When Weak Is Strong

How to cite: *Angew. Chem. Int. Ed.* **2023**, *62*, e202303390
doi.org/10.1002/anie.202303390

Fluorescent Probes

When Weak Is Strong: A Plea for Low-Affinity Binders for Optical Microscopy

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Abstract: The exploitation of low-affinity molecular interactions in protein labeling is an emerging topic in optical microscopy. Such non-covalent and low-affinity interactions can be realized with various concepts from chemistry and for different molecule classes, and lead to a constant renewal of fluorescence signals at target sites. Further benefits are a versatile use across microscopy methods, in 3D, live and many-target applications. In recent years, several classes of low-affinity labels were developed and a variety of powerful applications demonstrated. Still, this research field is underdeveloped, while the potential is huge.

1. Introduction

Labeling a target molecule with a fluorescent dye is a cornerstone of fluorescence microscopy. Two basic strategies exist: genetic labeling, e.g. the introduction of a fluorescent protein or a protein tag that is co-expressed with a target protein; secondary molecule labeling, e.g. with a fluorophore-labeled antibody or any other type of target-specific, fluorophore-labeled molecule. In both strategies, a fluorophore label is bound 'permanently' to a target molecule, either through a covalent bond, or through high-affinity interactions. There are three important consequences arising from such permanent fluorophore labels: i) the total detectable fluorescence signal, and thus observation time, is limited by the photobleaching of the fluorophore; ii) visualizing many targets in the same cell (often termed "multiplexing") is limited by a "spectral barrier", i.e. the number of fluorophores that can be discriminated by spectra, fluorescence lifetime, or other orthogonal parameters; and iii) the covalent labeling results in a perturbation of the system, changing the size, steric accessibility and physicochemical properties of the object labeled. A solution to this paradigm is to use fluorophore labels that exhibit a low affinity to a target molecule, while not compromising on the target specificity. Such labels bind to and unbind from a target in a transient and reversible way, while being kept in the imaging buffer which serves as a reservoir. This leads to a constant exchange of fluorophore labels at target sites. Since the concentration of labels in solution is typically many orders of magnitudes larger than the number of targets in a sample, the reservoir of intact labels is large and photobleaching is bypassed (Figure 1). Furthermore, non-permanent interactions facilitate the use of multiple labels by a simple buffer exchange, thereby bypassing the "spectral barrier" and enabling theoretically sequential imaging of an unlimited number of target molecules. Moreover, weak binding enables new imaging modalities in the field of super-resolution microscopy and single-molecule imaging by controlling the number of bound fluorophores through affinity and bulk concentration.

In recent years, different types of such low-affinity fluorophore labels have emerged (Figure 1), opening a new window of cellular imaging applications and beyond. However, this field is still in its infancy and the full potential of this approach is far from being harvested. Here, we review the current state of how low-affinity labels were integrated in fluorescence microscopy experiments, and present a roadmap for future developments in this promising research field.

2. Low-affinity labels may overcome several disadvantages of the classical covalent labeling procedures

Photobleaching is a common challenge for all fluorescence microscopy techniques. It is particularly detrimental when long time lapse imaging is desirable, for high-performance super-resolution techniques (e.g. stimulated emission depletion (STED) microscopy),[1] or when small numbers of fluorophores need to be imaged (e.g. single-molecule experiments). The impact of photobleaching can be minimized with low-affinity binders, which are kept in the imaging buffer that serves as a reservoir of 'fresh' (intact) fluorophore labels and allows for the continuous replenishment of fluorophore labels at target sites. To achieve this, it is key to correctly match the photobleaching rate with the exchange rate of the fluorophores. Probes with too high affinity would exchange too slowly resulting in significant photobleaching

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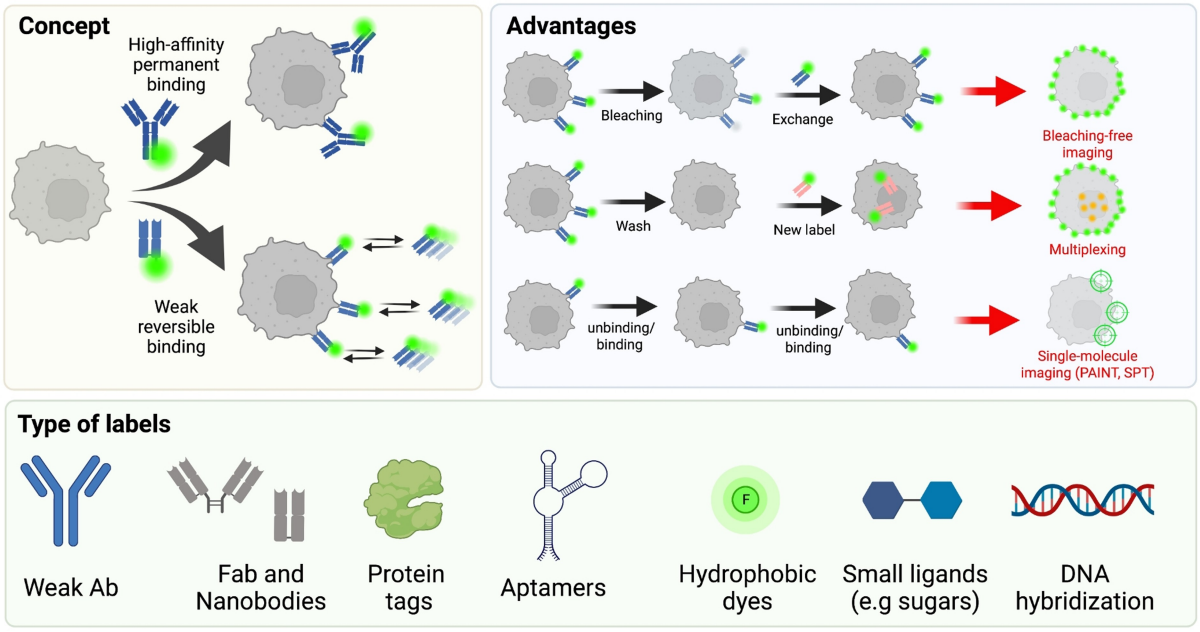

Figure 1. Concept, applications and types of low-affinity fluorophore labels. Conceptual visualization of high-affinity binding versus low-affinity and reversible binding (top left). Schematic depiction of the advantages of low-affinity cell labeling (top right). Summary of different types of low-affinity labels (bottom).

while probes that with a too low affinity will not guarantee enough signal. Moreover, the reservoir should contain a much larger number of probes as there are target sites. With these requirements met, exchangeable probes will provide a constant fluorescence signal over time, which enables long-time live cell microscopy, as well as multi-color confocal and super-resolution imaging of large 2D/3D samples with minimized out-of-plane photobleaching.[2,3] Yet, the application of low-affinity labels is not limited to fluorescence microscopy: photobleaching also limits the accessible observation time in other fluorescence methods, such as single-molecule Förster resonance energy transfer (FRET). Equally, photobleaching is a major obstacle in single-particle tracking (SPT), where a small number of target molecules is sparsely labeled and their movement is tracked.[4]

Low-affinity ligands enable advanced fluorescence methods where the tight control on the number of labels in the samples and their dynamics is crucial. For example, super-resolution microscopy techniques like "points accumulation for imaging in nanoscale topography" (PAINT)[5] rely on the reversible binding and detection of single fluorophores, building on weak interacting probes. Methods like super-resolution optical fluctuation imaging (SOFI)[6] or super-resolution radial fluctuation microscopy (SRRF)[7] are based on fluorescence fluctuations that can be enhanced by the binding and unbinding of low-affinity probes. In this context, unbinding virtually "turns off" the fluorescence emission of the probe, similarly to a photoswitching event; unbound probes diffuse fast compared to the image acquisition rate and will be not detected. The presence of unbound probes in the imaging buffer might however result in higher background, depending on the imaging method. If needed, a possible solution to higher background is the use of fluorogenic probes that will only fluoresce if bound to their target molecules.[8,9]

The experimental concept of keeping the labels in the imaging buffer enables simple multi-colour imaging protocols, by sequential washing in and out imaging buffers with

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different fluorophore labels. While classical microscopy is limited by spectral overlap of dyes to 3–4 colors (potentially expandable integrating orthogonal fluorescence parameters like lifetime), multiplexing experiments with low-affinity labels are not limited in the number of targets to be imaged. It has to be taken into account that this comes at the cost of time, as sequential imaging is necessary. Moreover, it is crucial to ensure that the previous probe is completely washed away to avoid cross talk between channels. Also, in this case the choice of the proper affinity is important as high-affinity probes would be difficult to completely remove or would require very long washing steps resulting in long measurements and shear-stress induced cell toxicity.

Low-affinity labels are interoperable, i.e. they are compatible with different advanced microscopy methods. So far, these labels were applied in combination with different super-resolution microscopy techniques, such as single-molecule localization microscopy (SMLM),[10] SOFI and STED, as well as with confocal and widefield microscopy (Figure 2). Specific experimental needs for the respective microscopy method can be easily realized by manipulating the concentration of the labels and their affinity for the target.

Another very important advantage is that low-affinity labels are intrinsically independent of any manipulation of the target cell, such as the expression of a fluorescent protein, or chemical fixation as required for antibody labelling. This implies two main advantages: i) minimal perturbation of the sample and thus more reliable results; ii) application to experiments where cell perturbation is not possible such as live cell imaging or imaging of cells that cannot be easily transfected.

However, the use of low-affinity labels is not free of challenges. The biggest requirement is the development of novel probes designed to exhibit reversible binding retaining high specificity for the target molecules. This is far from trivial as the range of affinities necessary (high-nM-to-μM) is rather narrow. Even better, a fine tuning of the kinetic constants (k_{on} and k_{off}) is desirable. Moreover, most of the screening processes currently used are designed to select for the highest affinity possible and are therefore not usable. Low-affinity labels simplify the imaging procedure by avoiding steps like cell fixation or covalent labeling, yet in order to get the best performance out of this approach, new technological implementations (e.g. microfluidic devices for multicolor imaging) and rounds of optimization (e.g. probe affinity and concentration) are necessary. It is also important to consider that intracellular targets will be significantly more challenging due to the need of membrane-permeable probes.

3. Types of low-affinity labels, underlying molecular interactions and their use for imaging

Low-affinity labels come in different chemical flavors and employ different types of intermolecular interactions. The first group are small-molecule organic dyes that act as direct target labels. One prominent example is Nile Red, a fluorogenic dye which turns fluorescent once it binds to lipid bilayers of cell membranes. Nile Red was also the first low-affinity fluorophore used in (super-resolution) fluorescence microscopy, in a method termed "points accumulation for imaging in nanoscale topography" (PAINT).[5] Recent work reported live-cell super-resolution microscopy STED microscopy with Nile Red, allowing for extended observation times without signal loss.[2] A further development is the redesign of Nile Red, rendering it sensitive to polarity, which allowed long-time live-cell super-resolution microscopy of endocytosis events.[11] Another target accessible with exchangeable small-molecule labels are amyloid fibers, which are bound by Thioflavin T[12] or the curcumin derivative CRANAD-2.[13]

A second group are molecules that consist of a low-affinity target-binding unit conjugated to a fluorophore. This group comprises, among others, conjugates of the DNA minor groove binder Hoechst with fluorophores, which were used for both PAINT[14] and STED microscopy;[2] fluorophore-peptide labels, such as the Lifeact peptide that targets cellular actin[15] and fluorophore-aptamer conjugates.[16] An interesting perspective is the use of physiological weak

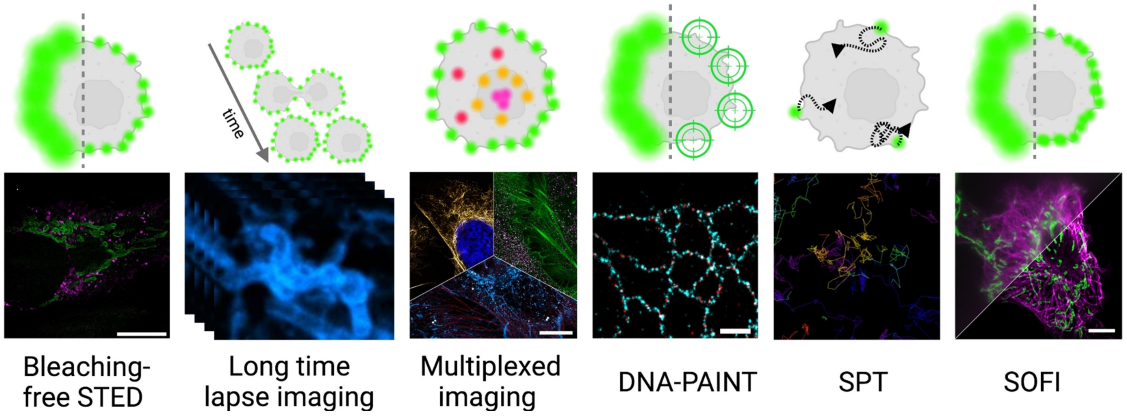

Figure 2. Selection of applications of low-affinity labels for diverse fluorescence microscopy methods (images adapted from Ref. [21, 25]).

interactions to perform reversible labeling. In this approach, a protein of interest is targeted with a fluorophore-labeled analog of its natural ligand. This approach was demonstrated for protein-protein interactions[17] and for glycoprotein-targeting labels that were used to map glycan-lectin interactions in live cells.[18]

A great repository for protein targeting are antibodies, developed and optimized for high specificity and target affinity. Targeted mutagenesis of antibodies allows increasing the dissociation rate without compromising the specificity, and by that, 'reprogramming' conventional antibodies into exchangeable labels.[19] Protein tags are another excellent option to genetically target proteins, with the additional benefits of live-cell imaging and stoichiometric protein labeling. For example, engineered mutants of the bacterial lipocalin Blc that bind a cell-permeable fluorogenic dye enabled live-cell fluorescence microscopy with a renewable signal.[20] Peptide-protein interactions have been tuned to perform low-affinity and reversible labeling for STED and SMLM imaging. For example the PDZ-domain has been used to map target proteins in fixed cells.[17] Recently, the HaloTag7 system was expanded towards orthogonal two-color live-cell imaging with exchangeable, cell-permeable and fluorogenic ligands,[8] and integrated into various super-resolution imaging modes[8,21] as well as in fast live cell imaging supported by a deep neural network.[22]

A third group builds on low-affinity DNA hybridization, which is achieved by designing a pair of sequence-complementary DNA oligonucleotides and the first one binding to a target (e.g., through conjugation to an antibody) and the second one conjugated to a fluorophore. Extensively used for single-molecule super-resolution microscopy in a method termed DNA-PAINT,[23,24] the concept of low-affinity DNA-hybridization has been transferred to other super-resolution methods.[25,26] Reversible DNA labels were also used in AI-enhanced single-molecule super-resolution microscopy with increased speed in image acquisition, benefitting from an adjustable and constant emitter density that matches the operation window of the neural network.[27] First attempts to bypass the limitation of the accessible observation time in single-molecule FRET experiments also made use of low-affinity DNA hybridization and demonstrated monitoring the dynamics of a Holliday junction for one hour.[28] Equally, DNA-mediated continuous fluorophore exchange enabled single-particle tracking with extended observation times.[29]

While the types of molecules and low-affinity interactions are different, there are common physicochemical and methodological characteristics: (i) dissociation constants are in the range of 100 nM to 100 μM (not limited to), and off-rates in the range of 1–100 s^{-1}; (ii) the concentration of the fluorophore label in the imaging buffer is an additional degree of freedom, typically kept between 0.1 nM and several hundred nM, allowing to match the degree of labeling to the specific imaging method.

4. A roadmap for low-affinity binders in imaging

In the previous sections we described the advantages and the current applications of low-affinity binders in fluorescence imaging. While the potential of this approach is very clear, there are still some hurdles that limit the widespread adoption of this method. So far most of the reports are anecdotal and common procedures are missing. In this section we want to propose a roadmap to tackle the open issues in this field and guide the development of imaging methods based on low-affinity labels (Figure 3).

First step would be the identification of key target applications where the use of low-affinity labels can make a substantial impact. Among the many possible, some seem more promising and more impactful. For example, limiting photobleaching is desirable in many imaging applications, however it is significantly more important in methods that intrinsically need to employ high illumination intensities. This is the case for high-performance super-resolution methods, such as STED and SMLM, where photobleaching is hampering long live cell time lapse imaging or complex 3D volumetric imaging. Low-affinity labels are beginning to help increase the observation time in live cell imaging. In addition, highly-multiplexed analyses are changing our view on biology, for example in the case of next generation sequencing, allowing to measure multiple cell features with single-cell resolution (e.g single cell RNA-seq) and perform multivariate analysis to study cell sub-populations imaging technologies that are capable of visualizing a large number of targets would enable phenotyping of cells or tissues with broad implications in cell biology and diagnostics. While

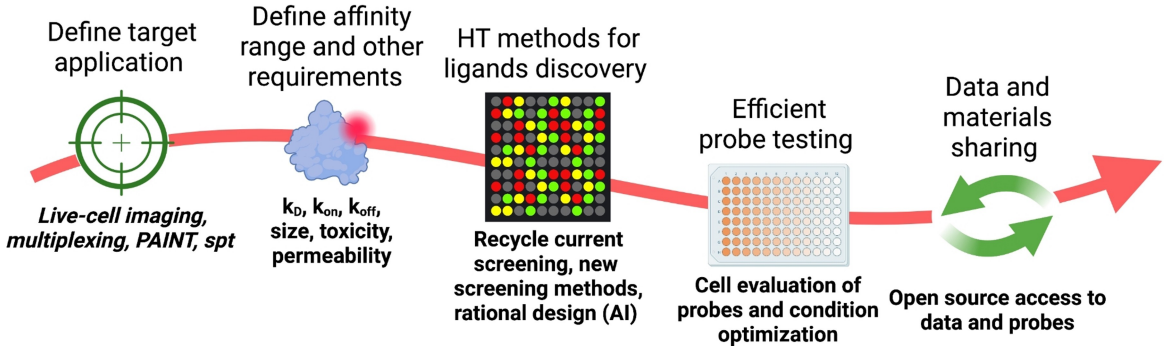

Figure 3. Proposed roadmap to identify, characterize and distribute low-affinity labels.

some approaches, mostly DNA-based, are available, the development of a palette of protein-specific low-affinity labels will increase the applicability of optical multiplexed phenotyping.

After the choice of a specific application of interest, the physicochemical requirements of the ideal probe should be clearly defined. Most important are the thermodynamic equilibrium constant (K_D) and the kinetic rate constants for binding (k_{on}) and unbinding (k_{off}). Here, every specific application will have its own optimum: for example, while binding times ($1/k_{off}$) around hundreds of milliseconds are used in single-molecule PAINT, longer binding times may be used for long time-lapse imaging and single-protein tracking.[30] Kinetic models predict ideal properties with respect to a specific experiment.

Despite the first promising examples, the largest bottleneck in this field is still the availability of probes with suitable affinity. All the design and screening efforts so far are mostly devoted to identifying high-affinity binders (e.g. antibodies with low-nM to pM affinities), while low-affinity binders are often discarded in the selection process. We believe that there is great potential in screening for low-affinity labels (10 nM–10 μM affinity) that can be used in a variety of optical microscopy applications. Before new and tailored screening methods are developed, much can be done to "recycle" the results of current screening. Current screening of affinity markers aims to select binders with the highest possible affinity (low nM-pM), and binders are screened out in several rounds because of their low affinity. While molecules that are low in specificity are discarded in the first rounds, those that are discarded in the last rounds are likely to be high in specificity but only of a little lower affinity, and they might fall within the right range for reversible labeling (high nM). Recycling the "garbage can" of the current screening may already provide several useful binders for low-affinity labeling.

An elegant example of low-affinity screening was recently reported by Watanabe and co-workers.[31] The authors devised a screening procedure for fast dissociating antibodies based on semi-automated TIRF imaging on 96-well plates. By imaging the residence time of single molecules, they can screen for bright times in the right regime for reversible labeling. Monoclonal antibodies against epitope tags and proteins involved in the cytoskeletal regulation (plastin and espin) were found and used for super-resolution imaging and light-sheet imaging. While screening procedures are probably the optimal solution for high-throughput ligand discovery, rational design of probes has also to be considered. Advances in molecular simulations, de novo protein design and artificial intelligence allows synthesizing proteins with tailored size, shape, function and binding affinity.[32] This may be a valid approach for specific targets as well as be combined with a screening experiment by providing a tailored pre-selected starting library for the selection.

It is worth mentioning that the success of this approach will also depend on the ability of the microscopy community to share resources towards the common goal of developing low-affinity labeling. The success of probes like fluorescent proteins or antibodies relies also on the availability of a large open-source library of DNA constructs (e.g., through community repositories) or wide commercial availability of products. Being able to share ligands within the community will speed up probe development and use. Moreover, sharing data acquired with these methods will help to standardize protocols.

5. Conclusion

Low-affinity fluorophore labels offer elegant solutions to bypass several barriers in fluorescence microscopy, enabling sub-diffraction spatial resolution, imaging of a large number of targets in the same cell, long-time live-cell imaging and minimized steric hindrance. These labels can be operated across microscopy modalities, and by that economize sample preparation and imaging experiments. However, this approach is still at its beginning and there are many challenges ahead before low-affinity binders can be used routinely in biophysics. A main challenge is the need to develop a robust process for effective screening of low-affinity probes. While this might require some effort, it comes with the benefit that such probes are simple to use, effective and economic, and open new experimental opportunities. With the advent of more low-affinity probes developed, the dissemination of experimental protocols and the reporting of exciting applications, these labels hold a great promise to answer so-far inaccessible questions in cell biology.

Acknowledgements

We are grateful to Dr. Marius Glogger for helpful discussions. We acknowledge funding by the Deutsche Forschungsgemeinschaft (DFG; grants SFB 902, SFB 1177, SFB 1507, GRK 2566, HE 6166/17-1) (M.H.), the LOEWE research network (FCI) (M.H.), the Volkswagen Foundation (grant 91069) (M.H.) and the Netherlands Science Organization (NWO) through the VIDI program 192.028 (L.A.). Open Access funding enabled and organized by Projekt DEAL.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Keywords: Exchangeable Fluorophore Labels · Fluorescence Microscopy · Fluorescent Probes · Super-Resolution Microscopy

[1] S. W. Hell, J. Wichmann, *Opt. Lett.* **1994**, *19*, 780–782.
[2] C. Spahn, J. B. Grimm, L. D. Lavis, M. Lampe, M. Heilemann, *Nano Lett.* **2019**, *19*, 500–505.
[3] K. K. Narayanasamy, A. Stojic, Y. Li, S. Sass, M. R. Hesse, N. S. Deussner-Helfmann, M. S. Dietz, T. Kuner, M. Klevanski, M. Heilemann, *Front. Synaptic Neurosci.* **2021**, *13*, 671288.
[4] H. Shen, L. J. Tauzin, R. Baiyasi, W. Wang, N. Moringo, B. Shuang, C. F. Landes, *Chem. Rev.* **2017**, *117*, 7331–7376.
[5] A. Sharonov, R. M. Hochstrasser, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 18911–18916.
[6] T. Dertinger, R. Colyer, R. Vogel, M. Heilemann, M. Sauer, J. Enderlein, S. Weiss, *Adv. Exp. Med. Biol.* **2012**, *733*, 17–21.
[7] N. Gustafsson, S. Culley, G. Ashdown, D. M. Owen, P. M. Pereira, R. Henriques, *Nat. Commun.* **2016**, *7*, 12471.
[8] J. Kompa, J. Bruins, M. Glogger, J. Wilhelm, M. S. Frei, M. Tarnawski, E. D'Este, M. Heilemann, J. Hiblot, K. Johnsson, *J. Am. Chem. Soc.* **2023**, *145*, 3075–3083.
[9] K. K. H. Chung, Z. Zhang, P. Kidd, Y. Zhang, N. D. Williams, B. Rollins, Y. Yang, C. Lin, D. Baddeley, J. Bewersdorf, *Nat. Methods* **2022**, *19*, 554–559.
[10] M. Sauer, M. Heilemann, *Chem. Rev.* **2017**, *117*, 7478–7509.
[11] P. Carravilla, A. Dasgupta, G. Zhurgenbayeva, D. I. Danylchuk, A. S. Klymchenko, E. Sezgin, C. Eggeling, *Biophys. Rep.* **2021**, *1*, None.
[12] J. Torra, P. Bondia, S. Gutierrez-Erlandsson, B. Sot, C. Flors, *Nanoscale* **2020**, *12*, 15050–15053.
[13] J. Torra, F. Viela, D. Megías, B. Sot, C. Flors, *Chem. Eur. J.* **2022**, *28*, e202200026.
[14] C. K. Spahn, M. Glaesmann, J. B. Grimm, A. X. Ayala, L. D. Lavis, M. Heilemann, *Sci. Rep.* **2018**, *8*, 14768.
[15] T. Kiuchi, M. Higuchi, A. Takamura, M. Maruoka, N. Watanabe, *Nat. Methods* **2015**, *12*, 893–893.
[16] P. Delcanale, D. Porciani, S. Pujals, A. Jurkevich, A. Chetrusca, K. D. Tawiah, D. H. Burke, L. Albertazzi, *Angew. Chem. Int. Ed.* **2020**, *59*, 18546–18555.
[17] R. P. Tas, L. Albertazzi, I. K. Voets, *Nano Lett.* **2021**, *21*, 9509–9516.
[18] R. Riera, T. P. Hogervorst, W. Doelman, Y. Ni, S. Pujals, E. Bolli, J. D. C. Codée, S. I. van Kasteren, L. Albertazzi, *Nat. Chem. Biol.* **2021**, *17*, 1281–1288.
[19] Q. Zhang, A. Miyamoto, S. Watanabe, T. Arimori, M. Sakai, M. Tomisaki, T. Kiuchi, J. Takagi, N. Watanabe, *Cells Rep. Methods* **2022**, *2*, 100301.
[20] N. G. Bozhanova, M. S. Baranov, N. V. Klementieva, K. S. Sarkisyan, A. S. Gavrikov, I. V. Yampolsky, E. V. Zagaynova, S. A. Lukyanov, K. A. Lukyanov, A. S. Mishin, *Chem. Sci.* **2017**, *8*, 7138–7142.
[21] M. Glogger, D. Wang, J. Kompa, A. Balakrishnan, J. Hiblot, H.-D. Barth, K. Johnsson, M. Heilemann, *ACS Nano* **2022**, *16*, 17991–17997.
[22] A. Saguy, O. Alalouf, N. Opatovski, S. Jang, M. Heilemann, Y. Shechtman, *bioRxiv* **2022**, 2022.07.01.498428.
[23] R. Jungmann, C. Steinhauer, M. Scheible, A. Kuzyk, P. Tinnefeld, F. C. Simmel, *Nano Lett.* **2010**, *10*, 4756–4761.
[24] J. Schnitzbauer, M. T. Strauss, T. Schlichthaerle, F. Schueder, R. Jungmann, *Nat. Protoc.* **2017**, *12*, 1198–1228.
[25] M. Glogger, C. Spahn, J. Enderlein, M. Heilemann, *Angew. Chem. Int. Ed.* **2021**, *60*, 6310–6313.
[26] C. Spahn, F. Hurter, M. Glaesmann, C. Karathanasis, M. Lampe, M. Heilemann, *Angew. Chem. Int. Ed.* **2019**, *58*, 18835–18838.
[27] K. K. Narayanasamy, J. V. Rahm, S. Tourani, M. Heilemann, *Nat. Commun.* **2022**, *13*, 5047.
[28] B. Vermeer, S. Schmid, *Nano Res.* **2022**, *15*, 9818–9830.
[29] F. Stehr, J. Stein, J. Bauer, C. Niederauer, R. Jungmann, K. Ganzinger, P. Schwille, *Nat. Commun.* **2021**, *12*, 4432.
[30] J. Piehler, M. Holtmannspötter, E. Wienbeuker, T. Dellmann, I. Watrinet, A. J. Garcia Saez, K. Johnsson, R. Kurre, *Angew. Chem. Int. Ed.* **2023**, *62*, e202219050.
[31] T. Miyoshi, Q. Zhang, T. Miyake, S. Watanabe, H. Ohnishi, J. Chen, H. D. Vishwasrao, O. Chakraborty, I. A. Belyantseva, B. J. Perrin, H. Shroff, T. B. Friedman, K. Omori, N. Watanabe, *Cell Rep.* **2021**, *34*, 108708.
[32] D. M. Mason, S. Friedensohn, C. R. Weber, C. Jordi, B. Wagner, S. M. Meng, R. A. Ehling, L. Bonati, J. Dahinden, P. Gainza, B. E. Correia, S. T. Reddy, *Nat. Biomed. Eng.* **2021**, *5*, 600–612.

Manuscript received: March 7, 2023
Accepted manuscript online: May 9, 2023
Version of record online: June 2, 2023