Electronic Supplementary Material (ESI) for Soft Matter.

Holographic Immunoassays: Direct Detection of Antibodies Binding to Colloidal Spheres

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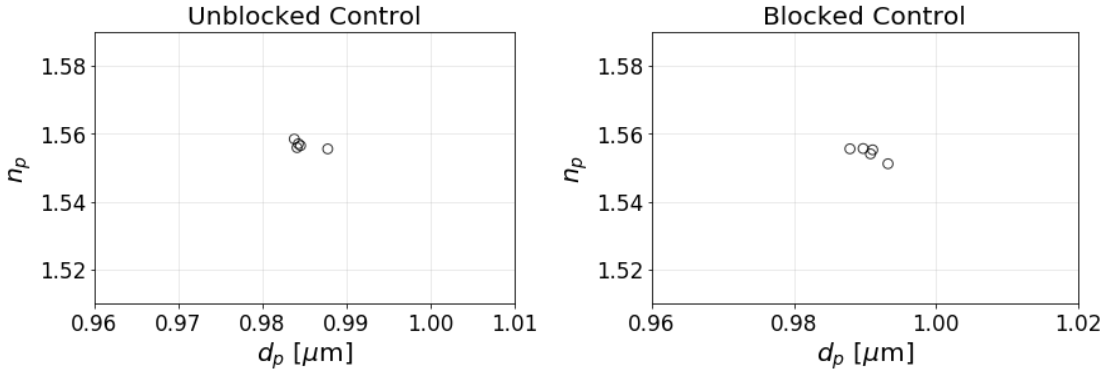
Supplementary Information: The Role of Surface Blocking in Holographic Immunoassays

The beads used for holographic immunoassays are functionalized with surface groups that specifically bind target antibodies and prevent other species from binding. Any gaps in the coverage of surface groups leave bare regions on a bead's surface that can bind molecules and nanoparticles nonspecifically. Nonspecific binding will increase the effective diameter of a probe bead and may influence the ability of holographic assays to detect and quantify specific binding. The deleterious effects of nonspecific binding can be minimized by ensuring the densest possible coverage of surface groups. Deficiencies in the coating can be remedied by blocking bare regions with molecules that bind strongly to the bare particle surface and hinder nonspecific binding by other species.

We have explored the use of bovine serum albumin (BSA) to block bare regions on commercially obtained polystyrene beads coated with protein A (Bangs Laboratories, catalog no. CP02000, lot no. 14540). Ideally, the coatings on these beads would be sufficiently dense that BSA would not bind to any great degree and blocking with BSA would have no effect on the outcome of holographic immunoassays. In practice, as illustrated by the holographic characterization data in Fig. 1, incubating the beads with 100 μg/mL of BSA increases the mean bead diameter by nearly 5 nm. This suggests that BSA indeed binds nonspecifically to these beads. Each data point in Fig. 1 represents the population-averaged properties of a sample of approximately 1000 beads. Not only does the mean diameter increase slightly after incubation with BSA, but the range of observed bead diameters also increases. This suggests that the polydispersity in the bare surface area on the beads exceeds the polydispersity in the beads. This may be due to patchy coverage of protein A on the surface.

Figure 1: Holographic measurements of the population-averaged bead diameter, d_p, and refractive index, n_p, before and after incubating commercial protein A probe beads with BSA. The measured diameter increases by nearly 5 nm after incubation indicating that BSA has bound nonspecifically to the beads' surfaces.

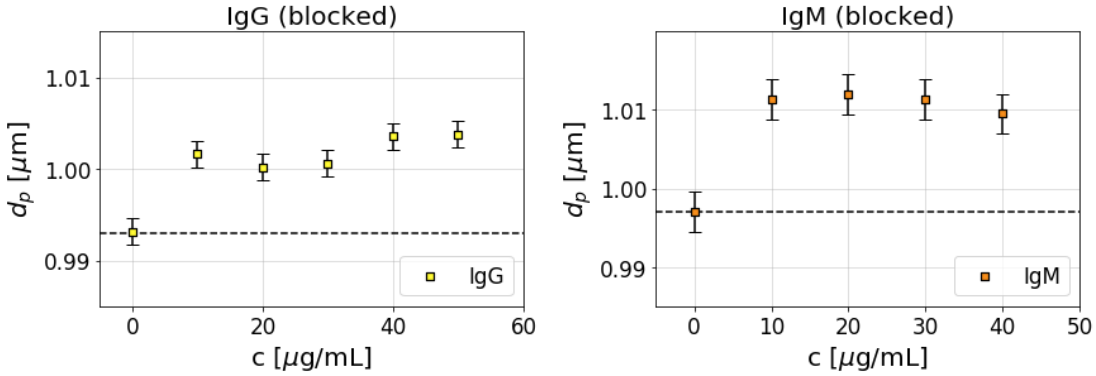


The benefit of blocking the probe beads can be appreciated by observing the blocked beads' response to incubation with a second species of non-target molecules. We performed holographic characterization results on probe beads incubated with alcohol dehydrogenase

(ADH) both before and after blocking with BSA. As shown in Fig. 3(c) of the main document, the diameter of the blocked beads does not increase when the beads are incubated with ADH indicating that ADH does not bind to either the protein A or the BSA on the surface of those beads. The unblocked beads, by contrast, grow by nearly 5 nm after incubation with ADH. This is roughly the same diameter shift that is observed during blocking by BSA, and is consistent with the interpretation that ADH binds nonspecifically to the same bare surface regions that can be blocked by BSA.

Having demonstrated that blocking by BSA prevents binding by non-target proteins, we next assess the effect of blocking on binding by target molecules. The data for IgG and IgM presented in Fig. 3 of the main manuscript were obtained with unblocked probe beads. Figure 2 below presents comparable binding assays performed with blocked probe beads. These beads are prepared in the titration buffer by incubating with a 1% solution of BSA in 1:1 ratio for 15-30 min at room temperature. The beads were then washed with the same buffer without BSA before incubating with antibody solution for 45 min. Each point represents results from a single set of measurements on a statistical sample of 1000 beads in 1 µL. As for the data presented in the main manuscript, incubation with antibodies yields a statistically significant increase in bead diameter, even after blocking. Unlike ADH, therefore, antibodies appear to bind to the blocked beads, presumably because they bind to protein A. The net increases in bead diameter observed in Fig. 2 are smaller than is observed for binding to unblocked beads. This is consistent with the interpretation that antibodies bind to both protein A and bare surface regions in the unblocked beads, but only bind to protein A in the blocked beads.

Figure 2: Holographic measurements of the increase in bead diameter, d_p - d_0, for BSA-blocked probe beads incubated for 45 minutes with different concentrations, c, of IgG and IgM. Each point represents population-average results for 1000 probe beads.



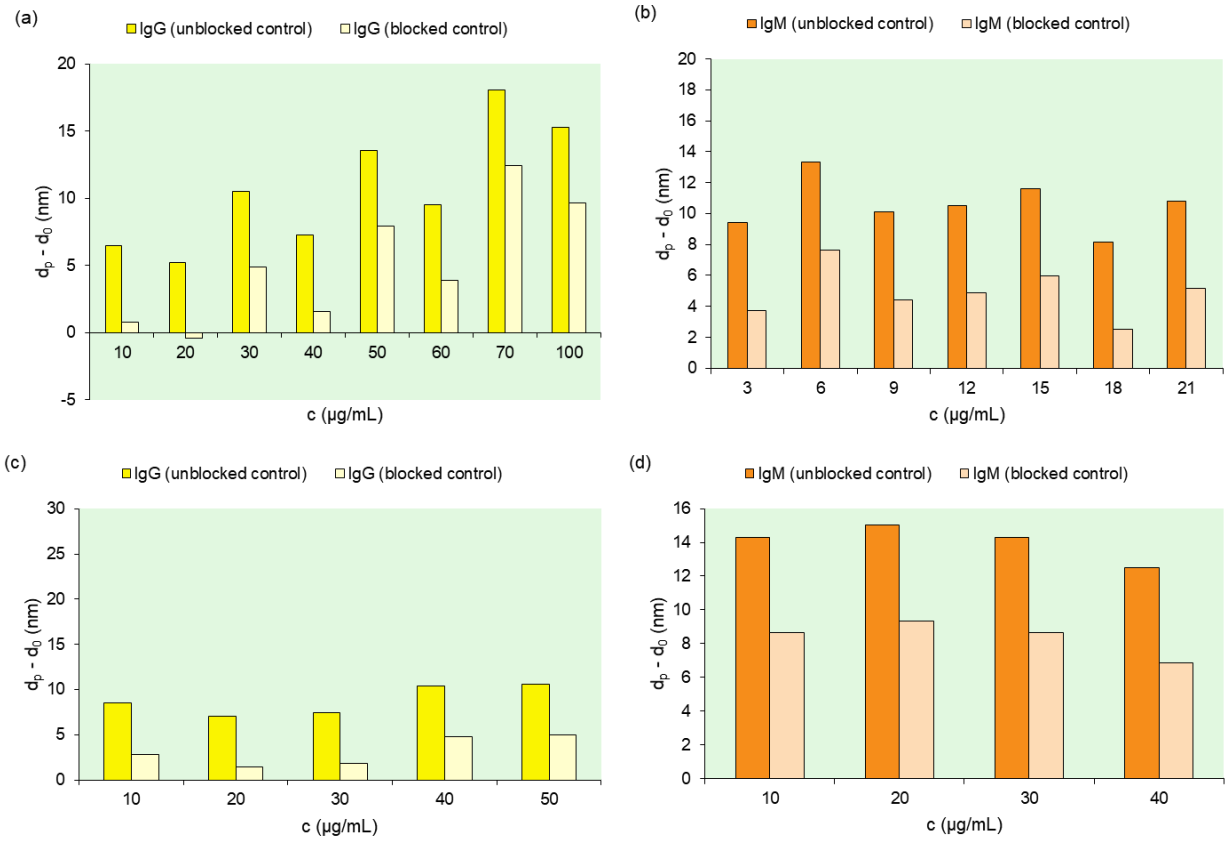
As in the measurements performed with unblocked beads, the diameter shift associated with IgM binding is comparable to the diameter shift associated with IgG binding, despite the nearly five-fold difference in the antibodies' molecular weights. Both sets of observations therefore support a model in which pentameric IgM binds flat to the surface of the beads, creating a molecular layer that is comparable in thickness to a coating of monomeric IgG.

The data in Fig. 2 are consistent with the rate constants reported in the main text. The observed binding rate therefore appears to be dominated by the rate of antibody binding to protein A rather than non-specific binding to bare regions on the particles' surfaces. This is reasonable if the beads' surfaces are predominantly covered with protein A, leaving comparatively small proportions bare. These data are not sufficiently finely sampled, however, to estimate the limit of

detection for holographic immunoassays on blocked spheres. The available data are consistent, nonetheless, with the limits reported in the main text that were obtained with unblocked beads.

Blocking with BSA decreases the observed increase in bead diameter due to antibody binding. This effect reflects both the increase in the control beads' diameter due to BSA binding and a comparable reduction in the acquisition of antibodies during the assay. Figure 3 presents a comparison between the shifts reported in Fig. 3 of the main text with the results obtained with blocked beads reported here. All diameter shifts in the main text are calculated by comparison with an unblocked control bead diameter, d_0. The diameter shifts presented in Fig. 3(a) and Fig. 3(b) were calculated by using this value to numerically determine the effect BSA blocking would have had. This demonstrates that regardless of which control measurement is used, a significant and proportional diameter shift is observed for the binding experiments that use either unblocked (a & b) or blocked (c & d) probe beads.

Figure 3: Comparisons of the diameter shifts observed in the binding assay using (a, b) unblocked probe beads and (c, d) blocked probe beads.



Pretreating the probe beads used for holographic immunoassays with a passive species such as BSA improves the selectivity of the assay by suppressing nonspecific binding. This would not be necessary if the surface coverage of binding sites were sufficiently dense to prevent nonspecific binding. Provided that non-functionalized regions constitute a sufficiently small proportion of the probe beads' surface area, the principal effect of nonspecific binding is to increase the apparent layer thickness, δ, which is treated in the main text as a

phenomenological parameter. This appears to be the case for the assays of IgG and IgM performed with this supply of commercial probe beads. Rate constants for antibody binding obtained in the main text with a much larger number of replicated assays on unblocked spheres are consistent with the results presented here on blocked spheres. The conclusions of the main text, therefore, are not changed by the implementation of blocking.