Supplementary Information for

Batch production of high-quality graphene grids for cryo-EM: cryo-EM structure of *methylococcus capsulatus* soluble methane monooxygenase hydroxylase

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Supplementary Movies

Movie S1. Batch-mode graphene grid production video

Movie S2. Tomogram of sMMOH particles on a graphene coated grid.

Movie S3. Tomogram of sMMOH particles on an Au Quantifoil grid.

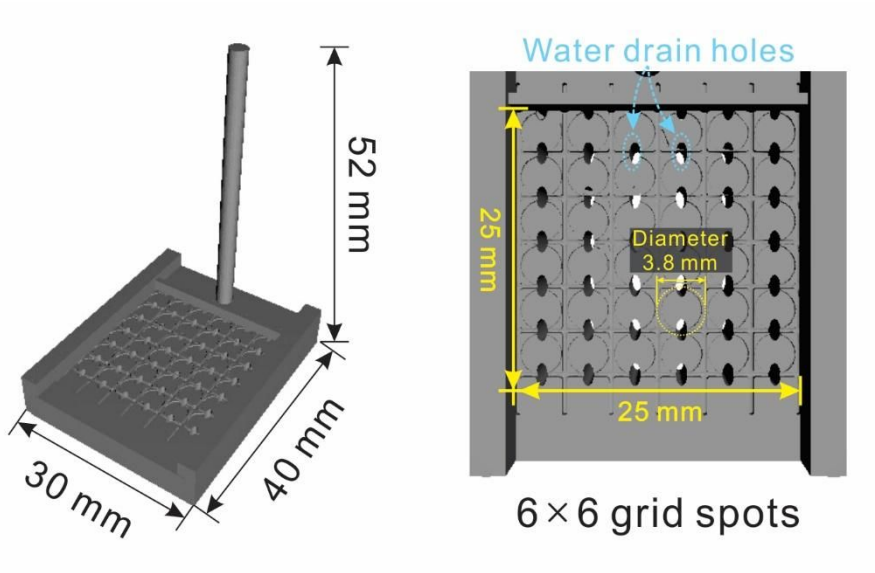

Figure S1.

Three-dimensional angle view (left) and top view (right) of the 3D-printed grid transfer tool (The Form 3 with the white resin, Formlabs) with scales. The total 36 grid holes (3.8 mm diameter with 0.2 mm depth) are positioned within 25 mm × 25 mm. A groove is positioned in between holes for cutting the graphene layer using tweezers after the drying step (100 °C, 30 min). The graphene grid then can be detached and transferred individually for acetone washing. To hold and position grids during the scooping step in water, we introduced the water drain hole in between grid holes (cyan dot circles). The cylinder bar attached on the grid transfer tool (52 mm height) is designed to handle the grid transfer tool under the water. This water drain hole further provides the space to pick the grid (after drying step) without bending it with the tweezers. The STL file for this grid transfer tool can be freely downloaded in the following link. The STL file for this grid transfer tool can be freely downloaded in the following link. (http://www.uscho-lab.com/research/ or https://drive.google.com/file/d/1Ypu8qxs3QKvii0EEzsLVLajQxGKETX9k/view?usp=sharing).

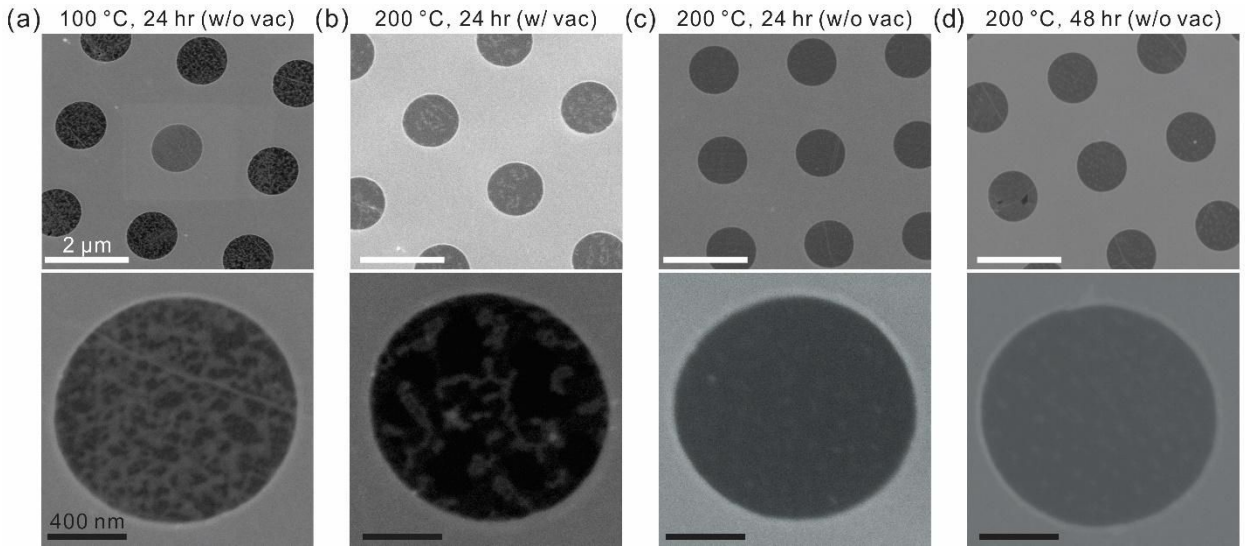

Figure S2.

SEM images of graphene grids (Au Quantifoil) made in different baking conditions of (a) 100 °C without vacuum, (b) 200 °C with vacuum, and (c) 200 °C without vacuum (d) 200 °C without vacuum for 48 hr. Bottom side images are magnified hole images of each condition.

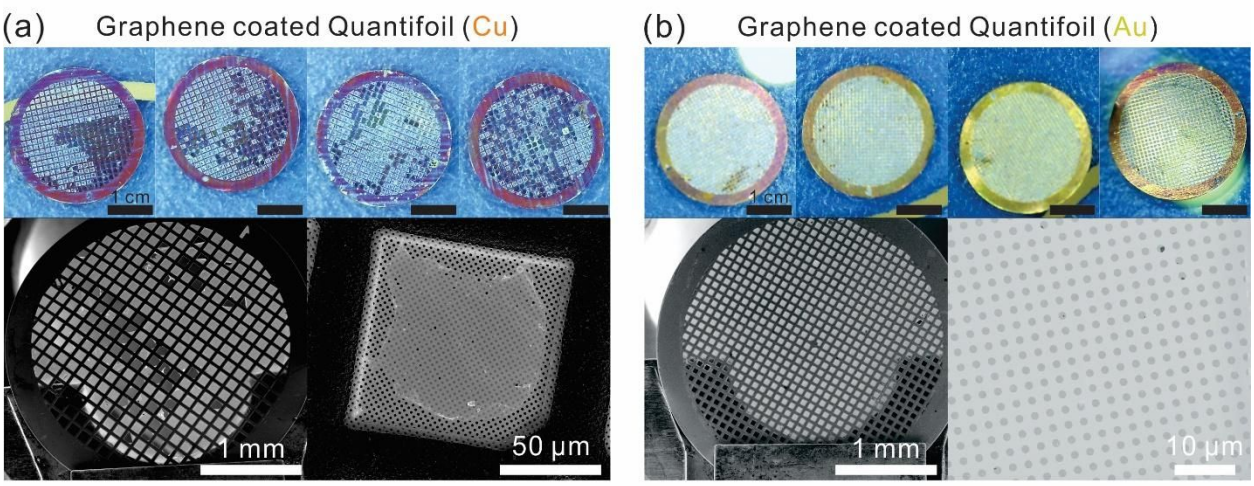

Figure S3.

Optical microscopy (OM) and SEM images in different magnifications of graphene-coated (a) Cu Quantifoil and (b) Au Quantifoil grids. As shown in (a), Cupper Quantifoil grids were heavily oxidized, and graphene layers were broken/shrunken during baking. Note that scale bars in magnified SEM image are in different scale.

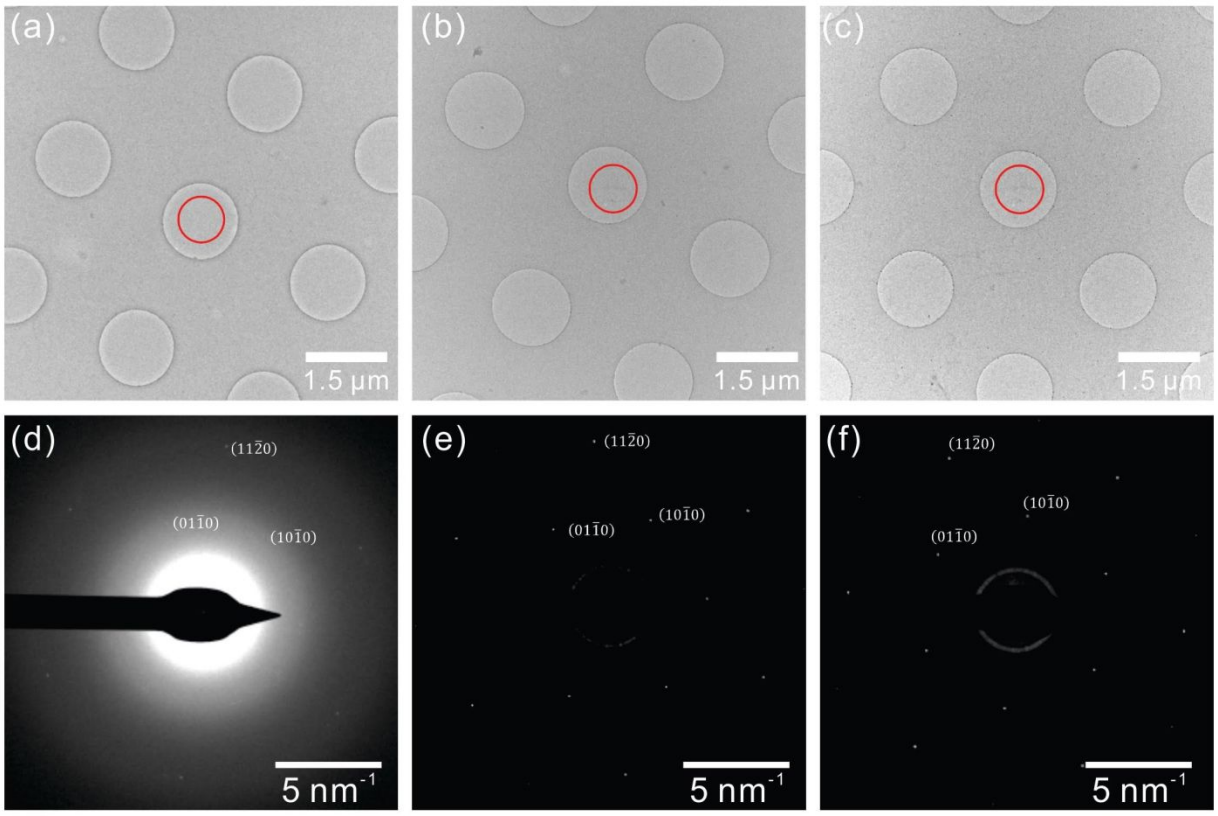

Figure S4.

(a-c) BF-TEM images and (d-f) SAED patterns of Au Quantifoil grids coated with (a, d) PMMA/graphene (b, e) Rinsed (acetone, 3 times) graphene (c, f) Baked (24 hr) graphene after rinsing. The red circle is SAED pattern obtained region with aperture size of 800 nm.

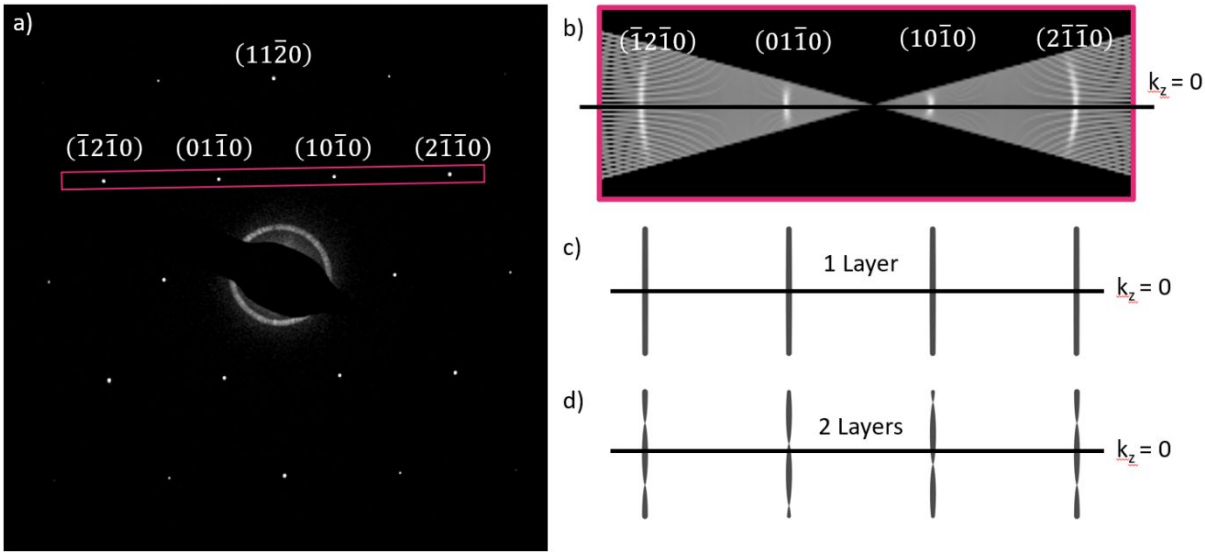

Figure S5.

a) Selected area electron diffraction of monolayer graphene b) 3D electron diffraction probes reciprocal out-of-plane (k_z) structure of Bragg rods. Schematic Bragg rods of c) mono- and d) double-layered graphene has distinctive out-of-plane features.

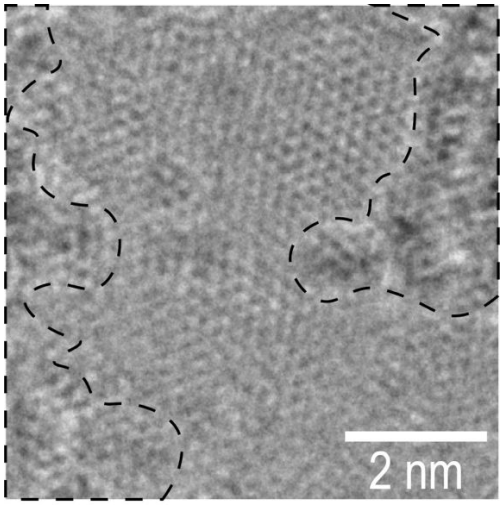

Figure S6.

High resolution TEM image of graphene grid. Central region is graphene and black-dashed regions with higher contrast might be originated from the graphene oxide and amorphous carbon residues.

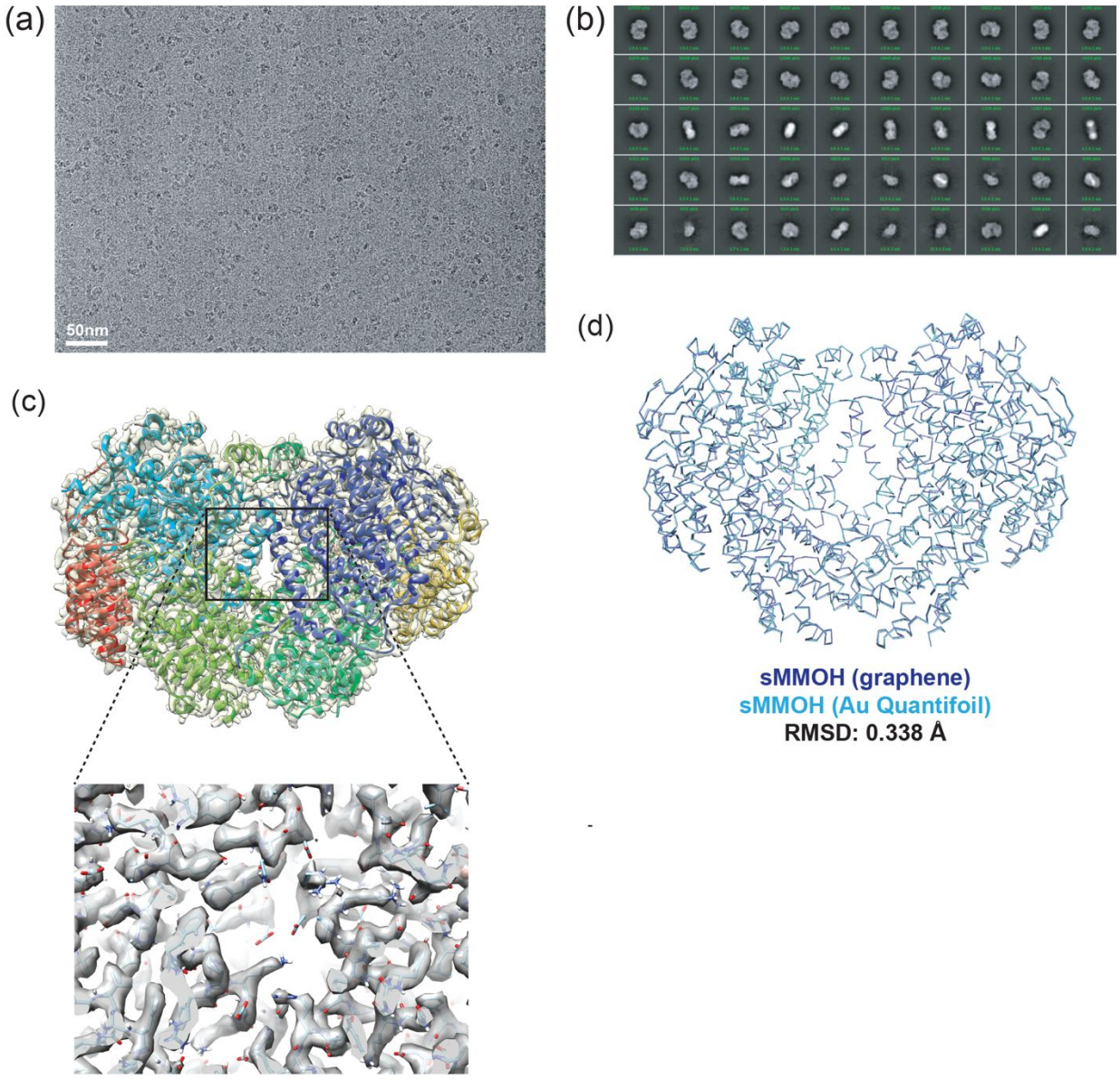

Figure S7.

(a) Representative microscopic image of *M. caps* sMMOH in the Au Quantifoil grid (300 KeV). (b) Top 50 2D classes selected from 200 classes of sMMOH in the Au Quantifoil grid. (c) 2.9 Å resolution cryo-EM map of sMMOH in the Au Quantifoil grid. Two MMOHα subunits are colored in blue/cyan, MMOHβ in green/light green, and MMOHγ in yellow/red. The central region of sMMOH is zoomed in to visualize the quality of the cryoEM map (the black square region). (d) Structural overlay of sMMOH cryo-EM structures determined using Au Quantifoil (cyan) and graphene (blue) grids. The Cα R.M.S. difference in between is 0.338 Å.

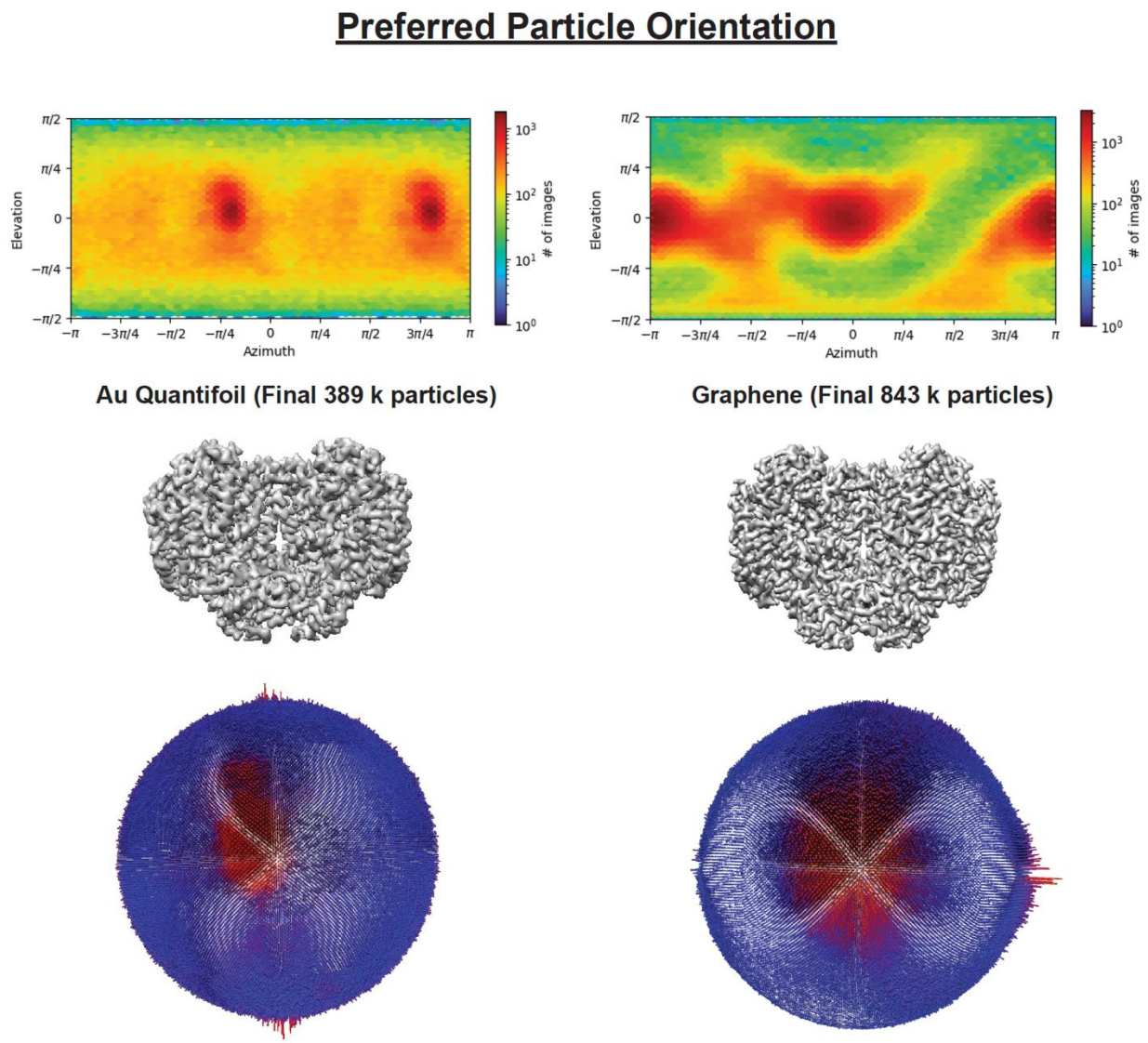

Figure S8.

The preferred particle orientation of sMMOH determined by Au Quantifoil (389 k particles) and graphene (843K particles) grids. The images were imported from cryoSPARC and RELION.

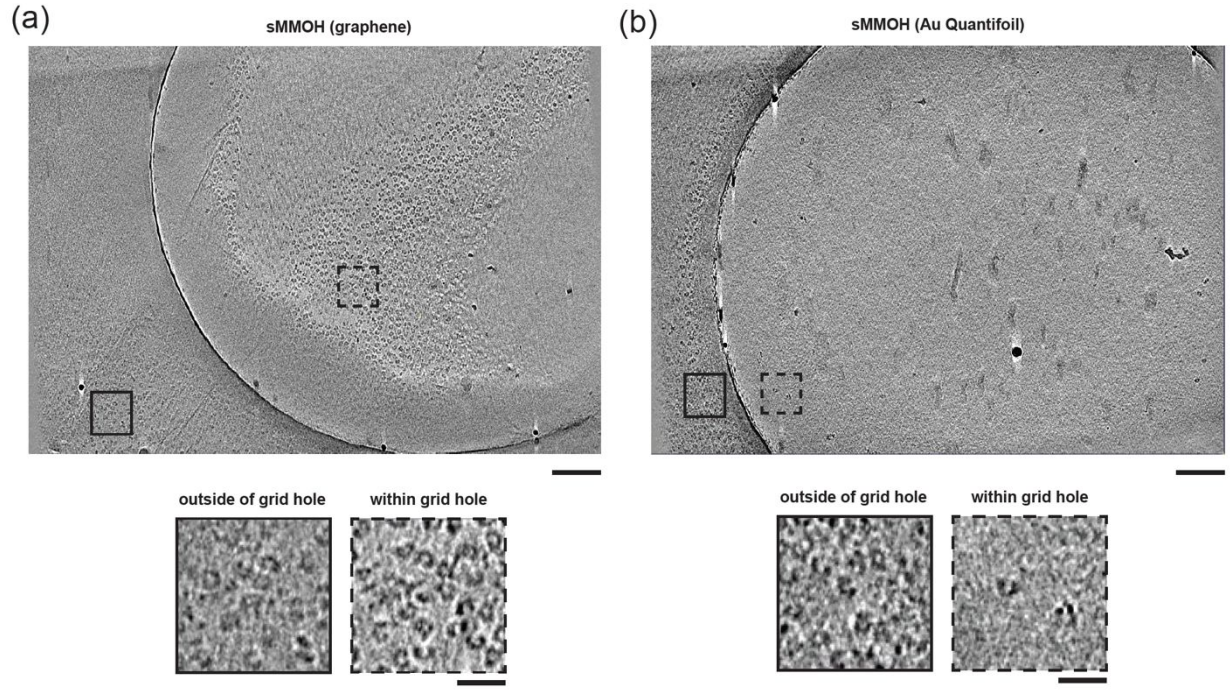

Figure S9.

Comparison of sMMOH particle distribution on plunge-frozen graphene-coated and unsupported Au Quantifoil grids. (a) Top: Slice through tomogram of a graphene-coated grid hole depicting a layer of sMMOH particles distributed in vitreous ice. Scale bar, 100nm. Bottom: Zoom of regions from the top panel. Solid box depicts particles from the outside of the grid hole. Dashed box depicts particles from within the grid hole. Scale bar, 25nm. (b) Top: Slice through a tomogram of unsupported Au Quantifoil grid hole depicting sMMOH particles mostly outside of the grid hole. Scale bar, 100nm. Bottom: Zoom of regions from the top panel. Solid box depicts particles from the outside of the grid hole. Dashed box depicts particles from within the grid hole. Scale bar, 25nm.

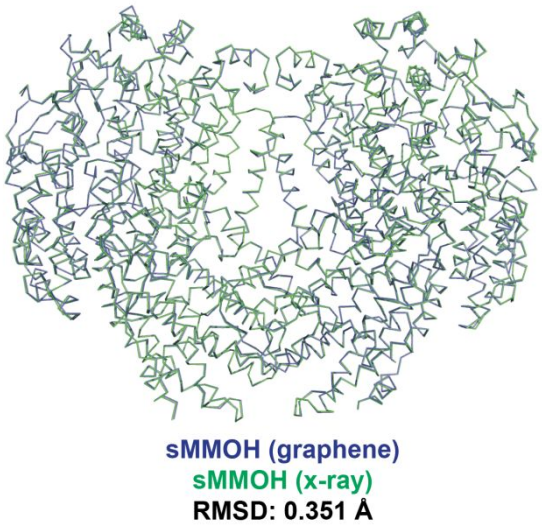

Figure S10.

The structural overlay of the x-ray crystal structure (green; PDB ID: 1MTY) and cryo-EM structure (blue; graphene grid) of sMMOH. The Cα R.M.S. difference of two structures was 0.351 Å.

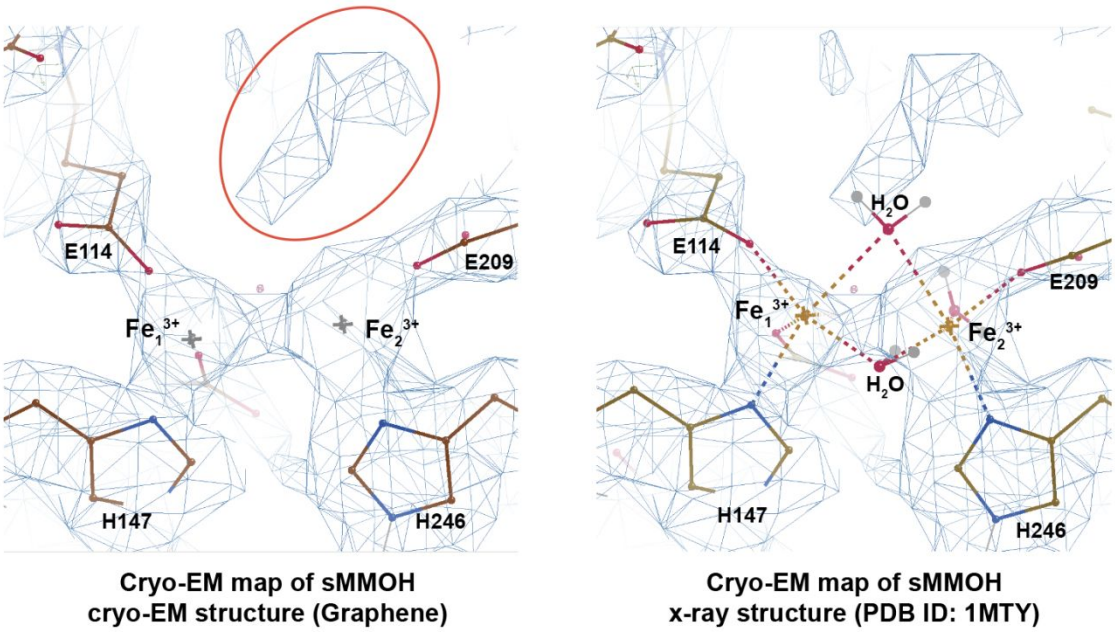

Figure S11.

Cryo-EM map of sMMOH at the di-iron center (graphene grid) was overlaid with the cryo-EM structure (left) and x-ray structure (right; PDB ID: 1MTY) of sMMOH. The extra unidentified cryo-EM density on top of the di-iron center is displayed and marked by red circle.

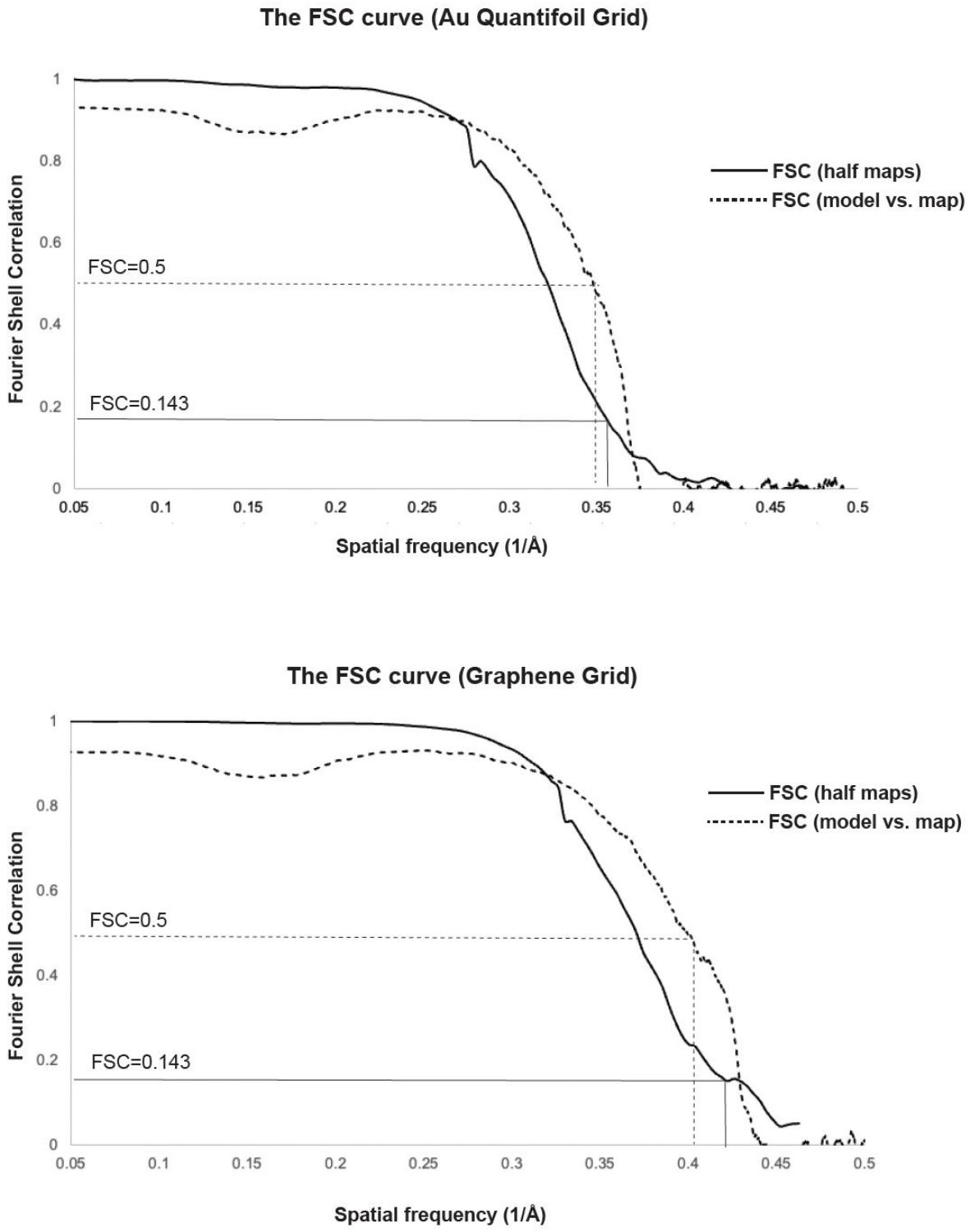

Figure S12.

Fourier Shell Correlation (FSC) curves of Au Quantifoil-grid dataset and Graphene-grid dataset. Solid lines indicate FSC curves of two half maps and dashed lines indicate FSC curves of model versus map.

	Peak type	Peak position (cm^{-1})	Integrated area	Integrated area ratio (%)
D band	Gaussian	1393.0	400000	56.9
G band	Gaussian	1553.4	228781	32.6
D peak	Gaussian	1586.0	8804	1.3
2D peak	Gaussian	2674.5	65148	9.3

Table S1. Deconvoluted peak results from graphene coated Quantifoil grid Raman spectra.

	Au Quantifoil grid	Graphene grid
Protein concentration	1.3 mg/ml	0.5 mg/ml
Total number of movie stacks	3075	3880
Total number of particles picked	2.1 M	4.2 M
Total number of particles for the final 3D reconstruction	389 k (18.5 %)	843 k (20.1%)
Resolution ($FSC_{0.143}$)	2.75 Å	2.32 Å
Resolution ($FSC_{0.5}^{map\ vs.\ model}$)	2.9 Å	2.5 Å

Table S2.

Comparison of statistics in cryo-EM structure determination (*M. caps* sMMOH)

	MMOH on Quantifoil	MMOH on Graphene
Data collection		
Microscope	Titan Krios K3	Titan Krios K3
Voltage (kV)	300	300
Defocus range (μm)	-0.6 to -1.9	-0.7 to -2.0
Pixel size (Å)	1.0275	1.059
Total dose (e^- / Å^2)	49	51
Particles (initial)	2.1 mil	4.2 mil
Particles (final)	389 K	843 K
Reconstruction		
Symmetry	C1	C1
Resolution (unmasked Å)	3.4	3.1
Resolution (mask Å)	2.75	2.32
Map-sharpening *B f*actor (Å^2)	127.7	97
Model composition		
Atoms (Hydrogen)	20577 (3206)	20577 (3206)
Protein residues	2113	2113
Water	0	0
Refinement		
Resolution (Å)	2.9	2.5
B factor (Å^2)		
Proteins	38.61	16.51
Ligand	48.15	25.06
Model-to-mal fit (CC)	0.86	0.86
R.m.s. deviations		
Bond (A)	0.009	0.008
Angles (°)	0.660	0.676
Validation		
Clashscore	12.52	12.20
Rotamer outliers (%)	0	3.25
Ramachandran plot (% favored)	96.62	97.67
Ramachandran plot (% outliers)	0.00	0.00
MolProbity score	1.83	2.06

Table S3.

Cryo-EM data collection, refinement, and validation statistics