Supplementary Information to

SplitCore: An exceptionally versatile viral nanoparticle for display of native whole proteins regardless of 3D structure
Andreas Walker, Claudia Skamel and Michael Nassal

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b) SplitCore CLPs lack c/e1 antigenicity but retain particle-specific epitopes.

Equal amounts of SplitCore (SC) and wild-type HBc CLPs, as 149 or 183 derivatives, were subjected to NAGE in 1% gels and blotted to PVDF membrane. Detectability of c/e1 and particle specific epitopes was assessed using monoclonal antibodies (mAb’s) 312 (Salfeld et al., 1989; Conway et al., 1998); 3105 (Belnap et al. 2003); 3120 (Conway et al., 2003); and 275 (Salfeld et al., 1989); reported epitopes are indicated below the HBc sequence. The dotted red line marks the separation site in the SplitCore proteins. Bound mAb’s were visualized using a peroxidase-conjugated secondary antibody and a chemiluminescent substrate.

(a) Enhanced assembly into T=3 particles by SplitCore proteins.

In >2% NAGE gels, particle diameter affects electrophoretic mobility of HBc CLPs (Newman et al., 2003). The indicated CLPs were compared by NAGE in 2.6% gels, followed by CB staining. HBC140, the shortest assembly-proficient HBC variant (Watts et al., 2002), generates ~50% T=3 CLPs and was used as reference. Enhanced T=3 formation by SplitCore proteins (SC-183, SC-149) is indicated by the increased intensity of the faster migrating bands, as confirmed by counting small vs. large particles on electron micrographs (percentages of T=3 CLPs indicated below the gels). The data confirm structural cross-talk (Böttcher et al., 2006) between the central helices and the C proximal multimerization sites (Fig. 1a).

(c) Drastically reduced reactivity with human α-HBcAg of SplitCore CLPs.

The Abbott Architect, Siemens Advia Centaur and Diasorin Liaison Anti-HBc are widely used chemiluminescent diagnostic assays to detect α-HBc antibodies. Antibodies bind to immobilized HBc, and are detected by acridinium-labeled α-human immunoglobulin (Architect) or HBc (Centaur), i.e. high signals indicate high level anti-HBc; in the Liaison assay, α-HBc in the sample competes with labeled α-HBc, i.e. low signals indicate high level α-HBc. Preincubation of the serum with recombinant HBc CLPs (rHBc) traps α-HBc (D. Huzly, M. Nassal, J. Vorreiter, V. Kapper-Falcone, D. Neumann-Haefelin, W. H. Gerlich; submitted). For the serum used, admixing rHBc to 150 ng/ml (Architect), 190 ng/ml (Centaur), and 330 ng/ml (Liaison) blocked the signal to 50%. Achieving the same inhibition with SplitCore CLPs required 13- to 14-fold (Architect, Centaur) or 80-fold (Liaison) higher concentrations; values for SplitCore CLPs presenting split GFP (SC-splitGFP), after correction for the lower molar core protein content, were 33-, 27- and 80-fold. These data are consistent with >90% of the human α-HBc response being directed against c/e1 (Salfeld et al., 1989) which is destroyed in the split constructs. RLU, relative light units. Curve-fitting was done by non-linear regression analysis as implemented in GraphPad Prism for Mac.
Supplementary Figure S2. SplitCore CLPs can natively display a heterologous protein fused to either coreN or coreC.

(a) Structures of HBc dimer and GFP (pdb: 1EMA; Ormö et al., 1996)) at approximately the same scale. GFP β-strand 11 is shown in lighter green. (b) Scheme of the split fusion proteins. (c) Overlapping stop/start codons as an alternative for co-expression of coreN and coreC from a bicistronic mRNA. This design was realized in construct coreN-GFP//stop-start//coreC, the others contained two RBSs (Fig. 1b). (d,e) Evidence for efficient formation of native GFP-displaying CLPs by all three constructs. The fusions shown were based on coreC183 with a Cys183Ser mutation plus a His6 tag; neither modification detectably affects capsid structure (Nassal et al., 1992; Vogel et al., 2005); comparable results were obtained with coreC149 derivatives. (d) Cosedimentation in sucrose gradients. Aliquots from gradient fractions (as in Fig. 1c) were analyzed by SDS-PAGE and CB staining (top), and by immunoblotting with mAb’s specific for coreN and coreC (10E11 and 10F10, respectively (Bichko et al., 1993)). The 8.9 kDa coreN fragment from coreN//RBS2//GFP-coreC, hardly visible by CB staining, was clearly detected by the ±-coreN mAb which also produced strong signals with coreN-GFP; conversely, GFP-coreC was detected with the ±-coreC mAb. The bands of ~50 kDa (*) reacting with both mAbs in all samples are probably translational read-through products. Potential proteolysis products of coreN-GFP are marked by **. (e) NAGE analysis. Aliquots of the gradient fractions were separated by NAGE, and GFP plus ethidium bromide stained nucleic acids were visualized by UV illumination (365 nm). Fractions 8 and 9 showed distinct green fluorescent bands (white arrows), as did a contiguous chain core-GFP fusion (lanes core-GFP) (Kratz et al., 1999). All splitCore-based CLPs displayed the same electrophoretic mobility (bottom gel) which was slightly higher than that of contiguous chain core-GFP CLPs. Presence of the coreC fragment was confirmed by immunoblotting with the α-coreC mAb (bottom).
Supplementary Figure S3. Equally efficient display of naturally dimeric OspC on SplitCore as on contiguous chain HBc.

(a) *B. burgdorferi* outer surface protein C (OspC) forms dimers in which all four termini protrude in close proximity from one side (pdb: 1GGQ; (Kumaran et al., 2001)). Despite nonmatching topologies of the four-helix-bundles in the OspC and HBc dimer, OspC insertion into c/e1 of contiguous chain HBc yields regular CLPs (Skamel et al., 2006). Here, one of the previously analyzed variants, OspCa, was fused to coreN. (b) SDS-PAGE analysis of sucrose gradient fractions. A sharp peak of fragments of the expected sizes occurred in fractions 7 to 9. (c) Negative staining EM. SplitCore and contiguous chain OspC CLPs were virtually indistinguishable. Hence SplitCore is as good a display platform for favorably structured heterologous proteins as conventional HBc.
Supplementary Figure. S4. Efficient expression of OspA fused to SplitCore coreN or coreC.

(a) Structure of OspA. In the extended structure (pdb: 1OSP (Li et al., 1997)) N and C terminus are >6nm apart. The neutralizing LA2 epitope comprises multiple residues in the C terminal region. A HBc dimer is shown for comparison.

(b,c) SDS-PAGE analysis of sucrose gradient fractions. Both SplitCore versions, with OspA fused to coreN (CoreN-OspA183H6) and to coreC (OspA-CoreC183H6), were well expressed and sedimented into the particle-typical fractions. For immunizations, the pooled fractions were dialyzed against TN150 buffer (10 mM Tris-HCl, 150 mM NaCl, pH 7.4) and resedimented through 10-60% sucrose gradients, then endotoxin was depleted by Triton X-114 phase partitioning (Liu et al., 1997) (single lanes on the right); final endotoxin content was <50 EU/mg of protein.
Supplementary Figure S5. Comparably efficient expression and CLP formation by coreC149- and coreC183-based SplitCoreN-CSP fusions.

(a) Scheme of the CSP fusions. (b) Sucrose gradient sedimentation profile for SplitCoreN-CSP149H6. (c) Further purified preparation used for immunizations. Material from fractions 8 to 11 from (b) was treated as described for SplitCore OspA. (d) Sucrose gradient sedimentation profile for SplitCoreN-CSP183H6. Equally good expression and CLP formation for core149 vs. core183 derivatives as seen here was also observed for all other SplitCore fusions examined.
Supplementary Figure S6. Split GFP presenting CLPs.

(a) Scheme of splitCore-splitGFP (SC-SGFP) complementation. The splitting site was moved into GFP, separating β-strands 10 and 11. Proper association should result in assembly-competent fluorescent subunits that expose two surface-accessible new termini for further derivatization. (b) Expression and assembly status. Gradient fractions from a double-RBS construct were analyzed by SDS-PAGE and NAGE (bottom). Fragments of the expected sizes cosedimented into fractions 8 to 11; material from fractions 8 and 9 migrated in NAGE as distinct green fluorescent band (UV) that also stained with CB; based on the molar extinction coefficient of GFP ($\varepsilon = 55,000 \text{ Mol}^{-1}\text{cm}^{-1}$) and total protein content, at least 75% of the GFP domains were properly folded. The non-migrating fluorescent material from fractions 10 to 12 probably represents aggregates containing folded GFP. Similar results were obtained with coreC183, and with double RBS constructs in which the order of the two cistrons was reversed (as shown in Fig. S7e). (c) Direct visualization of folded GFP containing material in the gradient tube from which the samples in (b) were derived. (d) Confirmation of CLP formation by negative staining EM. The two micrographs are from independent grids onto which material from fraction 8 was applied. Regularly shaped smaller (*) and larger (**) particles were abundant, with approximate diameters of ~38 nm and 42 nm, respectively.
Supplementary Figure S7. SplitCore-SplitGFP CLPs as a versatile fluorescent platform for display for differently structured heterologous proteins.

Schematic representations of the fusion proteins are shown on the left, expression cassettes and gradient profiles by SDS-PAGE in the center, and negative staining electron micrographs on the right. (a) OspC fused to coreN-GFPβ1-10. (b) OspC fused to GFPβ11-coreC. (c) OspA fused to coreN-GFPβ1-10. (d) One OspA each fused to coreN-GFPβ1-10 and GFPβ11-coreC. The broader distribution into higher gradient fractions correlates with the presence of a fraction of incomplete particles. (e) Attenuated Staphylococcus aureus enterotoxin B (SEB (Stiles et al., 2001)) fused to GFPβ11-coreC. Note that the cistron order in this expression construct is reversed. The right panel shows the structure of SEB (pdb: 3SEB (Papageorgiou et al., 1998)); N and C terminus are ~3.5 nm apart.
Supplementary Figure S8. The SplitCore approach is portable to a distant animal HBV core protein.

(a) Sequence alignment of HBV vs. woodchuck (WHV) and ground squirrel hepatitis virus (GSHV) core proteins. UniProt identifiers are P03146 (HBc), P69711 (WHc), P0C6J1-1 (GSHc). (b,c) WHV core protein split at an analogous position as HBc (splitWHc) remains assembly competent. (b) SDS-PAGE of sucrose gradient fractions. Two fragments of the sizes expected cosedimented into fractions 7 and 8; lane c, HBV SplitCore149H6. (c) NAGE analysis. NAGE followed by CB staining or immunoblotting with α-coreN mAb 10E11 showed distinctly migrating bands in fractions 6 to 9. Their mobility was comparable to that of SplitCore149H6 CLPs (lane c). The mAb recognizes both core proteins because its epitope resides in the conserved N terminal sequence (see (a)). (d) Negative staining EM of SplitWHc149. (e) Scheme of SplitWHc presenting the CSP repeat peptide (SplitWHc-NANP149) and full-length CSP CS27IVC (SplitWHc-CSP149). (f) Immunogenicity of SplitWHc CLPs. Immunogenicity in mice was assessed as described for SplitCore-CSP, with an overall similar outcome, except that the ratio of α-CSP (measured via NANP peptide) vs. α-carrier (α-WHc) response induced by splitWHc-CSP149 CLPs was even higher.
Supplementary Figure S9. SplitCore CLPs for display of surface-accessible free termini.

(a) Scheme of SplitCore CLPs displaying an artificial biotin acceptor peptide (BAP). The BAP sequence corresponds to peptide 85-10 in (Beckett et al., 1999) preceded by a G- and followed by a P-residue. The bold K denotes the target lysine for E. coli biotin-protein ligase BirA. The SplitCore-BAP149H6 construct was well expressed and formed CLPs. An aliquot of these CLPs was incubated with streptavidin and the reaction was sedimented through a 2 ml 10%-60% sucrose gradient. The distribution of SplitCore protein and streptavidin in the gradient was probed using α-coreN or α-streptavidin antibodies (α-Strep), and indicated cosedimentation of streptavidin with the CLPs. (b) Specificity control. HBc149H6 CLPs were treated as the SplitCore-BAP CLPs in (a). The lack of cosedimentation confirms that association in (a) was specific. Surface accessibility of free peptide ends, as provided by SplitCore but not contiguous chain HBc, is a prerequisite for various enzymatic as well as chemical modifications.
SUPPLEMENTARY PROTOCOLS

Reactivity of SplitCore vs. contiguous chain HBc CLPs with human α-HBc. Assays were performed at the Diagnostic Laboratory of the Virology Dept. of the University Hospital Freiburg. A single patient serum scoring positive for α-HBc in the Siemens ADVIA Centaur, Abbott Architect i1000, and DiaSorin LIAISON chemiluminescent anti-HBc assays was used throughout. To determine the amount of recombinant core protein required to block the α-HBc, serial dilutions of HBc183, SplitCore183, or SplitCore-SplitGFP CLPs were admixed to the serum to final concentrations from 0.1 ng/ml to 10 μg/ml; PBS served as negative control. Then all samples were retested in each assay as suggested by the manufacturers.

Vaccination with OspA-presenting CLPs. Immunizations were performed at the Max-Planck-Institute for Immunobiology, Freiburg, Germany, as approved by the local authorities and in compliance with German animal welfare legislation. Groups of 5 Balb/c mice each were inoculated into the tail-base with 10 μg coreN-OspA183H6 CLPs, OspA-CoreC183H6 CLPs, or LipOspA in 100 μl MPL-TDM adjuvant (Sigma) on day 0, 14, 18 and 49. Total α-OspA titers and LA2-specific antibodies in pooled sera from day 22 and 57 post immunization were determined by enzyme-linked immunosorbent assay (ELISA) as described (Nassal et al., 2005). For protection experiments, groups of six C.B-17 SCID (H-2d) mice were injected with day 57 immune sera from the vaccinated mice; transferred volumes per mouse contained the equivalent of 1 μg LA2 specific antibodies for coreN-OspA183H6 and LipOspA induced immune sera; of the low α-LA2 titered OspA-coreC183H6 immune sera, a volume equivalent to that of the coreN-OspA183H6 sera was used. Control mice received preimmunization sera instead. Two h later, mice were challenged with 10^4 borreliae (strain ZS7) per animal, and arthritic disease development was monitored over 52 days as described (Nassal et al., 2005). Additional data will be presented elsewhere (A. Walker, T. Stehle, R. Wallich, M. M. Simon, and M. Nassal; manuscript in preparation).

Vaccination with CSP and CSP repeat peptide presenting CLPs. The experiments were performed at the Vaccine Research Institute San Diego, USA. All animal care was performed according to National Institutes of Health standards as set forth in the Guide for the Care and Use of Laboratory Animals (1996). Groups of three (B10 x B10.s) F1 mice each were vaccinated by intraperitoneal injection of 20 μg of SplitCoreN-CSP149H6 CLPs or HBc149-NANP CLPs per animal in incomplete Freund’s adjuvant (IFA) as described (Billaud et al., 2007), and boosted once on day 56 with 10 μg antigen in IFA. Immune sera collected immediately before the boost and on day 98 were monitored for specific antibodies by ELISA using a synthetic (NANP)_5 peptide, recombinant CSP CS27IV protein (Cerami et al., 1992), or HBc183H6 CLPs as immobilized test antigens (Billaud et al., 2007); preimmune sera served as control. Titers were determined by serial dilutions of the sera; the highest dilution giving a three times higher OD_{492} reading than the equally diluted presera was defined as endpoint. Additional data will be presented elsewhere (A. Walker, J. Billaud, D. Milich and M. Nassal; manuscript in preparation).
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