

# The *Neisseria gonorrhoeae* methionine sulfoxide reductase (MsrA/B) is a surface exposed, immunogenic, vaccine candidate

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## Supplementary data

**Table S1. Primers used in this study.**

Primer name	Primer sequence 5'-3' *
1291msrFor	GCCGTCTGAAATGAAACACCGTACTTTCTTTTCCC
1291msrRev	TTCAGACGGCTTATTTCACTTTGCCCTTCAACGCG
msrexp_NdeIF	AAAATCCATATGAAAGGGACCGCGACCGTGCCGCA
msrexp_XhoIR	CCCTGACTCGAGTTATTTCACTTTGCCCTTC
pCTS32_Msr_AflIIFor	CTCGAGCTTAAGCCGGCGTTTCCTGTTTTTTC
pCTS32_Msr_SmaIRev	TGCGGCCCGGGTTATTTCACTTTGCCCTTCAACG
pComP_Msr_NdeIFor	ATCAAACATATGATGAAACACCGTACTTTCTTTTCC
pComP_Msr_NsiIRev	TGCGGCATGCATTTATTTCACTTTGCCCTTCAACG

**Table S2. Data for individual and pooled mice sera immunised with MsrA/B-Alum.**

<b>MsrA/B-Alum</b>	<b>ELISA titre vs MsrA/B</b>					<b>ELISA titre vs whole cells<sup>^</sup></b>			<b>SBA titre</b>		<b>OPA titre</b>	
<b>Mouse</b>	<b>IgG1</b>	<b>IgG2a</b>	<b>IgG2b</b>	<b>IgG3</b>	<b>IgM</b>	<b>WT</b>	<b>Δmsr</b>	<b>Δmsr_C</b>	<b>Pre</b>	<b>Post</b>	<b>Pre</b>	<b>Post</b>
<b>1</b>	655,360	200	800	200	12,800	128,000	2,000	512,000	-	-	-	-
<b>2</b>	2,621,440	200	3,200	200	51,200	256,000	2,000	512,000	-	-	-	-
<b>3</b>	1,310,720	200	800	200	6,400	64,000	2,000	256,000	-	-	-	-
<b>4</b>	1,310,720	200	1,600	200	6,400	512,000	2,000	1,024,000	-	-	-	-
<b>5</b>	655,360	200	1,600	200	3,200	56,000	2,000	512,000	-	-	-	-
<b>6</b>	1,310,720	200	200	200	3,200	128,000	2,000	512,000	-	-	-	-
<b>7</b>	1,310,720	200	800	200	3,200	128,000	2,000	256,000	-	-	-	-
<b>8</b>	1,310,720	200	6,400	200	12,800	512,000	2,000	1,024,000	-	-	-	-
<b>9</b>	655,360	200	400	200	51,200	128,000	2,000	512,000	-	-	-	-
<b>10</b>	2,621,440	200	200	200	3,200	128,000	2,000	512,000	-	-	-	-
<b>GMT</b>	1,222,945	200	919	200	8,445	155,496	2,000	512,000	-	-	-	-
<b>pool</b>									<50	<50	<50	<50

SBA titre; serum bactericidal titre (reciprocal of the lowest antibody dilution which induced more than 50% killing after 60 min). OPA titre, opsonophagocytic titre (reciprocal of the lowest antibody dilution which induced more than 50% killing after 90 min). GMT, geometric mean titre; -, not determined. <sup>^</sup> The titres of pre-immune sera against whole cell *N. gonorrhoeae* 1291 strains were  $\leq 200$ .

**Table S3. Data for individual and pooled mice sera immunised with MsrA/B-Freund's.**

<b>MsrA/B-Freund's</b>	<b>ELISA titre vs MsrA/B</b>					<b>ELISA titre vs whole cells<sup>^</sup></b>			<b>SBA titre*</b>		<b>OPA titre*</b>		<b>MsrA/B-Me(O) binding inhibition</b>	
<b>Mouse</b>	<b>IgG1</b>	<b>IgG2a</b>	<b>IgG2b</b>	<b>IgG3</b>	<b>IgM</b>	<b>WT</b>	<b>Δmsr</b>	<b>Δmsr_C</b>	<b>Pre</b>	<b>Post</b>	<b>Pre</b>	<b>Post</b>	<b>Pre</b>	<b>Post</b>
<b>1</b>	10,240,000	400	51,200	25,600	12,800	320,000	2,000	640,000	<50	50	<100	200	20.5%	41.9%
<b>2</b>	5,120,000	800	25,600	6,400	12,800	320,000	2,000	640,000	<50	50	100	200	4.6%	62.5%
<b>3</b>	10,240,000	400	25,600	6,400	6,400	320,000	2,000	640,000	<50	100	<100	200	5.7%	99.7%
<b>4</b>	10,240,000	800	25,600	12,800	12,800	320,000	2,000	640,000	<50	50	<100	200	9.0%	65.7%
<b>5</b>	10,240,000	800	12,800	6,400	6,400	80,000	2,000	160,000	<50	<50	<100	<100	6.2%	12.0%
<b>6</b>	20,480,000	400	12,800	1,600	6,400	40,000	2,000	160,000	50	200	100	800	3.2%	49.0%
<b>7</b>	10,240,000	200	12,800	6,400	12,800	160,000	2,000	320,000	<50	<50	<100	100	1.1%	54.1%
<b>8</b>	10,240,000	400	25,600	12,800	6,400	40,000	2,000	80,000	50	100	100	400	6.5%	54.7%
<b>9</b>	5,120,000	200	12,800	800	12,800	640,000	2,000	1,280,000	50	200	100	400	17.2%	64.9%
<b>10</b>	5,120,000	400	6,400	400	6,400	320,000	2,000	640,000	<50	50	<100	100	0.9%	55.9%
<b>GMT</b>	8914438	429	18102	4525	9051	246754	2000	393966	50	84	55	200		
<b>Mean</b>													7.5%	56.0%
<b>pool</b>									<100	100	<100	400	18.5%	67.9%

SBA titre; serum bactericidal titre (reciprocal of the lowest antibody dilution which induced more than 50% killing after 60 min). OPA titre, opsonophagocytic titre (reciprocal of the lowest antibody dilution which induced more than 50% killing after 90 min). GMT, geometric mean titre.

<sup>^</sup> The titres of pre-immune sera against whole cell *N. gonorrhoeae* 1291 strains was ≤200. \* When a final titre was not reached (i.e., <50 or <100) a value of the next 2-fold dilution (i.e., 25 or 50, respectively) was used to calculate the GMT.

D0322	EYAFSREYIDRLFKPGITVDVVSQEPFLSSADKTDGGCGWSPFTRPTDAKSVEYERDQPSFNMRHTEVRSHAADSRGLGHVPDGPDRDKAGGLRTCTINGASLKFIPLEQMADAAGTGALKGKGKVK	See
WHO_K	.	.
I291	.	.
MS-11	.	E
FA1090	.	.
75/i-12	.	.
SRR3348270	. Y . H .	S
999000027	.	.
316739890000441	.	E
08_590_00131	.	.
11CFX_C_051	K .	.
SRR3360766	.	.
316591EST_12_23	.	.
05AZI_T_001	.	.
SRR3348334	.	E
11CFX_C_043	.	E
315121EST_12_26	.	T
590_105	R .	H.
12CFX_C_026	.	.
31621IND_06_3583	.	.
SRR3336051	.	.
08_590_00144	.	E
SRR3360328	K .	.
MJA_2011_03-09	.	.
07AZI_T_019	.	.
D0347	.	E
SRR3360911	.	.
SRR3360841	.	E
08_590_48	.	.
SRR3350164	.	.
SRR3357142	.	.
Ngs1_55	.	.
13AZI_T_005	.	.
D0GK13	.	.
999000083	.	E
SRR3361355	.	.
Nm MC58	. Y . H .	.

**Figure S1. Conservation of gonococcal MsrA/B.**

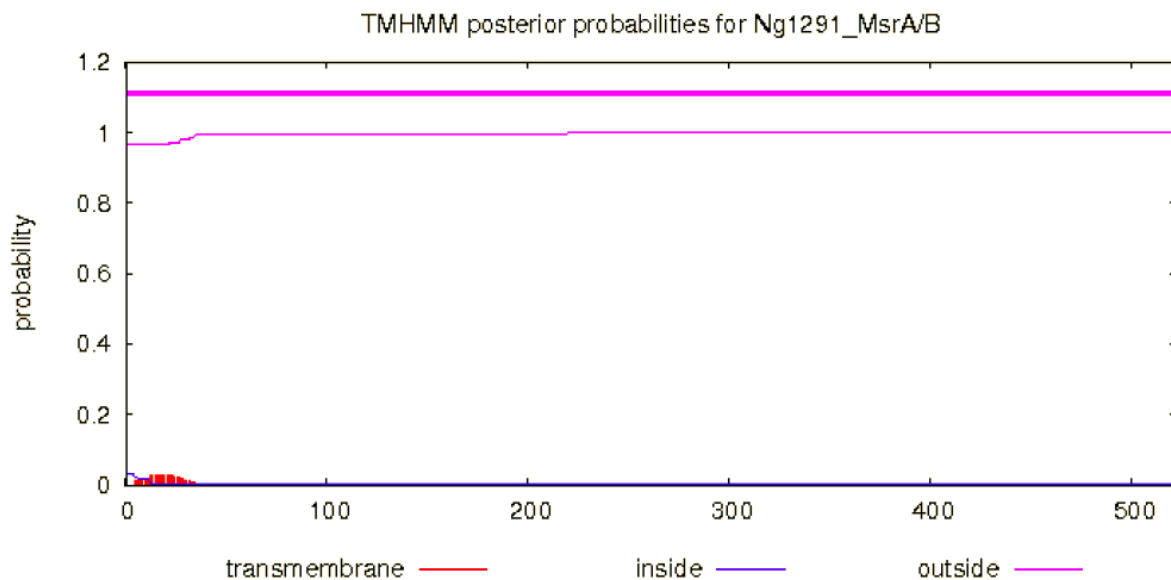
An alignment of the 35 unique MsrA/B variants of *N. gonorrhoeae* is shown, with the consensus sequence at the top. Matches to the consensus sequence are indicated by dots. A representative strain for each variant is shown and the number of strains with that variant is given in brackets. The *N. meningitidis* MC58 MsrA/B sequence is also included. The predicted signal peptide is shown, and \* indicate catalytic residues identified in *N. meningitidis* MsrA/B.

## TMHMM result

[HELP](#) with output formats

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```
# Ng1291_MsrA/B Length: 522
# Ng1291_MsrA/B Number of predicted TMHs: 0
# Ng1291_MsrA/B Exp number of AAs in TMHs: 0.651070000000000001
# Ng1291_MsrA/B Exp number, first 60 AAs: 0.61189
# Ng1291_MsrA/B Total prob of N-in: 0.03201
Ng1291_MsrA/B TMHMM2.0 outside 1 522
```

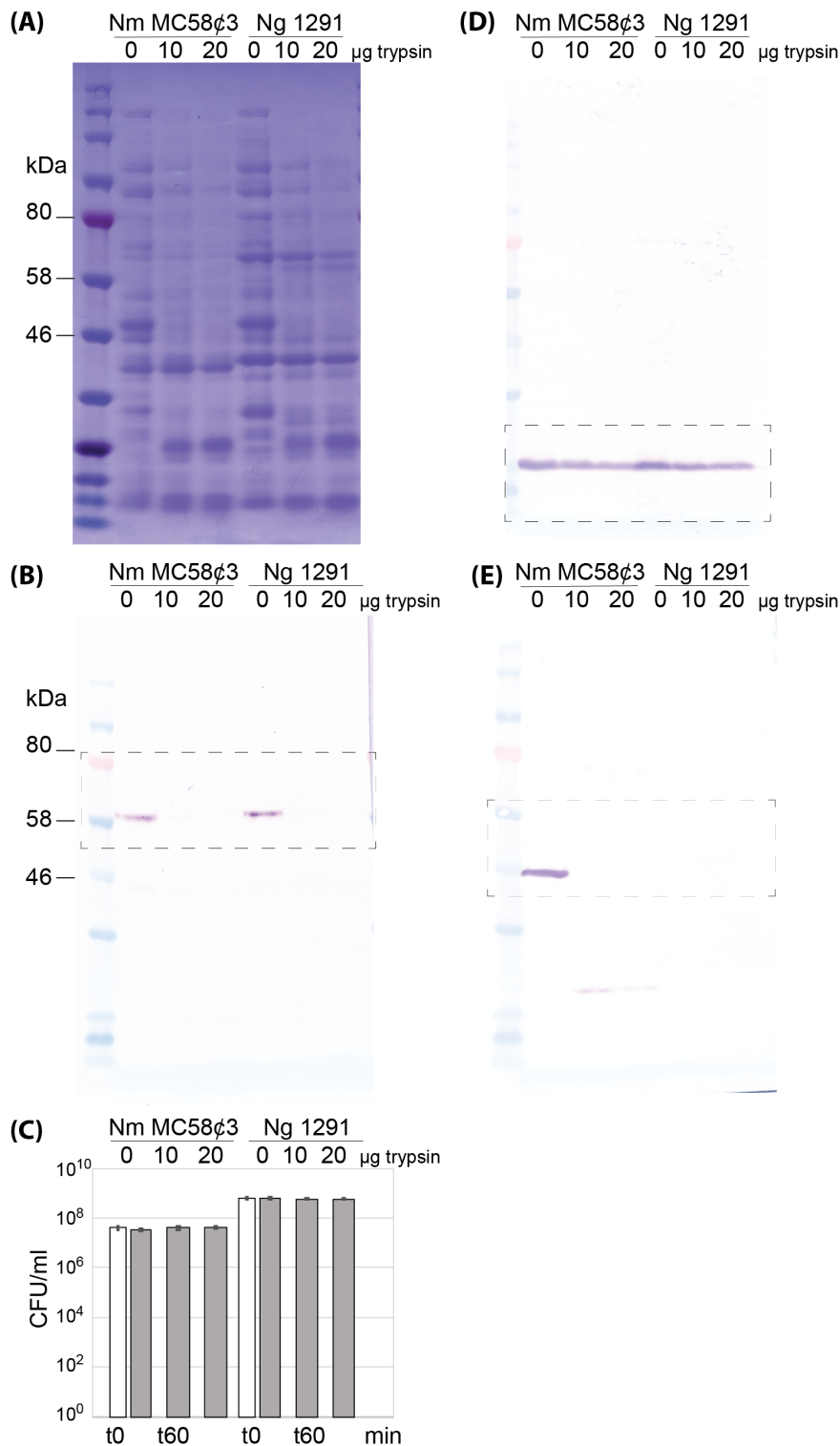


# [plot](#) in postscript, [script](#) for making the plot in gnuplot, [data](#) for plot

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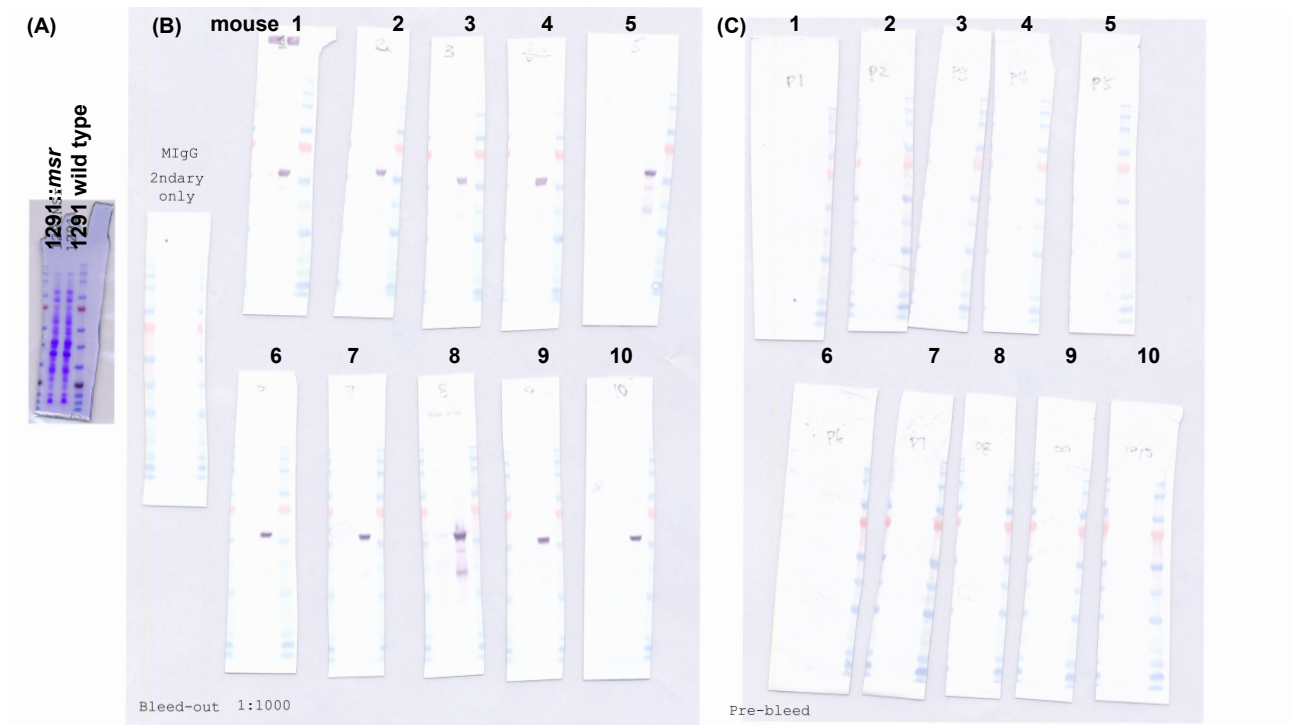
### Figure S2. Topology prediction of MsrA/B.

Topology prediction analysis using TMHMM (Krogh et al., 2001) indicates that MsrA/B does not have any transmembrane domains and that the whole protein is located outside of the membrane.



**Figure S3. Surface localisation of MsrA/B.**

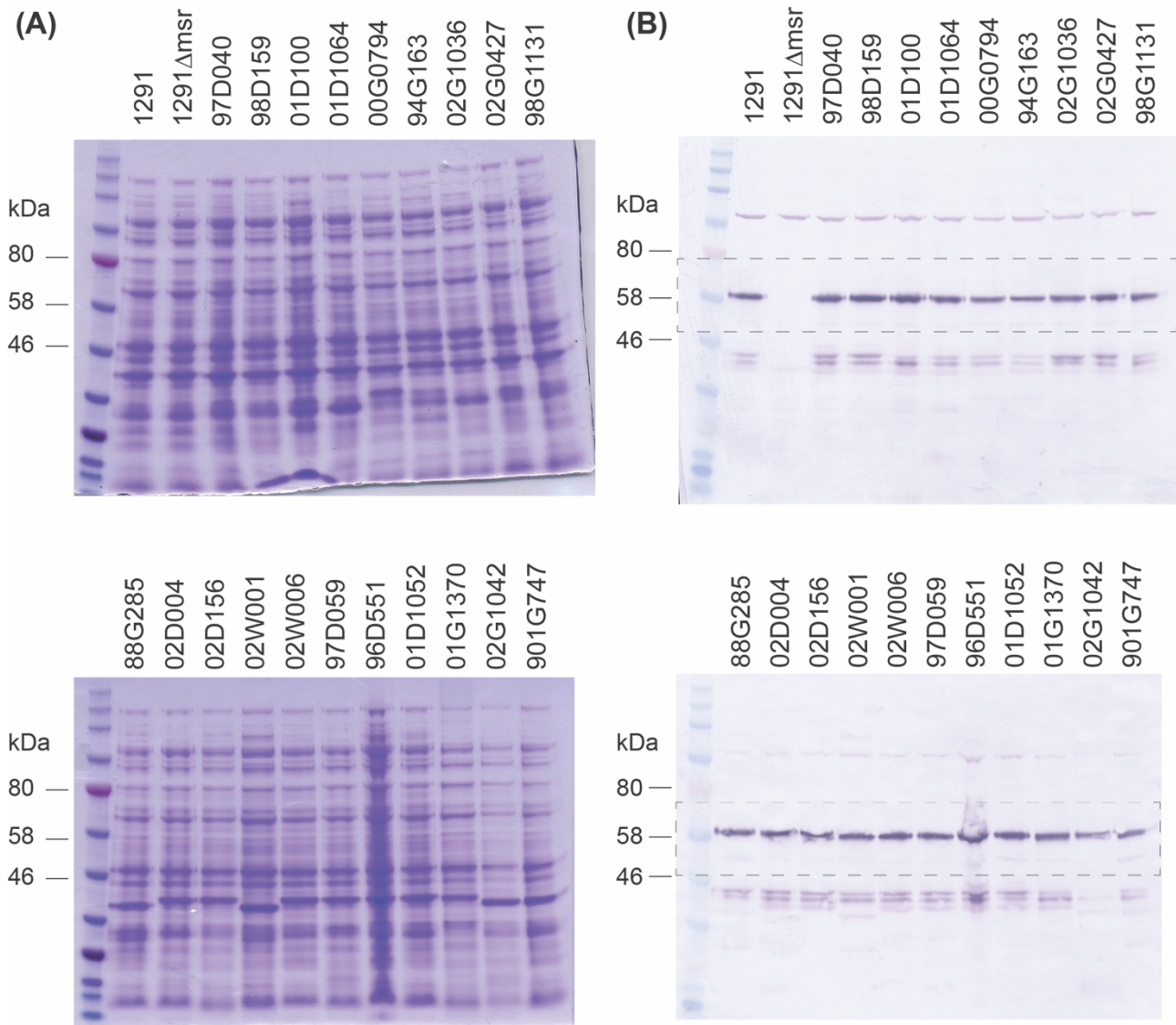
**(A)** Coomassie stained SDS-PAGE and **(B)** Western blot analysis of trypsin treated (20  $\mu$ g, 10 $\mu$ g) and untreated (0  $\mu$ g) whole cell *N. gonorrhoeae* 1291 and *N. meningitidis* MC58 $\phi$ 3, probed with anti-MsrA/B antibodies. The region of the Western blot shown in Figure 1 is boxed. **(C)** CFU/ml determined from samples taken at time 0 (t0) and at 60 min (t60) for each sample are shown below the Western blot. No significant differences were seen in CFUs/ml at t0 vs. 60mins, as assessed by a two-tailed unpaired Student's *t*-test ( $p > 1.5$ ), indicating that no cell lysis occurred during the assay. **(D-E)** Western blot analysis of samples from (A-C), probed with **(D)** antibodies to the intracellular protein GNA2091, and **(E)** antibodies to the extracellular protein PorA.



**Figure S4. Reactivity of mouse sera to MsrA/B.**

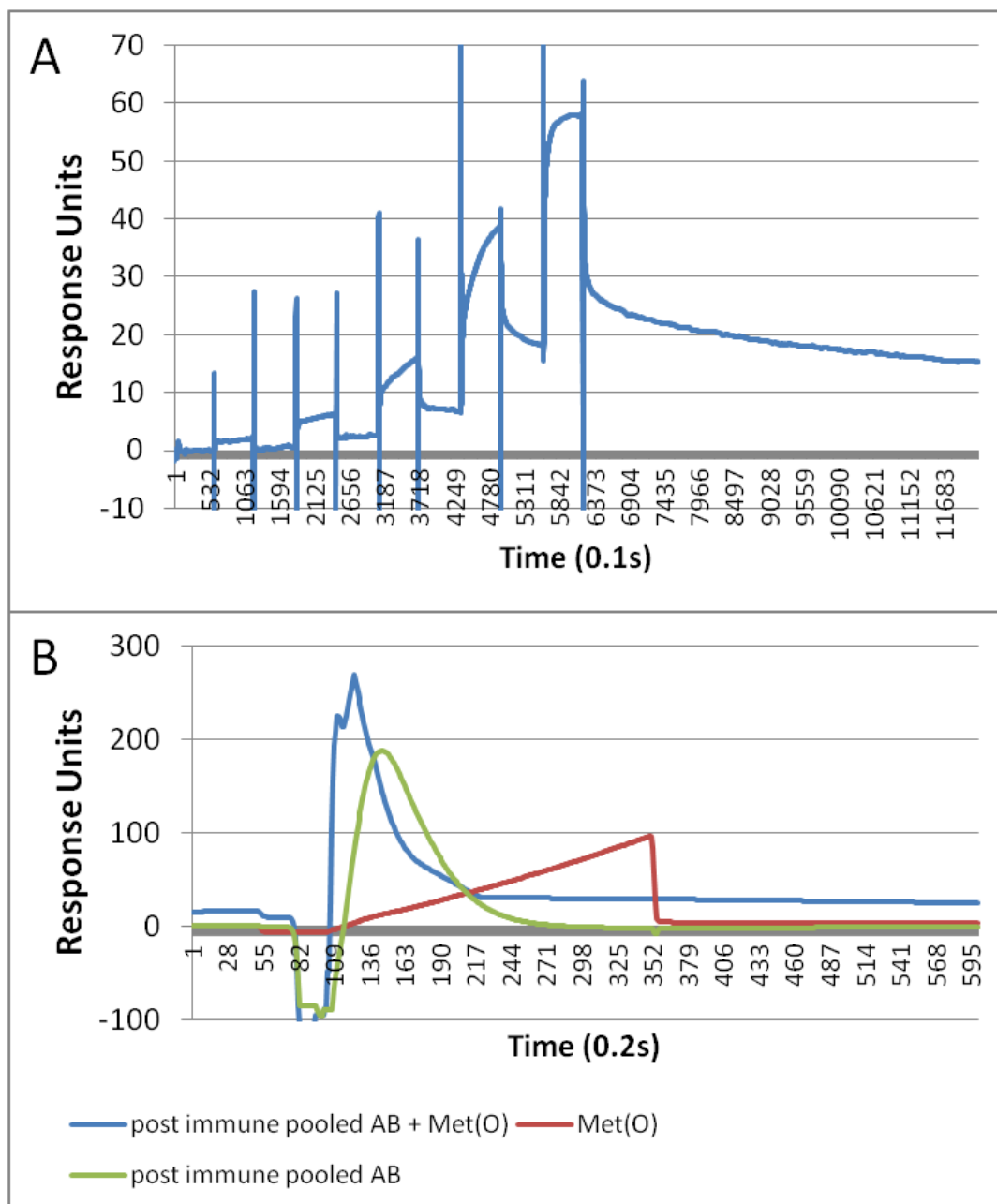
(A) Coomassie stained SDS-PAGE of *N. gonorrhoeae* 1291::msr and wild type whole cell lysates, and Western blot analysis of whole cell lysates probed with (B) anti-MsrA/B antibodies or (C) pre-immune sera from ten mice. There was no reactivity against MsrA/B in pre-immune sera, while an antibody response was generated by all mice that specifically recognizes MsrA/B in the wild-type strain but not the mutant strain.





**Figure S5. Expression of MsrA/B in a panel of gonococcal strains.**

(A) Coomassie stains SDS-PAGE and (B) Western blot analysis of MsrA/B expression in a panel of *N. gonorrhoeae* strains, including the 1291 wild type and *msr::kan* mutant (1291 $\Delta$ msr), and twenty clinical isolates from mucosal and disseminated gonococcal infections. The region of the Western blot shown in Figure 3 is boxed.



**Figure S6. Surface plasmon resonance (SPR) analysis of MsrA/B - Met(O) interaction.**

Representative sensorgrams from surface plasmon resonance analysis of recombinant MsrA/B binding to methionine sulfoxide (Met(O)). **(A)** Single cycle kinetics of Met(O) against MsrA/B. **(B)** Competition analysis of MsrA/B with pooled post immune serum (AB) demonstrating the direct competition between the pooled serum and Met(O).