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Physico-chemical characterization of the interaction of red fluorescent protein - DsRed with silica layers

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Abstract— The *Discosoma* Recombinant Red Fluorescent (DsRed) protein is the latest member of the family of fluorescent proteins. It holds great promise for applications in biotechnology and cell biology. However, before being used for rational engineering, knowledge on the underlying mechanisms relating the DsRed structural stability and adsorption properties on solid surfaces is highly demanded. The physico-chemical analysis performed in this study reveals that the interaction of DsRed with SiO_2 surfaces does not lead to protein denaturation. The secondary structure of DsRed is preserved after adsorption and dehydration. The measured contact angles of sessile droplets with different DsRed concentrations determine the interaction as hydrophilic one. The photoluminescence emission of dehydrated DsRed droplets is found to be slightly red-shifted, peaking at 590 nm.

***Topic* — Nano-Biomedicine**

***Index Terms/Keywords* — Thin SiO_2 layers, Proteins, DsRed**

I. Introduction

RECENT advances in biotechnology offer the possibility to explore chemical, physical and functional properties of proteins, in particular fluorescent proteins, in various applications such as biosensors, bioelectronics, drug delivery systems, *etc.*, [1]. The fluorescent proteins are a family of proteins of 25 – 30 kDa, mainly applied to study the organization and function of living systems; with the green fluorescent protein (GFP) as most extensively characterized member. The recently cloned from reef coral *Discosoma* sp. Recombinant Red Fluorescent (DsRed) protein [2] expresses the longest recorded yet excitation and emission maxima at 558 nm and 583 nm, respectively. DsRed holds great promise for biotechnology and cell biology as a spectrally distinct companion or substitute for the GFP.

The current technological achievements lead to exposure and interaction of proteins with non-biological organic and inorganic solid surfaces. Considering further development our choice is to go with DsRed protein and study its interaction with thin SiO_2 layers. Studying thin SiO_2 layers is based on their large use in microelectronics as diffusion barrier or thin electrical insulator, in plasmonics as host matrix, *etc.*, [3-6 and the references therein]. Most of the currently applied diagnostic methods are adapted to study the structural stability of proteins in solution. Nevertheless, some advanced diagnostic methods are based on probing proteins after dehydration, for example, with matrix assisted laser desorption/ionization (MALDI) [7] or the Fourier Transform InfraRed (FTIR) spectroscopy [8]. In this work we report results on the physico-chemical characterization of the interaction of DsRed protein with solid silica surfaces for a series of diluted protein solutions.

II. Experimental part

Thermally grown 100 nm-thick SiO_2 layers were used. Before being exposed to protein deposition, the SiO_2/Si substrates were consecutively cleaned in ethanol and acetone and rinsed in deionized water until attaining zero surface conductivity. DsRed was purchased from Biovision (at least 97% pure, in a freeze dried form). A stock solution of DsRed was made to a concentration of 1 mg/mL in water for injectable preparations (European Pharmacopeae, COOPER) with pH-value of 7.0. The pH-stability of the DsRed stock solution was repetitively controlled during measurements. The assays were performed at room temperature (23°C). The pH-behavior of DsRed was obtained for the range 3.0 – 11.0. Sessile droplets of DsRed with very small volume (3.8 ± 0.1 μL) were deposited on the surface of SiO_2 layers by using a Contact Angle Meter from GBX Scientific Instruments. Optical images of dehydrated sessile droplets were recorded with a digital microscope Keyence VHX-1000. The droplet diameter was measured on the images. The other parameters of the droplets: thickness, droplet boarder width and height were measured with a 2D-surface profilometer Alpha-Step IQ from KLA-Tencor. Morphological changes of DsRed were observed with a field emission Scanning Electron Microscopy (SEM), ZEISS CrossBeam 1540 XB. The photoluminescence emission of the DsRed was excited with an Ar^+ laser operating at 514 nm. The emitted light was dispersed using a Jobin Yvon spectrometer with a 150 grooves/mm grating.

III. Results and Discussion

When a droplet containing given concentration of proteins is brought to a contact with solid surface, the organization and protein behavior rely primarily on the contact angle hysteresis (CAH) at the solid-vapor and liquid-vapor interfaces [9, 10]. Figure 1 shows the DsRed behavior during dehydration for different protein concentrations. The linear decrease over time of the contact angle is common for all the studied DsRed concentrations. The droplet dehydration is more rapid for small concentrations of DsRed (up to 0.1 g/L). This effect is related to the large surface energy variation on the droplet profile close to the triple line (solid-liquid-vapor) and to the protein adsorption mechanisms induced by the liquid convective drive inside the droplet (Marangoni effect) [10].

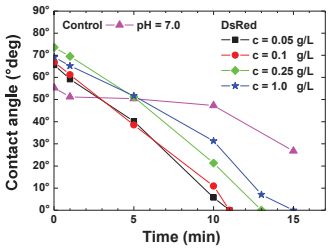
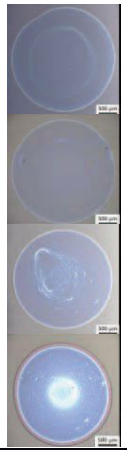


Fig. 1. Measured contact angles at 23°C as a function of time during sessile droplet dehydration for different DsRed concentrations.

As shown in Table II, all the droplet characteristics are increased when the concentration of DsRed is larger. It means that the DsRed adsorbs on the entire area covered by the droplet with preferential deposition close to the triple line, *i.e.* to the droplet boarder. The thickness of the adsorbed DsRed layer on solid surfaces can finely be controlled by the protein concentration.

TABLE II
CHARACTERISTICS OF DSRED SESSILE DROPLETS AT pH = 7.0 AND 23°C

DsRed (g/L)	Droplet image at 1s	Contact angle -θ	Optical image after dehydration	Dehydrated droplet[a]
control pH=7.0		54.3° ± 0.1°	n/a	n/a
0.05		65.4° ± 0.2°		d = 2.8 mm; e = 20 nm; l = 46 μm; h = 0.6 μm;
0.10		65.5° ± 1.6°		d = 2.8 mm; e = 30 nm; l = 67 μm; h = 1.4 μm;
0.25		73.7° ± 1.5°		d = 2.7 mm; e = 30 nm; l = 84 μm; h = 3.0 μm;
1.00		72.0° ± 2.7°		d = 2.6 mm; e = 60 nm; l = 200 μm; h = 5.2 μm.

[a]The DsRed sessile droplet characteristics after dehydration: d is the droplet diameter; e is the droplet thickness measured inside the droplet, just before the droplet boarder; l is the droplet boarder width; and h is the droplet boarder height. Scale bar on the images equals 500μm.

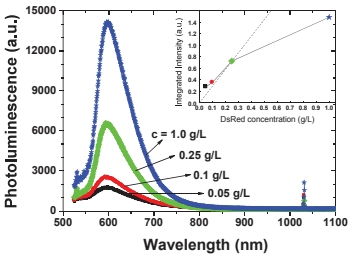


Fig. 2. Photoluminescence spectra of DsRed dehydrated droplets adsorbed on SiO_2 surface for different concentrations at pH = 7.0 and 23°C. The inset represents the integrated intensity of the photoluminescence peak as a function of the DsRed concentration.

The obtained photoluminescence spectra are presented in Fig. 2. The photoluminescence emission increases with increasing DsRed concentration. However, it does not scale up linearly most likely due to quenching phenomena (Fig. 2, inset). The photoluminescence emission band is peaking at 590 nm. The slight red-shift with respect to the DsRed emission in solution [11] can be attributed to conformation effects and to interaction of the DsRed with the thin silica layer.

IV. Conclusion

Physico-chemical analysis of the interactions of red fluorescent protein, DsRed with thermal SiO_2 surfaces shows that the thickness of the adsorbed DsRed protein layer can finely be tuned by the protein concentration. The measured contact angles of very small sessile droplets containing different concentration of DsRed determine the interaction as hydrophilic one. The DsRed proteins appear stable under pH-variations. The adsorption of DsRed on SiO_2 surfaces and the following dehydration processes do not lead to protein denaturation. The photoluminescence emission of dehydrated DsRed proteins adsorbed on SiO_2 layers is found to peak at 590 nm, which is slightly red-shifted compared to the reported value for a solution (583 nm). Potential modification of the DsRed excitation and emission spectra due to the DsRed-SiO_2 interactions in solution will be addressed further.

References

[1] D. M. Chudakov, M. V. Matz, S. Lukyanov, and K. A. Lukyanov, *Physiol. Rev.*, vol. 90, pp. 1103-1163, 2010.
[2] M. V. Matz, *et al*., *Nature Biotechnology*, vol. 17, pp. 969–973, 1999.
[3] E. A. Irene, in *Integrated Cirquits: Chemical and Physical Processing*, ACS Symposium Series, vol. 290, pp 31–46, 2009.
[4] W. B. Lacy, *et al.*, *Anal. Chem.*, vol. 68, pp. 1003-1011, 1996.
[5] R. Carles, C. Farcau, C. Bonafos, G. BenAssayag, M. Bayle, P. Benzo, J. Groenen, and A. Zwick, *ACS NANO*, vol. 5, pp. 8774-8782, 2011.
[6] A. Pugliara, C. Bonafos, R. Carles, B. Despax, and K. Makasheva, *Material Research Express*, vol. 2, pp. 065005, 2015.
[7] A. B. Hughes, *Amino Acids, Peptides and Proteins in Organic Chemistry*, Weinheim, Germany: Wiley-VCH Verlag & Co. KGaA, 2012.
[8] A. Barth, *Biochimica et Biophysica Acta*, vol. 1767, pp. 1073-1101, 2007.
[9] Y. L. Chen, C. A. Helm, and J. N. Israelachvili, *J. Phys. Chem*., vol. 95, pp. 10736-10747, 1991.
[10] J. R. Trantum *et al.*, *Langmuir*, vol. 29, pp. 6221−6231, 2013.
[11] M. F. Garcia-Parajo, M. Koopman, E. M. H. P. van Dijk, V. Subramaniam, and N. F. van Hulst, *PNAS*, vol. 98, pp. 14392–14397, 2001.