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Polyvalent Catalysts Operating on Polyvalent Substrates: A Model for Surface-Controlled Reactivity

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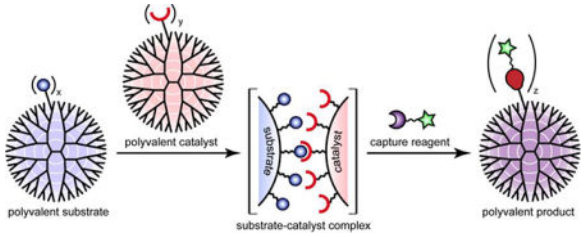
Abstract

Roll and rock. Unusually fast rates of nucleophilic catalysis of hydrazone ligation were observed using polyvalent anthranilic acid catalysts operating on polyvalent aldehyde substrates, with PAMAM dendrimers as the common platform. When presented in this way, the catalyst has a strong accelerating effect at concentrations 40–400 times lower than similar monovalent catalysts, and displays unique kinetic parameters. We attribute these properties to polyvalent engagement between the dendrimer surface groups, and a potential "rolling" effect giving rise to fast inter-particle kinetic turnover. The phenomenon is sensitive to the density of functional groups on each dendrimer, and insensitive to factors that promote or inhibit nonspecific particle aggregation. These findings constitute a rare experimental example of an underappreciated phenomenon in biological and chemical systems that are organized on interacting surfaces.

Entry for the Table of Contents



Keywords

polyvalency; multivalency; polyvalent catalysis; dendrimers; hydrazone formation; kinetics

Polyvalent binding is a powerful control element in biology and in laboratory-engineered molecular systems designed to engage biological surfaces.[1–3] Polyvalency may be regarded as a tool for the transfer of molecular information, such as by the induction of cellular signaling events to give a wide spectrum of responses. Chemical catalysis is another mechanism by which polyvalent recognition events can be magnified in their effect. While

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Dedication: To Professor K. Barry Sharpless on the occasion of his 75th birthday, with deepest thanks for peerless insight and inspiration - past, present, and future.

polyvalent catalysts have been intensively investigated for practical reasons in synthetic chemistry[4–12] – for example, to increase activity by virtue of high local concentration and recyclability by virtue of easier recovery – it has only rarely been tested as a vehicle for information transfer in model systems.[13]

However, interesting and illustrative examples involving polyvalent substrates under the heading of "cooperative" or "interfacial" catalysis do exist (see Supporting Information). In several of these cases, the importance of catalyst moving from one substrate to a nearby substrate ("scooting" or "hopping") is highlighted.[14–16] At the risk of oversimplification, these studies reveal that multiple copies of substrates are operated on by monovalent (solution-phase) catalysts in a manner sensitive to the average two-dimensional "concentration" (density) of the substrate. Polyvalent catalysts, in turn, exhibit the expected properties of high local concentration, which can be profound if the reaction mechanism requires the cooperative action of more than one molecular component to achieve optimal catalytic function. Yet the interaction of *polyvalent catalysts with polyvalent substrates* seems to have escaped intensive attention so far. Only polynucleotide phosphodiester hydrolysis by gold nanoparticle- or micelle-displayed catalysts has been repeatedly explored from this point of view.[5, 17–21] Significant advantages in rate and processivity have been identified, although most quantitative measurements have been reported using a monovalent model substrate.

We describe here the first exploration of polyvalent catalyst-substrate reactivity in which a bond forming event, rather than an irreversible cleavage reaction, is monitored. Like others,[11, 22] we think it likely that nature takes advantage of the principle in as yet unrealized ways, since living cells are full of surfaces decorated with all manner of functionality.

Figure 1A shows the overall kinetic scheme that inspired the experiments described below. The initial formation of a catalyst-substrate complex is followed by its dissociation or by capture/conversion to form a catalyst-product complex. We suggest that the overall catalytic rate can be greatly increased if dissociation of the catalyst-product complex (k_{-4}, or off-rate) is relatively slow compared to the rate of association of an adjacent catalytic site with an adjacent substrate unit (k_5). If this "rolling" process (analogous to the "scooting" of Berg and Jain[23] or the "hopping" of Dawson and Medintz[24]) occurs efficiently, catalyst and substrate would not be separated and diffusion limitations would be largely eliminated. If the scaffold is rigid, we expect the rolling effect to be most pronounced when catalytic residues are spaced over similar dimensions as the substrates. If the scaffold is flexible, more than one catalyst-substrate complex could be formed at the same time. This does not invalidate the essential nature of polyvalent-polyvalent catalysis, simultaneous catalyst-substrate engagement representing an extreme form of rolling.

To create this type of system, we employed polyamidoamine (PAMAM) dendrimers as conveniently modifiable polymer scaffolds.[25, 26] The condensation of hydrazines (capture reagent) with aldehydes (substrate), catalyzed by anthranilic acid derivatives[27] (catalyst, Figure 1B) was chosen as the test reaction because it is capable of generating a chromogenic signal and, at moderate catalyst concentrations, involves rate-limiting Schiff base formation, followed by rapid transimination with aryl hydrazine to yield the hydrazone product.[28, 29]

Such a kinetic scheme, as opposed to rate-limiting reagent capture, should be best suited to the generation of a polyvalent catalytic effect. The catalyst accelerates the dehydration step by a combination of nucleophilicity[28, 30–33] and intramolecular proton assistance[27, 34, 35]; the uncatalyzed reaction rate is also enhanced by a lowering of the pH of the reaction medium.[36]

Results and Discussion

Synthesis and fundamental reactivity

Polyvalent benzaldehyde substrate and anthranilate catalysts based on 2nd, 3rd, and 4th generation amine-terminated PAMAM dendrimers were prepared by a two-step acylation/CuAAC procedure as described in Supporting Information. These fully-loaded scaffolds displayed an average of 16, 31, and 61 functional groups, respectively (Figure 2). The latter two values were less than the nominal values of 32 and 64 attachments per dendrimer because commercial PAMAM samples suffer from generational (trailing generations and oligomers) and branching defects (missing arms and intramolecular loops) that are more severe for successive generations.[37]

In addition to varying scaffold size, the surface character of the functionalized G4 dendrimers was changed by the installation of different linkers (propargyl vs. tetraglyme) between the triazole and substrate/catalyst moieties to provide particles of different surface polarity proximity. Lastly, the average number of substrate or catalyst units per dendrimer (functional group density) was also varied by mixing functional (aldehyde or anthranilate) and non-functional (alcohol) components in the synthetic steps with precise control of average composition (Supporting Information). Monovalent and divalent analogues of the aldehyde substrate (**S, S_2**), anthranilate catalyst (**C, C_2**), and hydrazone (**P, P_2**) were also prepared for comparison (Figure 2).

Reaction with nitrobenzoxadiazole hydrazine **H**[38] gave rise to chromogenic hydrazone adducts, and reaction progress was monitored by absorbance at 520 nm as a function of time in a microtiter plate reader. Substrate and hydrazine concentrations were in the 25–100 μM range, and the reactions were performed at pH 5, near the optimal pH for anthranilate catalysis[27] in 0.1 M NaOAc buffer. Hydrazone and oxime ligations at the micromolar level are customarily performed using concentrations of aniline-derived nucleophilic catalyst of 1 mM or greater, but much lower concentrations of the polyvalent catalysts were required here. The observed reaction profiles were fit to the equation derived by Dawson that takes into account catalysis of both forward and reverse reactions.[30, 31] At the reagent concentrations used, completion was reached at less than 100% conversion of the limiting reagent, corresponding to K_{eq} values in the range of 2×10^4, as expected.[31] Representative data are shown in Figure 3 and Table 1; detailed experimental methods and results, including all kinetic runs and fits, are provided in Supporting Information.

In the absence of catalyst, the reaction of monovalent aldehyde **S** with **H** under these conditions proceeded at a rate of approximately 0.14 $M^{-1}s^{-1}$. The presence of monovalent catalyst at the low concentration of either 25 μM or 100 μM increased the reaction rate by a factor of approximately 3. Dendrimer-displayed aldehydes were better substrates than

others: in the absence of added catalyst, background rates of 1.3–6.6 M^1s^1 were observed, depending on which dendrimer scaffold was used. This is likely due to the high local concentration of aldehyde groups on these structures, but other examples of anomalous reactivity have been described.[39] Again, monovalent catalyst at 25 or 100 μM improved these reactions very little, increasing the rates three-fold at most.

In contrast, the use of the dendrimer-supported polyvalent catalysts resulted in much faster reactions with polyvalent, but not monovalent, substrates (Table 1). Rate constants of approximately 30–400 $M^{-1}s^{-1}$ were observed for the G2, G3, and G4 dendrimer systems, corresponding to rate enhancements of 13–28 fold relative to the rate of hydrazone formation from polyvalent substrate with monovalent catalyst, and approximately 90–1300 fold relative to the reaction involving monovalent substrate with monovalent catalyst. The same pattern was also observed at pH 6; although the absolute rates of all the reactions decreased relative to pH 5, the polyvalent display of catalyst and substrate afforded the same kinetic advantage (Supporting Information, Figure S4 and S8).

Similar levels of acceleration (approximately 70-fold) have been reported with monovalent catalysts operating on monovalent substrates, but requiring far higher concentrations of aniline[30] or 5-methoxyanthranilic acid[27] (at 10 mM and 1 mM, respectively). The use of either of these known catalysts at 25 μM gave similar results to monovalent **C** described above (data not shown). Note that substrate and catalyst concentrations are described here in terms of the functional groups; for example, when substrate **G4-pS$_{61}$** was used at an aldehyde concentration of 25 μM, the concentration of the fully-functionalized dendrimer was approximately 25/61 = 0.4 μM. Thus, in terms of the concentrations of reactive particles, the observed reaction rates are far in excess of any reported so far.

While G2, G3 and G4 dendrimer substrates and catalysts all showed strong rate accelerations when reacted with each other, the relative rates did not track with size. The G3-dendrimer imparted enhanced reactivity in all cases compared to G2 and G4 platforms (Figure 3B, Table 1), including as a polyvalent substrate reacting in the absence of catalyst (6.6 $M^{-1}s^{-1}$ *vs.* 2.6 $M^{-1}s^{-1}$ or 1.5 $M^{-1}s^{-1}$), in the presence of monovalent catalyst (14.0 $M^{-1}s^{-1}$ *vs.* 3.2 $M^{-1}s^{-1}$ or 2.3 $M^{-1}s^{-1}$), and as a polyvalent catalyst with the monovalent substrate (8.1 $M^{-1}s^{-1}$ *vs.* 1.1 $M^{-1}s^{-1}$ or 0.63 $M^{-1}s^{-1}$). As expected from these results, the G3 polyvalent-polyvalent combination was the fastest reaction measured here, with a rate constant of 391 $M^{-1}s^{-1}$ in the presence of 100 μM catalyst (3.2 μM of polyvalent G3-catalyst particle).

Particle aggregation

Several lines of evidence were inconsistent with nonspecific aggregation being the key factor in the observed acceleration of hydrazone formation, rather than a structure-based polyvalent catalytic effect of the type described in Figure 1A. (1) No aggregates were observed by dynamic light scattering for dendrimers bearing catalyst and substrate under the reaction conditions; only at concentrations approximately 100 times greater were aggregates detected. The minimum aggregate size detected by our instrumentation is approximately 5 nm. (2) G4 dendrimers with and without a tetraglyme spacer between the triazole and the aldehyde groups (designated **G4-S$_{61}$** and **G4-pS$_{61}$**) were prepared with the expectation that

they should differ significantly in their tendency to aggregate in aqueous solution. These scaffolds gave virtually identical results (Figure 3C vs. 3D, Table 1), suggesting that hydrophobicity of the dendrimer surface had little effect on polyvalent catalysis.[18] (3) The presence of unreactive hydrophilic or hydrophobic dendrimers added in excess had no effect on reaction kinetics (Supporting Information, Figure S10). If nonspecific aggregation were important, one or both of these added dendrimers would be expected to change the reaction rate.

Divalent vs. polyvalent substrates and catalysts

To gain a better appreciation for the roles of intramolecular association vs. polyvalency, divalent substrate and catalyst were prepared as the simplest tethered components. Figure 3E–G shows comparisons to reactions of monovalent, divalent and G2-based polyvalent reactants. Pairwise comparisons of rate (Supporting Information, Figure S20–S21) showed that multivalency had consistently greater effects for substrate than catalyst. Thus, divalent catalyst operated on various substrates only 1.5–2.7 times faster than monovalent catalyst, but the divalent substrate reacted 3.8–4.6 times faster than its monovalent counterpart in the presence of different catalysts. Similarly, the rate acceleration for polyvalent vs. divalent substrate (6.5–26.1 fold) was much greater than polyvalent vs. divalent catalyst (3.3–7.4 fold).

We suggest that this reflects the presence of two beneficial polyvalent effects for substrate: enhanced initial binding to catalyst (an advantage that can be described in terms of effective molarity[40, 41]) and enhanced processivity, or “rolling” (Figure 4). Polyvalent catalysts should benefit from the first but not the second. Overall, the intersection of polyvalent catalyst and substrate with each other is highly effective, especially when one considers that the concentrations of catalyst- and substrate-bearing particles are much less than those of the lower-valent species used in these comparisons.

Catalyst and substrate density on particles

Rates of hydrazone formation using G4 dendrimers bearing different numbers of substrate and catalyst units were measured, keeping the total concentration of substrate and catalyst moieties constant at 100 μM. Thus, particles bearing fewer functional units were used in larger amounts. The observed reaction rate constants were found to be highly sensitive to the functional group density on the scaffold (Figure 5). Relatively small decreases in rate were observed when cutting the occupancy of substrate or catalyst to three-quarters (61 to 46 per dendrimer), but a much greater loss was observed in going from three-quarters to half (46 to 31 per dendrimer, Figure 5A and 5B). The simultaneous variation of both substrate and catalyst loading produced a dramatic drop in catalytic rate at both the first and second dilution in functional group density (Figure 5C). Furthermore, polyvalent reactions involving 16 and 31 functional units on each scaffold were highly dependent on the scaffold size, as shown in Table 2. Thus, the reaction of **G2-S_{16}** mediated by **G2-C_{16}** was much faster than all reactions involving a similar number of reactive groups on the larger G4 scaffold (**G4-pS_{15} + G4-pC_{61}**; **G4-pS_{61} + G4-pC_{15}**; **G4-pS_{15} + G4-pC_{15}**). Similarly, **G3-S_{31} + G3-C_{31}** was a much faster reaction than reactions involving **G4-pS_{31}** or **G4-pC_{31}**. In contrast,

reactions between any of the fully-loaded dendrimers were all within a factor of 4 of each other (Supporting Information, Figure S4, S6, S8 and Table S1–S3).

This observed sharp and nonlinear dependence of reaction rate on functional group density is significantly different from that of "cooperative" catalysts, in which two or more functional groups on the same scaffold combine to produce a much more effective catalyst than if the functional groups are on different molecules. In such a case, for example, a two-armed catalytic assembly would be revealed by a constant second-order dependence of rate on density of catalyst-component groups on the scaffold.[11, 42] Figure 5 shows a different scenario in which the magnitude of rate acceleration varies across the range of functional group densities, implicating a structure-dependent phenomenon such as "rolling." A simple aggregation effect would be expected to produce a steadier drop in rate as the putative aggregates were diluted by the addition of more dendrimers bearing fewer functional groups.

Variations in concentration, temperature, and viscosity

The concentrations of polyvalent catalysts and polyvalent substrates were independently varied to survey the reaction rate dependence on these factors (Figure 6). For all three scaffolds, rate was found to increase with increasing catalyst concentration, although in a nonlinear manner (Figure 6, panels A–C). In contrast, for G4 and G3, but not G2 dendrimers, high substrate concentrations relative to catalyst were inhibitory (Figure 6, panels D–F).

These varying outcomes in the rate dependence on catalyst and substrate concentration would not be observed if each catalyst and substrate moiety was acting independently as in free solution. Furthermore, the inhibitory behavior of higher concentrations of the larger substrate dendrimers is consistent with a potentially deleterious consequence of polyvalent interactions. As represented in Figure 1, multiple catalyst and substrate groups on interacting scaffolds can engage with each other simultaneously. It is possible that some of the resulting imines may not be processed efficiently, since not all will be equally accessible to the hydrazine. This would constitute an inhibitory effect, expected to be more severe for larger dendrimers which should be more prone to simultaneous binding over large surface areas. The counterbalancing of this phenomenon with rate acceleration by rolling should produce an optimal formulation, in this preliminary study represented by the significantly faster reactivity of the G3-G3 poly-poly pair compared to G2-G2 and G4-G4.

Rates of hydrazone formation were also measured at different temperatures using polyvalent systems vs. monovalent analogues (Supporting Information, Figure S13 and Table S5). Most of these reactions, as well as the reaction of polyvalent substrate without catalyst, slowed down at increased temperatures, as previously observed by Bane and colleagues, and ascribed by them to the more favorable formation of imine intermediates at lower temperature.[43] [A similar inverse temperature dependence and formal negative enthalpy of activation have been reported for a different organocatalytic reaction (thiourea-catalyzed Mannich alkylation of imines), associated with the formation of key hydrogen bonds in the activated complex.[44]] In contrast, the very fast processing of polyvalent substrates in the G2 and G3 series were invariant with change in temperature between 25 and 45 °C (Figure S13, panels A and E).

This striking difference was reflected in kinetic parameters obtained by standard Eyring analysis, albeit over a limited temperature range (Supporting Information, Figure S13 and S14). Reactions involving polyvalent substrates with polyvalent catalysts showed substantially diminished entropic costs (less negative values of activation entropy), as expected for polyvalent assistance. When compared to each other, an apparent entropy-enthalpy compensation was also observed. While unlikely to be statistically significant, [45, 46] this is a uniquely interesting case: few studies of compensation have involved true catalysis (rather than the binding of catalysts), and none have examined activation parameters *vs.* variations in valency of substrates and catalysts.

Lastly, the relationship between solution viscosity and reaction rate was explored by measuring the rates of reactions of polyvalent **G3-S$_{31}$** substrate with hydrazine in the presence of varying catalysts (**G3-C$_{31}$, C$_2$, C**), in mixtures of glycerol and buffer up to 50% glycerol to increase solution viscosity.[47] While all observed rates were inversely dependent on glycerol content (Supporting information, Figure S23), the polyvalent catalyst was significantly less sensitive to increasing viscosity than the divalent or monovalent catalysts. The significant participation of a “rolling” interaction that does not require diffusion-induced collision of separated particles would be expected to produce such a result.

Conclusions

The interaction of polyvalent surfaces in a catalytic bond-forming function was shown here to be distinctive. It allowed for very rapid anthranilate-catalyzed hydrazone formation when both substrate and catalyst were displayed in a polyvalent manner, even though particle concentrations were low. Most interestingly, this type of process was found to be sensitive to functional group density, reaction viscosity, and temperature in unusual ways, consistent with the processive “rolling” of catalyst- and substrate-bearing particles with respect to each other. These findings illustrate a relatively simple way to enhance surface-based catalytic reactivity, and suggest the existence of a complex kinetic landscape with elements of homogeneous and heterogeneous catalytic features. We believe that additional examples await the construction of such systems in the laboratory and the discovery or appreciation of membrane- or biopolymer-arrayed catalysts and substrates in biology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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$^{-1}$ at pH 5.5; ref. 27). Since *p*-nitrobenzaldehyde should be a much more reactive electrophile than the 4-formylbenzamides, this suggests that the PAMAM dendrimer, and Dawson's peptide, may accelerate the reaction in other ways.

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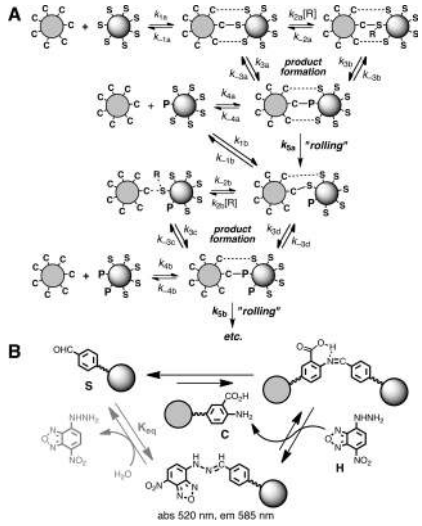

Figure 1.

Polyvalent catalysis of polyvalent substrates. (A) Overall catalytic scheme. Key: **C**=catalyst; **S**=substrate; **R**=capturing reagent such as nitrobenzoxadiazole hydrazine in the present case; **P**=product; $\boldsymbol{k_1}$, $\boldsymbol{k_{-1}}$ = initial association/dissociation rate constants; $\boldsymbol{k_3}$ = product-forming step; $\boldsymbol{k_{-3}}$ = catalyzed product decomposition step; $\boldsymbol{k_{-4}}$ = escape ≈ $\boldsymbol{k_{-1}}$; $\boldsymbol{k_5}$ = polyvalent association rate constant ("rolling" rate). Letter designations (k_{3a}, k_{3b}, etc.) denote the rates of the same fundamental step which may differ in different cycles, probably only slightly at early stages of reaction. Accelerated substrate transformation may occur if "rolling" (k_5) is much faster than diffusion controlled steps (k_{-4}, k_1). (B) Intermediates in the nucleophilic catalysis of hydrazone formation by monovalent or polyvalent anthranilic acid. In gray is shown the concomitant anthranilate-catalyzed hydrolysis of the hydrazone that establishes K_{eq}

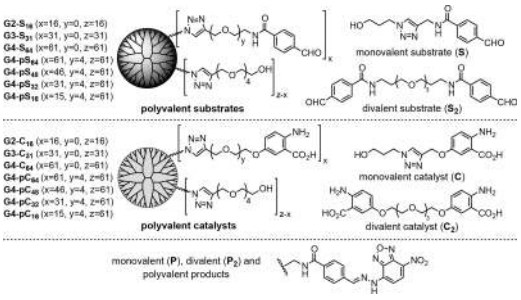

Figure 2.

Monovalent and dendrimer-based polyvalent substrates and catalysts. Functionalized dendrimers are designated by the following code: G#-$X_{valence}$, where G# is the dendrimer generation (G2, G3, or G4); X = functional unit displayed (S = benzaldehyde substrate with the short propargylamide linker from **2a**, pS = benzaldehyde substrate with the longer tetraglyme-based linker from **2b**, C = anthranilate with the short propargyl linker from **3a**, and pC = anthranilate with the longer tetraglyme-based linker from **3b**); valence = approximate number of substrate or catalyst units attached per dendrimer.

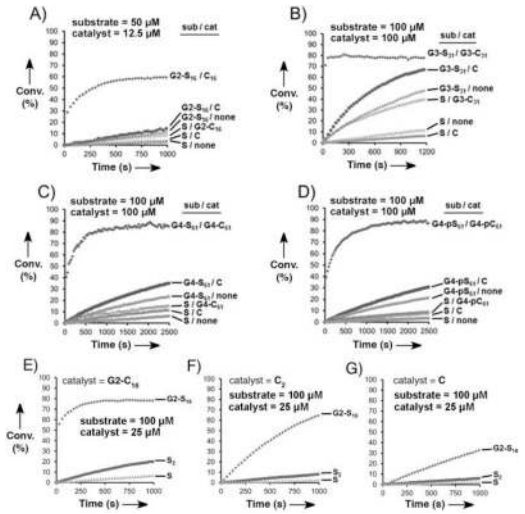

Figure 3.

Representative data for reactions of species shown in Figure 2. (A–D) Reactivity profiles of fully-loaded G2/short linker, G3/short linker, G4/short linker, and G4/long linker polyvalent catalysts and substrates in the presence of hydrazine H. (E–G) Reactions of monovalent, divalent, and G2-polyvalent substrates with monovalent, divalent, and G2-polyvalent versions of catalyst. All reactions were performed with 25 μM of hydrazine H; the concentrations of aldehyde and anthranilate groups are indicated in each panel.

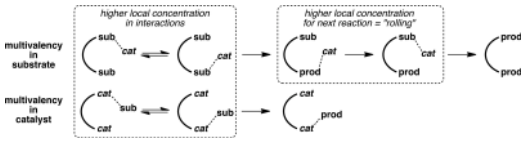

Figure 4.

Schematic representation of two different types of polyvalent advantage in catalysis. “sub” = substrate (aldehyde), “cat” = catalyst (anthranilate), “prod” = product (hydrazone).

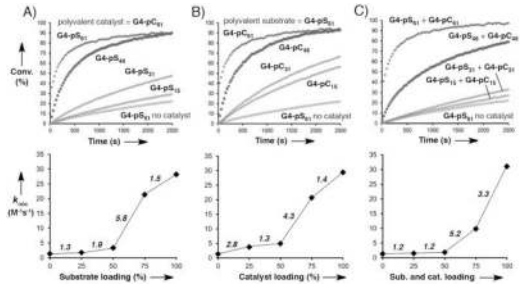

Figure 5.

Exploration of rate vs. density of substrate and catalyst on PAMAM scaffolds. (A) variation in substrate loading, (B) variation in catalyst loading, (C) simultaneous variation in substrate and catalyst loading. Plots of rates derived from these reactions are shown on the right; values in italics are the ratios of the rates of each reaction to the next reaction in the series with lower functional group density. All reactions were performed with 100 μM total substrate, 100 μM total catalyst, and 25 μM of hydrazine **H**. Similar results were observed in analogous experiments using 25 μM catalyst (Figure S16).

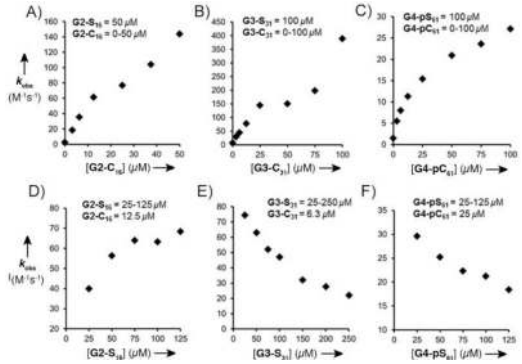

Figure 6.

Plots of observed rate constants *vs.* concentrations of fully-loaded polyvalent catalyst (A–C) or substrate (D–F), using G2/short linker, G3/short linker, and G4/long linker dendrimers. All reactions were performed with 25 μM of hydrazine **H**.

Table 1

Rate constants derived from plots shown in Figure 3A–D. [**H**] = 25 μM for all experiments.

Panel	Sub [a]	Cat [a]	k_{obs} ($M^{-1}s^{-1}$)	*rate ratio* [b]	*rate ratio* [c]
3A	**G2-S$_{16}$** [d]	**G2-C$_{16}$** [e]	66.5 ± 3.3	511.5	25.6
	G2-S$_{16}$ [d]	**C** [f]	3.2 ± 0.16	24.6	1.2
	G2-S$_{16}$ [d]	None	2.6 ± 0.13	20.2	1.0
	S	**G2-C$_{16}$** [e]	1.1 ± 0.05	8.5	0.8
	S	**C** [f]	0.41 ± 0.02	3.2	0.3
	S	none	0.13 ± 0.01	1.0	0.1
3B	**G3-S$_{31}$**	**G3-C$_{31}$**	391.0 ± 15.0	2607	59.2
	G3-S$_{31}$	**C**	14.0 ± 0.7	93.3	2.1
	G3-S$_{31}$	none	6.6 ± 0.3	44.0	1.0
	S	**G3-C$_{31}$**	8.1 ± 0.4	54.0	1.2
	S	**C**	0.3 ± 0.02	2.0	0.05
	S	none	0.15 ± 0.02	1.0	0.02
3C	**G4-S$_{61}$**	**G4-C$_{61}$**	30.1 ± 1.5	231.5	20.1
	G4-S$_{61}$	**C**	2.3 ± 0.11	17.7	1.5
	G4-S$_{61}$	none	1.5 ± 0.08	11.5	1.0
	S	**G4-C$_{61}$**	0.63 ± 0.03	6.0	0.4
	S	**C**	0.32 ± 0.02	3.2	0.2
	S	none	0.13 ± 0.01	1.0	0.09
3D	**G4-pS$_{61}$**	**G4-pC$_{61}$**	29.1 ± 1.5	223.8	22.3
	G4-pS$_{61}$	**C**	1.76 ± 0.09	13.5	1.4
	G4-pS$_{61}$	none	1.30 ± 0.08	10.0	1.0
	S	**G4-pC$_{61}$**	0.59 ± 0.04	4.5	0.5
	S	**C**	0.32 ± 0.02	2.5	0.2
	S	none	0.13 ± 0.01	1.0	0.1

[a] 100 μM unless otherwise noted.

[b] ratios of rates (k_{cat}/k_{uncat}) of the polyvalent reaction to the reaction of [**S** + **H**].

[c] k_{cat}/k_{uncat} relative to [polyvalent substrate + **H**] reaction.

[d] 50 μM.

[e] 12.5 μM.

[f] 25 μM.

Table 2

Comparison of reactions involving similar numbers of substrate and catalyst on different sized dendrimer scaffolds. Aldehyde and anthraniliate concentrations = 100 μM, unless noted otherwise.

Panel	Substrate	Catalyst [a]	k_{obs} ($M^{-1}s^{-1}$)	rate incr. [b]
5A	**G4-pS$_{15}$**	**G4-pC$_{61}$**	1.7 ± 0.09	1.1
5B	**G4-pS$_{61}$**	**G4-pC$_{15}$**	3.7 ± 0.19	2.5
5C	**G4-pS$_{15}$**	**G4-pC$_{15}$**	1.5 ± 0.08	1.0
S4F	**G2-S$_{16}$**	**G2-C$_{16}$** (50 μM)	143.9 ± 7.2	95.9
5A	**G4-pS$_{31}$**	**G4-pC$_{61}$**	3.3 ± 0.17	1.8
5B	**G4-pS$_{61}$**	**G4-pC$_{31}$**	4.8 ± 0.24	2.7
5C	**G4-pS$_{31}$**	**G4-pC$_{31}$**	1.8 ± 0.09	1.0
3B	**G3-S$_{31}$**	**G3-C$_{31}$**	391.0 ± 19.6	217.2

[a] 100 μM, unless noted otherwise.

[b] Fold rate constant increase relative to slowest reacting polyvalent scaffolds bearing similar number of reactive substrates and catalysts.