Supplementary Information

Engineered Silica Nanoparticles Act as Adjuvants to Enhance Allergic Airway Disease in Mice

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1. Design of Engineered Silica Nanoparticles

Materials

Plain SNP (LUDOX® TM-40 colloidal silica, 40 wt% suspension in H₂O), aminopropyltriethoxysilane (APTES), propargyl chloroformate and sodium azide were purchased from Sigma-Aldrich and used as received. Anhydrous dimethylformamide (DMF) was freshly distilled from powered BaO. Cu(PPh₃)Br [1] and 1-azido-2-(2-(2-(2-(methoxyethoxy)ethoxy)ethoxy)ethoxy)ethoxyethane (PEG-N₃) [2] were prepared as previously described in detail. Other reagents and solvents were used as received from the supplier.

Instruments and characterization

¹H nuclear magnetic resonance (NMR) and ¹³C NMR spectra were recorded in NMR solvent CDCl₃ on a Varian 300 MHz or VXR-500 MHz instrument. The CDCl₃ resonance was used as the internal standard for ¹³C NMR (δ = 77.0 ppm) and residual CHCl₃ for ¹H NMR (δ = 7.24 ppm). Fourier transform infrared (FTIR) spectra were recorded on a Mattson Galaxy series FTIR 3000. Thermogravimetric analyses (TGA) were obtained in air from a Perkin-Elmer TGA 7. Samples were held at 120 °C for 30 min to remove absorbed water from particle surfaces and then heated to 850 °C at a rate of 10 °C/min. All samples for FTIR and TGA were vacuum dried at room temperature for 24 h.

Dynamic Light Scattering (DLS) was performed in a Malvern NanoZS ZetaSizer with a 178 degree backscattering detection. Intensity average diameters were calculated from the autocorrelation function using Malvern’s Zetasizer Software 6.12. The samples for DLS analyses were sonicated prior to measuring the particle size (FS20H sonicator, Fischer Scientific). Centrifugation was performed at 6000 rpm on KµPrima-18R (Composite Rotor Inc.).

Surface Modification of Colloidal Silica Nanoparticles

As shown in figure S1, PEG-coated SNP were synthesized from plain SNP (commercially available LUDOX® TM-40 SNP) in three steps. To avoid aggregation, the modified SNP were purified by several steps of washing the nanoparticles with solvent, following by centrifugation. The intermediate particles (aSNP and aaSNP) were immediately further processed according the protocol and aggressive drying such as drying under vacuum [3] was avoided.
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Figure S1. Process of SNP PEG coating. SNP-coated SNP were synthesized from commercially available LTM40 silica nanoparticles in three steps. First, aminopropyltriethoxy silane (APTES) was condensed on the plain silica particles, resulting amine-modified SNP (aSNP). Then, particles were reacted with propargyl chloroformate to afford the alkyne-modified particles (aaSNP). Finally, particles were clicked with PEG-N₃ catalyzed by 10% of Cu(PPh₃)Br diisopropylamine to achieve PEG-coated SNP.

(1) Synthesis of amine-modified SNP (aSNP): Commercially available plain SNP (LUDOX™ TM-40 colloidal silica; 4.8 g in H₂O) were diluted with 100 mL of a 1:1 solution of EtOH/H₂O (v/v), and then aminopropyltriethoxy silane (APTES) (5 mL, 20 mmol) was drop wise added to the silica suspension. The mixture gradually evolved to a turbid suspension due to the formation of silane oligomers. After stirring for 3 days, the suspension was washed with EtOH and centrifuged (6000 rpm) for 20 - 40 min to remove the silane oligomer and any other impurities by. The purified product was kept in ethanol.

(2) Synthesis of alkyne-modified SNP (aaSNP): To remove ethanol and water, aSNP were washed and recovered by centrifugation (30 min) twice with reagent grade DMF, followed by washing three times with anhydrous DMF. The aSNP (30 mg, 1 equiv. of amine) were re-dispersed in a mixture of anhydrous DMF and triethylamine (3:1) by sonication (40 min) to obtain a homogeneous solution. After cooling in an ice bath for 30 min, propargyl chloroformate (2 mL, 1000 equiv.) was added drop wise and the solution turned to slight yellow. After addition of propargyl chloroformate, the ice bath was removed and the mixture was stirred for 24 h. The product was purified by several cycles of washing with CH₂Cl₂ and recovery by centrifugation, until the solvent was colorless and no sign of residual amine salts. The product was stored in CH₂Cl₂ till further modification.

(3) Synthesis of PEG-coated SNP by “Click” modification of aaSN: established aaSNP (38 mg, 1 equiv. of alkyne) were dispersed in 50 mL of DMF, followed by sonication for 30 min. An excess of PEG-N₃ (3.4 equiv.) and 0.2 mL of N,N-diisopropylethylamine were added to the suspension, followed by three freeze-pump-thaw processes to remove O₂. Solid Cu(PPh₃)Br (10% equiv.) was added followed by a final freeze-pump-thaw process. After purging with N₂,
the suspension was stirred at RT for 24 h. The suspension was centrifuged to recover the PEGylated SNP (2x10 mL). The purified particles were kept in distilled water. The nanoparticle concentration was measured by drying fixed amounts of the aqueous solution.

Changes in the SNP surface chemistry were tracked by FTIR and figure S2 shows the normalized FTIR spectra of SNP after each modification step. Since the IR band at 806 cm\(^{-1}\) is characteristic for silica, IR data were normalized allowing semi-quantitative comparison of the nanoparticles during the syntheses. For example, the spectrum of aSNP shows increased absorption at 2900–3300 cm\(^{-1}\) and a new band at 1431 cm\(^{-1}\), corresponding to the alkyl and amine groups of APTES. After treating aSNP with propargyl chloroformate, new absorption bands are seen at 1700 cm\(^{-1}\) due to C=O stretching, and triple bond stretching at 2127 cm\(^{-1}\), confirming successful alkyne functionalization on the particle surface (aaSNP) [4]. IR is less useful for determining PEGylation; the characteristic C-O bands are obscured by the strong IR bands of the particles, but bands from C-H bending and stretching increased.

![FTIR spectra of PEG-coated SNP and its precursors. The spectra are normalized by the absorbance at 888 cm\(^{-1}\), a band characteristic of SiO\(_2\).](image)

The TGA data in figure S3 and table 1 (main manuscript) confirm the step-by-step modification of silica particles. Since the TGA mass losses correspond to oxidation of the organic layer on the nanoparticles, the grafting density on the particle surface can be calculated. Table 1 in the article shows a 3.7% weight loss for amine-modified nanoparticles (aSNP), which corresponds to a grafting density of 2.7 group/nm\(^2\). After the alkyne modification (aaSNP), the additional mass loss (2.4%) indicate that about half of the amines were converted to the triple bonds, and the pegylation step (3.6%) corresponds to a grafting density of 0.7 PEG chain/nm\(^2\) and successful synthesis of water-soluble nanoparticles (PEG-coated SNP).
Figure S3. TGA data for PEG-coated SNP and its precursors. All samples were held at 120 °C for 30 min to remove absorbed water from particle surfaces, and then heated in air to 850 °C at a rate of 10 °C/min.

The DLS analysis shows that all nanoparticles have monomodal (Figure S4), but their size and size distribution depend on the surface chemistry of the particles, and the solvent used for the analysis. The average diameter of commercially available plain SNP is 30 nm (Table 1, article), showing a narrow particle distribution. Adding the amine to the particle surface increased the average diameter to 103 nm (DMF-NEt$_3$ 1:3) and after alkyne modification (hydrophobic) the average particle size was 126 nm (DMF). PEGylation made the particles water soluble, the average diameter decreased to 90 nm and the distribution narrowed as indicated by figure S4 and table 1.

Figure S4. DLS data (size distribution by intensity) for PEG-coated SNP and its precursors.
References

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2. Kitto HJ, Schwartz E, Nijemeisland M, Koepf M, Cornelissen JJLM, Rowan AE, Nolte RJM: Post-modification of helical dipeptido polyisocyanides using the “click” reaction. *J Mater Chem* 2008, 18:5615–5624.

3. Kar M, Vijayakumar PS, Prasad BLV, Sen Gupta S: Synthesis and characterization of poly-L-lysine-grafted silica nanoparticles synthesized via NCA polymerization and click chemistry. *Langmuir* 2010, 26:5772–5781.

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2. Supplementary Information Gene Expression Analysis

Figure S5. Heat map of gene expression array. Gene expression in lung tissue was analyzed with a 96-gene array. Relative increased gene expression towards control is indicated in red (≥2 fold expression) and relative decreased gene expression in green (≤2 fold). Black labels indicate no differences in gene expression. Increased gene expression in allergic and SNP exposed animals was found for various cytokines, chemokines and immune responsive genes as well as secretory mucus/surfactant genes. No changes greater than 2-fold were found for oxidative stress response, growth factors and different transcription factors.
### Table S1. TaqMan Gene Expression analysis. Detailed list of all genes and primers used for gene expression analysis with the TaqMan Gene Expression Assay from Applied Biosystems.

| Gene Symbol | Gene name | Gene Alias | Array Group    | Assay ID                  | NCBI Gene Reference |
|-------------|-----------|------------|----------------|---------------------------|---------------------|
| 18S         | 18S rRNA  | Endogenous | Mm03928990_g1  | NR_003286.1               |
| Actb        | Actin, beta | Endogenous | Mm00607939_s1  | NM_01101.3                |
| Arbp        | Acidic ribosomal phosphoprotein P0 | ARBP (Endog) | Mm01974474_gH  | NM_203736.1               |
| Gapd        | Glyceraldehyde-3-phosphate dehydrogenase | GAPDH | Endogenous | Mm99999915_g1 | NM_017008.3 |
| Gusb        | Glucuronidase, beta | Gus | Endogenous | Mm00446953_m1 | NM_010368.1 |
| Hprt1       | Hypoxanthine guanine phosphoribosyl transferase | Hprt | Endogenous | Mm00446968_m1 | NM_013566.2 |
| Ccl2        | Chemokine (C-C motif) ligand 2 | MCP-1, Scya-2 | Chemokine | Mm00441242_m1 | NM_011333.3 |
| Ccl3        | Chemokine (C-C motif) ligand 3 | MIP1-alpha | Chemokine | Mm00441258_m1 | NM_011337.2 |
| Ccl4        | Chemokine (C-C motif) ligand 4 | MIP1-beta | Chemokine | Mm00443111_m1 | NM_013652.2 |
| Ccl5        | Chemokine (C-C motif) ligand 5 | RANTES | Chemokine | Mm01302427_m1 | NM_013653.3 |
| Ccl8        | Chemokine (C-C motif) ligand 8 | MCP-2 | Chemokine | Mm01297183_m1 | NM_021443.2 |
| Ccl11       | Chemokine (C-C motif) ligand 11 | Eotaxin, Scya11 | Chemokine | Mm00441238_m1 | NM_011330.3 |
| Ccl12       | Chemokine (C-C motif) ligand 12 | MCP5 | Chemokine | Mm01211783_g1 | NM_011331.2 |
| Cxcl1       | Chemokine (C-X-C motif) ligand 1 | KC/CINC-1/IL8 | Chemokine | Mm00433859_m1 | NM_008176.3 |
| Cxcl2       | Chemokine (C-X-C motif) ligand 2 | MIP-2 | Chemokine | Mm00436450_m1 | NM_009140.2 |
| Cxcl3       | Chemokine (C-X-C motif) ligand 3 | MIP-2b, Dcip1, | Chemokine | Mm01701838_m1 | NM_203320.2 |
| Cxcl9       | Chemokine (C-X-C motif) ligand 9 | CMK, MIG, crg10 | Chemokine | Mm01345157_m1 | NM_008599.4 |
| Cxcl10      | Chemokine (C-X-C motif) ligand 10 | IP-10, Ifi10, gIP10 | Chemokine | Mm00445235_m1 | NM_021274.1 |
| Ifng        | Interferon gamma | IFN-g | Cytokine | Mm00801778_m1 | NM_008337.3 |
| Il1a        | Interleukin 1 alpha | Cytokine | Mm00439620_m1 | NM_010554.4 |
| Il1b        | Interleukin 1 beta | Cytokine | Mm00434228_m1 | NM_008361.3 |
| Il2         | Interleukin 2 | Cytokine | Mm00434256_m1 | NM_008366.2 |
| Il4         | Interleukin 4 | Cytokine | Mm00445259_m1 | NM_021283.2 |
| Il5         | Interleukin 5 | Cytokine | Mm00439646_m1 | NM_010558.1 |
| Il6         | Interleukin 6 | Cytokine | Mm00446190_m1 | NM_031168.1 |
| Il10        | Interleukin 10 | Cytokine | Mm00439616_m1 | NM_010548.1 |
| Il13        | Interleukin 13 | Cytokine | Mm00434204_m1 | NM_008355.3 |
| Il17a       | Interleukin 17A | Ctlia8 | Cytokine | Mm00439618_m1 | NM_010552.3 |
| Il23a       | Interleukin 23 | p19 | Cytokine | Mm00518984_m1 | NM_031252.2 |
| Tgfb1       | Transforming growth factor, beta 1 | TGF-beta1 | Cytokine | Mm01178820_m1 | NM_011577.1 |
| Tnfa        | Tumor necrosis factor alpha | TNF alpha | Cytokine | Mm00443258_m1 | NM_013693.2 |
| Il1rn       | Interleukin 1 receptor antagonist | Cytokine receptor | Mm01337566_m1 | NM_031167.5 |
| TgfbR1      | Transforming growth factor, beta receptor I | TbetaRI | Cytokine receptor | Mm00436971_m1 | NM_009370.2 |
| Timp1       | Tissue inhibitor of metalloproteinase 1 | Cytokine receptor | Mm00441818_m1 | NM_00104438.4 |
| Lrg1        | Immunoresponsive gene 1 | Inflammatory | Mm01224529_m1 | NM_008392.1 |
| Itln1       | Intelectin 1 (galactofuranose binding) | Itlna | Inflammatory | Mm00843942_sH | NM_010584.2 |
| Nlrp3       | NLR family, pyrin domain containing 3 | FCAS, FCU | Inflammatory | Mm00840904_m1 | NM_145827.3 |
| Ptafr       | Platelet-activating factor receptor | PAFR | Inflammatory | Mm02621061_m1 | NM_001081211.1 |
| Gene Symbol | Gene name              | Gene Alias          | Array Group | Assay ID            | NCBI Gene Reference |
|-------------|------------------------|---------------------|-------------|---------------------|---------------------|
| Retnla      | Resistin like alpha    | Fizz-1              | Inflamatory | Mm00445109_m1      | NM_020509.3         |
| Rsad2       | Radical S-adenosyl methionine domain containing 2 | | Inflamatory | Mm00491265_m1      | NM_021384.4         |
| Saa3        | Serum amyloid A3       | Saa-3               | Inflamatory | Mm00441203_m1      | NM_011315.3         |
| Serpin1     | Serine peptidase inhibitor | PAI-1              | Inflamatory | Mm01204469_m1      | NM_008871.2         |
| Tfi2        | Tissue factor pathway inhibitor 2 | | Inflamatory | Mm00436948_m1      | NM_009364.3         |
| Chi3f1      | Chitinase 3-like 1     | Brp39, Gp39         | Chitinase   | Mm00801477_m1      | NM_076959.3         |
| Chi3l3      | Chitinase 3-like 3     | YM1,ECF-L           | Chitinase   | Mm00657889_mH      | NM_09892.2          |
| Chi3l4      | Chitinase 3-like 4     | YM2                 | Chitinase   | Mm00840870_m1      | NM_145126.2         |
| Chia        | Chitinase, acidic      | AMCase              | Chitinase   | Mm00458221_m1      | NM_023186.3         |
| Ccnd1       | Cyclin D1              | CD1,Cyl-1           | Cell Cycle  | Mm03053889_s1      | NM_007631.2         |
| Cdkn1a      | Cyclin-dependent kinase inhibitor 1A | P21              | Cell Cycle  | Mm01303209_m1      | NM_007669.4         |
| Gas1        | Growth arrest specific 1 | Gas-1              | Cell Cycle  | Mm01700206_g1      | NM_008086.1         |
| Pdna        | Proliferating cell nuclear antigen | Pdna             | Cell Cycle  | Mm00448100_g1      | NM_011045.2         |
| Fgfl10      | Fibroblast growth factor 10 | FGF-10             | Growth factor | Mm00433275_m1      | NM_008002.4         |
| Tfif2       | Trefoil factor 2 (spasmolytic protein 1) | SP, mSP            | Growth factor | Mm00447491_m1      | NM_009363.3         |
| Vegfa       | Vascular endothelial growth factor A | VPF, Vegf          | Growth factor | Mm01281447_m1      | NM_00102520.3       |
| Egfr        | Epidermal growth factor receptor | Egfr              | Growth factor receptor | Mm00433023_m1      | NM_207655.2         |
| Pdgfrb      | Platelet derived growth factor receptor, beta | Pdgfr             | Growth factor receptor | Mm01262489_m1      | NM_001146268.1      |
| Accn3       | Amiloride-sensitive cation channel 3 | ASIC3             | Ion channel | Mm00805460_m1      | NM_183000.2         |
| Clca2       | Chloride channel calcium activated 2 | Ion channel | Mm00661630_m1      | NM_030601.2         |
| Clca3       | Chloride channel calcium activated 3 | Gob-5              | Ion channel | Mm00489959_m1      | NM_017474.1         |
| F2r         | Coagulation factor II (thrombin) receptor | Par1, ThrR       | Coagulation | Mm00438851_m1      | NM_010169.3         |
| Gja1        | Gap junction protein, alpha 1 | Cell adhesion | Mm00439105_m1      | NM_010288.3         |
| Icam1       | Intercellular adhesion molecule 1 | Cell adhesion | Mm00516023_m1      | NM_010493.2         |
| Arg2        | Arginase type II       | Oxidoreductase      | Mm00477592_m1      | NM_007905.3         |
| Cat         | Catalase               | Cas1,Cs-1           | Oxidoreductase | Mm00437992_m1      | NM_009804.2         |
| Cyp2e1      | Cytochrome P450, family 2e1 | Oxidoreductase | Mm00491127_m1      | NM_021282.2         |
| Mt1         | Metallothionein 1      | Oxidoreductase      | Mm00496660_g1      | NM_013602.3         |
| Nqo1        | NAD(P)H dehydrogenase, quinone 1 | Nmo-1, Ox-1        | Oxidoreductase | Mm00500821_m1      | NM_008706.5         |
| Sod1        | Superoxide dismutase 1, soluble | Oxidoreductase | Mm01344233_g1      | NM_011343.1         |
| Sod2        | Superoxide dismutase 2, mitochondrial | Oxidoreductase | Mm00449726_m1      | NM_013671.3         |
| Hmox        | Heme oxygenase (decycling) 1 | HO-1               | Oxidoreductase | Mm00516004_m1      | NM_010442.2         |
| Mmp12       | Matrix metalloproteinase 12 | Protease | Mm00500554_m1      | NM_008605.3         |
| Muc5ac      | Mucin 5, subtypes A & C | Secretory | Mm01276725_g1      | NM_010844.1         |
| Muc5b       | Mucin 5, subtype B     | Secretory | Mm00466376_m1      | NM_028801.2         |
| Sgb1a1      | Clara cell secretory protein | CCSP, CC10         | Secretory | Mm00442046_m1      | NM_011681.2         |
| SftpC       | Surfactant protein C   | SP-C                | Surfactant | Mm00488144_m1      | NM_011359.1         |
| SftpD       | Surfactant protein D   | SP-D                | Surfactant | Mm00486060_m1      | NM_009160.2         |
| Nos2        | Nitric oxide synthase 2, inducible | iNOS               | Synthase | Mm00440485_m1      | NM_010927.3         |
| Ptgs2       | Prostaglandin-endoperoxide synthase 2 | COX2              | Synthase | Mm004781374_m1     | NM_011198.3         |
| Gclc        | Glutamate-cysteine ligase, catalytic subunit | Synthase | Mm00802655_m1      | NM_010295.1         |
| Gstm1       | Glutathione S-transferase, mu 1 | Gstb1             | Transferase | Mm00833915_g1      | NM_010358.5         |
| Gene Symbol | Gene name                        | Gene Alias | Array Group | Assay ID            | NCBI Gene Reference |
|------------|---------------------------------|------------|-------------|---------------------|---------------------|
| Gstp1      | Glutathione S-transferase, pi 1 | GstpiB     | Transferase | Mm00496606_m1      | NM_013541.1        |
| Egr1       | Early growth response 1         | Egr        | Transcription factor | Mm00656724_m1 | NM_007913.5      |
| Gata3      | GATA binding protein 3          |            | Transcription factor | Mm00484683_m1 | NM_008091.3      |
| Spdef      | SAM pointed domain containing ets transcription factor | PDEF, Pse | Transcription factor | Mm00600221_m1 | NM_013891.4      |
| Stat3      | Signal transducer and activator of transcription 3 | Aprf    | Transcription factor | Mm01219775_m1 | NM_213659.2      |
| Stat6      | Signal transducer and activator of transcription 6 |        | Transcription factor | Mm01160477_m1 | NM_009284.2      |
| Tbx21      | T-box 21                        | T-bet, TBT1 | Transcription factor | Mm00450960_m1 | NM_019507.2      |
| Tslp       | Thymic stromal lymphopoietin    |            | Transcription factor | Mm00498739_m1 | NM_021367.1      |
| Ttf1       | Transcription termination factor, RNA polymerase I | | Transcription factor | Mm00657017_m1 | NM_009442.2      |
| Atf4       | Activating transcription factor 4 |       | Transcription factor | Mm00515324_m1 | NM_009716.2      |
| Foxa2      | Forkhead box A2                 | HNF3-beta  | Transcription factor | Mm00839704_mH | NM_010446.2      |
| Foxp3      | Forkhead box P3                 | JM2        | Transcription factor | Mm00475165_m1 | NM_054039.1      |
| Nfe2l2     | Nuclear factor, erythroid derived 2, like 2 | Nrf2     | Transcription factor | Mm00477784_m1 | NM_010902.3      |
| Pparg      | Peroxisome proliferator activated receptor gamma | PPAR-g | Transcription factor | Mm00440945_m1 | NM_001127330.1   |
| Socs1      | Suppressor of cytokine signaling 1 | SSI-1 | Transcription factor | Mm00782550_s1 | NM_009896.2      |
| Socs3      | Suppressor of cytokine signaling 3 | SOC3     | Transcription factor | Mm00545913_s1 | NM_007707.2      |
3. Gating Strategy for FACS

Figure S6. Cells from TBLN were stained for expression of surface molecules, including CD4, CD69, Cd11c, CD11b, MHC II, and Gr-1. Cells were first gated on singlets using FSC-A/FSC-H, then gated on total cells, including lymphocytes, monocytes, and granulocytes, using FSC-A/SSC-A. Subsequently, the total cell population was gated on CD4⁺, Gr-1⁻, CD11c⁺, or CD11c⁺CD11b⁻ population, respectively. The CD11c⁺CD11b⁻ population was further gated on Gr-1⁺ for AM and Gr-1⁻ for pDC.