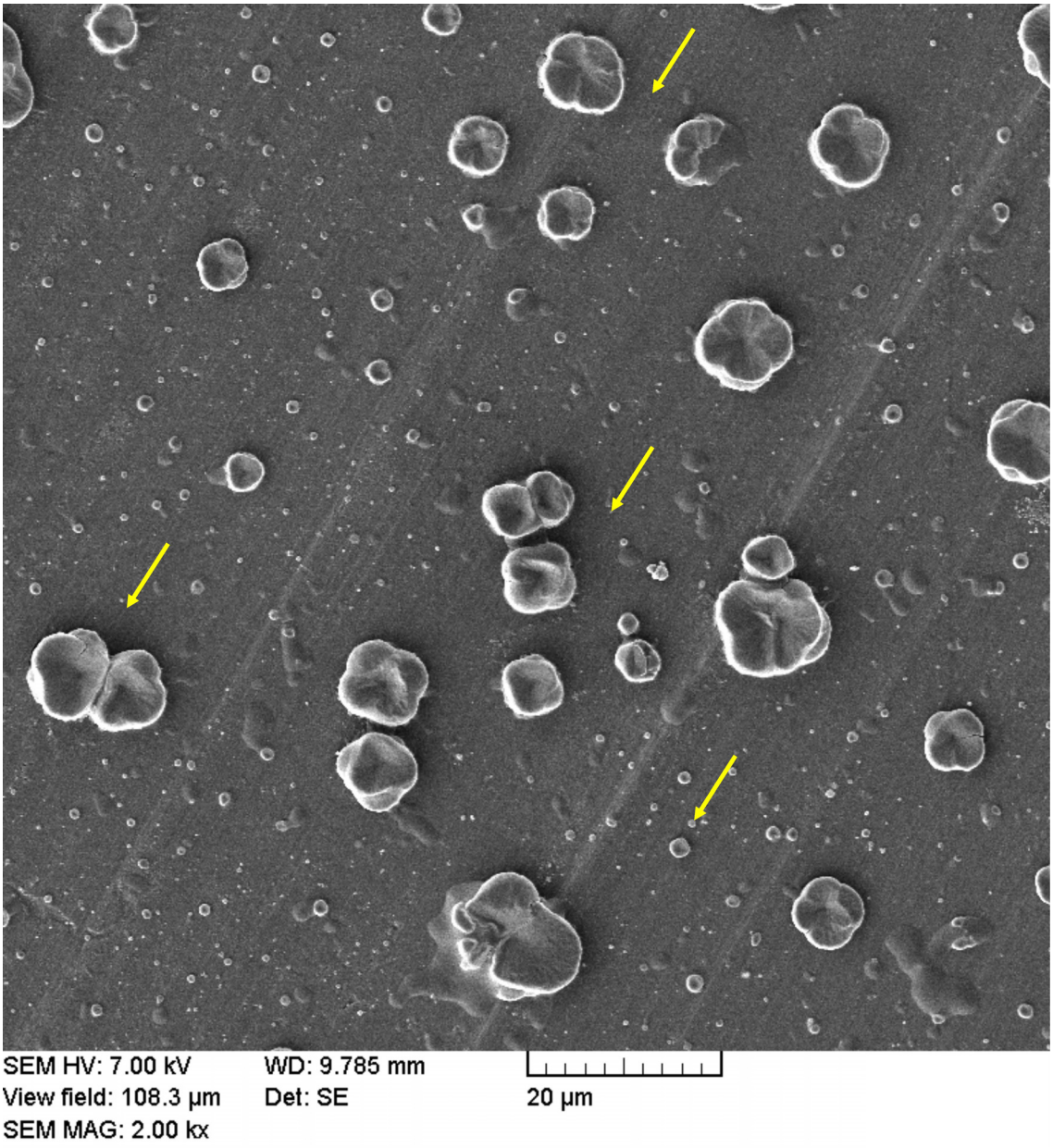
POPC/POPG [1/3 (mol/mol)]

Figure S22



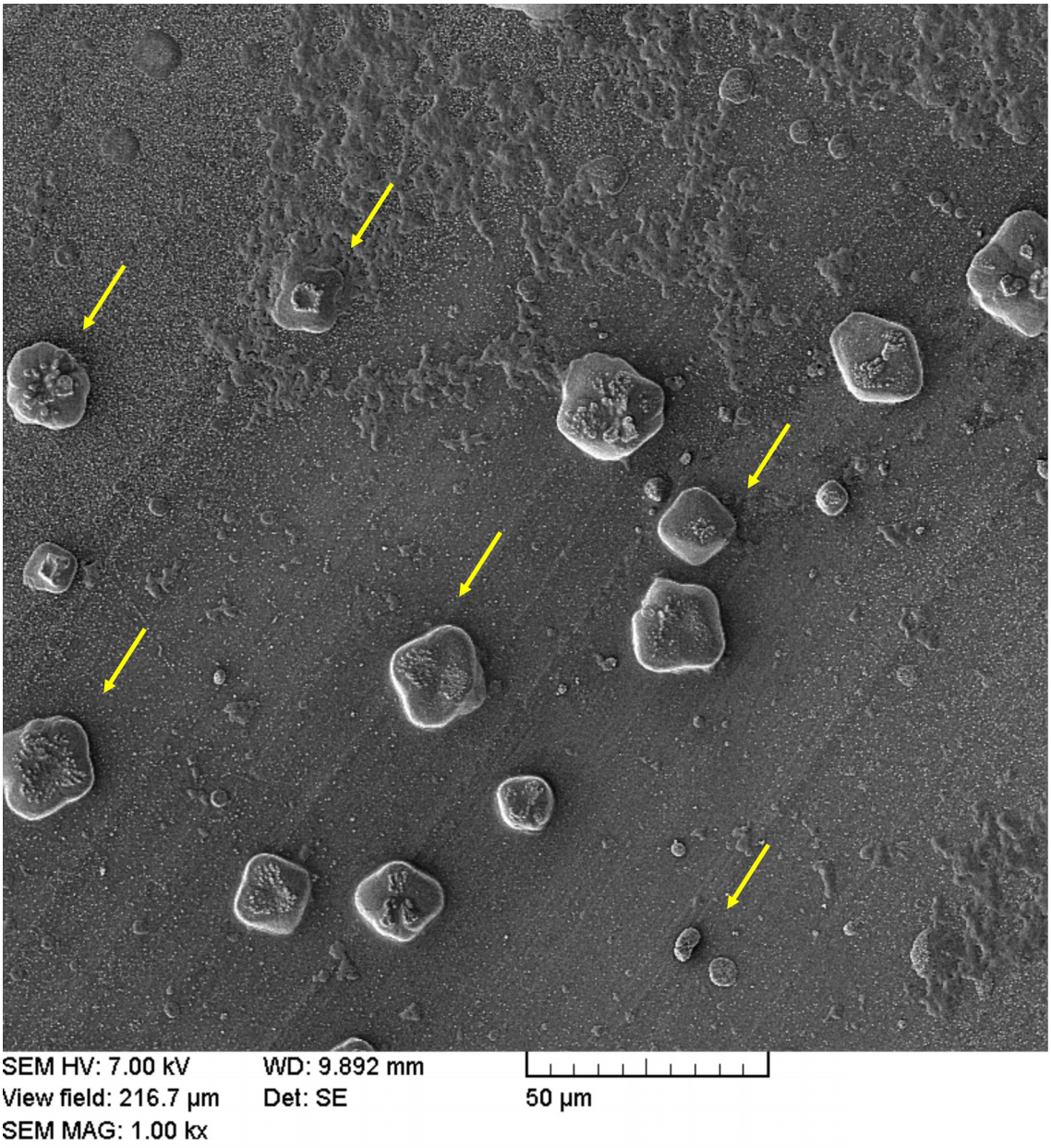
HHC-10:POPE/POPG [3/1 (mol/mol)]

Figure S23



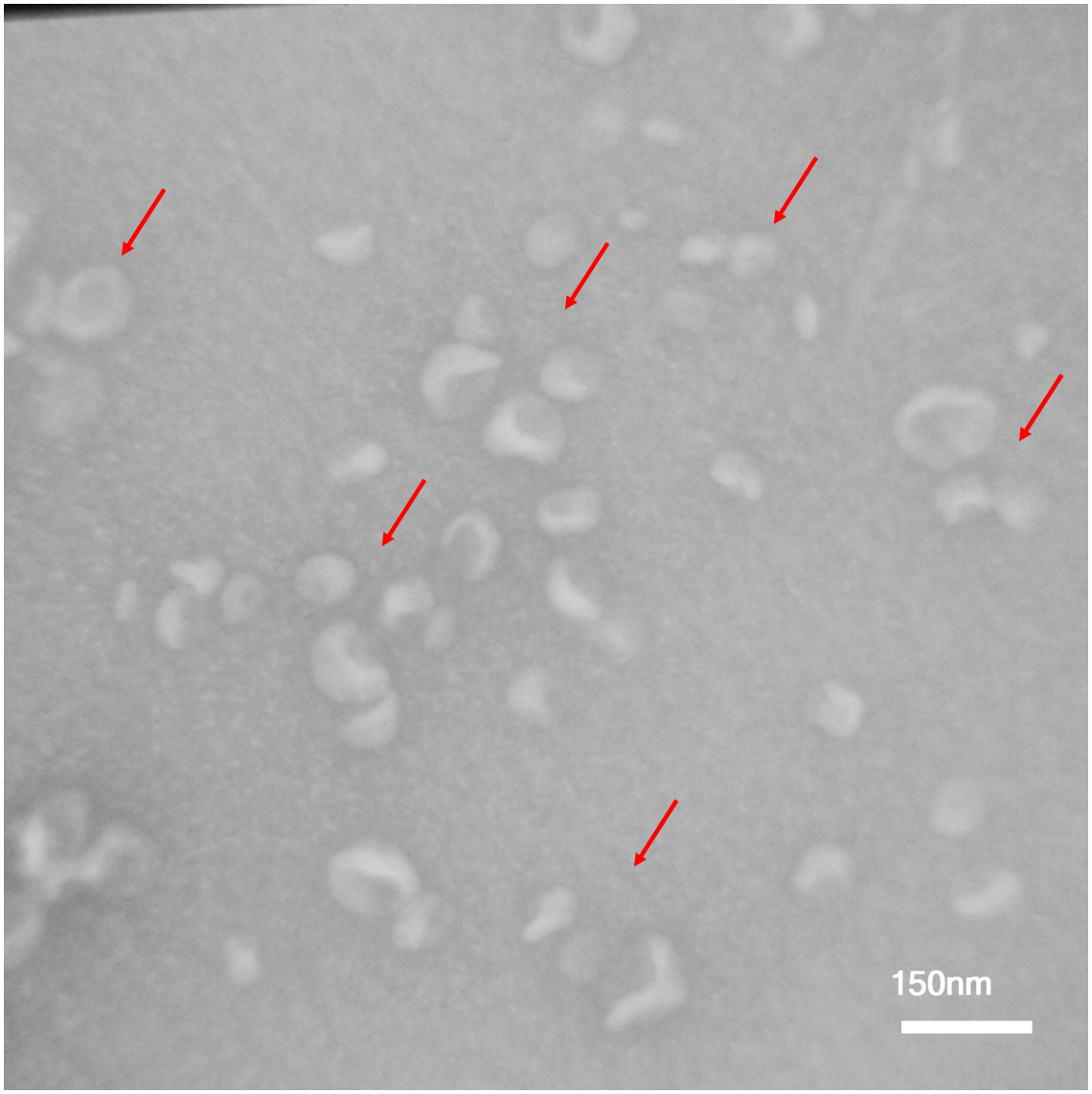
^{4Har}HHC-10:POPE/POPG [3/1 (mol/mol)]

Figure S24



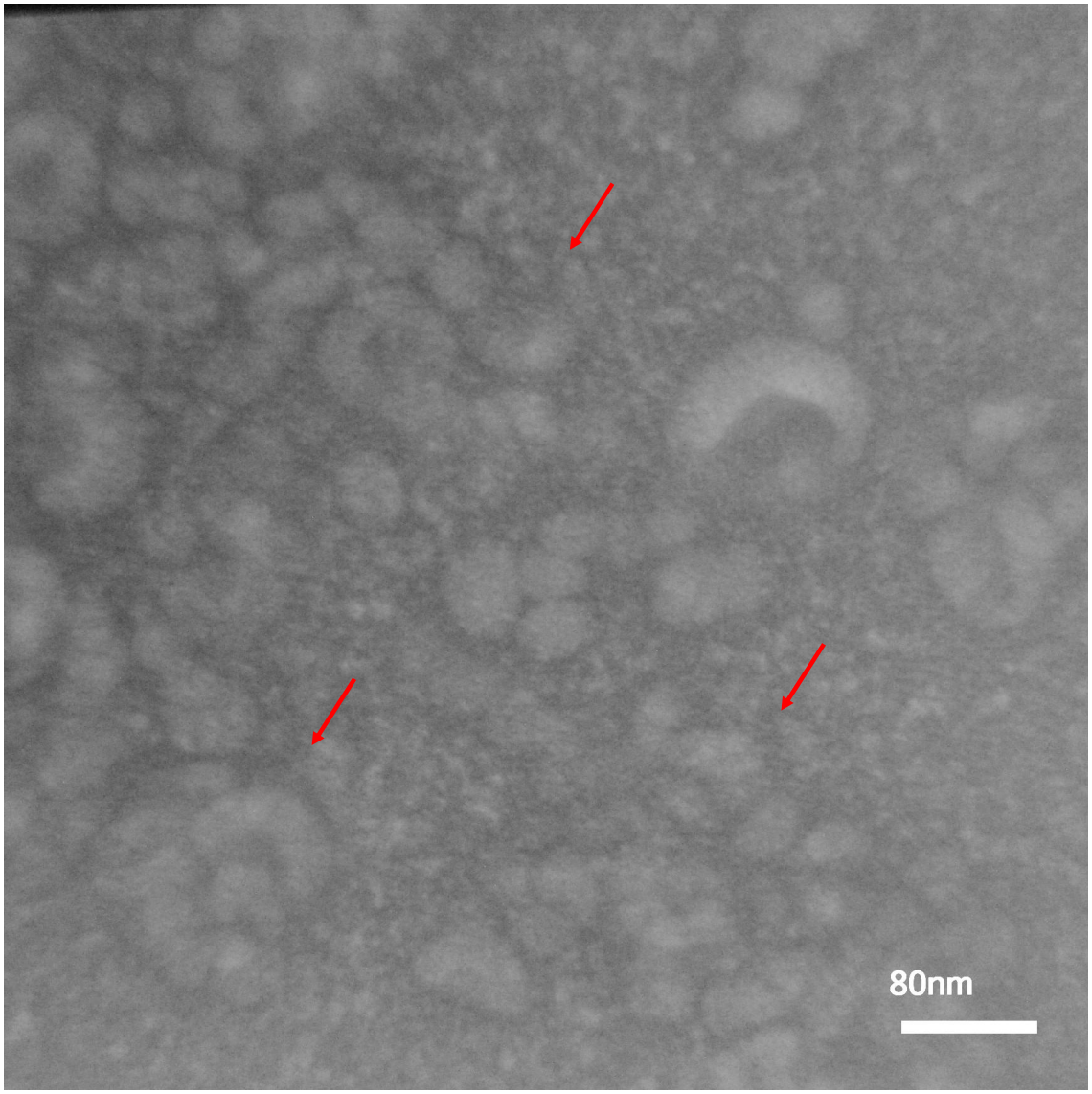
^{4Har}HHC-10:POPC/POPG [1/3 (mol/mol)]

Figure S25



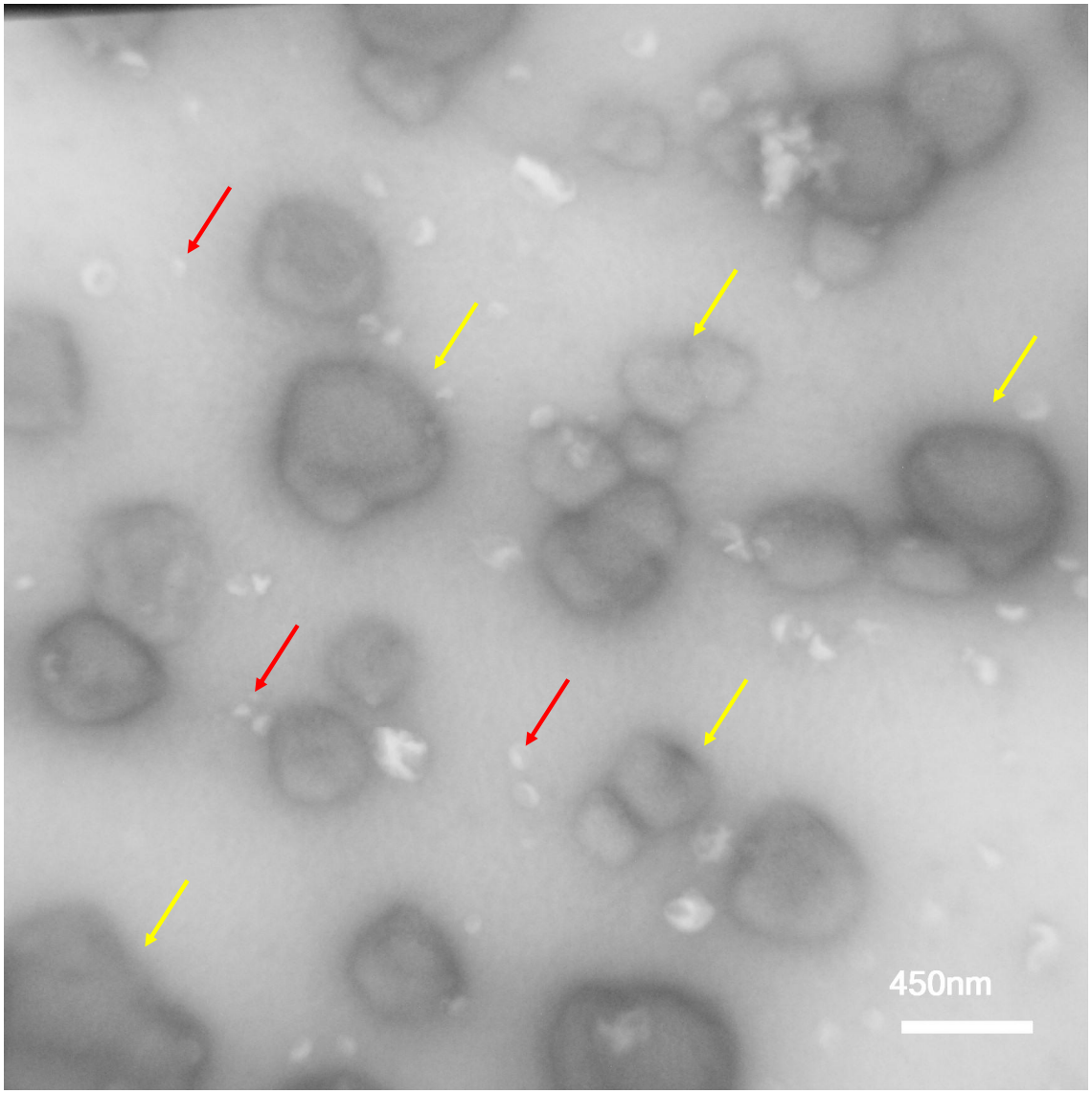
POPE/POPG [3/1 (mol/mol)]

Figure S26



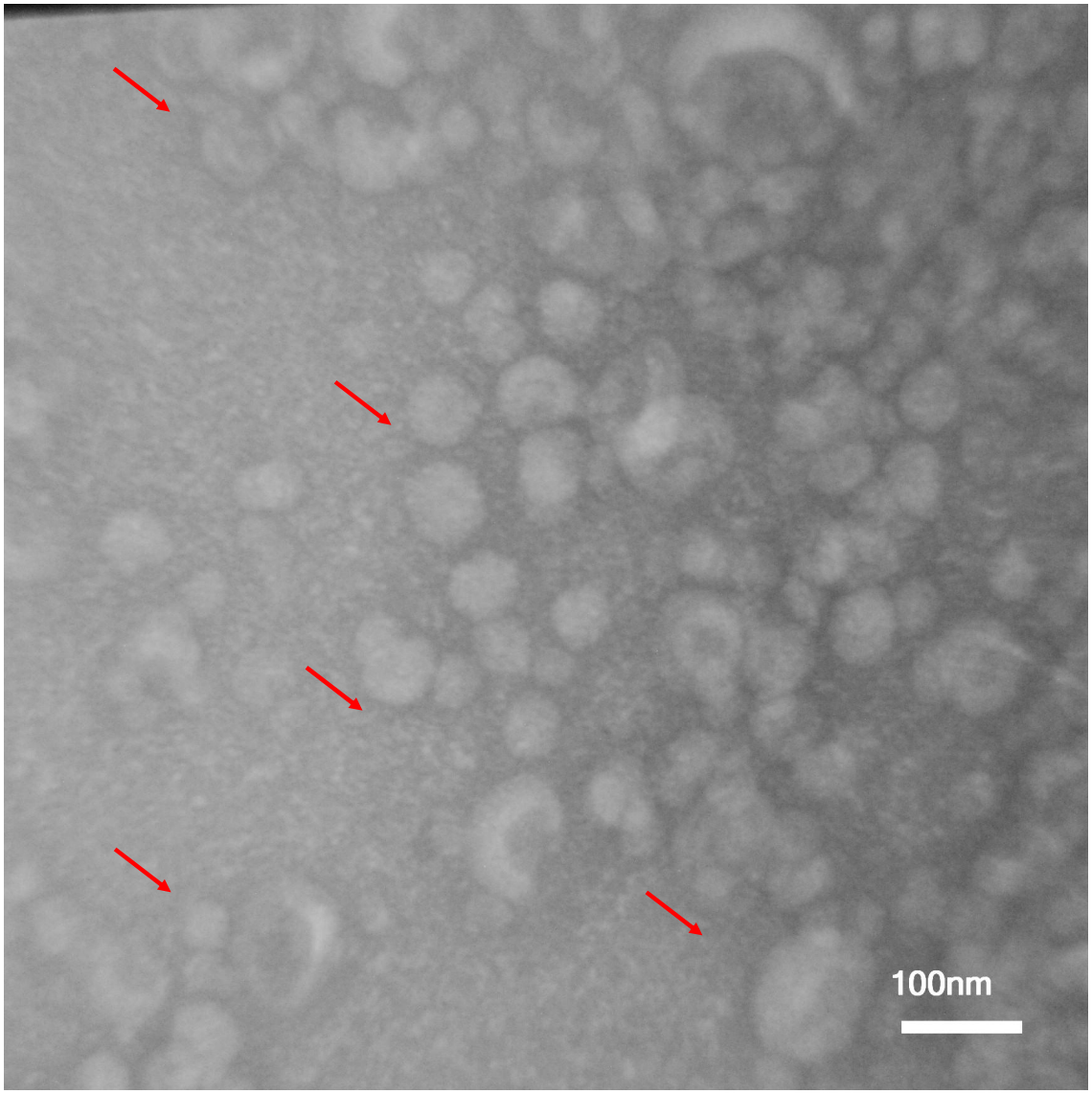
POPC/POPG [3/1 (mol/mol)]

Figure S27



^{4Har}HHC-10:POPE/POPG [3/1 (mol/mol)]

Figure S28



^{4Har}HHC-10:POPC/POPG [3/1 (mol/mol)]

Figure S29