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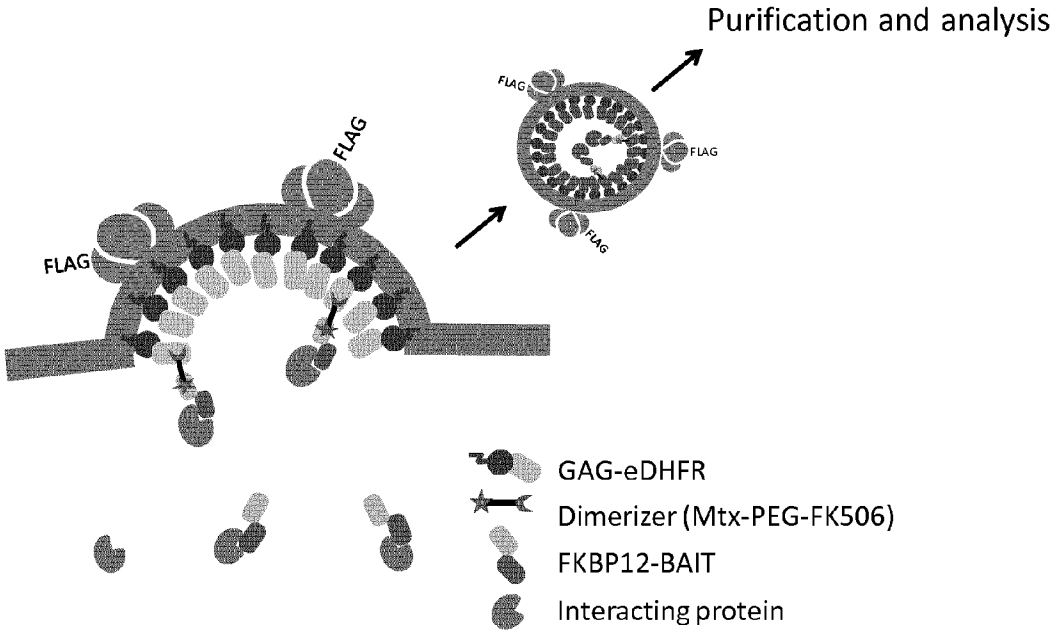

(57) Abrégé/Abstract:
The present invention relates to a virus-like particle, in which a protein complex is entrapped, ensuring the formation of the protein complex under physiological conditions, while protecting said protein complex during purification and identification. The invention relates further to the use of such virus like particle for the isolation and identification of protein complexes.

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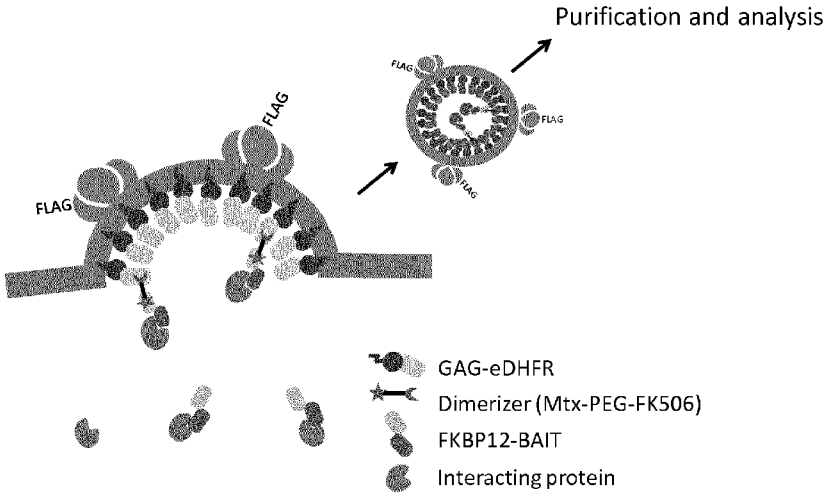
Figure 1



(57) Abstract: The present invention relates to a virus-like particle, in which a protein complex is entrapped, ensuring the formation of the protein complex under physiological conditions, while protecting said protein complex during purification and identification. The invention relates further to the use of such virus like particle for the isolation and identification of protein complexes.

VIRUS-LIKE PARTICLE BASED PROTEIN-PROTEIN INTERACTION

The present invention relates to a virus-like particle, in which a protein complex is entrapped, ensuring the formation of the protein complex under physiological conditions, while protecting said protein complex during purification and identification. The invention relates further to the use of such virus like particle for the isolation and identification of protein complexes.

Protein-protein interactions are an essential key in all biological processes, from the replication and expression of genes to the morphogenesis of organisms. Protein-protein interactions govern amongst others ligand-receptor interaction and the subsequent signaling pathway; they are important in assembly of enzyme subunits, in the formation of biological supramolecular structures such as ribosomes, filaments and virus particles and in antigen-antibody interactions.

Researchers have developed several approaches in attempts to identify protein-protein interactions. A major breakthrough was obtained by the introduction of the genetic approaches, of which the yeast two-hybrid (Fields and Song, 1989) is the most important one. Although this technique became widely used, it has several drawbacks. The fusion proteins need to be translocated to the nucleus, which is not always evident. Proteins with intrinsic transcription activation properties may cause false positives. Moreover, interactions that are dependent upon secondary modifications of the protein such as phosphorylation cannot be easily detected.

Several alternative systems have been developed to solve one or more of these problems.

Approaches based on phage display do avoid the nuclear translocation. WO9002809 describes how a binding protein can be displayed on the surface of a genetic package, such as a filamentous phage, wherein the gene encoding the binding protein is packaged inside the phage. Phages, which bear the binding protein that recognizes the target molecule are isolated and amplified. Several improvements of the phage display approach have been proposed, as described e.g. in WO9220791, WO9710330 and WO9732017.

However, all these methods suffer from the difficulties that are inherent at the phage display methodology: the proteins need to be exposed at the phage surface and are so exposed to an environment that is not physiological relevant for the *in vivo* interaction. Moreover, when screening a phage library, there will be a competition between the phages that results in a selection of the high affinity binders.

US5637463 describes an improvement of the yeast two-hybrid system, whereby can be screened for modification dependent protein-protein interactions. However, this method relies

on the co-expression of the modifying enzyme, which will exert its activity in the cytoplasm and may modify other enzymes than the one involved in the protein-protein interaction, which may on its turn affect the viability of the host organism.

An interesting evolution is described in US5776689, by the so-called protein recruitment system. Protein-protein interactions are detected by recruitment of a guanine nucleotide exchange factor (Sos) to the plasma membrane, where Sos activates a Ras reporter molecule. This results in the survival of the cell that otherwise would not survive in the culture conditions used. Although this method has certainly the advantage that the protein-protein interaction takes place under physiological conditions in the submembrane space, it has several drawbacks. Modification-dependent interactions cannot be detected. Moreover, the method is using the pleiotropic Ras pathway, which may cause technical complications such as the occurrence of false positives.

A major improvement in the detection of protein-protein interactions was disclosed in WO0190188, describing the so called Mappit system. The method, based on a cytokine receptor, allows not only a reliable detection of protein-protein interactions in mammalian cells, but also modification-dependent protein interactions can be detected, as well as complex three hybrid protein-protein interactions mediated by a small compound (Caligiuri et al., 2006). However, although very useful, the system is limited in sensitivity and some weak interactions cannot be detected. Moreover, as this is a membrane based system, nuclear interactions are normally not detected. Recently, a cytoplasmic interaction trap has been described, solving several of those shortcomings. However, all these 'genetic' systems rely on the overexpression of both interaction partners, which may result in false positives, due to the artificial increase in concentration of one or both of the interaction partners.

As an alternative for the genetic protein-protein interaction detection methods described above, biochemical or co-purification strategies combined with mass spectrometry-based proteomics (Paul et al., 2011; Gingras et al., 2007) can be used. For the co-purification strategies, a cell homogenate is typically prepared by a detergent-based lysis protocol, followed by capture using a (dual) tag approach (Gavin et al., 2002) or via specific antibodies (Malovannaya et al.) The protein complex extracted from the 'soup' of cell constituents is then expected to survive several washing steps, mostly to compensate for the sensitivity of contemporary MS instruments, before the actual analysis occurs. There are no clear guidelines on the extent of washing or on available buffers and their stringency. Most lysis and washing protocols are purely empirical in nature and were optimized using model interactions. It is therefore hard to speculate on the loss of factors during these steps (false negatives), or the possibility of false interactions due to loss of cellular integrity (false positives). Use of metabolic

labeling strategies allows separation between the proteins sticking to the purification matrix, and between the proteins that associate specifically to the bait protein. Depending on the purification conditions and the sensitivity of the MS instruments used, it is no exception to find more than 1000 proteins in the eluted fraction of a gel-free AP-MS experiment.

There is a further need for co-purification techniques, isolating the protein complexes in their physiological environment, but wherein the complex is protected during the further purification and analysis.

The evolutionary stress on viruses promotes highly condensed coding of information and maximal functionality for small genomes. Accordingly, for HIV-1 it is sufficient to express a single viral protein, the p55 GAG protein, to allow the efficient production of virus-like particles (VLPs) from cells (Gheysen et al., 1989;Shioda and Shibuta, 1990). The p55 GAG protein consists of different parts, which are processed by HIV protease upon maturation of the particle into a functional infectious particle. The N-terminal matrix protein part ensures binding to the membrane via myristoylation and ensures budding (Bryant and Ratner, 1990). The Capsid protein forms the cone shaped viral core after processing, while the nucleocapsid protein and the p6 protein bind to and protect the viral RNA. The p55 GAG protein is highly mobile before accumulation in cholesterol-rich regions of the membrane, where multimerization actually initiates the budding process (Gomez and Hope, 2006). A total of 4000-5000 GAG molecules are required to form a single particle with a size of about 145 nm (Briggs et al., 2004).

Surprisingly we found that the p55 GAG protein can be used to trap a bait protein together with its physiological binding partners into VLPs that are budded from human cells. The very mild 'extraction' or 'abduction' of the protein complex ensures the identification of relevant interacting proteins. After introduction of a simple one-step particle enrichment protocol to speed up the workflow, we found that this viral particle based protein – protein interaction trap approach (called "Virotrap") can be used for the detection of binary interactions. We also show the identification of new partners by the coupling of the Virotrap process to MS-based analysis.

A first aspect of the invention is an artificial virus-like particle (called "Virotrap particle"), comprising (1) a viral particle forming polypeptide, (2) a first interaction polypeptide and (3) a second interaction polypeptide, interacting with the first interacting polypeptide. As explained below, in one preferred embodiment the viral forming polypeptide and the first interacting polypeptide may be two different polypeptide domains of a fusion protein (i.e. a fusion protein consisting of at least two polypeptides derived from two different proteins); In another preferred embodiment, the viral particle forming polypeptide and the first interacting polypeptide are independent proteins. Besides the first and the second interaction polypeptide, the virus-like

particle may comprise other proteins, recruited to first and/or second interaction polypeptide, wherein all the proteins together form one protein complex. Polypeptide refers to a polymer of amino acids and does not refer to a specific length of the molecule. This term also includes post-translational modifications of the polypeptide, such as glycosylation, phosphorylation and acetylation. A virus like particle, as used here, is a particle consisting at least of a viral particle forming protein, but preferably without the viral DNA or RNA. Viral particle forming proteins, as used here, are known to the person skilled in the art and are proteins that allow the assembly of viral particles, and preferably budding of said particles of the cell. Examples of such particles have been described in the art and include, but are not limited to particles derived from virus families including Parvoviridae (sucha as adeno-associated virus), Retroviridae (such as HIV), and Flaviviridae (such as Hepatitis C virus). In a preferred embodiment, said particle forming polypeptide may be a modification of the natural occurring particle forming protein, such as a deletion and or mutation, as long as they do not inhibit the particle formation. Preferably, said deletion and/or mutation is reducing the binding of the particle forming polypeptide with host proteins. Preferably, said modification is a fusion protein. Preferably, the viral particle forming polypeptide, or its modification forms a viral structure, consisting of a hollow particle, in which the first and second interaction polypeptides are trapped. In a preferred embodiment, the first interaction polypeptide is anchored to said viral structure, ensuring the capturing of the protein complex formed by the first and the second interaction polypeptide into the inside of the virus-like particle. The anchoring may be direct, wherein the fusion partner of the viral particle forming polypeptide acts as first interaction polypeptide, or indirect, wherein an independent linker molecule binds to the viral particle forming polypeptide at one hand, and to a construct comprising the first interaction polypeptide at the other hand ("dimerizing linker") as illustrated in figure 1. As a non-limiting example, such a linker may be a molecule as illustrated in figure 1, or it may be a bispecific protein or peptide affinity ligand such as an antibody, or in preferred setting a bispecific nanobody or alphabody^{TM} binding to the viral particle forming polypeptide at one hand, and to the first interaction polypeptide at the other hand. In case of a direct anchoring, the viral forming polypeptide and the first interacting polypeptide are two different polypeptide domains of the same protein Preferably, said viral particle forming polypeptide is a HIV protein, even more preferably said viral particle forming polypeptide is the p55 GAG protein, or a modification or functional fragment thereof. A modification or functional fragment as used here is a modification or functional fragment that is still capable of forming virus-like particles that are capable of entrapping the protein complex according to the invention. Preferably, said modification is a fusion protein, even more preferably, the p55 GAG protein is fused to the first interaction polypeptide.

Another aspect of the invention is the use of an artificial virus like particle, according to the invention, for the detection of protein-protein interactions.

Still another aspect of the invention is a method for detecting protein-protein interactions, comprising (1) the expression of a viral particle forming polypeptide in a cell (2) recruiting a first interaction polypeptide to said viral particle forming polypeptide (3) recruiting a second interacting polypeptide to the first interaction polypeptide (4) isolation of the virus-like particles and (5) analysis of the entrapped protein complex. Preferably, said cell is a mammalian cell. Preferably, the analysis of the entrapped protein complex is an MS based analysis. It is clear for the person skilled in the art that protein-protein interactions of any nature can be detected with the method. As a non-limiting example, the method may be used to detect proteins involved in a signaling network, but it may also be used to detect antigen-antibody interactions, or other affinity binding proteins and their target(s).

The present invention as claimed relates to:

[1] An artificial virus-like particle comprising an HIV viral particle forming p55 Group Antigens (GAG) polypeptide, wherein the artificial virus-like particle budded from a cell, wherein a bait protein bound to a prey protein is trapped inside the artificial virus-like particle, wherein the prey protein is a native physiological binding partner of the bait protein, wherein the bait protein is anchored to the p55 GAG polypeptide, and wherein:

the bait protein is anchored to the p55 GAG polypeptide by in-frame fusion between the bait protein and the p55 GAG polypeptide, or

the bait protein is anchored to the p55 GAG polypeptide via a flexible hinge between the bait protein and the p55 GAG polypeptide, or

the bait protein is anchored to the p55 GAG polypeptide via a dimerizing linker which binds at one end to the p55 GAG polypeptide and which binds at the other end to the bait protein.

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[2] The artificial virus-like particle according to [1], wherein the bait protein is anchored to the p55 GAG polypeptide by in-frame fusion between the bait protein and the p55 GAG polypeptide.

[3] The artificial virus-like particle according to [1], wherein the bait protein is anchored to the p55 GAG polypeptide via a flexible hinge between the bait protein and the p55 GAG polypeptide.

[4] The artificial virus-like particle according to [1], wherein the bait protein is anchored to the p55 GAG polypeptide via a dimerizing linker which binds at one end to the p55 GAG polypeptide and which binds at the other end to the bait protein.

[5] The artificial virus-like particle according to [4], wherein the dimerizing linker binds to the p55 GAG polypeptide directly.

[6] The artificial virus-like particle according to [4], wherein the dimerizing linker binds to a first protein which is fused in-frame with the p55 GAG polypeptide.

[7] The artificial virus-like particle according to [6], wherein the first protein is dihydrofolate reductase (DHFR) and the dimerizing linker comprises methotrexate at one end, so that the dimerizing linker is linked to the p55 GAG polypeptide via binding between methotrexate and DHFR.

[8] The artificial virus-like particle according to [4] to [7], wherein the dimerizing linker binds to the bait protein directly.

[9] The artificial virus-like particle according to [4] to [7], wherein the dimerizing linker binds to a second protein which is fused in-frame with the bait protein.

[10] The artificial virus-like particle according to [9], wherein the second protein is FKBP12 protein and the dimerizing linker comprises FK506 at one end, so that the dimerizing linker is linked to the bait protein via binding between FK506 and FKBP12 protein.

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[11] The artificial virus-like particle according to [4] to [10], wherein the ends of the dimerizing linker are linked by polyethylene glycol.

[12] The artificial virus-like particle according to [1] to [11], wherein the bait protein and the prey protein are native physiological binding partners in the cytoplasm.

[13] The artificial virus-like particle according to [1] to [12] further comprising a purification tag on the surface of the particle.

[14] The artificial virus-like particle according to [13] wherein the purification tag is a tagged vesicular stomatitis virus- glycoprotein G (VSV-G) protein.

[15] The artificial virus-like particle according to [1] to [14] which is budded from a mammalian cell.

[16] The artificial virus-like particle according to [15], wherein the mammalian cell is a human cell.

[17] Use of an HIV viral particle forming p55 Group Antigens (GAG) polypeptide to detect at least one interaction between a bait protein and a prey protein, wherein the bait protein and the prey protein are native physiological binding partners, wherein the bait protein is anchored to the p55 GAG polypeptide, and wherein:

the bait protein is anchored to the p55 GAG polypeptide by in-frame fusion between the bait protein and the p55 GAG polypeptide, or

the bait protein is anchored to the p55 GAG polypeptide via a flexible hinge between the bait protein and the p55 GAG polypeptide, or

the bait protein is anchored to the p55 GAG polypeptide via a dimerizing linker which binds at one end to the p55 GAG polypeptide and which binds at the other end to the bait protein.

[18] Use of an HIV viral particle forming p55 Group Antigens (GAG) polypeptide to trap a bait protein together with its physiological binding partner(s) in a virus-like particle budded from a cell, wherein the bait protein is anchored to the p55 GAG polypeptide, and wherein:

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the bait protein is anchored to the p55 GAG polypeptide by in-frame fusion between the bait protein and the p55 GAG polypeptide, or

the bait protein is anchored to the p55 GAG polypeptide via a flexible hinge between the bait protein and the p55 GAG polypeptide, or

the bait protein is anchored to the p55 GAG polypeptide via a dimerizing linker which binds at one end to the p55 GAG polypeptide and which binds at the other end to the bait protein.

[19] The use according to [18], wherein the bait protein and its physiological binding partner(s) are native physiological binding partners in the cytoplasm.

[20] A method for detecting specific protein-protein interactions in an artificial virus-like particle budded from a cell, comprising:

(1) expressing in the cell an artificial virus-like particle comprising an HIV viral particle forming p55 Group Antigens (GAG) polypeptide to which a bait protein is anchored;

(2) recruiting a prey protein to the bait protein wherein the prey protein is a native physiological binding partner of the bait protein;

(3) isolating the artificial virus-like particles budded from the cell; and

(4) analyzing the bait-prey protein complex trapped inside the artificial virus-like particle for specific protein-protein interaction.

[21] The method according to [20], wherein:

the bait protein is anchored to the p55 GAG polypeptide by in-frame fusion between the bait protein and the p55 GAG polypeptide, or

the bait protein is anchored to the p55 GAG polypeptide via a flexible hinge between the bait protein and the p55 GAG polypeptide, or

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the bait protein is anchored to the p55 GAG polypeptide via a dimerizing linker which binds at one end to the p55 GAG polypeptide and which binds at the other end to the bait protein.

[22] The method according to [20], wherein the bait protein is anchored to the p55 GAG polypeptide by in-frame fusion between the bait protein and the p55 GAG polypeptide.

[23] The method according to [20], wherein the bait protein is anchored to the p55 GAG polypeptide via a flexible hinge between the bait protein and the p55 GAG polypeptide.

[24] The method according to [20], wherein the bait protein is anchored to the p55 GAG polypeptide via a dimerizing linker which binds at one end to the p55 GAG polypeptide and which binds at the other end to the bait protein.

[25] The method according to [24], wherein the dimerizing linker binds to the p55 GAG polypeptide directly.

[26] The method according to [24], wherein the dimerizing linker binds to a first protein which is fused in-frame with the p55 GAG polypeptide.

[27] The method according to [26], wherein the first protein is dihydrofolate reductase (DHFR) and the dimerizing linker comprises methotrexate at one end, so that the dimerizing linker is linked to the p55 GAG polypeptide via binding between methotrexate and DHFR.

[28] The method according to [24] to [27], wherein the dimerizing linker binds to the bait protein directly.

[29] The method according to [24] to [27], wherein the dimerizing linker binds to a second protein which is fused in-frame with the bait protein.

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[30] The method according to [29], wherein the second protein is FKBP12 protein and the dimerizing linker comprises FK506 at one end, so that the dimerizing linker is linked to the bait protein via binding between FK506 and FKBP12 protein.

[31] The method according to [24] to [30], wherein the ends of the dimerizing linker are linked by polyethylene glycol.

[32] The method according to [20] to [31], wherein the bait-prey protein complex trapped inside the artificial virus-like particles is analysed by mass spectrometry.

[33] The method according to [32] wherein the artificial virus-like particles are purified prior to analysis.

[34] The method according to [20] to [33] wherein specific protein-protein interactions are detected as compared to background protein-protein interactions from reference virus-like particles comprising HIV p55 GAG protein without the bait protein anchored to it, or as compared to background protein-protein interactions from reference virus-like particles comprising HIV p55 GAG protein to which is anchored another bait protein other than the bait protein recited in [20].

[35] A method of detecting a specific protein-protein interaction in a cell, the method comprising:

(1) expressing in the cell a recombinant fusion protein comprising an HIV p55 Group Antigens (GAG) protein and a bait protein, wherein the expressed fusion protein forms a protein-protein complex with an endogenously-expressed prey protein that is a native physiological binding partner of the bait protein;

(2) forming virus-like particles comprising the protein-protein complex;

(3) isolating virus-like particles budded from the cell; and

(4) detecting the specific interaction between the bait protein and the prey protein in the virus-like particles as compared to background protein-protein interactions from reference virus-like particles comprising HIV p55 GAG protein without the bait protein anchored to it, thereby detecting the specific protein-protein interaction in the cell.

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[36] A method of detecting a specific protein-protein interaction in a mammalian cell, the method comprising:

(1) expressing in the mammalian cell a recombinant fusion protein comprising an HIV p55 Group Antigens (GAG) protein and a bait protein, wherein the expressed fusion protein forms a protein-protein complex with an endogenously-expressed prey protein that is a native physiological binding partner of the bait protein;

(2) forming virus-like particles comprising the protein-protein complex;

(3) isolating virus-like particles budded from the mammalian cell; and

(4) detecting the specific interaction between the bait protein and the prey protein in the virus-like particles as compared to background protein-protein interactions from reference virus-like particles comprising HIV p55 GAG protein to which is anchored a bait protein different from the bait protein recited in (1), thereby detecting the specific protein-protein interaction in the mammalian cell.

[37] The use according to [18] or [19] wherein the cell is a mammalian cell.

[38] The use according to [37], wherein the mammalian cell is a human cell.

[39] The method according to [20] to [35] wherein the cell is a mammalian cell.

[40] The method according to [39], wherein the mammalian cell is a human cell.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Principle of conditional protein complex trapping in virotrap particles. A bait protein is fused in frame to FKBP12. Upon addition of Mtx-PEG-FK506 dimerizer, the FKBP12-bait fusion protein is recruited to the GAG-eDHFR fusion protein, leading to trapping of the bait and its interacting proteins in the virotrap particles that are formed by GAG multimerization and budding. Further purification of virotrap particles followed by proteomic analysis results in identification of the interacting proteins.

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Figure 2: Plasmid map for the pMET7-VT1-MCS vector. Transfer of the coding sequences for EGFP led to the pMET7-VT1-EGFP vector respectively, transfer of the GATEWAY® cassette resulted in the pMET7-VT1-GW, which then allowed transfer of the CSK1B coding sequence from a GATEWAY® entry clone, leading to pMET7-VT1-CKS1B. MCS: multi-cloning site.

Figure 3: Western blot showing the interaction between CSK1B bait and CDK2 prey upon addition of dimerizer (5 and 10 µM). HEK293T cells were transfected with the pMET7-VT1-CSK1B or pMET7-VT1-EGFP bait constructs, combined with the E-tagged CDK2 prey construct. After purification, the nanoparticles were directly brought in 2x SDS-PAGE loading buffer and loaded for SDS-PAGE separation and western blot analysis. The 3 upper panels show samples prepared from purified particles. The lower panels are lysate samples obtained from the producer cells confirming similar expression levels for the different components.

Figure 4: A: Schematic representation of the Virotrap strategy. Expression of a GAG-bait fusion protein results in the submembrane multimerization and consequent budding of virotrap particles from the cells. Interaction partners of the bait protein are also trapped in these

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virotrap particles and can be identified after purification and MS analysis. B. Supernatants from HEK293T cells transfected with different combination of bait proteins (GAG-EGFP control and GAG-Ras) and FLAG-tagged prey proteins (IRS2 and RAF), were harvested after 24 hours, and were processed by ultracentrifugation to pellet the particles. Particle pellets were separated by SDS-PAGE and probed after Western blotting with anti-FLAG antibodies. C. HEK293T cells were seeded in 6 well plates and transfected with bait and prey combinations as in (B). Both wildtype and E-tagged VSV-G glycoproteins were expressed to allow particle enrichment via a single step protocol from a 1 ml harvest. Western blotting of the eluted particles was performed with anti-FLAG and anti-GAG antibodies. D. Virotrap experiments for 2 interaction pairs in both directions. Single step purifications via anti-FLAG antibodies from 6 well transfections were loaded for Western blotting and probed with anti-Etag, anti-GAG and anti-Actin antibodies for both the enriched particles and the producer cell lysates. The expression of GAG-S100A1 and GAG-S100B was below the detection limit in the lysates. Note that expression of the GAG protein without a fused bait does not lead to detectable particle formation.

Figure 5: Detection of binary interactions with Virotrap. Comparison of the results for the positive (PRS) and the random reference set (RRS) obtained with the Virotrap system with other binary systems. All interactions from the PRS and RRS were explored by transfection in 6 well plates, processing by a single step purification protocol, and Western blot analysis of the eluted particles and lysates of the producer cells. The presence of prey proteins was revealed by anti-Etag antibodies on the VLP samples. The colored blocks show the Virotrap results for 30% positive interactions at the expense of 5% false positive signals in the RRS set. For the other methods, we used data from Braun et al., 2009

Figure 6: Identification of novel interaction partners using co-complex MS analysis. (A) Analysis of the CDK2 interactome using Virotrap. A total of 9 Virotrap experiments with on-bead VLP lysis was performed. The GAG-CDK2 identification list was challenged with identification lists from mock and GAG-EGFP controls, from GAG-FADD and from 5 additional Virotrap experiments. The CDK2 interactome (Right Panel Table) was obtained by removing all protein identifications found in the other experiments. *CKS1B was retained as it was also used as a GAG-BAIT construct in the reference experiments. Note that the CDK2-CKS1B interaction is a model interaction used for validation of the system. (B) The FADD interactome was obtained by adding 3 repeat experiments and controls using a specific elution protocol (scheme on the left). One of the FADD repeats was treated with TNFα during production. The protein list (right panel table) was obtained by considering only confident identifications (at least 2 peptides) and by removing all proteins identified in the 11 other experiments. The

numbers in brackets show the number of times the protein was identified in the 4 FADD experiments. *: interaction was shown with Casein Kinase 1 α (CSNK1A). **Affects FAS signaling. (C). Confirmation of the A20-FADD interaction. A20 was found in 2 FADD Virotrap experiments. The interaction was confirmed by co-immunoprecipitation experiments showing specific binding of A20 to immune-precipitated FADD (left panels), or of FADD to immune-precipitated A20 (right panels). Tagged proteins (FLAG and VSV tags) were expressed in HEK293T cells and were precipitated after lysis using paramagnetic anti-FLAG beads. The co-precipitated proteins were revealed by anti-VSV antibodies.

Figure 7: Amino acid sequence of Desmoglein 1 (SEQ ID NO: 26) with annotation of extracellular, transmembrane and cytoplasmic regions, and with mapping of the peptides identified in the Virotrap analysis with S100A1 bait protein.

EXAMPLES

Materials and methods

Plasmids and antibodies

The p55 GAG fusion constructs were generated by PCR amplification using primers Oligo1 and Oligo2 (see Table 1) of the p55 GAG coding sequence from the pCMV-dR8.74 packaging construct (Addgene) and by subsequent In-Fusion™ reaction (Clontech) in pMG1-Ras, a Ras expression vector used in the MAPPIT system (Eyckerman et al., 2001), resulting in a p55 GAG-RAS under control of the strong SRalpha promoter (pMET7-GAG-Ras). EGFP was transferred from pEGFP-C1 vector (Clontech) to generate the pMET7-GAG-EGFP construct. Using PCR-based cloning, a Gateway™ cassette was inserted to allow recombination assisted cloning. The complete set of positive and random reference clones were transferred in a single direction (no bait-prey swap) using standard GATEWAY™ cloning. Prey ORFs from these sets were transferred into a GATEWAY™-compatible pMET7 expression vector with a N-terminal E-tag fused in frame.

The pMD2.g pseudotyping vector was kindly provided by D. Trono. The pcDNA3-FLAG-VSV-G and pcDNA3-Etag-VSV-G are described elsewhere (Eyckerman et al., submitted).

Antibodies used for Western blot were anti-p24 GAG (Abcam), anti-FLAG (M2, Sigma Aldrich), anti-actin (Sigma Aldrich) and anti- E-tag (Phadia). Secondary antibodies were from LI-COR, and blots were digitally imaged using an ODYSSEY® Imager system (LI-COR).

Cell culture, production and purification of Virotrap particles.

HEK293T cells were cultured in a humidified atmosphere at 8% CO_2 using high glucose DMEM (Invitrogen) complemented with 10% FCS and antibiotics.

Cells were transfected overnight the day after seeding with a standard calcium phosphate transfection procedure. For ultracentrifugation experiments, we transfected 25 µg of bait vector (GAG-EGFP and GAG-Ras) normalized to 50 µg with a mock vector, in $6x10^6$ cells seeded the day before in $75cm^2$ bottles. For concentration of the virotrap particles, we harvested supernatant after 24 hrs, centrifuged samples for 3' at 1250xg to remove cellular debris and filtered the supernatant through 0.45 mm filters. The samples were then centrifuged in a Beckman ultracentrifuge using a Ti41 swinging bucket rotor at 22000 rpm. The supernatant was discarded and particle pellets were re-suspended directly in loading buffer for Western analysis.

For binary interaction assays, 650.000 HEK293T cells were seeded the day before transfection in 6 well plates. On the day of transfection, a DNA mixture was prepared containing the following: 3.5 µg bait construct (pMET7-GAG-bait), 0.8 µg prey construct (pMET7-E-tag prey or pMET7-FLAG-Raf), 0.7 µg pMD2.G and 1.4 µg pcDNA3-FLAG-VSV-G. Following overnight transfection, cells were washed once with PBS and 1 ml of fresh growth medium was added to the wells. Cellular debris was removed from the harvested supernatant by 3' centrifugation at 2000xg. The cleared medium was then incubated with 10 µl Dynabeads® MyOne™ Streptavidin T1 beads (Invitrogen)pre-loaded with 1 µg monoclonal ANTI-FLAG® BioM2-Biotin, Clone M2 (Sigma-Aldrich®) according to the manufacturer's protocol. After 2hrs binding at 4°C by end-over-end rotation, beads were washed 2 times with washing buffer (20mM HEPES pH 7.4, 150mM NaCl), and the captured particles were released directly in 35 µl 2x SDS-PAGE loading buffer. A 5' incubation step at 65°C before removal of the beads ensured complete release. After boiling, the samples were loaded on a 10% SDS-PAGE gel, or on commercial 4-12% gradient gels (Biorad), and after separation the proteins were transferred to Hybond™-C Extra nitrocellulose membranes (GE Healthcare). Lysates of the producer cells were prepared by direct addition of 200µl RIPA buffer (50mM TRIS.HCl pH 7.4, 150mM NaCl, 1% NP40, 1% sodium deoxycholate, 0.1% SDS + Complete protease inhibitor cocktail [Roche]) to the 6 well plates after washing of the cells in chilled PBS. The lysates were cleared by centrifugation at 13000xg, 4°C for 15' to remove the insoluble fraction.

The PRS and RRS were randomized and processed in sets of about 45 single PPI measurements. Each set was loaded on 2 4-12% gradient gels with 26 slots (Biorad). Each set of measurements also contained the GAG-EGFP expression control, a mock control and the interaction between GRAP2 and LCP2 as a positive control for Virotrap functionality. A single

pooled positive control for the GRAP2-LCP2 interaction was also loaded on each gel to allow cross-comparison between the gels. Bands were quantified by fluorescence signals with an Odyssey system (LICOR). The detection threshold was based on RRS signals and was determined for each individual gel.

For mass spectrometry, $4.75x10^6$ HEK293T cells seeded in 75 cm^2 bottles were transfected the next day with a total of 50 µg DNA. The following DNA quantities were used: GAG-bait 25 µg; mock vector 17.8 µg; 7.2 µg of a 50/50 pMD2.G - pcDNA3-FLAG-VSV-G mix. The cellular supernatant was harvested after 32 hrs and was centrifuged for 3' at 450xg to remove cellular debris. The cleared supernatant was then filtered using 0.45 µm filters (Millipore). A total of 100 µl MyOne™ Streptavidin T1 beads pre-loaded with 10 µl ANTI-FLAG® BioM2-Biotin antibody was used to bind the tagged particles. Particles were allowed to bind for 2hrs by end-over-end rotation. Bead-particle complexes were washed once with washing buffer and were then frozen overnight in lysis buffer (PBS, 0.4% CHAPS, 1.2M guanidine hydrochloride) to release and denature the trapped proteins. After lysis of the particles and removal of the beads, proteins were reduced (10 mM TCEP.HCl, 10 minutes at 37°C) and alkylated (20mM Iodoacetamide, 10 minutes at 37°C). Via NAP5 gel filtration columns (GE Healthcare), the protein sample was transferred to 10 mM ammonium bicarbonate buffer. Trypsin digest was performed overnight at 37°C using 0.5µg sequence-grade trypsin (Promega). Samples were vacuum dried and resuspended in 2% acetonitrile, separated by nano-LC and directly analyzed with a LTQ Orbitrap Velos instrument (Thermo Scientific). Searches were performed using the MASCOT algorithm at 99% confidence against the human Swissprot database complemented with HIV-1 and EGFP protein sequences.

Example 1: Generation of the conditional trapping construct

The p55 GAG fusion constructs were generated by PCR amplification using primers Oligo1 and Oligo2 (see Table 1) of the p55 GAG coding sequence from the pCMV-dR8.74 packaging construct (Addgene) and by subsequent In-Fusion™ reaction (Clontech) in a pMET7-gp130-RAS construct (Eyckerman et al., 2001). This resulted in a p55 GAG-fusion construct under control of the strong SRalpha promoter. The plasmid was designated pMET7-GAG-RAS. EGFP was transferred from pEGFP-C1 vector (Clontech) to generate the pMET7-GAG-EGFP construct. The eDHFR fragment was amplified from plasmid pSEL1-eDHFR (Caligiuri et al., 2006) with primers Oligo3 and Oligo4, digested with XhoI and XbaI and cloned in the SalI-XbaI opened pMET7-GAG-RAS backbone, which resulted in pMET7-GAG-eDHFR.The FKBP12 protein was amplified with primers Oligo5 and Oligo6 from pMG2-FKBP12 (Eyckerman et al., 2005). The PCR product was digested with NdeI and XbaI and cloned in the NdeI-XbaI opened pMET7-GAG-eDHFR vector, which resulted in the pMET7-GAG-eDHFR-FKBP12-MCS

construct. The reverse primer Oligo6 also encoded a flexible Gly-Gly-Ser hinge sequence and contained a number of restriction enzyme recognition sites. This multi-cloning site (MCS) allows different cloning strategies for the C-terminal fusion of a bait protein. The primers Oligo7 and Oligo8 were annealed and ligated into the NdeI-MluI opened pMET7-GAG-eDHFR-FKBP12-MCS construct to insert a FLAG tag sequence and a T2A auto-processing site. This resulted in pMET7-GAG-eDHFR-T2A-FLAG-FKBP12-MCS. The *Thosae asigna* 2A (T2A) auto-processing sequence ensures by a ribosomal skip mechanism (Szymczak et al., 2004) the complete cleavage of the fusion protein resulting in 2 protein fragments upon translation: the GAG-eDHFR part and the FKBP12-MCS part. The EcoRI site that was present within the eDHFR coding sequence was removed by using site-directed mutagenesis (Quickchange® Site-Directed Mutagenesis kit, Stratagene) with Oligo9 and Oligo10 on pMET7-GAG-eDHFR-T2A-FLAG-FKBP12-MCS, resulting in the pMET7-VT1-MCS construct.

The Gateway® cassette (Invitrogen) was amplified by primers Oligo11 and Oligo12 from pMG1-Gateway (Braun et al., 2009), and cloned via MfeI-XbaI in the EcoRI-XbaI opened pMET7-VT1-MCS plasmid, which resulted in the pMET7-VT1-GW destination vector. The coding sequence for CSK1B was transferred via the Gateway® LR reaction from the Positive Reference Set described in Braun et al. (Braun et al., 2009) into the pMET7-VT1-GW destination vector resulting in pMET7-VT1-CSK1B. The coding sequence for CDK2 was transferred by the LR reaction to a pMET7-Etag-Gateway® construct (Lievens *et al.*, unpublished) leading to pMET7-Etag-CDK2.

The pcDNA3-FLAG-VSV-G construct used for purification was generated as described (Eyckerman *et al.*, submitted). The pMD2.G construct expressing VSV-G under control of a strong CMV promoter was provided by Didier Trono (EPFL, Lausanne, Switzerland).

The chemical bivalent molecule or dimerizer consists out of methotrexate (Mtx) and FK506 linked via a PolyEthylene Glycol (PEG) linker, and was prepared as described in Caligiuri *et al* (Caligiuri et al., 2006).

Table 1: Oligonucleotides used for the generation of the pMET7-VT1 constructs.

Number	Sequence	Use	SEQ ID NO
Oligo1	CTCTAAAAGCTGCGGGGCCCGCTAGCGCCACCA TGGGTGCGAGAGCGTCAG	GAG amplification	1
Oligo2	TGTATTCGGTGAATTCTGAGCTCGTCGACCCGCC TTGTGACGAGGGGTCGCTGC	GAG amplification	2
Oligo3	GCGACTCGAGCGGAATCAGTCTGATTGCGG	eDHFR amplification	3
Oligo4	CGCTTCTAGATTACATATGGCCGCTGCCCCGCC GCTCCAGAATCTC	eDHFR amplification	4
Oligo5	GCGACATATGGGCACGCGTGTGCAGGTGGAAAC CATCTC	FKBP12 amplification	5

Number	Sequence	Use	SEQ ID NO
Oligo6	CGCTTCTAGATTACTCGAGTGCGGCCGCGAATTC TGAGCTCGTCGACCCGCCTTCCAGTTTTAGAAGC TCC	FKBP12 amplification	6
Oligo7	TATGGAGGGCAGAGGCAGCCTGCTGACCTGCGG CGACGTGGAGGAAAACCCCGGCCCCGATTACAA GGATGACGACGATAAGA	T2A and FLAG sequence annealing	7
Oligo8	CGCGTCTTATCGTCGTCATCCTTGTAATCGGGGC CGGGGTTTTCCTCCACGTCGCCGCAGGTCAGCA GGCTGCCTCTGCCCTCCA	T2A and FLAG sequence annealing	8
Oligo9	GAATCGGTATTCAGCGAGTTCCACGATGCTGATG	EcoRI mutagenesis	9
Oligo10	CATCAGCATCGTGGAACTCGCTGAATACCGATTC	EcoRI mutagenesis	10
Oligo11	CCCCAATTGACAAGTTTGTACAAAAAAGC	Gateway cassette amplification	11
Oligo12	GGGTCTAGATCAAACCACTTTGTACAAG	Gateway cassette amplification	12

Example 2: Production, harvest and western blot analysis

For production of virotrap particles, a co-transfection in HEK293T cells was performed via the Ca-Phosphate precipitation method. HEK293T cells were cultured in DMEM medium (Gibco) and 10%FCS at 37°C in a humidified atmosphere with 5% CO_2. The day before transfection, 650.000 cells were seeded in a 6-well plate. On the day of transfection, a DNA mixture was prepared containing the following:

0.7 µg pMD2.G

1.4 µg pcDNA3-FLAG-VSV-G

0.8 µg pMET7-Etag-CDK2

3.5 µg pMET7-VT1-CKS1B or pMET7-VT1-EGFP

15 µl of 2.5M $CaCl_2$

Water was added to a total volume of 150 µl.

The DNA mixture was then added dropwise to 150 µl 2xHeBs solution while vortexing. The transfection mix was brought on the cells and the precipitates were left overnight for transfection. Following transfection, the cells were washed once with PBS and 1 ml of fresh growth medium was added with either 5 or 10 µM of dimerizer, or without dimerizer. The production medium was harvested after 24hrs. Cellular debris was removed by 1' centrifugation at 2000 xg. The cleared medium was then incubated with 10 µl Dynabeads® MyOne™ Streptavidin T1 beads (Invitrogen) loaded with 1 µg monoclonal ANTI-FLAG® BioM2-Biotin, Clone M2 (Sigma-Aldrich®) according to the manufacturer's protocol. After 2hrs

binding at 4°C by end-over-end rotation, beads were washed 2 times with washing buffer (20mM HEPES pH 7.4, 150mM NaCl), and the captured nanoparticles were released directly in 35 µl 2x SDS-PAGE loading buffer. The samples were incubated for 5' at 65°C to ensure complete denaturation/release of the virotrap particles. After removal of the beads and boiling, the samples were loaded on a 10% SDS-PAGE gel, and after separation the proteins were transferred to Hybond™-C Extra nitrocellulose membranes (GE Healthcare). Lysates of the producer cells were prepared by direct addition of 200µl RIPA buffer (50mM TRIS.HCl pH 7.4, 150mM NaCl, 1% NP40, 1% sodium deoxycholate, 0.1% SDS + Complete protease inhibitor cocktail [Roche]) to the 6 well plates after washing of the cells in chilled PBS. The lysates were cleared by centrifugation at 13000 xg, 4°C for 15' to remove the insoluble fraction. Western blots were probed with mouse anti-E tag (Phadia, 1/1000), mouse anti-GAG (Abcam, 1/1000) or rabbit anti-VSV-G (Sigma/Aldrich, 1/5000). Secondary antibodies were from LI-COR, and blots were digitally imaged using an ODYSSEY® Imager system (LI-COR).

Example 3: The pMET7-VT1 construct

We first generated a plasmid for expression of the bait construct. We cloned the p55 GAG fragment in the pMET7 vector which drives expression via the strong SRα promoter. The *E. coli* derived Dihydrofolate reductase (DHFR) protein was fused C-terminally of GAG while the FK506-Binding Protein 12 (FKBP12) coding sequence was cloned via a T2A auto-processing site and a FLAG-tag, in frame and C-terminal of DHFR. The GAG-eDHFR-T2A-FKBP12 expression construct was followed by a multi-cloning site to allow efficient transfer of bait proteins into this expression vector (Figure 2).

A GATEWAY® cassette and an EGFP expression construct were inserted in the MCS of pMET7-VT1-MCS by standard cloning procedures. The CSK1B protein was transferred via the GATEWAY® LR reaction into the pMET7-VT1-GATEWAY® construct.

Example 4: Test of the CKS1B-CDK2 interaction

We make use of a well-described protein-protein interaction pair to test the conditional trapping in nanoparticles. The coding sequence for the CDC28 Protein Kinase Regulatory Subunit 1B (CKS1B) protein was fused to the FKBP12 in the VT1 vector. Addition of the chimeric dimerizer molecule, which consists of methotrexate (Mtx) and FK506 linked by a polyethylene glycol linker, would thus result in the recruitment of the CKS1B bait protein to the GAG protein and to the forming nanoparticles (Figure 3). We also transfected the pMET7-VT1-EGFP bait construct as a control for irrelevant associations. The interaction partner Cyclin-Dependent Kinase 2 (CDK2) which has a N-terminal E-tag sequence, was co-expressed with both the CSK1B bait protein and the EGFP control protein. We performed 3 separate transfections for

each bait construct in combination with the CDK2 prey. The first series was left untreated to verify dimerizer-independent interactions, while the second and third series were treated with 5 and 10 µM of dimerizer respectively. After enrichment and direct elution in SDS loading buffer buffer, the samples were loaded on a 10% PAGE gel and transferred to nitrocellulose membranes after migration. The membranes were first probed with antibodies directed against the E-tag revealing presence of the prey protein in dimerizer-treated and bait-specific conditions. Expression of GAG and VSV-G was verified by using specific antibodies. The expression of the prey (E-tag), GAG and VSV-G was also monitored in the lysates from the producer cells to ensure equal protein levels.

Clear dimerizer-specific recruitment of the prey construct to the particles can be shown in case of co-expression of the bait CSK1B and prey CDK2. Some weak background association independent from the dimerizer is observed when the EGFP bait is expressed.

Example 5: Evaluation of the viral- particle trap

To remove the homogenization step in classical AP-MS strategies, we reasoned that incorporation of a protein complex inside a secreted vesicle should 'trap' the interactions under native conditions and should protect the complex during the downstream purification process. As expression of the HIV1 p55 GAG protein allowed the formation of secreted particles, we explored the concept of packaged or 'wrapped' protein complexes by the generation of a plasmid for the expression of GAG fused in frame to a bait protein. A flexible hinge sequence was inserted to limit sterical interference. Figure 4A shows a schematic presentation of the Virotrap concept. The N-terminus of GAG is essential for membrane association through myristoylation and should thus remain available (Bryant and Ratner, 1990). All domains required for multimerization were still present in the expression construct. As a first PPI pair to evaluate the concept, we selected the H-Ras protein which lacked the myristoylation signal as a bait, combined with the cRAF prey protein. A GAG-EGFP construct and an IRS2 prey were used as irrelevant bait and prey respectively. Both preys contained a N-terminal FLAG tag to facilitate detection. A first method of particle enrichment was ultracentrifugation, a well-described strategy for the concentration of lentiviral particles for various cell biological applications. After co-expression of bait and prey proteins, cell supernatants were harvested, filtered and centrifuged. After removal of the supernatant, the pelleted particles were resuspended directly in loading buffer and loaded for SDS-PAGE. After Western blotting and revelation of the tagged cRAF protein, we could demonstrate clear enrichment of the prey protein only when the H-Ras bait protein was present (Figure 4B and 4C). Expression controls for the particles and the producer cell lysates showed comparable expression for all bait and prey constructs. These results showed that the interaction between H-Ras and cRAF can be

detected by co-packaging of bait and prey in secreted particles from live cells. A 'mock' empty vector for bait expression was also employed to exclude enrichment of the prey in exosomes that were also pelleted by the ultracentrifugation procedure.

To remove the tedious ultracentrifugation step, we have developed and optimized a single step enrichment protocol for particles in the supernatant. Briefly, co-expression of the classical VSV-G pseudotyping construct together with a tagged variant of this glycoprotein resulted in optimal presentation of the purification tag on the surface of the particles. Paramagnetic beads containing immune reagents for the affinity tag were then employed to capture the particles from the supernatant. We first confirmed the interaction between H-Ras and cRAF using this new purification strategy. In this case, the system was based on the co-expression of untagged VSV-G with E-tagged VSV-G for capture and purification. HEK293T cells were transfected in a 6-well format with GAG-RAS bait together with FLAG-RAF prey, and the controls used in the ultracentrifugation experiment. Virotrap particles were produced for 24hrs and were harvested in 1ml of supernatant. After purification using anti-E antibodies coupled to paramagnetic particles, SDS-page and western blotting, the preys were revealed using anti-FLAG antibodies. Clear and specific enrichment of cRAF-prey was shown for the H-Ras-bait protein. No or very little cRAF was revealed in case of a-specific bait, while no detectable irrelevant IRS2-prey was found for the H-Ras-bait.

We then explored the interaction between 2 protein pairs in both directions. These protein interactions were selected based on literature evidence on their confirmation in independent methods (Braun et al., 2009), and because both protein partners reside in the cytoplasm. In this case, we used the purification strategy with a FLAG-tagged VSV-G variant to replace the E-tagged VSV-G glycoprotein in the previous experiment. After purification by the one-step protocol using anti-FLAG resin, direct elution in PAGE loading buffer, and Western blotting for the E-tag, we could readily detect interactions between CDK2 and CKS1B and between S100A and S100B. By swapping bait and prey we were also able to show the interactions in both directions (Figure 4D). As controls we used irrelevant bait and prey constructs.

By design, the Virotrap system is ideally suited to study cytoplasmic interactions. To explore the uses and limitations and to compare to other existing technologies we have evaluated the concept by testing the human positive reference set (hsPRS-v1). The positive reference set consists of 92 PPIs that were selected based on literature data, while the random reference set is generated using 188 randomly selected proteins (hsRRS-v1; (Venkatesan et al., 2009). Both sets contain proteins from all cellular compartments to remove any bias in localization. All PPIs from the PRS were tested in the Virotrap technology by recombination-assisted transfer of one bait set in fusion to the GAG protein. Prey ORFs were transferred to an expression

vector resulting in N-terminally E-tagged fusion proteins. The PPIs were tested in a single direction implying no swap of bait and prey constructs. The same strategy was used for the RRS set, again without swapping of bait and prey proteins. All experiments were performed by transfection of bait and prey expression vectors in HEK293T cells in a 6 well format. One day after transfection, supernatants were harvested and processed using the one step purification protocol. Enriched particles were eluted from the paramagnetic beads in PAGE loading buffer and loaded on SDS-PAGE gels. A total of about 184 binary virotrap experiments was performed. Apart from positive and negative controls, the experiments were controlled for transfection and for immunoblotting efficiency. The presence of prey proteins in purified particles was revealed via anti-E tag immunoblots. The threshold of detection of true positives versus false positives was set for every individual gel. This led to the detection of 28 (31%) interactions in the PRS, while 5 (5%) interactions were detected in the RRS. We also verified expression of the bait protein fusions in the lysates of the producer cells, which showed that approximately 30% (56 out of 184 bait fusions) was not expressed at a detectable level. Although this could be explained by structural constraints in the fusion proteins or by interference with particle formation, we could only observe detectable expression for 25 out of 41 tested prey proteins (61%) of the prey proteins in the producer lysates, where only a N-terminal E-tag was inserted before the protein. We therefore believe that the current data provides an underestimate of the detectable interactions. Figure 5 shows the overlap between the Virotrap data and data obtained with other PPI methods for the PRS and RRS as published by Braun and colleagues (2009). 7 interactions out of the positive reference set can be detected with all methods, while 9 interactions are unique for the Virotrap method, proving the unique application window for the technology.

Example 6: Discovery of novel interaction partners using co-complex Virotrap

For the detection of novel interaction partners, we scaled up the purification procedure to compensate for a larger production scale.

We investigated the protein complexes of 2 cytosolic proteins in more detail: Cyclin-Dependent Kinase 2 (CDK2) and Fas-Associated via Death Domain (FADD). An important issue in typical MS co-complex strategies relates to the background. The background in Virotrap will contain GAG and VSV-G derived peptide sequences together with host binding partners, proteins implicated in budding, and serum proteins associated with the outside of the VLPs. To define these background proteins, we have included additional experiments in the design of this study to allow the construction of a comprehensive background list. In its simplest format, this list can be subtracted from the protein identifications that are specific for the bait (Figure 6A). We performed a total of 9 experiments (mock control, EGFP bait and 5 additional bait constructs).

Cells were co-transfected with bait proteins and constructs for purification. After harvest and purification, particles were lysed directly on the beads using a chaotropic lysis buffer. The lysates were then processed by reduction and alkylation, buffer exchange and trypsin digest. MS analysis followed by identification via MASCOT (99% confidence) resulted in the identification of between 140 (for LCP2 bait) to 277 (FADD) proteins by at least two unique peptides (Table 2). By comparing the identification lists, we were able to extract a background list of 306 proteins that were found in at least 2 of the lists for the different experiments (Table 3). By subtraction of this list from the CDK2 identification list, a limited set of 15 putative binding partners remains. Further removal of protein identifications that were also found with a single peptide in other experiments revealed a short list of 7 binding partners. For 4 of these candidates there is clear evidence in literature (Figure 6A). The list of unique proteins for FADD is more extensive (73 proteins), even after removal of proteins that were additionally found in one of the other experiments with a single peptide (35 proteins, Table 4). It is clear that this list contains real binding partners (Receptor interacting protein 1 RIPK1, Casein Kinase 1 alpha and epsilon) as well as unlikely binders. We therefore extended the analysis of FADD using additional Virotrap experiments using a specific elution protocol. In these experiments, we eluted the particles from the FLAG-antibody beads by competition with FLAG-peptide. The particles were then lysed in SDS, processed with detergent removal columns, and digested by trypsin. Controls included in these experiments were mock, EGFP bait and an expression construct of a GAG variant without a bait. We also performed 3 additional experiments for the FADD interactome (Figure 6B, Table 5 for overview). Combination of the identifications from these experiments and the previous 9 Virotrap assays resulted in a specific list of 3 candidate partners identified uniquely with at least 2 peptides in all 4 FADD experiments: Syndecan 4 (SDC4), Casein kinase 1 epsilon (CSNK1E) and RIPK1. The list of candidate partners identified in 2 out of 4 experiments contained also 2 additional kinases : YES1 and Cyclin-Dependent Kinase 1 (CDK1) (Figure 6B, Right panel table).

Relaxing the criteria where all identifications (including single peptide identifications) in fewer of the repeat samples were included, revealed FAS receptor as candidate interaction partner in the lists of identifications, while TRADD was also found in a single FADD experiment. A20 (TNFAIP3) was identified in our first experiment series, and could be confirmed upon treatment of the cells with TNFα during Virotrap particle production. The interaction between FADD and A20 was also shown by orthogonal co-immunoprecipitation (Co-IP) experiments (Figure 6C).

Table 2: Overview of the proteomics data obtained for different GAG-bait constructs.

Results were obtained by searches of MS/MS data against all human and bovine SWISSPROT accessions, complemented with HIV-1, EGFP and VSV-G sequences by using

MASCOT software. False discovery rates (FDRs) were determined by MASCOT searches against a database containing all sequences after reversion. Numbers are shown for all identified proteins (all) or proteins identified with at least 2 peptides (>1 peptide). Unique proteins were obtained by only considering protein identifications with at least 2 peptides after removal of proteins that were identified in one of the other Virotrap experiments.

	PRIDE Experiment Accession	# Spectra	# Peptide sequences	# Proteins (all)	# Proteins (> 1 peptide)	# Unique proteins (>1 peptide)	FDR (%)
Mock	28956	7529	1093	297	158	6	0.4
GAG-EGFP	28955	8142	1253	368	193	17	0.4
GAG-CDK2	28954	7618	1237	360	195	16	0.3
GAG-FADD	28959	6752	1889	463	277	73	0.7
GAG-TRAF3	28958	5461	1411	329	173	11	0.7
GAG-CKS1B	28953	7486	1246	374	218	33	0.4
GAG-LCP2	28951	7728	1264	260	140	9	0.4
GAG-GRAP2	28952	6768	1197	340	189	7	0.4
GAG-S100A	28957	6399	1361	311	184	21	0.3

Table 3 List of background proteins that were found in at least 2 out of 9 Virotrap experiments. Both SWISSPROT accessions and gene names are shown, as well as the number of Virotrap experiments containing the protein (x/9).

accession	Gene symbol	Times identified
A5A3E0	POTEF	9
A5PJE3	FGA	9
E1B7N2	LOC619094	9
E1B8G9	HIST3H2BB	9
E1B953	TUBB	9
E1B9F6	LOC100848359	9
E1B9K1	UBC	9
E1BH06	LOC617696	9
E1BJB1	TUBB2A	9
F1MI18	LOC506828	9
F1MNF8	LOC100141266	9
F1MNW4	ITIH2	9
F1MRD0	ACTB	9
F1MSZ6	SERPINC1	9
F1MYN5	FBLN1	9

accession	Gene symbol	Times identified
F1N5M2	GC	9
G3N2V5	HSP90AB1	9
G3X6N3	TF	9
G5E513	Bt.12809	9
O00560	SDCBP	9
O46375	TTR	9
P00735	F2	9
P01966	HBA	9
P02081	HBBF_BOVIN Hemoglobin fetal subunit beta OS=Bos taurus PE=1 SV=1	9
P02769	ALB	9
P07437	TUBB	9
P07900	HSP90AA1	9
P08107	HSPA1A	9
P08670	VIM	9
P11142	HSPA8	9
P12268	IMPDH2	9
P12763	AHSG	9
P15497	APOA1	9
P22626	HNRNPA2B1	9
P28800	SERPINF2	9
P34955	SERPINA1	9
P35580	MYH10	9
P56652	ITIH3	9
P81187	CFB	9
P81644	APOA2	9
Q00839	HNRNPU	9
Q03247	APOE	9
Q07020	RPL18	9
Q08431	MFGE8	9
Q13813	SPTAN1	9
Q3SZ57	AFP	9
Q3SZR3	ORM1	9
Q3ZBS7	VTN	9
Q58D62	FETUB	9
Q7SIH1	A2M	9
Q9BQA1	WDR77	9
A2I7M9	SERPINA3-2	8
F1MGU7	FGG	8
F1MMK9	AMBP	8
Q29443	TF	8
Q2KJF1	A1BG	8
Q3SZV7	HPX	8
F1MBQ8	DDX5	8
F2Z4C1	TUBA3C	8
G5E507	HSP90AB1	8
P17697	CLU	8
P35527	KRT9	8
P60709	ACTB	8
Q3SYW6	EIF3C	8
A1A4R1	HIST2H2AC	8

accession	Gene symbol	Times identified
P01045	KNG2	8
P26373	RPL13	8
12831136	gb|AAK08483.1|AF324493_2 gag polyprotein [HIV-1 vector pNL4-3]	8
A6NKZ8	YI016_HUMAN Putative tubulin beta chain-like protein ENSP00000290377 OS=Homo sapiens PE=5 SV=2	8
Q28107	F5	8
P04264	KRT1	8
P13645	KRT10	8
A6QLG5	RPS9	8
A7E350	PLG	8
E1BEG2	HNRNPA3	8
G8JKY0	RPS8	8
P15311	EZR	8
P39023	RPL3	8
P62424	RPL7A	8
A2I7N3	SERPINA3-7	7
A7E307	DDX17	7
F1MJH1	GSN	7
F1MY44	HNRNPM	7
G1K122	RBP4	7
O43175	PHGDH	7
P00003	FLAG-VSVG	7
P12259	F5	7
P61978	HNRNPK	7
Q9TTJ5	RGN	7
Q3Y5Z3	ADIPOQ	7
E1BF20	HNRNPH1	7
G5E5T5	G5E5T5_BOVIN Uncharacterized protein (Fragment) OS=Bos taurus PE=4 SV=1	7
296556483	gb|AAK08484.2|AF324493_3 pol polyprotein [HIV-1 vector pNL4-3]	7
E1B7J1	E1B7J1_BOVIN Elongation factor 1-alpha OS=Bos taurus PE=3 SV=1	7
E1BAK6	DAZAP1	7
F1MWU9	HSPA6	7
P40429	RPL13A	7
A5D9B4	HNRPH2	7
E1BHA5	E1BHA5_BOVIN Uncharacterized protein OS=Bos taurus PE=4 SV=1	7
P09651	HNRNPA1	7
P11940	PABPC1	7
P61353	RPL27	7
A2VE06	RPS4Y1	7
G3N262	G3N262_BOVIN Uncharacterized protein OS=Bos taurus PE=3 SV=1	7
P62269	RPS18	7
P26038	MSN	7
A7YW45	PRMT5	6
G3MYZ3	AFM	6
Q05443	LUM	6
F1MY85	C5	6
A6NHL2	TUBAL3	6

accession	Gene symbol	Times identified
P60842	EIF4A1	6
F1MQ37	MYH9	6
A6QPP2	SERPIND1	6
P35579	MYH9	6
P35908	KRT2	6
P29966	MARCKS	6
E1B7R4	EIF3A	6
P13639	EEF2	6
P12277	CKB	6
P23528	CFL1	6
E1B8G4	E1B8G4_BOVIN Uncharacterized protein OS=Bos taurus PE=3 SV=2	6
P62917	RPL8	6
Q8IX12	CCAR1	6
P04406	GAPDH	6
P14618	PKM	6
Q0VCZ3	YTHDF2	6
G3MX91	TARDBP	6
F1MI47	RBM14	5
A5D784	CPNE8	5
G3N0S9	LOC515150	5
F1MNV5	KNG1	5
Q2UVX4	C3	5
A5PK20	HIST1H1E	5
F1MYC9	SPTBN1	5
F1MVC0	CAD	5
E1BGR6	E1BGR6_BOVIN Uncharacterized protein OS=Bos taurus PE=3 SV=1	5
P62249	RPS16	5
P83731	RPL24	5
G3X861	G3X861_BOVIN Uncharacterized protein (Fragment) OS=Bos taurus PE=3 SV=1	5
P62280	RPS11	5
P08865	RPSA	5
A5PKD6	GNB4	5
F1MKC4	F1MKC4_BOVIN Uncharacterized protein OS=Bos taurus PE=3 SV=2	5
P26641	EEF1G	5
Q06830	PRDX1	5
P09543	CNP	5
F1MMD7	ITIH4	5
Q3T052	ITIH4	4
P02768	ALB	4
P55884	EIF3B	4
P02538	KRT6A	4
A7MAZ5	HIST1H1D	4
P35613	BSG	4
Q3SZH5	AGT	4
P39060	COL18A1	4
P60033	CD81	4
P62937	PPIA	4
Q01082	SPTBN1	4

accession	Gene symbol	Times identified
Q08E32	CHMP4B	4
A5PK61	H3F3C	4
Q13151	HNRNPA0	4
F1MB60	RPS26	4
P23396	RPS3	4
F1MMP5	ITIH1	4
O15372	EIF3H	4
P46777	RPL5	4
Q02543	RPL18A	4
G3X7A5	C3	4
E1BE42	E1BE42_BOVIN Uncharacterized protein OS=Bos taurus PE=3 SV=1	4
Q9Y265	RUVBL1	4
O18789	RPS2	4
G3N2F0	G3N2F0_BOVIN Elongation factor 1-alpha OS=Bos taurus PE=3 SV=1	4
P62913	RPL11	4
A6NMY6	ANXA2P2	4
E1BB17	HNRNPH3	4
P08779	KRT16	4
F6QVC9	ANXA5	4
E1BNB4	PABPC1L	4
A6H769	RPS7	4
E1BAT6	E1BAT6_BOVIN Uncharacterized protein OS=Bos taurus PE=3 SV=1	4
Q562R1	ACTBL2	4
Q8WUM4	PDCD6IP	4
P19338	NCL	3
Q3SYR0	SERPINA7	3
P01614	KV201_HUMAN Ig kappa chain V-II region Cum OS=Homo sapiens PE=1 SV=1	3
P17690	APOH	3
G3N361	NONO	3
P63243	GNB2L1	3
P84103	SRSF3	3
Q01130	SRSF2	3
G8JKV5	RPL14	3
F1MJM0	ZNF326	3
F1ML72	RPL34	3
F1MSD2	RUVBL2	3
O15371	EIF3D	3
P08621	SNRNP70	3
P18621	RPL17	3
G3X8B1	LOC613401	3
P62935	PPIA	3
O43242	PSMD3	3
P41252	IARS	3
P49327	FASN	3
Q3MHL4	AHCY	3
Q86YQ8	CPNE8	3
G5E604	G5E604_BOVIN Uncharacterized protein (Fragment) OS=Bos taurus PE=4 SV=1	3

accession	Gene symbol	Times identified
Q12906	ILF3	3
A7MB16	EIF3B	3
F1MH40	Bt.57604	3
E1BCL3	LOC507211	3
P54727	RAD23B	3
G3N2D7	IGLL1	3
A7MBG8	RUVBL1	3
F1MZ00	SNRPD3	3
F1N6C0	F1N6C0_BOVIN Uncharacterized protein OS=Bos taurus PE=4 SV=2	3
Q13310	PABPC4	3
F1MXE4	PSMD6	3
A4IFP7	ARF5	3
F1N0E5	CCT4	3
P46779	RPL28	3
A5PK39	TPP2	3
P78371	CCT2	3
P02786	TFRC	3
F1MPU0	CLTC	3
O14744	PRMT5	3
Q969P0	IGSF8	3
Q9P2B2	PTGFRN	3
P07224	PROS1	2
Q28085	CFH	2
F1MG05	EEF1G	2
F1MLW8	LOC100847119	2
E1BMJ0	LOC100847889	2
A0JND2	KRT80	2
B8Y9S9	FN1	2
P02656	APOC3	2
Q3MHN2	C9	2
F6QYV9	SSRP1	2
P02253	HIST1H1C	2
P07910	HNRNPC	2
P08758	ANXA5	2
P43243	MATR3	2
Q9Y2W1	THRAP3	2
E1BQ37	SFPQ	2
P11586	MTHFD1	2
A6QLT5	UBAP2L	2
E1B9M9	LOC525863	2
P20645	M6PR	2
Q96EP5	DAZAP1	2
P40227	CCT6A	2
P61024	CKS1B	2
G1K134	Bt.57435	2
P84090	ERH	2
P30101	PDIA3	2
F1MZ92	YBX1	2
A4IFC3	PABPC4	2
A5PK63	RPS17	2

accession	Gene symbol	Times identified
E1BCF5	RPL26L1	2
F1MHJ6	F1MHJ6_BOVIN 60S ribosomal protein L18a OS=Bos taurus PE=3 SV=2	2
F1MLH6	CALM2	2
P05543	SERPINA7	2
P13010	XRCC5	2
Q15366	PCBP2	2
E1BF81	SERPINA6	2
P16403	HIST1H1C	2
G5E531	TCP1	2
P02788	LTF	2
P08238	HSP90AB1	2
P62194	PSMC5	2
P04350	TUBB4A	2
P02533	KRT14	2
Q5D862	FLG2	2
D3IVZ2	DDX3Y	2
O75131	CPNE3	2
P57721	PCBP3	2
Q5VW32	BROX	2
E1BKM4	PDCD6IP	2
P60660	MYL6	2
F1MZV2	CHMP5	2
O75340	PDCD6	2
P29144	TPP2	2
A5D9H5	HNRPD	2
A6NIZ1	RP1BL_HUMAN Ras-related protein Rap-1b-like protein OS=Homo sapiens PE=2 SV=1	2
A7Z057	YWHAG	2
E1B726	PLG	2
E1B7T4	E1B7T4_BOVIN Uncharacterized protein OS=Bos taurus PE=3 SV=2	2
E1BK63	E1BK63_BOVIN Ribosomal protein L15 OS=Bos taurus PE=3 SV=1	2
E1BNR0	Bt.110587	2
G3MYE2	G3MYE2_BOVIN Uncharacterized protein (Fragment) OS=Bos taurus PE=3 SV=1	2
O14828	SCAMP3	2
P00004	VSVG	2
P05023	ATP1A1	2
P06733	ENO1	2
P08195	SLC3A2	2
P18124	RPL7	2
P27635	RPL10	2
P36578	RPL4	2
P49006	MARCKSL1	2
P52272	HNRNPM	2
P53985	SLC16A1	2
P61204	ARF3	2
P62258	YWHAE	2
P62847	RPS24	2
Q02878	RPL6	2

accession	Gene symbol	Times identified
Q14152	EIF3A	2
Q15758	SLC1A5	2
Q53EZ4	CEP55	2

Table 4: List of FADD interaction partners.

List of FADD interaction partners after removal of all proteins (including single peptide protein identifications) that were found in at least one of the other Virotrap experiments. Proteins in bold have been linked to FADD or to FAS signaling before.

	Accession	Protein
1	A2VDY3	CHMP4A
2	A4FUC2	HNRNPUL1
3	A6QLS9	RAB10
4	E1BAF6	PRRC2A
5	**F1MND1**	**CDC42**
6	F1MSI2	AGRN
7	F1MX61	SF3B1
8	G3N3Q3	G3N3Q3_BOVIN
9	G5E5V7	G5E5V7_BOVIN
10	O00232	PSMD12
11	O00299	CLIC1
12	O60884	DNAJA2
13	P01891	HLA-A
14	P05556	ITGB1
15	**P06493**	**CDK1**
16	P07195	LDHB
17	P11017	GNB2
18	**P21580**	**TNFAIP3**
19	**P31431**	**SDC4**
20	P31689	DNAJA1
21	P48643	CCT5
22	**P48729**	**CSNK1A1**
23	**P49674**	**CSNK1E**
24	P60174	TPI1
25	P61247	RPS3A
26	P62871	GNB1
27	P62888	RPL30
28	**Q13158**	**FADD**
29	**Q13546**	**RIPK1**
30	Q148F1	CFL2
31	Q8TB73	NDNF
32	Q99661	KIF2C
33	Q9NRX5	SERINC1
34	Q9UN37	VPS4A
35	Q9Y5K6	CD2AP

Table 5: Overview of the proteomics data obtained for different GAG-bait constructs using specific elution of particles from the purification beads.

Results were obtained by searches of MS data against all human and bovine SWISSPROT accessions, complemented with HIV-1, EGFP and VSV-G sequences. False discovery rates (FDRs) were determined by MASCOT searches against a database containing all sequences after inversion. *an alternative codon-optimized GAG construct without a bait was used to generate particles. **GAG-FADD Virotrap particles were produced in the presence of TNFα.

	PRIDE Experiment Accession	# Spectra	# Peptide sequences	# Proteins (all)	# Proteins (> 1 peptide)	# Unique proteins (>1 peptide)	FDR (%)
mock	28963	6706	557	169	95	14	0.6
sGAG*	28965	7661	595	255	117	18	0.4
GAG-EGFP	28964	6450	397	242	114	29	1.7
GAG-FADD	28962	7546	864	375	174	20	0.7
GAG-FADD	28960	8377	517	166	98	2	0.4
GAG-FADD**	28961	8388	717	231	121	9	0.5

Example 7: Identification of desmosomal components

By employing a similar background removal strategy for the S100A1 bait as for the CDK2 bait (i.e. removal of all a-specific proteins identified) in the first set of 9 experiments, we obtained a list of 10 putative interaction partners identified with at least two peptides (Table 6). Remarkably, 3 components of the desmosome can be found in this list (Desmoplakin DSP, Desmoglein 1 DSG1 and Junction Plakoglobin JUP), as well as 2 keratin proteins not found in other bait proteins (thus not constituting classical contaminating keratins). These keratins are known intermediate filament components that use the desmosome for anchoring (Kitajima, 2013). The link between S100 proteins and desmosomes is hinted in literature. The S100A10 and S100A11 can be found together with desmosomal proteins in the cornified envelope (Robinson et al., 1997). Various members of the S100 family of proteins have been implicated in inflammatory skin disorders affecting the integrity of the skin such as psoriasis (Eckert et al.,2004). In addition, it is clear that these calcium-binding proteins play an important role in metastasis of tumors, both in the primary tumor cells and the metastatic niche (Lukanidin and Sleeman, 2012). The S100A1 protein also plays an important role in striated

muscle and has been implicated in myocardial (dys)function and heart failure (Krause et al., 2009).

In figure 7, we show the mapping of the identified peptides of Desmoglein 1 on the amino acid sequence. We were able to identify peptides from both the extracellular part and the intracellular part of the protein. In Table 7, the identified peptides for Desmoglein 1 are shown with their MASCOT scores. This data clearly supports the fact that Virotrap allows the detection of transmembrane prey proteins.

Table 6: Putative interaction partners for the S100A1 bait protein. Desmosomal or intermediate filament proteins are annotated in the comments column.

Accession	Protein	Comment
P05089	ARG1	
P13647	KRT5	Intermediate filament
P14923	JUP	Desmosome component
P15924	DSP	Desmosome component
P23297	**S100A1**	**BAIT**
Q02413	DSG1	Desmosome component
Q6UWP8	SBSN	
Q8N1N4	KRT78	Intermediary filament
Q96P63	SERPINB12	
Q99816	TSG101	
Q9UK41	VPS28	

Table 7: the identified peptides for Desmoglein 1 (DSG1, Swissprot Accession Q02413) are shown with their respective MASCOT scores.

Peptide	start aa position	end aa position	modified peptide sequence	MASCOT score	SEQ ID NO
I	439	445	NH2-TGKLTLK-COOH	33	13
E	916	925	NH2-ESSNVVVTER-COOH	77	14
J1	326	332	NH2-TNVGILK-COOH	41	15
F	198	213	NH2-IIRQEPSDSPM(oxid)FIINR-COOH	57	16
A	129	144	NH2-ALNSMGQDLERPLELR-COOH	48	17
M+K	391	422	NH2-TYVVTGNMGSNDKVGDFVATDLDTGRPSTTVR-COOH	84	18
F	198	213	NH2-IIRQEPSDSPMFIINR-COOH	74	19
D	220	238	NH2-TM(oxid)NNFLDREQYGQYALAVR-COOH	73	20
L	423	438	NH2-YVMGNNPADLLAVDSR-COOH	107	21
D	220	238	NH2-TMNNFLDREQYGQYALAVR-COOH	75	22
J2	333	352	NH2-VVKPLDYEAMQSLQLSIGVR-COOH	49	23
G	87	105	NH2-ISGVGIDQPPYGIFVINQK-COOH	118	24
B	369	390	NH2-ASAISVTVLNVIEGPVFRPGSK-COOH	86	25

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SEQUENCE LISTING IN ELECTRONIC FORM

In accordance with Section 111(1) of the Patent Rules, this description contains a sequence listing in electronic form in ASCII text format (file: 29775-146 Seq 17-11-2014 v1.txt).

A copy of the sequence listing in electronic form is available from the Canadian Intellectual Property Office.

CLAIMS:

1. An artificial virus-like particle comprising an HIV viral particle forming p55 Group Antigens (GAG) polypeptide, wherein the artificial virus-like particle budded from a cell, wherein a bait protein bound to a prey protein is trapped inside the artificial virus-like particle, wherein the prey protein is a native physiological binding partner of the bait protein, wherein the bait protein is anchored to the p55 GAG polypeptide, and wherein:

the bait protein is anchored to the p55 GAG polypeptide by in-frame fusion between the bait protein and the p55 GAG polypeptide, or

the bait protein is anchored to the p55 GAG polypeptide via a flexible hinge between the bait protein and the p55 GAG polypeptide, or

the bait protein is anchored to the p55 GAG polypeptide via a dimerizing linker which binds at one end to the p55 GAG polypeptide and which binds at the other end to the bait protein.

2. The artificial virus-like particle according to claim 1, wherein the bait protein is anchored to the p55 GAG polypeptide by in-frame fusion between the bait protein and the p55 GAG polypeptide.

3. The artificial virus-like particle according to claim 1, wherein the bait protein is anchored to the p55 GAG polypeptide via a flexible hinge between the bait protein and the p55 GAG polypeptide.

4. The artificial virus-like particle according to claim 1, wherein the bait protein is anchored to the p55 GAG polypeptide via a dimerizing linker which binds at one end to the p55 GAG polypeptide and which binds at the other end to the bait protein.

5. The artificial virus-like particle according to claim 4, wherein the dimerizing linker binds to the p55 GAG polypeptide directly.

Date Reçue/Date Received 2021-03-05

6. The artificial virus-like particle according to claim 4, wherein the dimerizing linker binds to a first protein which is fused in-frame with the p55 GAG polypeptide.

7. The artificial virus-like particle according to claim 6, wherein the first protein is dihydrofolate reductase (DHFR) and the dimerizing linker comprises methotrexate at one end, so that the dimerizing linker is linked to the p55 GAG polypeptide via binding between methotrexate and DHFR.

8. The artificial virus-like particle according to any one of claims 4 to 7, wherein the dimerizing linker binds to the bait protein directly.

9. The artificial virus-like particle according to any one of claims 4 to 7, wherein the dimerizing linker binds to a second protein which is fused in-frame with the bait protein.

10. The artificial virus-like particle according to claim 9, wherein the second protein is FKBP12 protein and the dimerizing linker comprises FK506 at one end, so that the dimerizing linker is linked to the bait protein via binding between FK506 and FKBP12 protein.

11. The artificial virus-like particle according to any one of claims 4 to 10, wherein the ends of the dimerizing linker are linked by polyethylene glycol.

12. The artificial virus-like particle according to any one of claims 1 to 11, wherein the bait protein and the prey protein are native physiological binding partners in the cytoplasm.

13. The artificial virus-like particle according to any one of claims 1 to 12 further comprising a purification tag on the surface of the particle.

14. The artificial virus-like particle according to claim 13 wherein the purification tag is a tagged vesicular stomatitis virus- glycoprotein G (VSV-G) protein.

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15. The artificial virus-like particle according to any one of claims 1 to 14 which is budded from a mammalian cell.

16. The artificial virus-like particle according to claim 15, wherein the mammalian cell is a human cell.

17. Use of an HIV viral particle forming p55 Group Antigens (GAG) polypeptide to detect at least one interaction between a bait protein and a prey protein, wherein the bait protein and the prey protein are native physiological binding partners, wherein the bait protein is anchored to the p55 GAG polypeptide, and wherein:

the bait protein is anchored to the p55 GAG polypeptide by in-frame fusion between the bait protein and the p55 GAG polypeptide, or

the bait protein is anchored to the p55 GAG polypeptide via a flexible hinge between the bait protein and the p55 GAG polypeptide, or

the bait protein is anchored to the p55 GAG polypeptide via a dimerizing linker which binds at one end to the p55 GAG polypeptide and which binds at the other end to the bait protein.

18. Use of an HIV viral particle forming p55 Group Antigens (GAG) polypeptide to trap a bait protein together with its physiological binding partner(s) in a virus-like particle budded from a cell, wherein the bait protein is anchored to the p55 GAG polypeptide, and wherein:

the bait protein is anchored to the p55 GAG polypeptide by in-frame fusion between the bait protein and the p55 GAG polypeptide, or

the bait protein is anchored to the p55 GAG polypeptide via a flexible hinge between the bait protein and the p55 GAG polypeptide, or

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the bait protein is anchored to the p55 GAG polypeptide via a dimerizing linker which binds at one end to the p55 GAG polypeptide and which binds at the other end to the bait protein.

19. The use according to claim 18, wherein the bait protein and its physiological binding partner(s) are native physiological binding partners in the cytoplasm.

20. A method for detecting specific protein-protein interactions in an artificial virus-like particle budded from a cell, comprising:

(1) expressing in the cell an artificial virus-like particle comprising an HIV viral particle forming p55 Group Antigens (GAG) polypeptide to which a bait protein is anchored;

(2) recruiting a prey protein to the bait protein wherein the prey protein is a native physiological binding partner of the bait protein;

(3) isolating the artificial virus-like particles budded from the cell; and

(4) analyzing the bait-prey protein complex trapped inside the artificial virus-like particle for specific protein-protein interaction.

21. The method according to claim 20, wherein:

the bait protein is anchored to the p55 GAG polypeptide by in-frame fusion between the bait protein and the p55 GAG polypeptide, or

the bait protein is anchored to the p55 GAG polypeptide via a flexible hinge between the bait protein and the p55 GAG polypeptide, or

the bait protein is anchored to the p55 GAG polypeptide via a dimerizing linker which binds at one end to the p55 GAG polypeptide and which binds at the other end to the bait protein.

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22. The method according to claim 20, wherein the bait protein is anchored to the p55 GAG polypeptide by in-frame fusion between the bait protein and the p55 GAG polypeptide.

23. The method according to claim 20, wherein the bait protein is anchored to the p55 GAG polypeptide via a flexible hinge between the bait protein and the p55 GAG polypeptide.

24. The method according to claim 20, wherein the bait protein is anchored to the p55 GAG polypeptide via a dimerizing linker which binds at one end to the p55 GAG polypeptide and which binds at the other end to the bait protein.

25. The method according to claim 24, wherein the dimerizing linker binds to the p55 GAG polypeptide directly.

26. The method according to claim 24, wherein the dimerizing linker binds to a first protein which is fused in-frame with the p55 GAG polypeptide.

27. The method according to claim 26, wherein the first protein is dihydrofolate reductase (DHFR) and the dimerizing linker comprises methotrexate at one end, so that the dimerizing linker is linked to the p55 GAG polypeptide via binding between methotrexate and DHFR.

28. The method according to any one of claims 24 to 27, wherein the dimerizing linker binds to the bait protein directly.

29. The method according to any one of claims 24 to 27, wherein the dimerizing linker binds to a second protein which is fused in-frame with the bait protein.

30. The method according to claim 29, wherein the second protein is FKBP12 protein and the dimerizing linker comprises FK506 at one end, so that the dimerizing linker is linked to the bait protein via binding between FK506 and FKBP12 protein.

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31. The method according to any one of claims 24 to 30, wherein the ends of the dimerizing linker are linked by polyethylene glycol.

32. The method according to any one of claims 20 to 31, wherein the bait-prey protein complex trapped inside the artificial virus-like particles is analysed by mass spectrometry.

33. The method according to claim 32 wherein the artificial virus-like particles are purified prior to analysis.

34. The method according to any one of claims 20 to 33 wherein specific protein-protein interactions are detected as compared to background protein-protein interactions from reference virus-like particles comprising HIV p55 GAG protein without the bait protein anchored to it, or as compared to background protein-protein interactions from reference virus-like particles comprising HIV p55 GAG protein to which is anchored another bait protein other than the bait protein recited in claim 20.

35. A method of detecting a specific protein-protein interaction in a cell, the method comprising:

(1) expressing in the cell a recombinant fusion protein comprising an HIV p55 Group Antigens (GAG) protein and a bait protein, wherein the expressed fusion protein forms a protein-protein complex with an endogenously-expressed prey protein that is a native physiological binding partner of the bait protein;

(2) forming virus-like particles comprising the protein-protein complex;

(3) isolating virus-like particles budded from the cell; and

(4) detecting the specific interaction between the bait protein and the prey protein in the virus-like particles as compared to background protein-protein interactions from reference virus-like particles comprising HIV p55 GAG protein without the bait protein anchored to it, thereby detecting the specific protein-protein interaction in the cell.

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36. A method of detecting a specific protein-protein interaction in a mammalian cell, the method comprising:

(1) expressing in the mammalian cell a recombinant fusion protein comprising an HIV p55 Group Antigens (GAG) protein and a bait protein, wherein the expressed fusion protein forms a protein-protein complex with an endogenously-expressed prey protein that is a native physiological binding partner of the bait protein;

(2) forming virus-like particles comprising the protein-protein complex;

(3) isolating virus-like particles budded from the mammalian cell; and

(4) detecting the specific interaction between the bait protein and the prey protein in the virus-like particles as compared to background protein-protein interactions from reference virus-like particles comprising HIV p55 GAG protein to which is anchored a bait protein different from the bait protein recited in (1), thereby detecting the specific protein-protein interaction in the mammalian cell.

37. The use according to claims 18 or 19 wherein the cell is a mammalian cell.

38. The use according to claim 37, wherein the mammalian cell is a human cell.

39. The method according to any one of claims 20 to 35 wherein the cell is a mammalian cell.

40. The method according to claim 39, wherein the mammalian cell is a human cell.

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Figure 1

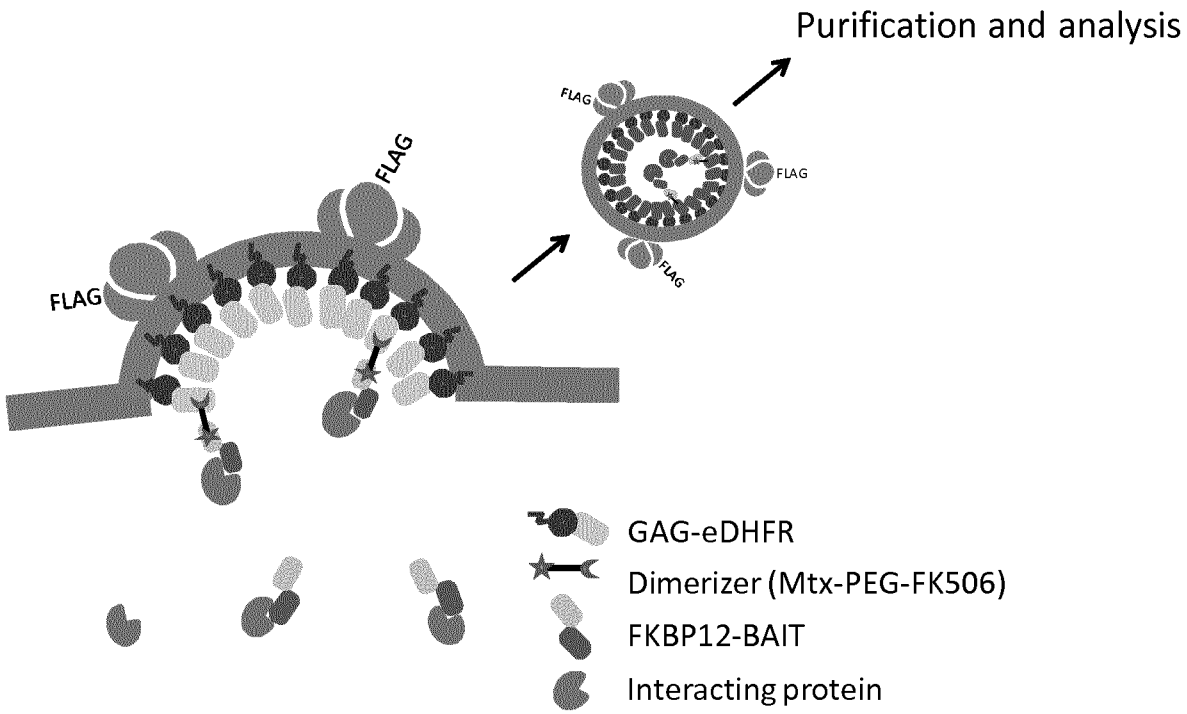


Figure 2

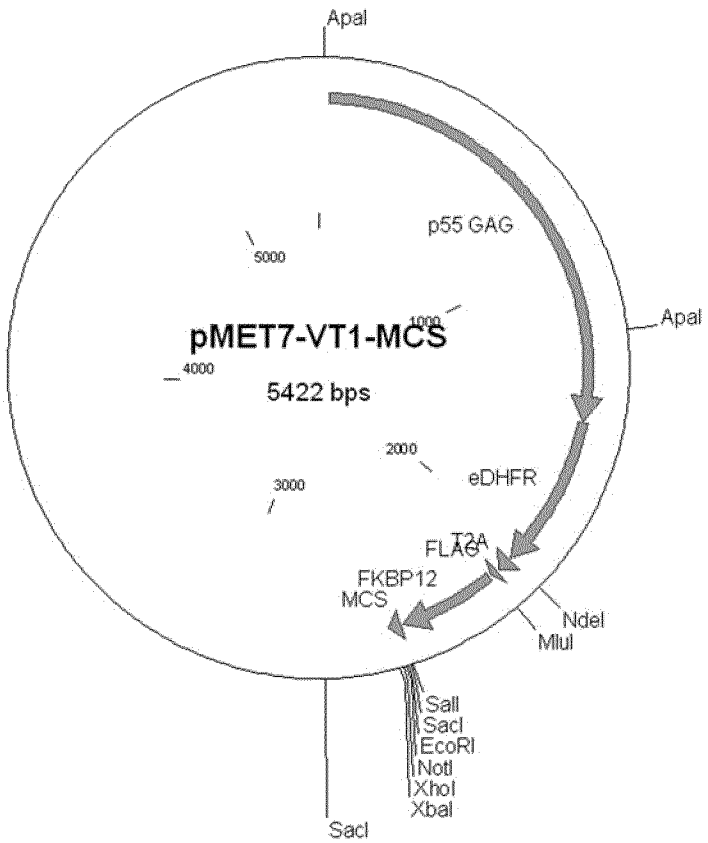


Figure 3

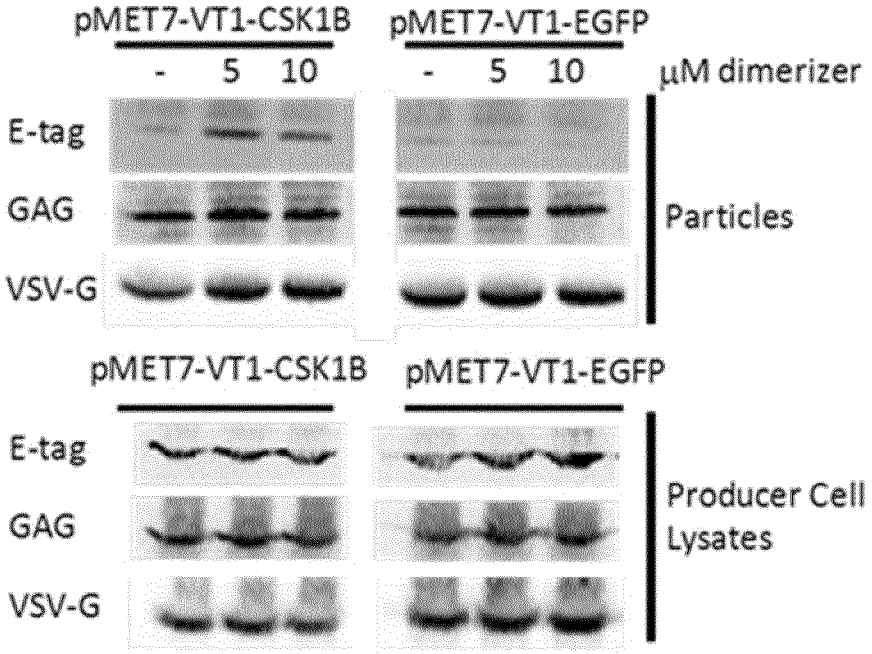
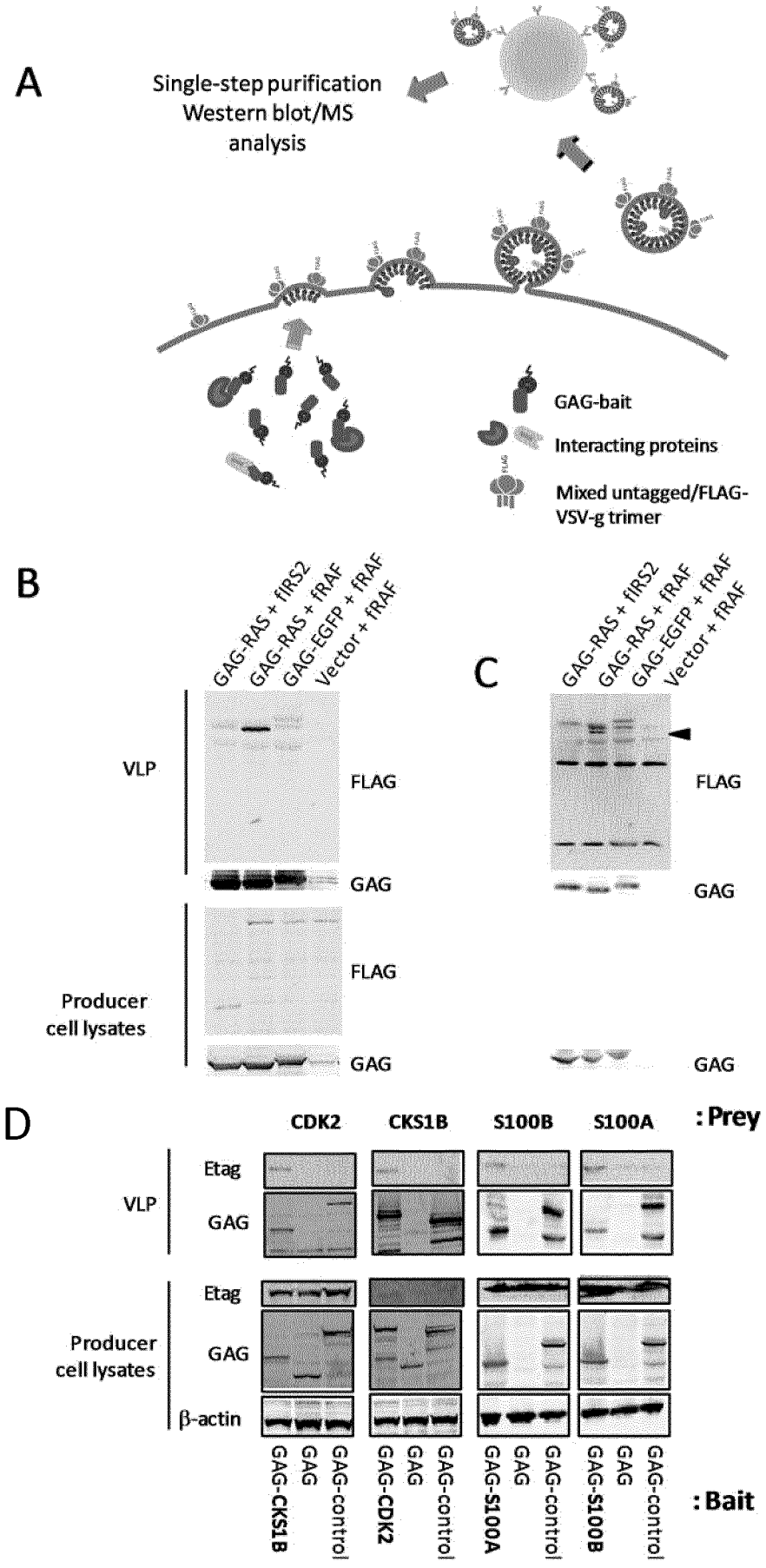

pMET7-VT1-CSK1B
pMET7-VT1-EGFP
- 5 10
- 5 10
µM dimerizer
E-tag
GAG
VSV-G
Particles
pMET7-VT1-CSK1B
pMET7-VT1-EGFP
E-tag
GAG
VSV-G
Producer Cell Lysates

Figure 4


A
Single-step purification
Western blot/MS
analysis
GAG-bait
Interacting proteins
Mixed untagged/FLAG-
VSV-g trimer
B
GAG-RAS + fIRS2
GAG-RAS + fRAF
GAG-EGFP + fRAF
Vector + fRAF
VLP
FLAG
GAG
Producer
cell lysates
FLAG
GAG
C
GAG-RAS + fIRS2
GAG-RAS + fRAF
GAG-EGFP + fRAF
Vector + fRAF
FLAG
GAG
GAG
D
CDK2
CKS1B
S100B
S100A
: Prey
VLP
Etag
GAG
Producer
cell lysates
Etag
GAG
β-actin
GAG-CKS1B
GAG
GAG-control
GAG-CDK2
GAG
GAG-control
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GAG
GAG-control
GAG-S100B
GAG
GAG-control
: Bait

Figure 5

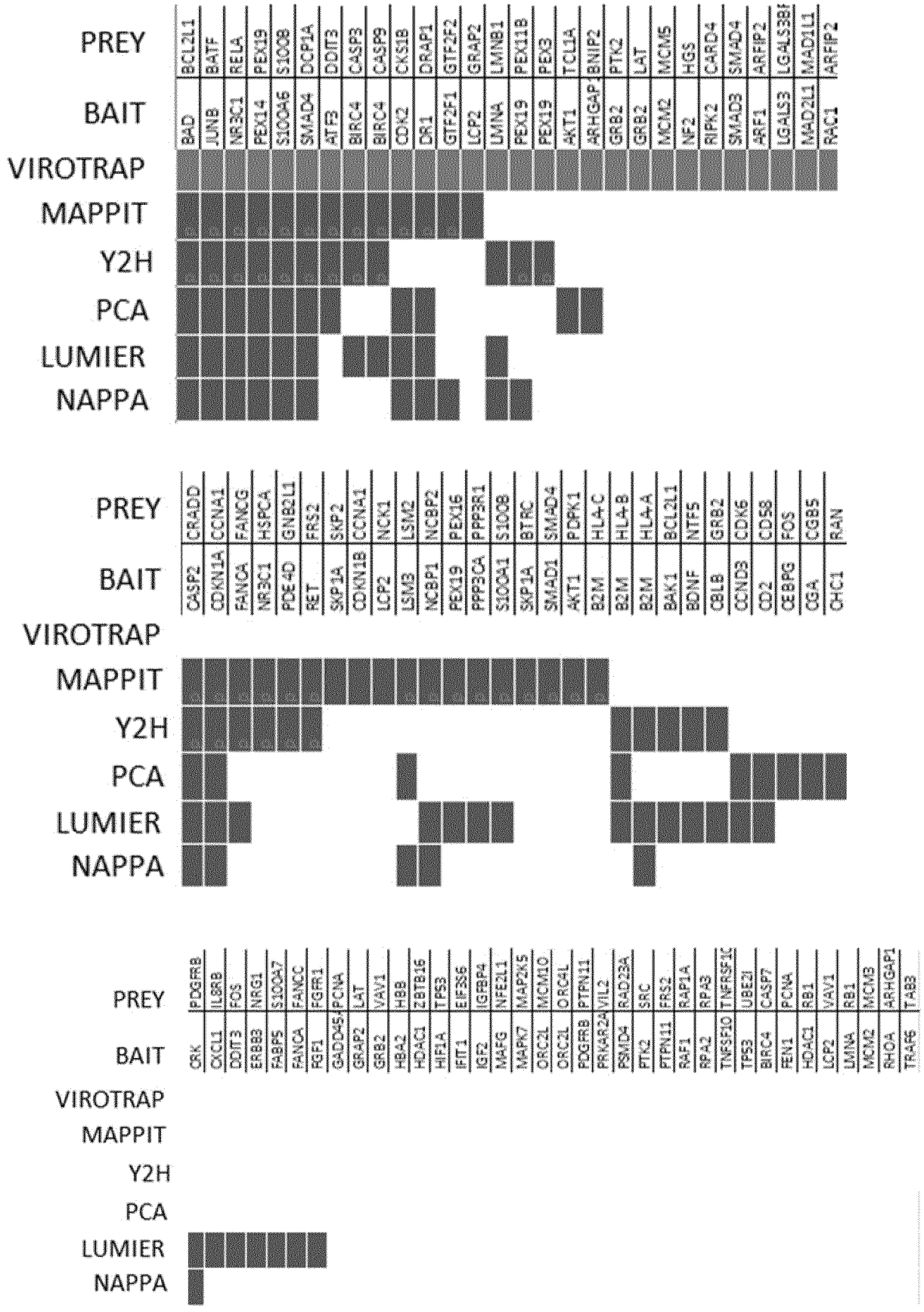
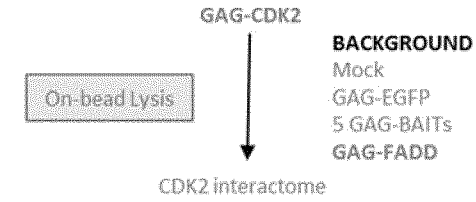

PREY
BAIT
VIROTRAP
MAPPIT
Y2H
PCA
LUMIER
NAPPA
BCL2L1 BATF RELA PEX19 S100B DCP1A DDIT3 CASP3 CASP9 CKS1B DRAP1 GTF2F2 GRAP2 LMNB1 PEX11B PEX3 TCL1A BNIP2 PTK2 LAT MCM5 HGS CARD4 SMAD4 ARFIP2 LGALS3BP MAD1L1 ARFIP2
BAD JUNB NR3C1 PEX14 S100A6 SMAD4 ATF3 BIRC4 BIRC4 CDK2 DR1 GTF2F1 LCP2 LMNA PEX19 PEX19 AKT1 ARHGAP1 GRB2 GRB2 MCM2 NF2 RIPK2 SMAD3 ARF1 LGALS3 MAD2L1 RAC1
CRADD CCNA1 FANCG HSPCA GNB2L1 FRS2 SKP2 CCNA1 NCK1 LSM2 NCBP2 PEX16 PPP3R1 S100B BTRC SMAD4 PDPK1 HLA-C HLA-B HLA-A BCL2L1 NTF5 GRB2 CDK6 CDS8 FOS CGB5 RAN
CASP2 CDKN1A FANCA NR3C1 PDE4D RET SKP1A CDKN1B LCP2 LSM3 NCBP1 PEX19 PPP3CA S100A1 SKP1A SMAD1 AKT1 B2M B2M B2M BAK1 BDNF CBLB CCND3 CD2 CEBPG CGA CHC1
PDGFRB IL8RB FOS NRG1 S100A7 FANCC FGFR1 PCNA LAT VAV1 HBB ZBTB16 TP53 EIF3S6 IGFBP4 NFE2L1 MAP2K5 MCM10 ORC4L ORC4L PTPN11 VIL2 RAD23A SRC FRS2 RAP1A RPA3 TNFRSF1C UBE2I CASP7 PCNA RB1 VAV1 RB1 MCM8 ARHGAP1 TAB3
CRK CXCL1 DDIT3 ERBB3 FABP5 FANCA FGF1 GADD45A GRAP2 GRB2 HBA2 HDAC1 HIF1A IFIT1 IGF2 MAFG MAPK7 ORC2L ORC2L PDGFRB PRKAR2A PSMD4 PTK2 PTPN11 RAF1 RPA2 TNFSF10 TP53 BIRC4 FEN1 HDAC1 LCP2 LMNA MCM2 RHOA TRAF6

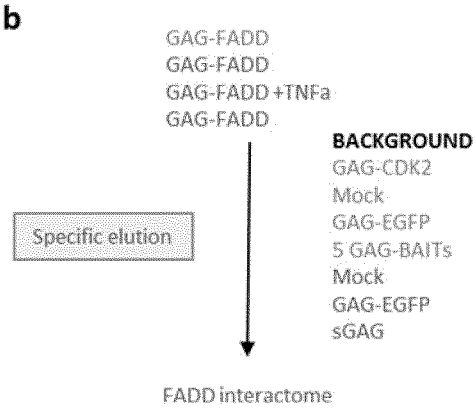
Figure 6

a



	Protein	Literature
1	CDK3	
2	Clusterin fr	
3	CCNA2	Nigg et al., 1995
4	**CDK2 (Bait)**	
5	PSMB6	
6	CKS2	Booher et al., 1989
7	CDC37	Prince et al., 2005
8*	CKS1B*	Booher et al., 1989

b



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1	SDC4 (4)	Wu et al., 2012***
2	CSNK1E (4)	Alappat et al., 2005**
3	**FADD (Bait) (4)**	
4	RIPK1 (4)	Varfolomeev et al., 1996
5	SERINC1 (3)	
6	KIF2C (3)	
7	YES1 (2)	Kleber et al., 2008***
8	CDK1 (2)	Matthess et al., 2010***
9	DSG2 (2)	
10	PTK7 (2)	
11	CHMP4A (2)	
12	SERINC3 (2)	

c

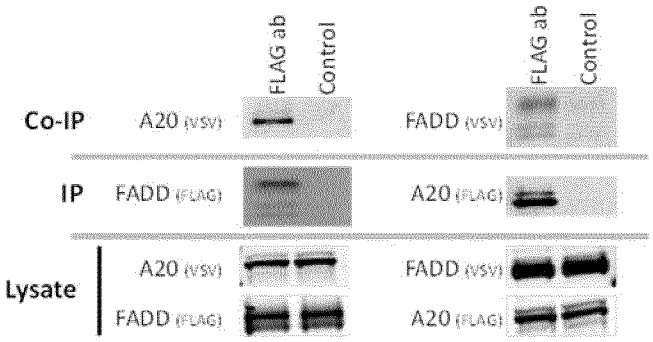

Figure 7


1 mdwsffrvva mlfiflvvve vnsefriqvr dyntkngtik whsirrqkre wikfaaacre gednskrnpi akihsdcaan qqvtyrisgv
>>...Signal Peptide....>>
>>.......Propeptide.......>>
>>..........Extracellular Region.....>
91 gidqppygif vinqktgein itsivdrevt pffiiycral nsmgqdlerp lelrvrvldi ndnppvfsma tfagqieens nantlvmiln
Peptide G
Peptide A
>.....Extracellular Region.....>
181 atdadepnnl nskiafkiir qepsdspmfi inrntgeirt mnnfldreqy gqyalavrgs drdggadgms aececnikil dvndnipyme
Peptide F
Peptide D
>.....Extracellular Region.....>
271 qssytieiqe ntlnsnllei rvidldeefs anwmaviffi sgnegnwfei emnertnvgi lkvvkpldye amqslqlsig vrnkaefhhs
Peptide J
>.....Extracellular Region.....>
361 imsqyklkas aisvtvlnvi egpvfrpgsk tyvvtgnmgs ndkvgdfvat dldtgrpstt vryvmgnnpa dllavdsrtg kltlknkvtk
Peptide B
Peptide M
Peptide K
Peptide L
Peptide I
>.....Extracellular Region.....>
451 eqynmlggky qgtilsiddn lqrtctgtin iniqsfgndd rtntepntki ttntgrqest sstnydtstt stdssqvyss epgngakdll
>.....Extracellular Region.....>
541 sdnvhfgpag igllimgflv lglvpflmic cdcggaprsa agfepvpecs dgaihswave gpqpeprdit tvipqippdn aniiecidns
>.....>> Extracellular Region
>>.....>> Transmembrane Region
>>.....Cytoplasmic Region.....>
631 gvytneyggr emqdlggger mtgfeltegv ktsgmpeicq eysgtlrrns mrecreggln mnfmesyfcq kayayadede grpsndclli
>.....Cytoplasmic Region.....>
721 ydiegvgspa gsvgccsfig edlddsfldt lgpkfkklad islgkesypd ldpswppqst epvclpqete pvvsghppis phfgtttvis
>.....Cytoplasmic Region.....>
811 estypsgpgv lhpkpildpl gygnvtvtes yttsdtlkps vhvhdnrpas nvvvtervvg pisgadlhgm lempdlrdgs nvivtervia
>.....Cytoplasmic Region.....>
901 pssslptslt ihhpressnv vvterviqpt sgmigslsmh pelanahnvi vtervvsgag vtgisgttgi sggigssglv gtsmgagsga
Peptide E
>.....Cytoplasmic Region.....>
991 lsgagisggg iglsslggta sighmrsssd hhfnqtigsa spstarsrit kystvqysk
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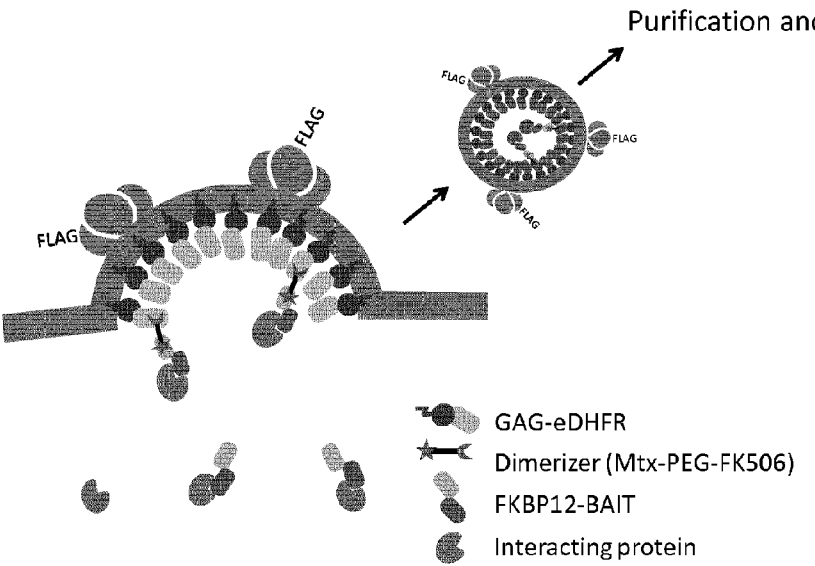
Purification and analysis
FLAG
FLAG
FLAG
FLAG
FLAG
GAG-eDHFR
Dimerizer (Mtx-PEG-FK506)
FKBP12-BAIT
Interacting protein