**Mapping sequential IgE-binding epitopes on major and minor egg allergens**

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# Supplementary Methods

## OVM and OVA epitope mapping

Several groups have attempted to quantify epitopes on OVM and OVA using in vitro enzymatic digestion assays [1-3], SPOT membranes [4-6], or peptide microarrays [7], with varying consistency across the results. Our goal was to create a comprehensive egg-epitope library that can be used with BBEA technology. The peptides were synthesized with 12 AA overlap and an estimated binding score at an individual AA level was calculated as a product of average nMFI and the proportion of subjects with positive signal (nMFI 2 standard deviations above the background signal). Neighboring AAs with a score above the median of all AAs constituted an epitope. Those epitopes were mapped to the conformational protein structure with PyMOL software (PDB 1OVA and modelled structure for OVM).

## OVM and sALB’s conformational structure reconstruction using homology modeling

Structure reconstruction of both OVM and sALB’s (available to download from https://github.com/msuprun/egg\_epitope\_mapping\_PDBfiles) was done using homology modeling via ModBase server (<https://modbase.compbio.ucsf.edu/>) with ModPipe [8]. Structure quality was assessed by Structural Analysis and Verification Server v5.0 (<https://servicesn.mbi.ucla.edu/SAVES/>) using ERRAT, PROCHECK, WHATCEHCK, and Verify 3D metrics [9]. The final OVM’s structure consisted of two models for the positions at AA 21-86 (template from Sus scrofa’s kazal inhibitor, PDB 1PCE, 38% sequence identity) and AA 104-210 (template from insect derived kazal inhibitor, PDB 1TBR, 35% sequence identity). sALB was modeled using the crystal structure of bovine serum albumin (PDB 4F5S, 44% sequence identity) as a template, with successful reconstruction of almost the whole protein (AA 29-611).

## Computational epitope predictions

We developed a predictor based on an ensemble of 12 B-cell epitope prediction tools (Table 1): BCIgEPred [10], CBTOPE [11], BCPred [12], ElliPro [13], DiscoTope [14], ABCpred [15], BepiPred 2.0 [16], and IEDB resources (<http://tools.iedb.org/main/bcell/>): Karplus-Schulz Flexibility, Parker Hydrophilicity [17], Emini Surface Accessibility [18], Chou-Fasman Beta-Turn [19], Kolaskar-Tongaonkar Antigenicity [20]. The prediction consisted of the following steps:

1. All 12 tools with their default settings were used to obtain AA-level predictions using an entire OVM and half of the OVA sequence for training, and the rest of the OVA for testing.
2. Random Forest algorithm (*caret* package v6.0) identified the best combination of those algorithms that predicted experimentally discovered OVM and OVA epitopes, and
3. this ensemble algorithm was used to detect epitopes on minor proteins, of which the top 5 OVT, 6 LYS, and 5 sALB candidates were synthesized. Additionally, 3 peptides for YGP42 protein were selected based on IEDB resources (due to the lack of structural templates); and for a newly identified allergen - YGP40 - 5 epitopes described by the Sogawa et al were included [21].
4. These peptides (Table E2) were evaluated for IgE binding from the same subjects used for the OVM and OVA epitope mapping.

## Egg epitope library

Several descriptive analyses were used to identify informative epitopes from OVM and OVA proteins, including unsupervised hierarchical clustering, with peptides having higher nMFI values grouped together. Epitopes with high variability across patients could be helpful when studying different allergy phenotypes. To assure such epitopes were included, additional selection kept peptides with a coefficient of variation (CV) >90% or extreme values (below 10th or above 90th percentile) of the 1st or 2nd components of of the principal component analysis (PCA). The final library consisted of 65 epitopes (Table E3).

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# Supplementary Tables

## **Table E1.** Sequence and structural details for allergenic egg proteins.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Protein** | **Protein Short Name** | **WHO/IUIS Allergen Name** | **UniProt ID** | **PDB ID** | **Amino Acid Sequence** |
| **Ovomucoid** | **OVM** | **Gal d 1** | P01005 | ModWeb\_Slow\_OVM21-86.11-01-2018.pdb AND ModWeb\_Slow\_OVM104-210.11-01-2018.pdb  <https://github.com/msuprun/egg_epitope_mapping_PDBfiles> | AEVDCSRFPNATDKEGKDVLVCNKDLRPICGTDGVTYTNDCLLCAYSIEFGTNISKEHDGECKETVPMNCSSYANTTSEDGKVMVLCNRAFNPVCGTDGVTYDNECLLCAHKVEQGASVDKRHDGGCRKELAAVSVDCSEYPKPDCTAEDRPLCGSDNKTYGNKCNFCNAVVESNGTLTLSHFGKC |
| **Ovalbumin** | **OVA** | **Gal d 2** | P01012 | 1OVA chain A | MGSIGAASMEFCFDVFKELKVHHANENIFYCPIAIMSALAMVYLGAKDSTRTQINKVVRFDKLPGFGDSIEAQCGTSVNVHSSLRDILNQITKPNDVYSFSLASRLYAEERYPILPEYLQCVKELYRGGLEPINFQTAADQARELINSWVESQTNGIIRNVLQPSSVDSQTAMVLVNAIVFKGLWEKAFKDEDTQAMPFRVTEQESKPVQMMYQIGLFRVASMASEKMKILELPFASGTMSMLVLLPDEVSGLEQLESIINFEKLTEWTSSNVMEERKIKVYLPRMKMEEKYNLTSVLMAMGITDVFSSSANLSGISSAESLKISQAVHAAHAEINEAGREVVGSAEAGVDAASVSEEFRADHPFLFCIKHIATNAVLFFGRCVSP |
| **Ovotransferrin** | **OVT** | **Gal d 3** | P02789 | 1N04 chain A | AAPPKSVIRWCTISSPEEKKCNNLRDLTQQERISLTCVQKATYLDCIKAIANNEADAISLDGGQAFEAGLAPYKLKPIAAEVYEHTEGSTTSYYAVAVVKKGTEFTVNDLQGKTSCHTGLGRSAGWNIPIGTLLHRGAIEWEGIESGSVEQAVAKFFSASCVPGATIEQKLCRQCKGDPKTKCARNAPYSGYSGAFHCLKDGKGDVAFVKHTTVNENAPDQKDEYELLCLDGSRQPVDNYKTCNWARVAAHAVVARDDNKVEDIWSFLSKAQSDFGVDTKSDFHLFGPPGKKDPVLKDLLFKDSAIMLKRVPSLMDSQLYLGFEYYSAIQSMRKDQLTPSPRENRIQWCAVGKDEKSKCDRWSVVSNGDVECTVVDETKDCIIKIMKGEADAVALDGGLVYTAGVCGLVPVMAERYDDESQCSKTDERPASYFAVAVARKDSNVNWNNLKGKKSCHTAVGRTAGWVIPMGLIHNRTGTCNFDEYFSEGCAPGSPPNSRLCQLCQGSGGIPPEKCVASSHEKYFGYTGALRCLVEKGDVAFIQHSTVEENTGGKNKADWAKNLQMDDFELLCTDGRRANVMDYRECNLAEVPTHAVVVRPEKANKIRDLLERQEKRFGVNGSEKSKFMMFESQNKDLLFKDLTKCLFKVREGTTYKEFLGDKFYTVISSLKTCNPSDILQMCSFLEGK |
| **Lysozyme** | **LYS** | **Gal d 4** | P00698 | 3B6L chain A | KVFGRCELAAAMKRHGLDNYRGYSLGNWVCAAKFESNFNTQATNRNTDGSTDYGILQINSRWWCNDGRTPGSRNLCNIPCSALLSSDITASVNCAKKIVSDGNGMNAWVAWRNRCKGTDVQAWIRGCRL |
| **Serum albumin** | **sALB** | **Gal d 5** | P19121 | ModWeb\_VerySlow\_2\_sALB29-611.11-07-2018.pdb  <https://github.com/msuprun/egg_epitope_mapping_PDBfiles> | FARDAEHKSEIAHRYNDLKEETFKAVAMITFAQYLQRCSYEGLSKLVKDVVDLAQKCVANEDAPECSKPLPSIILDEICQVEKLRDSYGAMADCCSKADPERNECFLSFKVSQPDFVQPYQRPASDVICQEYQDNRVSFLGHFIYSVARRHPFLYAPAILSFAVDFEHALQSCCKESDVGACLDTKEIVMREKAKGVSVKQQYFCGILKQFGDRVFQARQLIYLSQKYPKAPFSEVSKFVHDSIGVHKECCEGDMVECMDDMARMMSNLCSQQDVFSGKIKDCCEKPIVERSQCIMEAEFDEKPADLPSLVEKYIEDKEVCKSFEAGHDAFMAEFVYEYSRRHPEFSIQLIMRIAKGYESLLEKCCKTDNPAECYANAQEQLNQHIKETQDVVKTNCDLLHDHGEADFLKSILIRYTKKMPQVPTDLLLETGKKMTTIGTKCCQLGEDRRMACSEGYLSIVIHDTCRKQETTPINDNVSQCCSQLYANRRPCFTAMGVDTKYVPPPFNPDMFSFDEKLCSAPAEEREVGQMKLLINLIKRKPQMTEEQIKTIADGFTAMVDKCCKQSDINTCFGEEGANLIVQSRATLGIGA |
| **YGP40** | **YGP40** | See Sogawa et al. *Int Arch Allergy Immunol*. 2018 (reference #21 of Supplementary Materials file) | | | |
| **YGP42** | **YGP42** | **Gal d 6** | P87498 |  | PEIASQIAQEDQSTCEVSKGDFKTFDRMSFTCSFNKSCNVVVAQDCTEHPKFIITTRKVDHQSLSREVHINTSSANITICPAADSSLLVTCNKESVLSDSGVSEYEKDNIKIYKNGKTVIVEAPIHGLKNVNFDGEILKVTVASWMRGKTCGVCGNNDREKHNELLMPNHKLAHSCSAFVHSWVLLEETCSGGCKLQRRYVKLNRNPTIDGEESTCYSVDPVLKCMKDCTPIEKTSVKVGFHCFPKATAVSLLEWQRSSDKKSASEDVVESVDADIDCTCTGDCS |

## **Table E2. A**mino acid sequences of 24 top epitope candidates synthesized for testing with BBEA.

|  |  |
| --- | --- |
| **Protein** | **Sequence** |
| OVT\_1 | SSPEEKKCNNLRDLT |
| OVT\_2 | LRDLTQQERISLTCV |
| OVT\_3 | TIEQKLCRQCKGDPK |
| OVT\_4 | DQLTPSPRENRIQWC |
| OVT\_5 | VMDYRECNLAEVPTH |
| LYS\_1 | AAAMKRHGLDNYRGY |
| LYS\_2 | GYSLGNWVCAAKFES |
| LYS\_3 | RNTDGSTDYGILQIN |
| LYS\_4 | TDYGILQINSRWWCN |
| LYS\_5 | WCNDGRTPGSRNLCN |
| LYS\_6 | WVAWRNRCKGTDVQA |
| sALB\_1 | YQRPASDVICQEYQD |
| sALB\_2 | LEKCCKTDNPAECYA |
| sALB\_3 | KQETTPINDNVSQCC |
| sALB\_4 | GVDTKYVPPPFNPDM |
| sALB\_5 | MVDKCCKQSDINTCFGE |
| YGP42\_1 | VDHQSLSREVHINTS |
| YGP42\_2 | GVCGNNDREKHNELL |
| YGP42\_3 | SSDKKSASEDVVESV |
| YGP40\_1 | NYSMPANCYHILVQD |
| YGP40\_2 | VQDCSSELKFLVMMK |
| YGP40\_3 | CAKGCSATKTTPVTV |
| YGP40\_4 | CSATKTTPVTVGFHC |
| YGP40\_5 | FHCLPADSANSLTDK |

## **Table E3:** Amino acid sequences of 65 epitopes selected for the egg epitope library.

|  |  |  |
| --- | --- | --- |
| **Peptide** | **Protein** | **Sequence** |
| **OVM-001** | Ovomucoid | AEVDCSRFPNATDKE |
| **OVM-003** | Ovomucoid | RFPNATDKEGKDVLV |
| **OVM-004** | Ovomucoid | NATDKEGKDVLVCNK |
| **OVM-005** | Ovomucoid | DKEGKDVLVCNKDLR |
| **OVM-007** | Ovomucoid | VLVCNKDLRPICGTD |
| **OVM-008** | Ovomucoid | CNKDLRPICGTDGVT |
| **OVM-009** | Ovomucoid | DLRPICGTDGVTYTN |
| **OVM-010** | Ovomucoid | PICGTDGVTYTNDCL |
| **OVM-013** | Ovomucoid | YTNDCLLCAYSIEFG |
| **OVM-014** | Ovomucoid | DCLLCAYSIEFGTNI |
| **OVM-015** | Ovomucoid | LCAYSIEFGTNISKE |
| **OVM-018** | Ovomucoid | TNISKEHDGECKETV |
| **OVM-021** | Ovomucoid | ECKETVPMNCSSYAN |
| **OVM-023** | Ovomucoid | PMNCSSYANTTSEDG |
| **OVM-025** | Ovomucoid | YANTTSEDGKVMVLC |
| **OVM-027** | Ovomucoid | EDGKVMVLCNRAFNP |
| **OVM-028** | Ovomucoid | KVMVLCNRAFNPVCG |
| **OVM-031** | Ovomucoid | FNPVCGTDGVTYDNE |
| **OVM-032** | Ovomucoid | VCGTDGVTYDNECLL |
| **OVM-033** | Ovomucoid | TDGVTYDNECLLCAH |
| **OVM-036** | Ovomucoid | CLLCAHKVEQGASVD |
| **OVM-038** | Ovomucoid | KVEQGASVDKRHDGG |
| **OVM-040** | Ovomucoid | SVDKRHDGGCRKELA |
| **OVM-041** | Ovomucoid | KRHDGGCRKELAAVS |
| **OVM-042** | Ovomucoid | DGGCRKELAAVSVDC |
| **OVM-044** | Ovomucoid | ELAAVSVDCSEYPKP |
| **OVM-046** | Ovomucoid | VDCSEYPKPDCTAED |
| **OVM-048** | Ovomucoid | PKPDCTAEDRPLCGS |
| **OVM-050** | Ovomucoid | AEDRPLCGSDNKTYG |
| **OVM-052** | Ovomucoid | CGSDNKTYGNKCNFC |
| **OVM-054** | Ovomucoid | TYGNKCNFCNAVVES |
| **OVM-055** | Ovomucoid | NKCNFCNAVVESNGT |
| **OVM-056** | Ovomucoid | NFCNAVVESNGTLTL |
| **OVM-058** | Ovomucoid | VESNGTLTLSHFGKC |
| **OVA-001** | Ovalbumin | MGSIGAASMEFCFDV |
| **OVA-003** | Ovalbumin | ASMEFCFDVFKELKV |
| **OVA-006** | Ovalbumin | FKELKVHHANENIFY |
| **OVA-021** | Ovalbumin | DKLPGFGDSIEAQCG |
| **OVA-028** | Ovalbumin | SSLRDILNQITKPND |
| **OVA-040** | Ovalbumin | YLQCVKELYRGGLEP |
| **OVA-053** | Ovalbumin | IIRNVLQPSSVDSQT |
| **OVA-057** | Ovalbumin | SQTAXVLVNAIVFKG |
| **OVA-059** | Ovalbumin | LVNAIVFKGLWEKAF |
| **OVA-064** | Ovalbumin | KDEDTQAMPFRVTEQ |
| **OVA-068** | Ovalbumin | TEQESKPVQMMYQIG |
| **OVA-071** | Ovalbumin | MMYQIGLFRVASMAS |
| **OVA-075** | Ovalbumin | MASEKMKILELPFAS |
| **OVA-085** | Ovalbumin | LEQLESIINFEKLTE |
| **OVA-090** | Ovalbumin | WTSSNVMEERKIKVY |
| **OVA-095** | Ovalbumin | LPRMKMEEKYNLTSV |
| **OVA-108** | Ovalbumin | LKISQAVHAAHAEIN |
| **OVA-113** | Ovalbumin | EAGREVVGSAEAGVD |
| **OVA-120** | Ovalbumin | EFRADHPFLFCIKHI |
| **OVT\_1** | Ovotransferrin | SSPEEKKCNNLRDLT |
| **OVT\_3** | Ovotransferrin | TIEQKLCRQCKGDPK |
| **OVT\_5** | Ovotransferrin | VMDYRECNLAEVPTH |
| **LYS\_1** | Lysozyme | AAAMKRHGLDNYRGY |
| **LYS\_3** | Lysozyme | RNTDGSTDYGILQIN |
| **LYS\_6** | Lysozyme | WVAWRNRCKGTDVQA |
| **sALB\_1** | Serum albumin | LRDSYGAMADCCSKA |
| **sALB\_4** | Serum albumin | KQETTPINDNVSQCC |
| **sALB\_5** | Serum albumin | GVDTKYVPPPFNPDM |
| **YGP40\_1** | YGP40 | NYSMPANCYHILVQD |
| **YGP40\_5** | YGP40 | FHCLPADSANSLTDK |
| **YGP42\_1** | YGP42 | VDHQSLSREVHINTS |

# Supplementary Figures

## **Fig. E1.** Study schematic.



## **Fig. E2.** UnscalednMFI values ofIgE to all OVM (left) and OVA (right) peptides tested using BBEA in 38 egg allergic subjects and a negative pool (NP).



## **Fig. E3.** Review of the OVM and OVA epitope mapping literature.

OVM **(A)** and OVA **(B)** epitopes identified by groups using different technologies; bottom row represents epitopes mapped in the current study and correspond to Figure 1C,E.



## **Fig. E4.** Example of the OVM’s epitope selection for the 65-plex egg epitope library.

Peptides selected based on the unsupervised clustering (manhattan distance and mcquitty agglomeration algorithm) of column-scaled nMFI values **(A)** and extreme values of 1st and/or 2nd principal component (PC) **(B)**.



## **Fig. E5.** EMmean of the nMFI for each of the 65 ses-IgG4s in BER and BET children.

Colored bars represent significant difference from BER (red if higher and blue if lower than BER) based on FDR (red/dark blue) or p-value (pink/light blue). Stars atop of the bars represent significant difference from healthy subjects, based on the p-value.

