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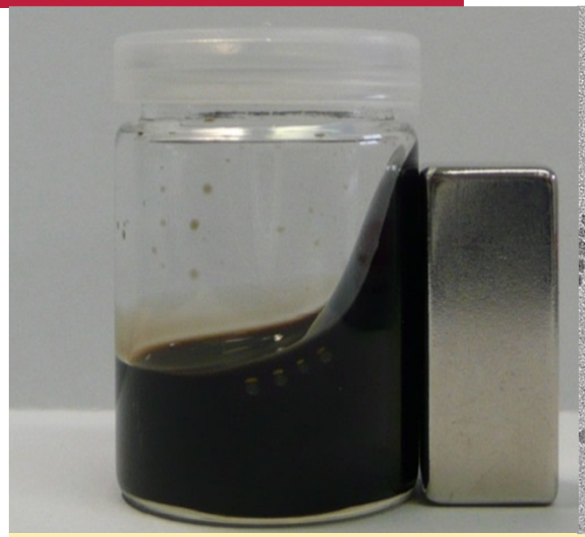
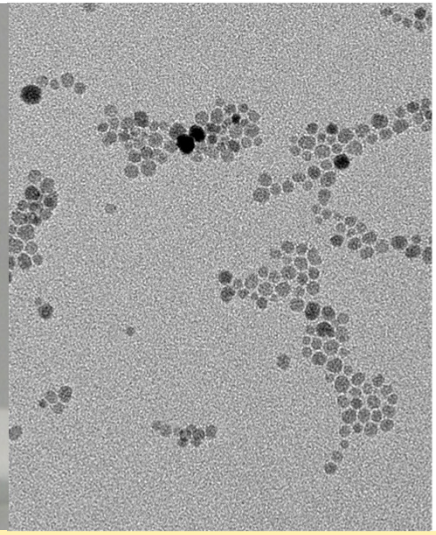
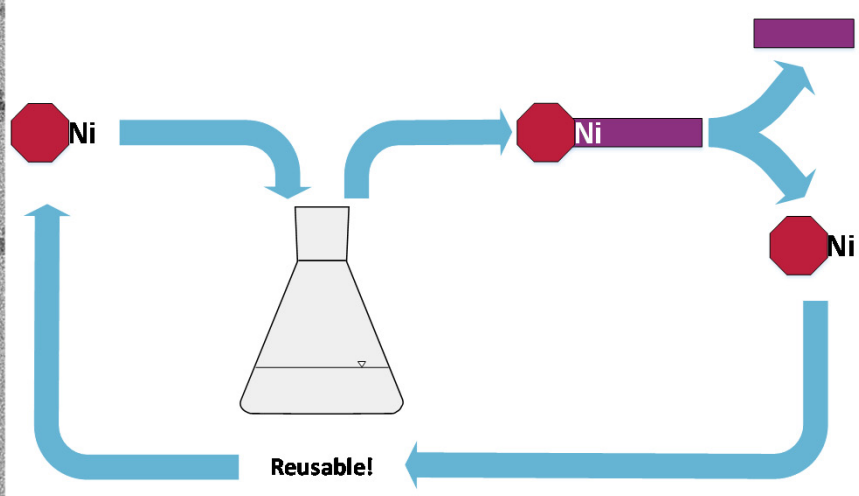






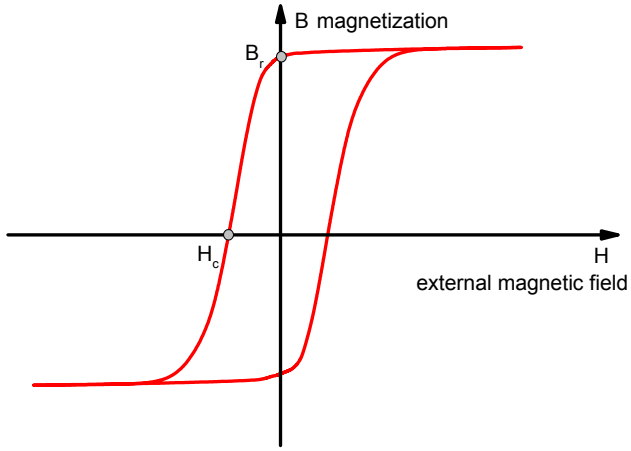
Application of Tailored Magnetic Nanoparticles for Protein Purification

L. Kleinfeldt, J. Gädke, I.-C. Masthoff, R. Biedendieck, R. Krull, G. Garnweitner

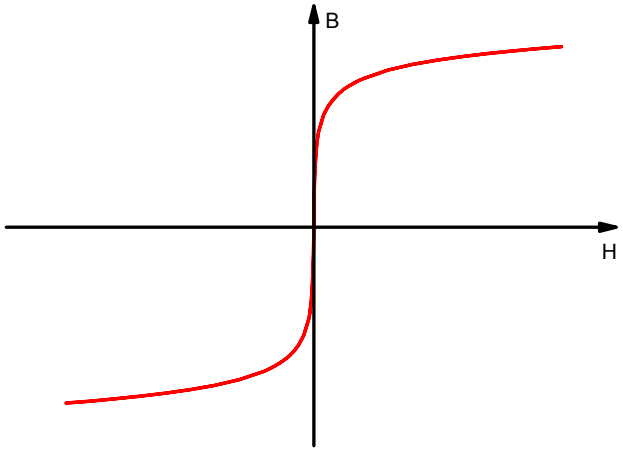
Contents

Introduction to magnetic nanoparticles

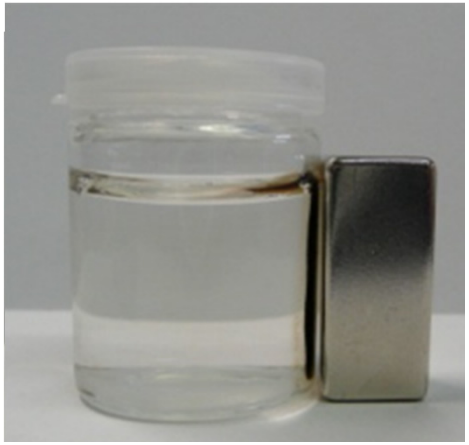
- Nanoparticles (<25 nm) of ferro-/ferrimagnetic materials show superparamagnetic behavior → no coercivity nor remanence
- This prevents an agglomeration by magnetic attractive forces
- Under an external field, high saturation magnetization is achieved
  → magnetic fluids (small, stable nanoparticles, high-η medium)
  → magnetic nanoparticle manipulation (larger nanoparticles, low-η medium)



ferro-/ferrimagnetic material

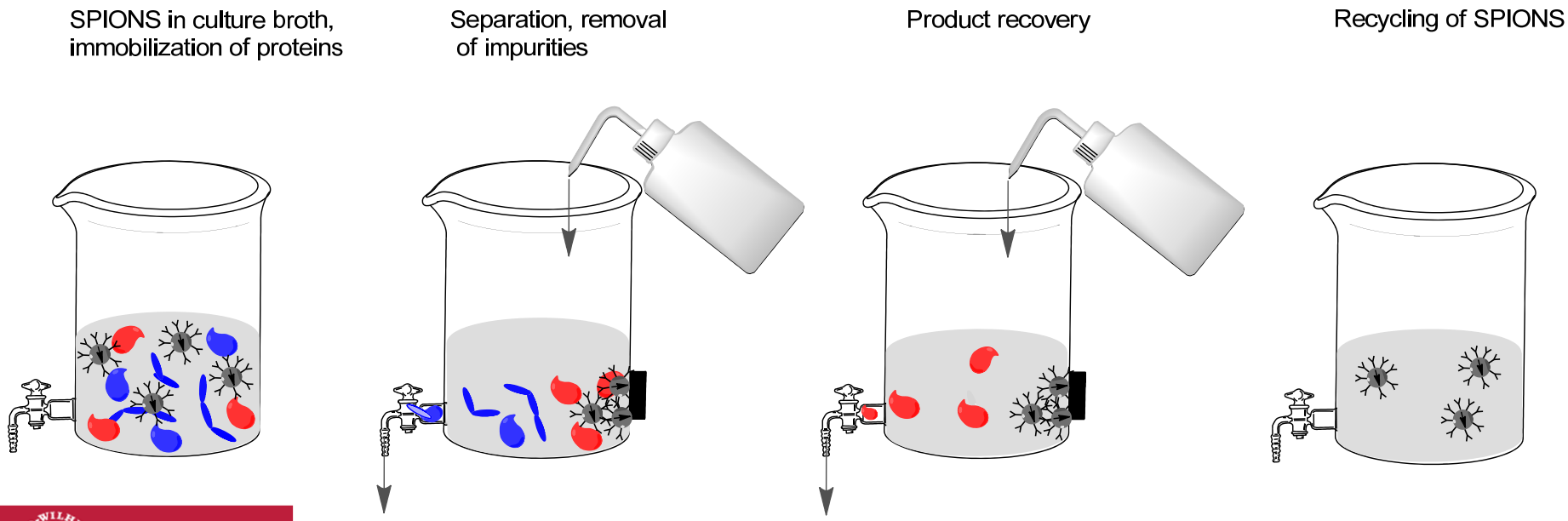


superparamagnetic material



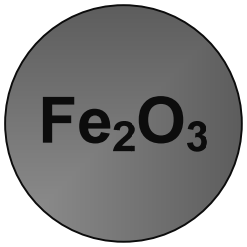
Aims and approach

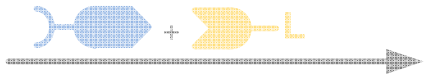
- Highly efficient, selective and low-cost magnetic separation system for the purification of 6-Histidine tagged recombinant proteins
- Selective separation of target protein in cell-free supernatant (*ex situ*) / during cultivation in a bioreactor *(in situ*)
- Tailor-made superparamagnetic iron oxide nanoparticles (SPIONs)
- Great simplification of downstream processing by reduction of processing steps
- Recyclable system for cost efficiency

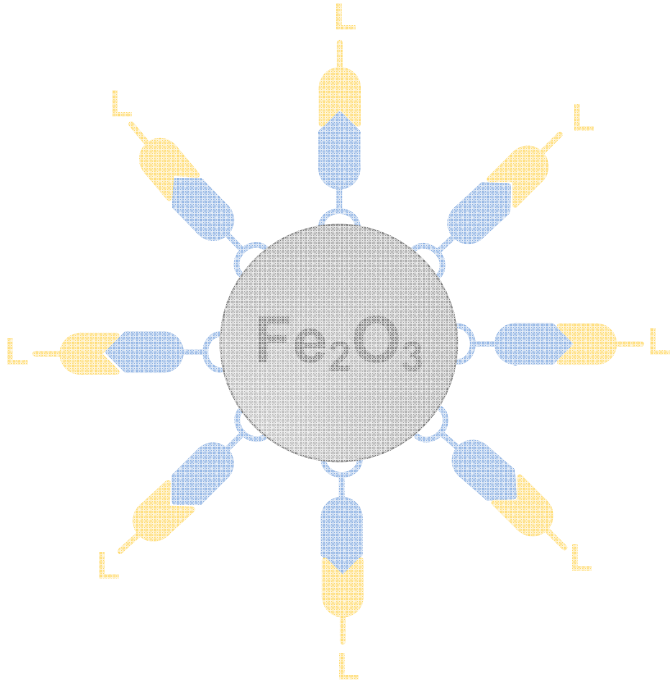


I.-C. Masthoff, J. Gädke, R. Krull, G. Garnweitner, *CITplus* 10 (2014)

Synthesis of SPIONs







1st Step

Synthesis of tailored core nanoparticles

2nd Step

Synthesis of tailored ligands for surface modification

3rd Step

Coupling of the ligand to the nanoparticle surface

Synthesis of SPIONs

iron oxide

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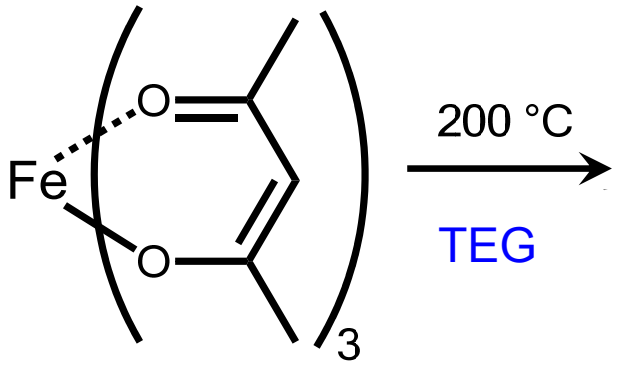
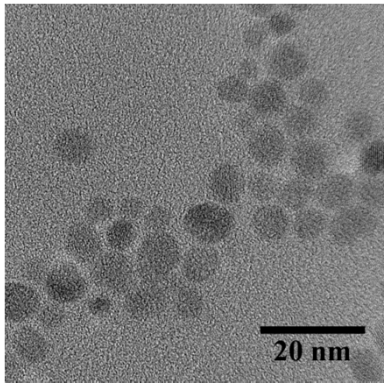


200 °C

TEG



Non-aqueous sol-gel synthesis in triethylene glycol (TEG)

- Formation of highly crystalline nanoparticles
- Synthesis in 1 L reactor established
- Spherical SPIONs of 8 nm with good homogeneity obtained
- Size and composition can be altered for tailoring of magnetic properties

I.-M. Grabs, C. Bradtmöller, D. Menzel, G. Garnweitner, *Cryst. Growth Des.* 12 (2012), 1469-1475.

Synthesis of SPIONs

iron oxide

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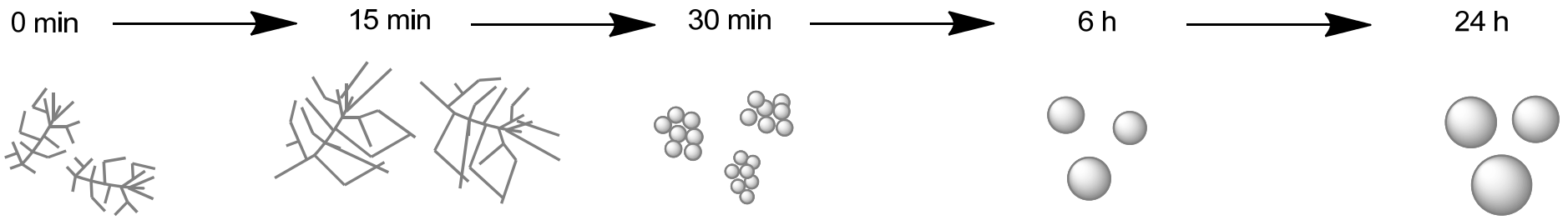
Analysis of particle formation and growth via kinetic studies in reactor system

- Formation via sol-gel-type process
- Organic content of particles diminishes over time
- Process conditions allow tuning of particle size to some extent
- Stable particle dispersions are obtained in TEG and H_2O



Loose network of partially reacted precursor molecules size ~ 5.6 nm

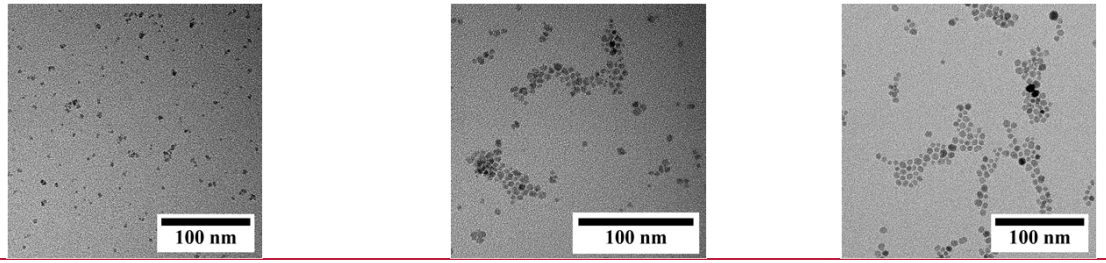
Growth of network size ~ 6.0 nm

Expulsion of organic compound from the network start of crystallization size ~ 4.9 nm

Crystalline particles size ~ 6.0 nm

Growth of particles size ~ 7.9 nm

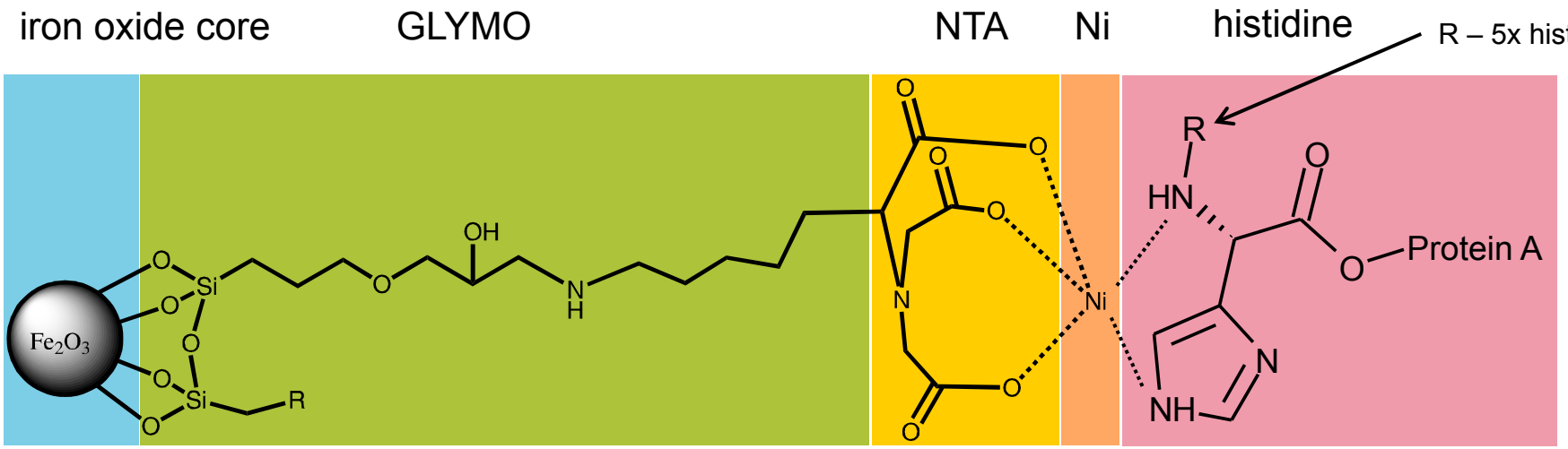
TEM Analyses after 2 h, 6 h, and 24 h



I.-C. Masthoff, M. Kraken, D. Menzel, F.J. Litterst, G. Garnweitner, *J. Sol-Gel Sci. Technol.* 77 (2016), 553-564.

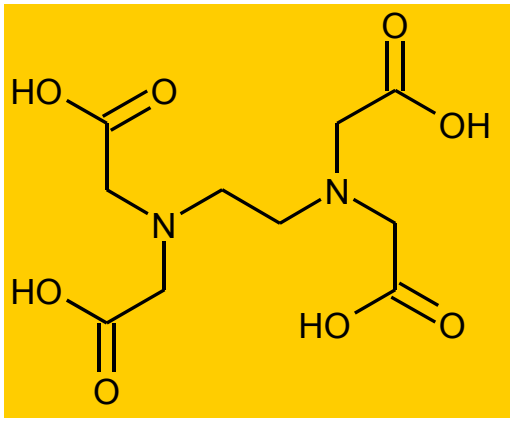
Functionalization of SPIONs

SPIONs - superparamagnetic iron oxide nanoparticles



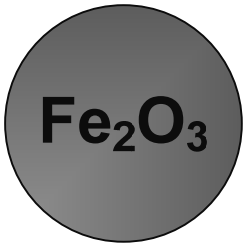
$k_{EDTA} > k_{NTA}\ (Ni^{2+})$

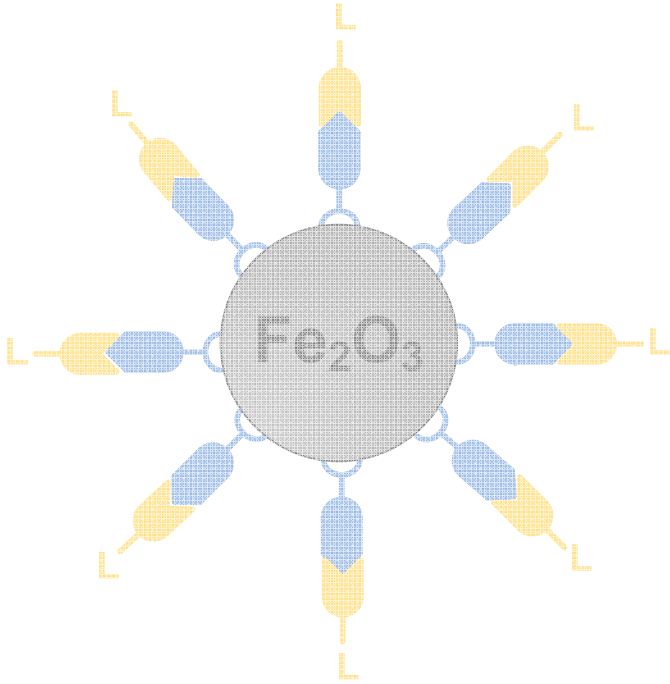
EDTA



GLYMO: 3-glycidyloxypropyltrimethoxysilane
NTA: nitrilotriacetic acid
EDTA: ethylenediaminetetraacetate

Functionalization of SPIONs





2nd Step

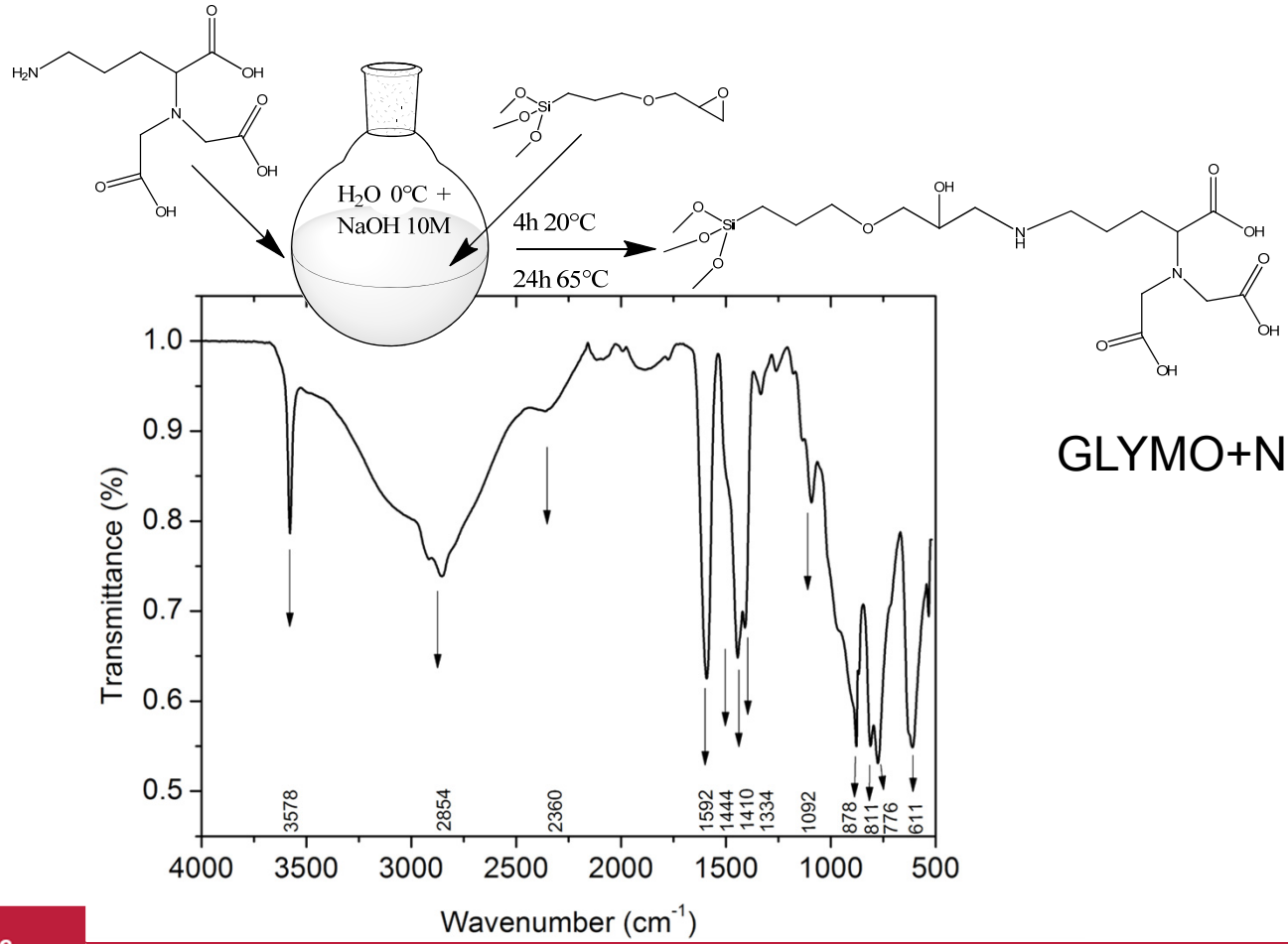
Synthesis of tailored ligands for surface modification

3rd Step

Coupling of the ligand to the nanoparticle surface

Functionalization of SPIONs

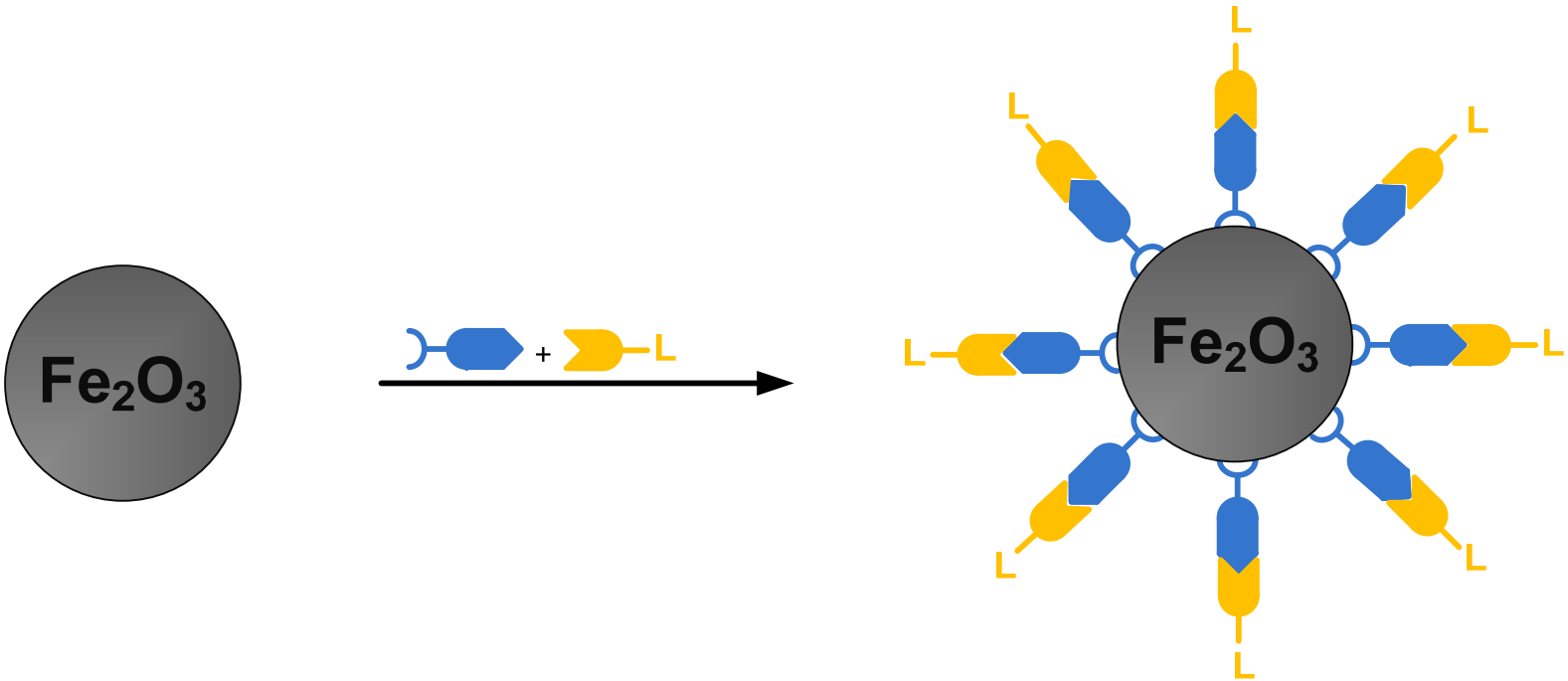
Coupling of (3-glycidyloxypropyl)trimethoxysilane (GLYMO) with N,N-bis(carboxymethyl)-L-lysine (NTA)



GLYMO+NTA = GNTA

I.-C. Masthoff, F. David, C. Wittmann, G. Garnweitner, *J. Nanopart. Res.* 16 (2013): 2164

Functionalization of SPIONs

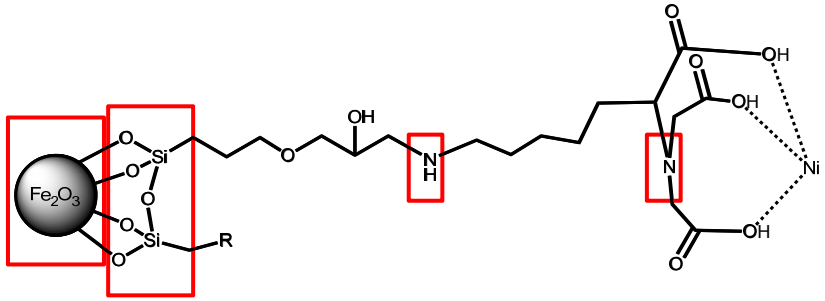


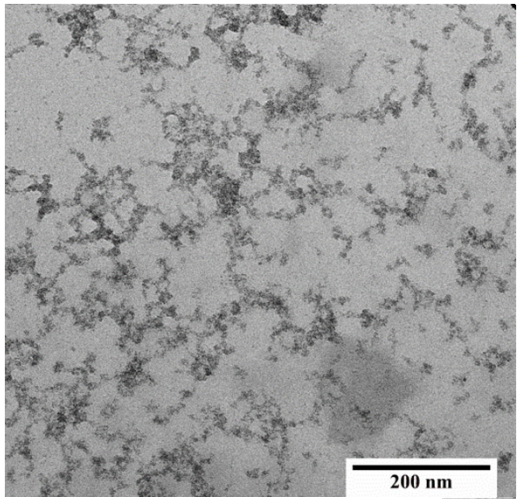
3rd Step

Coupling of the functional group to the surface of the particles

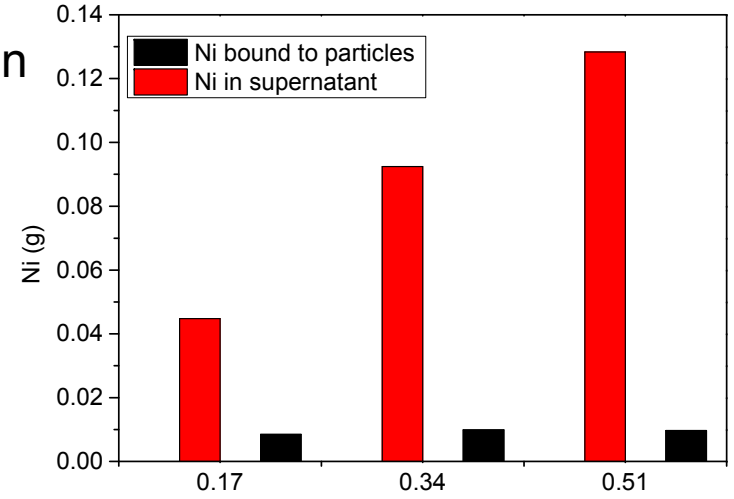
Functionalization of SPIONs

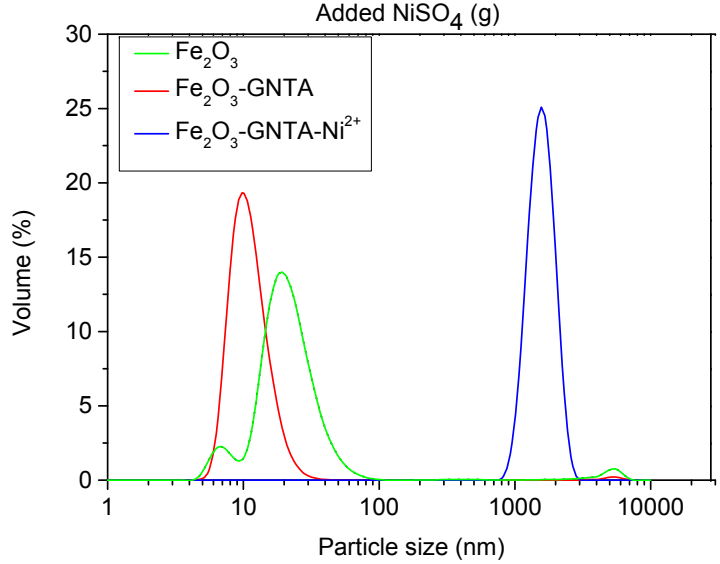
Characterization of particle functionalization





Particle with GNTA+Ni^{2+}





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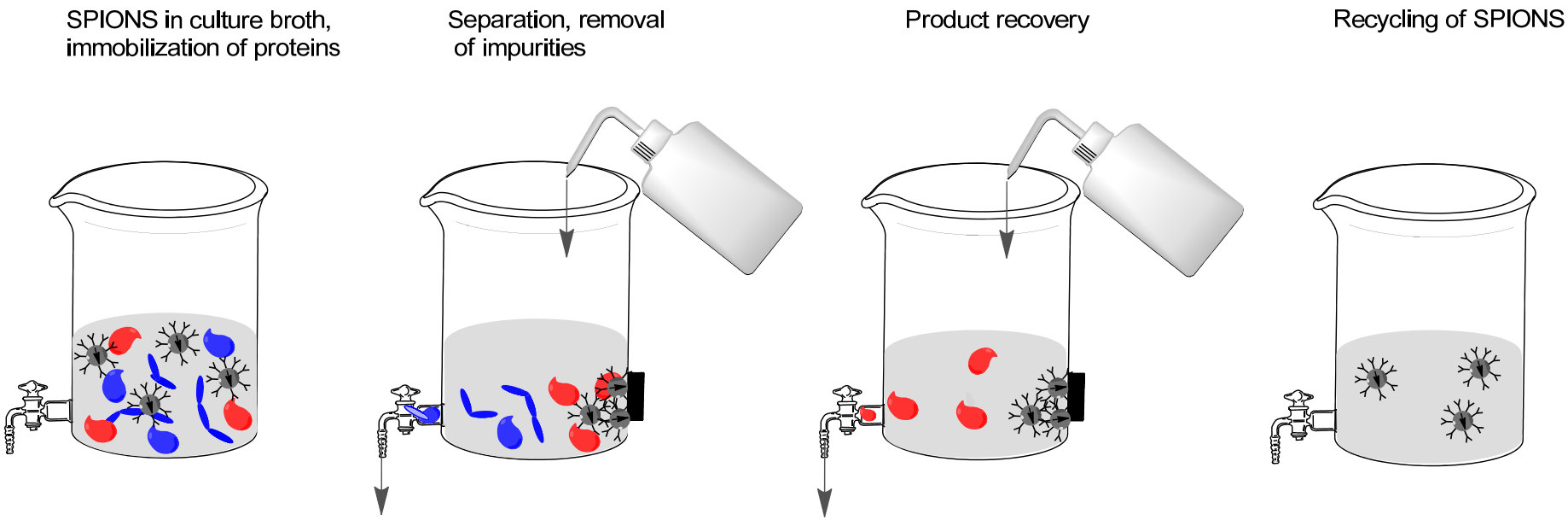
Purification and regeneration

- Model system: Recombinant Protein A from *Bacillus megaterium* cultures
- Selective separation of target protein in cell-free supernatant (*ex situ*) / **during cultivation in a bioreactor *(in situ*)**
- No adverse effects on bacterial productivity observed
- Use of SPIONs in multiple cycles



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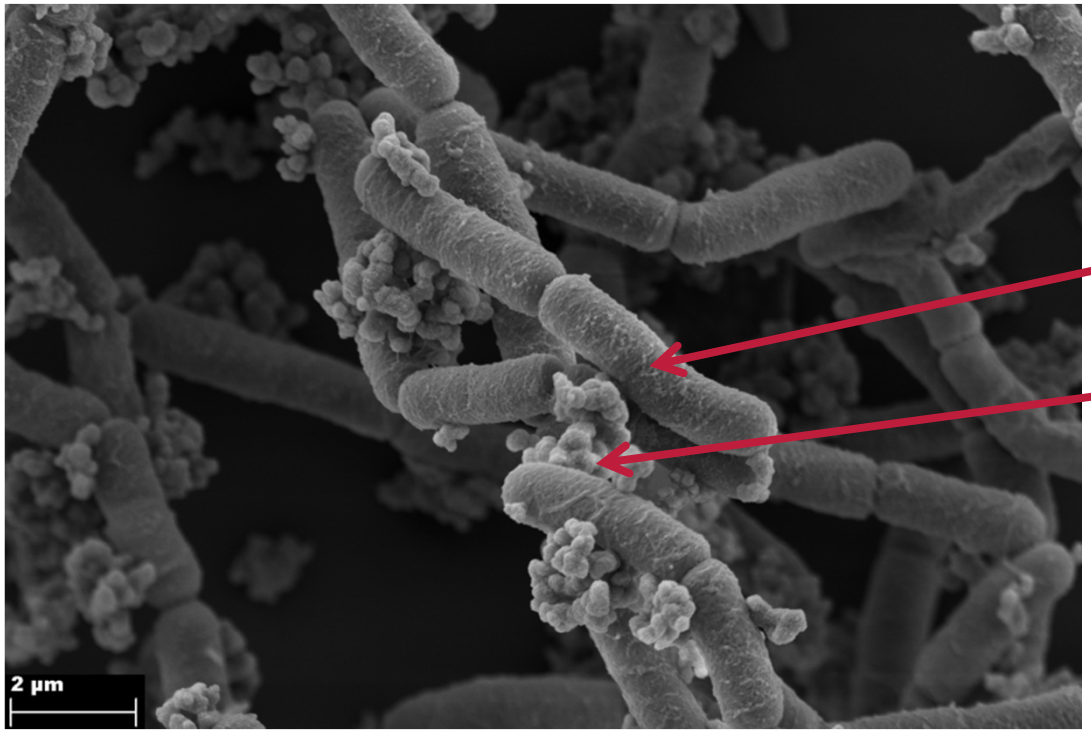
Purification and regeneration

Scanning electron microscopy comparison of SPIONs and bacteria



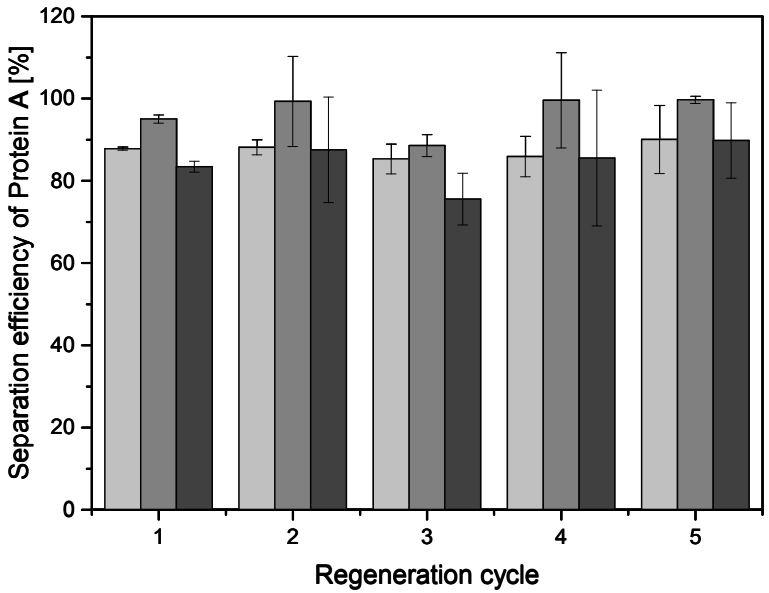
Bacillus megaterium (2 x 5 µm)

SPIONs in structurally defined agglomerates (1 µm)

2 mg SPIONs / mL culture in complex medium,
M. Rohde, HZI

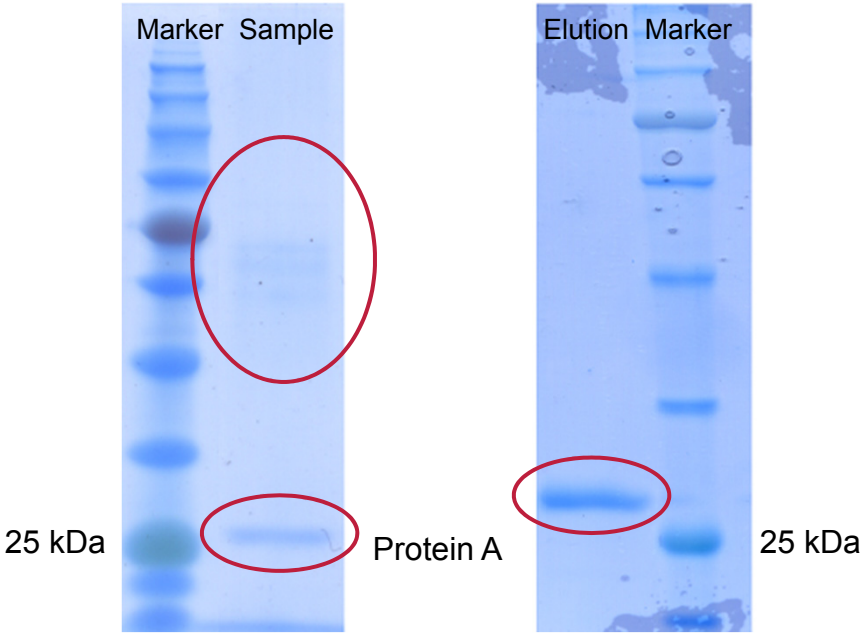
Purification and regeneration

In situ binding and separation efficiency



Constant SPION concentration of 500 µg/mL culture
100 % = 60 mg Protein A per liter (120 mg/g SPIONs)

Product bound from media

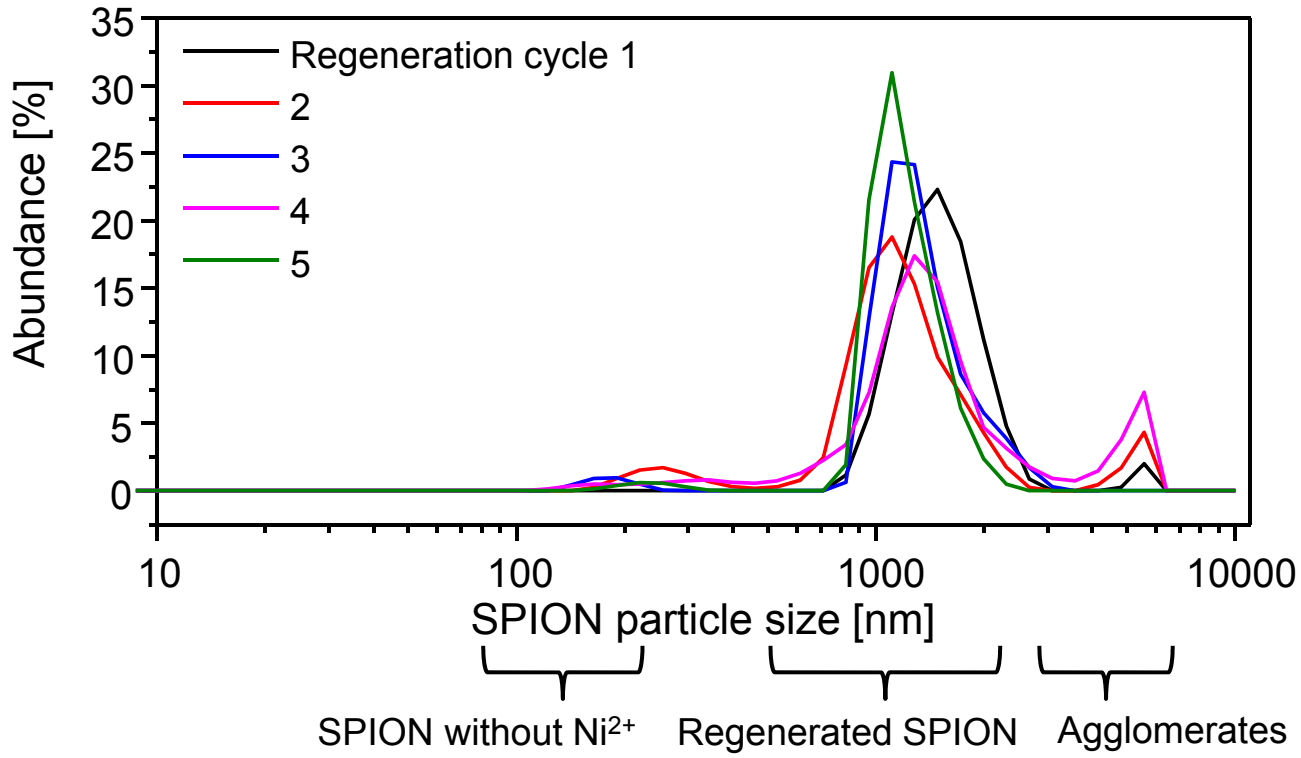


- Purity was determined using SDS-PAGE with a sensitivity of 5 ng per sample
- No protein impurity in the elution fraction

➢ Final purity of ~ 99.9 % regarding protein compounds.

Purification and regeneration

Dynamic light scattering (DLS) measurements of particles after recycling



- The size of the regenerated particles does not differ significantly between the cycles.
- Particles are present as defined agglomerates of ~ 1000 nm in diameter.

Purification and regeneration – challenges

- High separation efficiency can only be ensured when constant particle concentration is realized
- Loss of nanoparticles in each production and regeneration cycle
- Insufficient magnetic separation / incomplete precipitation due to varying colloidal stability

Summary

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SPIONs with tailored properties

- Non-aqueous synthesis as versatile and scalable strategy
- Process knowledge allows adjustment of properties
- Post-structuring into hierarchical aggregates

Functionalization strategy

- Use of designed linker allows covalent coupling
- Optimization of surface concentration
- Robust protocol for linker synthesis and coupling

Purification of recombinant proteins

- Metal complex-based protein binding strategy
- Purification and regeneration cycle established
- In situ purification successfully accomplished with high efficiency and purity

Acknowledgments

I.-C. Masthoff, L. Kleinfeldt, S. Zellmer, C. Schilde – iPAT

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P. Pfeiffer, J. Rösler – Institute for Materials Science, TU Braunschweig (SEM)

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Niedersächsisches Ministerium für Wissenschaft und Kultur

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