D1.2 – List of analytes, assays and fluorescence labels

Project Information

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PP	Restricted to other programme participants (incl. Commission Services)	
RE	Restricted to a group specified by the consortium (incl. Commission Services)	
CO	Confidential, only for the members of the consortium (incl. Commission Services)	

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V1.0	09/06/2021	First draft containing contributions from WSFR and CNR
V2.0	14/06/2021	Revision of the document after contribution of INN
V2.1	16/06/2021	General revision of the document
V3.0	23/06/2021	Final revision by WSFR, Innosieve and IZSVe

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1 Executive Summary

For the detection of relevant targets in the h-ALO project, bio-recognition elements are a crucial factor for success. In the h-ALO biosensor the selective bio-recognition elements, comprising DNA coding sequences, innovative aptamers and commercially available antibodies, are combined for the multiplexed detection of microbiological, pesticide/antiparasitic and heavy metals contaminations. The proposed multiplexing capability of the h-ALO sensor will enable early detection and monitoring of microbiological, pesticide/antiparasitic and heavy metal contaminations simultaneously in aquaponics water, raw milk, craft beer and organic honey. Therefore, the selected bio-recognition molecules need to be fit for purpose in every h-ALO defined matrix. The selection should focus on ease of applicability, sensitivity requirements (zero tolerance, MRL or industry set parameters), but most importantly, on availability. Additionally, fluorophore molecules suitable for incorporation and/or coupling to the aptamers and DNA probes are essential for operating the h-ALO sensor in the plasmonic-enhanced fluorescence (PEF) sensor mode.

For the selection of aptamers, suitable to detect heavy metals, a database with recent literature is compiled. Based on this literature, aptamers for the detection of mercury (Hg^{2+}), methyl-mercury (CH_3Hg^+), lead (Pb^{2+}) and cadmium (Cd^{2+}) are directly implemented or redesigned by adaptation of the most logical approach closest to the h-ALO sensor format (for the direct detection, or for the indirect complementary strand approach). For the immunoassay-based detection of pesticides a high TRL multiplex assay is introduced in the h-ALO project. This assay will be further extended on the basis of end-user demands (please consider D1.2) for the application in the h-ALO target-matrices: aquaponics water (carbamate pesticides), beer (glyphosate herbicide), milk (levamisol, triclabendazole and moxidectin antiparasitic pesticides) and honey (azoxystrobin pesticide and tetracyclines antibiotics group). For the PEF detection a database containing over 300 different fluorochromes is compiled. Based on the expected specifications of the h-ALO sensor (especially the Optoplasmonic Module), an initial selection of fluorochromes is made based on optical features such as absorption and emission maximum wavelengths, Stokes shift, extinction coefficient and fluorescence quantum yield. Moreover, parameters such as Excitation coefficient, Emission coefficient and PEF factor related to the entire process of optical excitation and emission of the Fluorophores within the detection scheme based on the nanoplasmonic grating are considered (please refer to D1.3). Eventually this selection has been further narrowed down to a final list of 6 suitable fluorochromes based on the ability of conjugation to aptamers and DNA oligo's.

2 Aptamers for heavy metals

2.1 Selection of aptamers

Aptamers are small, synthetic, single-stranded oligonucleotides (DNA) which can selectively bind chemical and biological targets. Aptamers form diverse, complex secondary structures, like multi-branched loops or G-quadruplexes. Most aptamers fold into their unique three-dimensional conformation upon binding their specific target. For the h-ALO project, aptamers for the detection of lead, cadmium and mercury are selected based on a literature study which focused on recent publications.

For the detection of Pb^{2+}, three different types of aptamers are described in literature: among these, the most common form is the G4-quadruplex. This type of aptamer consists of four G-rich sequences which form a G-quadruplex with Pb^{2+} ion due to a G-Pb-G interaction. For setting up the benchmark assays, and the implementation on the h-ALO sensor the G4-quadruplex format has been selected.

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Aptamers directed against Hg^{2+} and CH_3Hg^+ are rich in thymine (T) and readily form a T–Hg^{2+}–T or T-CH_3Hg^+-T configuration in the presence of Hg^{2+} or CH_3Hg^+ respectively. The binding force of DNA sequences to Hg^{2+} or CH_3Hg^+ is different, Hg^{2+} < CH_3Hg^+, and can be tuned by altering the number and locations of T-bases. Therefore, two different aptamers for the detection of Hg^{2+} and an aptamer optimised for detection of CH3Hg^+ will be implemented in the h-ALO project.

Cd^{2+} targeted aptamers are mainly based on one clone, Cd4. The Cd4 aptamer displays a high binding affinity to Cd^{2+} which is facilitated by a domain of 30 nucleotides that is rich in T and G nucleotides. This domain forms a secondary structure which consists of a stem-loop that is critical for binding the target. Different modifications of the CD4 aptamer are described for different sensing surfaces, but the aptamer that will be used in the h-ALO project consists of the critical domain of the original Cd4 aptamer which forms the stem-loop structure for binding of Cd^{2+}.

For the development of the benchmark aptamer-detection technology, the selected aptamers from literature have been optimized and ordered (Table 1). Each aptamer sequence is attached to a C-12 carbon spacer at the 5' end, to avoid sterical hindrance on the sensing surfaces. For the coupling to the sensing surfaces, the C-12 carbon spacer is coupled to an amino group (-NH_2). For an indirect heavy-metal detection approach, complementary sequences labeled with ATTO532 fluorochromes have been designed. For the future combination of aptamer- and antibody-based assays on the same h-ALO sensor surface, the complementary sequences have been designed for annealing temperatures lower than 60°C degrees. This to avoid the irreversible denaturation of antibodies.

Table 1. Selected and h-ALO adapted aptamer sequences and their complementary reporter strands

Heavy metal	Adapted aptamer sequence	Complementary strand sequence	Annealing temp (°C)
Lead	5'-NH2-C12-GGGTGGGTGGGTGGGT-3'	5'-ATTO532-ACCCACCCACCCAC-3'	41
Mercury	5'-NH2-C12-TTCTTTCTTCCCCTTCTTTCTT-3'	5'-ATTO532-AAGAAAGAAGGGGAAG-3'	51
Methyl-mercury	5'-NH2-C12-GTTCTTTGTTAAAAATTCTTTGTTC-3'	5'-ATTO532-GAACAAAGAATTTTTAACA-3'	49.2
Cadmium	5'-NH2-C12-ACTCTGGACTGTTGTGGTATTATTTTTGGTTGTGCAGTATG-3'	5'-ATTO532-CATACTGCACAACCAAA-3'	41.2
Random strand	5'-NH2-C12-AGACTAAGCTCAGACTCAGCTCAG-3'	5'-ATTO532-CTGAGCTGAGTCTGAGC-3'	54.1

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2.2 Fundamental literature database for the selection of aptamers

Fundamental literature about aptamer-based lead detection

1. Li, T.; Dong, S.; Wang, E. A Lead(II)-Driven DNA Molecular Device for Turn-On Fluorescence Detection of Lead(II) Ion with High Selectivity and Sensitivity. Journal of the American Chemical Society 2010, 132, 13156-13157, doi:10.1021/ja105849m.
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4. Gao, F.; Gao, C.; He, S.; Wang, Q.; Wu, A. Label-free electrochemical lead (II) aptasensor using thionine as the signaling molecule and graphene as signal-enhancing platform. Biosensors and Bioelectronics 2016, 81, 15-22, doi:https://doi.org/10.1016/j.bios.2016.01.096.
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7. Abu-Ali, H.; Nabok, A.; Smith, T.J. Development of Novel and Highly Specific ssDNA-Aptamer-Based Electrochemical Biosensor for Rapid Detection of Mercury (II) and Lead (II) Ions in Water. Chemosensors 2019, 7, 27.

Fundamental literature about aptamer-based mercury detection

1. Abu-Ali, H.; Nabok, A.; Smith, T.J. Development of Novel and Highly Specific ssDNA-Aptamer-Based Electrochemical Biosensor for Rapid Detection of Mercury (II) and Lead (II) Ions in Water. Chemosensors 2019, 7, 27.
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5. Liu, C.-W.; Tsai, T.-C.; Osawa, M.; Chang, H.-C.; Yang, R.-J. Aptamer-based sensor for quantitative detection of mercury (II) ions by attenuated total reflection surface enhanced infrared absorption spectroscopy. Analytica Chimica Acta 2018, 1033, 137-147, doi:https://doi.org/10.1016/j.aca.2018.05.037.
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Fundamental literature about aptamer-based cadmium detection

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5. Li, S.; Ma, X.; Pang, C.; Tian, H.; Xu, Z.; Yang, Y.; Lv, D.; Ge, H. Fluorometric aptasensor for cadmium(II) by using an aptamer-imprinted polymer as the recognition element. Microchimica Acta 2019, 186, 823, doi:10.1007/s00604-019-3886-7.
6. Gan, Y.; Liang, T.; Hu, Q.; Zhong, L.; Wang, X.; Wan, H.; Wang, P. In-situ detection of cadmium with aptamer functionalized gold nanoparticles based on smartphone-based colorimetric system. Talanta 2020, 208, 120231, doi:https://doi.org/10.1016/j.talanta.2019.120231.
7. Fakude, C.T.; Arotiba, O.A.; Mabuba, N. Electrochemical aptasensing of cadmium (II) on a carbon black-gold nano-platform. Journal of Electroanalytical Chemistry 2020, 858, 113796, doi:https://doi.org/10.1016/j.jelechem.2019.113796.

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2.3 Back-up strategy for heavy metal detection

Aptamer assays are widely researched, but until now there are no commercial aptamer assays available. Aptamers surely have advantages (e.g. cheap to produce, animal-friendly and highly stable) over the classic antibody used in detection assays. However, aptamers are more difficult to work with and highly platform-dependent (Table 2). The classic antibody approach is still considered the golden standard, but is under the strong attention of the European Union, since it is based on animal experiments. Therefore, the aptamer approach is chosen for the heavy metals. However, due to the higher risk factor, a back-up approach using classical antibodies is studied in case the aptamer approach does not work in the benchmark sensor and/or h-ALO sensor.

Table 2. Antibodies versus aptamers: comparison of different aspects

Aspect	Antibodies	Aptamers
Sensitivity	High through affinity	Medium through avidity
Track record	Reliable golden standard approach for a wide range of bio-sensor immunoassays	Innovative approach for selected bio-sensor immunoassays
Size	160 - 15 Kd	12 – 30 Kd
Production/maintenance costs	High	Low
Animal welfare	Animal experiments necessary	No animal experiments necessary
Engineering for improving	Highly labour intensive	Low labour intensive
Development time	Several months	Several weeks
Stability at room temperature	Medium	High
Available market applications	High	Low
Surface density on chip	Low	High
Regeneration/temperature stability	Medium	High
Modification during synthesis	Not possible	Possible
Commercial benchmark assays	yes	No

For this back-up strategy, a database of antibodies for heavy metals that are commercially available and produced within scientific collaboration is compiled. The selected antibodies are listed in Table 3. WFSR has good experiences with Unibiotest from Wuhan, so their antibodies will be included in an initial selection for setting up the heavy-metal detection back-up strategy.

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Table 3. Available antibodies for the detection of heavy metals

Supplier	Antibody directed against	Supplier	Antibody directed against
Unibiotest	Cadmium	Creative Biolabs	Lead
Unibiotest	Lead	Creative Biolabs	Cadmium
Unibiotest	Mercury	Creative Biolabs	Cadmium (clone 1H9)
Unibiotest	Methyl Mercury Chloride	Creative Biolabs	Nickel
Unibiotest	Copper	Creative Biolabs	Arsenic
Unibiotest	Chromium	Jiangnan University	Mercury
Creative Diagnostics	Mercury	Jiangnan University	Methyl Mercury Chloride
Creative Diagnostics	Cadmium	Jiangnan University	Copper
Creative Diagnostics	Lead	Jiangnan University	Chromium
Creative Diagnostics	Copper	Jiangnan University	Cadmium
Creative Diagnostics	Chromium	Jiangnan University	Lead
Creative Diagnostics	Methyl Mercury Chloride	Jiangnan University	Mercury (clone 2)
Invitrogen/ThermoFisher	Lead	Jiangnan University	Aluminium
LSBio	Lead	Jiangnan University	Nickel

3 Detection of pesticides by immunoassays

As a key-enabling approach in the design of the h-ALO sensor, multiplex detection of different typologies of analytes and of different compounds within the same typology of analyte was introduced. Considering the pesticides class, even though high TRL benchmark assay multiplex method is already in place, this detection tool still allows the easy addition of target compounds that would meet the end-users' needs (please refer to D1.1).
The current pesticide immunoassay multiplex detects 29 pesticides from 6 different pesticide classes, which are all highly detrimental to bees and insects (Table 4).

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Table 4. High TRL benchmark assays for the detection of pesticides

Group	Microsphere-code	Pesticides	Detection range (ng/mL)
Neonicotinoids	#66 IMI	Acetamiprid	0.1-10
		Clothianidin	0.1-10
		Imidacloprid	<0.1
		Imidaclothiz	0.1-10
		Nitenpyram	0.1-10
		Thiacloprid	0.1-10
Avermectins	#38 AVRM	Abamectin	<0.1
		Doramectin	0.1-10
		Emamectin	<0.1
		Eprinomectin	<0.1
		Ivermectin	0.1-10
Pyrethroids	#27 PYRS	Cyfluthrin	10-1000
		Cyhalothrin	10-1000
		Cypermethrin	10-1000
		Deltamethrin	10-1000
		Fenpropathrin	0.1-10
Phenyl pyrazole	#73 FPIR	Fipronil	<0.1
		Fipronil-sulfone	<0.1
Carbamates	#55 CABF	Carbofuran	0.1-10
		Carbosulfan	10-1000
		Isoprocarb	0.1-10
		Propoxur	0.1-10
	#30 CABY	Carbaryl	0.1-10
Organophosphorus	#64 PARA	Fenitrothion	0.1-10
		Methyl-parathion	0.1-10
		Methyl-paraxon	10-1000
		Parathion	<0.1
	#26 CHLP	Chlorpyrifos	0.1-10
		Triazophos	10-1000

The current multiplex covers most of the pesticides that were highlighted by the end-users and then reported in the h-ALO Grant Agreement. However, at the start of the project, a small desk study was undertaken in view of the Stakeholders Workshop at M5 in order to extend this multiplex with end-user-selected targets. Thus, an updated list of antibodies and antigen-conjugates has been drafted in order to meet end-users' needs. The extended list includes: levamisol, triclabendazole, moxidectin (additional antiparasitic targets for milk), but also the herbicide glyphosate (additional target for honey and craft beer), azoxystrobin and the antibiotics group tetracyclines (additional targets for honey).

After the round table between WSFR and the end-users committee (EUC) at the Stakeholders' Workshop (please refer to D1.1), additional targets are taken into consideration for addition to the pesticides list during the project.
The critical parameter for implementing new targets is strongly based on the availability of antibodies and their corresponding antigen-conjugates. These are crucial for developing new detection assays. Besides the availability of biorecognition molecules, the feasibility and

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workload are parameters that seriously need to be considered in the progress of WP2 and WP3 in order to define the best trade-off between the feasible realization of detection assays according to the technology-provider partners and the expected fit for purpose at the end-users' side.

4 Selection of microbial targets

An extensive study for selecting the microbial targets has been performed in close contact with the EUC by starting from the information that was already present in the project Grant Agreement. On the basis of the bilateral interactions between Innosieve and selected end-users, and of the outputs of the Stakeholders Workshop, the microbial targets have been confirmed, and additional targets are added for further investigation and back-up strategies. For each food chain, at least one microbial target of interest is selected.

Aquaponics water

Selected targets: *Escherichia coli* and *Salmonella enterica*. *Escherichia coli* is selected as the primary microbial target of interest. *Escherichia coli* is an indicator bacterium capable to estimate the level of faecal contamination in water systems. They are generally not very dangerous to human health, but since they are found in the intestinal tracts of warm-blooded animals, they are used to indicate the presence of a potential health risk. Additionally, *Salmonella enterica*, a bacterium dangerous to human health, is selected as a back-up target if no suitable antibody for *Escherichia coli* is available (if required).

Craft beer

Selected group target: *Lactobacillus* species. *Lactobacillus* species are the most frequently occurring beer-spoiling micro-organisms in craft beer. Their presence may cause off-flavours, turbidity, elevated carbon dioxide levels and acidity. An additional target that was indicated by the end-users is *Saccharomyces cerevisiae* var. *diastaticus*. Unfortunately, due to a combination of the nature of the organism (very close to the brewing yeast), the samples of the end-users (unfiltered craft beer), the currently most promising sample preparation protocol (separation-based on size) and the possible protocol of use of the h-ALO sensor, the detection of *Saccharomyces cerevisiae* var. *diastaticus* proves to be out of reach for unfiltered beer samples with a high background flora of living brewing yeast cells in the h-ALO sensor.

Raw milk

Selected targets: *Escherichia coli*, *Salmonella enterica* and *Listeria monocytogenes*. *Escherichia coli* is selected as the primary microbial target of interest. *Escherichia coli* is an indicator bacterium capable to estimate the level of faecal contamination. Therefore, it provides an excellent parameter to monitor milking hygiene and indicates the potential presence of human pathogens. *Salmonella enterica* and *Listeria monocytogenes* have been indicated as an additional target of interest by the end-users and are selected as possible other choices in case no effective antibody for *Escherichia coli* is available.

Honey

Selected target: *Clostridium botulinum* and *Escherichia coli. Clostridium botulinum* are selected as the primary microbial target of interest. The presence of *Clostridium botulinum* spores can

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lead to infant botulism. *Escherichia coli* and *B. cereus* are selected as a back-up targets in case spore-forming *Clostridium botulinum* bacteria are not specifically detectable by the h-ALO sensor or the benchmark technology.

5 Selection of fluorophores

An extensive desk study was undertaken by WFSR for the construction of a diversified initial database, containing the most commonly used fluorophores (Annex 1). From this initial list, CNR and PLASMORE selected a group of 14 fluorophores that meet the following optical/spectral requirements (please refer to D1.3):

- ☐ Absorption or excitation maximum should be at a wavelength >436 nm. This requirement is correlated to the stability/efficiency requirements for the light source (the Organic Light Emitting Diode, OLED). CNR has experience in the fabrication of OLEDs based on commercial deep blue emitters, also listed in D1.3. Such OLEDs would be suitable for excitation of fluorophores with absorption below 436 nm. However, both the efficiency and the stability of such OLEDs are practically low. For this reason, we decided to avoid deep blue OLEDs as light sources, and to discard consequently fluorophores absorbing in that spectral region (<436 nm).
- ☐ The Stokes Shift should be ≥60 nm. In the PEF detection mode, to improve the signal-to-noise ratio, two spectral features should be simultaneously optimized: i) the spectral overlap between the electroluminescence spectrum of the OLED and the fluorescence spectrum of the fluorophore should be minimized, so that the optical filter can block the light from the OLED back-reflected by the nano-plasmonic grating (NPG), before reaching the light detector (organic phototransistor, OPT) while allowing the light emitted by the fluorophores to reach the OPT; ii) the overlap between the OLED electroluminescence spectrum and the fluorophore absorption spectrum should be maximized in order to guarantee high optical excitation of the fluorophore. These requirements are possibly fulfilled by narrowing the emission spectrum of the OLED (i.e. using an OLED with a small full width at half maximum -FWHM-). However, only a few OLEDs show such a low FWHM, while most OLEDs that emit light in the visible range have a typical FWHM of around 50-60 nm on average. Therefore, selecting fluorophores with a large Stokes shift (≥ 60 nm) is a preferred option.
- ☐ The emission quantum yield (QY) should be ≥0.3. This ensures maximized emission intensity from the fluorophore, at a fixed excitation intensity. For the same reason, the Molar Extinction Coefficient, which is the fraction of light absorbed per units of concentration and light path, should be as large as possible, at least above 10^4 $mol^{-1} \cdot cm^{-1}$ at the maximum-absorption wavelength.

As a further requirement, the size and molecular weight of the fluorophore should be sufficiently low (i.e. molecular weight below 100 kDa). This requirement is necessary for fluorophores to be effectively bio-functionalized by chemical coupling with small recognition elements (as in the case of aptamers or DNA small fragments). A fluorophore with large molecular weight and large spatial hindrance could inhibit recognition at the binding sites of the NPG, hampering the selective detection of analytes through PEF. For this reason, the quantum dots QD 605 and QD 655, and R-Phycoerythrin (R-PE) are removed from the selected fluorophores. Finally, also the Fluorophore 4-(Dicyanomethylene)-2-Methyl-6-(p-Dimethylaminostyryl)-4H-Pyran (DCM) is removed from the final selection, due to low stability issues experienced at CNR in past experiments when handling this dye in air.

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Table 5. Selection of the best fluorophore options based on the optical/spectral requirements of; i) Maximum Absorption/Excitation Wavelength (Max Abs/Exc. WL); ii) Maximum Emission Wavelength (Max Em. WL), (iii) Stokes Shift; iv) Molar Extinction Coefficient; v) Quantum Yield.

Category	Fluorophore	Fluorophore short name	Max Abs/Exc. WL (nm)	Max Em. WL (nm)	Stokes Shift (nm)	Molar Extinction Coefficient ($mol^{-1} \cdot cm^{-1}$)	Quantum Yield
dye	4-(Dicyanomethylene)-2-Methyl-6-(p-Dimethylaminostyryl)-4H-Pyran	DCM	458	624	166	44900	0,6
dye	7-Benzylamino-4-Nitrobenz-2-Oxa-1,3-Diazole	BBD	459	529	70	19700	0,36
dye	4-(Dicyanomethylene)-2-Methyl-6-(p-Dimethylaminostyryl)-4H-Pyran	DCM	464	624	160	42000	0,43
dye	NBD-X (succinimidyl ester)	NBD-X	467	539	72	22000	
dye	FM 1-43	FM 1-43	479	598	119	40000	0,3
Phyco biliprotein	R-Phycoerythrin	R-PE	480	578	98	1960000	0,68
ATTO	ATTO 490LS	ATTO 490LS	496	661	165	40000	0,3
Chromeo	Chromeo P503 Py-Dye	Chromeo P503	503	600	97	24000	0,5
Chromeo	Chromeo P503 Dye BSA-conjugate	Chromeo P503 BSA	505	600	95	24000	0,5
dye	6-Carboxyrhodamine 6G, SE]	Dye 524	524	551	-	94000	-
quantum dot	eVolve 605	QD 605	605	-	-	-	-
ATTO	ATTO 611X	ATTO 611X	611	681	70	100000	0,35
quantum dot	eVolve 655	QD 655	655	-	-	-	-
dye	NIR820	NIR820	792	-	-	-	-

The final list which meets the above formulated requirements includes the following fluorophores:

- NIR 820
- ATTO611x
- Chromeo P503
- ATTO490LS
- FM1_43
- BBD
- Dye524
- NBDx

This list of fluorophores, together with their spectral/optical features and figures of merit, has shared within WFSR, CNR and PLASMORE for calculations on the best spectral matching between

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these fluorophores, the light source (OLED), and the NPG optical features for PEF detection, as reported in D1.3. In parallel, information on the possibility to chemically couple the selected fluorophores to the 5' or 3' end of DNA-aptamers and nucleotides has been collected from one of the main commercial providers of oligonucleotides, Eurogentec. The possibility for biofunctionalization is a further structural requirement which guides the final selection of fluorophores for their application to PEF detection. As a result, the list of selected fluorophores is reduced to ATTO490LS and Dye 524 because only these two fluorophores are suitable for modification of aptamers and oligo nucleotides. The list of fluorophores is further extended with ATTO620, ATTO700, ATT740 and ALEXA FLUOR 750. Although these fluorophores typically have a Stokes shift < 60 nm they do have the advantage that they can be coupled to aptamer/DNA fragments and therefore are also considered in the calculation for the PEF efficiency in D1.3.

Table 6. Final selection of fluorophores that meet all requested characteristics

Fluorophore	Max Abs/Exc. WL (nm)	Max Em. WL (nm)	Stokes Shift (nm)	Brightness Index (a.u.)	Molar Extinction Coefficient ($mol^{-1} \cdot cm^{-1}$)	Quantum Yield
ATTO 490LS	496	661	165	12	40000	0.3
5-CR6G, SE	522	550	28	n.s.	94000	n.s.
ATTO 620	618	642	24	60	120000	0.5
ATTO 700	699	719	20	30	120000	0.25
ATTO 740	740	764	24	n.s.	120000	0.1
Alexa Fluor 750	749	775	26	n.s.	290000	0.12

n.s. = not specified

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6 Conclusion

In general, the preliminary final list of target detection assays covers the major interest brought forward by the end-users represented in the EUC. Besides that, the selection also shows that the majority of the assays cover multiple food matrices (Table 7). A final selection of aptamers, and their corresponding complementary reporter oligo, is defined for lead, mercury, methyl-mercury and cadmium heavy metals. These 4 heavy metals are considered very relevant by the end users and comply with at least 3 out of the 4 relevant matrices in the h-ALO project (Table 7). Since these assays are also new for a benchmark assay, we have chosen two novel approaches, which will be developed on two different platforms. As is shown in table 7, the selection of antibodies for the detection of pesticides is rather extensive. The main reason, on one hand, is to try and fulfil the demand of the end users, while on the other hand, the h-ALO project benefits from the high TRL assays that are the background of partners in the Consortium. This means that for 10, out of the 13 pesticides and pesticide groups, the immunoassays are already available in the benchmark technology and only need to be transferred to the h-ALO sensing surface.

Based on end-users specific needs, a herbicide (glyphosate) and antibiotics (tetracyclines) detection assay have been added to the preliminary final selection. While some immunoassays are relevant for 3 out of 4 matrices, others are really specific to 1 matrix.

Considering the selection of microbial targets, 1 to 3 targets are selected for each matrix. For craft beer, *Lactobacillus* species is chosen as the main target, for milk and aquaponics water, *Escherichia coli* and for honey *Clostridium botulinum*. The additional targets for each matrix are reserved for second-choice strategies. Specific probe sequences will be reported in D3.4 (M18).

The desk study for suitable fluorophores for the h-ALO sensor results in a final selection of 6 fluorophores. This selection takes into account, all the specifications and constraints brought forward by the technology-provider partners. They might be considered as a trade-off between the demands for optics, spectral properties as well sensor surface compatibility and the incorporation to aptamers and DNA reporter strands.
The chosen analytes represent important targets for the safety of products belonging to the food chains of interest. Not all of them has legislative criteria reporting limits of concentration, however their absence would guarantee product safety and quality, and in most cases, they are of interest for producers and/or consumers. The specific maximum residue levels and limits for all target in each matrix will be reported in D6.3 according not only to relevant legislation, but also to requirements from guidelines and from scientific literature.

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Table 7. Preliminary final selection of analyte targets revised according to end-users' needs (AQW: aquaponic water, B: craft beer; H: raw milk; H: organic honey)

	Target	Assay format	Matrix	Benchmark technology	Readiness level[1] (%)
Heavy Metals	Lead	aptamer	AQW, B, M, H	Octet - Luminex	15
	Mercury	aptamer	AQW, B, H	Octet - Luminex	15
	Methyl-mercury	aptamer	AQW, B, H	Octet - Luminex	15
	Cadmium	aptamer	AQW, B, H	Octet - Luminex	15
	Lead	antibody	AQW, B, M, H	Luminex	5
	Mercury	antibody	AQW, B, H	Luminex	5
	Methyl-mercury	antibody	AQW, B, H	Luminex	5
	Cadmium	antibody	AQW, B, H	Luminex	5
Pesticides	Carbamates (group)	antibody	AQW	Luminex	80
	Carbamates (carbaryl)	antibody	AQW	Luminex	80
	Organophosphates (group)	antibody	B, M, H	Luminex	80
	Organophosphates (chlorpyrifos)	antibody	B, M, H	Luminex	80
	Neonicotinoids (group)	antibody	H	Luminex	80
	Neonicotinoids (thiamethoxam)	antibody	H	Luminex	80
	Fipronil	antibody	H	Luminex	80
Pesticides-antiparasitics	Pyrethroids (group)	antibody	M, H	Luminex	80
	Avermectins (group)	antibody	M, H	Luminex	90
	Avermectins (moxidectin)	antibody	M, H	Luminex	10
	Benzimidazoles (all)	antibody	M	Luminex	90
	Benzimidazoles (triclabendazole)	antibody	M	Luminex	5
	Levamisol	antibody	M	Luminex	5
Herbicide	Glyphosate	antibody	H, B	Luminex	10
Antibiotic	Tetracyclines	antibody	H	Luminex	90
Micro-organisms	*Escherichia coli*	SPC[2] or Antibody[3]	AQW, M[4], H[4]	*Sieve*-ID® technology	80
	Lactobacillus spp.	SPC[2]	B	*Sieve*-ID® technology	15
	Listeria monocytogenes	SPC[2] or Antibody[3]	M[4]	*Sieve*-ID® technology	25
	Salmonella enterica	SPC[2] or Antibody[3]	AQW, M[4]	*Sieve*-ID® technology	80
	Clostridium botulinum	SPC[2]	H[4]	*Sieve*-ID® technology	5
	Bacillus cereus	SPC[2]	H[4]	*Sieve*-ID® technology	0

[1] For the benchmark assay. [2] Solid Phase Cytometry only. [3] Solid Phase Cytometry with antibody coating. [4] Final selection of the bacterial target depends on the availability of suitable antibodies directed to structures on the outer bacterial membrane.

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7 Annex 1: Complete list of fluorophores

Category	Fluorophore	Abs or Ex max (nm)	Em max (nm)	Stokes Shift	Brightness Index	Extinction Coefficient	Quantum Yield
Alexa Fluor	Alexa Fluor 350	346	442	96		19000	
Alexa Fluor	Alexa Fluor 405	401	421	20		35000	
Alexa Fluor	Alexa Fluor 430	431	541	110	9	16000	0.55
Alexa Fluor	Alexa Fluor 488	495	519	24	67	71000	0.94
Alexa Fluor	Alexa Fluor 500	502	525	23			
Alexa Fluor	Alexa Fluor 514	517	542	25		80000	
Alexa Fluor	Alexa Fluor 532	532	553	21	65	81000	0.8
Alexa Fluor	Alexa Fluor 546	556	573	17	100	104000	0.96
Alexa Fluor	Alexa Fluor 555	555	565			155000	0.1
Alexa Fluor	Alexa Fluor 568	578	603	25	69	91300	0.75
Alexa Fluor	Alexa Fluor 594	590	617	27	47	73000	0.64
Alexa Fluor	Alexa Fluor 610	612	628	16		144000	
Alexa Fluor	Alexa Fluor 633	632	647	15		159000	
Alexa Fluor	Alexa Fluor 635	633	647	14		140000	
Alexa Fluor	Alexa Fluor 647	650	665	15		270000	0.33
Alexa Fluor	Alexa Fluor 660	663	690	27		132000	0.37
Alexa Fluor	Alexa Fluor 680	679	702	23		183000	0.36
Alexa Fluor	Alexa Fluor 700	702	723	21		205000	0.25
Alexa Fluor	Alexa Fluor 750	749	775	26		290000	0.12
Alexa Fluor	Alexa Fluor 790	782	805	23		260000	
amino acid	Trp	287	348	61	2	6000	0.31
amino acid	Tryptophan	220	354	134	0.66948	5579	0.12
amino acid	Tyr	275	303	28	0.3	1500	0.21
amino acid	Tyrosine	225	303	78	0.18265	1405	0.13
ATTO	ATTO 390	390	479	89	22	24000	0.9
ATTO	ATTO 425	436	484	48	40.5	45000	0.9
ATTO	ATTO 430LS	433	547	114	21	32000	0.65
ATTO	ATTO 465	465	507	42	41.25	75000	0.55
ATTO	ATTO 488	500	525	25	86.1	105000	0.82
ATTO	ATTO 490LS	496	661	165	12	40000	0.3
ATTO	ATTO 495	495	527	32	36	80000	0.45
ATTO	ATTO 520	524	545	21	99	110000	0.9
ATTO	ATTO 532	532	553	21	103.5	115000	0.9
ATTO	ATTO 550	553	576	23	96	120000	0.8
ATTO	ATTO 565	563	592	29	110.4	120000	0.92
ATTO	ATTO 590	594	624	30	96	120000	0.8
ATTO	ATTO 594	601	627	26		120000	0.85
ATTO	ATTO 610	614	634	20	77	110000	0.7
ATTO	ATTO 611X	611	681	70		100000	0.35
ATTO	ATTO 620	618	642	24	60	120000	0.5
ATTO	ATTO 635	635	658	23	30	120000	0.25
ATTO	ATTO 647	644	670	26	24	120000	0.2
ATTO	ATTO 647N	644	669	25		150000	
ATTO	ATTO 655	663	683	20	33	110000	0.3
ATTO	ATTO 680	680	700	20	36	120000	0.3
ATTO	ATTO 700	699	719	20	30	120000	0.25
ATTO	ATTO 725	729	752	23		120000	0.1
ATTO	ATTO 740	740	764	24		120000	0.1
ATTO	ATTO-Dino 1 (dsDNA)	490	531	41	125	179000	0.7
Brilliant	Brilliant Blue BB515	490	515	25			
Brilliant	Brilliant Ultraviolet BUV395	348	395	47			
Brilliant	Brilliant Ultraviolet BUV496	348	496	148			
Brilliant	Brilliant Ultraviolet BUV563	348	563	215			
Brilliant	Brilliant Ultraviolet BUV661	348	661	313			
Brilliant	Brilliant Ultraviolet BUV737	348	737	389			
Brilliant	Brilliant Ultraviolet BUV805	348	805	457			
Brilliant	Brilliant Violet BV421	405	421	16	1625	2500000	0.65
Brilliant	Brilliant Violet BV510	405	510	105	254	577000	0.44
Brilliant	Brilliant Violet BV570	405	570	165	184	2300000	0.08
Brilliant	Brilliant Violet BV605	405	603	198	696	2400000	0.29
Brilliant	Brilliant Violet BV650	405	645	240	425	2500000	0.17
Brilliant	Brilliant Violet BV711	405	711	306	420	2800000	0.15
Brilliant	Brilliant Violet BV785	405	785	380	100	2500000	0.04
Chromeo	Chromeo 488	488	517	29	20	73000	0.27
Chromeo	Chromeo 494	494	628	134	8	55000	0.15
Chromeo	Chromeo 505	505	526	21	21	70000	0.3
Chromeo	Chromeo 546	545	561	16	15	98800	0.15
Chromeo	Chromeo 642	642	660	18	38	180000	0.21
Chromeo	Chromeo P429 Dye BSA-conj	430	536	106	7.5	75000	0.1
Chromeo	Chromeo P429 Py-Dye	429	536	107	8	75000	0.1
Chromeo	Chromeo P503 Dye BSA-conj	505	600	95	12	24000	0.5
Chromeo	Chromeo P503 Py-Dye	503	600	97	12	24000	0.5
Chromeo	Chromeo P540 Dye BSA-conj	533	627	94	10	50000	0.2
Chromeo	Chromeo P540 Py-Dye	533	627	94	10	50000	0.2
Chromeo	Chromeo P543 BSA-conjugat	279	590	311	8.55	57000	0.15
Chromeo	Chromeo P543 Py-Dye	543	590	47	9	57000	0.15
dye	1-ANS	271	372	101	1.56	7800	0.2
dye	1,1'-Diethyl-4,4'-Carbocyanir	710	717	7	1.47	210000	0.007
dye	1,2-Diphenylacetylene	271	404	133	0.0926016	27560	0.00336
dye	1,4-Diphenylbutadiene	330	373	43	13.86	33000	0.42
dye	1,4-Diphenylbutadiyne	305	330	25	0.044758	27800	0.00161
dye	1,6-Diphenylhexatriene	353	425	72	66.144	84800	0.78
dye	2-Methylbenzoxazole	231	300	69	2.32	46400	0.05
dye	2,5-Diphenyloxazole	303	354	51	35.7	35700	1
dye	4-(Dicyanomethylene)-2-Met	458	624	166	26.94	44900	0.6
dye	4-(Dicyanomethylene)-2-Met	464	624	160	18.06	42000	0.43
dye	4-Dimethylamino-4'-Nitrostil	432	588	156	18.9	27000	0.7
dye	4'6-Diamidino-2-Phenylindol	353	465	112	15.66	27000	0.58
dye	4'6-Diamidino-2-Phenylindol	344	487	143	1.161	27000	0.043
dye	5-FAM	492	518	26	72.68	79000	0.92
dye	6-FAM	494	520	26		75000	0.9
dye	6-FAM dr	494	520	26		75000	0.9
dye	5-IAF	492	515	23			
dye	5-TAMRA	543	568	25	61.88	91000	0.68
dye	6-TAMRA	547	573	26			
dye	7-Benzylamino-4-Nitrobenz-	459	529	70	7.092	19700	0.36
dye	7-Methoxycoumarin-4-Aceti	220	382	162	2.1276	11820	0.18
dye	9,10-Bis(Phenylethynyl)Anthr	271	467	196	35.4	35400	1
dye	9,10-Diphenylanthracene	279	302	23	14	14000	1

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Category	Fluorophore	Abs or Ex max (nm)	Em max (nm)	Stokes Shift	Brightness Index	Extinction Coefficient	Quantum Yield
dye	Acridine Orange	271	520	249	5	27000	0.2
dye	Acridine Orange	271	520	249	5.4	27000	0.2
dye	Acridine Yellow	264	492	228	18.518	39400	0.47
dye	Anthracene	356	397	41	3.492	9700	0.36
dye	Auramine O	431	499	68	0.759	25300	0.03
dye	Benzene	255	303	48	0.01113	210	0.053
dye	Biphenyl	247	326	79	2.88	16000	0.18
dye	BO-PRO-1	280	481	201	9.28	58000	0.16
dye	BO-PRO-3	574	599	25	50.22	81000	0.62
dye	BOBO-1	256	481	225	25.08	114000	0.22
dye	BOBO-3	570	604	34	57.72	148000	0.39
dye	BODIPY 507/545	513	549	36	60	82800	0.73
dye	BODIPY FL	504	510	6	63	70000	0.9
dye	BODIPY TR	588	616	28	57	68000	0.84
dye	C3-Indocyanine	544	557	13	9.31	133000	0.07
dye	C3-Oxacyanine	485	497	12	7.45	149000	0.05
dye	C3-Thiacyanine Dye (EtOH)	559	571	12	6.3	126000	0.05
dye	C3-Thiacyanine Dye (PrOH)	560	572	12	8.05	161000	0.05
dye	C5-Indocyanine	638	657	19	80	200000	0.4
dye	C5-Oxacyanine	582	603	21	116.62	238000	0.49
dye	C5-Thiacyanine	656	674	18	87.15	249000	0.35
dye	C7-Indocyanine	743	771	28	67.2	240000	0.28
dye	C7-Oxacyanine	687	712	25	107.8	220000	0.49
dye	Calcein	494	516	22	63	81000	0.78
dye	Cascade Blue	378	423	45	14	26000	0.54
dye	CHOxAsH-CCXXCC	386	430	44	11.76	33600	0.35
dye	Coumarin 1	375	445	70	17.155	23500	0.73
dye	Coumarin 30	406	478	72	28.676	42800	0.67
dye	Coumarin 314	436	476	40	31.824	46800	0.68
dye	Coumarin 343	443	462	19	27.909	44300	0.63
dye	Coumarin 6	456	500	44	42.12	54000	0.78
dye	Cresyl Violet Perchlorate	603	622	19	44.82	83000	0.54
dye	Cresyl Violet Perchlorate	603	622	19	45	83000	0.54
dye	Crystal Violet (Glycerol)	592	638	46	2.128	112000	0.019
dye	Cy2	483	506	23	18	150000	0.12
dye	Cy3	547	570	23	6	150000	0.04
dye	Cy3.5	576	596	20	22.5	150000	0.15
dye	Cy3B	552	570	18	87	130000	0.67
dye	Cy5	649	670	21	70	250000	0.28
dye	Cy5.5	675	694	19	58	250000	0.23
dye	Cy7	753	767	14	56	200000	0.28
dye	Labeling-detection	550	564	14		150000	
dye	CyLyte Fluor 3, NHS ester	554	576	22			
dye	Dansyl Glycine (Dioxane)	262	492	230	2.838	4300	0.66
dye	Dansyl-X	333	518	185			
dye	DAPI (in DMSO)	353	465	112	16	27000	0.58
dye	DAPI (in H2O)	344	487	143	1	27000	0.04
dye	dichlorofluorescein	504	529	25	82.8	90000	0.92
dye	Dragon fly orange	554	576	22			
dye	DY-681	691	708	17			
dye	DY-781	782	800	18			
dye	Eosin Y	525	543	18	75	112000	0.67
dye	Eosin Y	525	543	18	75.04	112000	0.67
dye	Ethyl-p-Dimethylaminobenzc	309	330	21	6.7164	23160	0.29
dye	EYFP	514	527	13	51	84000	0.61
dye	FITC	495	514	19	80.96	88000	0.92
dye	FlAsH-CCXXCC	508	528	20	20.5	41000	0.5
dye	fluorescein	493	514	21	80.96	88000	0.92
dye	Fluorescein (EtOH)	500	540	40	89.531	92300	0.97
dye	Fluorescein F2- (pH >8)	490	515	25	71	76900	0.92
dye	Fluorescein FH- (pH 5.3)	472	515	43	11	29000	0.37
dye	Fluorescein-Dibase	225	514	289	72.917	92300	0.79
dye	fluoro-emerald	495	514	19	80.96	88000	0.92
dye	FM 1-43	479	598	119	12	40000	0.3
dye	Fura Red	435	655	220	0.533	41000	0.013
dye	Fura-2, Ca++ free	363	512	149	6	28000	0.23
dye	Fura-2, Ca++ saturated	335	505	170	17	34000	0.49
dye	Fura-2, Zn++ saturated	345	505	160	24	34000	0.69
dye	HEX	535	556	21		90000	0.7
dye	HiLyte Fluor 405	404	428	24		35000	0.54
dye	HiLyte Fluor 488	497	526	29	70.98	78000	0.91
dye	HiLyte Fluor 532	545	565	29		171000	0.26
dye	HiLyte Fluor 555	550	566	16	6	150000	0.04
dye	HiLyte Fluor 594	593	616	23		80,000	0.9
dye	HiLyte Fluor 647	649	673	24	67.5	250000	0.27
dye	HiLyte Fluor 680	678	702	24	19	190000	0.1
dye	HiLyte Plus 555	552	567	15	6	150000	0.04
dye	HiLyte Plus 647	649	668	19	67.5	250000	0.27
	HiLyte™ Fluor 750	750	782	32		275,000	0.12
dye	Hoechst 33258 (in DMF)	354	486	132	16	46000	0.35
dye	Hoechst 33258 (in H2O)	345	507	162	2	46000	0.03
dye	Hoechst-33258 (DMF)	354	486	132	16.1	46000	0.35
dye	Hoechst-33258 (H2O)	345	507	162	1.564	46000	0.034
dye	Indo-1, Ca++ free	346	475	129	13	33000	0.38
dye	Indo-1, Ca++ saturated	330	401	71	18	33000	0.56
dye	IRDye38	778	806	28	62	179000	0.35
dye	IRDye40	768	788	20	53	140000	0.38
dye	IRDye700	681	712	31	81	170000	0.48
dye	IRDye78	768	796	28	68	220000	0.31
dye	IRDye80	767	791	24	53	250000	0.21
dye	IRDye800	787	812	25	41	275000	0.15
dye	JOE	520	548	28	44	73000	0.6
dye	JOJO-1	530	545	15	75.24	171000	0.44
dye	LOLO-1	568	580	12	43.2	108000	0.4
dye	Lucifer Yellow CH	230	542	312	5	24200	0.21
dye	Lucifer Yellow CH	230	542	312	5.082	24200	0.21
dye	Merocyanine 540	559	579	20	53.82	138000	0.39
dye	Merocyanine 540	559	579	20	54	138000	0.39
dye	monobromobimane	301	490	189	1.5	5000	0.3
dye	N,N'-Difluoroboryl-1,9-Dime	503	521	18	3.744	48000	0.078

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D1.1 List of analytes, assays and fluorescence labels

Category	Fluorophore	Abs or Ex max (nm)	Em max (nm)	Stokes Shift	Brightness Index	Extinction Coefficient	Quantum Yield
dye	N,N'-Difluoroboryl-1,9-Dimethyl-5-Phenydipyrr	515	526	11	2.862	54000	0.053
dye	N,N'-Difluoroboryl-1,9-Dimethyl-5-Phenydipyrr	516	536	20	13.57	59000	0.23
dye	Naphthalene	220	322	102	1.38	6000	0.23
dye	NBD-X (succinimidyl ester)	467	539	72		22000	
dye	neo-Cy5 (DMSO)	656	675	19	49	195000	0.25
dye	Nile Blue (MeOH)	625	659	34	20.736	76800	0.27
dye	Nile Red	262	580	318	26.6	38000	0.7
dye	NIR1	761	796	35	62	268000	0.23
dye	NIR1	763	796	33	85	250000	0.34
dye	NIR2	664	684	20	61.64	268000	0.23
dye	NIR2	662	684	22	85	250000	0.34
dye	NIR3	750	777	27	77	275000	0.28
dye	NIR3	726	777	51	111.8	260000	0.43
dye	NIR4	629	671	42	77	275000	0.28
dye	NIR4	650	671	21	1112	260000	0.43
dye	NIR820	792			0		
dye	Oregon Green 488	496	516	20	68	76000	0.9
dye	Oregon Green 514	506	526	20	85	88000	0.96
dye	Oxazine 1	643	665	22	13.53	123000	0.11
dye	Oxazine 170	614	641	27	52.29	83000	0.63
dye	Oyster 645 (ethanol)	651	669	18	100	250000	0.4
dye	Oyster 656 (ethanol)	665	684	19	11	220000	0.5
dye	P-Quaterphenyl	293	363	70	36.49	41000	0.89
dye	P-Terphenyl	276	338	62	31.434	33800	0.93
dye	Pacific Blue	400	447	47	16	29500	0.55
dye	Perylene	253	435	182	36.19	38500	0.94
dye	Phenol	220	292	72	0.1755	2340	0.075
dye	Phenylalanine	222	279	57	0.00429	195	0.022
dye	Phthalocyanine	699	701	2	97.2	162000	0.6
dye	Pinacyanol-Iodide	604	621	17	0.128	128000	0.001
dye	Piroxicam	325	476	151	0.455	13000	0.035
dye	PO-PRO-1	437	455	18	19.5	50000	0.39
dye	PO-PRO-3	539	567	28	50.16	88000	0.57
dye	POPO-1	431	456	25	55.2	92000	0.6
dye	POPOP	256	407	151	44	47000	0.93
dye	Proflavin (pH 7)	261	511	250	13.226	38900	0.34
dye	Pyrene	241	381	140	17.28	54000	0.32
dye	Quinine Sulfate (0.05M H2SO4)	256	450	194	3.1122	5700	0.546
dye	Quinine Sulfate (0.5M H2SO4)	256	451	195	3.1122	5700	0.546
dye	Quinine sulfate (in 0.5M H2SO4)	256	451	195	3	5700	0.55
dye	Rhodamine 110	496	520	24	71	80000	0.89
dye	Rhodamine 123	512	531	19	76.68	85200	0.9
dye	Rhodamine 6G	530	552	22	110	116000	0.95
dye	Rhodamine B	543	565	22	74	106000	0.7
dye	Rhodamine B	543	565	22	74.2	106000	0.7
dye	5(6)-CR110 [5-(and-6)-Carboxyrhodamine 110,	498	521	23		76000	
dye	5-CR6G, SE [5-Carboxyrhodamine 6G, SE]	522	550	28		94000	
dye	6-CR6G, SE [6-Carboxyrhodamine 6G, SE]	524	551			94000	
dye	Riboflavin	220	531	311	9.9	33000	0.3
dye	Rose bengal	559	571	12	10	90400	0.11
dye	5(6)-ROX	568	591	27			
dye	SNIR1	666	695	29	52.32	218000	0.24
dye	SNIR2	764	803	39	24.64	224000	0.11
dye	SNIR3	667	697	30	59	245000	0.24
dye	SNIR4	765	803	38	30.94	238000	0.13
dye	Spectrum FRed	650	676	26	70	250000	0.28
dye	Squarylium dye III	628	646	18	200.85	309000	0.65
dye	Star 440 SXP	436	515	79	15	22700	0.68
dye	Star 470 SXP	472	624	152	4	29000	0.12
dye	Star 488	503	524	21	57	64500	0.89
dye	Star 512	511	530	19	69	84000	0.82
dye	Star 520SXP	515	612	97	3	60000	0.05
dye	Star 580	587	607	20	65	72000	0.9
dye	Star 600	604	627	23	32	43500	0.73
dye	Star 635	639	654	15	32	63000	0.51
dye	Star 635P	635	651	16	115	125000	0.92
dye	Star Red	638	655	17	191	212000	0.9
dye	Stilbene	294	345	51	1.45	29000	0.05
dye	Sulforhodamine 101	576	591	15	125	139000	0.9
dye	SYTO 11	257	527	270	36.75	75000	0.49
dye	SYTO 13	257	509	252	29.6	74000	0.4
dye	SYTO 17	258	634	376	18.48	88000	0.21
dye	SYTOX Green	504	523	19	35.51	67000	0.53
dye	SYTOX Orange	257	570	313	71.1	79000	0.9
dye	6-TET	520	535	15			
dye	Tetra-t-Butylazaporphine	339	626	287	67.2	320000	0.21
dye	Tetra-t-Butylnaphthalocyanine	785	793	8	0.26915	26915	0.01
dye	Tetramethylrhodamine	544	572	28			
dye	5-TMRIA [Tetramethylrhodamine-5-iodoaceta	541	567	26			
dye	Tetrakis(2,6-Dichlorophenyl)Porphyrin	419	716	297	0.5681	299000	0.0019
dye	Texas Red	586	605	19	83	108000	0.77
dye	Texas Red-X	583	603	20	104	116000	0.9
dye	TMR	540	565	25	65	95000	0.68
dye	TO-PRO-1	515	531	16	15.75	63000	0.25
dye	TO-PRO-3	642	661	19	11.22	102000	0.11
dye	Toluene	220	290	70	0.48688	2864	0.17
dye	TOTO-1	514	533	19	39.78	117000	0.34
dye	TOTO-3	261	660	399	9.24	154000	0.06
dye	Tris(2,2'-Bipyridyl)Ruthenium(II)	286	625	339	0.6132	14600	0.042
dye	Yakima yellow	530	550	20		83800	0.96
dye	YO-PRO-3	613	631	18	16	100000	0.16
dye	YOYO-1	491	509	18	51.428	98900	0.52
dye		231	631	400	25.05	167000	0.15
dye	Zinc Phthalocyanine	674	678	4	84.54	281800	0.3
fluorescent protein	DsRed (Campbell Tsien 2003)	559	583	24	45.03	57000	0.79
fluorescent protein	DsRed Dimer2 (Campbell Tsien 2003)	553	579	26	81.6	120000	0.68
fluorescent protein	DsRed Dimer2 (Campbell Tsien 2003)	553	579	26	41.4	60000	0.69
fluorescent protein	DsRed-Express T1 (Campbell Tsien 2003)	277	584	307	17.85	35000	0.51
fluorescent protein	Emerald (Tsien1998)	491	509	18	39.1	57500	0.68
fluorescent protein	H9-40 (Tsien1998)	398	511	113	18.56	29000	0.64

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Category	Fluorophore	Abs or Ex max (nm)	Em max (nm)	Stokes Shift	Brightness Index	Extinction Coefficient	Quantum Yield
fluorescent protein	KikGR Green	507	517	10	37.59	53700	0.7
fluorescent protein	KikGR Red	359	593	234	22.815	35100	0.65
fluorescent protein	mApple	567	592	25	36.75	75000	0.49
fluorescent protein	mBanana	540	553	13	4.2	6000	0.7
fluorescent protein	mCherry	587	610	23	15.84	72000	0.22
fluorescent protein	mHoneyDew	478	562	84	2.04	17000	0.12
fluorescent protein	mKate2	589	633	44	25	62500	0.4
fluorescent protein	mOrange	546	562	16	48.99	71000	0.69
fluorescent protein	mOrange2	549	565	16	34.8	58000	0.6
fluorescent protein	mPlum	589	649	60	4.1	41000	0.1
fluorescent protein	mRaspberry	597	624	27	12.9	86000	0.15
fluorescent protein	mRFP1 (Campbell Tsien 2003)	585	607	22	11	44000	0.25
fluorescent protein	mStrawberry	574	596	22	26.1	90000	0.29
fluorescent protein	mTangerine	568	585	17	11.4	38000	0.3
fluorescent protein	P4-3 (Tsien1998)	380	446	66	6.69	22300	0.3
fluorescent protein	PA-GFP (post-activation)	502	517	15	13.746	17400	0.79
fluorescent protein	R14H4 RFP	589	648	59	4	40000	0.1
fluorescent protein	TagRFP-T	557	584	27	33.21	81000	0.41
fluorescent protein	tdTomato	554	581	27	47.61	69000	0.69
fluorescent protein	Topaz (Tsien1998)	514	527	13	19.5	32500	0.6
fluorescent protein	W1B ECFP (Tsien1998)	431	476	45	13	32500	0.4
fluorescent protein	WEGFP (post-activation)	482	505	23	13.88	34700	0.4
fluorescent protein	WTGFP (Tsien1998)	395	511	116	19.75	25000	0.79
phycobiliprotein	Allophycocyanin (APC)	650	660	10	476	700000	0.68
phycobiliprotein	APC	651	660	9	476	700000	0.68
phycobiliprotein	B-phycoerythrin (B-PE)	545	575	30	2362	2410000	0.98
phycobiliprotein	R-Phycoerythrin (R-PE)	480	578	98	1333	1960000	0.68
porphyrin	Magnesium Octaethylporphyrin (CH2Cl2)	407	581	174	61.245	408300	0.15
porphyrin	Magnesium Octaethylporphyrin (Toluene)	410	582	172	61.245	408300	0.15
porphyrin	Magnesium Phthalocyanine (PrOH)	668	671	3	66.196	87100	0.76
porphyrin	Magnesium Phthalocyanine (Pyridine)	674	678	4	41.808	87100	0.48
porphyrin	Magnesium Tetramesitylporphyrin	427	664	237	75.939	446700	0.17
porphyrin	Magnesium Tetraphenylporphyrin	426	663	237	85.5	570000	0.15
porphyrin	Octaethylporphyrin	400	623	223	20.67	159000	0.13
porphyrin	Porphin	396	683	287	11.223	261000	0.043
porphyrin	Tetrakis(o-Aminophenyl)Porphyrin	406	654	248	15.106	166000	0.091
porphyrin	Tetramesitylporphyrin	427	721	294	37.576	427000	0.088
porphyrin	Tetraphenylporphyrin	419	649	230	44	400000	0.11
porphyrin	Tetraphenylporphyrin (Diprotonated)	240	687	447	60.34	431000	0.14
porphyrin	Zinc Octaethylporphyrin	404	571	167	18.765	417000	0.045
porphyrin	Zinc Tetramesitylporphyrin	421	643	222	11.935	385000	0.031
porphyrin	Zinc Tetraphenylporphyrin	423	645	222	18.942	574000	0.033
qdot	QD525	300	525	225	284	710000	0.4
qdot	QD565	300	565	265	760	1900000	0.4
qdot	QD585	300	585	285	1400	3500000	0.4
qdot	QD605	300	605	305	1760	4400000	0.4
qdot	QD655	250	655	405	5460	9100000	0.6
quantum dot	eVolve 605	605					
quantum dot	eVolve 655	655					
quantum dot	QD525	348	525	177	192	320000	0.6
quantum dot	QD565	348	565	217	440	1100000	0.4
quantum dot	QD585	348	585	237	880	2200000	0.4
quantum dot	QD605	348	605	257	960	2400000	0.4
quantum dot	QD655	348	655	307	2280	5700000	0.4
quantum dot	QD705	348	705	357			

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