

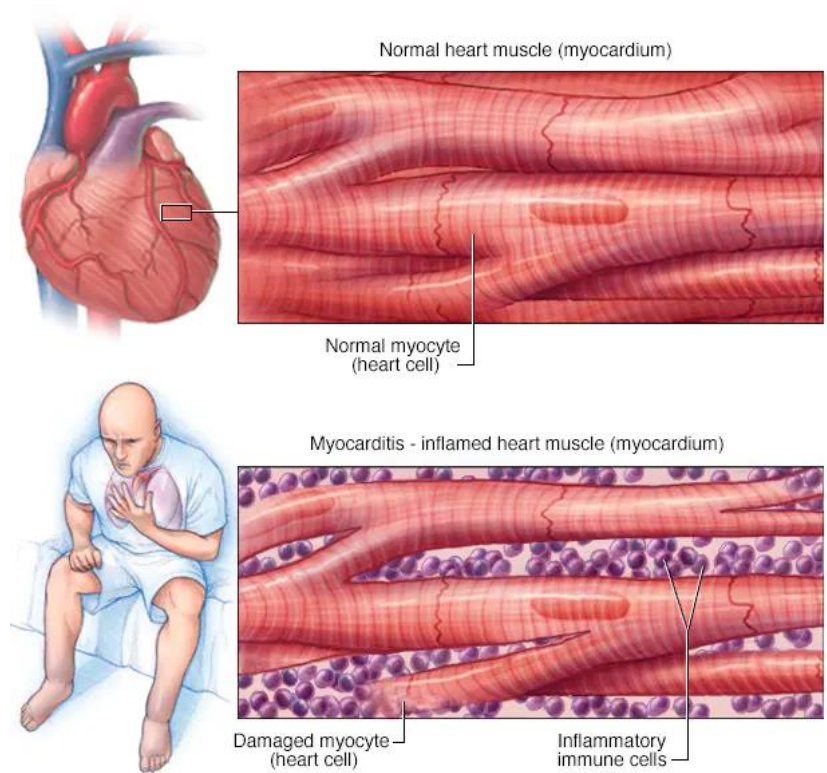


PEPMatch: Homology of SARS-CoV-2 Spike Sequences to Myocarditis Antigens

Presented by: Daniel Marrama, Bioinformatics Research Tech

What is myocarditis?

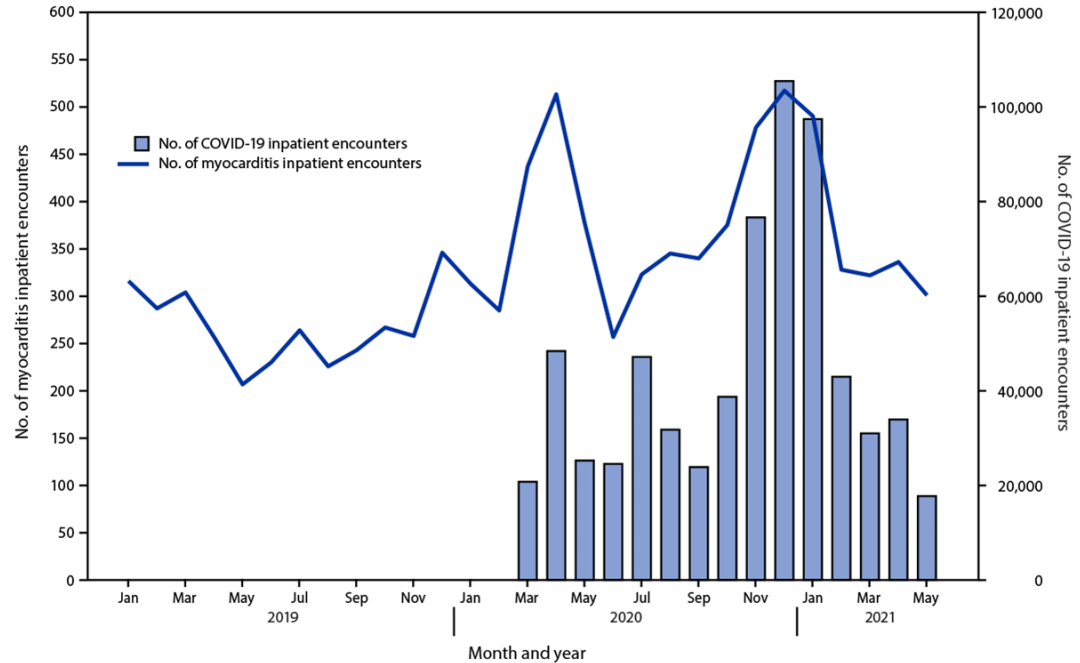
- Inflammatory condition affecting the heart muscle
- Causes shortness of breath, chest pain, and arrhythmias
- Complications can include cardiomyopathy or cardiac arrest
- Third most common cause of death for young males
- Typically follows a viral infection



Myocarditis. Mayo Clinic. <https://www.mayoclinic.org/diseases-conditions/myocarditis/multimedia/img-20456507>

Incidence of Myocarditis Associated with COVID-19 Infection

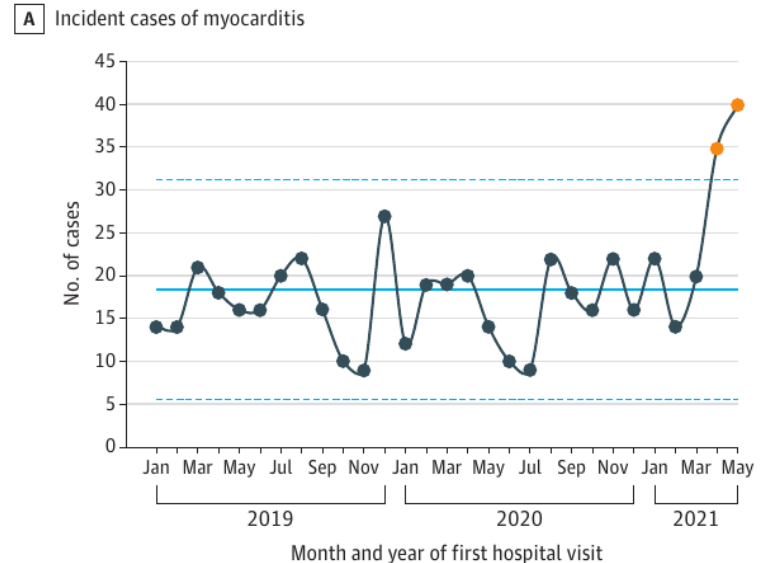
- CDC reported 42% increase of myocarditis between 2019 to 2020 after reviewing data from >900 hospitals
- 89.6% of patients with COVID-19 and myocarditis received both diagnoses within the same month



Incidence of Myocarditis after COVID-19 mRNA Vaccination

- In recent JAMA letter, data from 40 hospitals in the US revealed an increased incidence of myocarditis in early to mid 2021
- 20 patients had vaccine-related (onset within days of vaccination) myocarditis and 37 had pericarditis
 - Myocarditis: 11 received Moderna; 9 received Pfizer
 - Pericarditis: 12 Moderna, 23 Pfizer, and 2 J&J
 - Most myocarditis incidents occurred after 2nd dose (16/20)
 - Median onset was 3.5 days after vaccination and median discharge was 2 days

Figure. Monthly Number of Inpatient and Emergency Department Cases of Myocarditis and Pericarditis at 40 Hospitals in the Western US



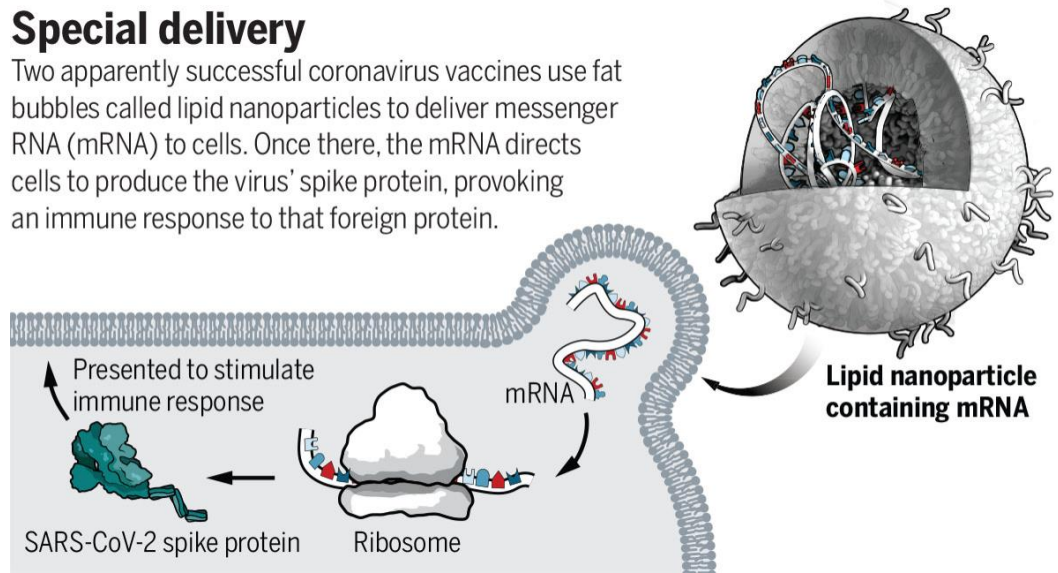
Diaz, George et al.
JAMA (2021)

mRNA COVID-19 Vaccines

- mRNA encoding for spike protein is delivered in nanoparticles to deltoid muscle cells
- This foreign protein is presented to immune cells for recognition to stimulate a response
- Only concerned with spike antigen for this analysis

Special delivery

Two apparently successful coronavirus vaccines use fat bubbles called lipid nanoparticles to deliver messenger RNA (mRNA) to cells. Once there, the mRNA directs cells to produce the virus' spike protein, provoking an immune response to that foreign protein.



















Wadman, Meredith *Science* (2020)

Are spike epitopes significantly homologous to myocarditis-associated antigens?

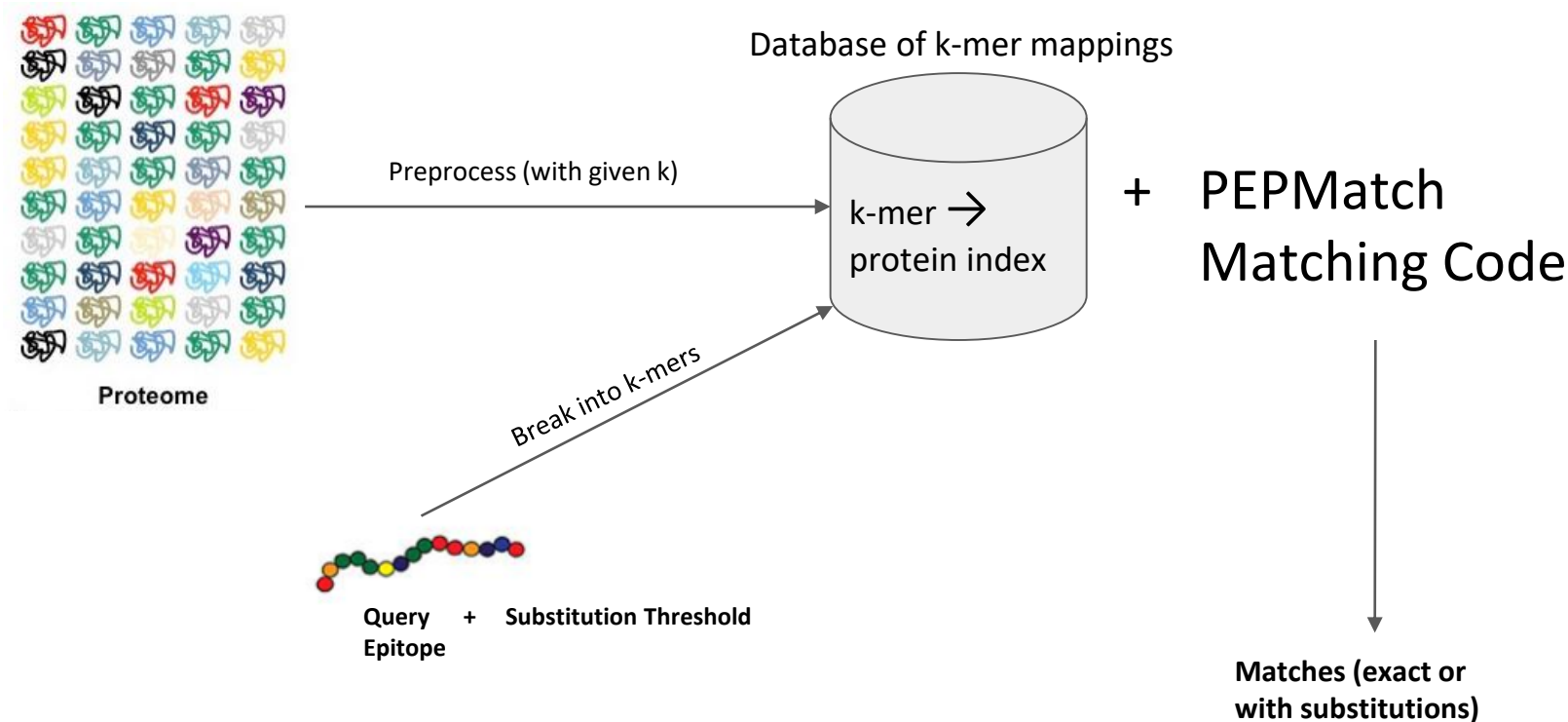
1. Collect list of antigens
 - a. From the IEDB and myocarditis literature
2. Search for peptide matches using PEPMatch within antigens
 - a. 9-mers for CD8+ T cells
 - b. 15-mers for CD4+ T cells
 - c. # of mismatches for cross-reactivity is still uncertain so we search down to ~50% homology
3. Shuffle spike peptides for control and repeat
4. Perform Fisher's exact test to determine if spike peptides are more likely to be found in these antigens as opposed to randomly shuffled peptides
5. Repeat 1-4 with 1,000 randomly selected protein sets of 35 proteins

Antigen Collection

- Search for human epitopes related to myocarditis in IEDB revealed 66 epitopes mapping back to 8 antigens
- Review literature on autoimmune myocarditis listed an additional 23 known associated antigens
- Authors noted several other suspected antigens bringing the total list to 35 cardiac proteins

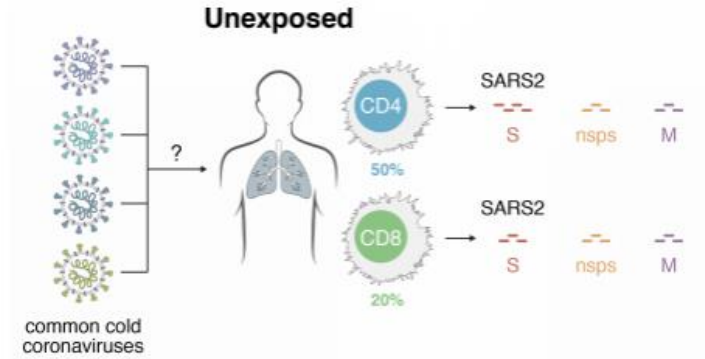
Epitopes (66)	Antigens (8)
8 Records Found	
Antigen 	
	Myosin-7  
	Myosin-6  
	Transmembrane protease serine 4 (UniProt:G3V124) 
	Muscarinic acetylcholine receptor M2  
	Myosin-binding protein C, cardiac-type (UniProt:Q14896)  
	Beta-2-glycoprotein 1  
	Myosin-binding protein C, fast-type  
	Laminin subunit alpha-1  
8 Records Found	

PEPMatch Tool Overview



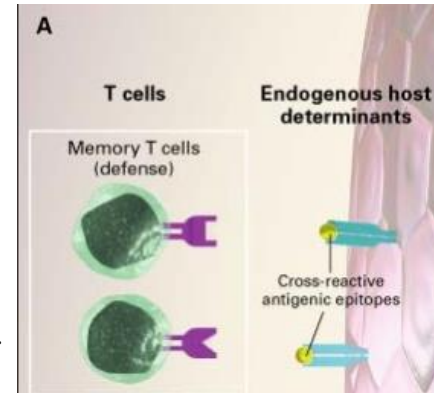
PEPMatch - Applications

Epitope Cross-Reactivity In Infection



In Autoimmunity

Grifoni, Alba et al. Cell (2020)



Albert, Lori et al. NEJM (1999)

Curation for IEDB

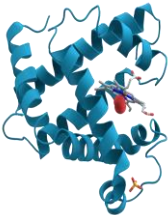
Reference Paper



Epitope



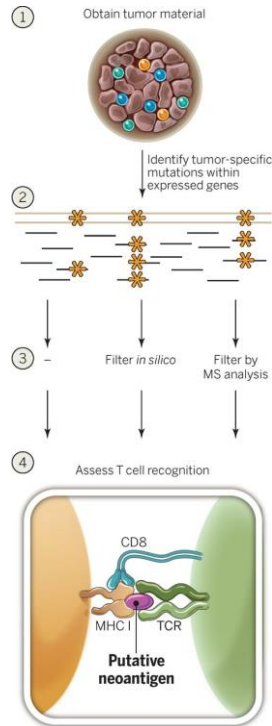
Sourced from a protein



In an organism



Neopeptide Similarity For Vaccine Candidates



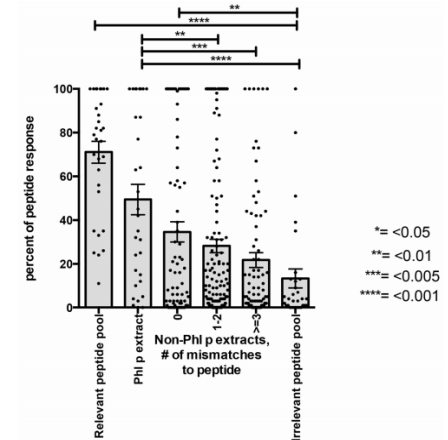
Schumacher TN, et al. Science. (2015)

Conservation Analyses using PEPMatch

- Milk allergens → conserved epitopes in human proteome might drive stronger reactions
- Fungi allergens → establish relationship of conservation and immunogenicity between two fungus species: *Alternaria alternata* and *Aspergillus fumigatus*
- Other infectious agents and their antigens conserved across many other pathogens
- Autoimmunity
 - COVID-19 infection → cross-reactive T cells causing immune thrombocytopenic purpura (ITP)?
 - **COVID-19 vaccination → autoimmune myocarditis caused by spike epitopes produced by mRNA vaccines?**

T-cell epitope conservation across allergen species is a major determinant of immunogenicity

Luise Westernberg¹, Véronique Schulten¹, Jason A Greenbaum¹, Sara Natali³, Victoria Tripple¹, Denise M. McKinney¹, April Frazier¹, Heidi Hofer², Michael Wallner², Federica Sallusto^{3,4}, Alessandro Sette¹, and Bjoern Peters¹



Westernberg, Luise et al. Journal of Allergy and Clinical Immunology (2016)

PEPMatch Search of Spike and Shuffled Peptides

- 1,265 9-mers with 4 mismatches searched in preprocessed cardiac proteins
 - Up to and including 4 mismatches = 56% homology
- 1,259 15-mers with 7 mismatches searched in preprocessed cardiac proteins
 - Up to and including 7 mismatches = 53% homology
- Repeated these searches with randomly shuffled peptides

```
from pepmatch import Matcher  
Matcher('native_spike_9mers.fasta', '9606', max_mismatches = 4, split = 2, output_format='csv').match()
```

Fisher's Exact Test: 9-mers

- Close, but not significant for spike homology at $\geq 67\%$
- No significance at $\geq 56\%$ or $\geq 78\%$
- No spike matches in cardiac proteins at $\geq 89\%$

SARS-CoV-2 Spike 9-mers vs. Shuffled 9-mers in Cardiac Proteins

Homology $\geq 56\%$

Fisher's Exact Test: Odds Ratio: 1.059, p-value: 0.526

	Homologous	Non-Homologous	Total
Spike Peptides	857	408	1265
Shuffled Peptides	841	424	1265
Total	1698	832	2530

SARS-CoV-2 Spike 9-mers vs. Shuffled 9-mers in Cardiac Proteins

Homology $\geq 67\%$

Fisher's Exact Test: Odds Ratio: 1.426, p-value: 0.06

	Homologous	Non-Homologous	Total
Spike Peptides	77	1188	1265
Shuffled Peptides	55	1210	1265
Total	132	2398	2530

SARS-CoV-2 Spike 9-mers vs. Shuffled 9-mers in Cardiac Proteins

Homology $\geq 78\%$

Fisher's Exact Test: Odds Ratio: 3.005, p-value: 0.625

	Homologous	Non-Homologous	Total
Spike Peptides	3	1262	1265
Shuffled Peptides	1	1264	1265
Total	4	2526	2530

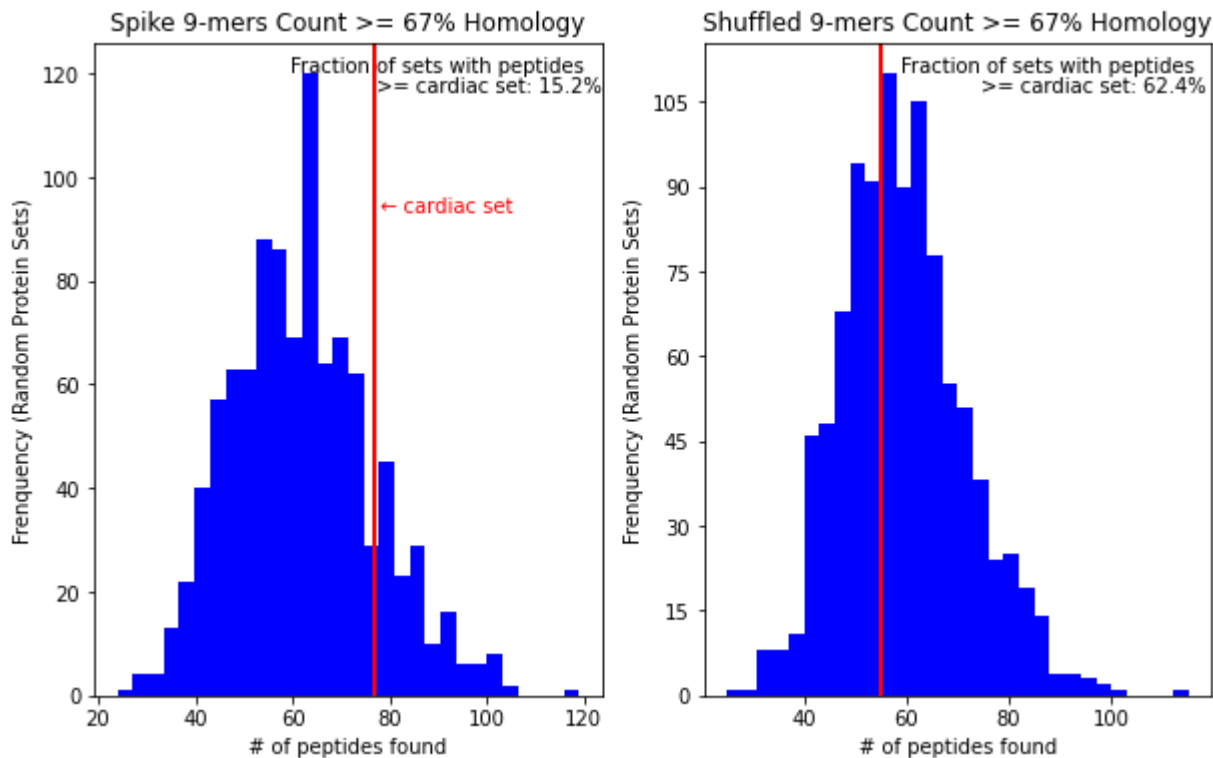
Fisher's Exact Test: 15-mers

- Spike peptides not significantly homologous in cardiac proteins at $\geq 53\%$ homology (or 7 mismatches)
- No spike peptide matches in cardiac proteins at 60% homology or more

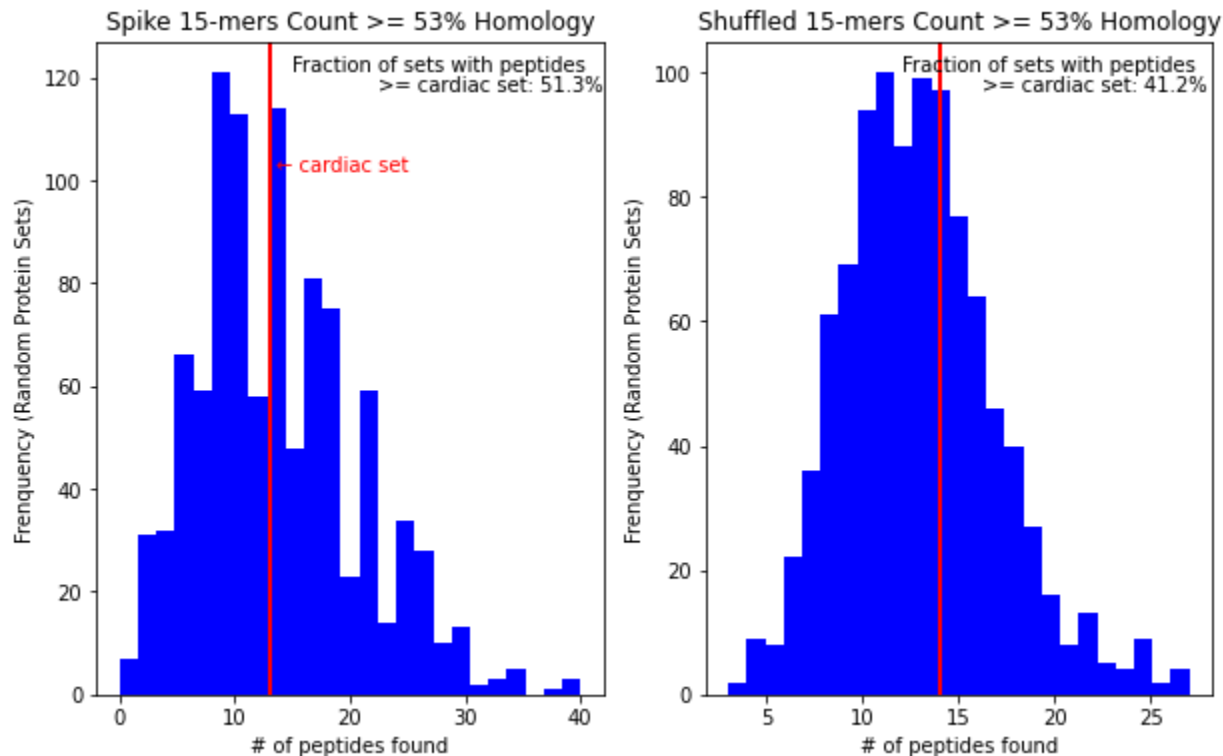
SARS-CoV-2 Spike 15-mers vs. Shuffled 15-mers in Cardiac Proteins
Homology $\geq 53\%$
Fisher's Exact Test: Odds Ratio: 0.928, p-value: 1.0

	Homologous	Non-Homologous	Total
Spike Peptides	13	1246	1259
Shuffled Peptides	14	1245	1259
Total	27	2491	2518

Distribution of Spike Matches in 1,000 Random Protein Sets



Distribution of Spike Matches in 1,000 Random Protein Sets



Conclusion and Caveats

- Spike peptides not significantly homologous within myocarditis-associated antigens compared with shuffled peptides as control at any homology level
 - For both CD4+ or CD8+ T cell epitopes
- Weak or no trend for spike peptides found in myocarditis-associated antigens compared with randomly select protein sets

- 3-D discontinuous B cell epitopes not taken into account
- Does not exclude that some individuals may still have a cross-reactive event - does HLA typing play a role?
- Most cases resolve quickly - is this more consistent with an innate immune response rather than adaptive immune response?
 - All myocarditis incidences from the JAMA paper found patients were discharged after a median of 2 days
 - None had previous history of autoimmune disease

Paper live on the IEDB



Help

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Analysis Resource

See our latest homology analysis of SARS-CoV-2 spike sequences to myocarditis-associated antigens [here](#) (updated 10/3).

Thank you
for listening!

Any questions?