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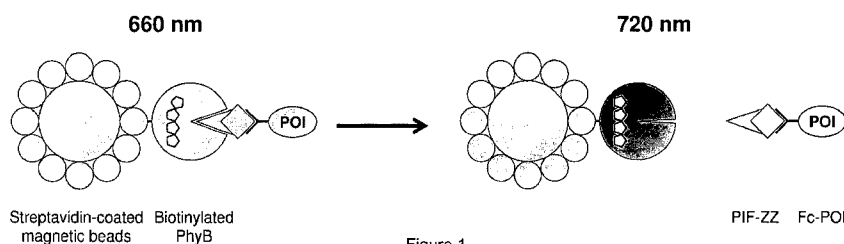


Figure 1

(57) **Abstract:** The present invention relates to a system for the caging and controlled release of at least one protein of interest, said system comprising e.g. a phytochrome B that is coupled to a capture reagent and e.g. a phytochrome interacting factor to which the at least one protein of interest is coupled. The present invention further relates to a method for the caging and controlled release of at least one protein of interest, said method using said system, as well as a kit comprising e.g. a phytochrome B that is coupled to a capture reagent and e.g. a phytochrome interacting factor that is coupled to a binding domain. Moreover, the present invention relates to the use of said system for the controlled release of at least one protein of interest. The system and method of the present invention can be used for the purification of the protein of interest.



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SYSTEM FOR THE CAGING AND CONTROLLED RELEASE OF PROTEINS

Description

The present invention relates to a system for the caging and controlled release of at least one protein of interest, said system comprising a first protein that is coupled to a capture reagent and a second protein that is coupled to said protein of interest, wherein said first and second proteins can bind to each other in a light-dependent manner. The present invention further relates to a method for the caging and controlled release of at least one protein of interest, said method using said system, as well as a kit comprising a first protein that is coupled to a capture reagent and a second protein that is coupled to a binding domain, wherein said first and second proteins can bind to each other in a light-dependent manner.

The caging and spatiotemporally controlled release of proteins of interest (POIs) has a wide range of potential uses in basic research, as well as in applied science, e.g. in novel diagnostic and therapeutic methods.

The trapping of biological compounds in cages and the subsequent controlled uncaging of them at the site of interest allows for a nearly instantaneous manipulation of the bioactive compound concentration. The caging of small signaling molecules, such as second messengers, neurotransmitters, and nucleotides, has revolutionized biological research by providing a means to spatiotemporally regulate and monitor a wide range of cellular processes. Caging based on chemical modification of the molecule using a photo-removable protective group has been successfully applied to a multitude of small signaling molecules. The same strategy has also been applied to chemically synthesizable peptides. However, it is in most cases not compatible with large proteins. Instead, large proteins require a tailored caging procedure for each individual protein of interest, and the caging is often complicated due to difficulties in achieving site-specific chemical modification. Considering the wide range of biological signaling processes relying on non-chemically synthesizable proteins, there is a demand for

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a method enabling the caging of these in a generic manner. Such a method would be an excellent tool for the manipulation of protein-regulated processes, e.g. in developmental biology and tissue engineering.

Alternative methods for the controlled release of proteins based on protein immobilization or interactive biohybrid materials have also been reported. Such methods have the advantage of being generically applicable, *i.e.* they are not limited to solely one protein. Specifically, protein immobilization strategies based on the interaction of a drug-responsive bacterial repressor tag with DNA have been used for the isolation, quantification and labeling of target proteins, as well as in diagnostics and drug discovery processes. Further, interactive biohybrid materials provide the spatiotemporally controlled release of proteins via the administration of small molecule inducers. In this context, hydrogels based on protein-protein or protein-DNA interactions have been developed. However, as hydrogels require implantation, they are mainly suitable for localized protein release. Additionally, the small size of the hydrogel limits the amount of payload protein.

Therefore, the technical problem underlying the present invention is to provide a simple, cheap, versatile and generically applicable system for the caging and spatiotemporally controlled release of arbitrary POIs that is not restricted to small protein payloads or localized release.

The solution to the above technical problem is achieved by the embodiments characterized in the claims.

In particular, in a first aspect, the present invention relates to a system for the caging and controlled release of at least one protein of interest (POI), said system comprising:

- (a) a first protein that is coupled to a capture reagent; and
- (b) a second protein that is coupled to said at least one POI;

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wherein said first and second proteins can bind to each other in a light-dependent manner.

The system of the present invention is characterized in that the POI can be reversibly caged by illumination with light of a suitable wavelength or wavelength range that mediates binding of said first and second proteins to each other, and reversibly released in a spatiotemporally controlled manner by illumination with light of another suitable wavelength or wavelength range that abolishes binding of said first and second proteins to each other (Fig. 1).

The term "system" as used herein relates to any entity containing the constructs (a) and (b) as defined above. In particular, said term is intended to encompass any container, plurality of containers, package, kit, vial or the like comprising said constructs. Further, the term "system" encompasses any solution, plurality of solutions, complex, plurality of complexes, reaction mixture, biological entity or cell comprising said constructs.

The term "caging" as used herein relates to any process of reversibly immobilizing the at least one POI on said capture reagent. The term "controlled release" as used herein relates to any process of intentionally releasing the at least one POI from said capture reagent in a spatiotemporally controlled manner, *i.e.* at a predetermined time and at a predetermined location.

The term "wherein said first and second proteins can bind to each other in a light-dependent manner" as used herein is intended to describe the fact that the interaction and dissociation of said proteins from each other can be controlled by light. In particular, illuminating said proteins with light of a suitable wavelength or wavelength range induces a conformational change in one or both of said proteins that allows the binding of said proteins to each other. Illuminating said proteins with light of a different suitable wavelength or wavelength range induces a conformational change in one or both of said proteins that abolishes the binding of said proteins to each other and, thus, lead to the dissociation of said proteins.

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The POI to be caged and released in the context of the present invention is not particularly limited. This is due to the fact that the usability and efficacy of the system of the present invention is independent of the nature of the POI to a large degree. Accordingly, any POI can be used as long as it can be coupled to said second protein. Moreover, more than one and up to a multitude of POIs can be used at the same time. In preferred embodiments, the POI is selected from the group consisting of pharmaceutically active proteins, antibodies, and antibody fragments.

The first and second proteins used in the context of the present invention are not particularly limited, provided that said proteins are capable of binding to each other in a light-dependent manner as described above. Suitable protein pairs are known in the art. The following Table 1 shows a non-limiting list of suitable proteins, wherein one of said first and second proteins can be taken from column 1, and the other of said first and second proteins can be taken from column 2 within the same group. In this context, it is emphasized that said first protein can be taken from column 1 and said second protein can be taken from column 2 in the same group, or said first protein can be taken from column 2 and said second protein can be taken from column 1 in the same group. The listed proteins are known in the art and can be derived from diverse organisms, preferably from phototroph or phototropic organisms such as e.g. plants or cyanobacteria.

In a particular embodiment, one of said first and second proteins is selected from the group consisting of Phytochrome A (PhyA), Phytochrome B (PhyB), Phytochrome C (PhyC), Phytochrome D (PhyD), and Phytochrome E (PhyE); and the other of said first and second proteins is selected from the group consisting of FHL, FHY1, Phytochrome interacting factor 1 (PIF1), PIF3, PIF4, PIF5, PIF6, PIF7, PIF8, COP1, CRY1, FyPP, IAA17, PsIAA4, NDPK2, PAPP5, PIL2, PIL5, and PIL6. By way of example, said first protein could be a Phytochrome, whereas said second protein could be a Phytochrome interacting factor. Alternatively, said first protein could be a Phytochrome interacting factor, whereas said second protein could be a Phytochrome.

Table 1: Non-limiting list of proteins that can be used as first and second proteins in the context of the present invention

	Column 1	Column 2
Group 1	Phytochrome A (PhyA)	FHL
	PhyB	FHY1
	PhyC	Phytochrome interacting factor 1 (PIF1)
	PhyD	PIF3
	PhyE	PIF4
		PIF5
		PIF6
		PIF7
		PIF8
		COP1
		CRY1
		FyPP
		IAA17
		PsIAA4
		NDPK2
		PAPP5
		PIL2
		PIL5
		PIL6
Group 2	CRY1	CIB1
	CRY2	SPA1
Group 3	FKF1	GI
Group 4	Cph1	Cph1
Group 5	Dronpa	Dronpa
Group 6	UVR8	UVR8
		COP1
		RUP1
		RUP2
Group 7	LOV2	J α
	AsLOV2	ePDZ
Group 8	VVD	VVD

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In another particular embodiment, one of said first and second proteins is selected from the group consisting of CRY1 and CRY2; and the other of said first and second proteins is CIB1 or SPA1.

In another particular embodiment, one of said first and second proteins is FKF1; and the other of said first and second proteins is GI.

In another particular embodiment, both of said first and second proteins are Cph1.

In another particular embodiment, both of said first and second proteins are Dronpa.

In another particular embodiment, one of said first and second proteins is UVR8; and the other of said first and second proteins is selected from the group consisting of UVR8, COP1, RUP1, and RUP2.

In another particular embodiment, one of said first and second proteins is selected from the group consisting of LOV2 and AsLOV2; and the other of said first and second proteins is selected from the group consisting of J α and ePDZ.

In another particular embodiment, both of said first and second proteins are VVD.

In a particularly preferred embodiment, one of said first and second proteins is a Phytochrome, preferably PhyB; more preferably *Arabidopsis thaliana* PhyB; and most preferably a polypeptide consisting of the first 651 N-terminal amino acids of one of said PhyBs, *i.e.* a C-terminally truncated PhyB; and the other of said first and second proteins is a PIF; preferably an *Arabidopsis thaliana* PIF; more preferably PIF3 or PIF6; more preferably *Arabidopsis thaliana* PIF3 or PIF6; and most preferably a polypeptide consisting of the first 100 N-terminal amino acids of one of said PIFs, *i.e.* a C-terminally truncated PIF.

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The capture reagent to be used in the context of the present invention is not particularly limited and suitable capture reagents are known in the art. In a preferred embodiment, the capture reagent is selected from the group consisting of magnetic beads and agarose. In a preferred embodiment, the first protein is coupled to the capture reagent via the interaction between biotin and streptavidin, *i.e.* the first protein is biotinylated, preferably via a biotinylatable tag (such as e.g. AVITagTM) that is coupled thereto, and the capture reagent is coupled to streptavidin. Methods for the biotinylation of proteins, as well as methods for the coupling of streptavidin to a capture reagent are known in the art.

According to the present invention, the second protein is coupled to the at least one POI. Means of coupling the two proteins are not particularly limited and are known in the art. In a particular embodiment, the POI is directly coupled to the second protein, e.g. by a linker, preferably a cleavable linker. In another particular embodiment, the second protein is coupled to a first binding domain, the at least one POI is coupled to a second binding domain, and the second protein is coupled to the at least one POI via the interaction between the first and second binding domains.

The binding domains to be used in the context of the present invention are complementary binding domains, *i.e.* the first and second binding domains bind to each other. Suitable binding domains are not particularly limited and are known in the art. In a particular embodiment, one binding domain is a domain binding the F_c portion of an antibody, and the other, complementary binding domain is the F_c portion of an antibody. A specific example of a domain binding the F_c portion of an antibody is the ZZ domain of protein A derived from *Staphylococcus aureus* which binds to the F_c portion of immunoglobulin G (IgG).

In another particular embodiment, the above second binding domain is not coupled directly to the POI, but to a protein binding molecule, wherein said at least one POI is bound to said protein binding molecule. In an alternative embodiment, the protein binding molecule is directly coupled to said second protein. Suitable

protein binding molecules are not particularly limited and are known in the art. Respective examples include antibodies, antibody fragments, aptamers, Affibodies[®], *i.e.* artificial antigen-binding peptides derived from *Staphylococcus aureus* Protein A, and DARPINs (Designed Ankyrin Repeat Proteins), *i.e.* artificial antigen-binding proteins.

In one embodiment, the system of the present invention is for use in the controlled release of at least one POI as defined above.

In a second aspect, the present invention relates to a method for the caging and controlled release of at least one POI, said method comprising the steps of:

- (a) providing a system as defined in any one of claim 1 to 8; and
- (b1) caging said at least one POI by illuminating said system with light of a specific wavelength or wavelength range that mediates binding of said first and second proteins to each other;
- (b2) releasing said at least one POI by illuminating said system with light of a specific wavelength or wavelength range that abolishes binding of said first and second proteins to each other.

In this aspect, the terms “system”, “caging”, and “controlled release” are as defined above. Moreover, the at least one POI is preferably as defined above.

In the method of the present invention, reversible caging of the at least one POI is effected by illuminating the system of the present invention with light of a specific wavelength or wavelength range that mediates binding of said first and second proteins to each other. At such wavelengths, the first and second proteins bind to each other in a reversible manner. By way of example, in case the first and second proteins are PhyB and a PIF, the system is illuminated with light that induces a reversible conformational change to the Pfr state of PhyB, *i.e.* the state in which PhyB is able to bind PIF in a reversible manner. Suitable wavelengths in this respect are known in the art, and include for the example of PhyB and PIF

e.g. between 500 and 720 nm, preferably between 620 and 700 nm, and more preferably about 660 nm. As a consequence of a respective illumination, the at least one POI is bound to the capture reagent via PhyB and PIF (Fig. 1). Further, the reversible release of the at least one POI is effected by illuminating the system of the present invention with light of a specific wavelength or wavelength range that abolishes binding of said first and second proteins to each other. By way of example, in case the first and second proteins are PhyB and a PIF, the system is illuminated with light that induces a reversible conformational change to the Pr state of PhyB, *i.e.* the state in which PhyB is not able to bind PIF. Suitable wavelengths in this respect are known in the art, and include for the example of PhyB and PIF e.g. more than 720 nm, preferably about 740 nm. As a consequence of a respective illumination, the at least one POI is released from the capture reagent (Fig. 1).

The method of the present invention can be used for the light-controlled purification of said at least one POI by caging said at least one POI in step (b1), thereby immobilizing said at least one POI, and eluting said at least one POI from the capture reagent in step (b2). In this context, several existing protein purification methods have the drawback that elution of the purified protein from the carrier material requires harsh elution conditions such as shifts in non-physiologic pH ranges. With the method of the present invention, POIs can be bound to and eluted from the carrier material just by illumination with light of a specific wavelength or wavelength range. For this purpose, the binding of the POI to the capture reagent can be triggered by illumination with light of a specific wavelength or wavelength range that triggers the interaction between said first and second proteins. After washing the capture reagent to remove unbound proteins, the POI can be eluted from the capture reagent by illumination with light of a specific wavelength or wavelength range that triggers the dissociation between said first and second proteins.

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In a third aspect, the present invention relates to a kit comprising:

- (a) a first protein that is coupled to a capture reagent, and
- (b) a second protein,

wherein said first and second proteins can bind to each other in a light-dependent manner.

In a preferred embodiment, said second protein is coupled to a suitable binding domain. Alternatively, said second protein is coupled to a protein binding molecule, or directly to the POI.

In a further embodiment, said second protein is coupled to a suitable first binding domain, and the kit further comprises either a POI that is coupled to a suitable second binding domain or a protein binding molecule that is coupled to a suitable second binding domain, wherein said first and second binding domain bind to each other.

In this aspect, the first and second proteins, the capture reagent, the protein binding molecule, as well as the binding domains, are preferably as defined above.

The kit of the present invention can further comprise suitable buffers and/or suitable disposables.

The system, method and kit of the present invention advantageously eliminate the need to optimize the caging procedure for each individual POI, and is therefore simpler, cheaper and more versatile than existing solutions. The ability to cage one or more POIs in a generic fashion allows e.g. the exploration of a variety of biological signaling processes, or the regulation of a wide range of biological processes within both basic and applied science. Suitable application areas are for instance tissue engineering and tumor therapy.

The Figures show:

Figure 1:

Light-controllable bead cage

Biotinylated PhyB-AVITag was coupled to streptavidin-coated magnetic beads. The fusion protein of PIF and the immunoglobulin G (IgG) binding ZZ domain of Protein A interacts at 660 nm with PhyB-AVITag and is released upon 720 nm illumination. Due to the interaction of the Fc-tag with the ZZ domain, any Fc tagged POI can be released from the bead cage by 720 nm light.

Figure 2:

Purification of (A) PhyB-AVITag (74kDa) and (B) ZZ-PIF3 and ZZ-PIF6 (both 27 kDa) by Ni²⁺-based affinity chromatography

For all purified proteins, a Coomassie-stained SDS-PAGE gel is shown.

Figure 3:

Interaction of PhyB-AVITag with avidin

Purified PhyB-AVITag (dark curve) and PhyB-AVITag with excess of avidin (light curve) were separated by size exclusion chromatography (upper panel). Selected fractions were visualized using a Streptavidin-HRP conjugate (lower panel). The peak at 14.04 ml corresponds to PhyB-AVITag with a calculated mass of 77 kDa (actual mass: 74 kDa). The peak at 15.39 ml relates to avidin with a calculated mass of 39 kDa (actual mass: 67 kDa) and the peak at 12.88 ml corresponds to the complex of PhyB-AVITag with avidin and a calculated mass of 137 kDa.

Figure 4:

Light-induced release of SEAP_{Fc} from the bead cage

The beads were coupled with ZZ-PIF3 or ZZ-PIF6 and SEAP_{Fc} and were incubated for 30 min either at 660 nm or 720 nm light. The release of SEAP_{Fc} was determined by measuring the SEAP activity in the supernatant.

The present invention will be further illustrated in the following examples without being limited thereto.

Examples

Experimental procedures:

Production of biotinylated and hexahistidine-tagged PhyB-AVITag.

Arabidopsis thaliana phytochrome B (PhyB) (amino acids 1-651) was amplified from pAL149 using oligonucleotides oMH1 and oMH38 and ligated (NotI, EcoRI) into p83 thereby replacing Cph1. With the used oligonucleotide oMH38, an AVITag and a hexahistidine tag was fused to the C-terminus of the protein. The plasmid was transformed together with the PCB-synthesizing plasmid p171 coding for *Synechocystis* PCC 6803 Ho and PcyA into *E. coli* BL21 StarTM (DE3) (Invitrogen, Carlsbad, CA, cat. no. C6010-03) and protein expression was induced at OD₆₀₀ = 0.8 with 1 mM IPTG and 0.4 % arabinose. The protein was expressed at 18°C for 20 h in the dark and the cells were harvested by centrifugation at 6,500 x g for 8 min. The cell pellet was resuspended in Ni-lysis buffer (50 mM NaH₂PO₄ pH 8.0, 300 mM NaCl, 10 mM imidazole), disrupted using a French Press (APV 2000, APV Manufacturing, Bydgoszcz, Poland) at 1000 bar and centrifuged at 30,000 x g for 30 min to remove cell debris. The supernatant was loaded onto a column containing Ni-NTA agarose (Qiagen, Hilden, Germany, cat. no. 30210), washed with 20 column volumes of Ni-wash buffer (50 mM NaH₂PO₄ pH 8.0, 300 mM NaCl, 20 mM imidazole) and eluted in 3 column volumes of Ni-elution buffer (50 mM NaH₂PO₄ pH 8.0, 300 mM NaCl, 250 mM imidazole).

Production of hexahistidine-tagged ZZ-PIF3 and ZZ-PIF6.

The ZZ-domain of protein A derived from *Staphylococcus aureus* was amplified from pRG052 using oligos oMH45/oMH46. *A. thaliana* PIF3 (amino acids 1-100) was synthesized by GeneArt (Life Technologies, Darmstadt, Germany), ligated (NdeI, HindIII) into pWW301 and amplified from this plasmid using oligos oMH48/oMH36. *A. thaliana* PIF6 (amino acids 1-100) was amplified from pAL175 using oligos oMH47/oMH6. The ZZ-containing fragment was joined with the PIF3 or PIF6 fragment by PCR (oligos oMH45/oMH36 or oMH45/oMH6, respectively)

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and ligated (NdeI, HindIII) into pWW301. The plasmids were transformed into *E. coli* BL21 StarTM (DE3)pLysS (Invitrogen, Carlsbad, CA, cat. no. C6020-03) and expression was induced at OD₆₀₀ = 0.6 with 1 mM IPTG. After expression of the protein at 37°C for 3 h the protein was purified by Ni²⁺-affinity chromatography as described for PhyB-AVITag.

Production of SEAP_{Fc}.

The reporter protein SEAP_{Fc} (an Fc-tagged version of the human placental secreted alkaline phosphatase) was produced as known in the art. After harvesting the supernatant containing SEAP_{Fc}, the supernatant was not further purified but biotin-depleted by incubating with 40 µl streptavidin-agarose suspension (Novagen, San Diego, CA, cat. no. 69203) per ml medium at 4°C overnight.

Sequences of used oligonucleotides.

Name	Sequence (5' – 3')	SEQ ID NO:
oMH1	TTCCGAATTCATTAAAGAGGAGAAATTAAGTATGGTTTCCGGAGTCGG GGGTAG	1
oMH6	AAGGAAGCTTTTAATGGTGATGGTGATGATGGTCAACATGTTTATTGCT TTCC	2
oMH36	CGGGCTTTGTTAGCAGCCGGATC	3
oMH38	TGACGCGGCCGCTTAATGGTGATGGTGATGATGTTCTGCGCCATTTCGAT TTTCTGAGCTTCGAAGATGTCGTTCCAGACCGCTACCAGAACCTGCACC TAACTCATCAATCCCCTG	4
oMH45	CCTTCATATGGCGCAACACGATGAAGC	5
oMH46	CATACCAGCAGAACCTGCGGAGCCAGCACCGCTCGAATTCGCGTCTA CTTTCGG	6
oMH47	GGCTCCGCAGGTTCTGCTGGTATGATGTTCTTACCAACCGATTATTG	7
oMH48	GGCTCCGCAGGTTCTGCTGGTATGCCGCTGTTTGAAGTGTTCG	8

Western Blot.

Biotinylated proteins immobilized on a PVDF membrane were detected using a Streptavidin-HRP conjugate (1:2,000; Pierce, Rockford, IL, cat. no. N100).

Size exclusion chromatography.

All size exclusion experiments were performed on an ÄKTAprime plus (GE Healthcare, Freiburg, Germany) fast protein liquid chromatography system using a

Superdex 200 10/300 GL (GE Healthcare, Freiburg, Germany) column and SE buffer (20 mM Tris-HCl pH 8.0, 150 mM NaCl, 5 mM β -mercaptoethanol). To verify the biotinylation of PhyB-AVITag, the protein was incubated with excess of avidin (Pierce, Rockford, IL, cat. no. 21121) and separated by size exclusion chromatography. As protein standard, gel filtration standard (Bio-Rad, Hercules, CA, cat. no. 151-1901) was used.

Preparation of the cage.

Streptavidin-coated magnetic beads (Pierce, Rockford, IL, cat. no. 88816) were washed once with wash buffer (20 mM Tris-HCl pH 8.0, 150 mM NaCl, 5 mM β -mercaptoethanol, 0.05% (v/v) Tween-20, 1% (w/v) bovine serum albumin (BSA) (Fluka, Rotkreuz, Switzerland, cat. no. 05479)) before they were incubated with excess of PhyB-AVITag in wash buffer overnight at 4°C in the dark. After washing the beads three times with wash buffer, the beads were incubated with excess of ZZ-PIF3 or ZZ-PIF6 in wash buffer at room temperature for 2 h under 660 nm illumination. All following steps were performed under 660 nm illumination to prevent release of the ZZ-PIF adapter proteins from the beads. The beads were washed three times for 30 min with wash buffer and were afterwards incubated with biotin-depleted cell culture medium containing SEAP_{FC} supplemented with 5 mM β -mercaptoethanol at room temperature. After washing the beads three times with wash buffer to remove unbound SEAP_{FC}, the bead cage was ready for the 720 nm light-induced SEAP_{FC} release. The activity of released SEAP_{FC} was measured using a p-nitrophenylphosphate-based light absorbance kinetic assay as known in the art.

Example 1:

Generation of a system according to the present invention

In a system of the present invention, a POI is coupled via the light-dependent PhyB/PIF interaction to a bead cage from which it is released upon 720 nm illumination (Figure 1). For this purpose, biotinylated PhyB-AVITag was coupled to

streptavidin-coated magnetic beads. A fusion protein between PIF3 or PIF6 and the immunoglobulin G (IgG) binding ZZ domain of Protein A derived from *Staphylococcus aureus* was engineered that interacts at 660 nm with the immobilized PhyB-AVITag and is released upon 720 nm illumination. Due to the interaction of the Fc-tag with the ZZ domain, any Fc tagged protein of interest can be trapped to the bead cage and released by 720 nm light within seconds.

For the coupling of PhyB to streptavidin coated magnetic beads, a biotinylated version of PhyB was generated. Therefore, the coding sequence for an AVITag and a hexahistidine tag was fused to the C-terminus of the N-terminal 651 amino acids of PhyB. The protein was coexpressed with PCB-synthesizing enzymes in *E. coli* and purified by Ni^{2+} -based affinity chromatography (Figure 2A). The biotinylation of PhyB-AVITag by the endogenous BirA biotin ligase of *E. coli* was verified (i) by observing the interaction with avidin using size exclusion chromatography (Figure 3) and (ii) by detecting the biotinylated protein immobilized on a PVDF membrane with an Streptavidin-HRP conjugate (Figure 3).

The adapter proteins ZZ-PIF3 and ZZ-PIF6 were expressed in *E. coli* and purified by Ni^{2+} -based affinity chromatography (Figure 2B).

Example 2:

Characterization of a system according to the present invention

For characterization purposes, an Fc-tagged secreted alkaline phosphatase (SEAP_{Fc}) was used as the caged POI. The release of SEAP_{Fc} from the beads was determined after 30 min of illumination with 660 nm or 720 nm light using either the ZZ-PIF3 or the ZZ-PIF6 adapter protein (Figure 4). For both adapter proteins, a complete release of SEAP_{Fc} could be achieved upon 720 nm illumination while the leakiness of the system at 660 nm was ~15% and ~17% SEAP_{Fc} release for ZZ-PIF3 and ZZ-PIF6, respectively.

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Claims

1. A system for the caging and controlled release of at least one protein of interest (POI), said system comprising:
 - (a) a first protein that is coupled to a capture reagent; and
 - (b) a second protein that is coupled to said at least one POI;wherein said first and second proteins can bind to each other in a light-dependent manner.
2. The system of claim 1, wherein
 - (i) one of said first and second proteins is selected from the group consisting of Phytochrome A (PhyA), Phytochrome B (PhyB), Phytochrome C (PhyC), Phytochrome D (PhyD), and Phytochrome E (PhyE); and
 - (ii) the other of said first and second proteins is selected from the group consisting of FHL, FHY1, Phytochrome interacting factor 1 (PIF1), PIF3, PIF4, PIF5, PIF6, PIF7, PIF8, COP1, CRY1, FyPP, IAA17, PsIAA4, NDPK2, PAPP5, PIL2, PIL5, and PIL6.
3. The system of claim 1 or claim 2, wherein
 - (i) one of said first and second proteins is PhyB; preferably *Arabidopsis thaliana* PhyB; more preferably a polypeptide consisting of the first 651 N-terminal amino acids of one of said PhyBs; and
 - (ii) the other of said first and second proteins is a PIF; preferably an *Arabidopsis thaliana* PIF; more preferably PIF3 or PIF6; more preferably *Arabidopsis thaliana* PIF3 or PIF6; and most preferably a polypeptide consisting of the first 100 N-terminal amino acids of one of said PIFs.
4. The system of any one of claims 1 to 3, wherein
 - (i) said second protein is coupled to a first binding domain;

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- (ii) said at least one POI is coupled to a second binding domain; and
 - (iii) said second protein is coupled to said at least one POI via the interaction between said first binding domain and said second binding domain.
5. The system of claim 4, wherein
- (i) said second binding domain is coupled to a protein binding molecule; and
 - (ii) said at least one POI is bound to said protein binding molecule.
6. The system of any one of claims 1 to 3, wherein
- (i) said second protein is directly coupled to a protein binding molecule; and
 - (ii) said at least one POI is bound to said protein binding molecule.
7. The system of claim 5 or claim 6, wherein said protein binding molecule is selected from the group consisting of antibodies, antibody fragments, aptamers, affibodies, and DARPin.
8. The system of any one of claims 1 to 7, wherein said POI is selected from the group consisting of pharmaceutically active proteins, antibodies, and antibody fragments.
9. The system of any one of claims 1 to 8 for use in the controlled release of at least one POI.
10. A method for the caging and controlled release of at least one protein of interest (POI), said method comprising the steps of:
- (a) providing a system as defined in any one of claim 1 to 8; and
 - (b1) caging said at least one POI by illuminating said system with light of a specific wavelength or wavelength range that mediates binding of said first and second proteins to each other;

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- (b2) releasing said at least one POI by illuminating said system with light of a specific wavelength or wavelength range that abolishes binding of said first and second proteins to each other.
11. The method of claim 10, which is used for the light-controlled purification of said at least one POI by caging said at least one POI in step (b1), thereby immobilizing said at least one POI, and eluting said at least one POI from the capture reagent in step (b2).
12. A kit comprising:
- (a) a first protein that is coupled to a capture reagent; and
 - (b) a second protein;
- wherein said first and second proteins can bind to each other in a light-dependent manner.
13. The kit of claim 12, wherein said second protein is coupled to one of a suitable binding domain, a protein binding molecule, and said POI.
14. The kit of claim 12, wherein said second protein is coupled to a suitable first binding domain, and the kit further comprises either a POI that is coupled to a suitable second binding domain or a protein binding molecule that is coupled to a suitable second binding domain, wherein said first and second binding domain bind to each other.
15. The kit of any one of claims 12 to 14, wherein
- (i) one of said first and second proteins is selected from the group consisting of Phytochrome A (PhyA), Phytochrome B (PhyB), Phytochrome C (PhyC), Phytochrome D (PhyD), and Phytochrome E (PhyE); and
 - (ii) the other of said first and second proteins is selected from the group consisting of FHL, FHY1, Phytochrome interacting factor 1 (PIF1), PIF3,

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PIF4, PIF5, PIF6, PIF7, PIF8, COP1, CRY1, FyPP, IAA17, PsIAA4, NDPK2, PAPP5, PIL2, PIL5, and PIL6.

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Figures

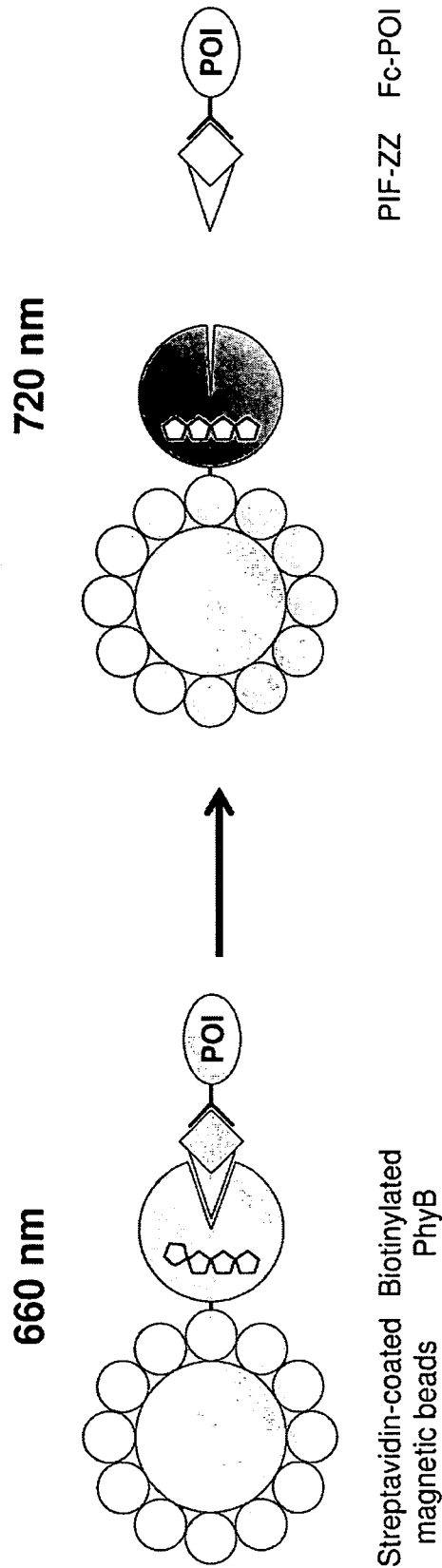


Figure 1

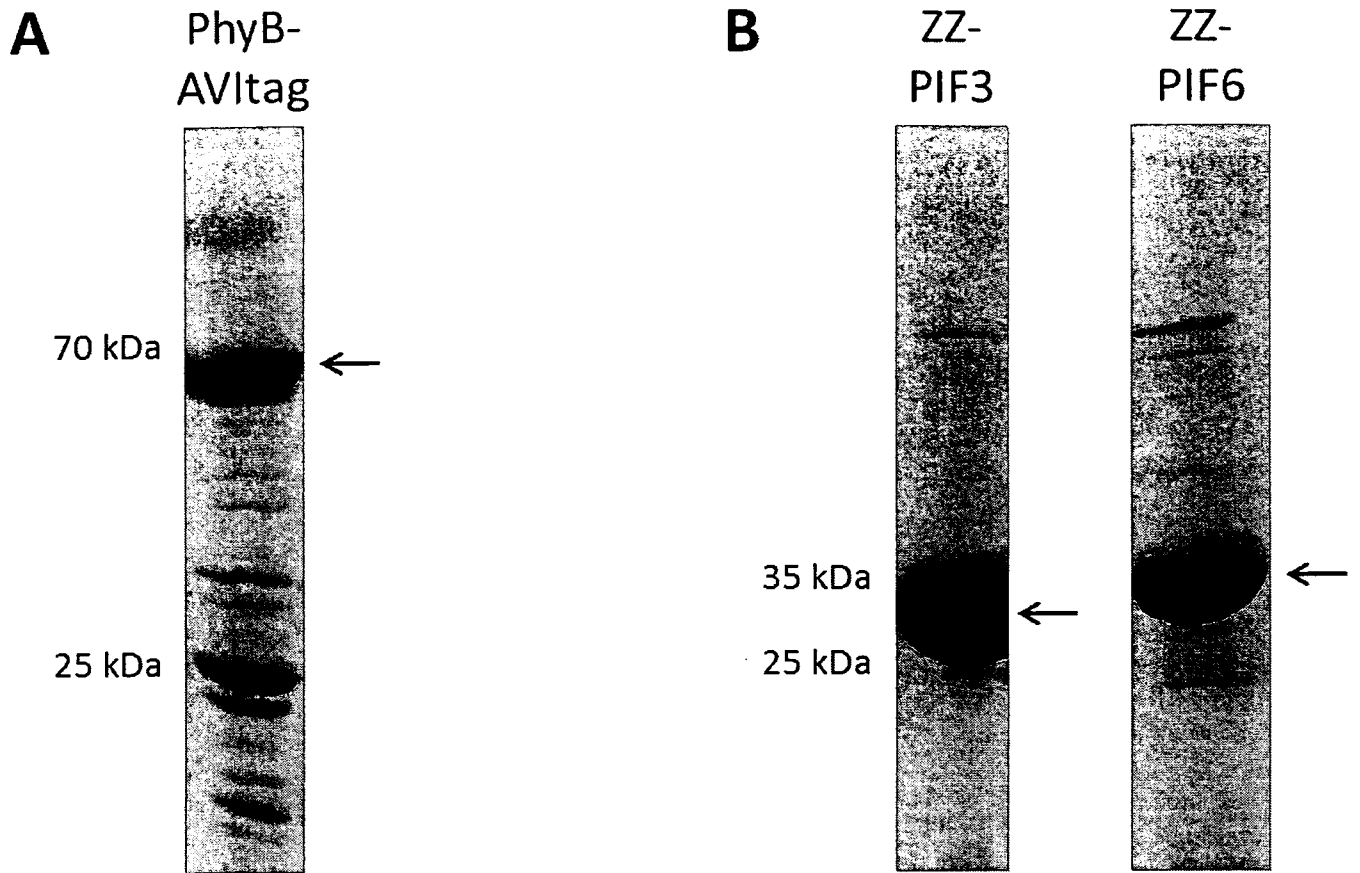


Figure 2

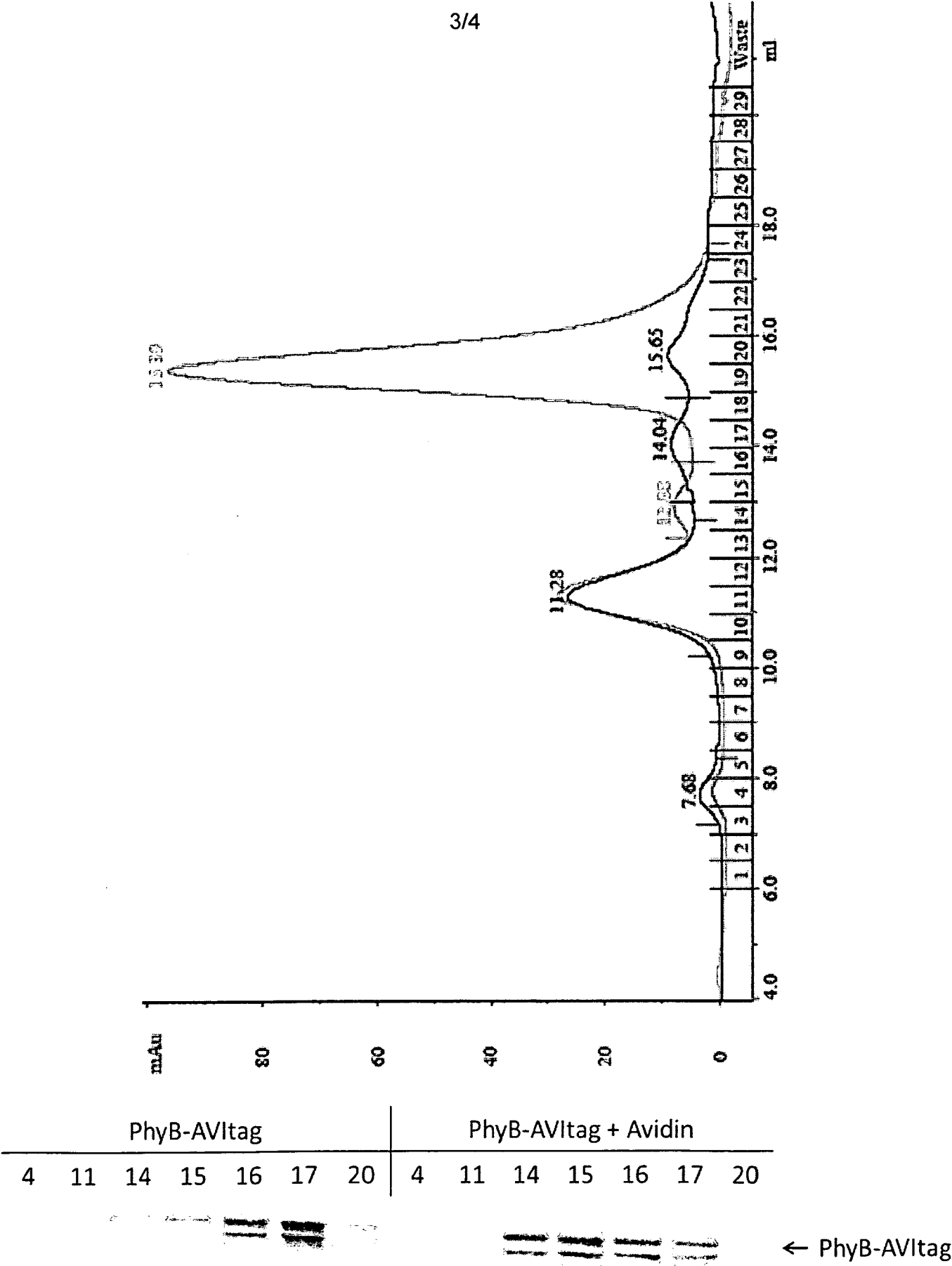


Figure 3

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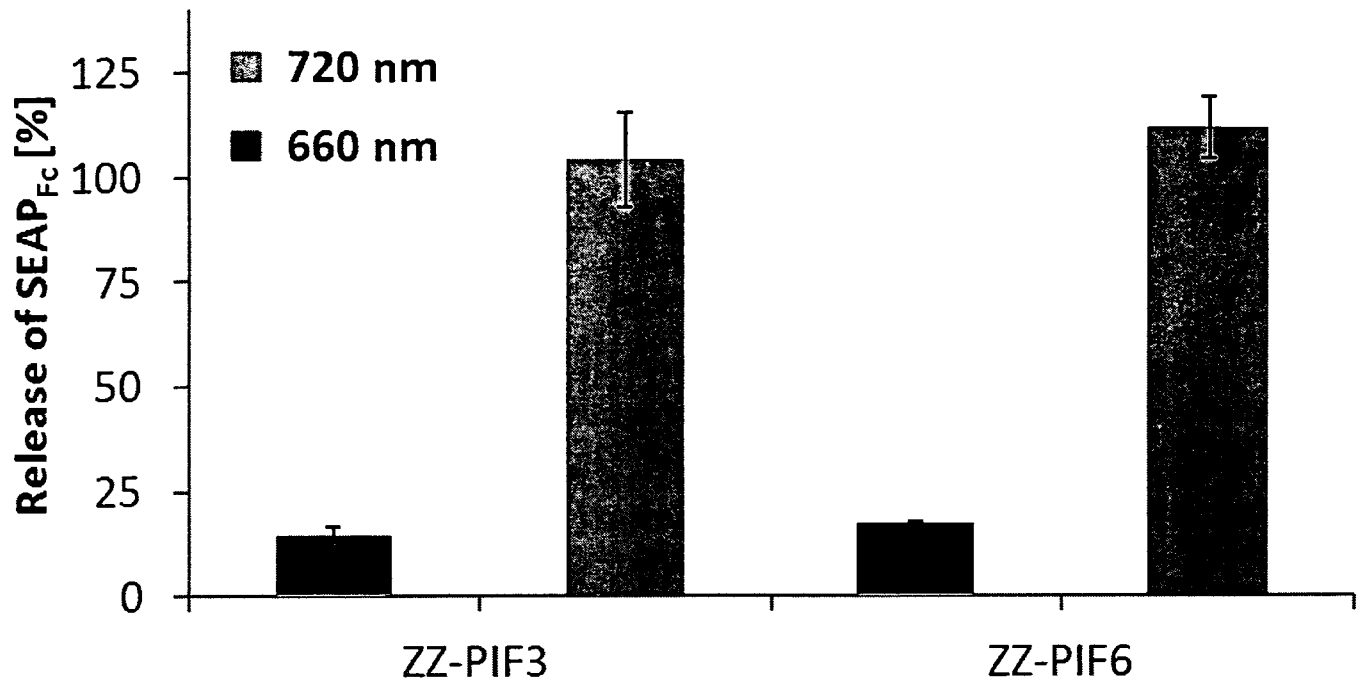


Figure 4

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2013/000393

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
 - a. (means)

☒

 on paper

☒

 in electronic form
 - b. (time)

☒

 in the international application as filed

☒

 together with the international application in electronic form

☐

 subsequently to this Authority for the purpose of search
2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
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A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/68
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/151948 A2 (UNIV CALIFORNIA [US]; VOIGT CHRISTOPHER A [US]; LEVSKAYA ANSELM [US];) 17 December 2009 (2009-12-17)	1-3, 8-10,12, 15
Y	the whole document	4-7,11, 13,14
Y	----- GE Lifesciences: "Recombinant Protein Purification Principles and Methods", November 2012 (2012-11), XP055061291, Retrieved from the Internet: URL:http://www.gelifesciences.com/gehcls_i mages/GELS/Related Content/Files/1336168762999/litdoc18114275 _20130204103715.pdf [retrieved on 2013-04-26] page 11 ----- -/-	4-7,11, 13,14



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search

30 April 2013

Date of mailing of the international search report

14/05/2013

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Rutz, Berthold

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/000393

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2013/000393

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Jared E. Toettcher ET AL: "Light Control of Plasma Membrane Recruitment Using the Phy-PIF System" In: "Protein Engineering for Therapeutics, Part B", 1 January 2011 (2011-01-01), Elsevier, XP055060550, ISSN: 0076-6879 ISBN: 978-0-12-396962-0 vol. 497, pages 409-423, DOI: 10.1016/B978-0-12-385075-1.00017-2, -----	1-15
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Information on patent family members

International application No

PCT/EP2013/000393

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