WORKSHOP

London, 18th January 2017

WP 2

Magnetic Nanoparticles for DARPin Conjugation

Cordula Grüttner

micromod Partikeltechnologie GmbH

Development and Production of Nano- and Micropartices since 1994

Production of IVD (*in vitro*-Diagnostic)-Components (GMP)

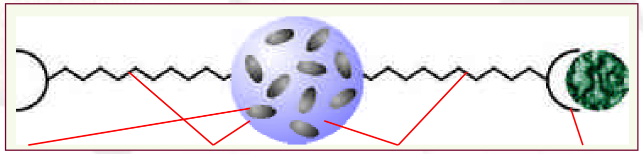
Collaboration with international distributors

70 % Turnover by orders from USA and Japan

Certified according to EN ISO 13485:2012/AC:2012

www.micromod.de

Modular Design of Nano- and Microparticles



physical	physico-chemical	chemical	biochemical function
magnetic properties, density, porosity	fluorescence, dyeing, radiolabelling	functional groups and sequences, spacers, grafted chains	target molecules, antibodies, conjugates, chelators
Separation Detection / Sensoring Heating	**Detection Treatment**	**Selective bond formation or other interaction Self organization**	

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Targeting Applications with MNPs

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MR Imaging

Particle type	Antibody	Target area	Reference
nanomag®-CLD-spio	J591	prostate cancer	Abdolahi, M. et al. Contr. Media & Mol. Imag. 2013, **8,** 2175-184
nanomag®-CLD-spio	C595	ovarian cancer	Shahbazi-Gahrouei, D. et al. J. Med. Phys. 2013, **38**(4), 198-204
nanomag®-CLD-spio	C595	breast cancer	Shanehsazzadeh,S. et al. Contr. Media & Mol. Imag. 2014, **10,** 225-236
BNF-Starch	anti-GD2 hu14.18K322A	human neuroblastoma	Baiu, D.C. et al. Nanomedicine, 2015, **10**(19), 2973-88

Hyperthermia

Particle type	Antibody	Target area	Reference
BNF-Dextran	Herceptin	breast cancer	Zhang, J. et al. Int. J. Hyperthermia, 2011, **27**(7), 682-697
nanomag®-CLD-spio	Herceptin	breast cancer	Ndong, C. et al. PLoS ONE, 2015, **10**(2), e0115636.
BNF-Starch	anti-Muc-1di-scFv-cysteine	breast cancer	Natarajan, A. et al. Cancer Biother. Radiopharm., 2008, **23**(1), 82-91.

Dartrix — Heating rates of perimag®

Determination of heating properties of several lots of plain perimag®
by Paul Southern, RCL

Lot	SAR [W/g Fe]	ILP [nHm^2/kg]
0181278	68,8	6,4
0151378	57,0	5,3
0231378	59,1	5,5
0051478	62,7	5,8
0171478	65,3	6,1

Field: 3300 A/m
Frequency: 989 kHz

→ High and reproducible heating parameters in the RCL system as basis for the choice of this particle type for DARPin conjugation

Dartrix — Zeta potential - pH dependence of functionalized perimag®

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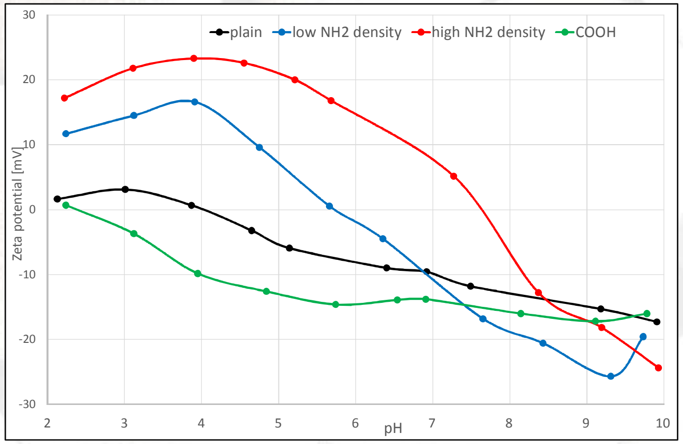

Dartrix — Functionalized perimag® for DARPin Conjugation

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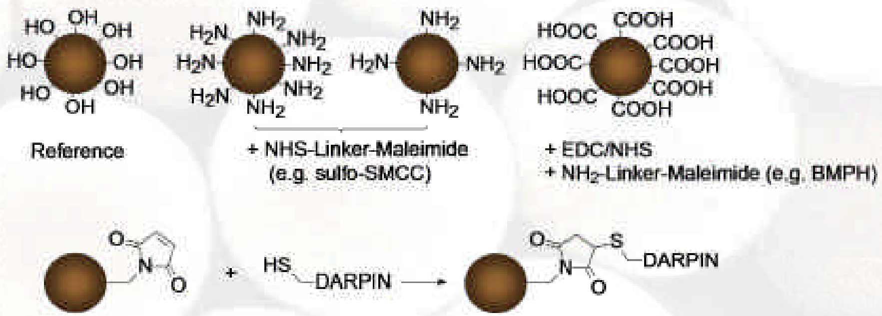

Particle Surface	Z-Average [nm]	Polydispersity Index
Plain (OH)	117	0.249
NH_2 (high density)	130	0.229
NH_2 (low density)	118	0.262
COOH	104	0.155

Dartrix — DARPin Conjugation to Maleimide Functionalized perimag®

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New DARPins from UZH:

Construct	pI	Negative charges	Positive charges
GCG-PAS300-E69	5.18	34	14
GCG-PAS300-Off7	5.26	27	11
GCG-PAS900-E69	5.18	34	14
GCG-PAS900-Off7	5.26	27	11
GCG-XTEN288-E69	4.31	82	14
GCG-XTEN288-Off7	4.25	75	11
GCG-XTEN864-E69	3.88	178	14
GCG-XTEN864-Off7	3.8	171	11

Reference: Cysteine

Dartrix — Characterization of new DARTRIX particles

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Surface	c (Fe) [mg/ml]	Z_{AVE} [nm] (PCS)	PDI	ZP [mV]	c (DARPin) [µg/mg Fe)
c-PAS300-E69	4,6	142	0.24	-13,4	18
c-PAS300-Off7	4,7	144	0.21	-13,5	16
c-PAS900-E69	4,4	139	0,22	-11,9	18
c-PAS900-Off7	5,8	142	0,21	-12,8	16
c-XTEN288-E69	4,8	140	0,22	-20,4	21
c-XTEN288-Off7	5,1	146	0,22	-20,4	21
c-XTEN864-E69	5,6	146	0,23	-20,0	17
c-XTEN864-Off7	5,5	143	0,20	-22,6	19
Cysteine (reference)	4,8	137	0,21	-15,0	12
NH_2 (reference)	8,2	142	0,24	+0,02	0

- Similar size of of all DARPin conjugates (143 +/- 4 nm)
- More negative ZP of all XTEN derivatives compared to PAS derivatives and reference

Dartrix — Comparison of zeta potential – pH functions of perimag® with PAS and XTEN DARPins

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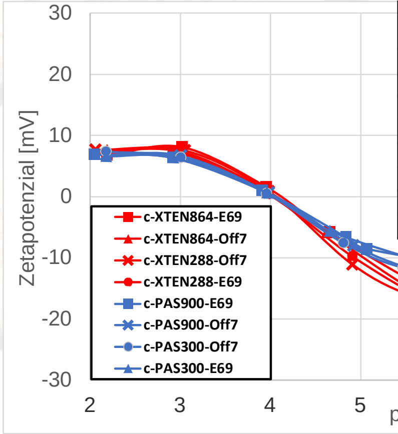

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GCG-XTEN864-E69	3.88	178	14
GCG-XTEN864-Off7	3.8	171	11

More negative ZP of all XTEN derivatives compared to PAS derivatives in the neutral and basic pH range

Dartrix — Zeta potential – pH Functions of perimag® with PAS DARPins

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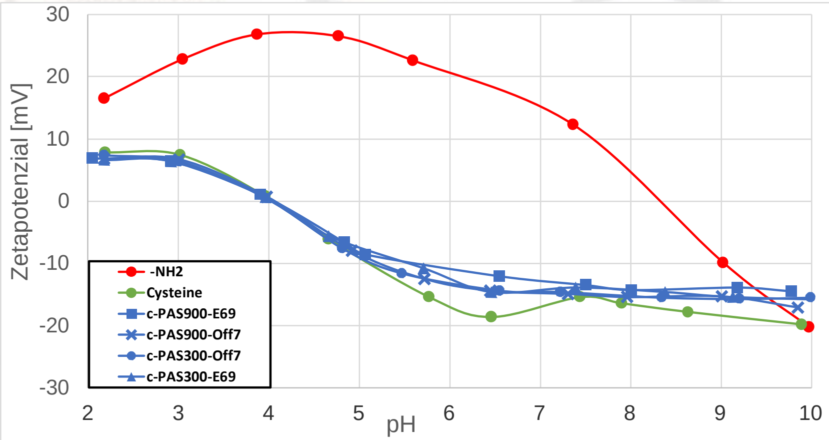

Isoelectric point of all PAS conjugates: 4.0

Dartrix — Summary

1. High and reproducible heating rates of perimag® particles
2. Successful conjugation of PAS- and XTEN DARPins
3. Selective Binding of DARTRIX particles to target cells at UCL
4. Good correlation of zeta potential data of DARTRIX particles with charges of DARPins
5. Particle production under controlled hygienic conditions