FIGURE S1

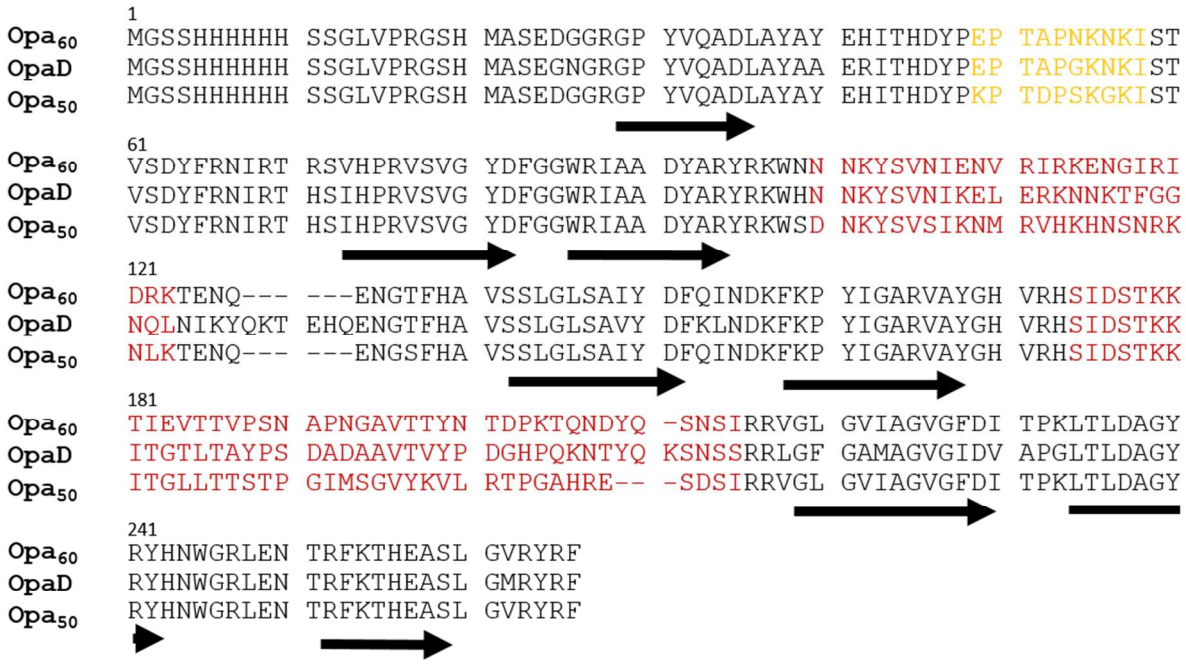


Figure S1. Sequence alignment of Opa_{60} from Gc strain MS11, OpaD from Gc strain FA1090, and Opa_{50} from Gc strain MS11. Semivariable regions are highlighted in yellow and hypervariable regions are highlighted in red. Black arrows indicate β-strands which form the membrane spanning barrel.

FIGURE S2

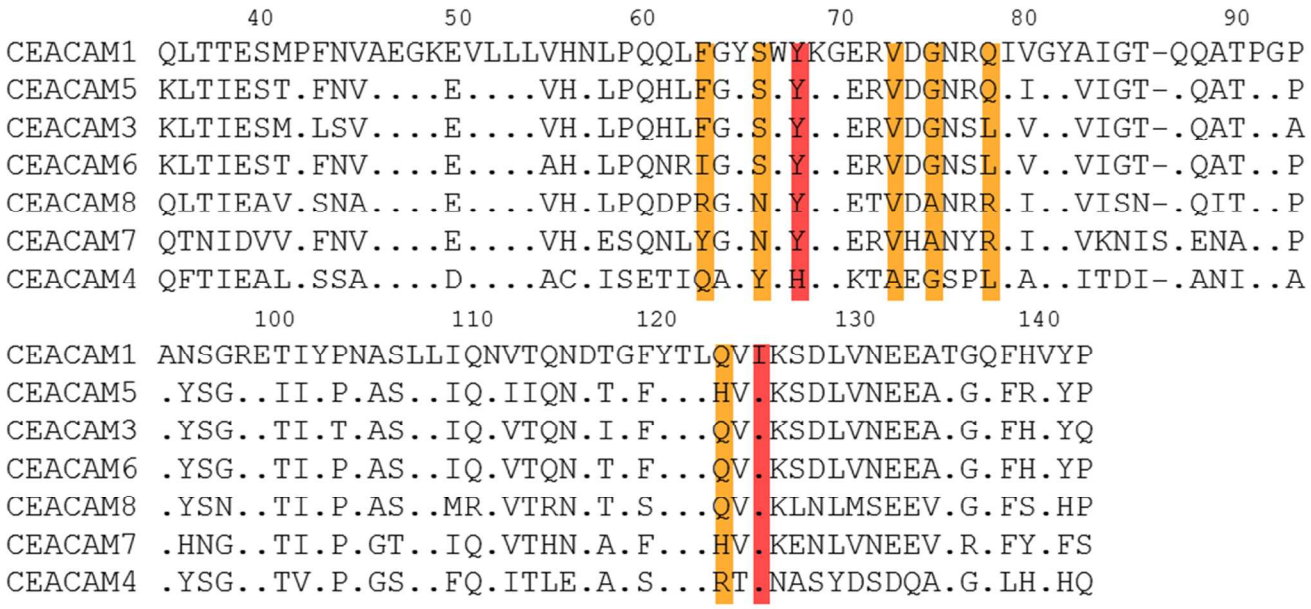


Figure S2. Sequence alignment of human NCCMs 1, 3, 4, 5, 6, 7, and 8. Essential residues for binding all Opa proteins are highlighted with red boxes and residues that are important to some Opa protein interactions with orange boxes (Figure 1A and 5). Residue numbering corresponds to CEACAM1 sequence, UniProt ID P13688-1.

FIGURE S3

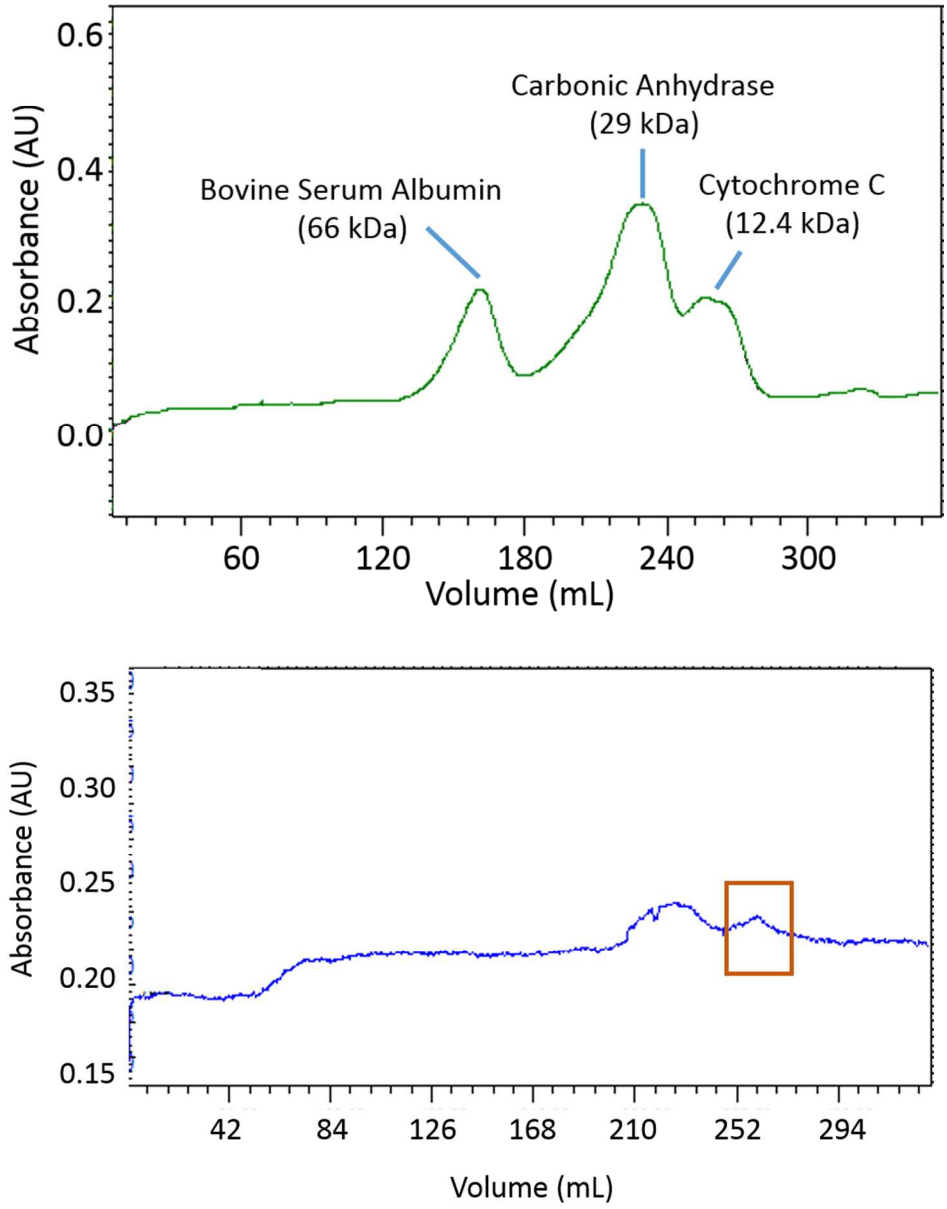


Figure S3. SEC chromatogram of molecular weight standards (top) and NCCM1 (bottom). Following GST tag cleavage, the NCCM1 was purified using SEC. Species were present with molecular weights corresponding to ~25 kDa (approximate MW of GST, TEV, and NCCM dimer), and ~12 kDa (approximate MW of NCCM monomer). A second passage through the SEC column was conducted immediately following (bottom), and fractions containing the monomeric NCCM (MW ~ 12 kDa) as asessed by SDS-PAGE were pooled and concentrated (orange box). Absorbance is at 280 nm.

FIGURE S4

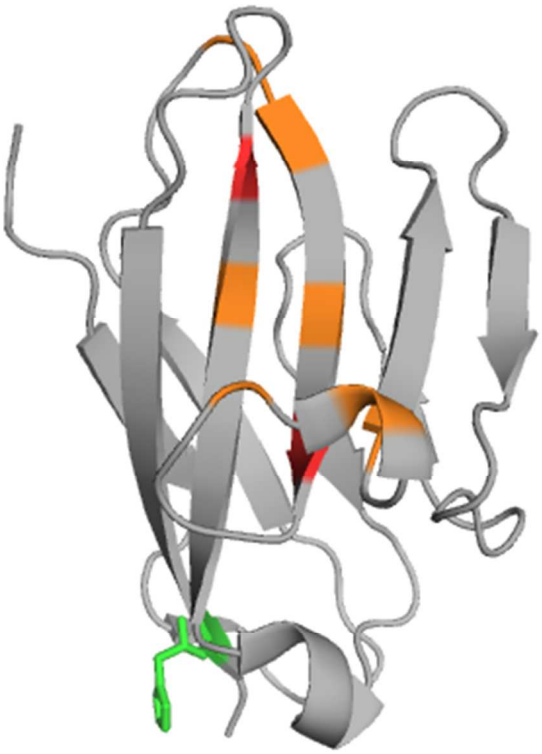
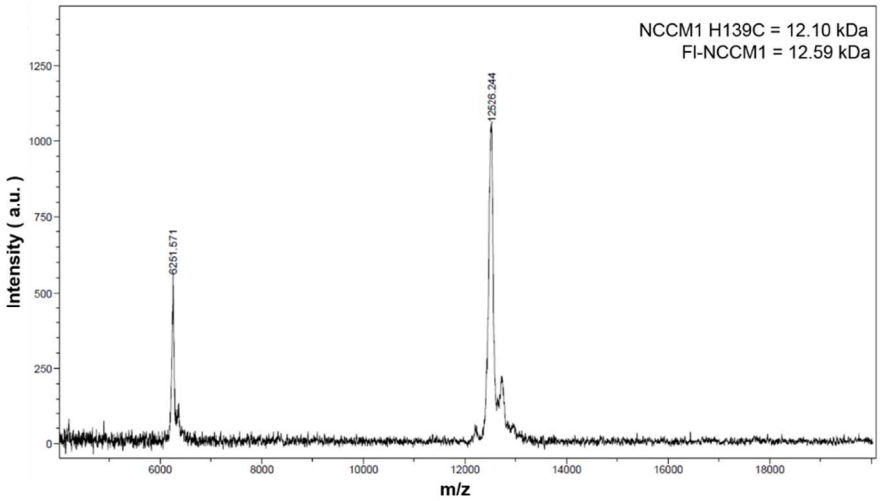


Figure S4. Ribbon representation of NCCM1 H139. The cysteine mutant (H139C), is located on the opposite side of the Opa-binding face. Opa-binding residues are colored as in Figure S2, H139 is colored green.

FIGURE S5

A



B

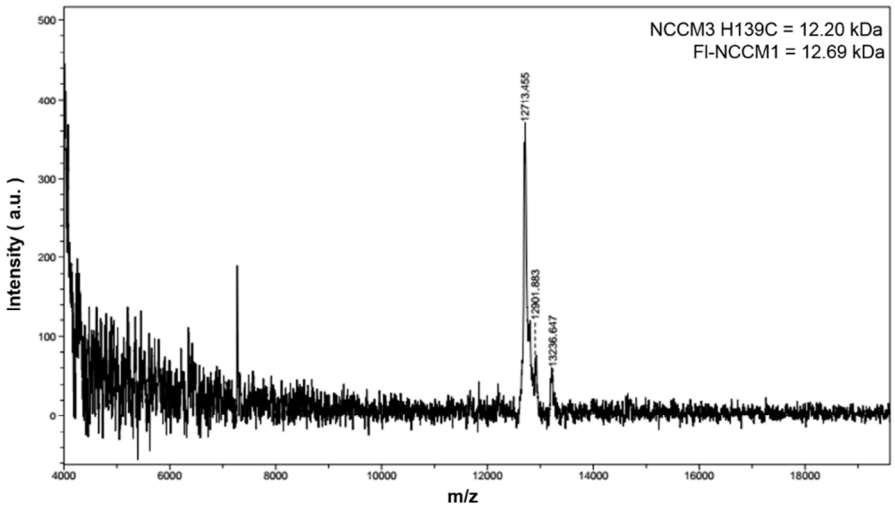


Figure S5. MALDI spectra of fluoresently labled (Fl-) NCCM1 (A) and NCCM3 (B). These spectra indicate that there is >95 % labeling efficiency of the AMS fluorophore (MW = 536.4 Da) with both NCCM1 and NCCM3.

FIGURE S6

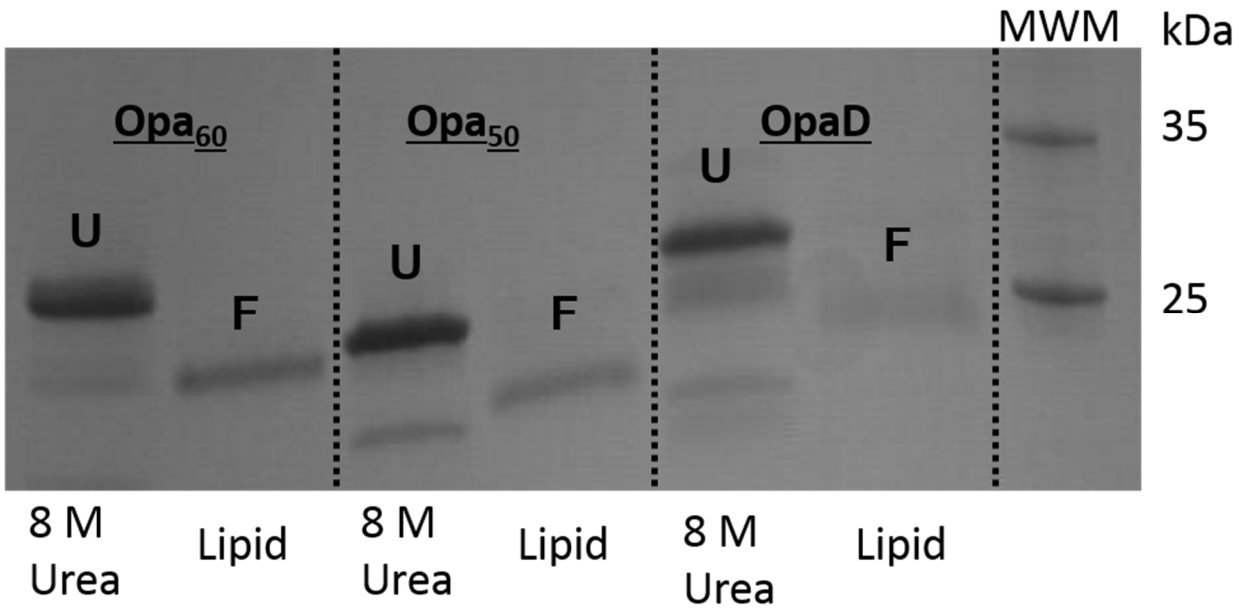


Figure S6. SDS-PAGE of Opa proteins. Folded Opa proteins (lanes labeled lipid) migrate at an apparent lower molecular weight than unfolded Opa proteins (8 M Urea) allowing folding to be assessed using SDS-PAGE. Fully folded samples were used for all experiments in this manuscript.

FIGURE S7

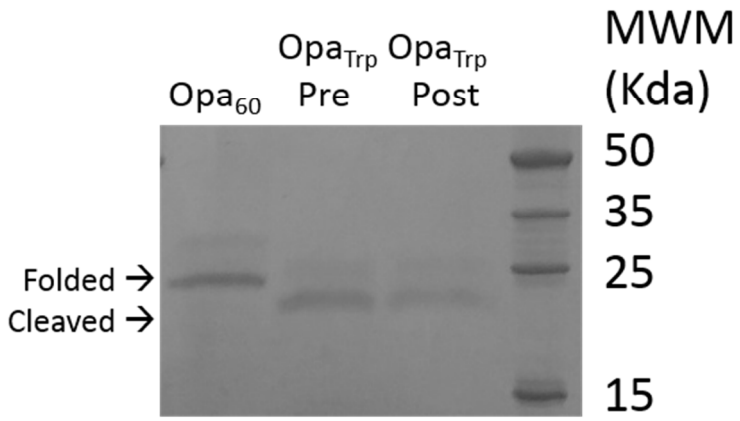


Figure S7. SDS-PAGE of trypsin cleavage of Opa_{60} in liposomes. Lane 1 contains folded Opa_{60} protein in liposomes, before the addition of trypsin (Opa_{60}). Lane 2 is the sample after the addition of trypsin and incubation (~ 4 h), before the trypsin was removed (Opa_{Trp} Pre). Lane 3 contains the flow through after passing over the benzamidine column (Opa_{Trp} Post) and lane 4 contains the molecular weight marker (MWM).

FIGURE S8

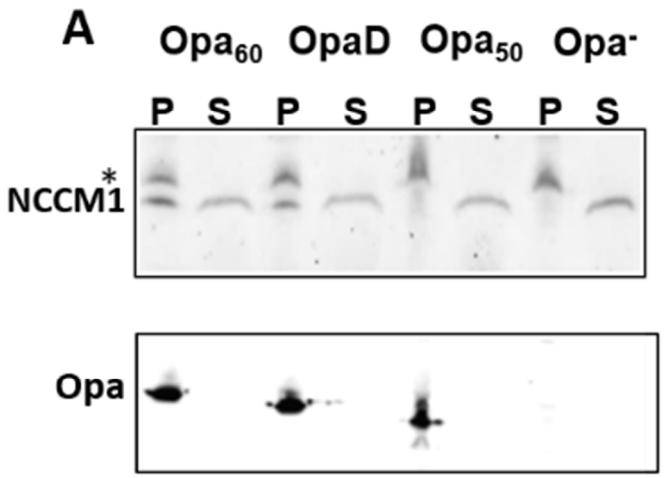


Figure S8. Immunoblot of pull-down assay using Opa expressing Gc and NCCM1that has been cleaved and purified from the GST tag. Bacteria expressing OpaD, Opa_{50}, or Opa_{60}, or Opa^- Gc, were incubated with NCCM1 (3 mg/mL), and samples were centrifuged for pellet (P) and supernatant (S) immunoblot assessment. Opa_{60} and OpaD Gc interacted with cleaved NCCM1 similarly to GST-NCCM1 (compare to Figure 2) indicating the GST tag does not interfere with NCCM binding to Opa proteins. There is less GST-NCCM1 (Figure 2) present in the supernantant samples compared to the cleaved NCCM1, which indicates there may be some aggregation of the cleaved NCCM1. Folded Opa proteins migrate at a lower apparent molecular weight than unfolded Opa proteins. As such, the two bands correspond to folded (lower band) and unfolded (upper band) protein in the Opa immunoblots. *Higher molecular weight bands in the Gc pellet samples of the CEACAM blots indicate nonspecific CEACAM antibody reactivity with antigens on the surface of Gc

FIGURE S9

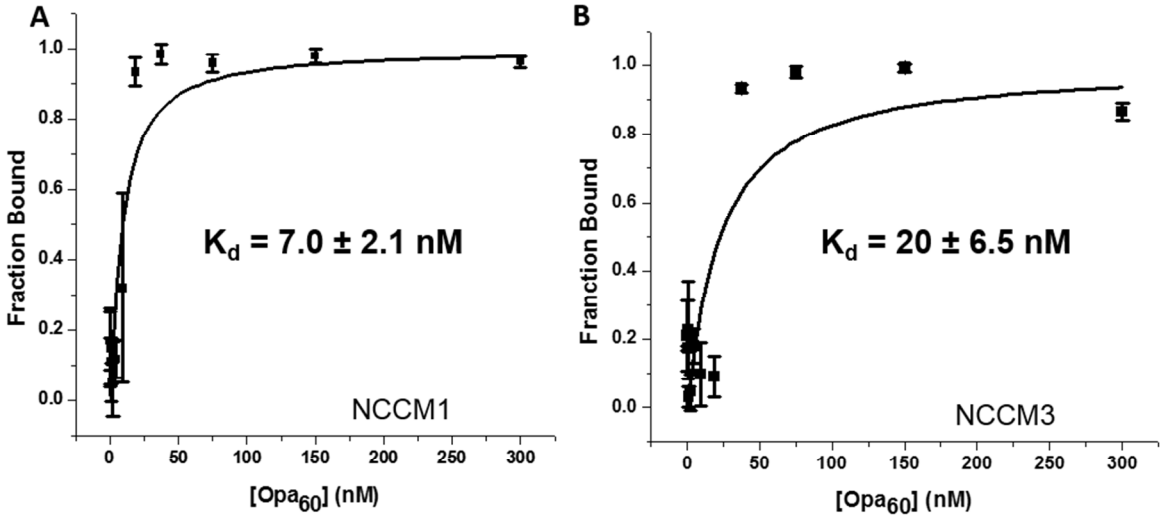


Figure S9. Competition FP assays with Opa_{60}. FP experiments were carried out as previously described with Opa_{60} proteoliposomes, incorporating half fl-labeled and half unlabeled NCCM1 (A) or NCCM3 (B). The total [NCCM] remained 5 nM. In both cases, the K_d values approximately doubled, indicating that the interaction is specific.