DOI: 10.1002/cbic.201200164

Pan-Selective Aptamers for the Family of Small GTPases

Michael Hons,[a, b] Björn Niebel,[a] Nora Karnowski,[a] Benjamin Weiche,[a] and Michael Famulok*[a]

The members of the large family of small-GTP-binding proteins (GTPases) function as molecular switches and are involved in regulation of a wide variety of cell processing events through signal cascades. Their molecular masses typically range between 20–40 kDa. Small GTPases cycle between an inactive GDP-bound conformation and an active GTP-bound state.[1] Only in their active conformation can GTPases interact with effector proteins to induce downstream signalling events. The cycling is regulated by guanine nucleotide exchange factors (GEFs) that induce the release of bound GDP to be replaced by GTP, and by GTPase-activating proteins (GAPs) that accelerate the GTPase activity of small GTPases.[2] The Ras superfamily is structurally classified into at least five families: Ras, Rho, Rab, Sar1/Arf and Ran. The 36 Ras family members are key mediators of extracellular signal transduction and regulate multiple downstream effects, mainly by modulation of gene expression. They also directly or indirectly regulate cell proliferation, differentiation, and survival. Rho proteins, the second branch of the Ras superfamily comprising at least 20 members, also serve as key regulators of extracellular-stimuli-transducers that mainly direct actin reorganisation, cell-cycle progression and gene expression and thus are involved in cancer progression.[3] The Rab family consists of 61 members[4] and is known to regulate intracellular vesicle transport and the trafficking of proteins between different organelles of the endocytic and secretory pathways.[5] They are involved in vesicle formation, budding, transport, fusion and release events.[6] In humans, 27 genes for Arf family proteins have been found.[7] The Arf family is involved mainly in vesicle budding, but also in endocytic recycling and cytoskeletal reorganization. Ran is the most frequent small GTPase in the cell, with only one protein in this family in humans. It is better understood for its role in nucleo-cytoplasmatic transport of both RNA and protein.[8] Furthermore, it regulates mitotic spindle formation, DNA replication and nuclear envelope assembly.[9] Many human diseases are related to small GTPases such as some cancers and immune and neurological ailments. All small GTPases share an ~20 kDa conserved G domain that contains a set of GDP/GTP-binding motifs (G1–G5).[6]

[a] M. Hons,[+] Dr. B. Niebel,[+] N. Karnowski, B. Weiche, Prof. Dr. M. Famulok
Life and Medical Sciences (LIMES) Institute
Chemical Biology & Medicinal Chemistry Unit, University of Bonn
Gerhard-Domagk-Strasse 1,53121 Bonn (Germany)
E-mail: m.famulok@uni-bonn.de

[b] M. Hons[+]
Present address:
Research Group of 3D Electron Cryomicroscopy
Max Planck Institute of Biophysical Chemistry
37077 Göttingen (Germany)

[+] These authors contributed equally to this work

Although there has been much progress in understanding the biological roles of small GTPases, many unsolved questions remain, especially regarding their control and downstream effector functions. This highlights the necessity for specific inhibitory molecules targeting small GTPases and their application as tools for investigating and understanding the molecular mechanism of small GTPases in greater detail. GTPase-binding small molecules have been described, among them the compounds NSC23766, EHT1864, "arabinose-derived compound 2", 6-thio-GTP, or the Secin series, which inhibit GEF activity and thereby effector functions of the small GTPases.[10] An alternative strategy is the generation of aptamers that target small GTPases directly. This strategy provides rapid access to molecular inhibitors based on folded nucleic acids ("intramers")[11] that can be expressed intracellularly to analyze the consequences of target inhibition in an in vivo context. So far, aptamers have been described that either target specific regions of the GTPase[12] or that bind to mutants representing a stabilized conformation of the molecule.[13] Recently, an aptamer that targets active GTP bound Cdc42 has been reported.[14] Less closely related GTPases have been targeted by RNA aptamers equally infrequently: we have described RNA aptamers that bind the bacterial elongation factor SelB,[15] and one example describes the isolation of an aptamer for a G protein-coupled receptor.[16] Given the huge number of family members of the GTPase superfamily, the lack of identified aptamers is surprising and efforts to find new sequences able to bind GTPases are desirable. Here we describe the identification of two RNA aptamers that bind different family members of the large Ras superfamily of small GTPases. We show that these two related sequences selectively bind to a huge variety of small GTPases without targeting non-GTPase proteins, thereby exhibiting pan-selectivity towards small-GTP-binding proteins.

These RNA-aptamers were identified serendipitously during a selection experiment that targeted the RhoGEF domain of the GEF Vav1. In addition to Vav1-binding aptamers that will be described elsewhere, the enriched RNA library contained at least two orphan sequences that did not bind Vav1, but exhibited an unexpected specificity for members of the Ras superfamily of small GTPases. We termed these sequences V63 and V88, respectively. As shown in Figure 1A, V63 and V88 share some conserved sequences (shadowed in magenta), but based on their calculated mfold secondary structures, a common folding motif does not become apparent. Due to their interesting binding behaviour, we decided to further analyse these aptamers in greater detail. In an initial analysis we conducted a series of filter-retention analyses using at least one member of the five known subfamilies Ras (KRas, RalA), Ran, Rab (Rab1, Rab5c), Rho (Rac1, RhoA, Cdc42) and Arf (Arf1, Arf6) as binding partners for radiolabelled V63 and V88, respectively. In addition, several non-GTPase proteins were used as negative con-

 © 2012 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

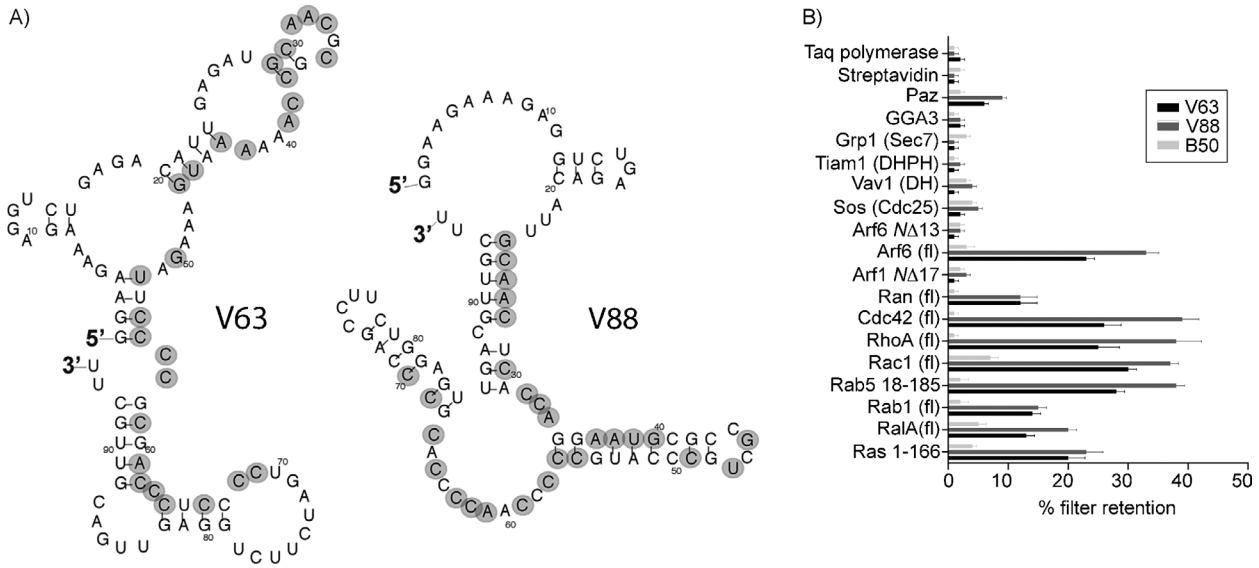

Figure 1. A) mfold secondary structure predictions for V63 and V88. The grey shaded bases represent the consensus sequences common in both aptamers. B) Normalized filter retention values of 5′-[^{32}P]-labeled V63, V88 in comparison with the unselected RNA library B50, incubated with the respective proteins (4 μM).

trols. The filter-binding analysis is summarised in Figure 1 B. Indeed, all full-length versions of the small GTPases employed in this assay exhibited binding to both aptamers V63 and V88, while their binding to the unselected RNA library B50 was strongly reduced. No significant filter retention of V63 and V88 was observed for the various control proteins, indicating specific association with small GTPases (Figure 1).

Strikingly, neither V63 nor V88 bound to the N-terminally truncated forms of the Arf1 and Arf6—*N*-Δ17-Arf1 or *N*-Δ13-Arf6, respectively—whereas their binding to the full-length versions of both Arf proteins was comparable to the other GTPases. Binding affinities for all members of the Ras superfamily of small GTPases are in the range 0.5–2.5 μM (Table 1). All GTPases were tested in their GDP-bound state, unless otherwise stated. While V63 showed the highest affinity for Ran1, Cdc42 and RhoA, the aptamer V88 binds Rac1 and Cdc42 in the mid-nanomolar range [Table 1, Figure 2].

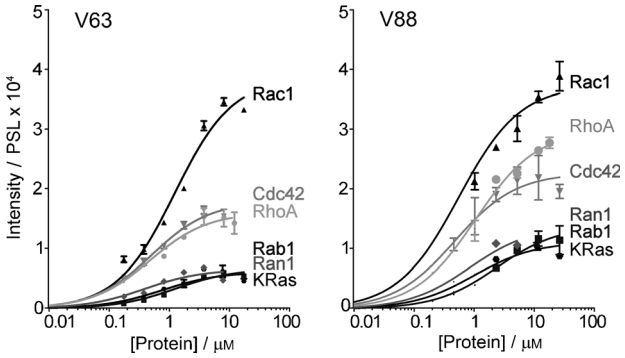

Figure 2. Filter retention analysis of in vitro GDP/GTPγS-loaded Rac1 and Arf6 with [^{32}P] end-labeled V63 and V88 (5 −10 nM).

Table 1. K_d values of aptamer V63 and V88 for small GTPases [μM].

Protein[a]	V63[b]	V88[b]	Protein[a]	V63[b]	V88[b]
Ran1	0.6 ± 0.2	1.1 ± 0.2	Rac1	1.8 ± 0.3	0.7 ± 0.3
Cdc42	0.8 ± 0.1	0.6 ± 0.2	Rab1	1.9 ± 0.3	2.5 ± 0.6
RhoA	0.8 ± 0.2	1.3 ± 0.1	KRas	1.2 ± 0.4	1.2 ± 0.3

[a] All GTPases were tested in their GDP-bound state [b] both aptamers were 5′-[^{32}P]-labeled.

Subsequently, we investigated whether V63 and V88 bind preferentially to the active GTP-bound, or to the inactive GDP-bound conformation of GTPases. We preloaded Arf6 and Rac1 with either GDP or GTPγS in vitro and analysed binding to the aptamers. Although the affinity after the in vitro loading process decreased slightly for V88, neither aptamers show distinctive specificity towards one conformation of Rac1 or Arf6 (Table 2).

Table 2. K_d values of aptamer V63 and V88 for small GTPases in GTP or GDP-bound form [μM].

Protein	loaded nucleotide	V63[a]	V88[a]
Rac1	GTP	1.0 ± 0.2	2.0 ± 0.1
	GDP	0.7 ± 0.3	2.1 ± 0.2
Arf6	GTP	1.4 ± 0.3	1.4 ± 0.4
	GDP	1.1 ± 0.2	1.7 ± 0.2

[a] Both aptamers contained a 5′-[^{32}P] label.

We then studied the effect of the aptamers on the effector recognition of the GTPase. This knowledge is important because many effector proteins recognize their respective GTPases in the activated GTP-bound state but not in the deactivated

 © 2012 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

state; this is the case for, for example, the ARF-effector GGA3 or the Rac1-effector Pak1. Both V63 and V88 recognize GTPases independently of their activation state, indicating that the GTPase effector binding site is not a major recognition motif for the aptamers. To further support this interpretation, we employed a recently developed assay based on luminescence oxygen channelling using the GTPase Rac1 as a test system (Figure 3 A).[17] Rac1 is sandwiched between a specific C-terminally interacting Rac1 monoclonal antibody (Rac1-mAB) and the recognition of the effector protein Pak1 within the interswitch region. This allowed analysis of the effect of V63 and V88 on both effector binding and interaction with the specific antibody at the C terminus. Figure 3 B shows that both aptamers have no effect on the effector interaction of the GTPase Rac1 and do not interfere with C-terminal antibody recognition.

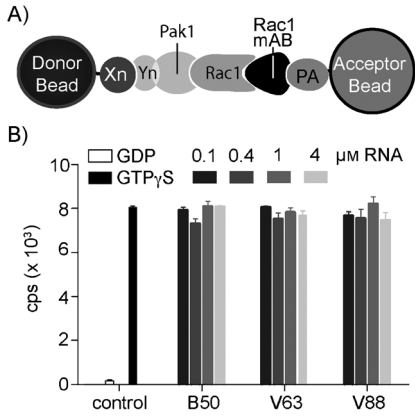

Figure 3. Luminescent oxygen channelling assay. A) Sensor assembly. PA: protein A coupled to acceptor beads; Rac1 mAB: anti-Rac1 rabbit IgG; Yn: biotin-GST-Pak1; Xn: streptavidin coupled to donor beads. B) Streptavidin donor beads and Protein A acceptor beads were incubated with biotinylated GST-Pak1 (amino acids 70–117), Rac-specific antibody (C-11) and GTPγS-loaded Rac1. As a control, we used the parent RNA library B50. RNA: nucleic acids V63, V88, or B50 at indicated concentrations.

The intrinsic and GEF-mediated guanine nucleotide exchange activities of GTPases are other features that might be altered upon aptamer complexation. To investigate this, we performed a mant-GTP-based guanine nucleotide exchange assay with Rac2. The affinities for Rac2 were again determined by filter retention analysis and found to be 1.8 ± 0.2 μM for V63 and 1.6 ± 0.3 μM for V88, well within the K_d values measured for the other GTPases. We first monitored the intrinsic exchange activity at increasing concentrations of V63, V88 and B50 (Figure 4 A). Interestingly, the exchange activity on Rac2 remained unaltered at increasing concentrations of the aptamers. The same effect was observed for the GEF-mediated exchange. After addition of the RhoGEF Tiam1 (amino acids 1033–1406), the intrinsic exchange activity increased fourfold but still remained unaffected by the addition of up to 4 μM V63, V88 or RNA library (Figure 4 B), thus providing one of the rare examples in which an aptamer binds to its target with high affinity without affecting its biological activity.

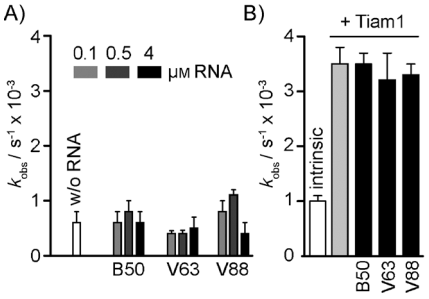

Figure 4. Mant-GTP guanine nucleotide exchange assay of Rac2. A) Intrinsic, GEF-independent exchange activity on Rac2 at increasing concentrations of V63, V88 and B50 B) Influence of V63 and V88 on the GEF (Tiam1)-triggered mant-GTP association on Rac2. B50, V63, and V88 were used at 4 μM concentration. The corresponding nucleotide exchange rate (1 s^{-1}) was calculated from an exponential fit of the fluorescence data.

V63 and V88 are the first RNA aptamers that show pan-specificity for the Ras superfamily of small GTPases. Due to their sequence identity of about 60% both aptamers likely bind the same motif in their target structures. Small GTPases are structurally most conserved within the phosphate recognition loop (G-1) and the base recognition loop (G-4),[18] V63 and V88 presumably target one of these motifs, or both. The β/γ phosphate-coordinating G-1 loop, also known as the Walker A motif, is not only present in GTPases but part of many nucleotide-binding proteins,[19] for example, *Thermus aquaticus* DNA (Taq) polymerase. Since we do not observe binding of V63 and V88 to the Taq polymerase (Figure 1), it appears unlikely that the Walker A motif is the predominant recognition element for the aptamers. This hypothesis is further supported by the observation that V63 and V88 do not discriminate between the GDP- and GTP-bound states of the tested GTPases (Table 2), which would have been expected for exclusive G-1 loop binders. These findings might argue for the G4-loop as a promising motif required for binding of V63 and V88. Unfortunately, however, we could not directly test this hypothesis because we found the deletion of the respective amino acids of the G-4 loop in Rac1 and Cdc42 to result in misfolding and insolubility of the overexpressed proteins under various expression conditions.

Another structural motif found in a subset of small GTPases is an α-helical region localized at the N terminus. In a cellular environment, this region is modified with lipid moieties that mediate membrane recruitment of GTPases like Arf or Rab. The influence of the N-terminal helix on the GTPase activity has been shown for Arf1[20] and the crystal structure of full-length Arf6 shows that this structure is positioned in the interswitch region, increasing conformational stability of the GTPase when not bound to phospholipids.[21] Removing this helix in Arf1 and Arf6 has detrimental effects on aptamer recognition. However, N-terminally truncated Rab5c is bound by V63 and V88,[22] indicating that the lack of binding to N-terminally truncated Arf proteins is due to broader conformational defects in this special class of small GTPases. The finding that V63 and V88 neither affect the intrinsic nor the GEF-catalysed guanine nucleotide exchange reaction on Rac2 is consistent with the lack of

 © 2012 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

GDP/GTP discrimination by both aptamers. Furthermore, the results obtained in the effector assay that sandwiches the GTPase effector region and the C terminus of Rac1 (Figure 3) show that V63 and V88 do not interfere with antibody recognition at the C terminus of Rac1. Given the high sequence variability in the relatively unstructured C terminus of small GTPases, we expect that this will also be the case for other members of this protein family. Small GTPases are structurally well conserved within the phosphate recognition loop (G-1) and the base recognition loop (G-4).[18] Taken together, and having excluded binding of both aptamers to most of the other structurally conserved motifs present in the target proteins, our results propose either the G-1 loop, the G-4 loop or both as recognition elements for V63 and V88. Future experiments will further narrow down the binding site of the aptamers on the G domain and analyse the influence of the aptamers on isoprenoid lipid modification of small GTPases, which is important for membrane localization.[23]

The pan-selectivity of V63 and V88 opens up a number of interesting applications. For example, both aptamers may serve as a starting point for their affinity maturation for individual GTPase members by Darwinian molecular evolution.[24] This would provide even more rapid access to specific probes for members of the vastly diverse class of small GTPases. Moreover, small-molecule probes such as NSC23766, EHT1864, 6-thio-GTP or others are often associated with functional inhibition and are therefore not suitable for investigating unperturbed proteins.[10a,b,d,25] For studying small-GTPase activity in real time, non-invasive probes have been developed that are based on FRET-coupled effector constructs.[26] However, these approaches require overexpression of the FRET probe, leading to potential effects on the endogenous function of the target protein. An alternative strategy that is less prone to these artefacts could be the use of non-inhibiting aptamer ligands such as V63 and/or V88 that are fused to RNA sequences which could eventually be fluorescently labelled and thereby would allow real-time imaging of the target molecule.[27] Furthermore, the specific tethering of modulatory molecules to specific GTPases could reduce side effects of inhibitors/activators that arise due to limited specificity of the modulating molecule itself. Considering the importance of small GTPases in cell signalling and the wide applicability of aptamer and small-molecule conjugates in different experimental setups,[28] V63 and V88 hold promise as useful agents in a variety of fields in both basic and applied research. Finally both aptamers may serve as RNA-scaffolds that can be integrated as artificial switches between the on and off state of the GTPase into nucleic acid-based or biohybrid nanoarchitectures.[29]

Experimental Section

In silico modeling of RNA sequences. The RNA sequences of V63 and V88 have been analysed with the mfold program package, which is available on the internet: http://mfold.rna.albany.edu.

Filter retention analysis. [^{32}P] end-labeled RNA (5–10 nM) was incubated in 1× PBS containing $MgCl_2$ (1 mM) with increasing concentrations of the respective proteins in a total volume of 25 μL. After incubation at 23 °C for 30 min, the RNA–protein complex was vacuum filtered through a moistened nitrocellulose membrane and washed with buffer [1× PBS, $MgCl_2$ (1 mM), total volume 1 mL]. The membrane was transferred to a cassette and exposed to a phosphorimager screen overnight and quantified the next day on the phosphorimager (Fujifilm BAS-2500).

Guanine nucleotide-exchange assay. The GTPase (Rac2, final conc. 2 μM) was incubated in guanine nucleotide exchange buffer [1× PBS, $MgCl_2$ (3 mM), DTT (1 mM)] with mant-GTP (400 nM, Jena Bioscience, Jena, Germany) and RNA at the corresponding concentration for 30 min at 23 °C in a total volume of 40 μL. After incubation for 5 min in the plate reader (Mithras LB-840, Berthold, Germany) the exchange reaction was initiated by injecting 10 μL of the exchange factor (Tiam1, final conc. 200 nM) or buffer control. The fluorescence was measured at 20 s intervals (λ_{ex} = 340 nm, λ_{em} = 460 nm) for 1800 s.

In vitro GTPγS or GDP Treatment. EDTA (0.5 M, pH 8.0, 2 μL) was added to Rac1 and Arf6 (25–100 μM, 100 μL) and mixed. GTPγS (10 mM, 1 μL) or GDP (10 mM, 1 μL) were used for loading. The mixture was incubated at 30 °C for 20 min with constant agitation before terminating with $MgCl_2$ (1 M, 5 μL) on ice.

Luminescence oxygen-based GTPase activation assay. All measurements were made on a LB940 Mithras plate reader (Berthold Instruments, Bad Wildbach, Germany) equipped with the AlphaScreen module. Both excitation and emission was set to 0.5 s. Bead reagents were pipetted under subdued light to prevent bleaching. The basic protocol includes the preparation of two mixtures: 1) Bead/antibody mix: streptavidin donor and protein A acceptor beads were diluted 1:50 (100 μg mL^{-1}) and mixed with a 1:100 dilution of the anti-Rac1-antibody (clone c-13, Santa Cruz Biotechnology) in 1× PBS containing $MgCl_2$ (3 mM) and preincubated for 90 min at 4 °C in the dark. 2) GTPase/RNA/effector mix: in vitro loaded GTPase (400 nM) was incubated with biotinylated GST-PAK1-effector (amino acids 70–117, 100 nM) and the respective RNA dilutions in binding buffer [1× PBS, $MgCl_2$ (3 mM)] for 90 min at 4 °C. Equal amounts (10 μL) of the mixtures were introduced into the wells of a 384-well ProxiPlate (Perkin–Elmer) and incubated for 30 min at RT before measurement at 25 °C.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (DFG), the Collaborative Research Center SFB 645, and the NRW Research School LIMES Chemical Biology.

Keywords: aptamers · GTP binding proteins · pan-selectivity · RNA recognition · small GTPases

[1] D. Vigil, J. Cherfils, K. L. Rossman, C. J. Der, *Nat. Rev. Cancer* **2010**, *10*, 842–857.
[2] J. L. Bos, H. Rehmann, A. Wittinghofer, *Cell* **2007**, *129*, 865–877.
[3] a) S. Etienne-Manneville, A. Hall, *Nature* **2002**, *420*, 629–635; b) R. Karlsson, E. D. Pedersen, Z. Wang, C. Brakebusch, *Biochim. Biophys. Acta Rev. Cancer* **2009**, *1796*, 91–98.
[4] J. B. Pereira-Leal, M. C. Seabra, *J. Mol. Biol.* **2001**, *313*, 889–901.
[5] M. Zerial, H. McBride, *Nat. Rev. Mol. Cell Biol.* **2001**, *2*, 107–117.
[6] K. Wennerberg, K. L. Rossman, C. J. Der, *J. Cell Sci.* **2005**, *118*, 843–846.
[7] J. G. Donaldson, C. L. Jackson, *Nat. Rev. Mol. Cell Biol.* **2011**, *12*, 362–375.
[8] a) K. Weis, *Cell* **2003**, *112*, 441–451; b) D. Görlich, M. J. Seewald, K. Ribbeck, *EMBO J.* **2003**, *22*, 1088–1100.

 © 2012 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

[9] H. Y. Li, K. Cao, Y. Zheng, *Trends Cell Biol.* **2003**, *13*, 553 – 557.
[10] a) D. Poppe, I. Tiede, G. Fritz, C. Becker, B. Bartsch, S. Wirtz, D. Strand, S. Tanaka, P. R. Galle, X. R. Bustelo, M. F. Neurath, *J. Immunol.* **2006**, *176*, 640 – 651; b) H. Akbar, J. Cancelas, D. A. Williams, J. Zheng, Y. Zheng, *Methods Enzymol.* **2006**, *406*, 554 – 565; c) A. Palmioli, E. Sacco, C. Airoldi, F. Di Nicolantonio, A. D'Urzo, S. Shirasawa, T. Sasazuki, A. Di Domizio, L. De Gioia, E. Martegani, A. Bardelli, F. Peri, M. Vanoni, *Biochem. Biophys. Res. Commun.* **2009**, *386*, 593 – 597; d) A. Shutes, C. Onesto, V. Picard, B. Leblond, F. Schweighoffer, C. J. Der, *J Biol Chem.* **2007**, *282*, 35666 – 35678; e) M. R. Ahmadian, T. Zor, D. Vogt, W. Kabsch, Z. Selinger, A. Wittinghofer, K. Scheffzek, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 7065 – 7070; f) J. C. Zeeh, M. Zeghouf, C. Grauffel, B. Guibert, E. Martin, A. Dejaegere, J. Cherfils, *J. Biol. Chem.* **2006**, *281*, 11805 – 11814; g) B. Albertoni, J. S. Hannam, D. Ackermann, A. Schmitz, M. Famulok, *Chem. Commun.* **2012**, *48*, 1272 – 1274; h) X. Bi, A. Schmitz, A. M. Hayallah, J. N. Song, M. Famulok, *Angew. Chem.* **2008**, *120*, 9707 – 9710; *Angew. Chem. Int. Ed.* **2008**, *47*, 9565 – 9568; i) M. Hafner, E. Vianini, B. Albertoni, L. Marchetti, I. Grune, C. Gloeckner, M. Famulok, *Nat. Protoc.* **2008**, *3*, 579 – 587.
[11] a) G. Mayer, M. Blind, W. Nagel, T. Bohm, T. Knorr, C. L. Jackson, W. Kolanus, M. Famulok, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 4961 – 4965; b) M. G. Theis, A. Knorre, B. Kellersch, J. Moelleken, F. Wieland, W. Kolanus, M. Famulok, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 11221 – 11226; c) M. Famulok, G. Mayer, *ChemBioChem* **2005**, *6*, 19 – 26; d) M. Famulok, M. Blind, G. Mayer, *Chem. Biol.* **2001**, *8*, 931 – 939.
[12] Y. Tanaka, K. Akagi, Y. Nakamura, T. Kozu, *Oligonucleotides* **2007**, *17*, 12 – 21.
[13] S. Jeong, S. R. Han, Y. J. Lee, J. H. Kim, S. W. Lee, *Oligonucleotides* **2010**, *20*, 155 – 161.
[14] J. Tok, J. Lai, T. Leung, S. F. Li, *Electrophoresis* **2010**, *31*, 2055 – 2062.
[15] a) S. J. Klug, A. Hüttenhofer, M. Famulok, *Rna* **1999**, *5*, 1180 – 1190; b) S. J. Klug, A. Hüttenhofer, M. Kromayer, M. Famulok, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 6676 – 6681.
[16] D. A. Daniels, A. K. Sohal, S. Rees, R. Grisshammer, *Anal. Biochem.* **2002**, *305*, 214 – 226.
[17] a) B. Niebel, C. Lentz, M. Pofahl, G. Mayer, A. Hoerauf, K. M. Pfarr, M. Famulok, *Chemistry* **2010**, *16*, 11100 – 11107; b) B. Niebel, B. Weiche, A. L. Mueller, D. Y. Li, N. Karnowski, M. Famulok, *Chem. Commun.* **2011**, *47*, 7521 – 7523.
[18] S. R. Sprang, *Annu. Rev. Biochem.* **1997**, *66*, 639 – 678.
[19] a) J. E. Walker, M. Saraste, M. J. Runswick, N. J. Gay, *EMBO J.* **1982**, *1*, 945 – 951; b) C. Ramakrishnan, V. S. Dani, T. Ramasarma, *Protein Eng.* **2002**, *15*, 783 – 798.
[20] R. A. Kahn, P. Randazzo, T. Serafini, O. Weiss, C. Rulka, J. Clark, M. Amherdt, P. Roller, L. Orci, J. E. Rothman, *J. Biol. Chem.* **1992**, *267*, 13039 – 13046.
[21] J. Menetrey, E. Macia, S. Pasqualato, M. Franco, J. Cherfils, *Nat. Struct. Mol. Biol.* **2000**, *7*, 466 – 469.
[22] J. X. Hong, X. Zhang, J. Moss, M. Vaughan, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 3056 – 3059.
[23] P. H. Liang, T. P. Ko, A. H. Wang, *Eur. J. Biochem.* **2002**, *269*, 3339 – 3354.
[24] M. S. Ahmed, G. Mayer, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3793 – 3796.
[25] Z. Surviladze, A. Waller, Y. Wu, E. Romero, B. S. Edwards, A. Wandinger-Ness, L. A. Sklar, *J. Biomol. Screening* **2010**, *15*, 10 – 20.
[26] N. Mochizuki, S. Yamashita, K. Kurokawa, Y. Ohba, T. Nagai, A. Miyawaki, M. Matsuda, *Nature* **2001**, *411*, 1065 – 1068.
[27] J. S. Paige, K. Y. Wu, S. R. Jaffrey, *Science* **2011**, *333*, 642 – 646.
[28] a) A. Bill, A. Schmitz, B. Albertoni, J. N. Song, L. C. Heukamp, D. Walrafen, F. Thorwirth, P. J. Verveer, S. Zimmer, L. Meffert, A. Schreiber, S. Chatterjee, R. K. Thomas, R. T. Ullrich, T. Lang, M. Famulok, *Cell* **2010**, *143*, 201 – 211; b) M. Hafner, A. Schmitz, I. Grune, S. G. Srivatsan, B. Paul, W. Kolanus, T. Quast, E. Kremmer, I. Bauer, M. Famulok, *Nature* **2006**, *444*, 941 – 944; c) G. Mayer, *Angew. Chem.* **2009**, *121*, 2710 – 2727; *Angew. Chem. Int. Ed.* **2009**, *48*, 2672 – 2689; d) D. Stumpfe, A. Bill, N. Novak, G. Loch, H. Blockus, H. Geppert, T. Becker, A. Schmitz, M. Hoch, W. Kolanus, M. Famulok, J. Bajorath, *ACS Chem. Biol.* **2010**, *5*, 839 – 849; e) A. Bill, H. Blockus, D. Stumpfe, J. Bajorath, A. Schmitz, M. Famulok, *J. Am. Chem. Soc.* **2011**, *133*, 8372 – 8379.
[29] a) D. Ackermann, T. L. Schmidt, J. S. Hannam, C. S. Purohit, A. Heckel, M. Famulok, *Nat. Nanotechnol.* **2010**, *5*, 436 – 442; b) K. A. Afonin, E. Bindewald, A. J. Yaghoubian, N. Voss, E. Jacovetty, B. A. Shapiro, L. Jaeger, *Nat. Nanotechnol.* **2010**, *5*, 676 – 682.

Received: March 7, 2012
Published online on June 11, 2012

 © 2012 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim