Bioengineered SP1 Optimized for Perovskites Heterostructures Growth and Stabilization

Classical nucleation theory distinguishes homogeneous and heterogeneous nucleation. The activation barrier for heterogeneous nucleation is lower than that for homogeneous nucleation, as the foreign surface effectively "catalyzes" the nucleation process. The extent of this reduction and the possibilities it opens for bioengineered SP1 proteins and halide perovskite nanocrystals are at the base of this proposal.

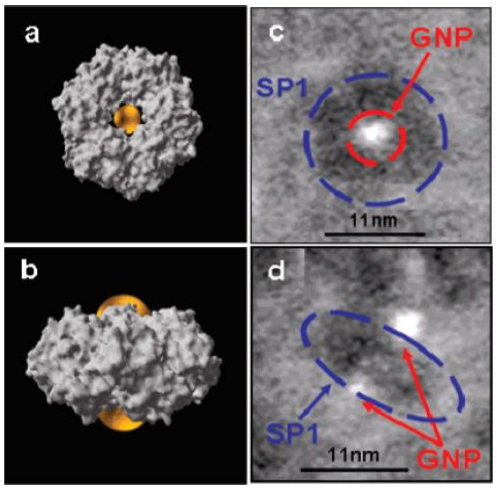

Fig 1. 6His-SP1 and commercially available 1.8nm gold NPs (GNP) hybrids. (a,b) Computer simulations of the SP1-GNP hybrid top and side views, respectively. (c,d) HAADF-STEM images of the 6His-SP1-GNP hybrid top and side views, respectively.

In the last few decades, methodologies for synthesizing and characterizing NPs have greatly evolved. Nanoelements hold unique properties compared to the bulk, which can be tailored through tight control of their sizes and shapes. most notable localized surface plasmons and excitonic quantum size effect. These have been shown to enhance catalysis[1,2] and biosensing[3] In this proposal, we focus on combining the bioengineering of proteins to catalyze the growth of semiconductor halide perovskites nanocrystals.

SP1 is a homo-oligomeric (12-mer) stress protein isolated from aspen plants (*Populus tremula*), which shows great stability even under harsh conditions[4]. It creates a ring-shaped structure with a central inner pore of 2-3nm[5] that could serve as a template for the synthesis of a variety of metals or semiconductor-based NPs[6,7]. The SP1 pore size is smaller than similar protein-based carriers, e.g. ferritin, and exhibits better stability[5]. The former allows the formation of smaller NPs, which are preferred for catalysis and other optoelectronic applications. In 2008, it was demonstrated that commercially available 1.8nm AuNPs can interact with the SP1 variant that has a 6-histidine tag (6His-SP1) translated in the inner pore of the protein[7] (Fig 1). In 2014, Liu *et al.* utilized the electrostatic interactions between negatively charged wild-type SP1 rings and positively charged cadmium telluride (CdTe) QDs (in different sizes) for the creation of highly ordered SP1-CdTe nanowires, [8] (Fig. 2). These nanostructures were demonstrated as a promising scaffold for the development of an artificial light-harvesting system.

It has been shown that peptides are capable of directing the mineralization of inorganic NPs[9] including perovskites-based materials.[10] That was achieved due to a unique high affinity of short peptide sequences to a specific element[9,11], e.g. gold, silver, cadmium, or lead. These peptides are referred to as metal-binding peptides (MBP). These peptides are commonly developed by phage display technique in a combinatorial approach[12]. Peptides that exhibit selective affinity for a specific metal after several rounds of panning are further characterized as templates for inorganic growth and as nanomaterial nucleation sites. These peptides can either be examined alone as ligands for metal NPs synthesis or fused

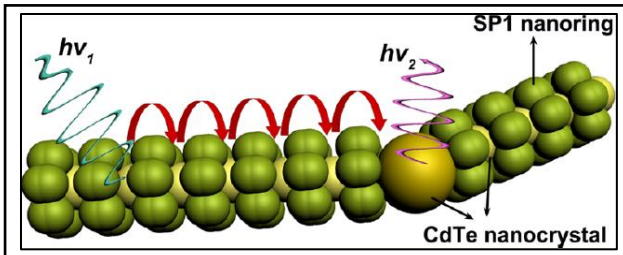

Fig. 2. Schematic illustration of highly ordered SP1-CdTe nanowires for the development of an artificial light-harvesting system.

to a protein scaffold. The latter allows the formation of nanomaterials with better controllable size and shape since the structure of the protein scaffold physically dictates the dimensions of the formed QD or NP.[9]

The SP1 monomers can be bioengineered to allow conditioning of the internal pocket, where the NP synthesis is targeted while maintaining its stability and unique structure[5]. Therefore, MBP could be introduced in the inner cavity of the protein, allowing high affinity and selectivity for the formation of defined NPs. The combination of highly ordered and stable structures alongside engineerable properties makes the SP1 protein a great platform for NPs synthesis, which can be applied in many fields such as diagnostics, drug delivery, photonics, and catalysis. The desired added peptide is translated and displayed in each SP1 subunit; therefore, 12 identical peptides are presented in each oligomer.

Lead halide perovskites (LHPs) APbX3 (where A=CH3NH3+, Cs+; X=Cl-, Br-, I-) are attracting much interest due to their potential in optoelectronic applications, such as light-emitting diodes, photovoltaic cells, photodetectors.[13–15] They have been synthesized also in the form of colloidal nanoparticles, presenting high photoluminescence quantum yield (PLQY)[16–18] have made them popular options as efficient chromophores for lasing and tagging applications. Transitioning these materials from bench to market Pb toxicity is a significant hurdle, especially when combined with the infamous instability of this material to humidity and excess heat.[19]

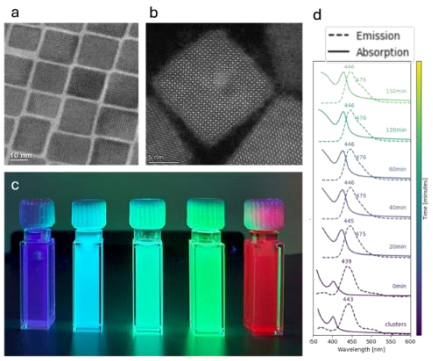

Fig 3. (a, b) low and high resultion micrographs of hallide perovskite nanocrystals of CsPbBr3 composotion. (c) UV excited collidial suspension of nanocrystals, By varying the halide anion from Br to Cl, I or their mixtures one, bandgao tunability throught the visible spectrum is demonstrated. (d) prelimenary results showing spectroscpic studyies of CsPbBr3 cluster formation suitable for the growth of heterostcrures.

Here we propose to address this challenge by employing bioengineered SP1 that presents peptides that exhibit selective affinity for lead (or other metals used in perovskites such as Sn, Ag, In, Na, Sb) on their cavity surfaces and will be used to catalyze perovskite NPs growth specifically, for the growth of halide perovskites heterostructures, using a few unit cells clusters with the composition of CsPbBr3 was shown to produce facile growth of heterostructures in the case of CdSe. [20]

We will follow a similar approach for designing the synthesis of *SP1/CsPbBr3 heterostructure.* Such heterostructured products may serve as a new strategy to capture and neutralize hazardous lead in lead-halide-degraded fragments. The SP1 cavity variants consist of versatile binding chemistries, which could enable capturing and in turn passivating the lead presence. High-efficiency passivation will enable safe deployment of halide perovskites in devices that otherwise may be restricted by regulators.

This biocompatible encapsulation is both interesting and novel. It may also be more affordable and less impactful on the environment, especially if it is considered an add-on material to be sprayed on top of existing lead-based solar cells. This will help to prevent and mitigate future hazards caused by lead leakage.

Aims

A. *Develop a room temperature SP1/CsPbBr3 heterostructure synthesis.*
B. *Characterization of the formed NPs in terms of crystallinity, homogeneity, and quantum yield.*
C. *Examined the biohybrid stability for different chemical environments or while absorbed on electrode surfaces.*

Detailed plan.

We intend to investigate the optimal conditions for heterostructure growth by adding bioengineered SP1 into a solution of CsPbBr3 nanoclusters, followed by injecting the last precursor dissolved in heated ODE. The different reaction temperatures and times will be tested for reaction optimization. The study will be based on absorption and PL measurements using a UV-VIS Bio-Tek micro-plate spectrometer and our new robotic, synthetic lab for high throughput parameter screen (solvents, SP1 variants, binding groups, etc).

The SP1 variants will be overexpressed and purified. Then, we intend to lyophilize the proteins before redissolving them in the tested solvent. We wish to examine the ability of the SP1 to enable perovskite growth in its cavity. The following expressed sequences at the protein cavity will be tested: (HHHHHH; SVTQNKY; MHGKTQATSGTIQS; GDVHHHGRHGAEHADI; YHHHHHH; YYHHHHHH; YDWLLAK.

These presented peptide sequences comprising mainly binding sites to metals, e.g., histidine and lysine, or carboxylic sites that can coordinate with lead ions. Subsequentially, these interactions or coordination may induce the formation of perovskite clusters or cluster growth at the protein cavity. The outer-sphere protein structure has great potential to improve the stability of the formed perovskites, as was shown for CdS NPs.[21]

We intend to overexpress the SP1 variants in E. Coli (BL21) and utilize the purified SP1proteins for automatic screening to identify perovskite growth. For that, the proteins will be added to different solvent mixtures that should enable cluster formation. It should be noted that SP1 stability under the harsh conditions of organic solvents has been tested and showed good promise[4,22] The formed biohybrids will be then characterized and their ability to act as light absorbers for photoanodic or photocathodic reactions will be tested. For that, an amorphous TiO_2 protecting layer will be added on top of electrodes/biohybrid conjugates.

Preliminary results

We recently examined the utilization of SP1 variants for the synthesis of Au, Ag, Pd[23], and CdS NPs.[21,24] As presented in Fig 4, the use of SP1- CdS metal binding peptide enabled CdS NPs growth in the protein cavity.[21,24] Excluding the SP1 drive to large-size particles that precipitate out. By TEM imaging we could determine the NPs size and distribution in the SP1 biohybrid. Furthermore, HR-TEM measurements confirmed that the 4.7nm synthesized NPs are crystalline. The samples were stained with uranyl acetate to image the biohybrid structure. As expected, the NPs were indeed grown in the SP1 cavity.

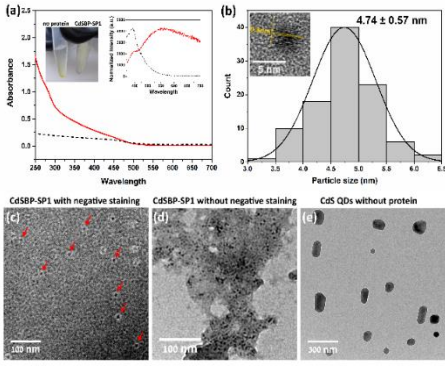

Fig 4. Characterization of the biosynthesized CdS QDs. (a) UV-Vis absorption spectra (b) Particle size distribution of the biosynthesized CdS QDs based on TEM analysis. Inset, HR-TEM (c) and (d) TEM micrograph of the CdSBP-SP1 capped CdS QDs (indicated by red arrows) with and without negative staining, respectively. (e) TEM micrograph of a control sample without CdSBP-SP1.

Alternatively, for metal NPs growth, sodium borohydride, natural reducing agent, or activated tyrosine were used. Fig. 5 presents the formation of Ag NPs using a di-tyrosine sequence activated by high pH and without reducing agent addition. These results confirm the SP1's unique ability to act as a template for NPs synthesis.

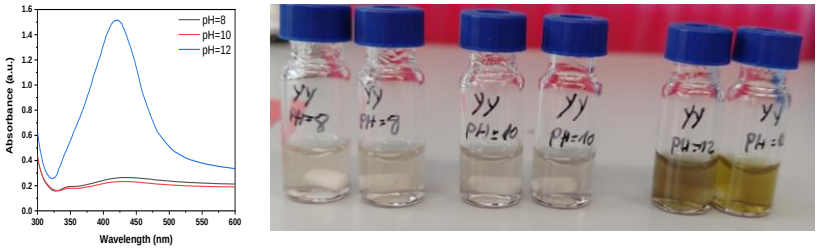

Fig. 5. Silver NPs formation using SP1 with fused YYHHHHHH as nucleation and reducing site.

References

(1) Waiskopf, N.; Ben-Shahar, Y.; Banin, U. Photocatalytic Hybrid Semiconductor–Metal Nanoparticles; from Synergistic Properties to Emerging Applications. *Advanced Materials* **2018**, *30* (41), 1706697. https://doi.org/10.1002/adma.201706697.

(2) Karani, A.; Yang, L.; Bai, S.; Futscher, M. H.; Snaith, H. J.; Ehrler, B.; Greenham, N. C.; Di, D. Perovskite/Colloidal Quantum Dot Tandem Solar Cells: Theoretical Modeling and Monolithic Structure. *ACS Energy Lett.* **2018**, *3* (4), 869–874. https://doi.org/10.1021/acsenergylett.8b00207.

(3) Liu, W.; Howarth, M.; Greytak, A. B.; Zheng, Y.; Nocera, D. G.; Ting, A. Y.; Bawendi, M. G. Compact Biocompatible Quantum Dots Functionalized for Cellular Imaging. *J. Am. Chem. Soc.* **2008**, *130* (4), 1274–1284. https://doi.org/10.1021/ja076069p.

(4) Wang, W.-X.; Pelah, D.; Alergand, T.; Shoseyov, O.; Altman, A. Characterization of SP1, a Stress-Responsive, Boiling-Soluble, Homo-Oligomeric Protein from Aspen. *Plant Physiol.* **2002**, *130* (2), 865–875. https://doi.org/10.1104/pp.002436.

(5) Arie Altman, W.-X. W. Characterization of SP1, a Stress-Responsive, Boiling-Soluble, Homo-Oligomeric Protein from Aspen. *Plant Physiology* **2002**, *130*, 865–875.

(6) Oded Shoseyov, S. B. Constrained Synthesis and Organization of Catalytically Active Metal Nanoparticles by Self-Assembled Protein Templates. *Advanced Materials* **2009**, *21*, 3515–3519.

(7) Danny Porath, I. M. SP1 Protein-Based Nanostructures and Arrays. *Nano Letters* **2008**, *8* (2), 473–477.

(8) Miao, L.; Han, J.; Zhang, H.; Zhao, L.; Si, C.; Zhang, X.; Hou, C.; Luo, Q.; Xu, J.; Liu, J. Quantum-Dot-Induced Self-Assembly of Cricoid Protein for Light Harvesting. *ACS Nano* **2014**, *8* (4), 3743–3751. https://doi.org/10.1021/nn500414u.

(9) Chen, C.-L.; Rosi, N. L. Peptide-Based Methods for the Preparation of Nanostructured Inorganic Materials. *Angewandte Chemie International Edition* **2010**, *49* (11), 1924–1942. https://doi.org/10.1002/anie.200903572.

(10) Luo, B.; Naghadeh, S. B.; Allen, A.; Li, X.; Zhang, J. Z. Peptide-Passivated Lead Halide Perovskite Nanocrystals Based on Synergistic Effect between Amino and Carboxylic Functional Groups. *Advanced Functional Materials* **2017**, *27* (6), 1604018. https://doi.org/10.1002/adfm.201604018.

(11) Sapsford, K. E.; Algar, W. R.; Berti, L.; Gemmill, K. B.; Casey, B. J.; Oh, E.; Stewart, M. H.; Medintz, I. L. Functionalizing Nanoparticles with Biological Molecules: Developing Chemistries That Facilitate Nanotechnology. *Chem. Rev.* **2013**, *113* (3), 1904–2074. https://doi.org/10.1021/cr300143v.

(12) Stanley Brown. Metal Recognition by Repeating Polypeptides. *nature biotechnology* **1997**, *15*, 269–272.
(13) Clinckemalie, L.; Valli, D.; Roeffaers, M. B. J.; Hofkens, J.; Pradhan, B.; Debroye, E. Challenges and Opportunities for CsPbBr3 Perovskites in Low- and High-Energy Radiation Detection. *ACS Energy Lett.* **2021**, *6* (4), 1290–1314. https://doi.org/10.1021/acsenergylett.1c00007.
(14) Isikgor, F. H.; Zhumagali, S.; T. Merino, L. V.; De Bastiani, M.; McCulloch, I.; De Wolf, S. Molecular Engineering of Contact Interfaces for High-Performance Perovskite Solar Cells. *Nat Rev Mater* **2023**, *8* (2), 89–108. https://doi.org/10.1038/s41578-022-00503-3.
(15) Zhou, Y.; Chen, J.; Bakr, O. M.; Mohammed, O. F. Metal Halide Perovskites for X-Ray Imaging Scintillators and Detectors. *ACS Energy Lett.* **2021**, *6* (2), 739–768. https://doi.org/10.1021/acsenergylett.0c02430.
(16) Protesescu, L.; Yakunin, S.; Bodnarchuk, M. I.; Krieg, F.; Caputo, R.; Hendon, C. H.; Yang, R. X.; Walsh, A.; Kovalenko, M. V. Nanocrystals of Cesium Lead Halide Perovskites (CsPbX3, X = Cl, Br, and I): Novel Optoelectronic Materials Showing Bright Emission with Wide Color Gamut. *Nano Lett.* **2015**, *15* (6), 3692–3696. https://doi.org/10.1021/nl5048779.
(17) Bekenstein, Y.; Koscher, B. A.; Eaton, S. W.; Yang, P.; Alivisatos, A. P. Highly Luminescent Colloidal Nanoplates of Perovskite Cesium Lead Halide and Their Oriented Assemblies. *J. Am. Chem. Soc.* **2015**, *137* (51), 16008–16011. https://doi.org/10.1021/jacs.5b11199.
(18) Koscher, B. A.; Bronstein, N. D.; Olshansky, J. H.; Bekenstein, Y.; Alivisatos, A. P. Surface- vs Diffusion-Limited Mechanisms of Anion Exchange in CsPbBr3 Nanocrystal Cubes Revealed through Kinetic Studies. *J. Am. Chem. Soc.* **2016**, *138* (37), 12065–12068. https://doi.org/10.1021/jacs.6b08178.
(19) Hailegnaw, B.; Kirmayer, S.; Edri, E.; Hodes, G.; Cahen, D. Rain on Methylammonium Lead Iodide Based Perovskites: Possible Environmental Effects of Perovskite Solar Cells. *J. Phys. Chem. Lett.* **2015**, *6* (9), 1543–1547. https://doi.org/10.1021/acs.jpclett.5b00504.
(20) Qiu, H.; Li, F.; He, S.; Shi, R.; Han, Y.; Abudukeremu, H.; Zhang, L.; Zhang, Y.; Wang, S.; Liu, W.; Ma, C.; Fang, H.; Long, R.; Wu, K.; Zhang, H.; Li, J. Epitaxial CsPbBr3/CdS Janus Nanocrystal Heterostructures for Efficient Charge Separation. *Advanced Science* **2023**, *10* (13), 2206560. https://doi.org/10.1002/advs.202206560.
(21) Bachar, O.; Meirovich, M. M.; Zeibaq, Y.; Yehezkeli, O. Protein-Mediated Biosynthesis of Semiconductor Nanocrystals for Photocatalytic NAD(P)H Regeneration and Chiral Amine Production. *Angewandte Chemie International Edition* **2022**, *61* (23), e202202457. https://doi.org/10.1002/anie.202202457.
(22) Zeibaq, Y.; Bachar, O.; Sklyar, J.; Adir, N.; Yehezkeli, O. A Bioengineered Stable Protein 1-Hemin Complex with Enhanced Peroxidase-Like Catalytic Properties. *Small Science n/a* (n/a), 2400025. https://doi.org/10.1002/smsc.202400025.
(23) Bachar, O.; Moshe Meirovich, M.; Kurzion, R.; Yehezkeli, O. In Vivo and in Vitro Protein Mediated Synthesis of Palladium Nanoparticles for Hydrogenation Reactions. *Chemical Communications* **2020**, *56* (76), 11211–11214. https://doi.org/10.1039/D0CC04812G.
(24) Bachar, O.; Meirovich, M. M.; Yehezkeli, O. Integrated Biotic–Abiotic Solar Driven NADPH Regeneration Platform in Escherichia Coli for Chemical Biomanufacturing Applications. *Advanced Functional Materials n/a* (n/a), 2314443. https://doi.org/10.1002/adfm.202314443.