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This version of the electronic supplementary information (ESI) replaces the version first published on 6th February 2017. Following further experiments the authors have added new SDS-PAGE and MALDI-TOF data

# ApAGP Fabricated Silver Nanoparticles Induce Amendment of Murine Macrophage Polarization

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## Electronic supplementary information (ESI)

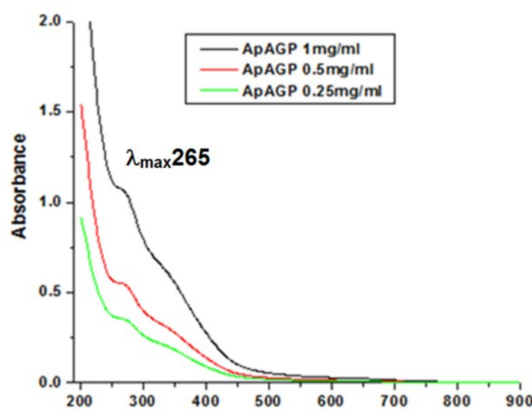


**Fig. S1A.** UV-Vis. Spectrum scan of ApAGP dissolved in PBS at different concentrations (1, 0.5 & 0.25 mg/ml).

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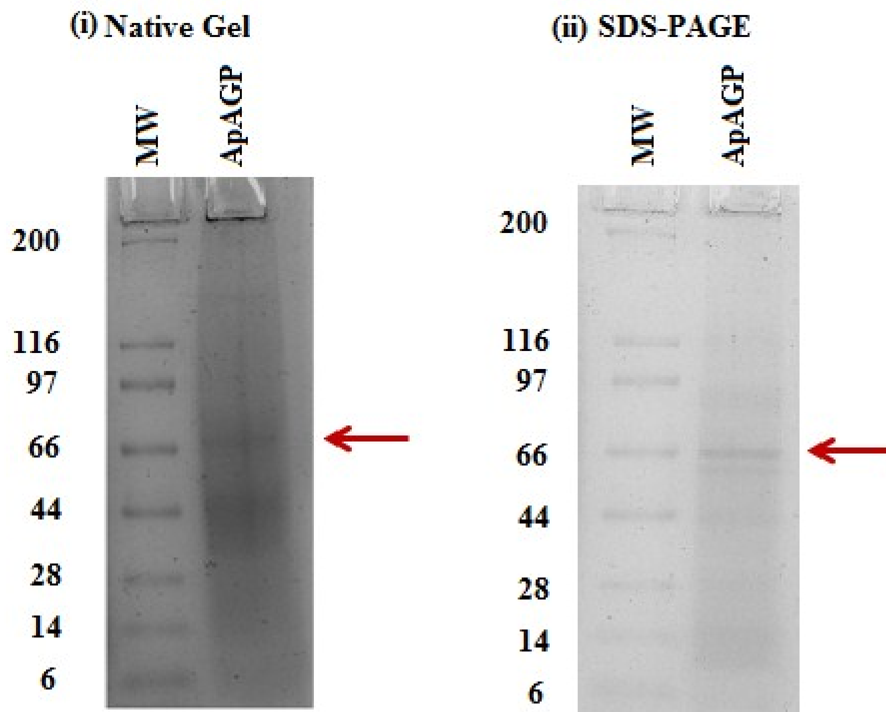


**Fig. S1B. (i) Native PAGE, and (ii) SDS-PAGE gel image of ApAGP.**

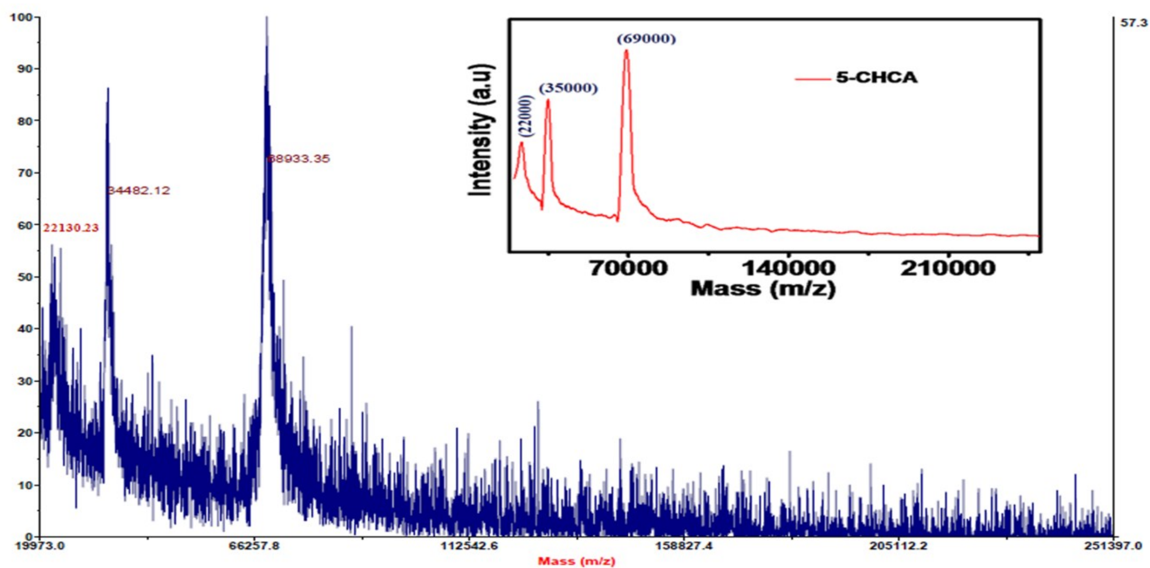


**Figure S1C: MALDI-TOF analysis of ApAGP.** The isolated ApAGP was characterized by mass spectrometry on an Applied Biosystems 4800 Plus MALDI-TOF/TOF instrument [matrix: α-cyano-4 hydroxycinnamic acid (CHCA), external calibration]. The sample were not fly properly even after repeated trial, hence the signal-to-noise ratio is poor. According to the

spectrum, the ApAGP is ~68 kDa protein indicated by the major peak. Another two additional peaks in the spectrum are the doubly-charged protein (~34 kDa) and the triply-charged protein (~22 kDa), respectively.

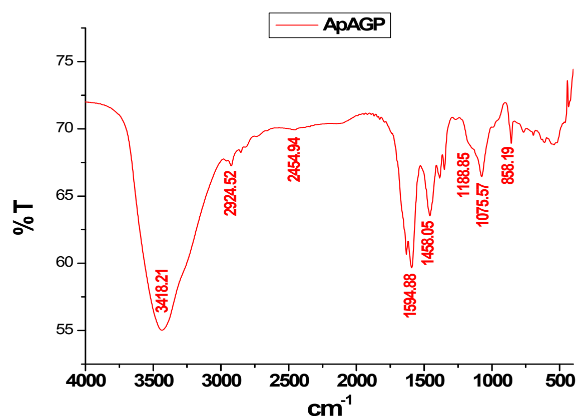


**Figure S1C: FT-IR spectrum of ApAGP.** In order to characterize different functional groups of the isolated APAGP we took the IR-spectra of the purified APAGP on KBr film. Appearance of the strong bands at 1075.57 $cm^{-1}$ correspond to the presence of pyranose and (or) furanose ring system, whereas, the band at and 1188.85 $cm^{-1}$ corresponds to the -C-O-C- stretching vibration, which is the characteristics of carbohydrate molecules. Presence of the amide linkage (of a protein) confirmed by the bands that appear at 1458.05 $cm^{-1}$ (amide III mode) and 1594.88 $cm^{-1}$ (amide I mode). Additionally, appearance of a weak band at 2454.94 $cm^{-1}$ (S-H stretching) clearly shows the presence of amino acid with -SH bond. Finally, the presence of band at 2924.52 $cm^{-1}$ corresponds the –C-H stretching vibration and the broad band appeared at 3418.21 $cm^{-1}$ represents the existence of hydroxyl groups (preferably of the carbohydrate part) which are involved in intermolecular hydrogen bonding.

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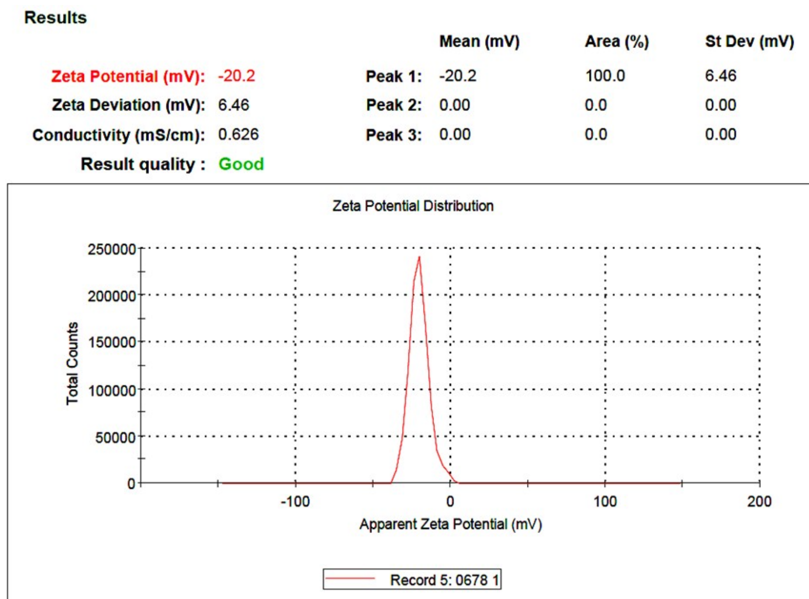


**Fig. S2.** Zeta potential distribution of SNP-ApAGP.

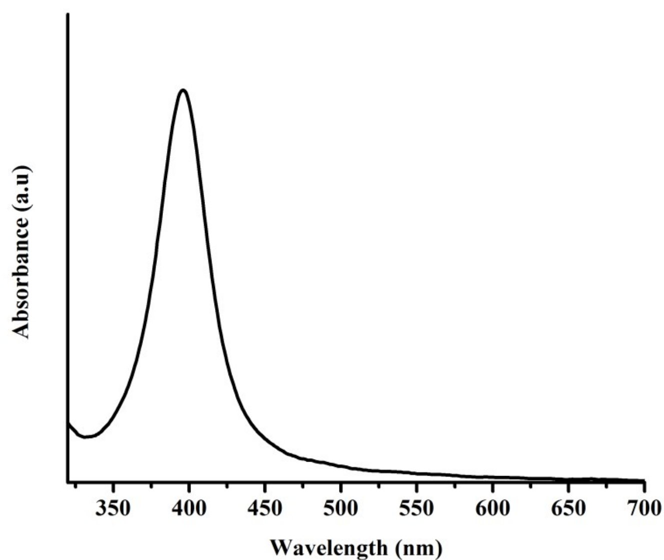


**Figure S3A:** Absorption spectrum of control SNPs (stabilized with citrate).

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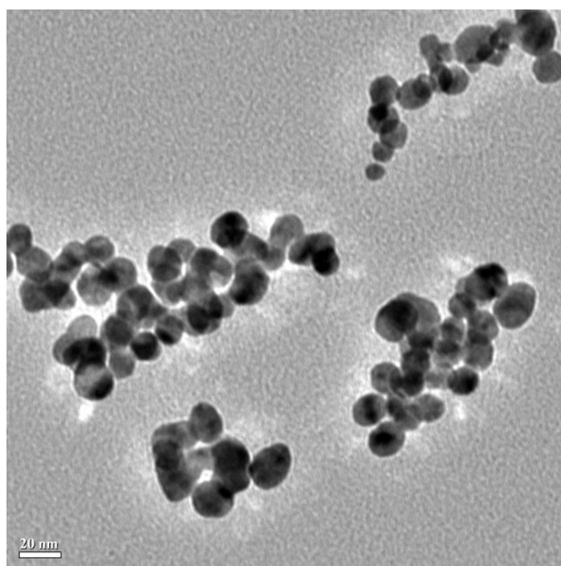


**Figure S3B:** TEM images of control SNPs (stabilized with citrate).

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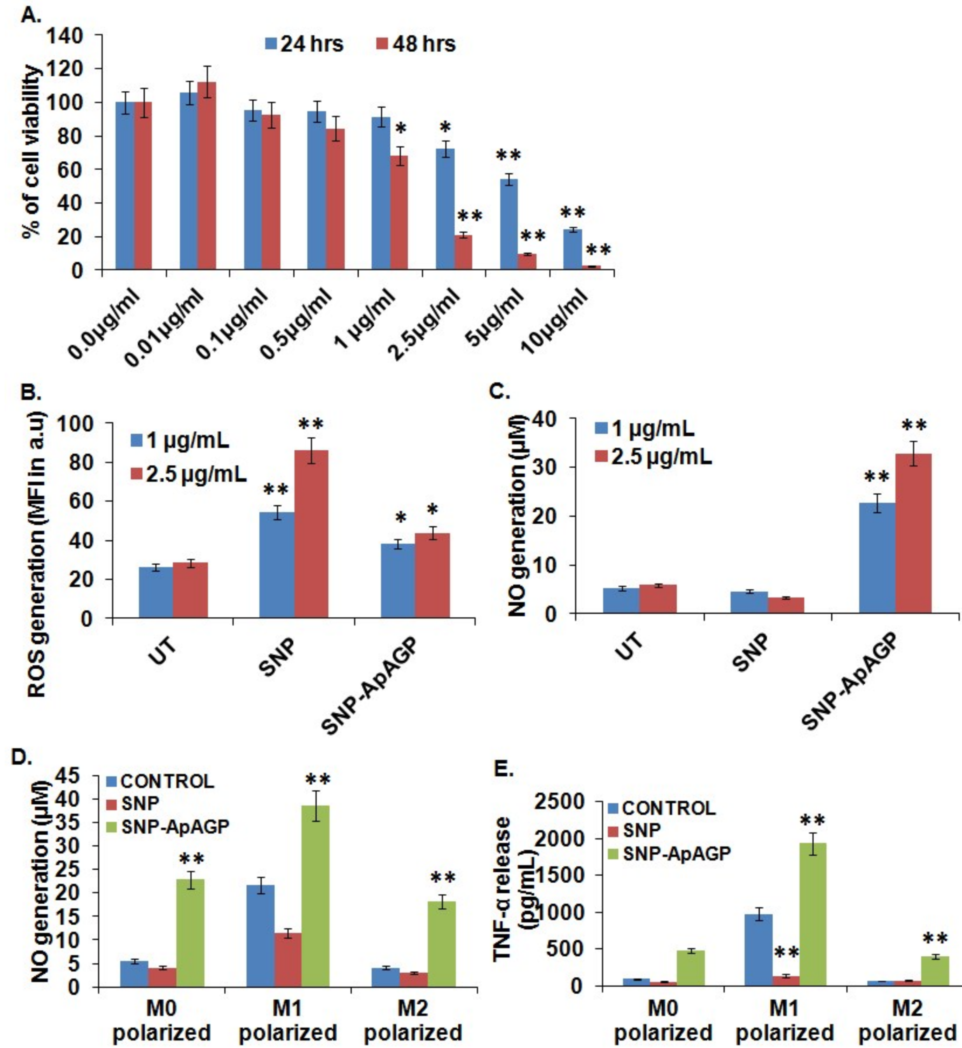


**Figure S4: Effect of SNP alone on mouse peritoneal macrophages.** A) % of cell viability of peritoneal macrophages due to SNP alone treatment. B-C) Comparative ROS and NO generation by peritoneal macrophages due to SNP alone and SNP-ApAGP treatment. D-E) NO generation and TNF-α release by M0, M1 and M2 polarized peritoneal macrophages during treatment with SNP alone (1 µg/mL) and SNP-ApAGP (1 µg/mL). The experiment was repeated three times independently and data are shown as mean ± SD. Asterisk indicates significant differences compared with the untreated control group (*$p$<0.05 and **$p$<0.01).

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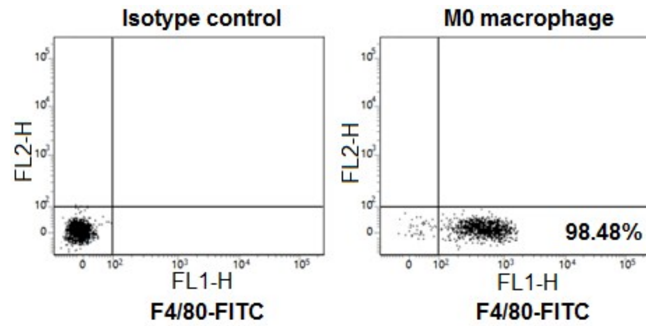


**Fig: S5. Characterization of isolated peritoneal macrophages.** Isolated and cultured peritoneal macrophages ($2\times10^6$) were stained with anti-mouse F4/80-FITC (BioLegend) and analyzed in a Becton-Dickinson FACS verse flow cytometer using CellQuest software. We found that 98.48% cells were F4/80 positive (cell surface marker of macrophage).

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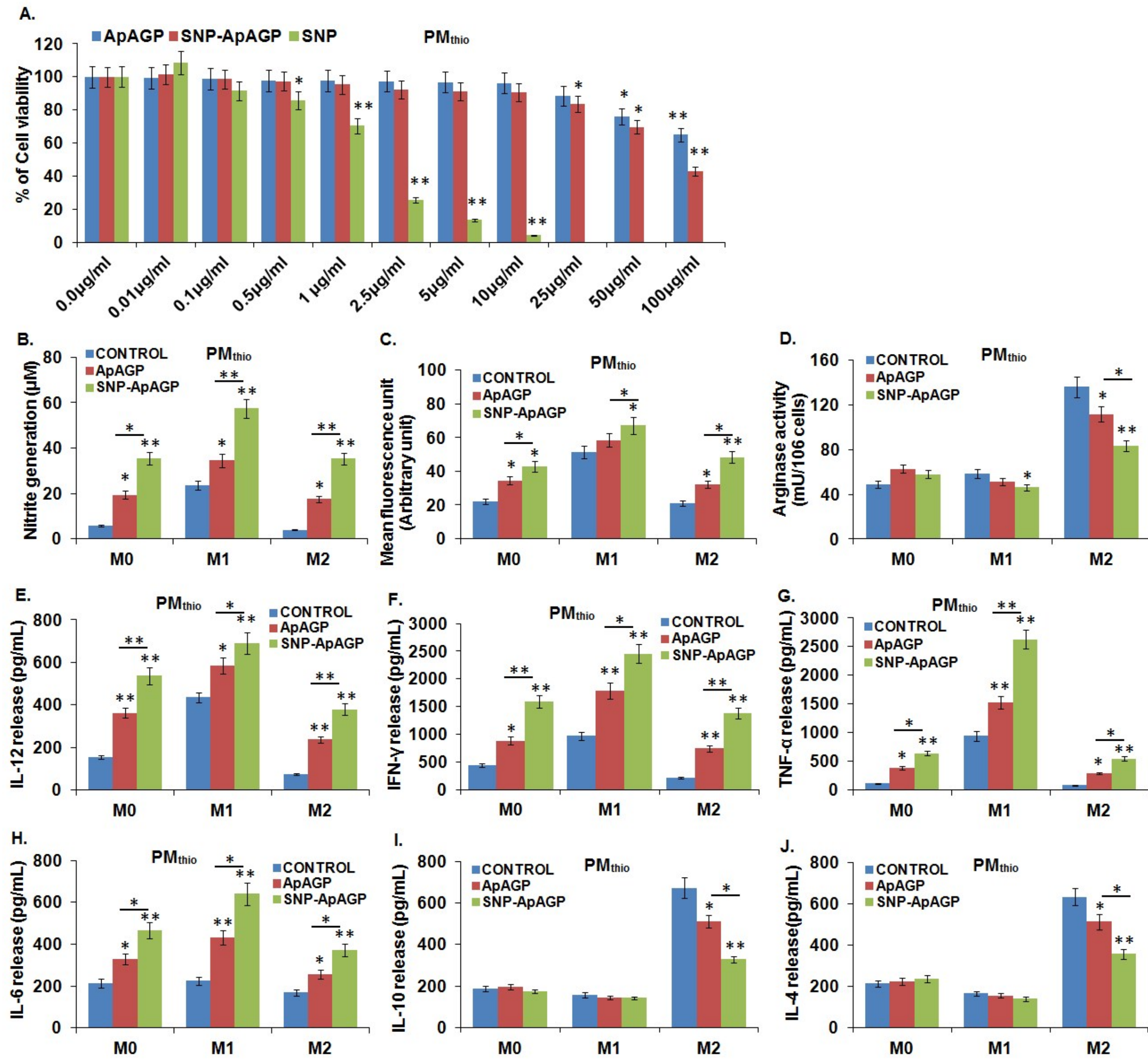


**Fig: S6: A) Cell cytotoxicity, B) NO generation, C) ROS generation, D) Arginase 1 activity, E) IL-12 release, F) IFN-γ release, G) TNF-α release, H) IL-6 release, I) IL-10 release, and J) IL-4 release by thioglycolate-elicited peritoneal macrophages.** Peritoneal macrophages were isolated by 4% thioglycolate intraperitoneal injections followed by harvesting after 5 days as described earlier (Charan Raja MR *et al.,* RSC Advances 2016; Bandyopadhyay S *et al.,* PLOS One 2015). Macrophages were cultured in RPM I1640 with 10% FBS and antibiotics for 48 h prior to all experiments.