SUPPLEMENTARY INFORMATION

Polymersomes decorated with SARS-CoV-2 spike protein receptor binding domain elicit robust humoral and cellular immunity

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Supplementary Figure S1. Synthesis and characterization of N₃-PEG-PPS.
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Chemical Synthesis and Characterization
Supplementary Figure S1 | Synthesis and characterization of $N_3$-PEG-PPS. a, Synthetic route, b, $^1$H NMR spectrum, c, gel permeation chromatography (GPC) trace, and d, summary of physiochemical properties of $N_3$-PEG-PPS.

Supplementary Figure S2 | Synthesis and characterization of RBD-linker. a, Synthetic route, b, MALDI of RBD-linker and free RBD, c, SDS PAGE of free RBD, RBD-linker, RBD$_{surf}$ before size exclusion chromatography (SEC) and purified RBD$_{surf}$ post-SEC.
Supplementary Figure S3 | Synthesis and characterization of PEG-PPS. a, Synthetic route, b, ¹H NMR spectrum, c, gel permeation chromatography (GPC) trace, and d, summary of physiochemical properties of PEG-PPS.

Supplementary Figure S4 | Additional cryoEM images of PS. Black box indicates magnified region in Figure 1b.
Supplementary Figure S5 | PS stability by SDS PAGE after > 180 d at 4 °C. SDS PAGE of a, RBDsurf and b, RBDencap. Lanes 1-6 represent RBD standard curve values of 400, 200, 100, 50, 25, and 12.5 µg/mL. Lane 7 contains of RBDsurf disrupted with Triton X. Lane 8 contains of RBDencap disrupted with Triton X, and Lane 9 contains undisrupted RBDencap.

Supplementary Figure S6 | RBD binding to HEK-hACE2 and HEK-293 cells. a, Mean fluorescence intensity (MFI) of AF647-labeled RBDsurf and empty PS bound to HEK-hACE2 cells characterized by flow cytometry. b, MFI of AF647-labeled RBDsurf and empty PS indicating an absence of binding to control HEK-293 (HEK) cells. Data plotted as mean ± SD for n = 2 replicates.
**Supplementary Figure S7 | MPLA PS as a TLR4 agonist.** Linear concentration-dependent stimulation of HEK-Blue™ TLR4 reporter cells with MPLA PS. Data plotted as mean ± SD for n = 3 replicates.

![Graph showing linear concentration-dependent stimulation of HEK-Blue™ TLR4 reporter cells with MPLA PS.](image)

R² = 0.9754

p < 0.0001

**Supplementary Figure S8 | In vitro activity of MPLA PS.** Dose-dependent secretion of a, IL-6, b, IL-1α, and c, IL-1β from cultured murine bone marrow-derived dendritic cells stimulated by free MPLA, MPLA PS, or empty PS. Data plotted as mean ± SD for n = 3 replicates.

![Graphs showing dose-dependent secretion of IL-6, IL-1α, and IL-1β](image)
Supplementary Figure S9 | ELISA absorbance vs. dilution curves. Absorbance vs. dilution for RBD-specific ELISAs for a, total IgG over time and b, IgG subtypes on d28. Log-transformed curves were quantified by AUC in Figure 2. Data plotted as mean ± SD and represent 1 of 2 experiments with n = 5 mice each.

Supplementary Figure S10 | Presence of IgA antibodies. a, Absorbance vs. dilution for RBD-specific IgA ELISAs. b, AUC from (a). Data plotted as mean ± SD and represent 1 of 2 experiments with n = 5 mice each. Symbols in (b) represent individual mice. Comparisons to unadjuvanted RBD_free were made using one-way ANOVA with Dunn's post-test.
Supplementary Figure S11 | IgG antibody and viral neutralization titers. **a**, Aggregate RBD-specific IgG antibody titers 1 week post-boost based on ELISA. Values below the LLOQ (= 2) are plotted as LLOQ/2. Titers were determined as the -log of the lowest plasma dilution for which \((OD_{450} - OD_{570}) - \text{(average of blanks + 4 \times standard deviation of blanks)} > 0.01\). \(P\) values represent comparisons to unadjuvanted RBD<sub>free</sub>. **b**) Viral neutralization titers for RBD<sub>surf</sub> + MPLA PS across three different cohorts of \(n = 5\) mice, indicating experiment reproducibility. Values below the LLOQ (= 2.11) are plotted as LLOQ/2.; \(ns\) \(p = 0.11\). Symbols represent individual mice. Comparisons were made using a Kruskal-Wallis nonparametric test with Dunn’s post-test.

Supplementary Figure S12 | Representative peptide array images. Boxes represent region of peptide array specific to the RBD of the Spike protein. Peptide arrays quantified in Figure 3c.
Supplementary Figure S13 | Analysis of plasma by mice vaccinated with RBD_{surf} + RBD_{encap} + MPLA PS and RBD_{free} + MPLA_{free}. Mice received a priming dose on day 0 with a boost on day 21, and plasma was taken weekly to monitor production of RBD-specific antibodies. a, AUC of absorbance curve of RBD-specific IgG ELISAs for mice vaccinated with either 5 μg RBD_{encap} + 5 μg RBD_{surf} + MPLA PS or 10 μg RBD_{free} + MPLA_{free}. Data plotted as mean ± SD for n = 5 mice. b, Neutralization of SARS-CoV-2 infection of Vero E6 cells in vitro. Data plotted as mean ± SEM for n = 5 mice. c, Epitope mapping using 15-amino-acid peptides with a 5-amino-acid shift, spanning the entire RBD sequence with representative images of peptide arrays.

Supplementary Figure S14 | Representative T follicular helper cell gating strategy.
Supplementary Figure S15 | Representative B cell gating strategy.

Supplementary Figure S16 | Total and naïve B cells in vaccinated mice 1 week post-boost. 

**a**, Total B cells (B220⁺ CD19⁺) and **b**, naïve B cells in dLNs. Data plotted as mean ± SD and represent 1 of 2 experiments with n = 5 mice each. Symbols represent individual mice. Comparisons to unadjuvanted RBD_free were made using one-way ANOVA with Dunn’s post-test.
Supplementary Figure S17 | Representative tetramer staining.

Supplementary Figure S18 | RBD-specific cells in vaccinated mice 1 week post-boost. Tetramer⁺ cells in dLNs on d28. Data plotted as mean ± SD and represent 1 of 2 experiments with n = 5 mice each. Symbols represent individual mice. Comparisons to unadjuvanted RBDfree were made using one-way ANOVA with Dunn’s post-test.
Supplementary Figure S19 | Representative intracellular cytokine gating strategy.

Supplementary Figure S20 | Th2-type cytokines secreted upon ex vivo stimulation with RBD. Lymph node cells isolated from the dLNs of PS vaccinated mice 1 week post-boost were restimulated ex vivo with full RBD protein. After 3 d, levels of IL-4, IL-5, and IL-13 secreted into the supernatant were measured. Data plotted as mean ± SD and represent 1 of 2 experiments with n = 5 mice each. Symbols represent individual mice. Comparisons to unadjuvanted RBD_free were made using one-way ANOVA with Dunn’s post-test.
**Supplementary Table S1 | Summary of loading capacities of PS.**

| Polymer (mg mL\(^{-1}\)) | Cargo (mg mL\(^{-1}\)) | Loading (wt%) |
|--------------------------|-------------------------|---------------|
| RBD\(_{surf}\)           | 7.96                    | 0.127         | 1.57         |
| RBD\(_{encap}\)          | 7.00                    | 0.125         | 1.75         |
| MPLA PS                  | 3.49                    | 0.241         | 6.46         |

Polymer concentration was determined by GPC, RBD concentration in RBD\(_{surf}\) was determined by CBQCA protein quantitation assay, RBD concentration in RBD\(_{encap}\) was determined by SDS PAGE, and MPLA concentration was determined by mass spectrometry.

**Supplementary Table S2 | Probes and antibodies for T\(_h\) cell panel.**

| Marker         | Color    | Vendor                        |
|----------------|----------|-------------------------------|
| Viability Dye | eFluor 780 | Invitrogen 65-0865-14         |
| CD4           | BV496    | BD Horizon 612952             |
| CD3           | BUV737   | BD Optibuild 741788           |
| CD44          | PerCpCy5.5 | Invitrogen 45-0441-82         |
| CXCR5         | BV421    | Biolegend 145512              |
| ICOS          | BUV396   | BD Horizon 565885             |
| Bcl6          | PE-Cy7   | Biolegend 358512              |

**Supplementary Table S3 | Probes and antibodies for RBD-specific B cell panel.**

| Marker         | Color             | Vendor                        |
|----------------|-------------------|-------------------------------|
| Viability Dye | Violet fluorescent reactive dye | Invitrogen L34964A            |
| RBD-tetramer  | PE                | -                             |
| RBD-tetramer  | APC               | -                             |
| F4/80 (Dump)  | FITC              | Biolegend 123107              |
| CD11c (Dump)  | FITC              | Biolegend 117306              |
| Ly6c(Dump)    | FITC              | Invitrogen 53-5932-82         |
| Ly6g (Dump)   | FITC              | Invitrogen 11-9668-82         |
| CD4 (Dump)    | FITC              | Biolegend 100406              |
| CD8a (Dump)   | FITC              | Biolegend 100706              |
| B220          | BUV496            | BD Horizon 612950             |
| CD19          | BUV396            | BD Horizon 565965             |
| CD138         | BV605             | Biolegend 142531              |
| IgM           | BV786             | BD Optibuild 743328           |
| IgD           | PE-Cy7            | Biolegend 405720              |
| CD38          | APC-Cy7           | Biolegend 102727              |
| GL7           | PerCP-Cy5.5       | Invitrogen 46-5902-82         |
Supplementary Table S4 | Probes and antibodies for restimulation panel.

| Marker      | Color     | Vendor           |
|-------------|-----------|------------------|
| Viability Dye | eFluor 455 (UV) | Invitrogen 65-0868-14 |
| CD3        | BUV395    | BD Horizon 563565 |
| CD4        | BV786     | BD Horizon 563331 |
| CD8        | BV421     | BD Horizon 563898 |
| IFNα       | APC       | Biolegend 505810 |
| TNFα       | BV605     | Biolegend 506329 |
| IL-2        | PE        | BD Pharmigen 554428 |

Chemical Synthesis and Characterization

**N₃-PEG-PPS.** N₃-PEG-PPS was synthesized by first dissolving N₃-PEG₂₄-SH (1 eq, MW ~1000 g mol⁻¹; Nanosoft Polymers) in degassed, anhydrous THF and deprotonating the thiol group by addition of sodium methoxide (NaOMe; 1.1 eq) under nitrogen gas. Propylene sulfide (40 eq) was added by syringe, and the reaction proceeded until completion at the desirable degree of polymerization of PPS, as determined by ¹H NMR. The polymer was precipitated multiple times in ice cold methanol to obtain the final product, N₃-PEG₂₄-PPS₄₀, characterized by ¹H NMR (400 MHz Bruker DRX spectrometer equipped with a BBO probe, using Topspin 1.3) and gel permeation chromatography (GPC; Tosoh EcoSEC size exclusion chromatography system with a Tosoh SuperAW3000 column). ¹H-NMR (400 MHz, CDCl₃) of N₃-PEG-PPS, δ 1.37 (s, PPS, 3H), 2.63 (m, PPS, 1H), 2.91 (m, PPS, 2H), 3.39 (t, -CH₂-N₃, 2H), 3.65 (m, PEG).

**PEG-PPS.** PEG-PPS was synthesized as previously described¹. Briefly, benzyl mercaptan (1 eq.) in degassed, anhydrous THF (20 mM) was deprotonated with NaOMe (1.1 eq.). Under nitrogen protection, propylene sulfide (39 eq) was rapidly added by syringe, and the reaction proceeded until completion at the desirable degree of polymerization of PPS, as determined by ¹H NMR. Subsequently, mPEG₁₇-mesylate (synthesized in-house as previously described¹) was added, and the mixture was allowed to react overnight. The polymer was precipitated multiple times in ice cold methanol to obtain the final product, PEG₁₇-PPS₃₀, characterized by ¹H NMR and GPC. ¹H-NMR (400 MHz, CDCl₃) of mPEG-PPS, δ 1.37 (s, PPS, 3H), 2.63 (m, PPS, 1H), 2.91 (m, PPS, 2H), 3.38 (m, -OCH₃, 3H), 3.65 (m, PEG), 7.32 (m, benzyl, 4H).

¹Scott, E. A. *et al.* Dendritic cell activation and T cell priming with adjuvant- and antigen-loaded oxidation-sensitive polymersomes. *Biomaterials* **33**, 6211–6219 (2012).