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In Silico Prediction of B-Cell Epitopes in the Outer Membrane Proteins of *Leptospira interrogans*

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ABSTRACT

Leptospirosis is a zoonosis of global reach, and the proteins on the outer surface of its causative spirochaete, *Leptospira interrogans*, are the natural targets for serodiagnosis and for subunit vaccines. Predicting which parts of those proteins an antibody can recognise, before any peptide is made, is attractive because it promises to narrow a long list of candidates cheaply. This narrative review asks how far in silico prediction of B-cell epitopes can be trusted when it is applied to the outer membrane proteins of *L. interrogans*, and it reports no new predictions. It draws on published work on leptospiral surface biology, on the sequence-based and structure-based tools in common use, and on benchmarking studies of their accuracy. Well-studied antigens such as LipL32, the immunoglobulin-like Lig proteins and OmpL1 show how predicted regions can be set against experimental findings, but they also show the limits: a region can be antigenic without protecting an animal, and an abundant protein can prove dispensable for infection. Linear propensity scales perform only modestly, machine-learning predictors depend on their training sets, and structure-based tools depend on the quality of the model they are given. The review concludes that prediction is best used to rank and prioritise candidates by consensus, never to declare one, and it lists the experimental steps that any predicted epitope still has to survive.

Keywords: B-cell epitope; *Leptospira interrogans*; Outer membrane proteins; In silico prediction; Reverse vaccinology; Narrative review

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Article Class: Narrative Review

1. Introduction

Leptospirosis is one of the most widespread zoonoses, and it falls hardest on people who live and work close to animals, floodwater and poor sanitation (1,

2). The disease is caused by pathogenic spirochaetes of the genus *Leptospira*, of which *L. interrogans* is the species studied in most detail (3, 4). Diagnosis still leans on serology and culture, and licensed



vaccines are whole-cell preparations that protect mainly against the serovar they contain; a defined surface antigen would be cleaner on both counts (5).

This is where computation enters. Once the genome of *L. interrogans* had been sequenced (6), it became possible to list every predicted outer membrane protein and to ask which might make a good antigen, a strategy usually called reverse vaccinology (7, 8, 9). For *Leptospira* that exercise has been attempted (10). The finer question is which residues of a chosen protein an antibody will recognise, and the many tools that claim to answer it do not always agree.

The title of this article is a methods question, and the article treats it as one. No local data are reported and no new predictions are presented; instead the review sets out what is known about the leptospiral outer membrane, how B-cell epitope predictors work and how well they have performed when tested fairly, and what a sensible workflow looks like for *L. interrogans*. The aim is to be clear about what prediction can offer and what it cannot.

2. Literature Review

2.1 The Leptospiral Outer Membrane

Leptospira is not a typical Gram-negative bacterium. Its outer membrane carries lipopolysaccharide, but it also has features reminiscent of other spirochaetes, and its proteins are a mixture of lipoproteins anchored in the membrane and integral proteins that span it (11, 12). Only the parts of these proteins that are exposed on the cell surface can meet an antibody during infection, so any epitope study begins by deciding which proteins are really on the outside.

Proteomic work has helped with that decision. Fractionation of intact cells produced a set of candidates (13), and a

later approach combined computational prediction of membrane-spanning structure with tests of surface exposure (14). Not every protein predicted to lie on the surface behaved that way when tested, which is a useful warning for what follows.

2.2 Antigens With an Experimental Record

A few proteins have been studied well enough to serve as reference points, and they are summarised in Table 1. LipL32 is the abundant surface lipoprotein, expressed during mammalian infection and found in pathogenic species (15). Its crystal structure is known (16), and its carboxy-terminal region was reported to be the main immunogenic domain and to bind collagen and fibronectin (17). It has also served in serological tests (18). Yet a knockout mutant still caused disease in animal models, so abundance and immunogenicity did not make the protein essential (19).

The immunoglobulin-like Lig proteins tell a related story from a different angle. They carry repeated immunoglobulin-like domains, bind host matrix proteins, and are expressed more strongly under host-like osmotic conditions (20). Fragments of them protected hamsters (21, 22) and marked acute infection in patients (23). The integral protein OmpL1 gave better protection when combined with the lipoprotein LipL41 than either gave alone (24).

2.3 What an Epitope Is, and Why Prediction Is Hard

A B-cell epitope is the part of an antigen that an antibody contacts. Most real epitopes are discontinuous: residues far apart in the sequence come together on the folded surface (25). A linear peptide is therefore an approximation, and a good one only when the region is exposed, flexible and presented in a similar shape in



the intact protein.

Table 1. Outer membrane proteins of pathogenic *Leptospira* often discussed in epitope studies.

Protein	Kind	What the literature reports
LipL32	Surface lipoprotein	Abundant, expressed in infection (15); dispensable in a knockout (19)
LigA, LigB	Immunoglobulin-like repeats	Bind matrix proteins (20); protective in hamsters (21, 22)
OmpL1	Integral outer membrane protein	Protective together with LipL41 (24)
LipL41	Surface lipoprotein	Synergy with OmpL1 (24)
Other surface proteins	Mixed	Identified by proteomics (13, 14)

Note: Qualitative summary of the cited literature; findings from one serovar or model do not automatically transfer to others.

2.4 Families of Prediction Method

The earliest predictors were single-property scales. Hydrophilicity (26), surface accessibility (27) and combined antigenicity profiles (28) were all proposed to pick out surface regions. They are simple and fast, which is why they remain popular. Table 2 sets the main families side by side.

A systematic benchmark found that such scales performed little better than chance (29). Machine-learning predictors followed, trained on collections of known epitopes (30, 31), and structure-based tools were built to find surface patches on solved or modelled proteins (32, 33). A separate class, exemplified by VaxiJen, predicts whether a whole protein is likely to be a protective antigen and says nothing about where the epitope lies (34). Each family fails in its own way. Their scores cannot be compared directly.

Table 2. Families of B-cell epitope prediction and their usual weak points.

Approach	Input	Usual weak point
Propensity scales	Sequence only	Barely better than chance in benchmarks (29)
Machine learning (linear)	Sequence, trained on known epitopes	Depends on training and test data (30, 31)
Structure-based (conformational)	Solved or modelled structure	Only as good as the model (32, 33)
Antigen screening	Sequence features	Predicts antigens, not epitopes (34)

Note: Weak points are those discussed in the cited benchmarking literature, not attributed to any single tool.

3. Materials and Methods

3.1 Scope

This is a narrative review. It asks one question in two parts: what do published sources show about the reliability of in silico B-cell epitope prediction, and what do they imply for the outer membrane proteins of *L. interrogans*? It presents no predictions for any specific protein and uses no local dataset.

3.2 Literature Assembly

Sources were gathered from the leptospiral literature, from the bioinformatics literature on epitope and localisation prediction, and from benchmarking studies, with the emphasis on work published up to 2012. We did not run a formal systematic search, did not register a protocol and did not screen records in duplicate. The selection therefore reflects our own judgement and will have missed relevant papers.

3.3 Appraisal Framework

Each source was read for three things: whether the claim was tested against experimental data, whether the test set was independent of the training set, and



whether protection or only binding was reported. The framework is informal and no scores were calculated. None of these steps is hard, but each is easy to skip when time is short.

4. Results

4.1 From Genome to Candidate Proteins

A cautious workflow begins with the proteome rather than the epitope. Localisation predictors such as PSORTb are used to separate surface and outer membrane proteins from cytoplasmic ones (35). Signal peptide detection (36) and transmembrane topology prediction (37) refine the list. Sequence searches (38) then show which candidates are conserved among pathogenic serovars, because a region found in one strain only is of limited use. Figure 1 shows how these steps feed one another.

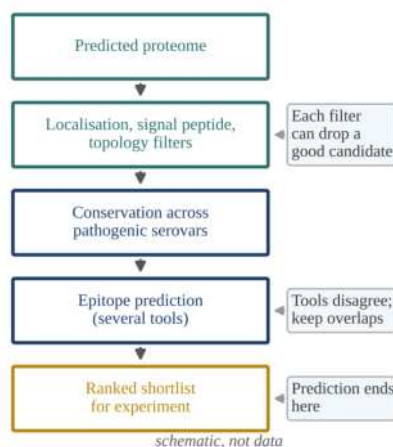


Figure 1. From genome to a ranked list of candidate regions (schematic, not data).

Each stage trims the list, but each also introduces error, and localisation tools trained on other bacteria may handle spirochaete lipoproteins less well (14). The output is a shortlist, not a verdict. A bench scientist reading such a list needs to know which entries deserve an expensive experiment. Read this way, a failed prediction is information rather than embarrassment.

4.2 Linear Epitope Prediction

For each shortlisted protein, several linear predictors can be run. The review found a consistent pattern in the literature: the tools agree on a few regions and disagree on many more. Benchmarks with independent test data report modest accuracy for every method, and better datasets have helped as much as better algorithms (39, 40). Such differences look small on paper, yet they decide which peptides are worth synthesising. This is why the same protein can look promising in one report and unremarkable in the next.

The practical consequence is that overlap between methods is more informative than any single high score. Figure 2 illustrates the idea with an invented sequence: where several independent tracks highlight the same stretch, that stretch earns attention, whereas a peak that appears on one track only deserves suspicion. The point is not that a tool is poor, only that its score travels less far than its output suggests.

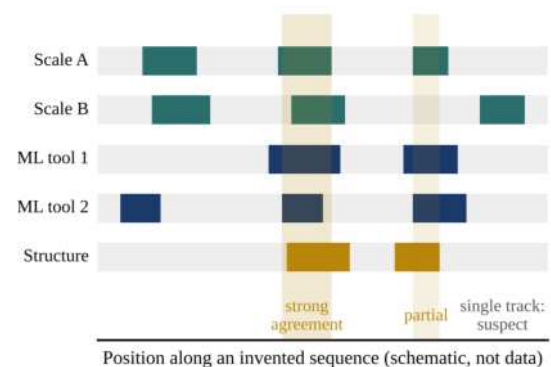


Figure 2. Agreement between predictors along a protein sequence (schematic, not data).

4.3 Conformational Epitopes and Structure Modelling

Structure-based tools require coordinates. For LipL32 these exist (16), but for most leptospiral outer membrane proteins they do not, and a homology model must stand in. SWISS-MODEL and similar servers produce usable models when a close template is available (41); with a distant



template the surface can be wrong in exactly the places that matter. A structure modelled from a distant template behaves differently from one that has been solved. ElliPro then searches the surface for protruding patches (33), and residue-level methods score single positions (32).

Integral outer membrane proteins add a further difficulty: only the extracellular loops are accessible, and a predictor that ignores the membrane can return epitopes buried in the bilayer. Table 3 collects the most common ways that predictions go astray and the checks that guard against them.

Table 3. Pitfalls in in silico epitope work and checks that address them.

Pitfall	Consequence	Check
Single tool used	Result reflects one method's bias (39)	Compare several tools
Modelled structure trusted	Surface patches are artefacts (41)	Report template and fit
Localisation assumed	Periplasmic protein enters the list (35)	Predict location first
Antigenic taken as protective	Wasted peptide work (19)	Test in an infection model
Strain variation ignored	Epitope absent elsewhere (38)	Align across serovars

Note: The checks are good practice drawn from the cited methodological literature, not a validated protocol.

4.4 From Prediction to Evidence

Prediction is only the first rung. The experimental record for *Leptospira* shows how far a protein can travel from a prediction to protection, and how often it stops short. LipL32 is the cautionary case: it is abundant, immunogenic and well characterised, yet it proved dispensable for infection (19). Antibody binding to a peptide, which is what most epitope experiments measure, says little about

whether that antibody can limit the infection of a living host.

Figure 3 arranges the rungs. A region that is predicted can be tested for binding to patient or immunised animal sera, then for antibody function, then for protection in an animal model such as the hamster (21, 22). Nothing in this reading argues against prediction; it argues for testing what is predicted.

5. Discussion

5.1 Interpretation

The central finding of this review is conditional. In silico prediction is a reasonable way to rank regions of a surface protein for follow-up, particularly when several independent methods agree and when the protein is already known to be surface-exposed. That is a modest claim, and it is the one the evidence will currently bear. It is not a way to declare an epitope. Performance on independent data is modest (29, 40), structure-based methods need good models (33, 41), and antigenicity does not imply protection (19). Reviews of this kind are more useful when they say where the evidence stops than when they smooth it over.



Fewer candidates reach each higher rung (schematic, not data)

Figure 3. Evidence ladder from prediction to protection (schematic, not data).

5.2 Limitations

This review is narrative and shares the weaknesses of the form. The sources were chosen by us, no formal search was run, and benchmark conclusions drawn from



other organisms may not carry over to spirochaete proteins. Software also changes quickly, and we report no experimental or predicted results of our own.

5.3 Future Work

Three needs stand out. First, benchmark sets built from leptospiral antigens, with the epitopes mapped by experiment and the test proteins held apart from the training data, would show how well generic tools perform on this genus. Second, outer membrane proteins would benefit from predictors that respect membrane topology. Third, a programme that carries a few shortlisted regions through all the rungs of [Figure 3](#), and reports failures as carefully as successes, would beat another list of candidates. It is a habit worth keeping even when the answer seems obvious.

6. Conclusion

Computational prediction of B-cell epitopes can shorten the list of regions worth testing in the outer membrane proteins of *L. interrogans*, and it costs little to run. The published evidence, however, shows that individual methods are only modestly accurate, that structure-based tools depend on the quality of the model, and that antigenic regions are not always protective. Used as a ranking device and followed by experiment, prediction is helpful. Used as an answer, it is not. These points apply to any surface antigen, not only to those of *Leptospira*.

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9. Conflict of Interest

The authors declare no conflict of interest.

10. Author Contributions

S.D. conceived the review and wrote the sections on leptospiral biology and antigens. K.S. wrote the sections on prediction methods and workflow. M.C. wrote the sections on structure modelling and evidence appraisal. All authors revised the manuscript and approved the final version.

11. Ethics Statement

This is a review of published literature. No human participants or animals were involved and no ethical approval was required.

12. Data Availability

No new data were generated. All sources are cited in the reference list.

13. References

1. Levett, P. N. (2001). Leptospirosis. *Clinical Microbiology Reviews*, 14(2), 296–326. DOI: [10.1128/CMR.14.2.296-326.2001](https://doi.org/10.1128/CMR.14.2.296-326.2001)
2. Bharti, A. R., Nally, J. E., Ricaldi, J. N., Matthias, M. A., Diaz, M. M., Lovett, M. A., ... Vinetz, J. M. (2003). Leptospirosis: A zoonotic disease of global importance. *The Lancet Infectious Diseases*, 3(12), 757–771. DOI: [10.1016/S1473-3099\(03\)00830-2](https://doi.org/10.1016/S1473-3099(03)00830-2)
3. Adler, B., & de la Peña Moctezuma, A. (2010). *Leptospira* and leptospirosis. *Veterinary Microbiology*, 140(3–4), 287–296. DOI: [10.1016/j.vetmic.2009.03.012](https://doi.org/10.1016/j.vetmic.2009.03.012)
4. Ko, A. I., Goarant, C., & Picardeau, M. (2009). *Leptospira*: The dawn of the molecular genetics era for an emerging zoonotic pathogen. *Nature Reviews Microbiology*, 7(10), 736–747. DOI: [10.1038/nrmicro2208](https://doi.org/10.1038/nrmicro2208)
5. Dellagostin, O. A., Grassmann, A. A., Hartwig, D. D., Félix, S. R., da Silva, É. F., & McBride, A. J. A. (2011). Recombinant vaccines against leptospirosis. *Human Vaccines*, 7(11), 1215–1224. DOI: [10.4161/hv.7.11.17944](https://doi.org/10.4161/hv.7.11.17944)
6. Ren, S. X., Fu, G., Jiang, X. G., Zeng, R., Miao, Y. G., Xu, H., ... Zhao, G. P. (2003). Unique physiological and pathogenic features of *Leptospira interrogans* revealed by whole-genome sequencing. *Nature*, 422(6934),



- 888–893. DOI: [10.1038/nature01597](https://doi.org/10.1038/nature01597)
7. Rappuoli, R. (2000). Reverse vaccinology. *Current Opinion in Microbiology*, 3(5), 445–450. DOI: [10.1016/S1369-5274\(00\)00119-3](https://doi.org/10.1016/S1369-5274(00)00119-3)
 8. Pizza, M., Scarlato, V., Masignani, V., Giuliani, M. M., Aricò, B., Comanducci, M., ... Rappuoli, R. (2000). Identification of vaccine candidates against serogroup B meningococcus by whole-genome sequencing. *Science*, 287(5459), 1816–1820. DOI: [10.1126/science.287.5459.1816](https://doi.org/10.1126/science.287.5459.1816)
 9. Sette, A., & Rappuoli, R. (2010). Reverse vaccinology: Developing vaccines in the era of genomics. *Immunity*, 33(4), 530–541. DOI: [10.1016/j.immuni.2010.09.017](https://doi.org/10.1016/j.immuni.2010.09.017)
 10. Gamberini, M., Gómez, R. M., Atzingen, M. V., Martins, E. A. L., Vasconcellos, S. A., Romero, E. C., ... Nascimento, A. L. T. O. (2005). Whole-genome analysis of *Leptospira interrogans* to identify potential vaccine candidates against leptospirosis. *FEMS Microbiology Letters*, 244(2), 305–313. DOI: [10.1016/j.femsle.2005.02.004](https://doi.org/10.1016/j.femsle.2005.02.004)
 11. Cullen, P. A., Haake, D. A., & Adler, B. (2004). Outer membrane proteins of pathogenic spirochetes. *FEMS Microbiology Reviews*, 28(3), 291–318. DOI: [10.1016/j.femsre.2003.10.004](https://doi.org/10.1016/j.femsre.2003.10.004)
 12. Haake, D. A., & Matsunaga, J. (2010). *Leptospira*: A spirochaete with a hybrid outer membrane. *Molecular Microbiology*, 77(4), 805–814. DOI: [10.1111/j.1365-2958.2010.07262.x](https://doi.org/10.1111/j.1365-2958.2010.07262.x)
 13. Cullen, P. A., Xu, X., Matsunaga, J., Sanchez, Y., Ko, A. I., Haake, D. A., & Adler, B. (2005). Surfaceome of *Leptospira* spp. *Infection and Immunity*, 73(8), 4853–4863. DOI: [10.1128/IAI.73.8.4853-4863.2005](https://doi.org/10.1128/IAI.73.8.4853-4863.2005)
 14. Pinne, M., & Haake, D. A. (2009). A comprehensive approach to identification of surface-exposed, outer membrane-spanning proteins of *Leptospira interrogans*. *PLoS ONE*, 4(6), e6071. DOI: [10.1371/journal.pone.0006071](https://doi.org/10.1371/journal.pone.0006071)
 15. Haake, D. A., Chao, G., Zuerner, R. L., Barnett, J. K., Barnett, D., Mazel, M., ... Bolin, C. A. (2000). The leptospiral major outer membrane protein LipL32 is a lipoprotein expressed during mammalian infection. *Infection and Immunity*, 68(4), 2276–2285. DOI: [10.1128/IAI.68.4.2276-2285.2000](https://doi.org/10.1128/IAI.68.4.2276-2285.2000)
 16. Vivian, J. P., Beddoe, T., McAlister, A. D., Wilce, M. C. J., Zaker-Tabrizi, L., Troy, S., ... Rossjohn, J. (2009). Crystal structure of LipL32, the most abundant surface protein of pathogenic *Leptospira* spp. *Journal of Molecular Biology*, 387(5), 1229–1238. DOI: [10.1016/j.jmb.2009.02.049](https://doi.org/10.1016/j.jmb.2009.02.049)
 17. Hauk, P., Macedo, F., Romero, E. C., Vasconcellos, S. A., de Moraes, Z. M., Barbosa, A. S., & Ho, P. L. (2008). In LipL32, the major leptospiral lipoprotein, the C terminus is the primary immunogenic domain and mediates interaction with collagen IV and plasma fibronectin. *Infection and Immunity*, 76(6), 2642–2650. DOI: [10.1128/IAI.01639-07](https://doi.org/10.1128/IAI.01639-07)
 18. Flannery, B., Costa, D., Carvalho, F. P., Guerreiro, H., Matsunaga, J., Da Silva, E. D., ... Ko, A. I. (2001). Evaluation of recombinant *Leptospira* antigen-based enzyme-linked immunosorbent assays for the serodiagnosis of leptospirosis. *Journal of Clinical Microbiology*, 39(9), 3303–3310. DOI: [10.1128/JCM.39.9.3303-3310.2001](https://doi.org/10.1128/JCM.39.9.3303-3310.2001)
 19. Murray, G. L., Srikram, A., Hoke, D. E., Wunder, E. A., Henry, R., Lo, M., ... Adler, B. (2009). Major surface protein LipL32 is not required for either acute or chronic infection with *Leptospira interrogans*. *Infection and Immunity*, 77(3), 952–958. DOI: [10.1128/IAI.01370-08](https://doi.org/10.1128/IAI.01370-08)
 20. Choy, H. A., Kelley, M. M., Chen, T. L., Møller, A. K., Matsunaga, J., & Haake, D. A. (2007). Physiological osmotic induction of *Leptospira interrogans* adhesion: LigA and LigB bind extracellular matrix proteins and fibrinogen. *Infection and Immunity*, 75(5), 2441–2450. DOI: [10.1128/IAI.01635-06](https://doi.org/10.1128/IAI.01635-06)
 21. Koizumi, N., & Watanabe, H. (2004). Leptospiral immunoglobulin-like proteins elicit protective immunity. *Vaccine*, 22(11–12), 1545–1552. DOI: [10.1016/j.vaccine.2003.10.007](https://doi.org/10.1016/j.vaccine.2003.10.007)
 22. Silva, E. F., Medeiros, M. A., McBride, A. J. A., Matsunaga, J., Esteves, G. S., Ramos, J. G. R., ... Ko, A. I. (2007). The terminal portion of leptospiral immunoglobulin-like protein LigA confers protective immunity against lethal infection in the hamster model of leptospirosis. *Vaccine*, 25(33), 6277–6286. DOI: [10.1016/j.vaccine.2007.05.053](https://doi.org/10.1016/j.vaccine.2007.05.053)
 23. Croda, J., Ramos, J. G. R., Matsunaga, J., Queiroz, A., Homma, A., Riley, L. W., ... Ko, A. I. (2007). *Leptospira* immunoglobulin-like proteins as a serodiagnostic marker for acute leptospirosis. *Journal of Clinical Microbiology*, 45(5), 1528–1534. DOI: [10.1128/JCM.02344-06](https://doi.org/10.1128/JCM.02344-06)
 24. Haake, D. A., Mazel, M. K., McCoy, A. M., Milward, F., Chao, G., Matsunaga, J., & Wagar, E. A. (1999). Leptospiral outer membrane proteins OmpL1 and LipL41 exhibit synergistic immunoprotection. *Infection and Immunity*, 67(12), 6572–6582. DOI: [10.1128/IAI.67.12.6572-6582.1999](https://doi.org/10.1128/IAI.67.12.6572-6582.1999)
 25. Barlow, D. J., Edwards, M. S., & Thornton, J. M. (1986). Continuous and discontinuous protein antigenic determinants. *Nature*, 322(6081), 747–749. DOI: [10.1038/322747a0](https://doi.org/10.1038/322747a0)
 26. Hopp, T. P., & Woods, K. R. (1981). Prediction of protein antigenic determinants from amino acid sequences. *Proceedings of the National Academy of Sciences*, 78(6), 3824–3828. DOI: [10.1073/pnas.78.6.3824](https://doi.org/10.1073/pnas.78.6.3824)



- [10.1073/pnas.78.6.3824](https://doi.org/10.1073/pnas.78.6.3824)
27. Emini, E. A., Hughes, J. V., Perlow, D. S., & Boger, J. (1985). Induction of hepatitis A virus-neutralizing antibody by a virus-specific synthetic peptide. *Journal of Virology*, 55(3), 836–839. DOI: [10.1128/JVI.55.3.836-839.1985](https://doi.org/10.1128/JVI.55.3.836-839.1985)
 28. Kolaskar, A. S., & Tongaonkar, P. C. (1990). A semi-empirical method for prediction of antigenic determinants on protein antigens. *FEBS Letters*, 276(1–2), 172–174. DOI: [10.1016/0014-5793\(90\)80535-Q](https://doi.org/10.1016/0014-5793(90)80535-Q)
 29. Blythe, M. J., & Flower, D. R. (2005). Benchmarking B cell epitope prediction: Underperformance of existing methods. *Protein Science*, 14(1), 246–248. DOI: [10.1110/ps.041059505](https://doi.org/10.1110/ps.041059505)
 30. Larsen, J. E. P., Lund, O., & Nielsen, M. (2006). Improved method for predicting linear B-cell epitopes. *Immunome Research*, 2, 2. DOI: [10.1186/1745-7580-2-2](https://doi.org/10.1186/1745-7580-2-2)
 31. Saha, S., & Raghava, G. P. S. (2006). Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. *Proteins*, 65(1), 40–48. DOI: [10.1002/prot.21078](https://doi.org/10.1002/prot.21078)
 32. Haste Andersen, P., Nielsen, M., & Lund, O. (2006). Prediction of residues in discontinuous B-cell epitopes using protein 3D structures. *Protein Science*, 15(11), 2558–2567. DOI: [10.1110/ps.062405906](https://doi.org/10.1110/ps.062405906)
 33. Ponomarenko, J., Bui, H. H., Li, W., Fusseder, N., Bourne, P. E., Sette, A., & Peters, B. (2008). ElliPro: A new structure-based tool for the prediction of antibody epitopes. *BMC Bioinformatics*, 9, 514. DOI: [10.1186/1471-2105-9-514](https://doi.org/10.1186/1471-2105-9-514)
 34. Doytchinova, I. A., & Flower, D. R. (2007). VaxiJen: A server for prediction of protective antigens, tumour antigens and subunit vaccines. *BMC Bioinformatics*, 8, 4. DOI: [10.1186/1471-2105-8-4](https://doi.org/10.1186/1471-2105-8-4)
 35. Yu, N. Y., Wagner, J. R., Laird, M. R., Melli, G., Rey, S., Lo, R., ... Brinkman, F. S. L. (2010). PSORTb 3.0: Improved protein subcellular localization prediction with refined localization subcategories and predictive capabilities for all prokaryotes. *Bioinformatics*, 26(13), 1608–1615. DOI: [10.1093/bioinformatics/btq249](https://doi.org/10.1093/bioinformatics/btq249)
 36. Petersen, T. N., Brunak, S., von Heijne, G., & Nielsen, H. (2011). SignalP 4.0: Discriminating signal peptides from transmembrane regions. *Nature Methods*, 8(10), 785–786. DOI: [10.1038/nmeth.1701](https://doi.org/10.1038/nmeth.1701)
 37. Krogh, A., Larsson, B., von Heijne, G., & Sonnhammer, E. L. L. (2001). Predicting transmembrane protein topology with a hidden Markov model: Application to complete genomes. *Journal of Molecular Biology*, 305(3), 567–580. DOI: [10.1006/jmbi.2000.4315](https://doi.org/10.1006/jmbi.2000.4315)
 38. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, 215(3), 403–410. DOI: [10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
 39. Greenbaum, J. A., Andersen, P. H., Blythe, M., Bui, H. H., Cachau, R. E., Crowe, J., ... Peters, B. (2007). Towards a consensus on datasets and evaluation metrics for developing B-cell epitope prediction tools. *Journal of Molecular Recognition*, 20(2), 75–82. DOI: [10.1002/jmr.815](https://doi.org/10.1002/jmr.815)
 40. Kringelum, J. V., Lundegaard, C., Lund, O., & Nielsen, M. (2012). Reliable B cell epitope predictions: Impacts of method development and improved benchmarking. *PLoS Computational Biology*, 8(12), e1002829. DOI: [10.1371/journal.pcbi.1002829](https://doi.org/10.1371/journal.pcbi.1002829)
 41. Arnold, K., Bordoli, L., Kopp, J., & Schwede, T. (2006). The SWISS-MODEL workspace: A web-based environment for protein structure homology modelling. *Bioinformatics*, 22(2), 195–201. DOI: [10.1093/bioinformatics/bti770](https://doi.org/10.1093/bioinformatics/bti770)