Supplementary Information

Directed self-assembly of proteins into discrete radial patterns

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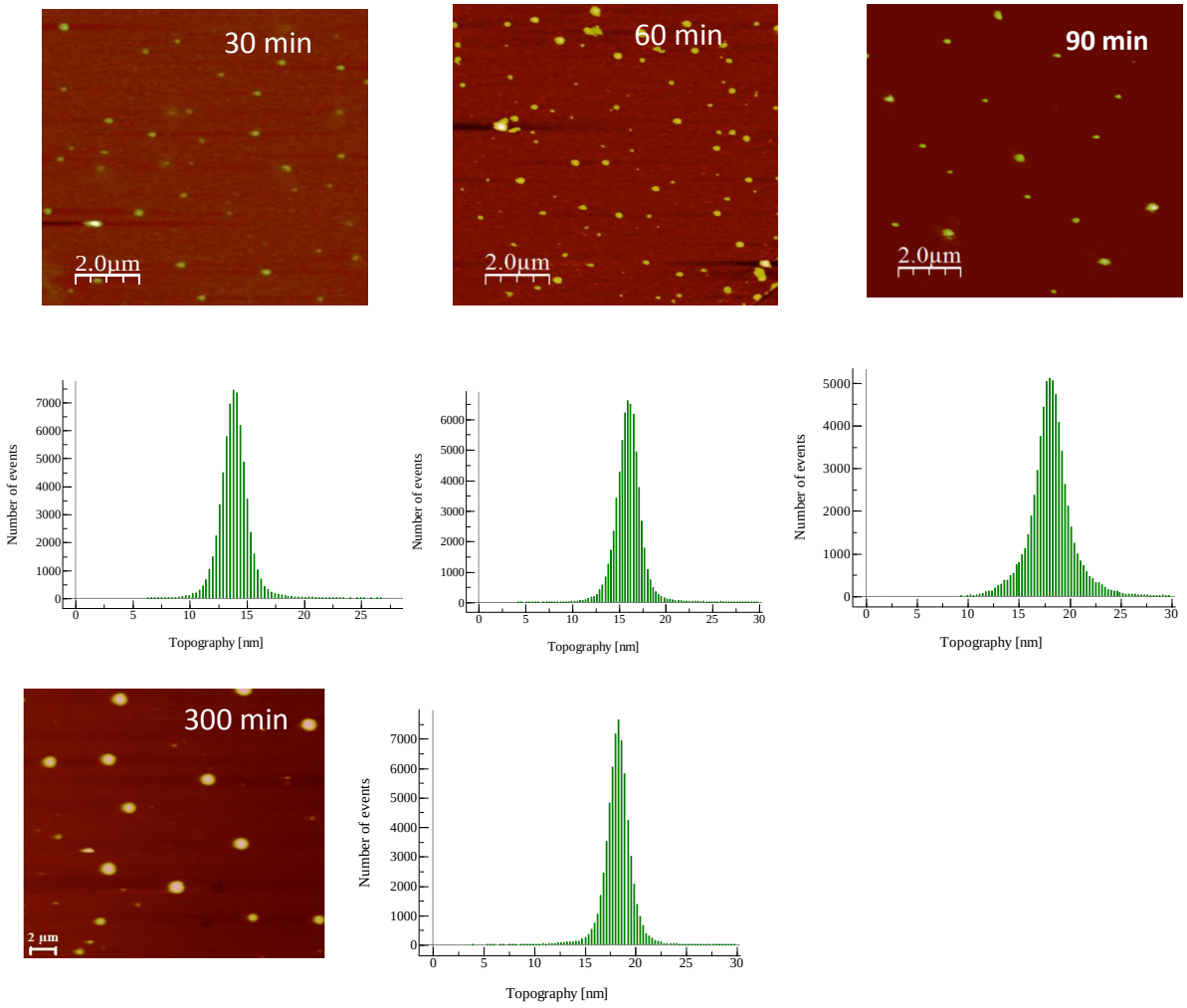
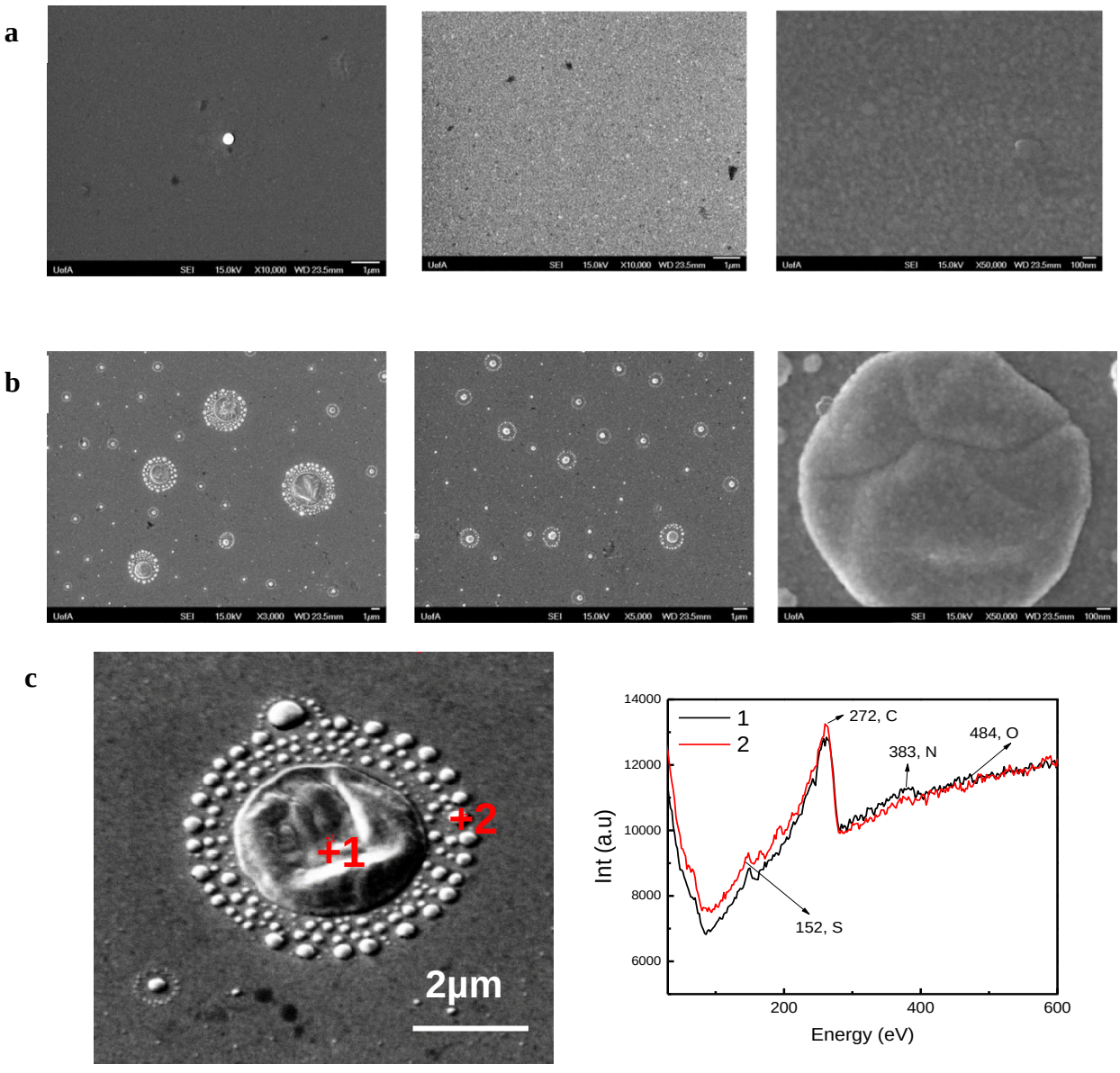
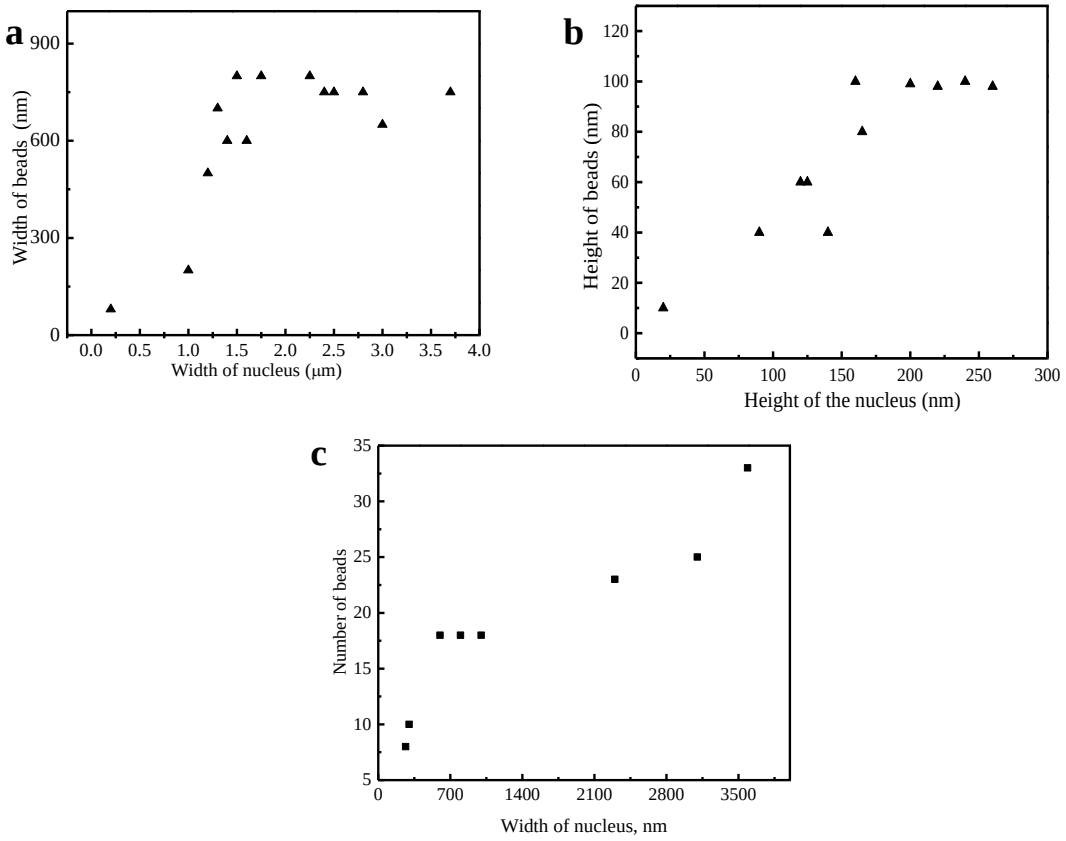


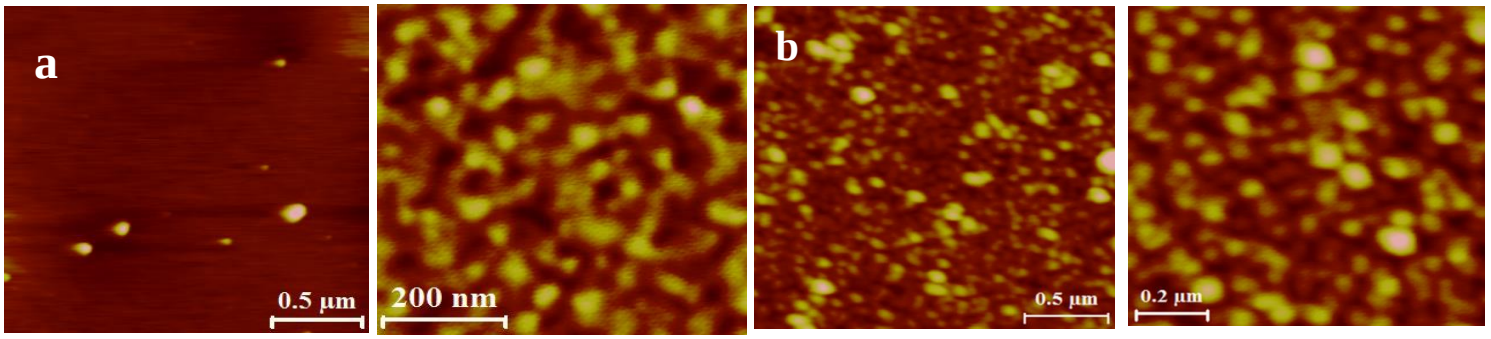
Supplementary Fig. S1 AFM images of PEG islands at different incubation times with corresponding height histograms.



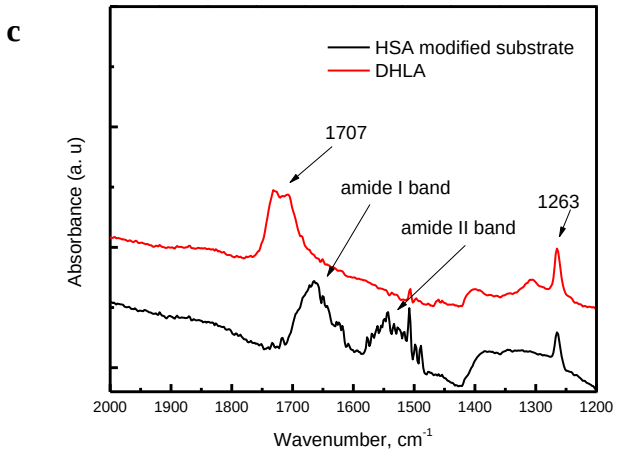
Supplementary Fig. S2 SEM image of PEG and HSA ring pattern on gold surface (a) PEG modified substrate does not show any patterns on the surface. (b) HSA ring patterns show degeneration of ring patterns after 3 days in air due to dehydration of the surface (c) Auger elemental analysis of the ring pattern shows significant peak for C at 272 eV which shows the elemental presence.



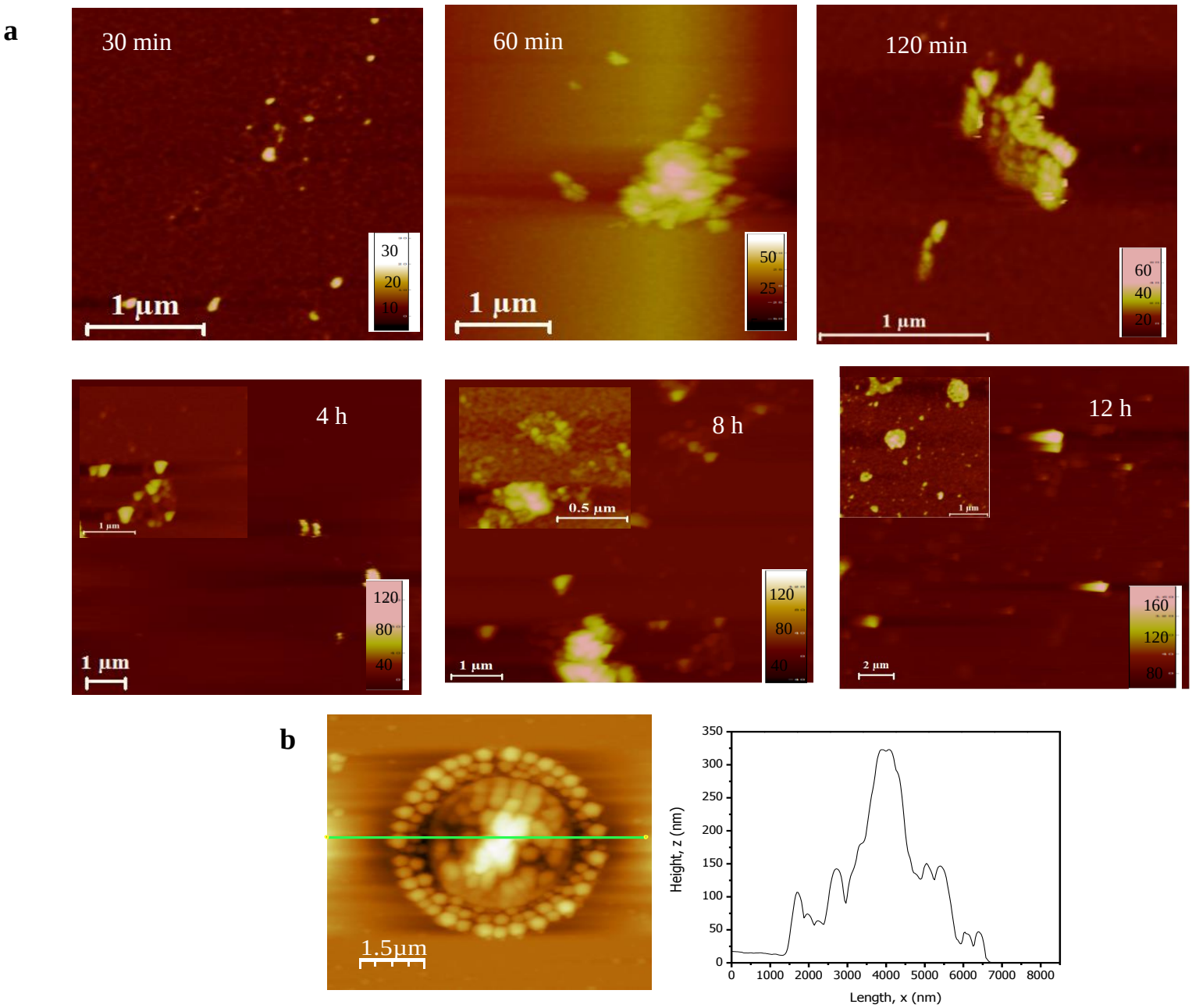
Supplementary Fig. S3 AFM analysis of the of self-assembled ring patterns after 15 h. (a) Variation of width of nucleus and width of the beads. (b) Relation between growth in height of nucleus and height of beads. (c) Relation between width of the nucleus and number of beads. The diameter of the beads increased with width of the nucleus size up to 2 μm and remained constant at 800 ± 100 nm for nucleus sizes greater than 2 μm. Similarly, the height of the beads increased with the increased height of the nucleus up to 200 nm and was constant at 100 ± 20 nm for nucleus heights greater than 200 nm. On the basis of the analysis of the AFM images, the number of beads around the nucleus was dependent upon the size of the nucleus; when increasing the size of the nucleus, the number of beads increased (Supplementary Fig. S3 C). The number of beads was approximately 8 for nucleus sizes below 300 nm and increased up to 10 for 300 nm. It remained constant at 18 for nucleus sizes between 500 nm and 1.5 μm and again increased to 33 for the inner concentric rings for nucleus sizes greater than 3 μm.



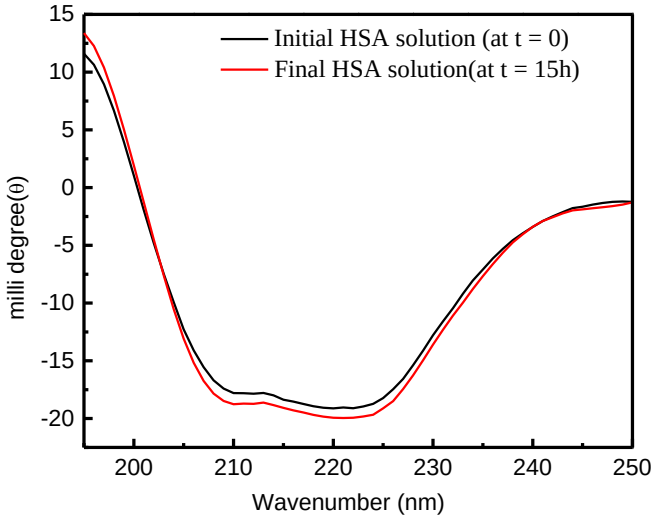
DHLA modified substrate HSA conjugated with DHLA modified surface



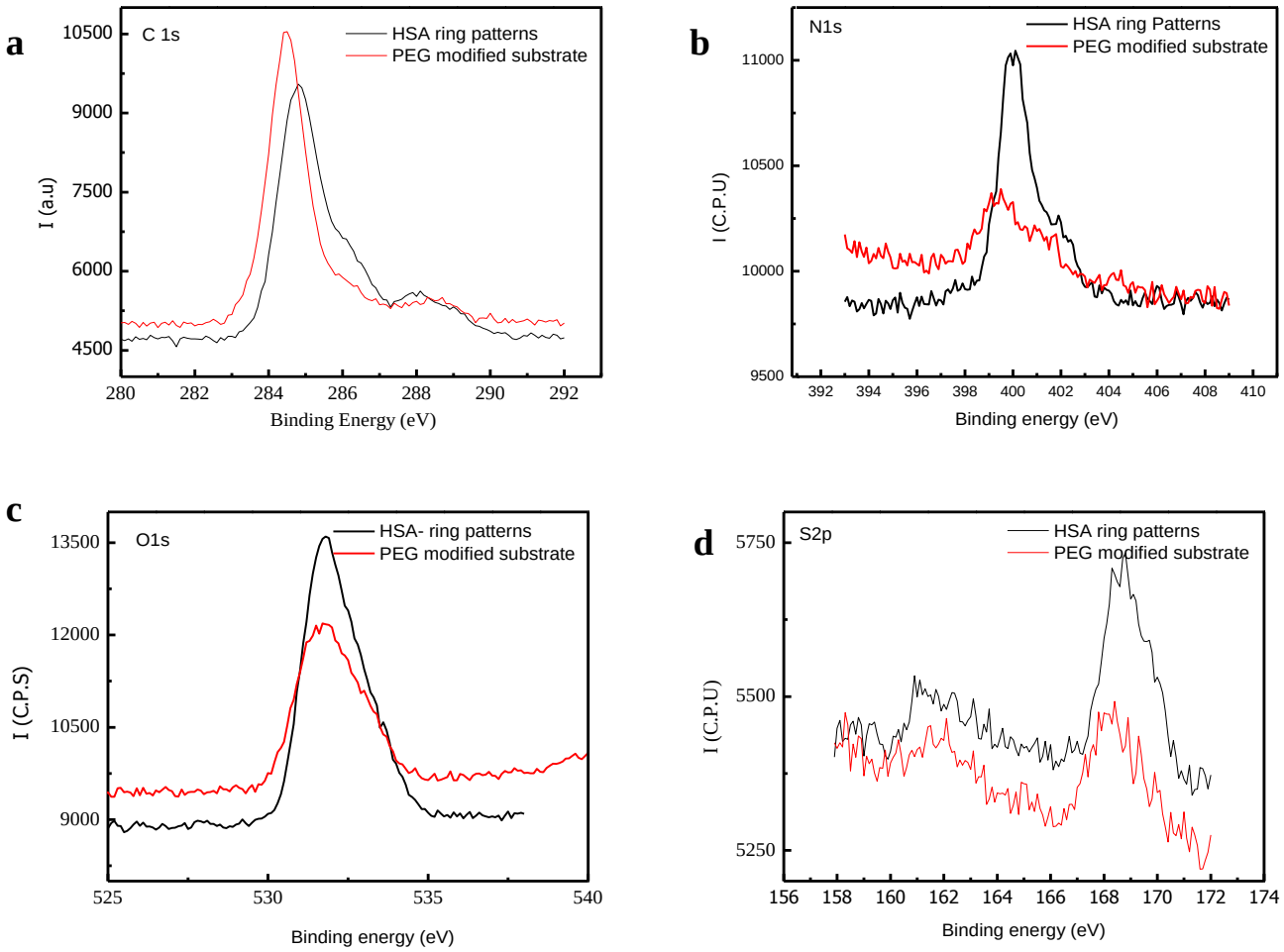
Supplementary Fig. S4 AFM images of DHLA and HSAmodified substrates with Spectroscopic analysis. (a) AFM images of dihydrolipoic acid (DHLA) modified gold substrate at a scale of 500 and 200 nm. These images show inhomogeneous SAM formation on the gold substrate using DHLA as surface ligand (b) AFM images of HSA conjugated DHLA modified substrate at a scale of 500 and 200 nm after incubation time of 15 h. (c) Variable angle FTIR spectra of DHLA and HSA conjugated substrate showing characteristic IR peaks. DHLA Shows a characteristic peak of carboxylate (C=O) moiety at 1707 cm^{-1} and 1263 cm^{-1} shows C-H wagging.[1,2] HSA conjugated surface shows amide I band at 1664 cm^{-1} and amide II band at 1544 cm^{-1}.[2]



Supplementary Fig. S5 AFM images of HSA conjugation on the substrate with increasing incubation time. a) AFM images showing the topography of the surface and increase in height of the overall aggregates with increased incubation time. Inset at time 4, 8 and 12 h shows the aggregation of protein towards ring pattern formation. b) Topography image and the line profile of the complete ring structure generated after 15 h. The increase in height of the nucleus is significant around 300 nm due to aggregation of protein.

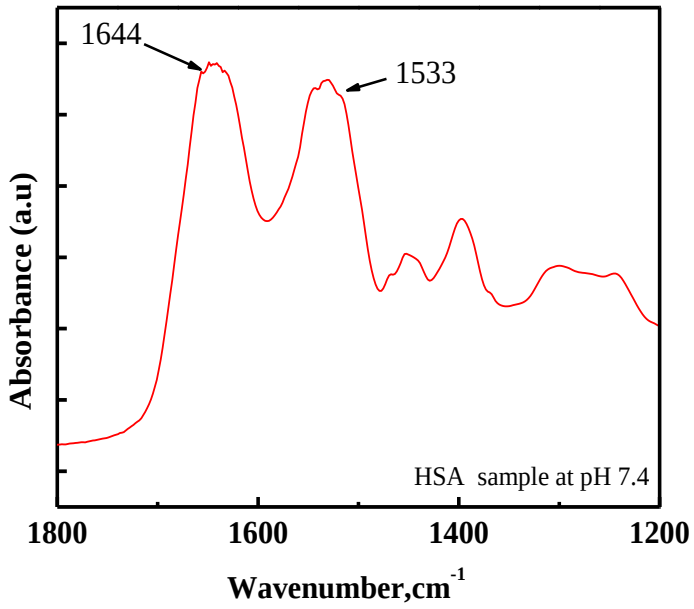


Supplementary Fig. S6 Circular dichroism spectra of HSA before and after incubation. The CD spectra show the characteristic bands at 210 and 222 nm, which correspond to typical α-helix structureof HSA in solution.[2] No change or shift in bands is observed for the solution which confirms no significant change in conformation of the protein in solution during incubation .



Supplementary Fig. S7. Survey region XPS spectra for HSA ring patterns and PEG modified subsrate. (a) C1s region for self-assembled protein patterns and PEG modified surface composition is indicated in the integrated areas. There is a significant hump in the HSA pattern at 286 eV showing backbone carbonyl groups present on the substrate as discussed in the manuscript (b) N1s region for self-assembled patterns and PEG modified surface composition is indicated in the integrated areas. (c) O1s region for self-assembled protein patterns and PEG modified surface composition is indicated in the integrated areas (d) S2p region for self-assembled protein patterns and PEG modified surface composition is indicated in the integrated areas. There is substantial presence of N, O and S in the ring structures as compared to PEG substrate.

a



b

Table 1

Incubation time	**Amide I (cm^{-1}) (major structure)**	**Amide II (cm^{-1})**
30 min	1657(α-helix)	1540
1h	1657 (α-helix)	1557
2h	1658(α-helix)	1548
4h	1660 (α-helix or β-turns/ random coil)	1537
8h	1665 (β-turns/random coil)	1544
15h	1662, 1621(α-helix, β-sheet/aggregates)	1554

Supplementary Fig. S8 Fourier Transform Infrared Spectroscopic analysis of HSA with increasing incubation time. a) Attenuated total reflection (ATR) IR spectra of native HSA showing characteristic amide I and II peaks at 1644 and 1533 cm^{-1} . b) Table 1 shows the change in the characteristic IR peaks of the HSA with increasing incubation time. There is a shift in the amide I band from the native HSA protein from α-helix towards random coil or β-turns after 2h.[1]

References

1. Jackson, M. & Mantsch, H. H. The use and misuse of FTIR spectroscopy in the determination of protein structure. *Crit. Rev. Biochem. Mol. Biol*. **30** 95–120 (1995)

2. Pribie, R., VanStokkum, I. H. M., Chapman, D., Haris, P. I. & Bolemendal, M. Protein Secondary Structure from Fourier Transform Infrared and/or Circular Dichroism Spectra *Anal. Biochem.* **214**, 366-378 (1993)