Molecular recognition of oxoanions based on guanidinium receptors

Pascal Blondeau,[a] Margarita Segura,[a] Ruth Pérez-Fernández†[a] and Javier de Mendoza*[ab]

Received 1st September 2006
First published as an Advance Article on the web 9th November 2006
DOI: 10.1039/b603089k

Guanidinium is a versatile functional group with unique properties. In biological systems, hydrogen-bonding and electrostatic interactions involving the arginine side chains of proteins are critical to stabilise complexes between proteins and nucleic acids, carbohydrates or other proteins. Leading examples of artificial receptors for carboxylates, phosphates and other oxoanions, such as sulfate or nitrate are highlighted in this *tutorial review*, addressed to readers interested in biology, chemistry and supramolecular chemistry.

1. Introduction

Nature frequently uses guanidinium moieties to coordinate different anion groups. Present in the side chain of the amino acid arginine, the guanidinium group forms strong ion-pairs with oxoanions such as carboxylates or phosphates in enzymes and antibodies, and it also contributes to the stabilisation of protein tertiary structures *via* internal salt bridges, mainly with carboxylates.[1] Not surprisingly, guanidinium-based compounds are found in many drugs and have been extensively used in molecular recognition studies, leading to the design and synthesis of various receptors for anions.[2]

The capacity of the guanidinium group to bind oxoanions is due to its geometrical Y-shaped, planar orientation, which directs the hydrogen bonding, and to its high pK_a value (around 12–13),[3] which ensures protonation over a wide pH range. The positive charge is delocalized over the three nitrogen atoms, and four out of the five hydrogen bond donors present in the guanidinium group of arginine can complement bidentate oxoanion acceptors, along the two edges available (Fig. 1). This accounts for the geometrical versatility of the binding modes. From the energy point of view, binding to oxoanions results from both ion-pairing and hydrogen bonding, and this turns out to be a difficult challenge in highly polar solvents or in water. In fact, the binding energy arises from the difference of the energy released by the host–guest interactions and the energy penalty necessary to remove the solvation shell around the host, which is quite high in water.

In proteins, the guanidinium–oxoanion interaction usually occurs inside hydrophobic pockets or in areas of low dielectric constant. On the contrary, in artificial synthetic systems designed to work in water or polar solvents, complexation takes place in an environment more exposed to solvation effects which compete with the donor and acceptor sites, causing a substantial decrease of the binding. This is usually overcome by increasing the number of charges or hydrogen bond donors or by the design of more sophisticated receptors

[a]*Institute of Chemical Research of Catalonia (ICIQ), 43007 Tarragona, Spain*
[b]*Department of Organic Chemistry, Universidad Autónoma de Madrid, 28049 Madrid, Spain*
† Current address: Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, United Kingdom.

Pascal Blondeau

Pascal Blondeau was born in Le Mans, France in 1978. He completed his MSc degree at University of Montpellier, France, in 2002 working on hybrid self-organized materials applied to transport biomimetism. He is currently doing his PhD under the supervision of Prof. Javier de Mendoza at ICIQ in Tarragona. His research interests involve the design and synthesis of chiral guanidinium receptors for molecular recognition of anions, enantioselective recognition of carboxylic acid derivatives as well as transport of amino acids.

Margarita Segura

Margarita Segura was born in Leiden, The Netherlands in 1969. She received her MSc degree in chemistry (1993) from the University of Granada, Spain and her PhD degree (1998) from the Universidad Autónoma (Madrid), supervised by Prof. Javier de Mendoza, working on molecular recognition of oxoanions from phosphodiesters, uronic acids and dipeptides. After postdoctoral research at the University of Parma, Italy (1998–2000) working on carbohydrate recognition in water in the group of Prof. Rocco Ungaro, she again joined de Mendoza's group in Madrid as a postdoctoral researcher (2000–2003) leading a project on supramolecular donor–acceptor electroactive systems linked by multiple hydrogen-bonding. Since 2004 she has been the Group Coordinator at Prof. de Mendoza's group at ICIQ in Tarragona.

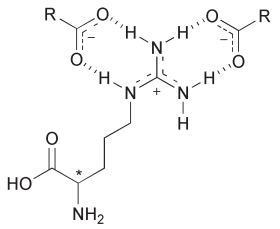


Fig. 1 The guanidinium group of arginine and its two possible binding modes with carboxylates.

where the access to the solvent is restricted. In this review, several examples on how this has been achieved in natural systems and in artificial guanidinium receptors are provided.

2. Guanidinium–oxoanion ion pairs in proteins and nucleic acids

2.1. Proteins

Protein structure has been at the forefront of research studies with the goal of better understanding the function of these biomolecules in the chemistry, physiology and pathology of the cell. Proteins are remarkably flexible and susceptible to the influence of the environment. Both intramolecular and intermolecular interactions involving the protein and the solvent define the native conformation.[4] To perform its function, a protein has to fold properly, a task where the various intra-protein or inter-protein interactions, as well as the interactions of the protein with metals or other molecules (such as co-factors, lipids or carbohydrates), are essential elements of control. An illustrative example of misfolding is the prion protein, which results in aggregated copies of the protein causing the "Mad Cow Disease" deadly condition.[5] Hydrogen-bonded salt bridges, such as those involving guanidinium–carboxylate, are relevant contributors to α-helical stabilization and sometimes destabilization of peptides and proteins.[6]

Guanidinium salt bridges play also important roles in enzyme active sites. Typical examples are carboxypeptidase A,[7a] creatine kinase,[7b] fumarate reductase,[7c] and malate dehydrogenase.[7d]

Protein–protein hetero-dimerization processes are often mediated by salt bridges involving arginine on one molecule and phosphorylated amino acids on the other. For example, phosphorylation of the OH group of a serine residue in a receptor enables the simultaneous interaction with two adjacent arginine residues of another receptor. On the other hand, phosphorylation of serines (or threonines) adjacent to the arginines of the same molecule slows down the attraction between the receptors.[8]

A related case is the involvement of the guanidinium group in cell–cell and cell–matrix adhesion motifs such as the tripeptide sequence RGD (arginine-glycine-aspartate). Adhesive proteins like fibronectin, osteopontin, vitronectin and collagens display the RGD sequence at their cell recognition site in extracellular matrices,[9] which is recognized by at least one member of the structural related integrins, a family of α,β hetero-dimeric transmembrane cellular receptors (Fig. 2).[10] On the cytoplasmatic side of the plasma membrane, the receptors connect the extracellular matrix to the cytoskeleton.

Thus, osteopontin (OPN), a multifunctional phosphorylated glycoprotein recognized as a key molecule in a multitude of biological processes such as bone mineralization or cancer metastasis, contains an integrin-binding RGD sequence. A significant regulation of OPN function is mediated through post-translational phosphorylation and glycosylation, a process that is essential for osteoclast attachment.[11] Osteoclasts are cells that actively reabsorb old bones so that a new bone may be replaced. Osteoporosis (bone loss) occurs when osteoclasts reabsorb bone faster than the osteoblasts cells are producing it.

Ruth Pérez-Fernández

Ruth Pérez was born in Madrid, Spain in 1975. She obtained her degree in chemistry (organic chemistry) at the Universidad Autónoma (Madrid). She was awarded a European PhD at the same university in October 2005 under the supervision of Prof. J. de Mendoza and Prof. P. Prados working in the field of protein–ligand interactions, self-assembly and dynamic evolution based on guanidinium–oxoanion interactions. In November 2005 she joined Prof. Jeremy Sanders' group at the University of Cambridge as a postdoctoral researcher and her current work concerns molecular recognition using a dynamic combinatorial approach.

Javier de Mendoza

Javier de Mendoza (Barcelona, Spain 1944) is Professor of Organic Chemistry at Universidad Autónoma (Madrid) and Group Leader at the ICIQ (Tarragona). He obtained MSc and PhD degrees in Pharmacy (Barcelona, 1967 and 1971). He has been Assistant Professor (1971–1975), Associate Professor (1975–1980) and Full Professor at the Universities of Barcelona, Bilbao, Alcalá de Henares and Autónoma (Madrid). He pursued a postdoctoral stay (1971–1972) in Montpellier (France) under the supervision of Prof. Robert Jacquier and Dr José Elguero. He is Chevalier de l'Ordre du Mérite of France since 1994 and he was awarded in 1999 with the Research National Prize and Medal of the Spanish Royal Chemical Society. Javier de Mendoza pioneered the introduction of Supramolecular Chemistry in Spain and his current research interests range from molecular recognition to calixarene chemistry, self-assembly and catalysts design.

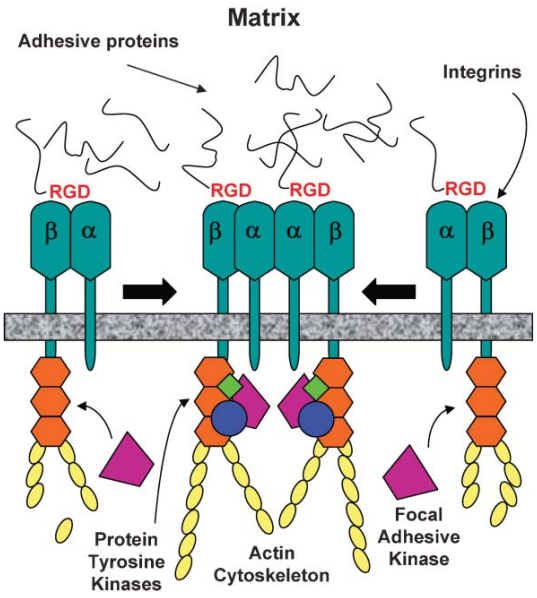


Fig. 2 Scheme of the binding of the RGD sequence to integrins in cell–cell and cell–matrix adhesion processes.

2.2. Nucleic acids

Proteins that interact with nucleic acids have a key role in biological processes. They are necessary for the control of the genetic information, replication, packaging and protection. Arginine is again essential for the interaction of proteins with DNA. In the nucleosome, in which the DNA winds around the arginine-rich histone, the amino acid side chains clearly show a direct interaction with the DNA phosphodiester chains (Fig. 3).[12] Due to the diversity of binding modes in this system, the 39 arginine residues present in the four histone proteins forming the nucleosome core may be divided into three groups: a first group of 20 arginine residues involved in histone–histone interactions not contacting DNA, followed by 7 arginines which enter the minor groove of DNA and are essential for histone–DNA binding; and a final group of 12 arginines which show direct guanidinium–phosphate salt bridge interaction. Methylation of arginine residues in the histone core leads to a conformational change allowing DNA transcription.[13] In this way, the transcription of genes can be regulated.

3. Guanidinium-based artificial receptors for oxoanions

Lehn and co-workers first reported in the late 1970's guanidinium-containing macrocycles for the recognition of phosphate PO_4^{3-} in water.[14] The weak association constants (K_a = 50 (**1**), 158 (**2**) and 251 (**3**) M^{-1}, pH titrations) can be explained in terms of the more delocalised charge of guanidinium over ammonium and accounts for the electrostatic prevailing interaction.

The guanidinium can be incorporated into a bicyclic framework (Fig. 4a) in order to improve its solubility in apolar solvents, where the hydrogen bonds are stronger, and to avoid the *anti* conformation, not suitable for hydrogen-bonding to oxoanions (Fig. 4b). As a result, the hydration of the cation is reduced and the conformational freedom restricted. Inserted into a decaline framework, the guanidinium cation becomes therefore an almost ideal complement for oxoanions, since both NH protons are docking sites for the two *syn* lone pairs of the oxoanion. The resulting ionic

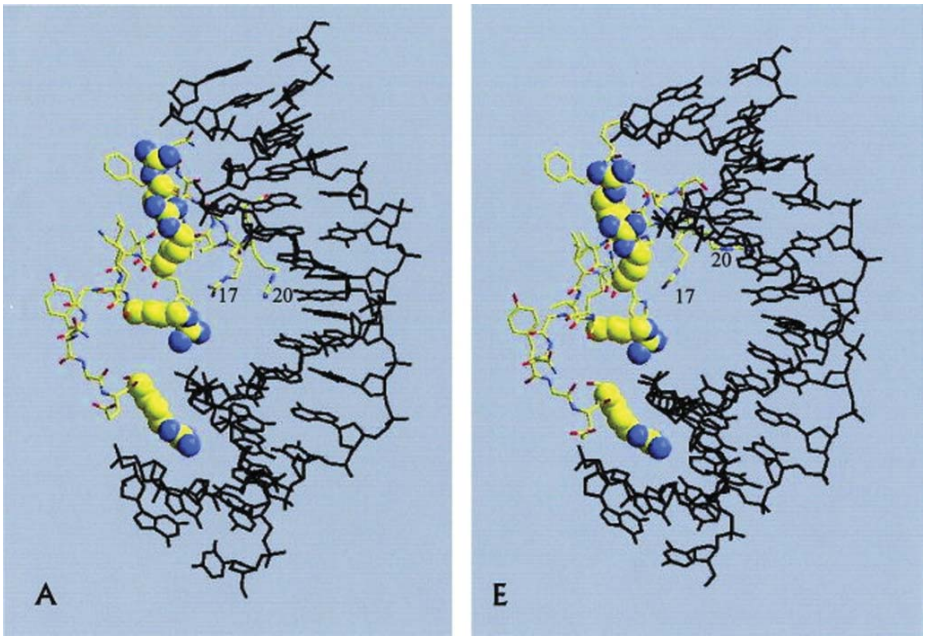
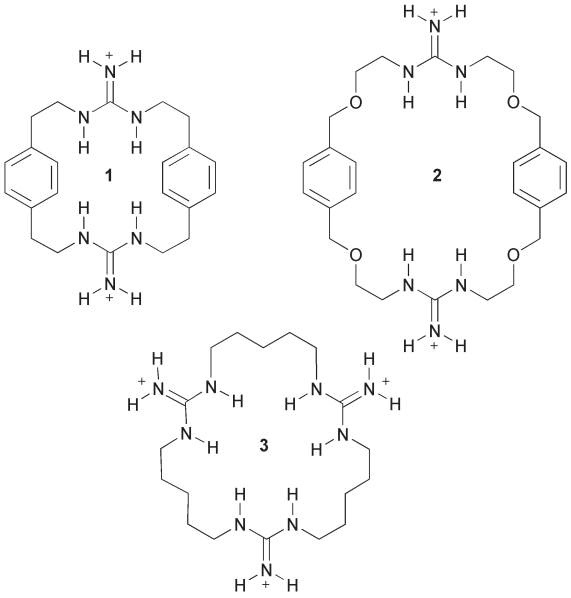


Fig. 3 Stereoview of a double stranded DNA interacting with arginines of H2A histones along the major groove. (Reprinted with permission from Subirana *et al.*[12] *Biopolymers*, 2003, **69**, 432–439. Copyright (2003) Wiley Periodicals, Inc.)



DD-AA (donor-donor–acceptor-acceptor) hydrogen-bonded complex is particularly stable and geometrically well defined. Due to the large pK_a difference between guanidinium and carboxylic acids (*ca.* 9 pK_a units in water) a trans-protonation that would destroy the salt bridge and give a less robust AD-DA hydrogen bond interaction[15] is unlikely, although it could occur in non-polar solvents, where the differences in pK_a are substantially reduced. Finally, C_2 symmetry can be introduced into the molecule by two stereogenic centres at the vicinal atoms, allowing chiral recognition of the oxoanion guest. Such a chiral bicyclic guanidinium binding subunit can be conveniently prepared in multigram quantities in nine steps from chiral amino acids (asparagine and methionine).[16]

The association constant between bicyclic guanidinium derivatives and carboxylates are quite high in chloroform or apolar solvents. Thus, UV titrations between **4** (tetraphenylborate salt) and tetrabutylammonium (TBA) *p*-nitrobenzoate

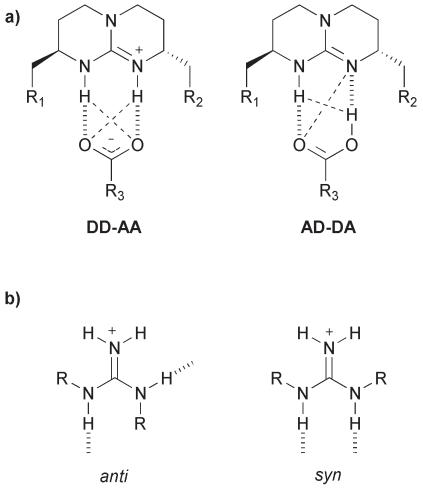
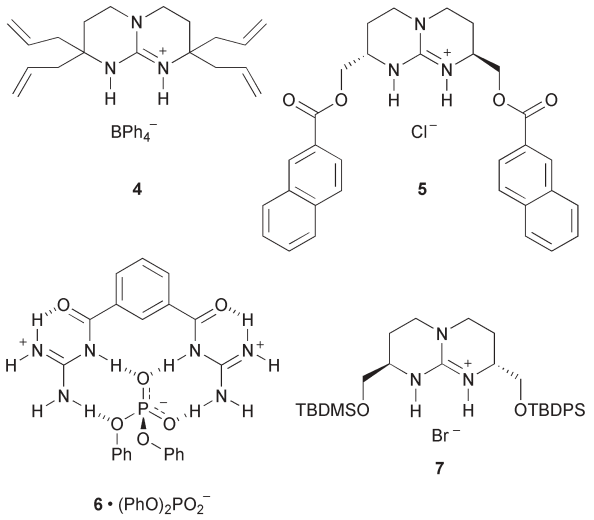


Fig. 4 a) Chiral bicyclic guanidinium receptor. b) *Anti* and *syn* conformations of guanidinium group.

gave $K_a = 7 \times 10^6$ M^{-1}.[17] The crystal structure of an acetate salt confirmed the formation of two strong symmetric hydrogen bonds between the host and the guest (N···O 2.850 Å). This first binding study confirmed the good match of oxoanions by guanidinium receptors through ion pair and a linear array of hydrogen bonds in apolar solvents.

We developed receptor **5** (chloride as counterion) for aromatic carboxylates, but the stability constant with TBA *p*-nitrobenzoate was much lower ($K_a = 1.6 \times 10^3$ M^{-1}, ^{1}H NMR titrations in $CDCl_3$).[18] This example illustrates the competition with the initial counterion and the importance of the counterion in binding strength: in this case the tetraphenylborate counterion results in significantly weaker binding than chloride. Thus, poorly coordinating counterions such as hexafluorophosphate or tetraphenylborate are necessary if strong binding constants are desired.

The strong deshielding of the NH signals in the ^{1}H NMR spectrum of **5**·*p*-nitrobenzoate indicates the presence of hydrogen bonds. Moreover, stacking interactions between the naphthoyl side arms and the *p*-nitrophenyl moiety are evidenced by the shifting of the aromatic signals. Despite their ionic character, hosts **4** and **5** are insoluble in water but soluble in chlorinated solvents. Thus, liquid–liquid extractions of water solutions of carboxylate salts give quantitatively the ion pair in the organic solvent, free from any competing ion.



Hamilton and co-workers synthesised bis-acylguanidinium salt **6** as a receptor for phosphodiesters. The binding constant with TBA diphenylphosphate ($K_a = 4.6 \times 10^4$ M^{-1}, measured by UV in CH_3CN), was one order of magnitude higher than with a simpler benzoylguanidinium tetraphenylborate.[19] The carbonyl groups contribute to the binding in two ways: they increase the acidity of the guanidinium NHs (but not to such an extent that trans-protonation can occur) and they pre-organise the host by intramolecular hydrogen bonds (chelation effect). The combination of these two factors and the additional hydrogen bonding from the guanidinium groups allows strong complexation in more polar solvents, such as acetonitrile.

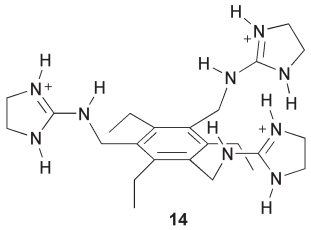
Schmidtchen studied guanidinium–carboxylate interactions by isothermal titration calorimetry (ITC).[20] The isotherm

binding curve between **7** (bromide) and tetraethylammonium acetate in acetonitrile ($K_a = 2.0 \times 10^5$ M^{-1}) revealed that the process was both entropically and enthalpically favourable for a 1 : 1 complex. Although thermodynamic parameters could be determined in both CH_3CN and DMSO, the reaction in MeOH produced too little heat to allow quantification of the association constant. This result shows that the stabilization of the guanidinium–carboxylate is not only due to the strong electrostatic interactions (ΔH°) but also to a favourable release of solvent molecules (ΔS°), which strongly emphasises the importance of solvation in host–guest interactions, a factor often neglected in receptor design.

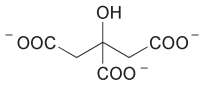
The thermodynamic aspects of dicarboxylate recognition by artificial receptors with increasingly acidic hydrogen bond donor groups such as two ureas (**8**), thioureas (**9**), or guanidiniums (**10** and **11**) in polar solvents (from DMSO to water) were studied by Hamilton (Fig. 5).[21]

As expected, association constants with carboxylate groups (**12** and **13**) increase with hydrogen acidity but are decreased in more polar solvents. While guanidinium–carboxylate association in DMSO is enthalpically driven, in more polar solvents such as methanol or water the association becomes an entropically driven process due to the liberation of solvent molecules upon binding.

Anslyn and co-workers developed receptor **14**, with three guanidinium moieties into a 1,3,5-triethyl-2,4,6-trimethylbenzene preorganized tripod platform,[22] showing selective binding towards citrate **15** in pure water ($K_a = 6.9 \times 10^3$ M^{-1}, ^{1}H NMR titrations). The host was able to complex citrate even from a crude extract of orange juice, which highlights its selectivity relative to other carboxylates. This receptor shows how the solvent competition can be overcome by accumulation of hydrogen bond donors (three guanidinium subunits) in a suitable fashion.

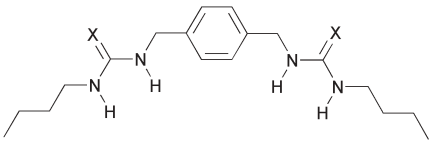


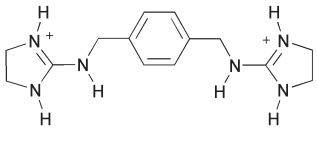
14

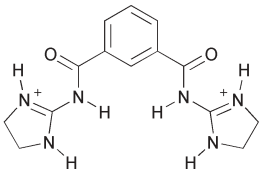


15

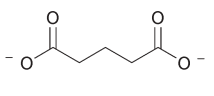
The same principles inspired Schmuck's 2-(guanidiniocarbonyl)-1*H*-pyrroles (Fig. 6), designed to complex carboxylate groups in highly competitive media, such as water.[23] Whereas the simple guanidinium cation **16** ($pK_a = 13$) does not show any sign of complexation with carboxylates in aqueous DMSO, the increased acidity of the acylguanidinium **17** ($pK_a = 7$–8), rises the binding affinity ($K_a = 50$ M^{-1}). An additional hydrogen bond from the pyrrole NH (as in **18**) increases the association significantly ($K_a = 130$ M^{-1}) and the additional amide group (**19**) adds a further hydrogen donor well oriented to reach the *anti* oxygen lone pair ($K_a = 770$ M^{-1}). The predicted geometries have been confirmed by X-ray



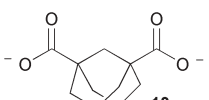




		ΔG kcal mol^{-1}	ΔH kcal mol^{-1}	ΔS cal mol^{-1} K^{-1}
			DMSO	
8 X = O	**12**	-3.1	-2.5	+5.9
	13	-3.3	-4.5	+0.1
9 X = S	**12**	-3.9	-4.1	+4.3
	13	-4.1	-5.9	+0.6
			MeOH	
10	**12**	-3.4	+3.7	+28
	13	-3.9	+4.0	+32
			MeOH	
11	**12**	-3.8	+4.0	+31
	13	-4.1	+4.4	+34



12



13

Fig. 5 Urea- (**8**), thiourea- (**9**) and guanidinium-based (**10–11**) receptors and association data for dicarboxylates **12** and **13** by isothermal titration calorimetry.

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.