CHARACTERIZATION OF DISPERSIBILITY AND STABILITY OF TiO$_2$ NANOPARTICLES IN CELL CULTURE MEDIA WITH STATIC MULTIPLE LIGHT SCATTERING (SMLS)

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STABILITY & SIZE
MICRORHEOLOGY
RHEOLOGY ON CHIP

- Nanoparticles (NPs) are widely used in industries

  Promising properties : *high surface area, high reactivity, small size*

  Cosmetic   *Food*   *Drug delivery*

STABILITY & SIZE

Introduction

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- But can enter living organisms & potential hazard on human health : *inflammations, cytotoxicity response,...*

STABILITY & SIZE

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  => **In vitro nanotox. studies aim to evaluate NPs toxicity**

STABILITY & SIZE

- *In vitro* nanotoxicological studies require the dispersion of NPS in relevant matrix (e.g. water, cell culture media…)
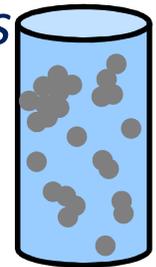
STABILITY & SIZE

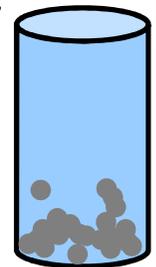# Introduction

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Before injection, dispersions must be…

well dispersed:
Size effect on cells

STABILITY & SIZE

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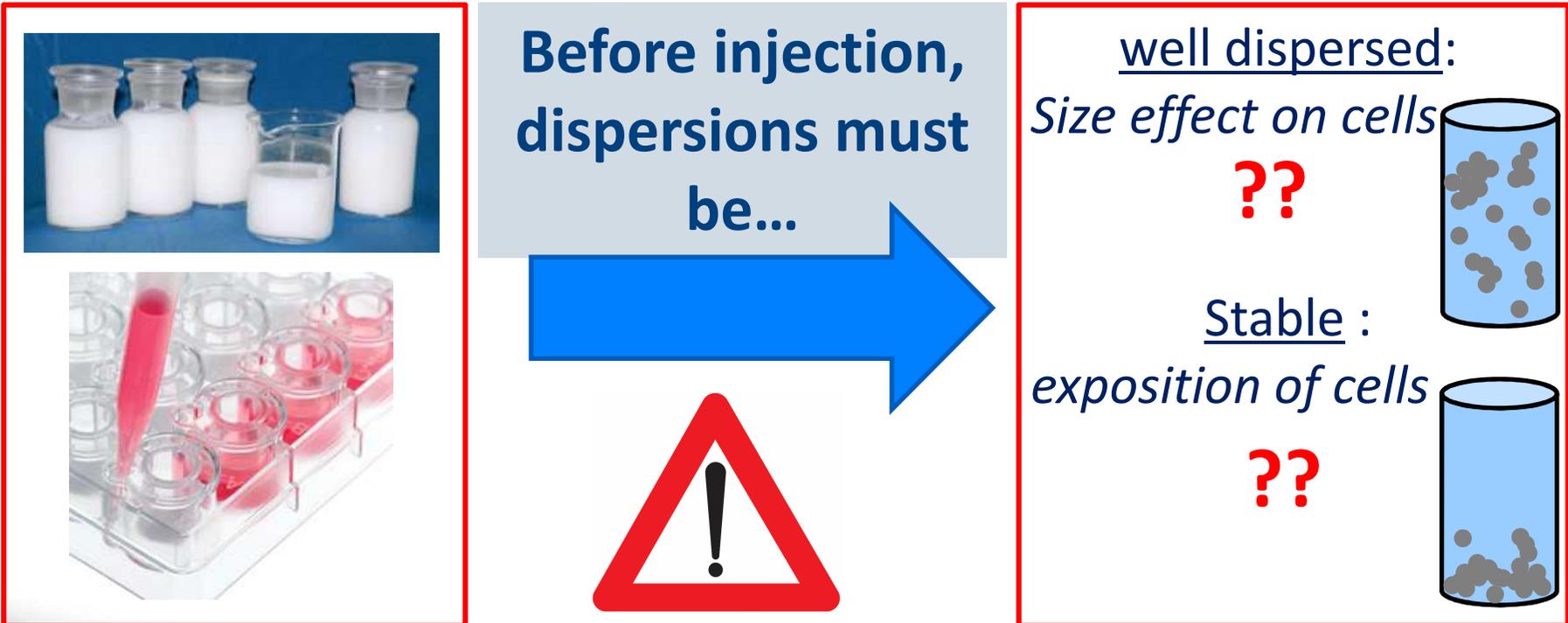
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Stable :
exposition of cells

STABILITY & SIZE
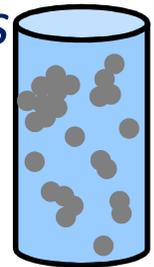
- *In vitro* nanotoxicological studies require the dispersion of NPS in relevant matrix (e.g. water, cell culture media…)

Before injection, dispersions must be…

well dispersed:
Size effect on cells
??

Stable :
exposition of cells
??

what are the cells truly exposed to?

STABILITY & SIZE

Materials and methods
preparation of the NPs dispersions

- Method:

  Preparation based on NanoREG protocol "*A common European approach to the regulatory testing of Manufactured Nanomaterials*"
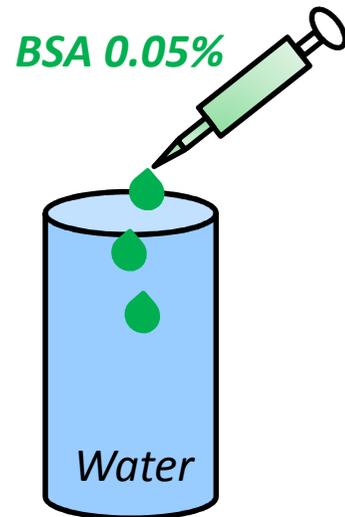
- Materials:

  - **Nanoparticles:** Titanium dioxide (**TiO$_2$**) particles P25 from *Evonik* – primary size 21nm, 80% anatase

  - **Cell culture media**: High glucose Dulbecco Modified Eagle Medium (**DMEM**) from *Sigma Aldrich*

  - **Proteins**: Bovine Serum Albumin (**BSA**) from *Sigma Aldrich*

Materials and methods
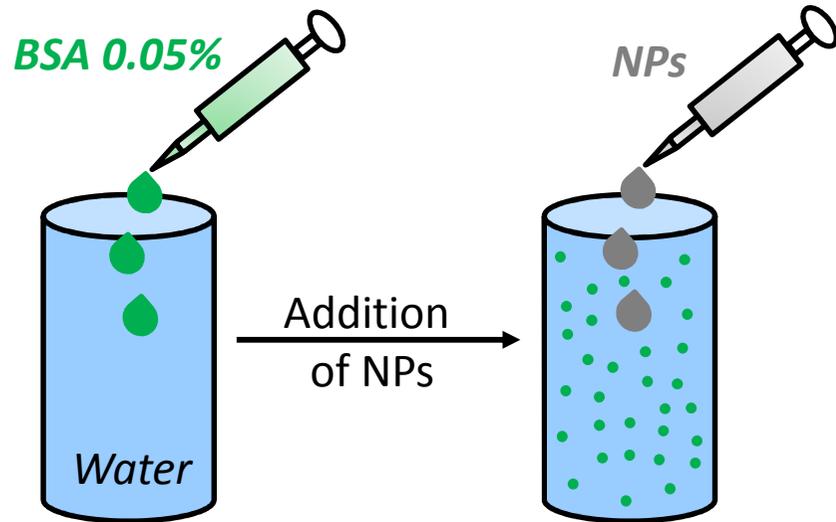## *preparation of the NPs dispersions*

- NanoREG protocol : 4 steps procedure

BSA 0.05%

Water

Materials and methods
preparation of the NPs dispersions

- NanoREG protocol : 4 steps procedure

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NPs

Addition of NPs →

Water

preparation of the NPs dispersions

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STABILITY & SIZE

Materials and methods
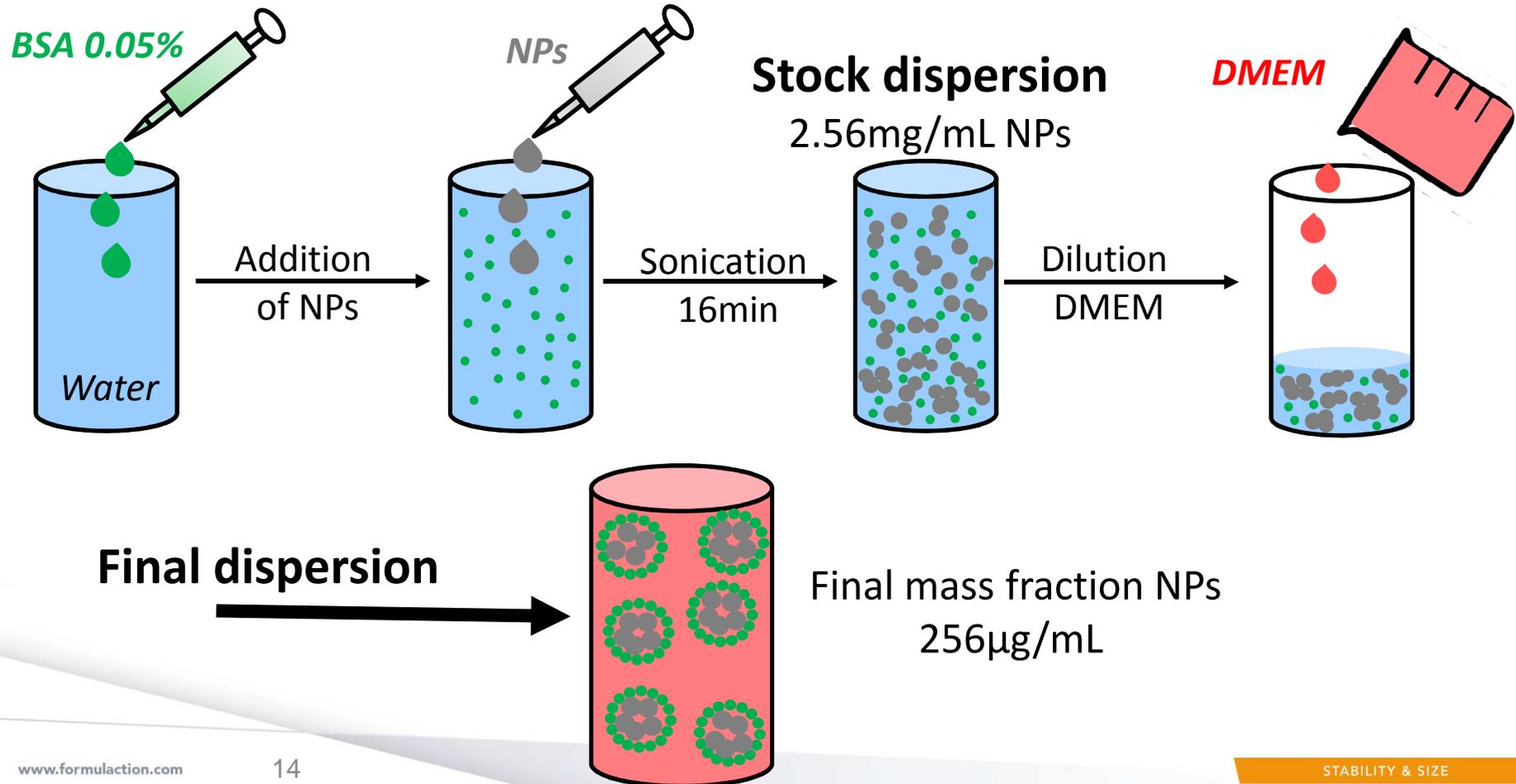## *preparation of the NPs dispersions*

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STABILITY & SIZE

Materials and methods
preparation of the NPs dispersions

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BSA 0.05%

NPs

Stock dispersion
2.56mg/mL NPs

DMEM

Water

Addition
of NPs

Sonication
16min

Dilution
DMEM

Final dispersion

Final mass fraction NPs
256µg/mL
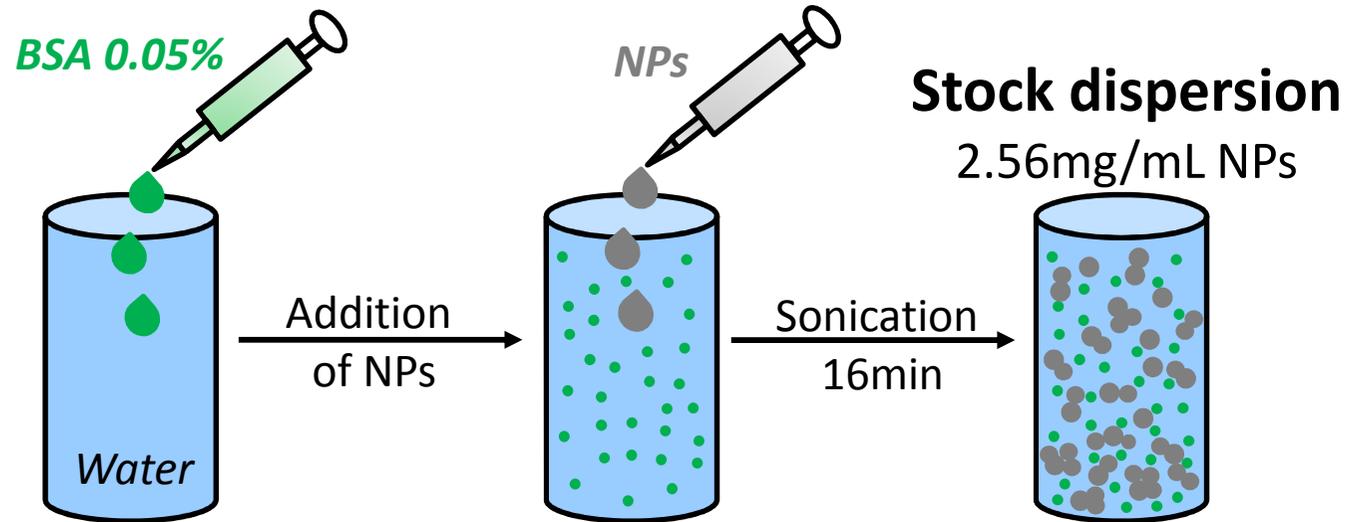
STABILITY & SIZE

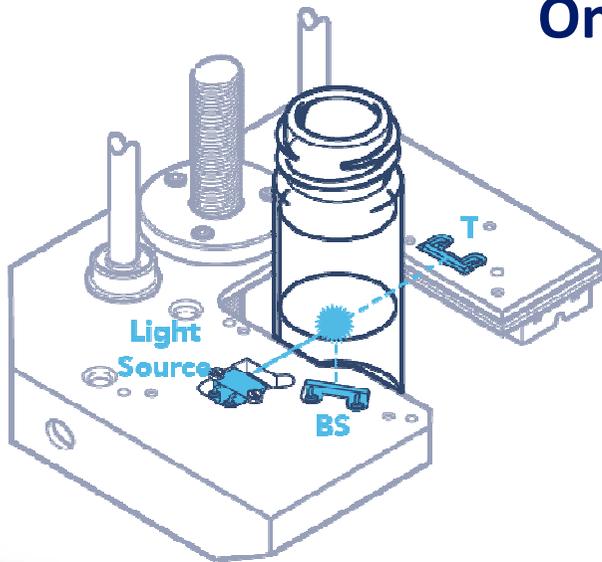# Characterization of dispersibility and stability

- SMLS technique (Turbiscan®): tool to characterize both stability and dispersibility in colloidal systems
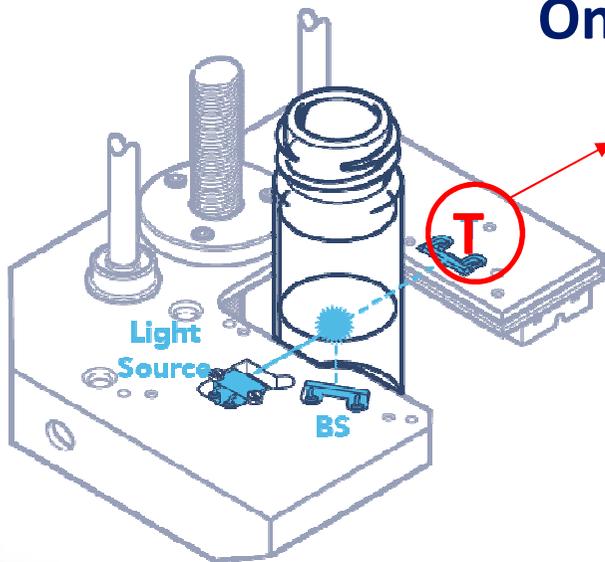
STABILITY & SIZE

Static multiple light scattering SMLS principle

- SMLS technique (Turbiscan®): tool to characterize both stability and dispersibility in colloidal systems

One light source and two sensors:

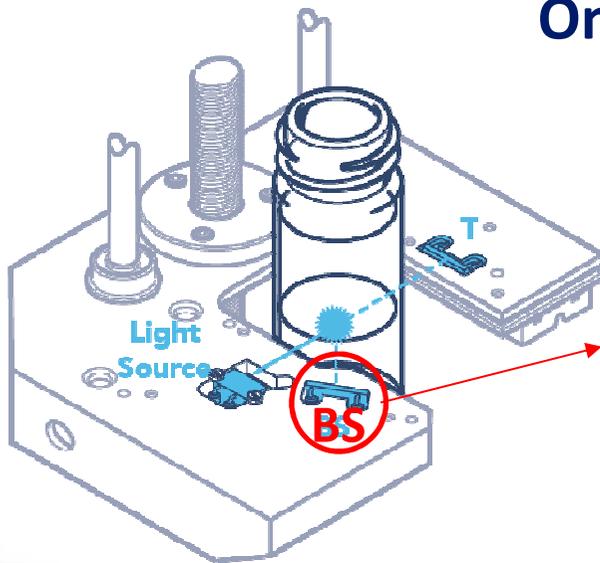# Static multiple light scattering SMLS principle

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Static multiple light scattering SMLS principle

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- Backscattering sensor **BS** *for* <u>concentrated media</u>

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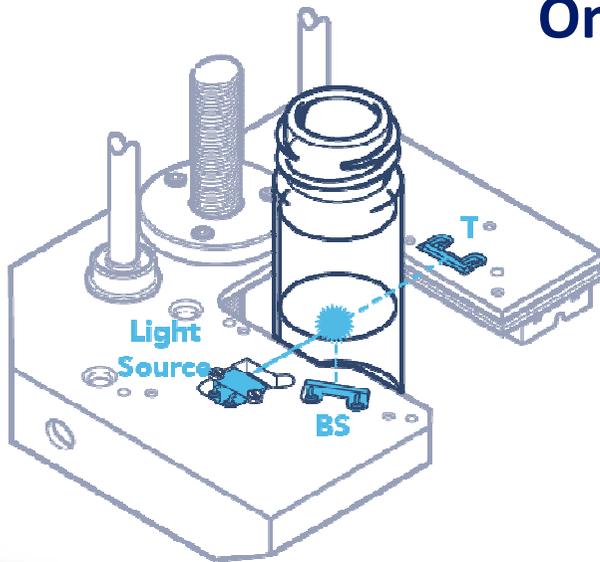
- Transmission sensor **T** for <u>dilute media</u>

$$T = f(d, \phi)$$

- Backscattering sensor **BS** *for* <u>concentrated media</u>

$$BS = f(d, \phi)$$

T and BS signals are used to determine:

- Particles mean size d
- Particles volume fraction ϕ

STABILITY & SIZE

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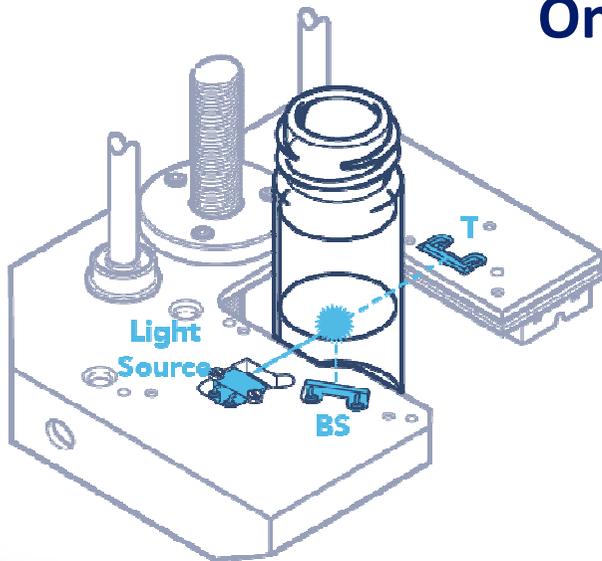
$$T = T_0 \exp\left(\frac{-3r_i \phi Q_e(d)}{d}\right)$$

- Backscattering sensor **BS** *for* <u>concentrated media</u>

$$BS = \sqrt{\frac{3\phi(1-g(d))Q_e(d)}{2d}}$$

T and BS signals are used to determine:

- Particles mean size d
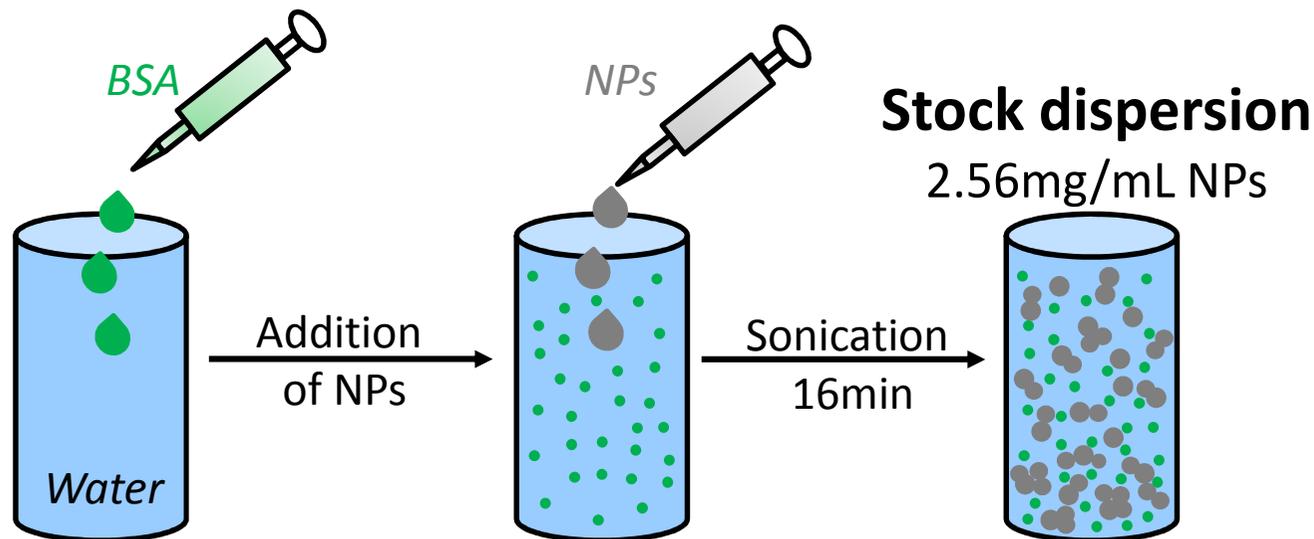- Particles volume fraction ϕ

STABILITY & SIZE

Static multiple light scattering SMLS principle

- SMLS technique (Turbiscan®): tool to characterize both stability and dispersibility in colloidal systems

BS and T scans repeated over time and height to characterize physical instabilities (e.g. sedimentation)
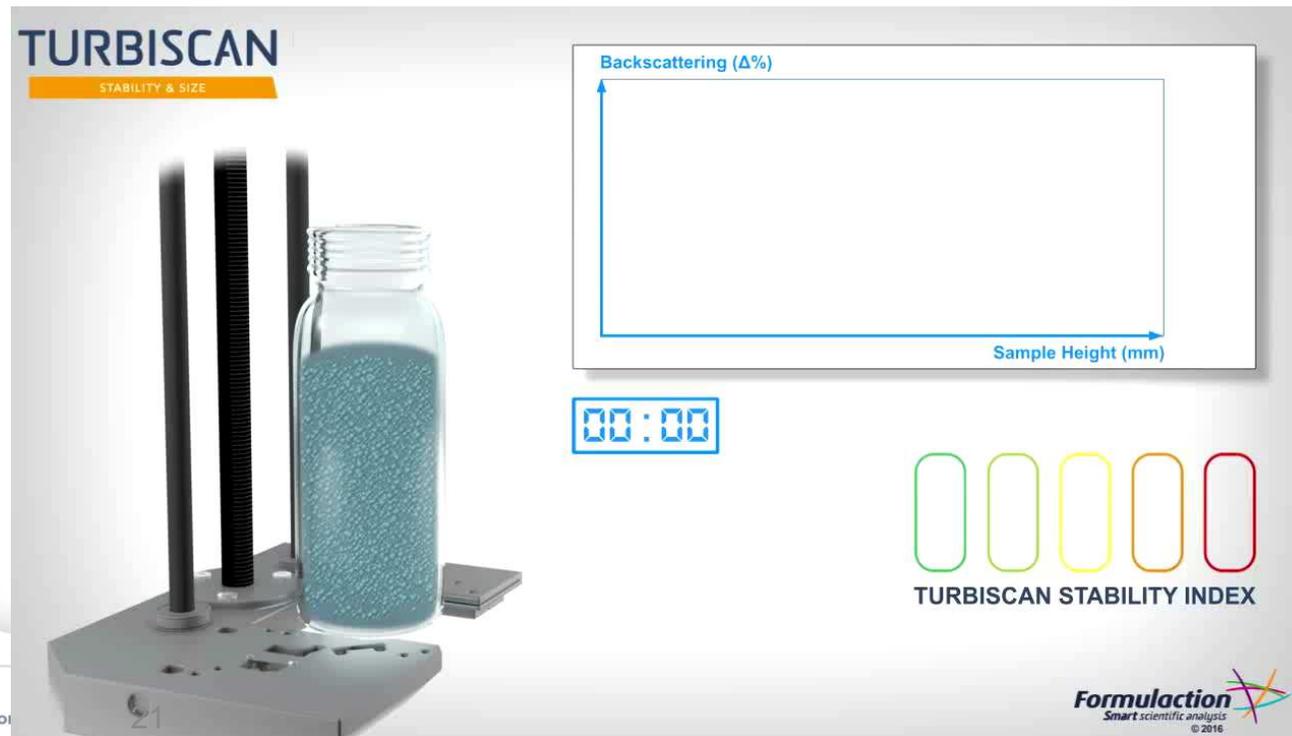
CHARACTERIZATION OF STOCK DISPERSION WITH TURBISCAN

BSA

NPs

Stock dispersion
2.56mg/mL NPs

Water

Addition
of NPs

Sonication
16min

STABILITY & SIZE

Turbiscan results for TiO2 stock dispersions

- BSA concentration influence on <u>initial mean size (t=0)</u>

<u>SMLS average size</u>

$$d = \frac{3\phi(1-g)Q_e}{2BS^2}$$

STABILITY & SIZE

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- No noticeable influence on mean size

- Mean size around 150nm

STABILITY & SIZE

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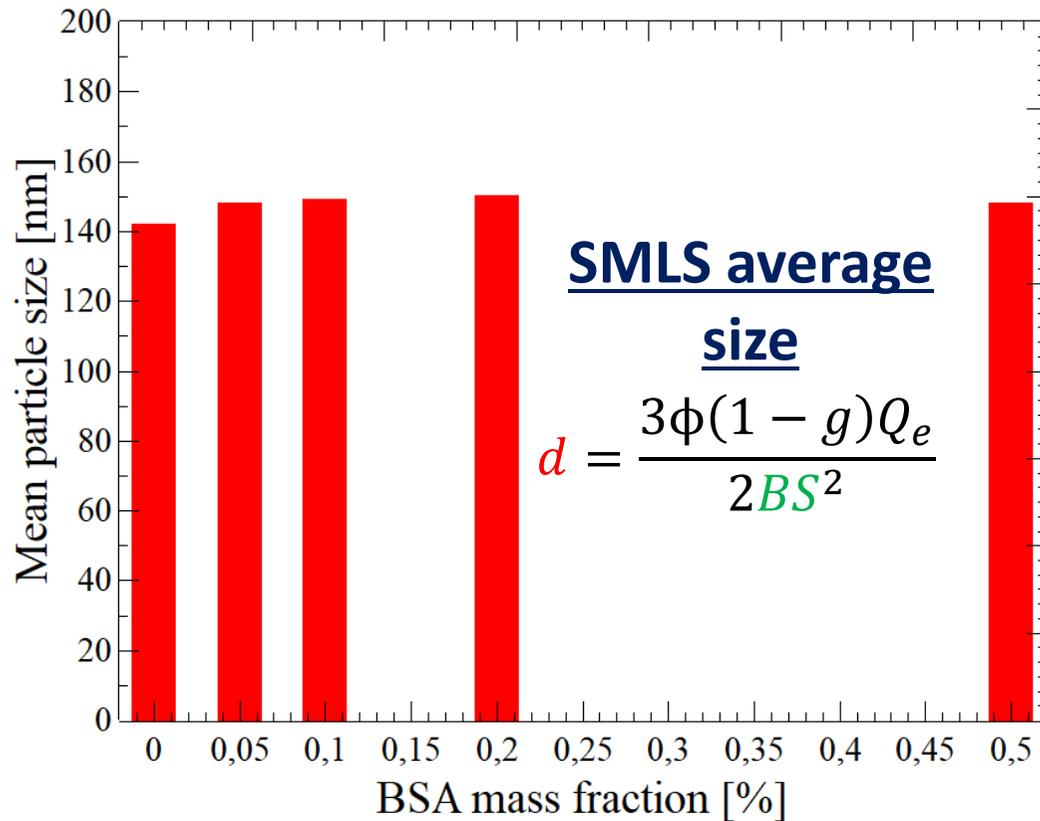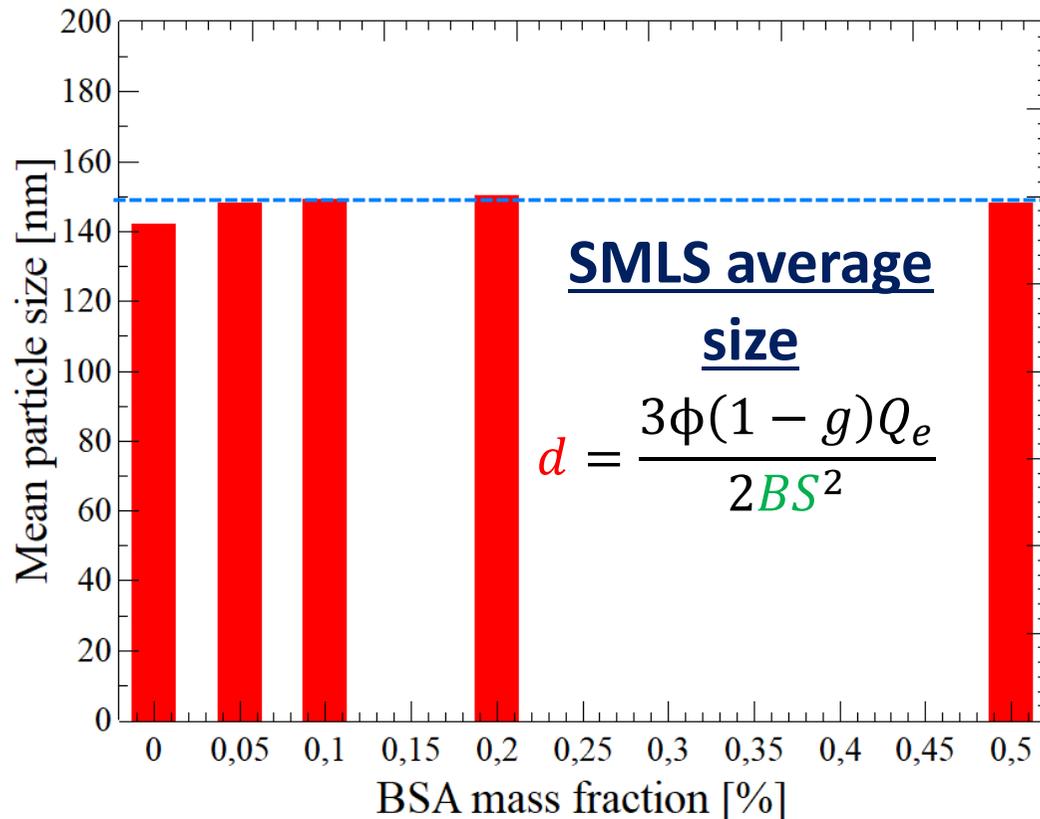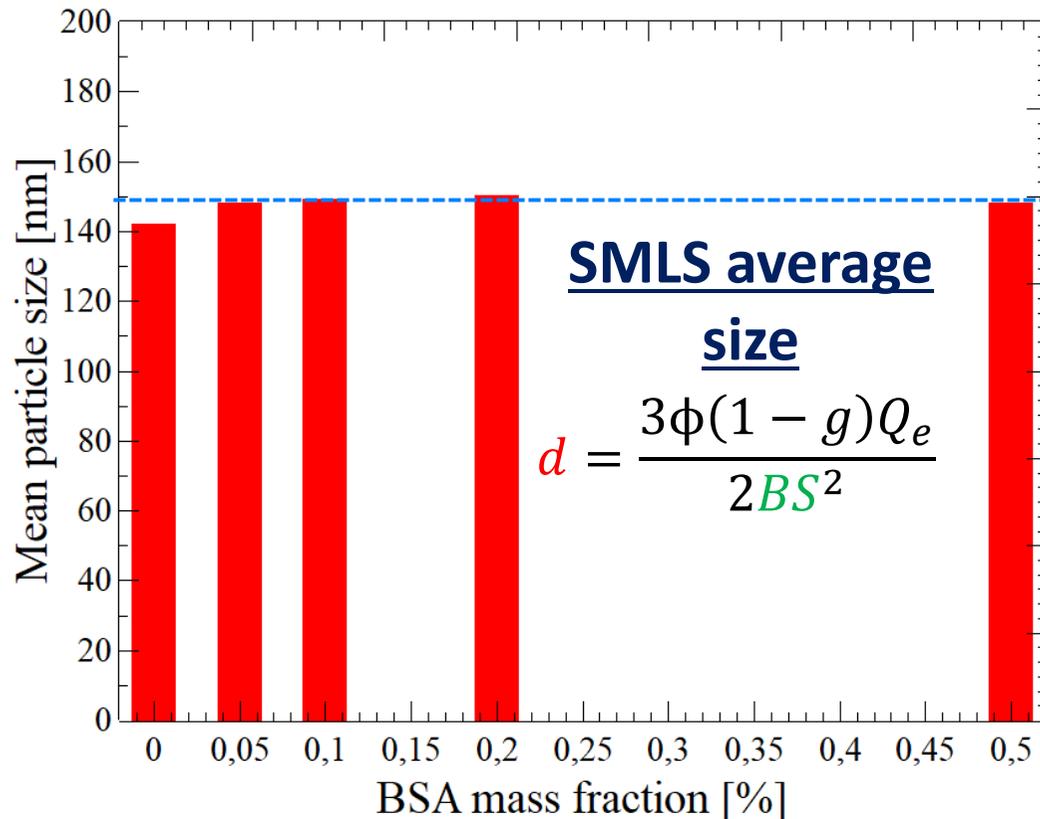
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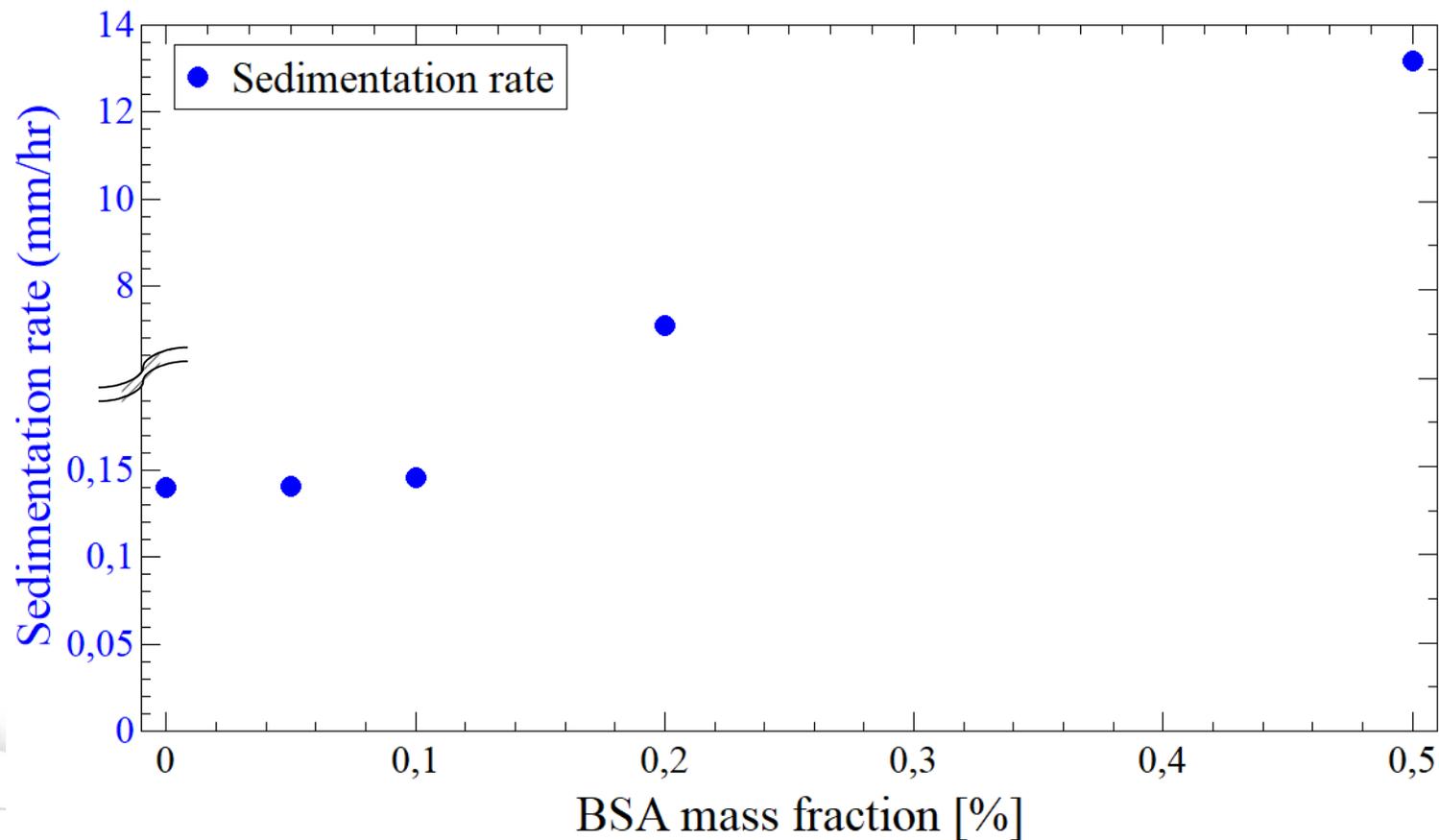
- At pH=7.2, electrostatic forces are predominant*

No interactions

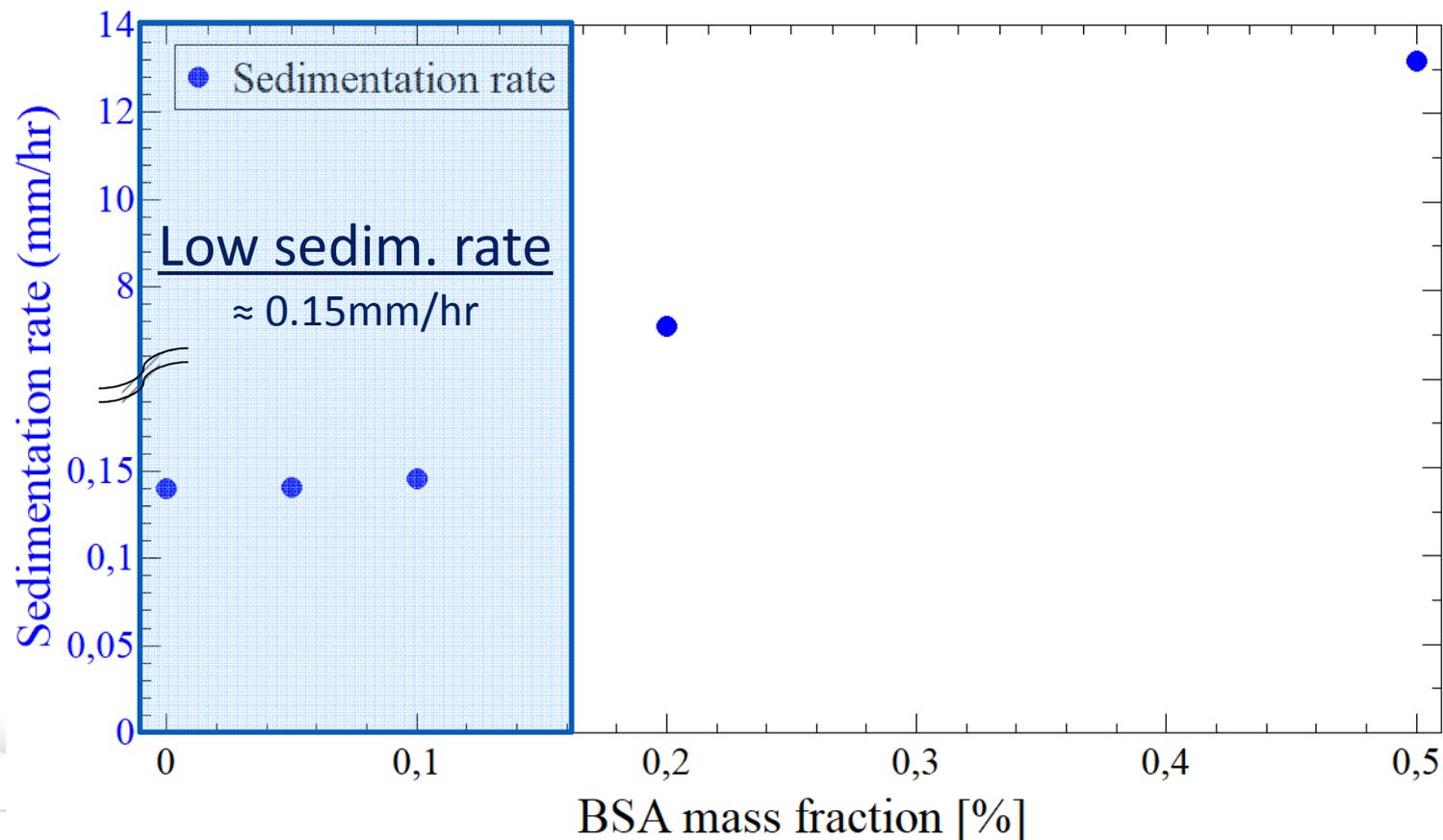*JI, Zhaoxia et *al.. Environmental science & technology*, 2010, vol. 44, no 19, p. 7309-7314

STABILITY & SIZE

Turbiscan results for TiO2 stock dispersions

- BSA concentration influence on <u>24h sedimentation rate</u>

STABILITY & SIZE

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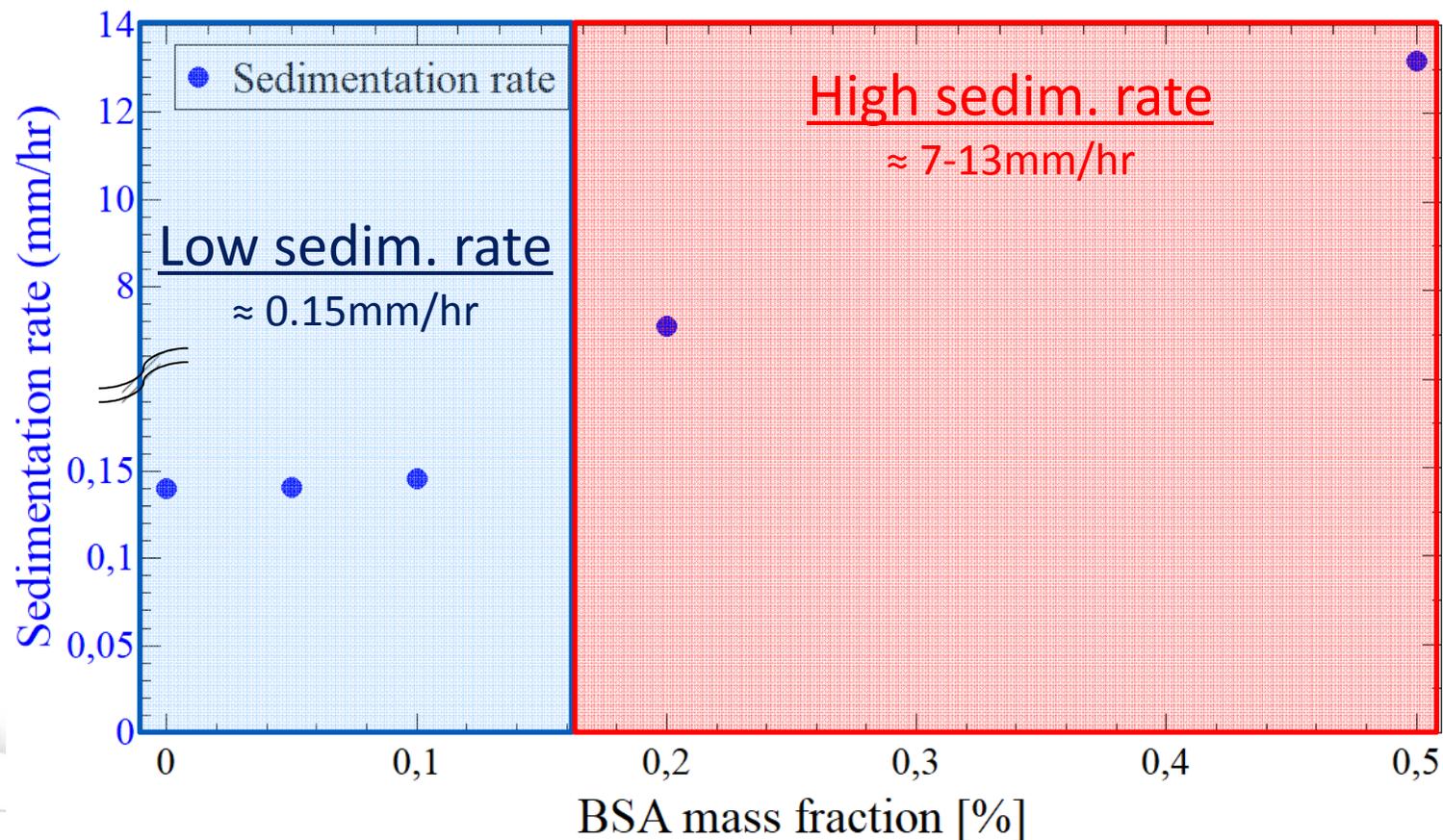
STABILITY & SIZE

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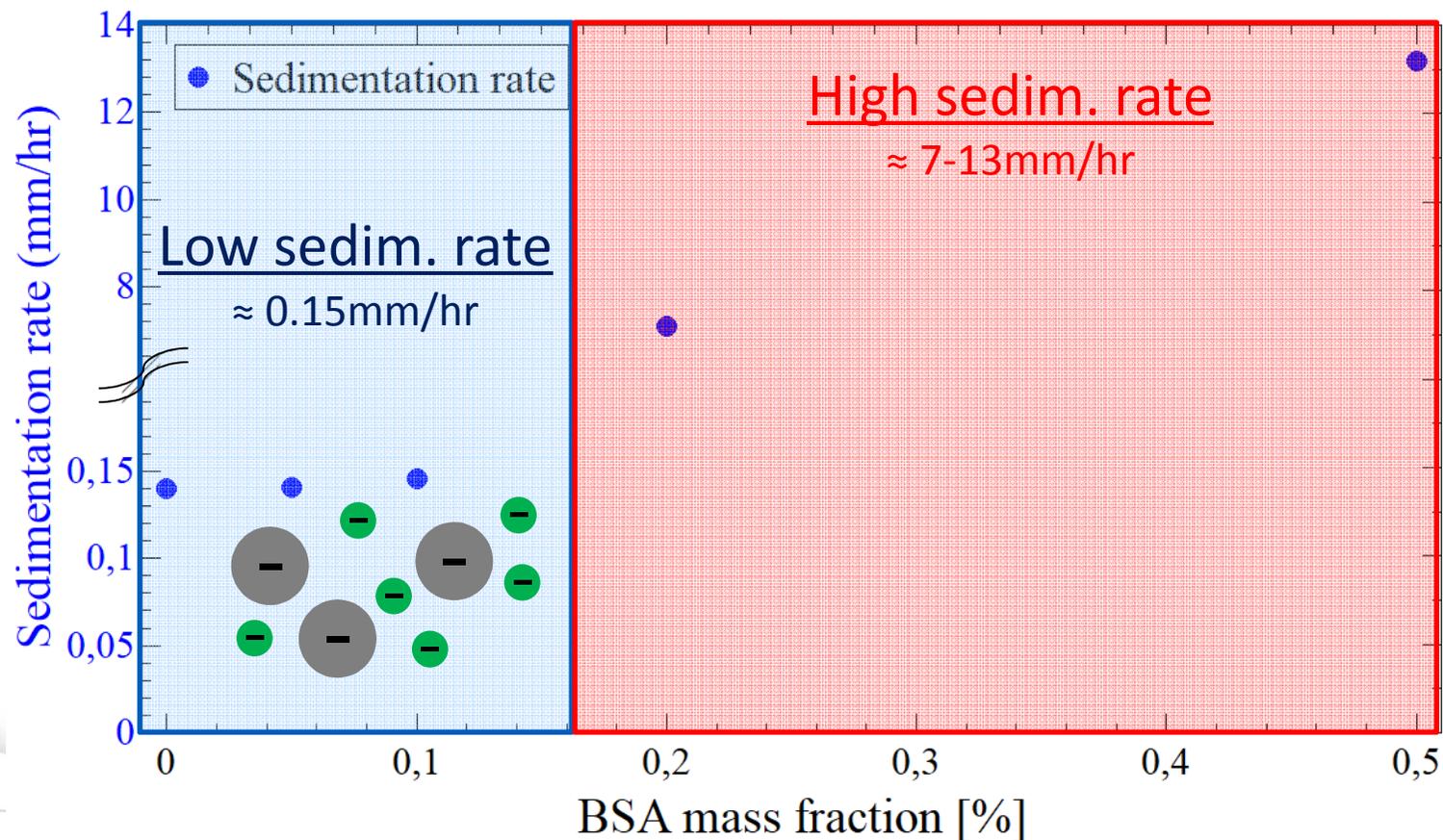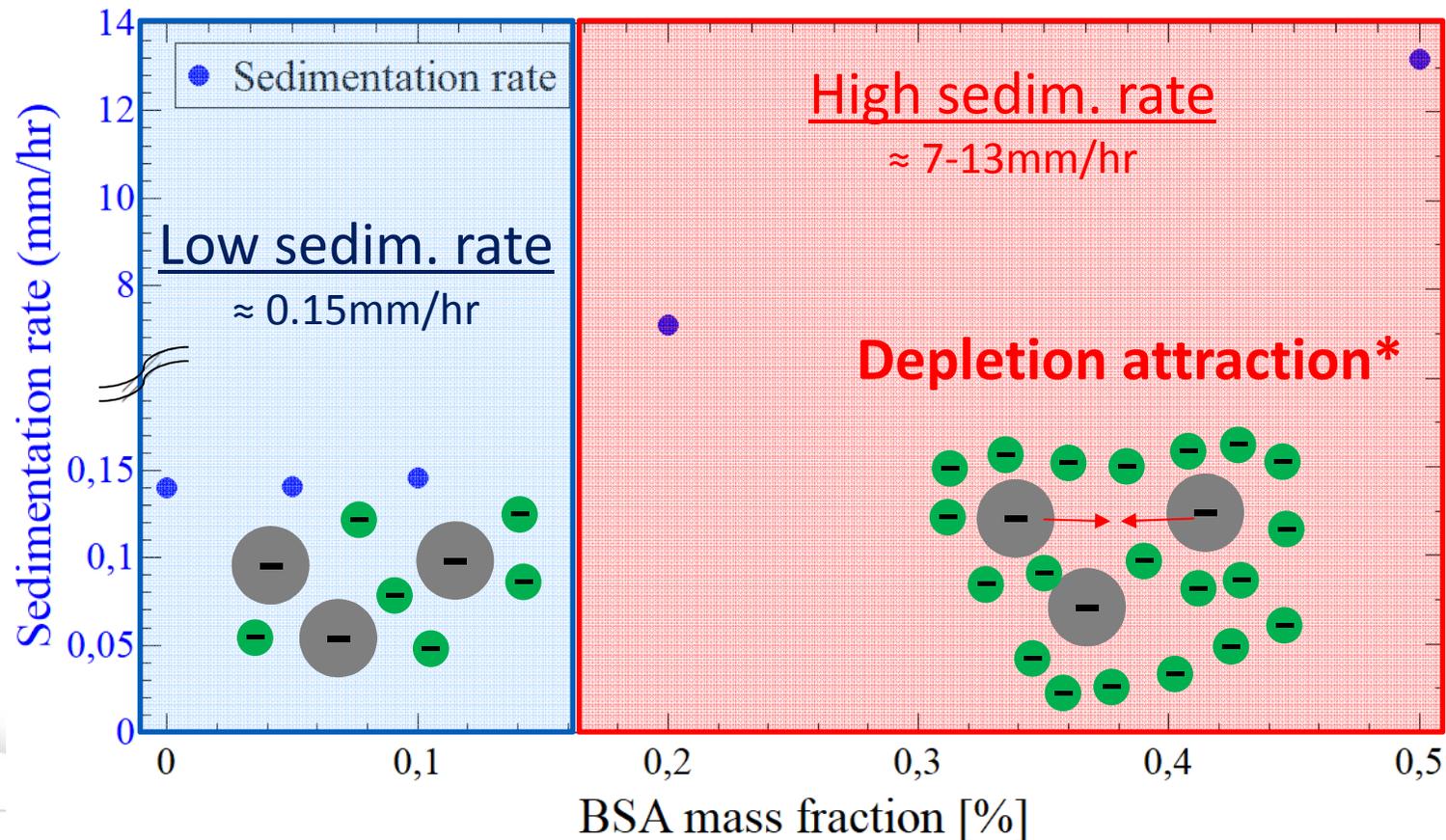- BSA concentration influence on <u>24h sedimentation rate</u>
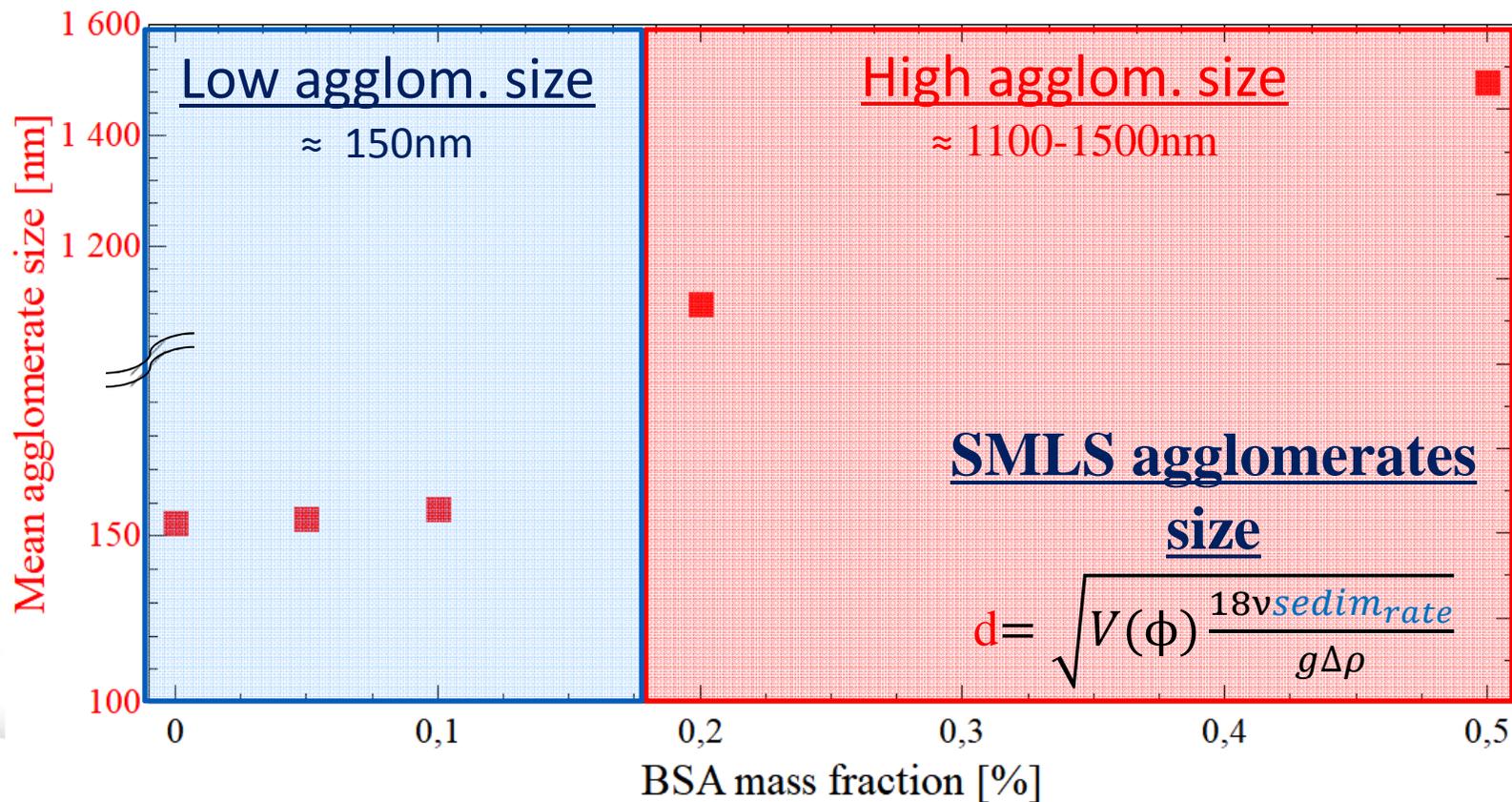
Turbiscan results for TiO2 stock dispersions

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*Yadav, I *et al*. *Langmuir*, 2017, vol. 33, no 5, p. 1227-1238.
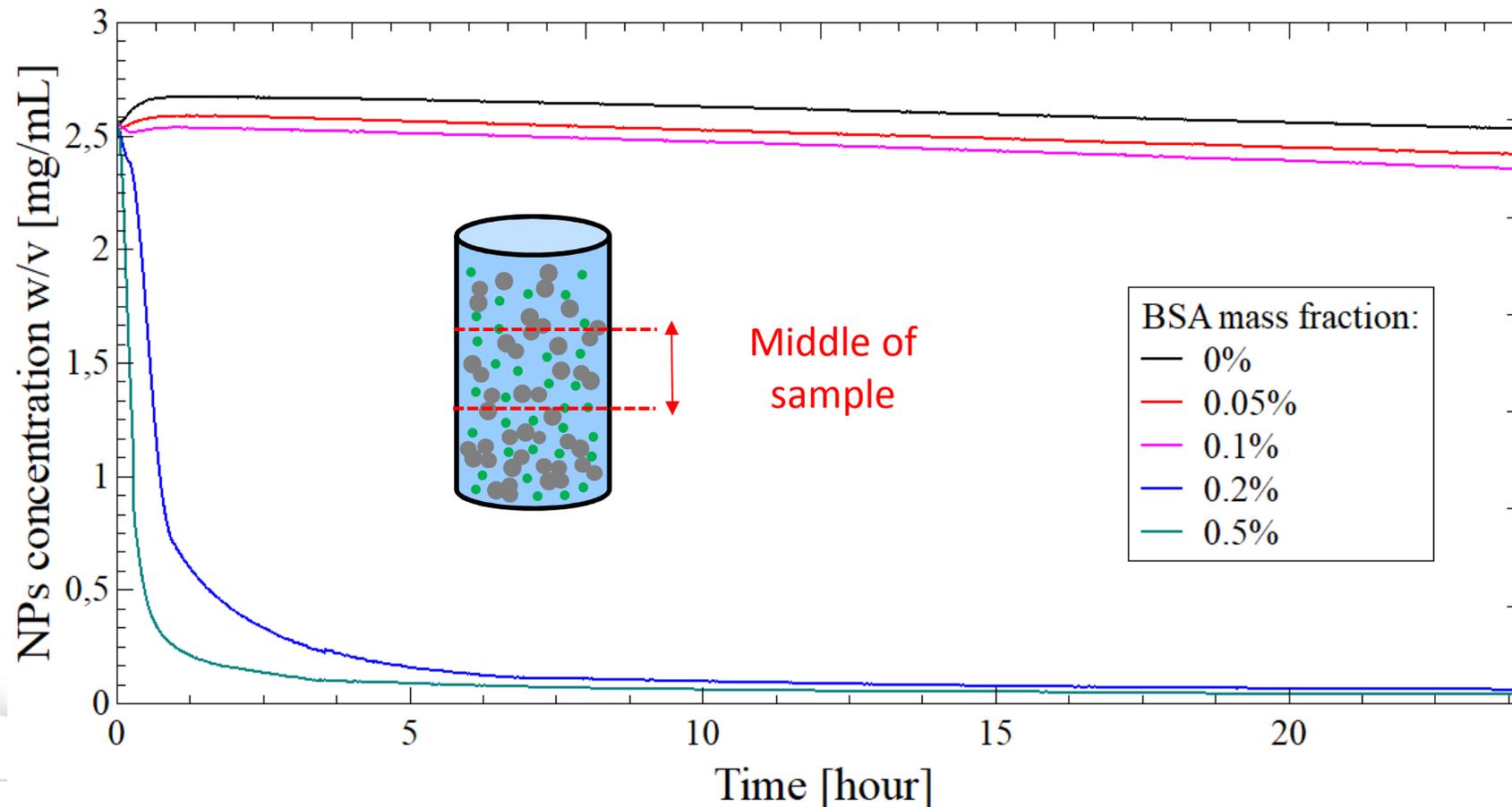
STABILITY & SIZE

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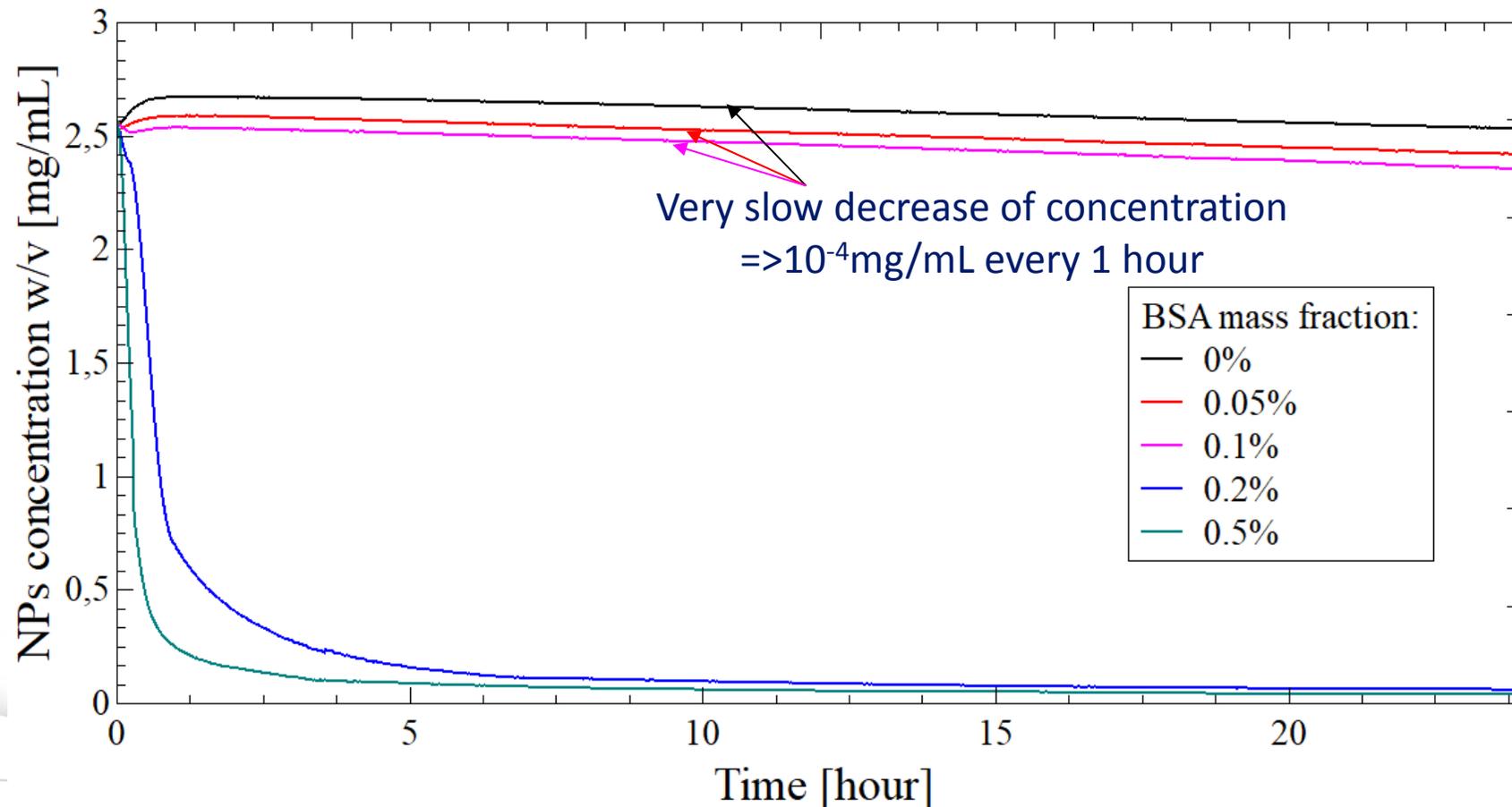- BSA concentration influence on <u>agglomerate size</u>

Turbiscan results for TiO2 stock dispersions

- BSA conc. influence on <u>middle sample concentration of NPS</u>

Turbiscan results for TiO2 stock dispersions

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Very slow decrease of concentration
=>10^{-4}mg/mL every 1 hour

BSA mass fraction:
— 0%
— 0.05%
— 0.1%
— 0.2%
— 0.5%

- BSA conc. influence on <u>middle sample concentration of NPS</u>

Very slow decrease of concentration
=>10^{-4}mg/mL every 1 hour

High decrease of concentration
=>0.9mg/mL every 1 hour

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STABILITY & SIZE

- BSA conc. influence on <u>middle sample concentration of NPS</u>

- For BSA mass fraction $<$ 0.2%, stock dispersions well dispersed and stable for long time

  → NanoREG perfectly adapted with 0.05% BSA
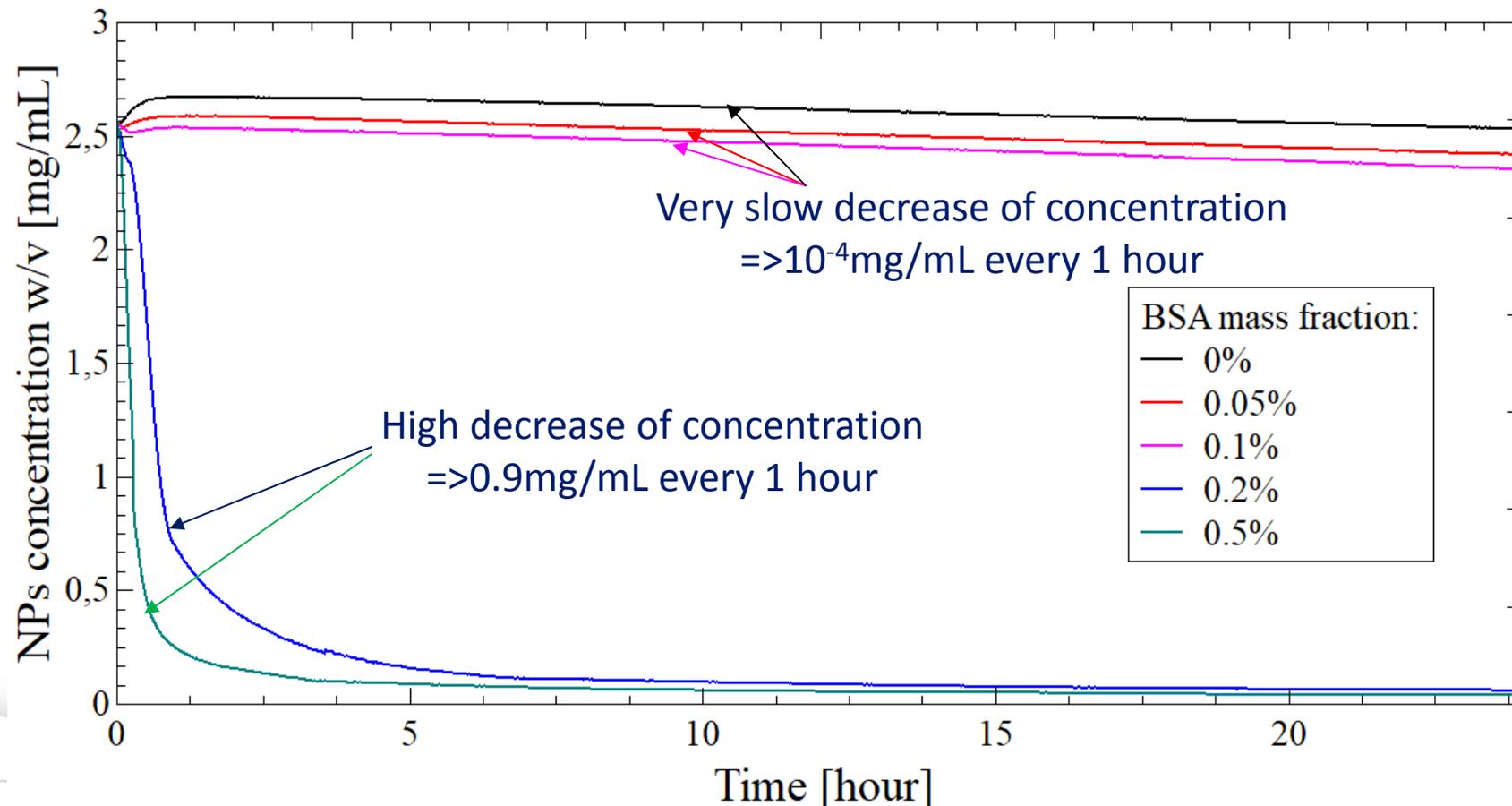
Conclusions on stock dispersion

- <u>For BSA mass fraction $< 0.2\%$,</u> stock dispersions well dispersed and stable for long time
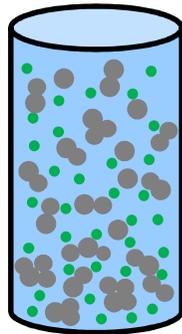
  → NanoREG perfectly adapted with 0.05% BSA

- <u>For BSA mass fraction $\geq 0.2\%$</u>, Agglomeration and destabilization of stock dispersions in 5min!
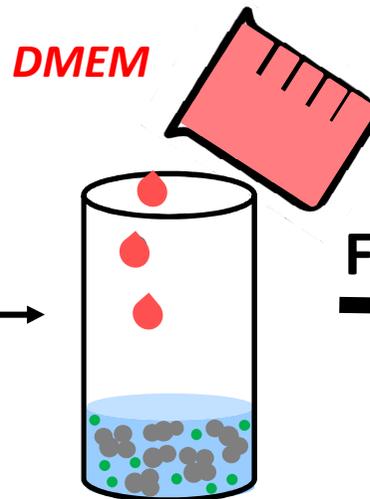
  → Stock dispersion must be diluted very quickly in cell culture medium

STABILITY & SIZE

CHARACTERIZATION OF STOCK DISPERSION DILUTIONS WITH TURBISCAN

Stock dispersion
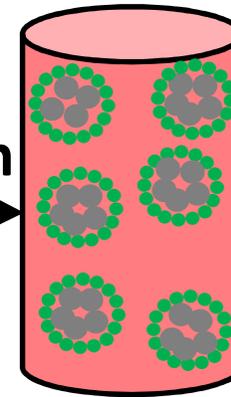
2.56mg/mL NPs

DMEM

Dilution
DMEM

Final dispersion

Final mass fraction NPs
256µg/mL

STABILITY & SIZE

Turbiscan results for cell culture media

- BSA conc. (in stock disp.) influence on <u>initial mean size (t=0)</u>

SMLS average size

$$d = \frac{3\phi(1-g)Q_e}{2BS^2}$$
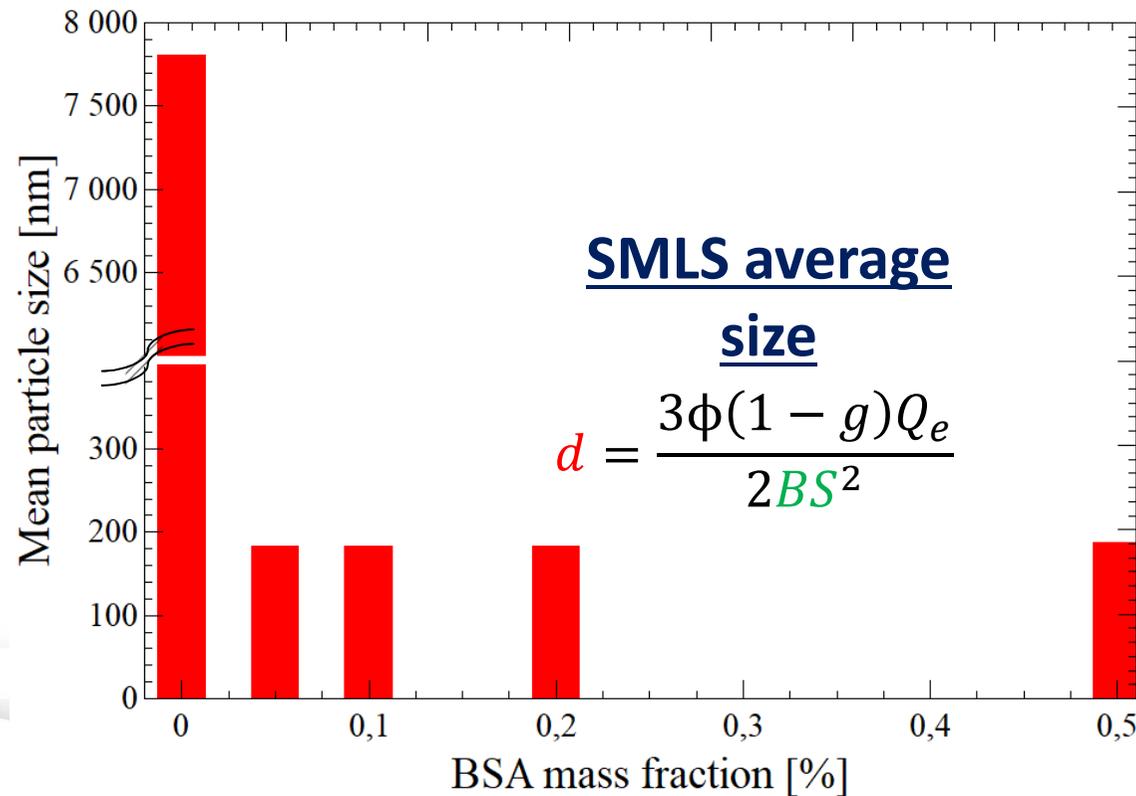
Turbiscan results for cell culture media

- BSA conc. (in stock disp.) influence on <u>initial mean size (t=0)</u>

- Immediate agglomeration without BSA*

*ZHANG, X *et al. Journal of nanoscience and nanotechnology*, 2010, vol. 10, no 8, p. 5213-5219.

STABILITY & SIZE

Turbiscan results for cell culture media

- BSA conc. (in stock disp.) influence on <u>initial mean size (t=0)</u>
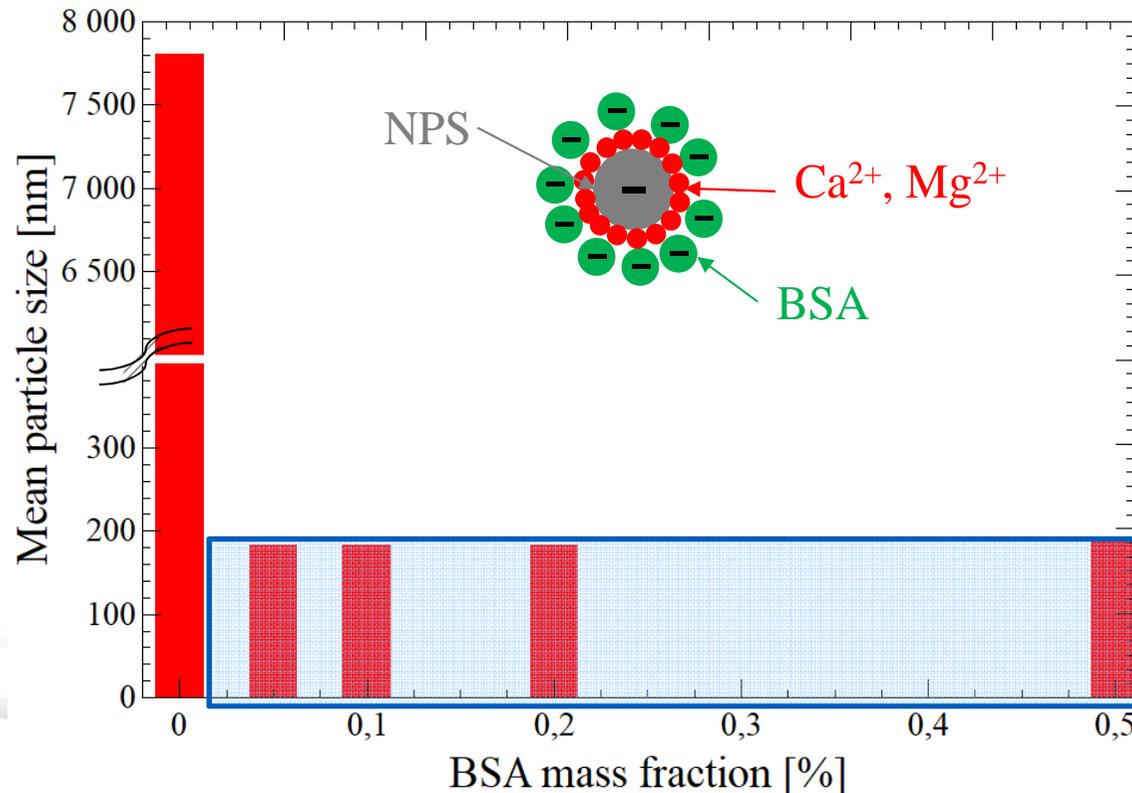
- Immediate agglomeration without BSA

- Addition of BSA greatly improve dispersibility in DMEM (protein corona*)

- Mean size around 180nm

*HORIE, M *et al. Chemical research in toxicology*, 2009, vol. 22, no 3, p. 543-553.
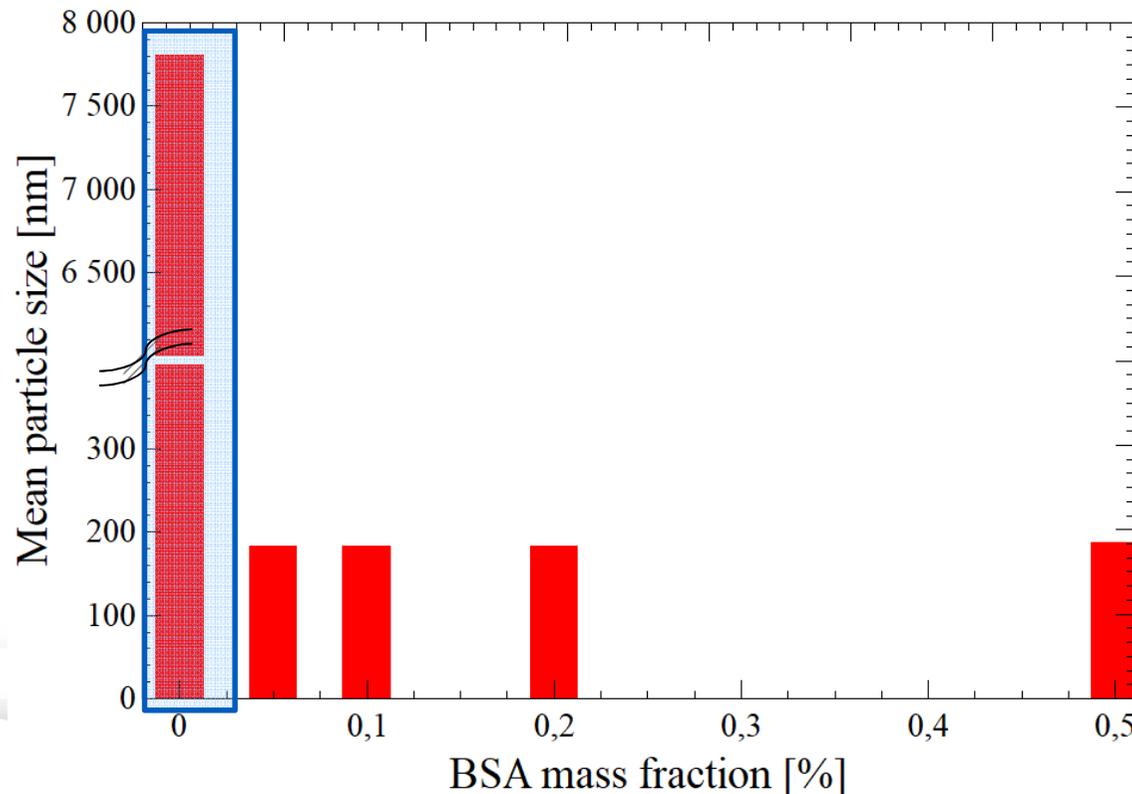
Turbiscan results for cell culture media

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Turbiscan results for cell culture media

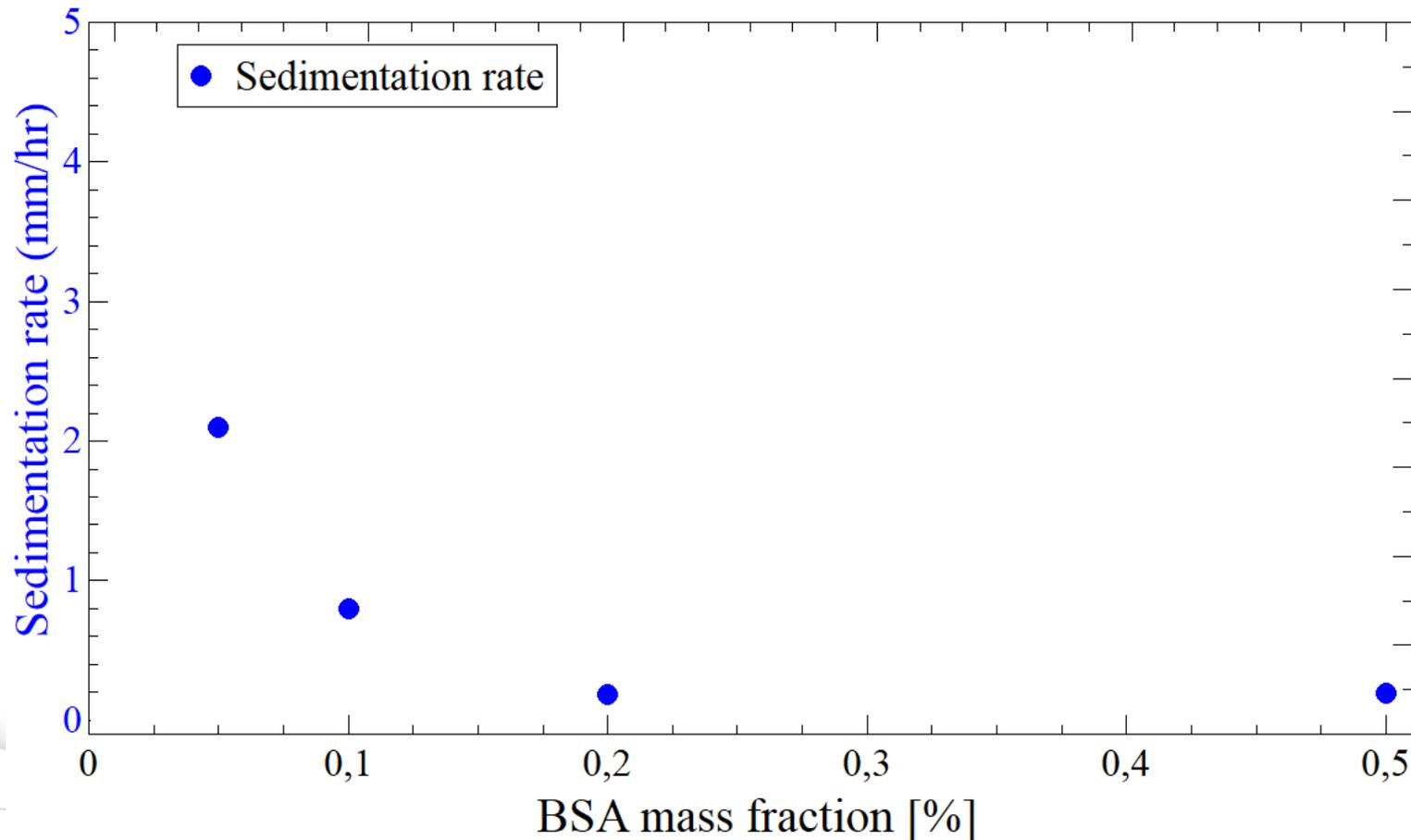- BSA conc. (in stock disp.) influence on <u>agglomerate size</u>
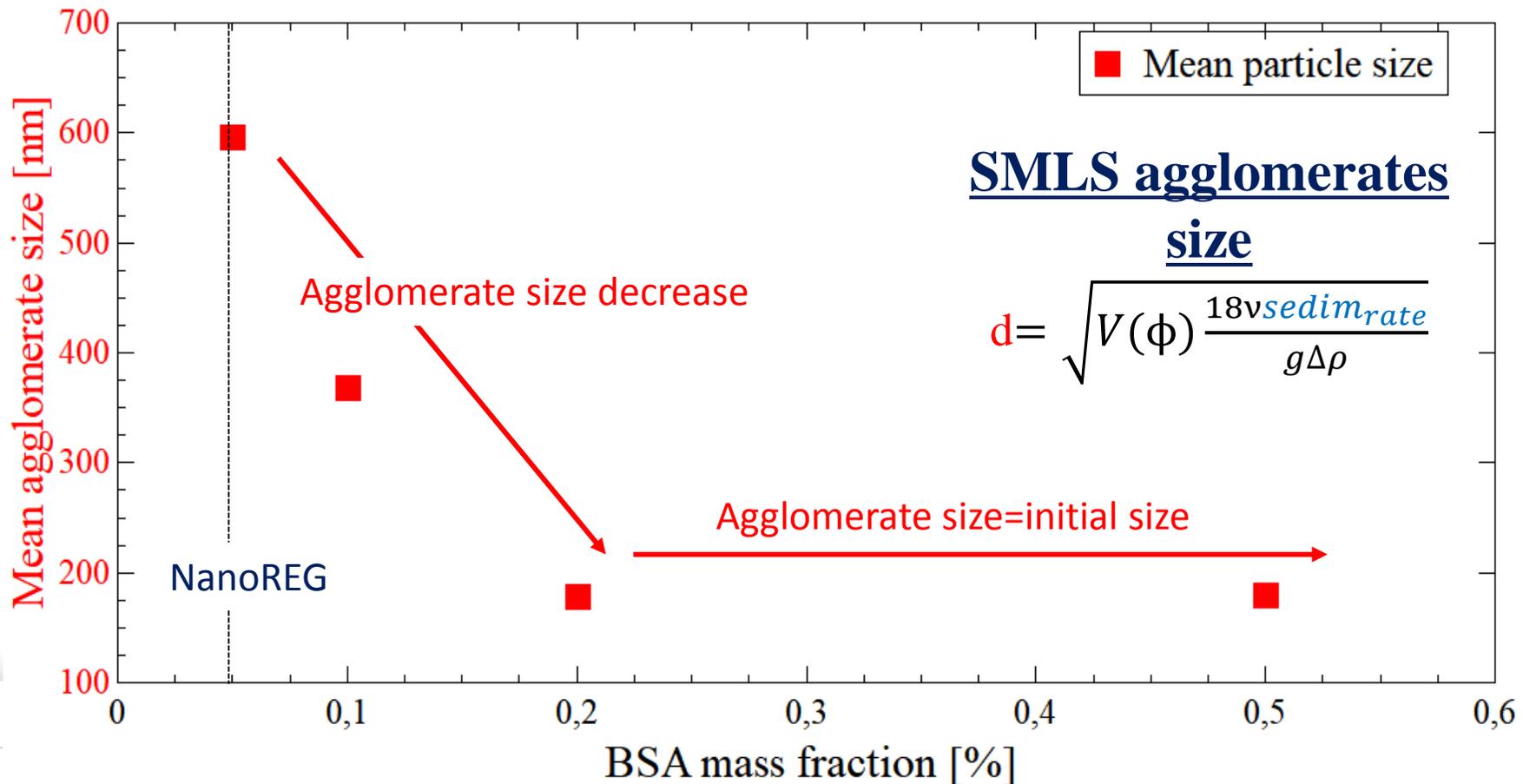
SMLS agglomerates size

$$d = \sqrt{V(\phi)\,\frac{18\nu\, sedim_{rate}}{g\Delta\rho}}$$

Mean particle size

Agglomerate size decrease

Agglomerate size=initial size

NanoREG

Mean agglomerate size [nm]

BSA mass fraction [%]

STABILITY & SIZE

- BSA conc. (in stock disp.) on <u>bottom sample concentration</u>

Turbiscan results for cell culture media

- BSA conc. (in stock disp.) on <u>bottom sample concentration</u>

NanoREG

In 10 hours, <u>delivered dose to adherent cells=</u>
- for BSA<0.2% <u>initial administered dose x10</u>
- for BSA≥0.2% <u>initial administered dose x2</u>

BSA mass fraction:
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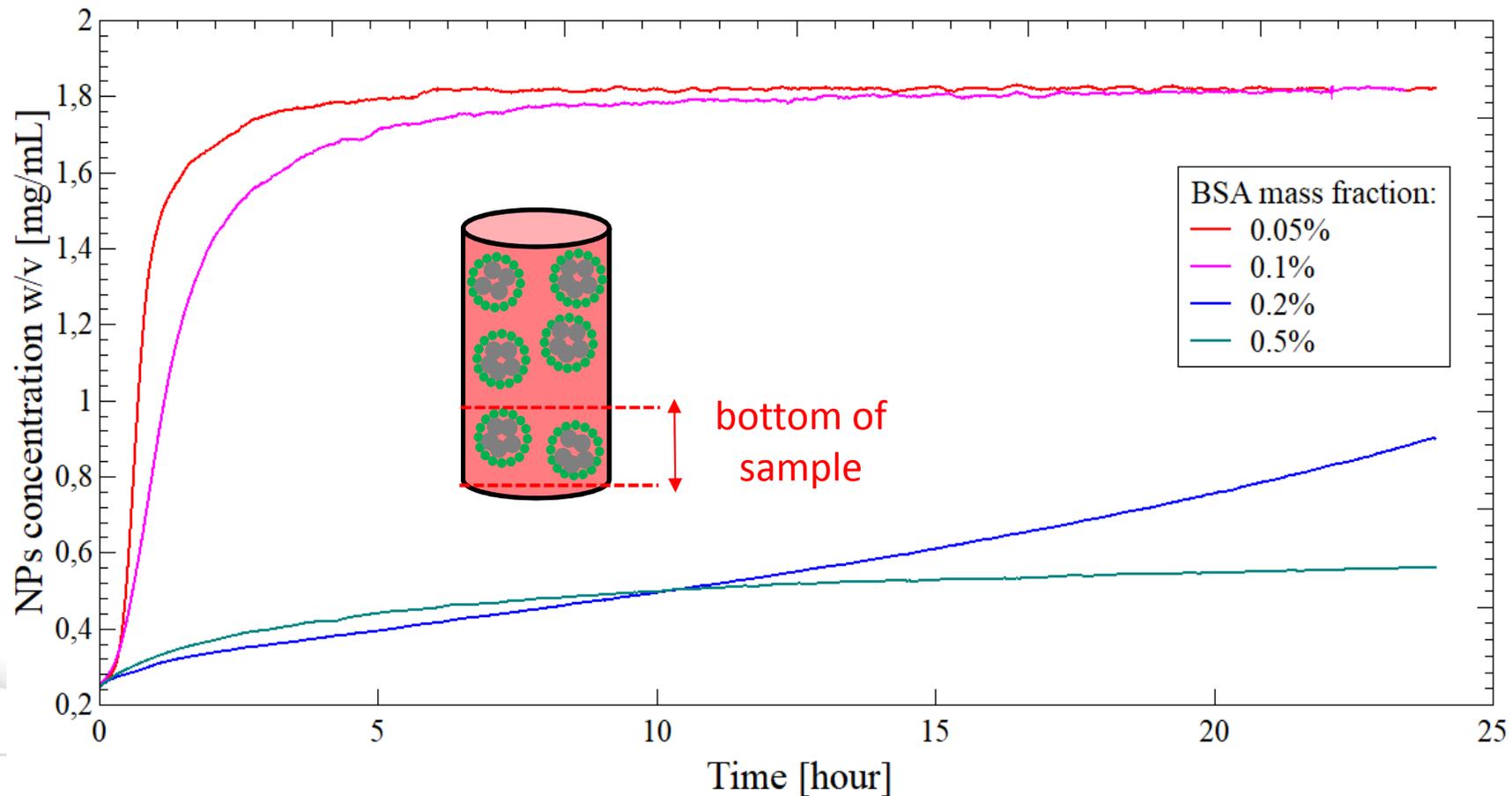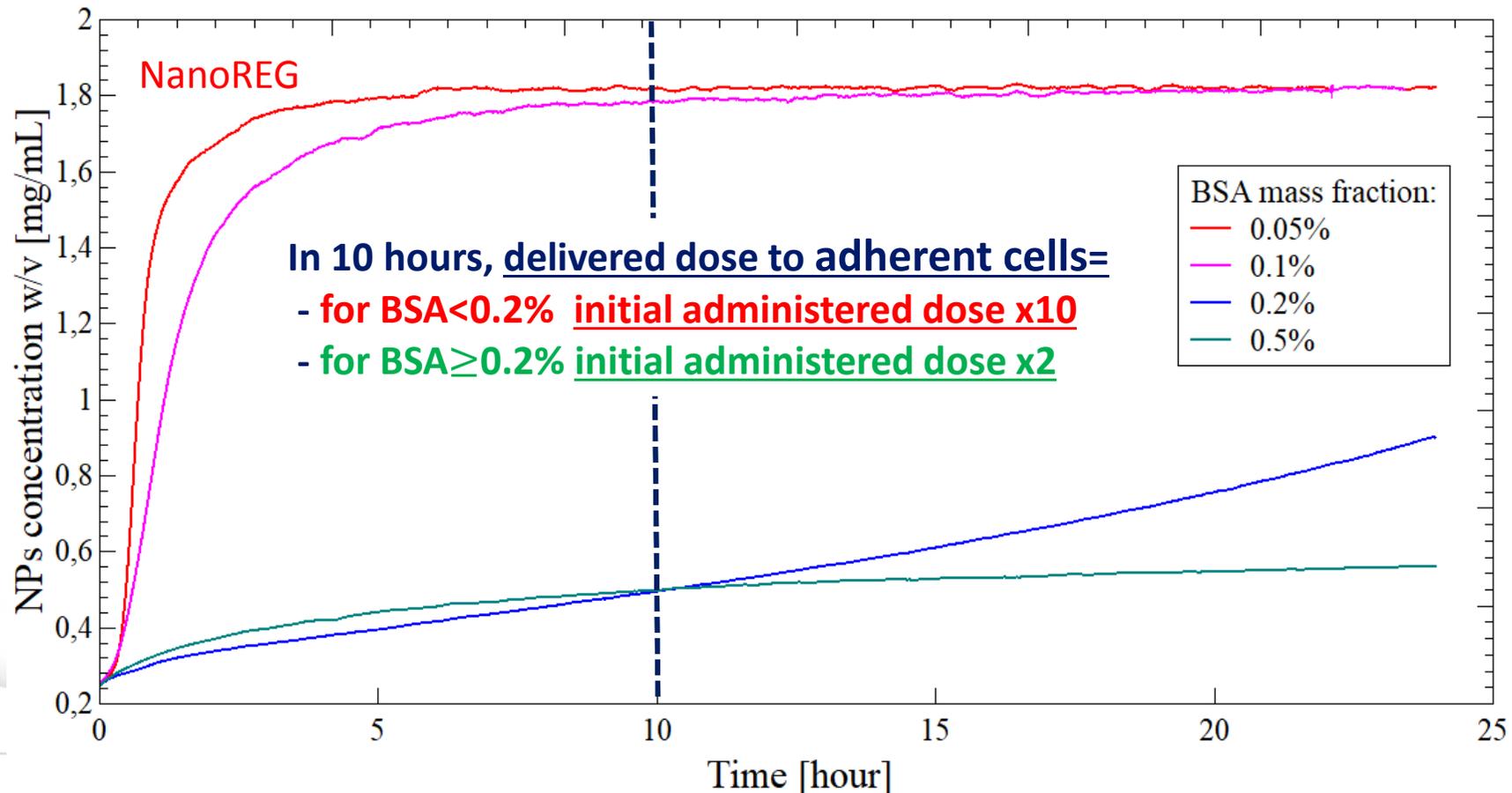
Conclusions and discussions

- Characterization of both **dispersibility and stability** of stock dispersions and dilutions with SMLS

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  But depletion after 5min in dispersion stock!

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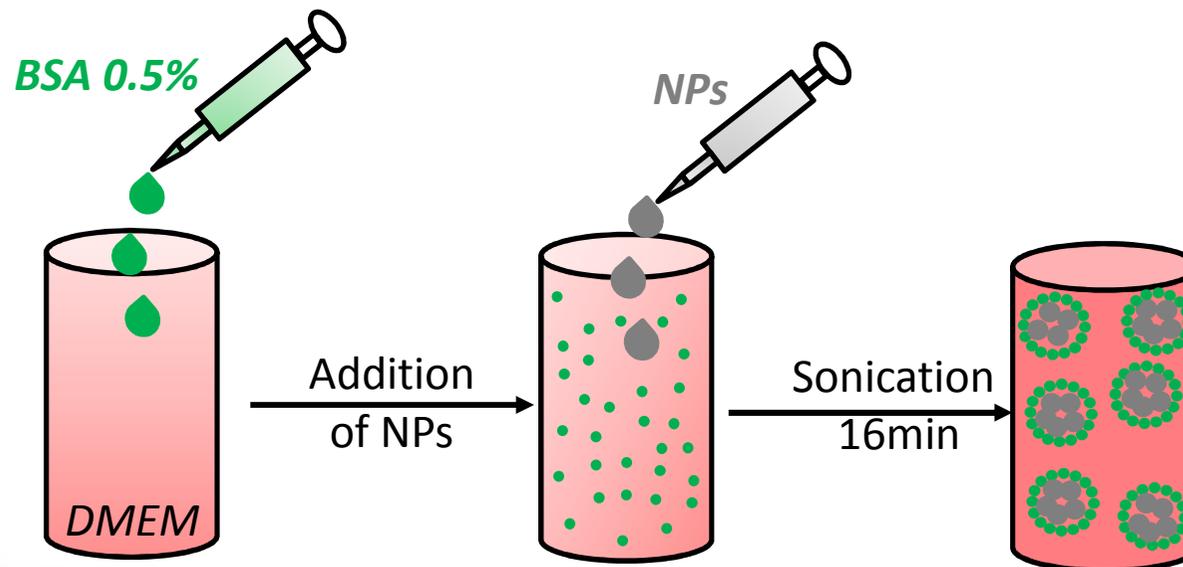
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  =>Maybe not the best NPs preparation in cell culture?*

BSA 0.5%

NPs

Addition of NPs

Sonication 16min

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JI, Zhaoxia et al. Environmental science & technology, 2010, vol. 44, no 19, p. 7309-7314

STABILITY & SIZE

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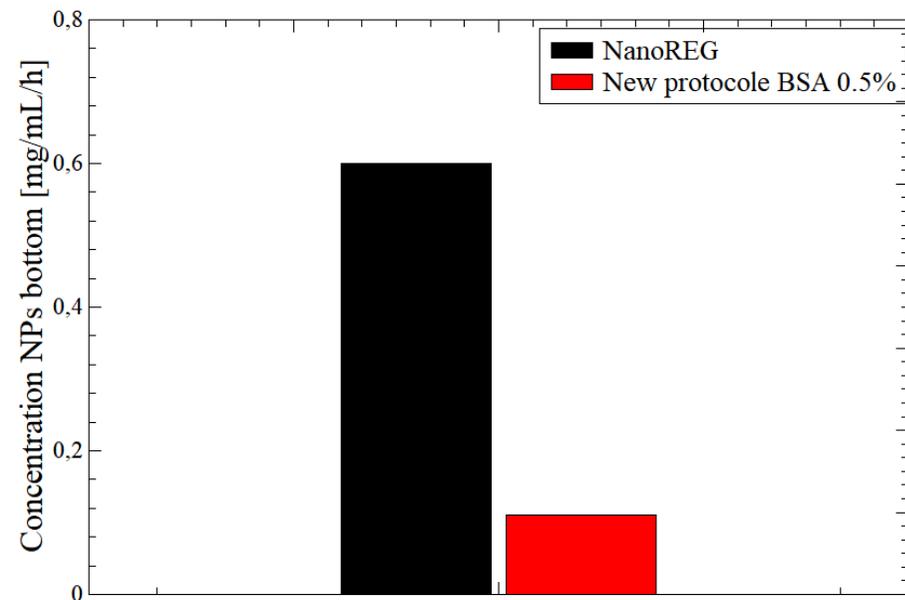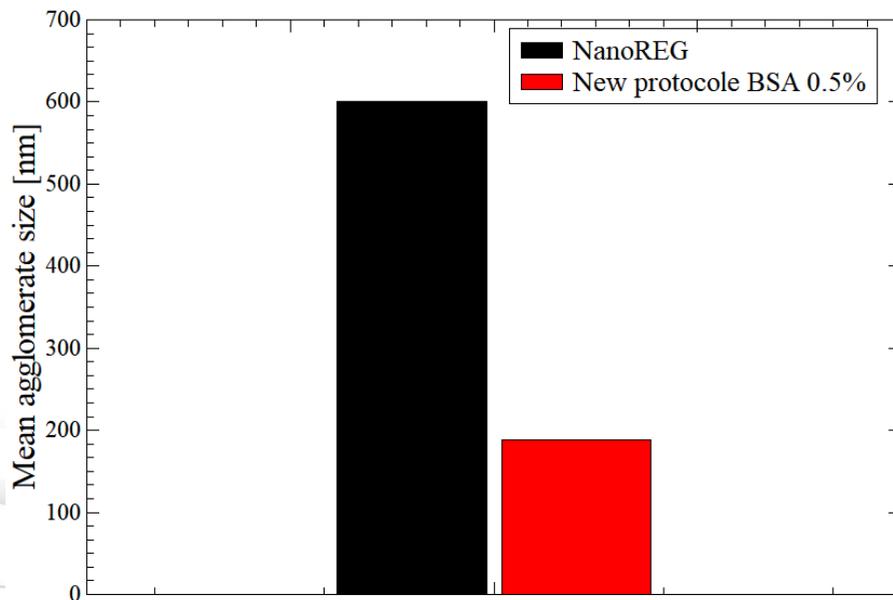 But depletion after 5min in dispersion stock!

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Thank you for your attention!

Few references that already use Turbiscan in nanotoxicity studies:

- Yoon, D., et al. (2011). **Agglomeration, sedimentation, and cellular toxicity of alumina nanoparticles in cell culture medium**. Journal of Nanoparticle Research, 13(6), 2543-2551

- Fangueiro, J. F., et al. (2014). **Design of cationic lipid nanoparticles for ocular delivery: development, characterization and cytotoxicity.** International journal of pharmaceutics, 461(1-2), 64-73.

- Calderó, G., et al. (2011). **Formation of polymeric nano-emulsions by a low-energy method and their use for nanoparticle preparation**. Journal of colloid and interface science, 353(2), 406-411.

- Carrière, M., et al. (2014). **Impact of titanium dioxide nanoparticle dispersion state and dispersion method on their toxicity towards A549 lung cells and Escherichia coli bacteria.** Journal of translational toxicology, 1(1), 10-20.

- Mendes, A. N., et al. (2012). **Preparation and cytotoxicity of poly (methyl methacrylate) nanoparticles for drug encapsulation**. In Macromolecular Symposia. Weinheim: WILEY-VCH Verlag.

- Wiśniewska, et al.(2014). **Comparison of stability properties of poly (acrylic acid) adsorbed on the surface of silica, alumina and mixed silica-alumina nanoparticles—application of turbidimetry method**. Open Chemistry, 12(4), 476-479.

Characterization of dispersibility and stability

- Devices to control stock dispersion and dilutions

	TEM	Optical absorbance	Spectroscopy	SAXS	X-ray diffraction
Dispersion	Yes	No	No	Yes	Yes
Sedimentation rate	No	Yes	Yes	No	No
Agglomerates size	Yes	No	No	No	No

Characterization of dispersibility and stability

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- Use of at least two devices (e.g. TEM + Optical absorbance)*

*ALLOUNI, Zouhir E., CIMPAN, Mihaela R., HØL, Paul J., et al. Agglomeration and sedimentation of TiO2 nanoparticles in cell culture medium. Colloids and surfaces B: Biointerfaces, 2009, vol. 68, no 1, p. 83-87.

STABILITY & SIZE

Characterization of dispersibility and stability

- Devices to control stock dispersion and dilutions

	TEM	Optical absorbance	Spectroscopy	SAXS	X-ray diffraction	DLS
Dispersion	Yes	No	No	Yes	Yes	Yes but
Sedimentation rate	No	Yes	Yes	No	No	Yes but
Agglomerates size	Yes	No	No	No	No	Yes but

- Use of at least two devices (e.g. TEM + Optical absorbance)

- Or use of DLS for both dispersibility and stability*

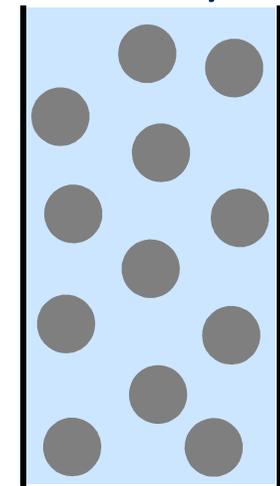*NanoREG protocol "*A common European approach to the regulatory testing of Manufactured Nanomaterials*"

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- Devices to control stock dispersion and dilutions

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Dispersion	Yes	No	No	Yes	Yes	Yes but
Sedimentation rate	No	Yes	Yes	No	No	Yes but
Agglomerates size	Yes	No	No	No	No	Yes but

- Use of at least two devices (e.g. TEM + Optical absorbance)

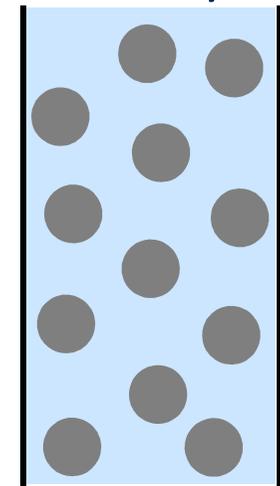- Or use of DLS for both dispersibility and stability

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STABILITY & SIZE

Characterization of dispersibility and stability

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→ But if one instability only!
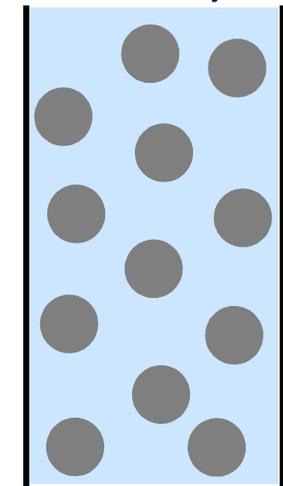
STABILITY & SIZE

Static multiple light scattering SMLS principle

- Examples for diluted dispersions – transmission

Transmitted signal during agglomeration

NPs

T

Height

T signal

t=0
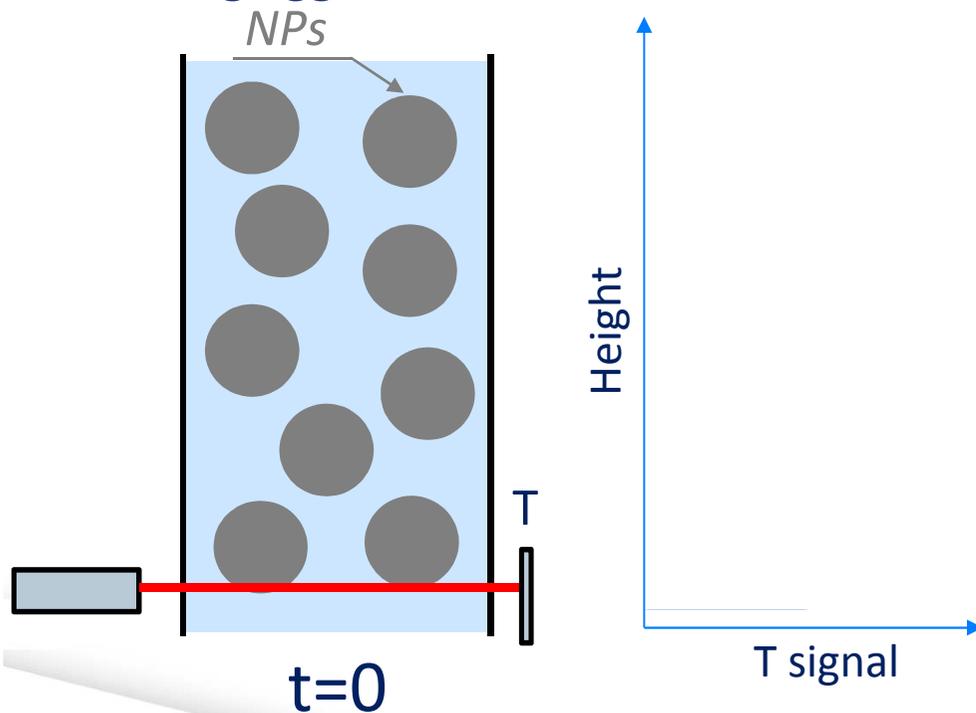
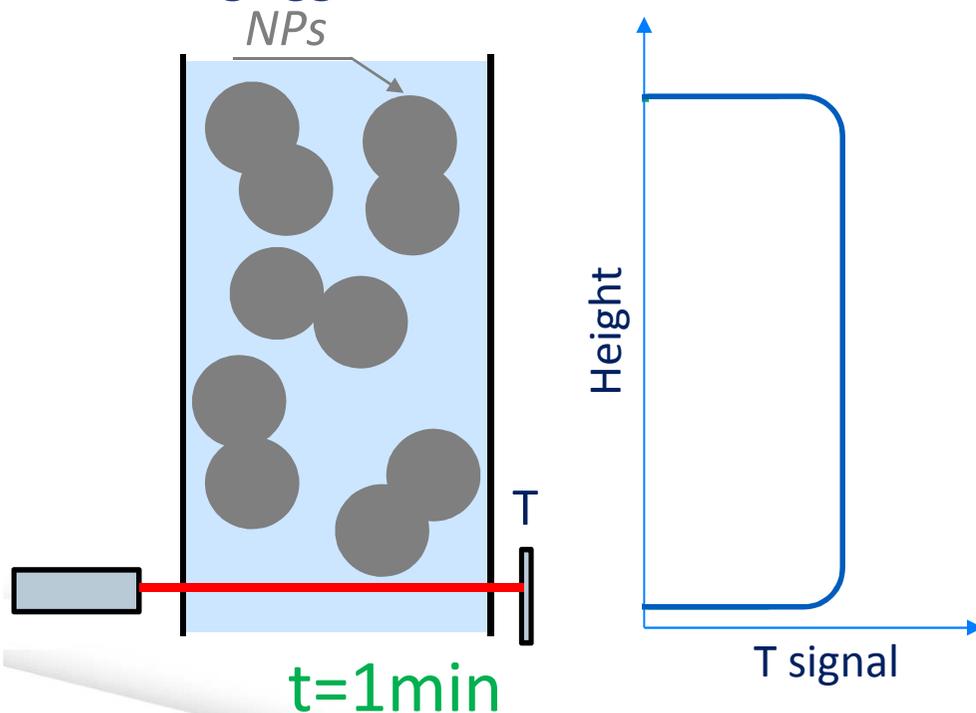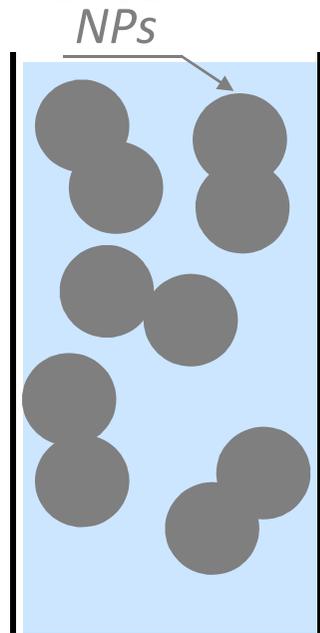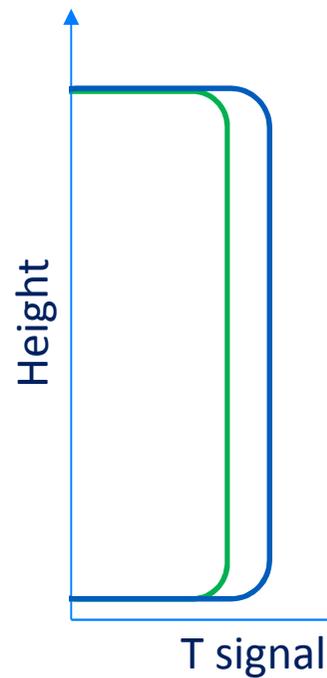- Examples for diluted dispersions – transmission

**Transmitted signal
during agglomeration**

t=1min

Static multiple light scattering SMLS principle
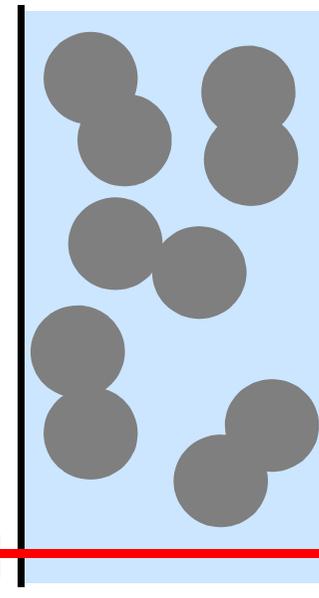
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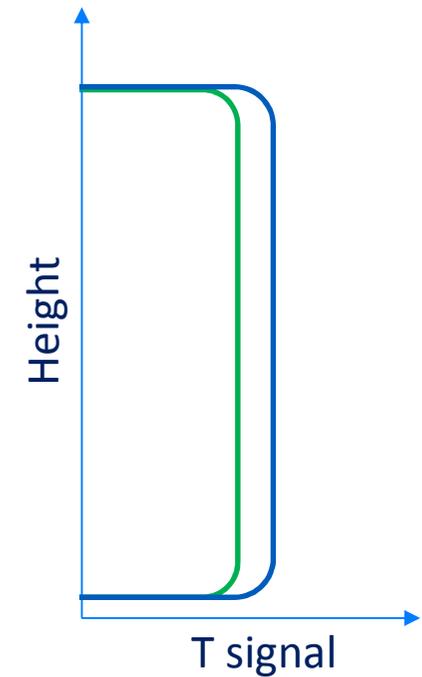
NPs

Height

T signal

t=1min

Transmitted signal during sedimentation
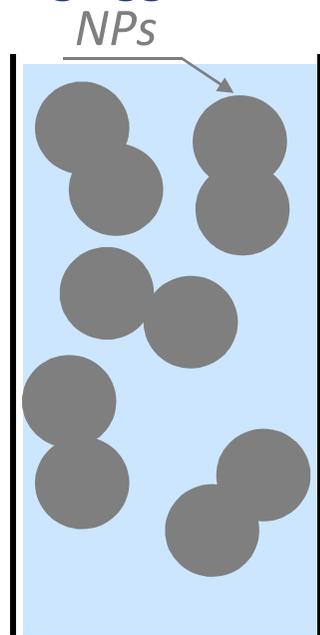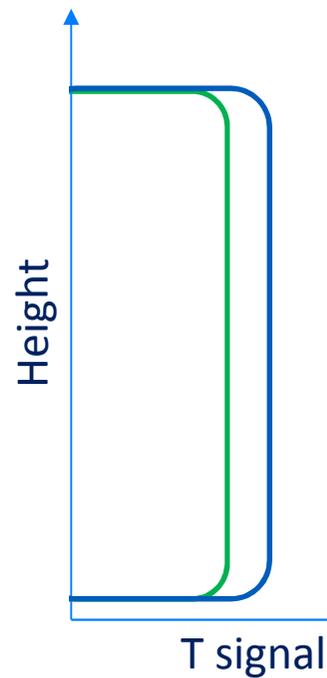
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T

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STABILITY & SIZE

Static multiple light scattering SMLS principle

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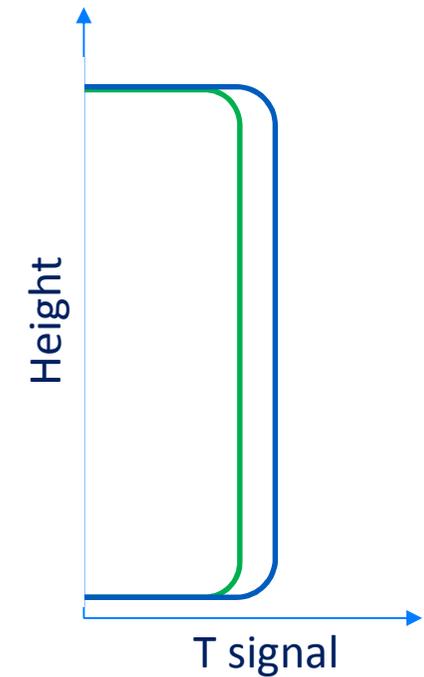
NPs

Height

T signal

t=1min

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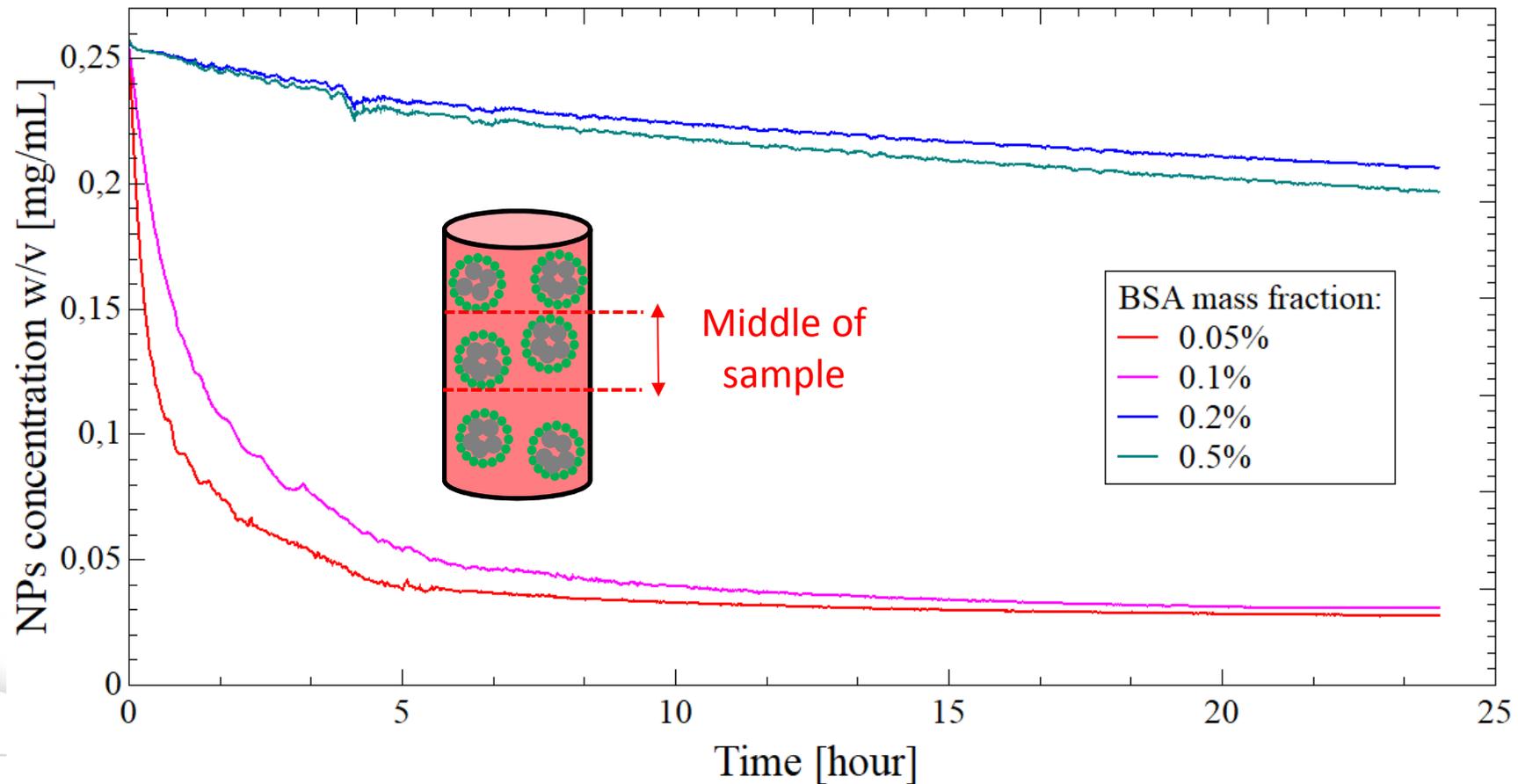
Height

T signal

T

t=20min
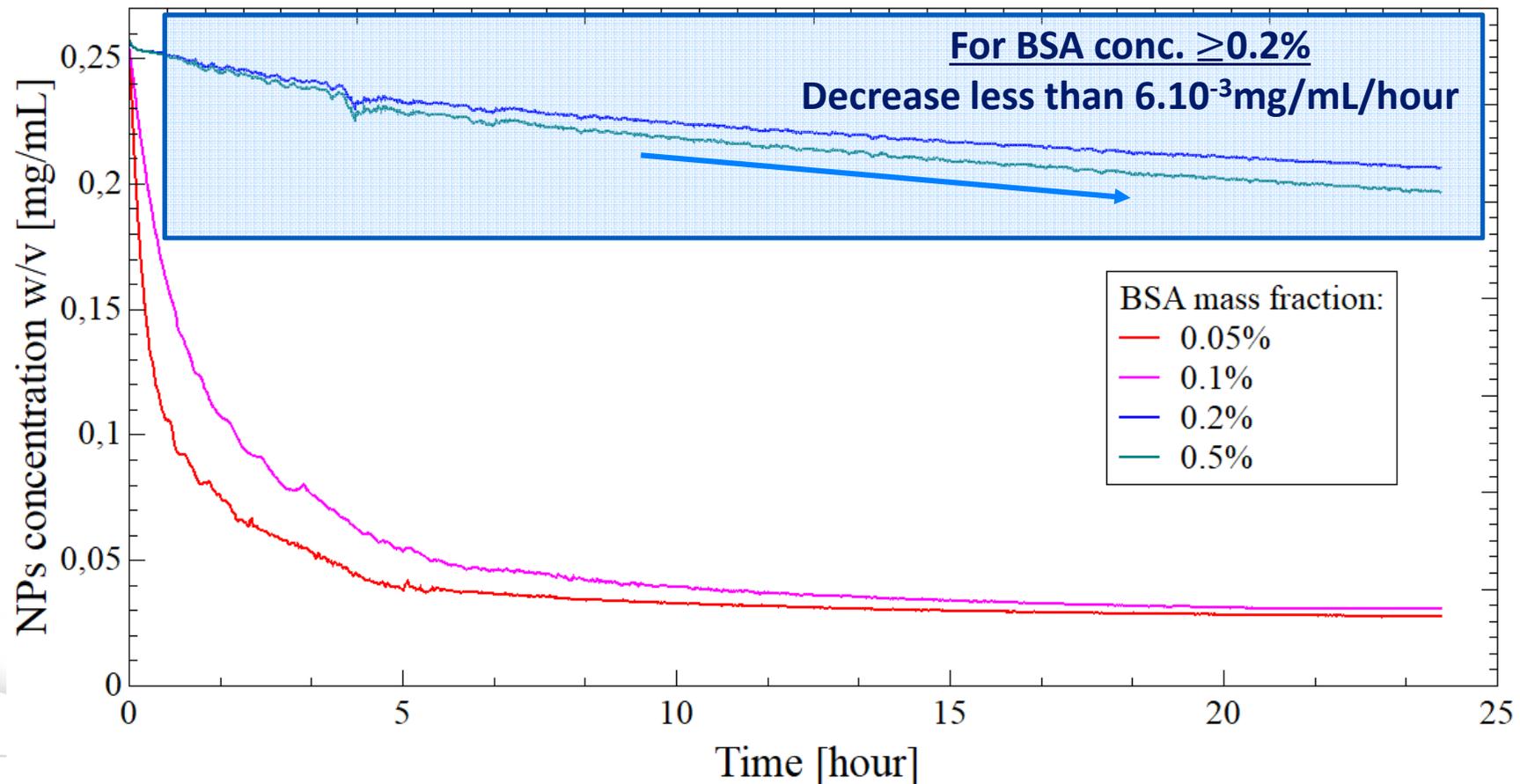
STABILITY & SIZE

Turbiscan results for cell culture media

- BSA concentration influence on <u>middle sample concentration</u>

Turbiscan results for cell culture media

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For BSA conc. ≥0.2%
Decrease less than 6.10^{-3}mg/mL/hour

BSA mass fraction:
— 0.05%
— 0.1%
— 0.2%
— 0.5%

Turbiscan results for cell culture media

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For BSA conc. <0.2%
>3.10⁻²mg/mL/hour

BSA mass fraction:
- 0.05%
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NPs concentration w/v [mg/mL]

Time [hour]
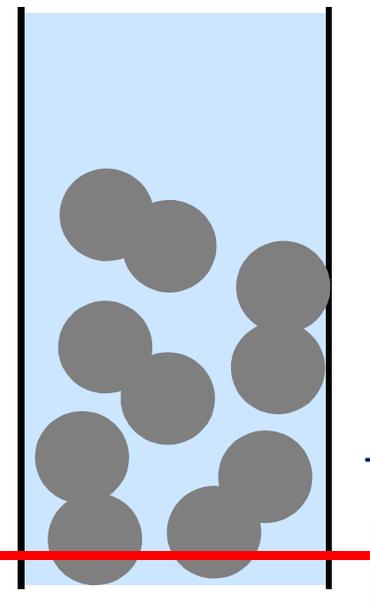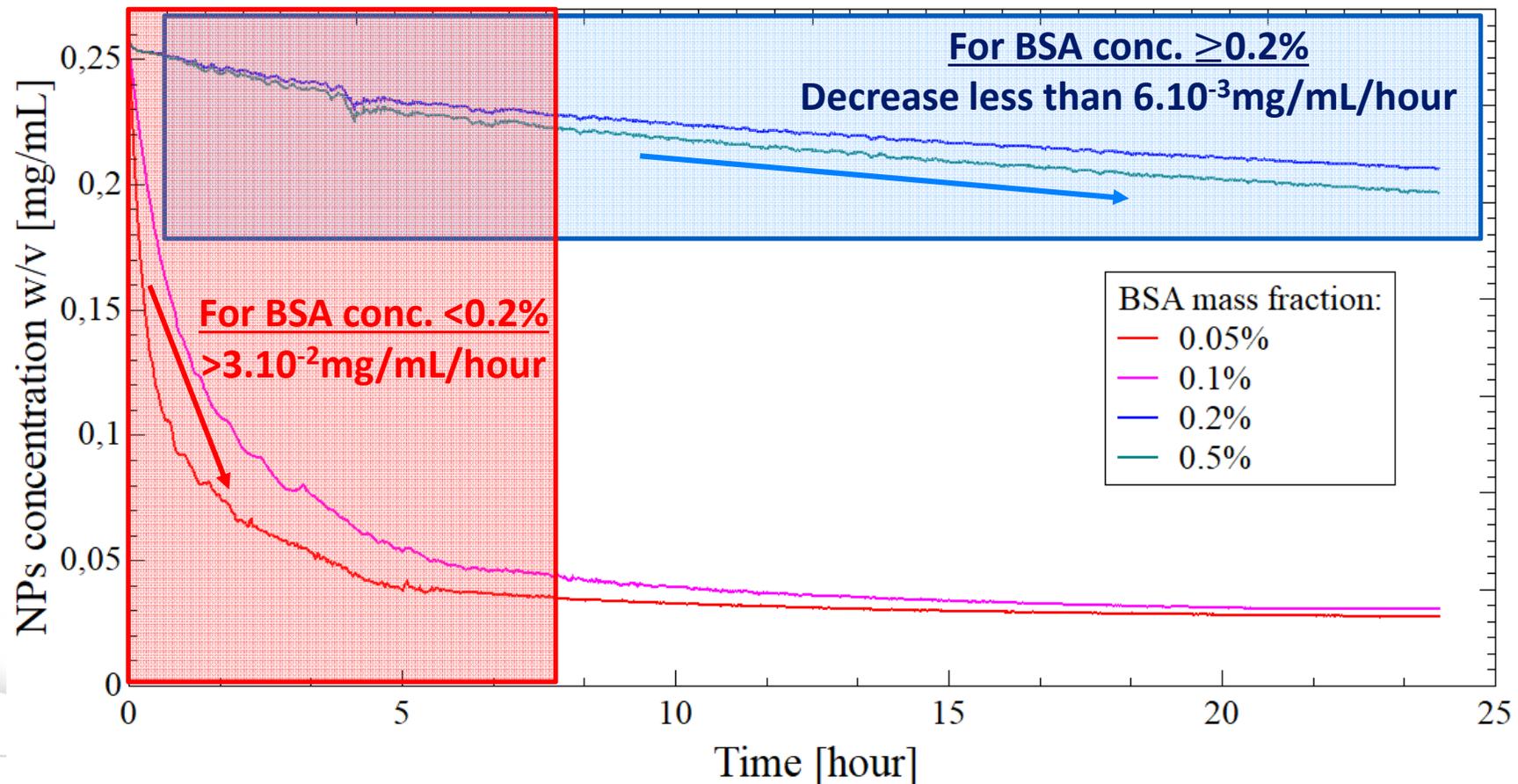
- Phosphate concentration influence on <u>agglomerate size</u>

    Fixed BSA concentration 0.2%
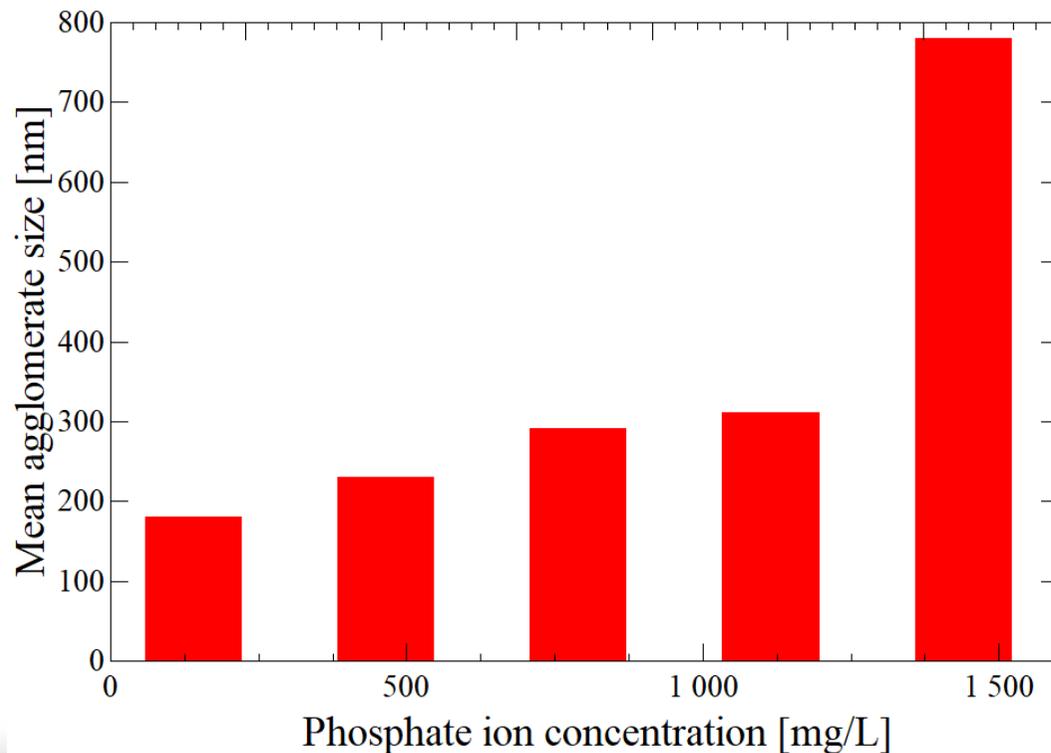
XU, Z et al,. Bovine Serum Albumin Adsorption on TiO2 Nanoparticle Surfaces: Effects of pH and Coadsorption of Phosphate on Protein–Surface Interactions and Protein Structure. *The Journal of Physical Chemistry C*, 2017, vol. 121, no 39, p. 21763-21771.

Conclusions and discussions

- Thanks to SMLS, we have **characterized both dispersibility and stability** of stock dispersions and dilutions

- **Best stability and dispersibility of NPS in DMEM** was obtained for **BSA conc. = 0.5%**

But depletion after 5min in dispersion stock!

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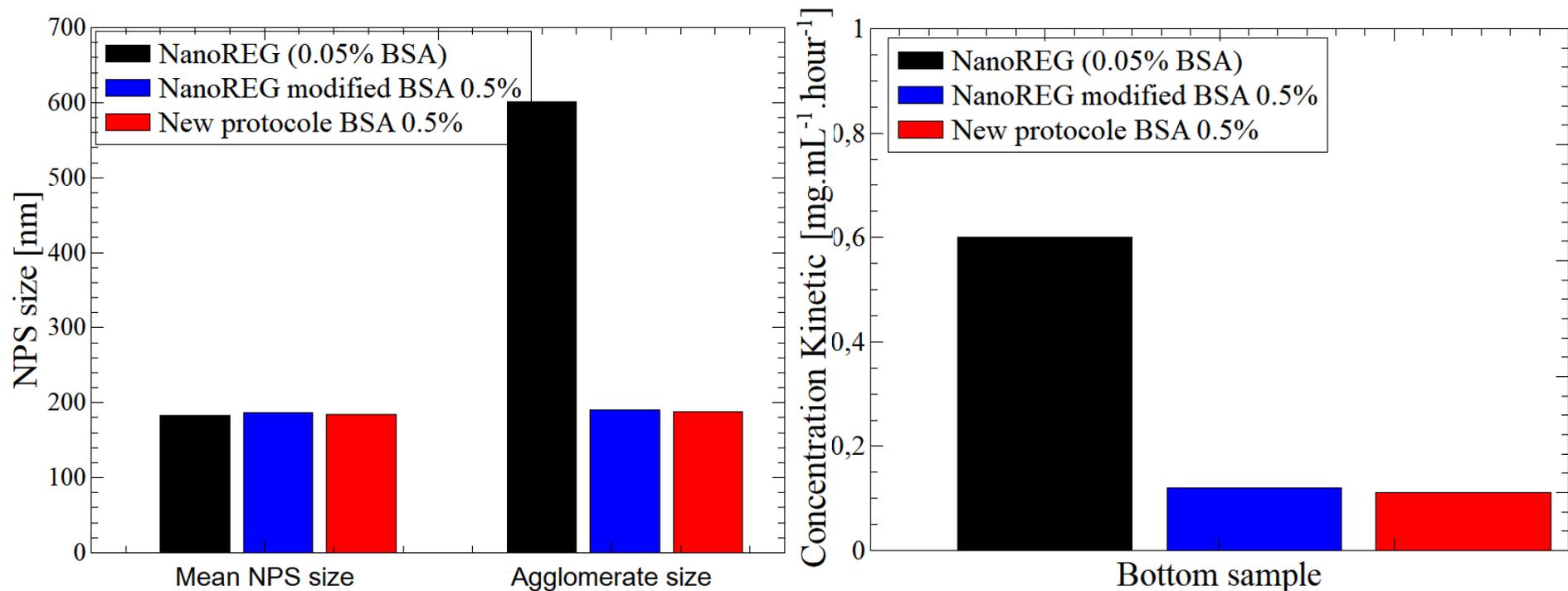
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=>What if some steps of the protocol are changed?

STABILITY & SIZE

Conclusions and discussions

- What if some steps of the protocol are changed?

- Better results than NanoREG protocol in term of dispersibility and stability in cell culture medium

STABILITY & SIZE

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Conclusions and discussions

- Thanks to SMLS, we have **characterized both dispersibility and stability** of stock dispersions and dilutions

- Behavior of NPs in stock dispersions and in cell culture media has been quantified thanks to:

  - Mean initial NPs size

  - Mean size of agglomerate

  - Sedimentation rate

  - Concentration kinetic (bottom and middle sample)