Supporting information

for

Optimizing the Cu-RDRP of *N*-(2-hydroxypropyl) methacrylamide toward biomedical applications

Vladimír Raus*and Libor Kostka

Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Heyrovsky Sq. 2, 162 06 Prague 6, Czech Republic

E-mail: raus@imc.cas.cz

EXPERIMENTAL

Materials

Methyl 2-chloropropionate (MCP; Aldrich, ≥ 99%), 2-chloropropionitrile (CPN; Aldrich, 95%), 2-bromopropionitrile (BPN; Aldrich, 97%), α-chlorophenylacetate (ECPA; Aldrich, 97%), CuCl (Aldrich, 99%), $CuCl_2$ (Aldrich, 99%), CuBr (Fluka, ≥ 98%), $CuBr_2$ (Fluka, ≥ 99%), NaCl (p.a., Lach-Ner, Czech Republic), Q-water (Millipore), dimethyl sulfoxide (DMSO; Aldrich, absolute, over molecular sieve), and Cuprisorb (Seachem) were used as received. 1,1,4,7,7-Pentamethyldiethylenetriamine (PMDETA; Aldrich, 99%) was vacuum distilled before use and kept under argon at 4°C. Tris[2-(dimethylamino)ethyl]amine (Me_6TREN) was synthesized according to the literature[1] and kept under argon at 4°C. Tris(2-pyridylmethyl)amine (TPMA) was synthesized as a yellow-brownish solid according to a literature protocol[1] and purified by repeated recrystallizations from diethyl ether and hexane to obtain a white crystalline product that was stored at 4°C. *N*-(2-Hydroxypropyl)methacrylamide (HPMA) was synthetized by a modification of the protocol involving the reaction of methacryloyl chloride with 1-aminopropan-2-ol in dichlormethane in the presence of sodium carbonate, as described in literature.[2] The solvents were bubbled with argon for at least 1 hour and stored under argon.

Cu(0)-RDRP of HPMA

In a typical experiment, CuCl (0.0138 g, 0.1397 mmol) and $CuCl_2$ (0.0094 g, 0.06985 mmol) were placed into a reaction flask equipped with a magnetic stirring bar and a three-way stopcock connected to an argon/vacuum inlet. After thorough de-oxygenation by several vacuum-argon cycles, degassed water (1 ml) was added. Subsequently, PMDETA (44 µl, 0.2096 mmol) was added, and the dark blue mixture was stirred for 30 min. Afterwards, solution of HPMA (2 g, 13.97 mmol) was added, the flask was cooled in an ice bath, and the polymerization was started by adding CPN (12 µl, 0.1397 mmol). After 1 h, the flask was removed from the ice bath, and the mixture was further stirred at r.t. After additional 9 h, the experiment was ended, the flask was opened to air, and the mixture was diluted with 10 ml of water. An aliquot of the diluted mixture was purified for SEC MALLS analysis by stirring for ca 2 hours with a small amount of the Cuprisorb resin until the discoloration disappeared. Both fractions were then precipitated in a 20-fold excess of acetone/diethylether 2:1 (v/v), the solids were collected by filtration, washed with acetone/diethylether 2:1 (v/v), and dried at 40°C in vacuum. Conversion was determined gravimetrically.

Copolymerization of HPMA and conjugation of Doxorubicin

Copolymerization of HPMA with the co-monomer (Figure S2) was performed using the standard polymerization procedure. Before further use, the copolymer was purified by dialysis (SpectraPor membrane, MWCO = 1000), yielding, upon freeze drying, a white powdered product with no observable discoloration from traces of copper salts.

Determination of the co-monomer content in the copolymer was based on the determination of deprotected hydrazide group content by a modified TNBSA assay, as described previously.[3, 4] The subsequent conjugation of Doxorubicin was carried out according to the literature.[4]

Characterization

The number-average molecular weights (M_n), weight-average molecular weights (M_w), and dispersities (Đ) of the polymers were determined using size-exclusion chromatography (SEC) on an HPLC Shimadzu system equipped with a SPD-M20A photodiode array detector (Shimadzu, Japan), an Optilab®rEX differential refractometer, and a multi-angle light scattering DAWN® HELEOS II detector (Wyatt Technology, USA), using 0.3 M sodium acetate buffer at pH 6.5 (20 %) and methanol (80 %, v/v) as the mobile phase with the flow rate of 0.6 $ml.min^{-1}$. The combination of TSKgel® AW3000+AW4000 column was used. The ASTRA software and the refractive index increment dn/dc = 0.167 mL/g were used for the calculation of molecular weights. ^1H NMR spectra (for conversion determination in the kinetic experiment) were measured in DMSO-d6 using a Bruker DPX 300 spectrometer at 300.1 MHz.

The content of Doxorubicin in the copolymer was determined spectrophotometrically at 488 nm.[4]

Table S1. Optimization of polymerization conditions: sub-stoichiometric concentrations of the catalytic system (standard conditions: HPMA/I = 100:1, monomer/H_2O = 2/3 (g/ml), 30 min pre-disproportionation, r.t.)

No.	Initiation and catalytic system	Stoichiometry	Time (h)	Conv. (%)	M_n (theor.)	M_n (GPC)	Đ
1[a]	CPN/CuCl/PMDETA	1/0.2/0.3	20	90	12 900	69 800	1.88
2[b]	CPN/CuCl/PMDETA	1/0.15/0.225	10	71	10 200	93 700	2.22
3[a]	CPN/CuCl/TPMA	1/0.2/0.3	24	10	1 400	3 800	1.55
4	CPN/CuCl/HMTETA	1/0.15/0.225	10	85	12 200	79 400	1.66
5[b]	MCP/CuCl/PMDETA	1/0.15/0.225	10	78	11 200	81 500	1.61
6	MCP/CuCl/HMTETA	1/0.1/0.1	3	60	8 600	205 300	1.58
7	MCP/CuCl/$CuCl_2$/PMDETA	1/0.5/0.25/0.75	10	69	9 900	26 100	1.43

[a] monomer/solvent = 1/2 (g/ml)

[b] monomer/solvent = 3/4 (g/ml)

Table S2. Optimization of polymerization conditions: stoichiometric concentrations of the catalytic system (standard conditions: HPMA/I = 100:1, HPMA/H_20 = 2/3 (g/ml), 30 min pre-disproportionation, r.t.)

No.	Initiation and catalytic system	Stoichiometry	Time (h)	Conv. (%)	M_n (theor.)	M_n (GPC)	Đ
1	CPN/CuCl/PMDETA	1/1/1	10	76	10 900	15 000	1.37
2	CPN/CuCl/Me_6TREN	1/1/1	10	59	8 400	15 400	3.39
3	CPN/CuCl/HMTETA	1/1/1	10	41	5 900	16 200	1.67
4	CPN/CuCl/TPMA	1/1/1	10	40	5 700	11 600	2.27
5	MCP/CuCl/PMDETA	1/1/1	10	83	11 900	21 400	1.45
6	MCP/CuCl/Me_6TREN	1/1/1	10	65	9 300	13 700	3.03
7	MCP/CuCl/HMTETA	1/1/1	2	96	13 700	41 300	1.59
8	MCP/CuCl/TPMA	1/1/1	10	51	7 400	13 300	1.90
9	CPN/CuCl/$CuCl_2$/PMDETA	1/1/0.5/1.5	10	74	10 600	22 100	1.35
10	MCP/CuCl/$CuCl_2$/PMDETA	1/1/0.5/1.5	10	90	12 900	18 900	1.41

Table S3. Optimization of polymerization conditions: supplementary experiments (standard conditions: HPMA/I = 100:1, monomer/H_2O = 2/3 (g/ml), 30 min pre-disproportionation)

No.	Initiation and catalytic system	Stoichiometry	T (°C)	Time (h)	Conv. (%)	M_n (theor.)	M_n (GPC)	Đ
1	CPN/CuCl/PMDETA	1/1/1	0, rt	10	74	10 600	19 600	1.34
2	CPN/CuCl/$CuCl_2$/PMDETA	1/1/0.5/1.5	0, rt	24	85	12 200	18 100	1.24
3	CPN/CuCl/$CuCl_2$/PMDETA	1/1/0.5/1.5	0, 60	6	72	10 300	19 000	1.19
4[a]	CPN/CuCl/$CuCl_2$/PMDETA	1/1/0.5/1.5	0	10	79	11 300	21 000	1.83
5	CPN/CuCl/$CuCl_2$/PMDETA	1/1/0.5/3	0, rt	10	86	12 300	22 700	1.30
6	BPN/CuCl/$CuCl_2$/PMDETA	1/1/0.5/1.5	0, rt	10	49	7 000	14 600	2.71
7	CPN/CuBr/$CuBr_2$/PMDETA	1/1/0.5/1.5	0, rt	10	44	6300	15 000	1.85
8	CPN/CuCl/$CuCl_2$/PMDETA	1/0.9/0.6/1.5	0, rt	10	90	12 900	20 800	1.22
9	CPN/CuCl/$CuCl_2$/PMDETA	1/0.8/0.7/1.5	0, rt	10	80	11 500	15 700	1.27
10[b]	CPN/CuCl/$CuCl_2$/PMDETA	1/1/0.5/1.5	0, rt	24	61	8 700	17 300	1.19
11[c]	CPN/CuCl/$CuCl_2$/PMDETA	1/1/0.5/1.5	0, rt	25	0	n.d.	n.d	n.d
12	MCP/CuCl/$CuCl_2$/ Me_6TREN	1/1/0.5/1.5	rt	10	85	12 200	22 900	2.77
13	MCP/CuCl/$CuCl_2$/HMTETA	1/1/0.5/1.5	rt	8	92	13 200	30 500	1.40
14	MCP/CuCl/$CuCl_2$/PMDETA	1/1/0.5/1.5	0, rt	2	92	13 200	35 300	1.40
15	MCP/CuCl/$CuCl_2$/PMDETA	1/0.8/0.7/1.5	0, rt	10	96	13 700	30 800	1.41
16	MCP/CuCl/$CuCl_2$/PMDETA	1/2/1/3	0, rt	11	89	12 300	23 300	1.44
17	MCP/CuCl/$CuCl_2$/PMDETA	1/2/1/3	rt	10	79	11 300	16 900	1.76
18	MCP/CuCl/$CuCl_2$/PMDETA	1/1.6/1.4/3	rt	10	83	11 900	16 800	1.67
19[d]	CPN/CuCl/$CuCl_2$/PMDETA	1/1/0.5/1.5	0, rt	10	88	12 600	17 500	1.23
20[d]	CPN/CuCl/$CuCl_2$/PMDETA	1/2/1/3	0, rt	10	85	12 200	38 800	1.18

[a] cooled to 0°C for the whole polymerization duration

[b] conducted in 0.67 M NaCl

[c] conducted in DMSO

[d] conducted in 25% (v/v) DMSO

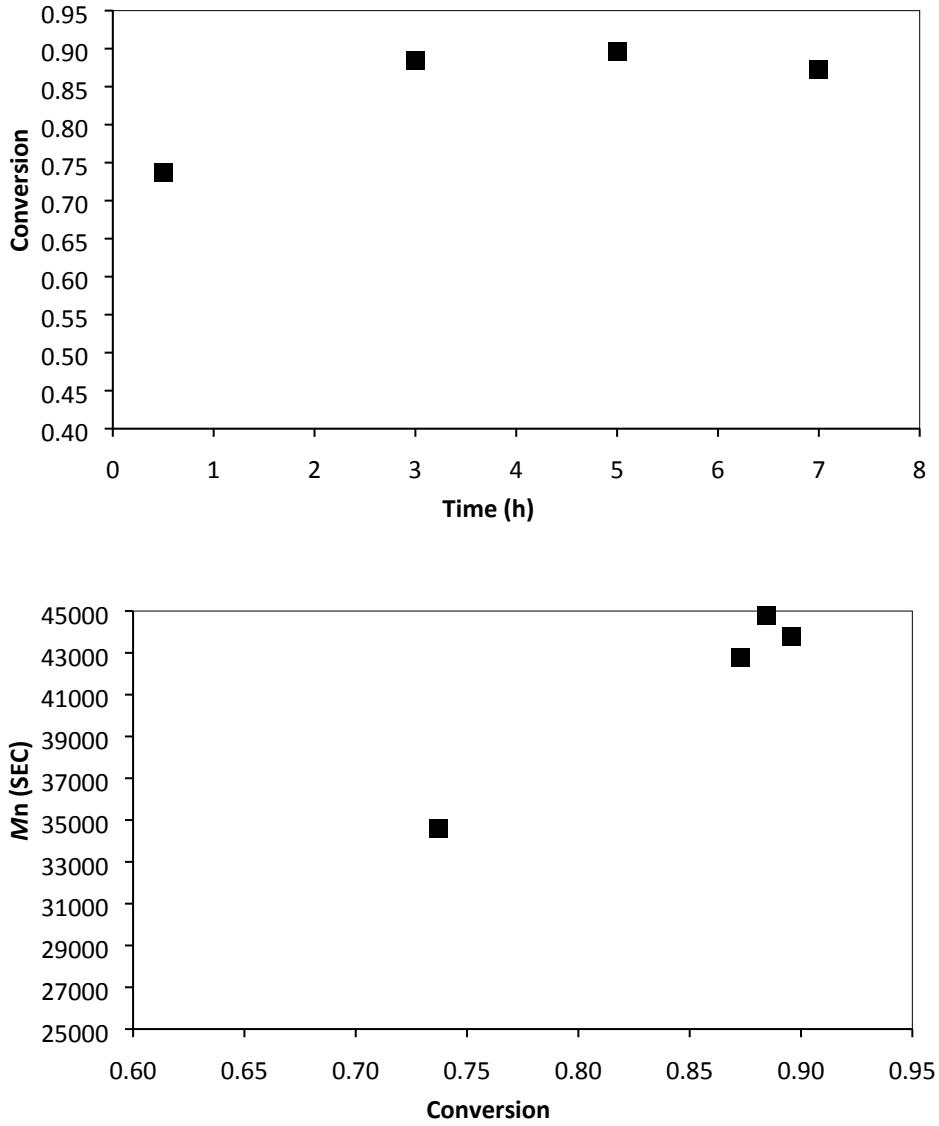

Figure S1. Kinetics of HPMA polymerization (CPN/CuCl/$CuCl_2$/PMDETA = 1/2/1/3, HPMA/I = 100:1, monomer/H_2O = 2/3 (g/ml), 30 min pre-disproportionation, 0°C + r.t.).

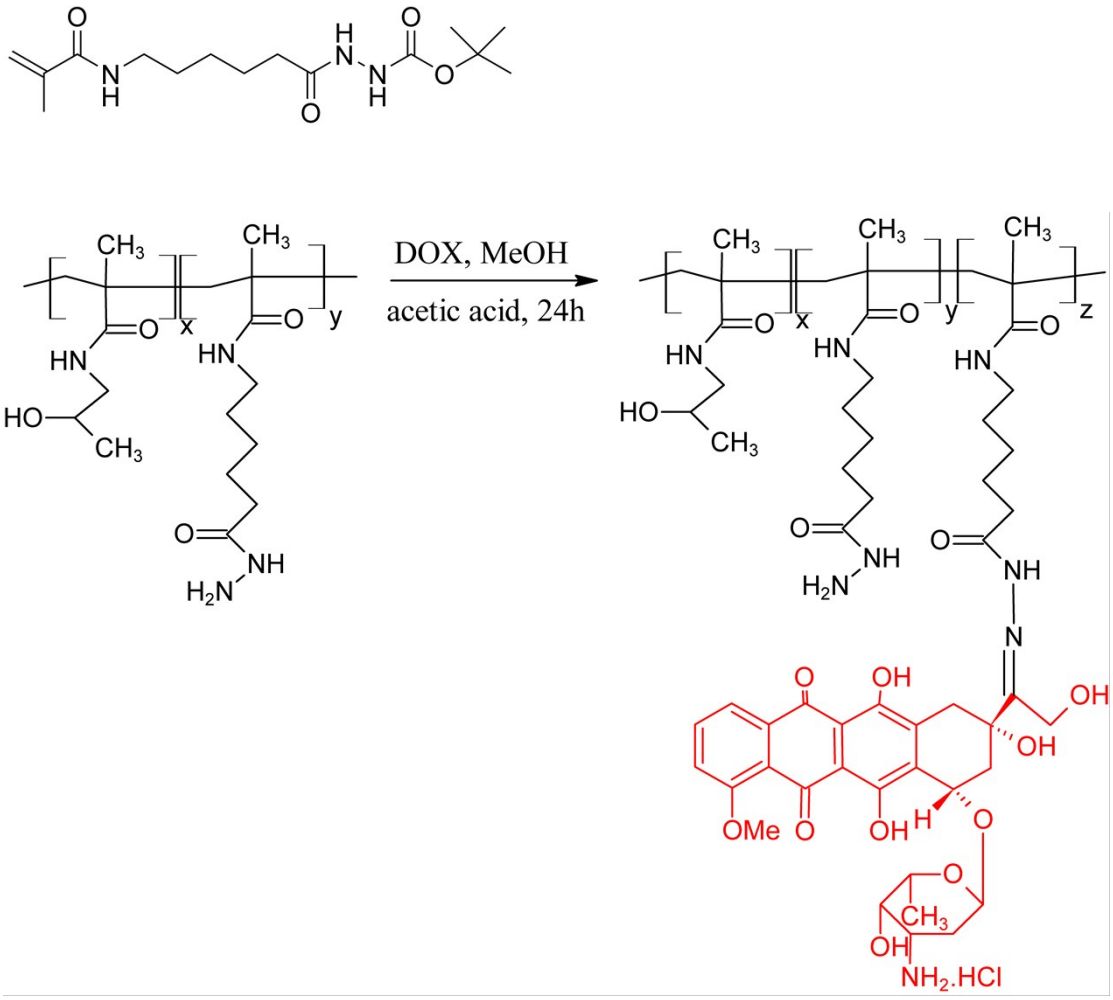

Figure S2. Structure of the co-monomer (up) and the conjugation strategy employed after hydrazide de-protection, with Doxorubicin shown in red (bottom).

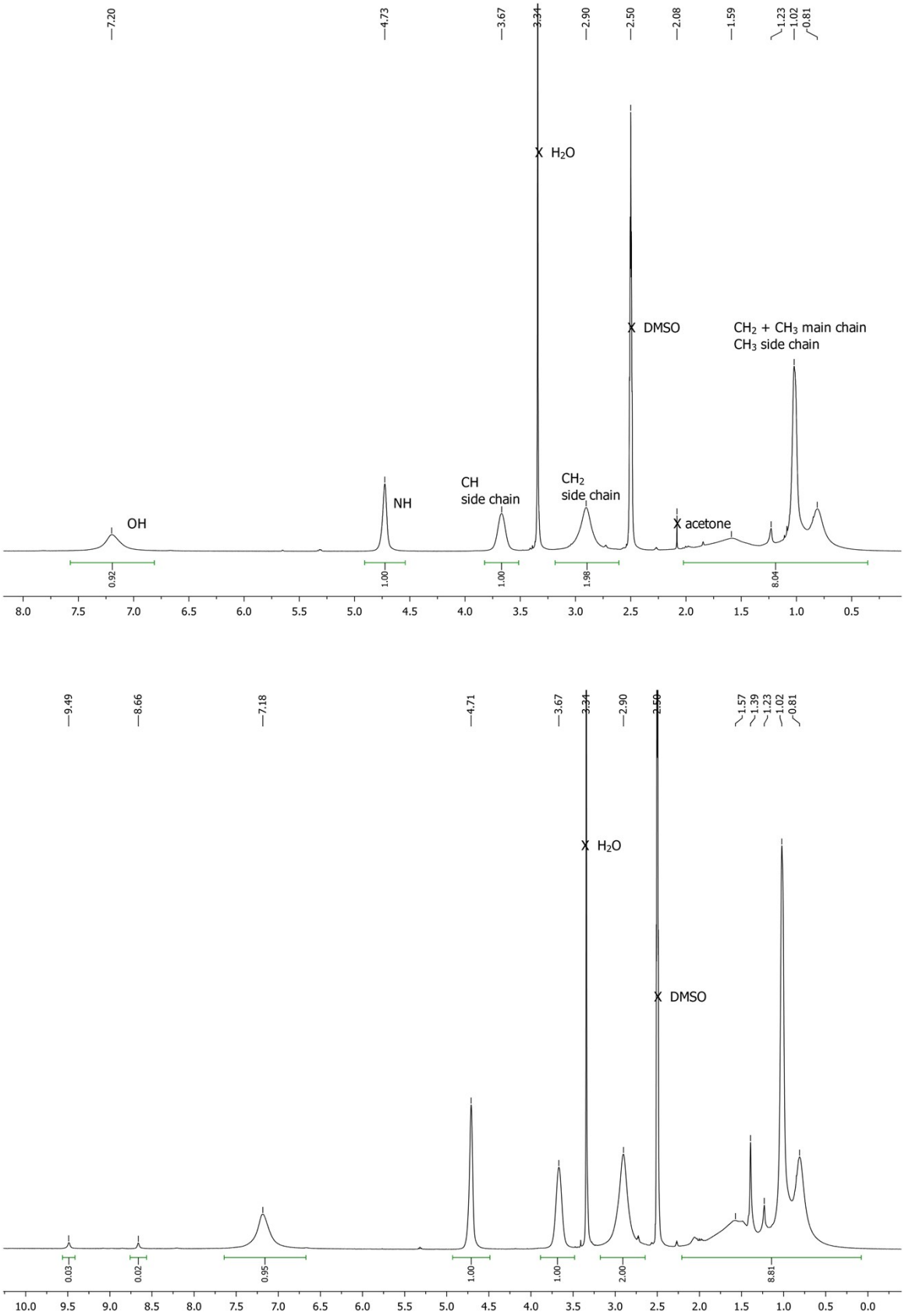

Figure S3. ^{1}H NMR spectra of typical poly(HPMA) (up) and of the copolymer of HPMA with the protected hydrazide (bottom); measured in DMSO-d6.

References

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3. T. Etrych, M. Jeĺınková, B. Říhová and K. Ulbrich, *J. Controlled Release*, 2001, **73**, 89-102.
4. H. Kostková, T. Etrych, B. Říhová, L. Kostka, L. Starovoytová, M. Kovář and K. Ulbrich, *Macromol. Biosci.*, 2013, **13**, 1648-1660.