Supporting Information for Publication

Quantification of *Borrelia burgdorferi* membrane proteins in human serum is a new concept for detection of bacterial infection

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Table S1. Donors of control and Lyme disease serum. Serum samples from donors with Lyme disease were collected on their 1st visit and on their 3rd visit two months later.

Patient (#)	Age (y)	Gender	Lyme disease
1	47	F	yes
2	61	M	yes
3	23	M	yes
4	62	M	no
5	31	F	no
6	24	F	no

Table S2. Identification of proteins in silver-stained gel spots numbered in Figure 1

Spot and protein[a]	Mass (kDa)	pI	Sequence coverage (%)	Score[b]
1. integral outer membrane protein P66 (H7C7N8)	68.1	6.04	61	461(76)
2. aminopeptidase 1 (P0C925)	51.5	5.77	70	540(76)
3. basic membrane protein A (Q45010)	36.9	5.17	83	553(76)
4. outer surface protein A (P0CL66)	29.4	8.77	73	347(76)
5. chaperone protein Dnak (P0C922)	69.2	5.17	57	685(76)
6. 60 kDa chaperonin (P0C923)	58.9	5.18	69	609(76)
7. enolase (O51312)	47.3	5.38	59	337(76)
8. glyceraldehyde 3-phosphate dehydrogenase (P46795)	36.2	7.74	78	434(76)

[a]The number in parentless is an UniProtKB entry identifier.

[b]MOWSE score from Mascot software (http://www.matrixscience.com/search_form_select.html). The number in parentless is the score at which statistical significance (p<0.05) occurred for that particular search.

Figure S1. Design of a QconCAT. Selected tryptic peptide sequences (in *red*) with their natural 6 mer flanking sequences (in *black*) are shown for 7 *B. burgdorferi* proteins. These sequences were arranged in QconCAT in the following order: ospC-apeA-hup-bmpA-p66-fla. Additional sequences at the N- and C-terminus are shown in *green*.

ospC
VLLAVKEVEALLSSIDEIAAKAIGKKIVVLAVKEVETLLASIDELAKAIGKKIKAIGKKIGNNGLEAN
QSKNTSLLSVLLAVKEVEALLSSIDELAKAIGKKI
apeA
IQRNKKSDEIVEGENLKILIGSLPIETKEKNKVK
ospA
KNKDGKYDLIATVDKLELKGTAKEVLKGYVLEGTLTAEKTTLVVKAKEVLKSYVLEGTLTAEKT
TLVVKTTLVVKEGTVTLSKNISKSGKGTSDKNNGSGTLEGEKTDKSKVKLTIADDLSQTKFEIF
KEGKTTLKVTEGTVVLSKNILKSGNWDSKSSTLTISVNSQKTKNLVFTITVQKYDSAGTNLEGK
AVEITT
hup
RPKVTKSDIVDQISLNIKNNNLKLGRLNARNPQTGEYVKVLDHHVAYFRPGKDLKERV
bmpA
VGMTFRAQEGAFLTGYIAARLSKTGKTKDVGRALNIFTSNHLKTNTFEG
p66
PMTGFKSTYYGFPSNDRAVRGTIGYKLPKLDLTFAIGGTGTGNRNQENDK
TWKPIKNLLDQNEDTKSVIAET
fla
AINASRNNGINAANLSKTQEKLSMISDQRANLGAFQNRLESIKD

QconCAT (ospC-apeA-ospA-hup-bmpA-p66-fla)
MEVLLAVKEVEALLSSIDEIAAKAIGKKIVVLAVKEVETLLASIDELAKAIGKKIKAIGKKIGNNGLE
ANQSKNTSLLSVLLAVKEVEALLSSIDELAKAIGKKIQRNKKSDEIVEGENLKILIGSLPIETKEKN
KVKNKDGKYDLIATVDKLELKGTAKEVLKGYVLEGTLTAEKTTLVVKAKEVLKSYVLEGTLTAE
KTTLVVKTTLVVKEGTVTLSKNISKSGKGTSDKNNGSGTLEGEKTDKSKVKLTIADDLSQTKFE
IFKEGKTTLKVTEGTVVLSKNILKSGNWDSKSSTLTISVNSQKTKNLVFTITVQKYDSAGTNLEG
KAVEITTRPKVTKSDIVDQISLNIKNNNLKLGRLNARNPQTGEYVKVLDHHVAYFRPGKDLKER
VGMTFRAQEGAFLTGYIAARLSKTGKTKDVGRALNIFTSNHLKTNTFEGPMTGFKSTYYGFPS
NDRAVRGTIGYKLPKLDLTFAIGGTGTGNRNQENDKTWKPIKNLLDQNEDTKSVIAETAINASR
NNGINAANLSKTQEKLSMISDQRANLGAFQNRLESIKDKLAAALEHHHHHH

Number of amino acids: 570
Molecular weight: 62082.4, Theoretical pI: 9.60
Instability index: The instability index (II) is computed to be 9.79
This classifies the protein as stable.
Grand average of hydropathicity (GRAVY): -0.39

Figure S2. Characterization of ^{15}N-labeled QconCAT. *A*, 8.5% SDS-PAGE of purified QconCAT with molecular weight standards on the *left*. *B*, Linear mode MALDI spectrum of purified QconCAT. The measured *m/z* $[M + H]^+$ value was 62,094.2. This matches well with the expected *m/z* $[M + H]^+$ value of 62,083.4 and confirms expression and purification of the full-length QconCAT. *C*, The isotope incorporation was determined at the peptide level after digestion of the purified QconCAT with trypsin. A MALDI spectrum of representative peptide is shown. MALDI spectra of three peptides were imported to Isotopic Enrichment Calculator (http://www.nist.gov/mml/bmd/bioanalytical/isoenrichcalc.cfm) and the mean value was higher than 99% of ^{15}N incorporation. This was accepted as a complete labeling and no correction was applied to the data.

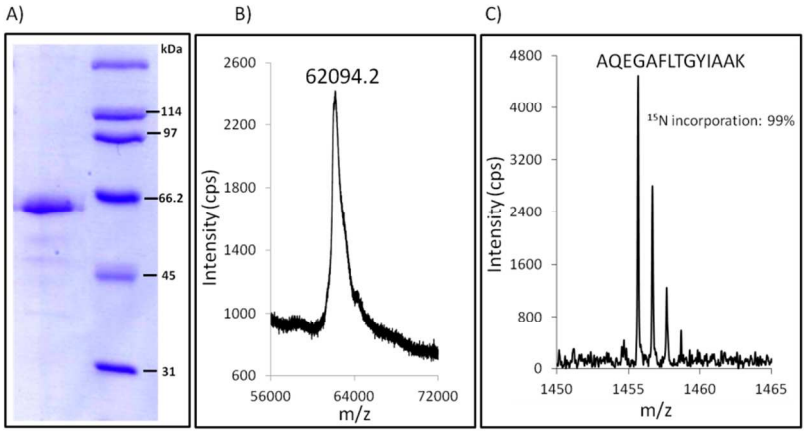

Figure S3. Two-dimensional PAGE pattern of *B. burgdorferi* membrane proteins before (A) and after (B) 0.1 mol/L Na_2CO_3 wash. The first and second dimensions were performed on 7-cm pH 3-10 immobilized pH gradient strips and 8-16% mini-PROTEAN TGX gels, respectively. After separation, proteins were detected by silver staining. The proteins identified in the numbered spots are: integral outer membrane protein P66 (#1), aminopeptidase 1 (#2), basic membrane protein A (#3), outer surface protein A (#4).