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Model systems for flavoenzyme activity: aromatic stacking in sol-gel matrices

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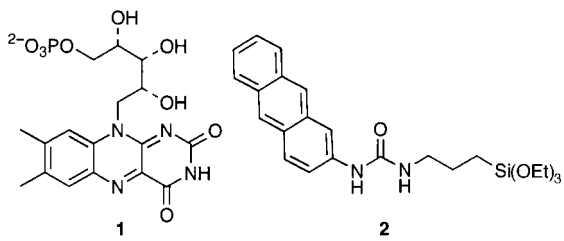
Silicate matrices effectively replicate both the isolation and preorganization found in the active sites of flavoenzymes.

The peptide scaffolding found in proteins performs two crucial roles in defining the active site environment. First, it provides isolation of this active site from unwanted interactions with water and other entities. Second, it presents reactive functionality in the geometry required for efficient recognition and catalysis. While researchers have been able to use solution-based model systems to explore key aspects of both isolation[1] and recognition,[2] the creation of models that simultaneously replicate both functions remains an intimidating prospect.[3]

One means of obtaining isolation is through the use of nanoporous[4] and mesoporous[5] media. In recent research we have demonstrated efficient hydrogen bond recognition of flavin mononucleotide (FMN)[6] in sol-gel silicates.[7] In these systems, incorporation of the flavin into the cybotactic region of the silicate effectively modeled the isolation provided by the active site. Hydrogen bond recognition of the flavin was then provided by doping with a diaminopyridine-based receptor, replicating the enzyme-cofactor hydrogen bonding found in the flavoenzymes.[8]

Aromatic–aromatic interactions are an important motif in flavoenzyme architecture (Fig. 1). As with hydrogen bonding, aromatic stacking plays a dual role in flavoenzyme function, providing efficient recognition of the flavin cofactor, and serving to modulate its reactivity.[9] In recent investigations, we created effective solution-phase models for enzyme–cofactor aromatic stacking.[10] We report here the use of silicate sol-gels to provide both isolation and preorganization for flavin–aromatic complexes, effectively replicating the role of the protein scaffolding.

Flavin-containing sols were prepared *via* the addition of dilute acid and tetraethyl orthosilicate (TEOS) to an aqueous solution of flavin mononucleotide (FMN) **1**, followed by



1 **2**

sonication to homogeneity. Aromatic stacking interactions were probed by addition of varying quantities of anthracene derivative *2* to the sol.[12] Sols were then poured into cuvettes and sealed: wet gels formed within 100 h.

The optical transparency of the silicate matrix allowed direct spectroscopic observation of aromatic stacking. As shown in Fig. 2, increasing quantities of receptor **2** resulted in decreased flavin fluorescence emission. This is consistent with solution-based studies, where we have established that aromatic stacking between the electron-rich anthracene and the electron-poor flavin effectively quenches flavin fluorescence.[13]

During the course of hydrolysis and gelation, there is negligible change (< 2%) in the volume of our sol-gels. We can therefore calculate an approximate pore volume by calculating

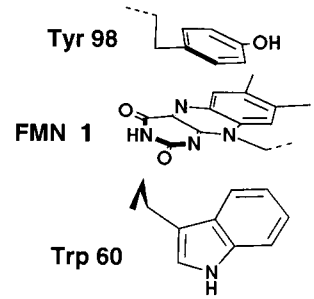
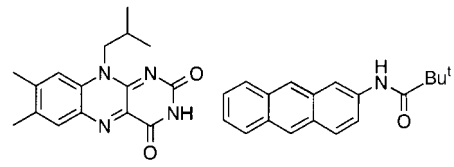


Fig. 1 Flavin binding site of the flavodoxin isolated from *Desulfovibrio vulgaris* (ref. 11).

the quantity of EtOH released during the hydrolysis of the orthosilicate. Assuming complete hydrolysis of TEOS, the final gel will be 17% silicate and 83% EtOH. Given this volume, the association constant for the FMN **1**–receptor **2** complex can be fitted to a 1 : 1 binding isotherm,[14] providing an estimated binding constant of 200 ± 50 M^{-1} (Fig. 3).[15] This is considerably higher than that observed for N^{10}-isobutylflavin **3**[16] with acylated aminoanthracene **4** (< 3 M^{-1}), demonstrating



3 **4**

an active role of the silicate matrix in preorganizing the flavin **1**–receptor **3** complex in the sol-gel. This result is consistent with our previous studies,[17] further demonstrating this unusual matrix-assisted recognition enhancement.

In summary, we have demonstrated that aromatic stacking to a sol-gel-bound flavin can be established through addition of an

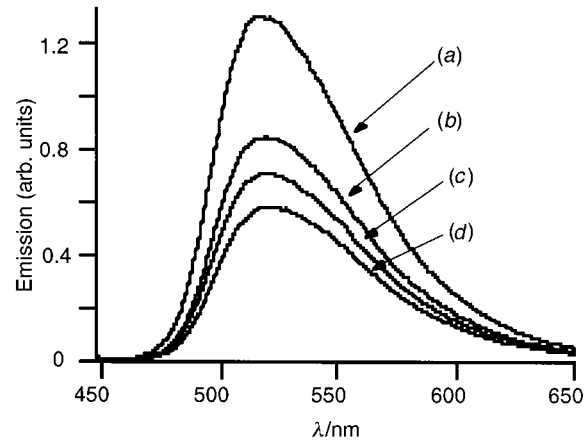


Fig. 2 Flavin fluorescence emission profiles of sol-gels containing FMN **1** and receptor **2**: (*a*) 0, (*b*) 10, (*c*) 20 and (*d*) 30 equiv. **2**. [FMN] = 0.0196 mmol dm^{-3}. Excitation wavelength = 445 nm; uncertainty of fluorescence values = ±5%.

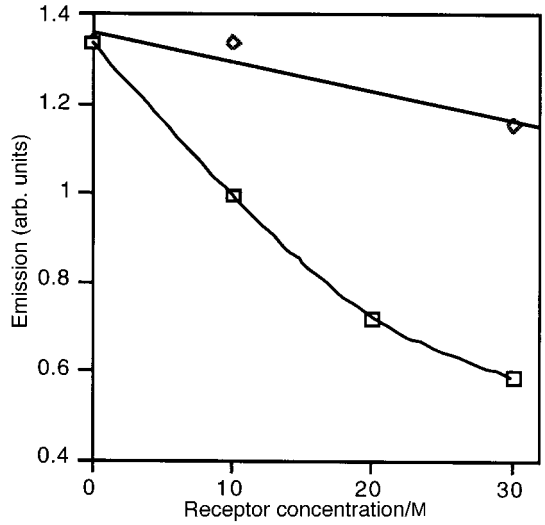


Fig. 3 Plot of flavin (**1**/**3**) fluorescence emission *vs.* receptor (**2**/**4**) concentration (□) **1** + **2** in sol-gel and (◇) **3** + **4** in EtOH. [FMN] = 0.0196 mmol dm^{-3}, [**3**] = 0.236 mmol dm^{-3} (calculated concentration of FMN inside the porous sol-gel matrix).

anthracene-based receptor. This interaction is greatly enhanced in the sol-gel relative to solution-phase studies. This indicates that the silicate matrix replicates the two-fold role of the protein scaffolding of flavoenzymes: isolation of the active site,[18] and preorganization of the active site functionality. Application of silicate sol-gels to the creation of functional flavoenzyme models is currently underway, and will be reported in due course.

Notes and references

1 D. J. Cram, *Nature*, 1992, **356**, 29; D. J. Cram, R. Jaeger and K. Deshayes, *J. Am. Chem. Soc.*, 1993, **115**, 10 111; R. Breslow, *Acc. Chem. Res*., 1995, **28**, 146; N. Branda, R. Wyeler and J. Rebek, *Science*, 1994, **263**, 1222; R. G. Chapman, G. Olovsson, J. Trotter and J. C. Sherman, *J. Am. Chem. Soc.*, 1998, **120**, 6252. For examples of molecular imprinting, see: B. Santora, A. Larsen and M. Gagne, *Organometallics*, 1998; **17**, 3138; S. Lee, I. Ichinose and T. Kunitake, *Langmuir*, 1998, **14**, 2857.

2 E. Gallo and S. Gellman, *J. Am. Chem. Soc.*, 1993, **115**, 9774; S. Zimmerman and T. Murray, *Tetrahedron Lett.*, 1994, 4077; J. Nowick, J. Chen and G. Noronha, *J. Am. Chem. Soc.*, 1993, **115**, 7636; G. Das and A. Hamilton, *J. Am. Chem. Soc.*, 1994, **116**, 11 139.

3 For dendritic systems that combine isolation and recognition. see C. Gorman, B. Parkhurst, W. Y. Su and K.-Y. Chen, *J. Am. Chem. Soc.*, 1997, **119**, 1141; S. Zimmerman, Y. Wang, P. Bharathi and J. Moore, *J. Am. Chem. Soc.*, 1998, **120**, 2172.

4 K. Balkus, L. Ball, B. Gnade and J. Anthony, *Chem. Mater.*, 1997, **9**, 380; Y. Xu and C. Langford, *J. Phys. Chem. B*, 1997, **101**, 3115; K. Yoon, Y. Park and J. Kochi, *J. Am. Chem. Soc.*, 1996, **118**, 12 710; S. Vasenkov and H. Frei, *J. Am. Chem. Soc.*, 1998, **120**, 4031. For a recent review see: R. J. Gorte, *Recent Advances and New Horizons in Zeolite Science and Technology. Studies in Surface Science and Catalysis, No. 102*, ed. H. Chon, S. Woo and S.-E. Park, Elsevier, Amsterdam, 1996.

5 D. Avnir, D. Levy and R. Reisfeld, *J. Phys. Chem.*, 1984, **88**, 5956; U. Narang, J. D. Jordan, F. V. Bright and P. N. Prasad, *J. Phys. Chem.*, 1994, **98**, 8101; F. Nishida, J. M. McKiernan, B. Dunn, J. I. Zink, C. J. Brinker and A. J. Hurd, *J. Am. Ceram. Soc.*, 1995, **78**, 1640; L. M. Ilharco, A. M. Santos, M. J. Silva and J. M. G. Martinho, *Langmuir*, 1995, **11**, 2419; K. Y. Parish, D. Habibi and V. Mohammadi, *J. Organomet. Chem.*, 1989, **369**, 17; A. Slama-Schwok, D. Avnir and M. Ottolenghi, *J. Am. Chem. Soc.*, 1991, **113**, 3984; A. Rosenfeld, D. Avnir and J. Blum, *J. Chem. Soc., Chem. Commun.*, 1993, 583. For recent reviews, see D. Avnir, *Acc. Chem. Res.*, 1995, **28**, 328; B. Dunn and J. I. Zink, *Chem. Mater.*, 1997, **9**, 2280.

6 For other studies of encapsulated biomolecules, see: L. Ellerby, C. Nishida, F. Nishida, S. Yamanaka, B. Dunn, J. Valentine and J. I. Zink, *Science*, 1992, **255**, 1113; S. Braun, S. Shtelzer, S. Rappoport, D. Avnir and M. Ottolenghi, *J. Non-Cryst. Solids*, 1992, **147** and **148**, 739; I. Pankratov and O. Lev, *J. Electroanal. Chem.*, 1995, **393**, 35.

7 For a detailed review of the fundamentals of sol-gel technology, see: C. J. Brinker and G. W. Scherer, *Sol-Gel Science*, Academic Press, San Diego, 1990; L. Hench and J. West, *Chem. Rev.*, 1990, **90**, 33; A. M. Buckley and M. Greenblatt, *J. Chem. Educ.*, 1994, **71**, 599.

8 E. Breinlinger, A. Niemz and V. Rotello, *J. Am. Chem. Soc.*, 1995, **117**, 5379.

9 B. Stockman, T. Richardson and R. Swenson, *Biochemistry*, 1994, **33**, 15 298.

10 E. Breinlinger and V. Rotello, *J. Am. Chem. Soc.*, 1997, **119**, 1165.

11 W. Watt, A. Tulinsky, R. P. Swenson and K. D. Watenpaugh, *J. Mol. Biol.*, 1991, **218**, 195.

12 Synthesized in 75% yield from 2-aminoanthracene and 3-(triethoxysilyl)propyl isocyanate.

13 *N*-Alkylurea analogs of **2** shows essentially identical quenching behavior as for **2**, demonstrating that hydrogen bonding to the urea is not involved in flavin recognition.

14 Solution studies have shown that additional anthracene–flavin interactions have little effect on flavin fluorescence, allowing selective monitoring of the 1 : 1 complex. The large excesses of receptor **2** used should minimize the effect of 2 : 1 receptor flavin binding on the receptor available for the first binding event.

15 The uncertainty presented is the asymptotic standard error. As this system is not in the standard state, the calculated K_a value is for comparison purpose only.

16 N^{10}-Isobutylflavin **3** was used for these studies due to the low solubility of **1** in EtOH.

17 M. Greaves and V. Rotello, *J. Am. Chem. Soc.*, 1997, **119**, 10 569.

18 At the concentrations of flavin **1** used in these sol-gel experiments, there is no deviation from ideal fluorescence dependence on concentration. This indicates that there are no flavin–flavin interactions occurring, demonstrating site isolation of the flavin species.

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