

ChemComm

Chemical Communications

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: I. Alfonso, *Chem. Commun.*, 2024, DOI: 10.1039/D4CC03163F.



This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.

ARTICLE

Supramolecular Chemical Biology: Designed Receptors and Dynamic Chemical Systems

Ignacio Alfonso*^aReceived 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

Supramolecular chemistry focuses on the study of species joined by non-covalent interactions, therefore dynamic and relatively ill defined structures. Despite being a well-developed field, it has to face important challenges when dealing with the selective recognition of biomolecules in highly competitive biomimetic media. However, supramolecular interactions reside at the core of chemical biology systems, since many processes in Nature are governed by weak, non-covalent, strongly dynamic contacts. Therefore, there is a natural connection between these two research fields, which are not frequently related or sharing interests. In this feature article, I will highlight our most recent results in the molecular recognition of biologically relevant species, following different conceptual approaches from the most conventional design of elaborated receptors to the less popular dynamic combinatorial chemistry methodology. Selected illustrative examples from other groups will be also included. The discussion has been mainly focused on systems with potential biomedical applications.

Introduction

Supramolecular chemistry is a mature discipline that has found applications in different fields of science and technology, from materials science, energy or biomedicine.¹ From its foundation, supramolecular chemistry has used Nature as a source of inspiration since natural chemical species are often held by non-covalent contacts.² Just to mention some examples, we can recall the complementary base pairing of nucleic acids, the self-assembly of lipids in cell membranes and the specific recognition of proteins towards small molecules (enzyme substrates or cofactors) or other proteins (microtubules or protein-protein interaction regions). Thus, also supramolecular concepts and approaches are at the heart of chemical processes within biological environment.³ On the other hand, in the last decades, the study and modulation of biological processes with chemical tools has experienced an important development, as a way to improve their understanding at molecular level.⁴ This knowledge paves the way towards more personalized and selective tools for both early diagnosis and efficient therapies.⁵ Accordingly, there is a close connection between the concepts and methodological approaches of supramolecular chemistry and chemical biology.^{6,7} By promoting a parallel development in fundamental ideas and in experimental or theoretical approaches, both subdisciplines would be mutually benefitted and improved.⁸ This feature article proposes to facilitate a

dialogue between these subareas for a common development in order to achieve some of the current goals in chemical biology by taking advantage from the supramolecular chemistry knowledge. As a payback, the main challenges in supramolecular chemistry could be faced by considering biomimetic benchmark systems, also leading to real-life applications in different areas related to healthcare and well-being.

Main challenges of supramolecular chemistry in biological systems

In spite of the important development of supramolecular chemistry since its early definition, challenges are still evident. Thus, for the molecular recognition or self-assembly of non-covalent complexes, these must desolvate in order to expose the corresponding and complementary binding epitopes (Fig.1). This unavoidable need becomes a true hurdle in the case of biological environments. The main solvent in biology is water, a highly polar, H-bond donor and acceptor solvent and accordingly very competitive for most polar non-covalent interactions.⁹ Thus, hydration energies are often very high for bio-relevant as targets, meaning a first barrier to overcome for molecular recognition to be done in pure water.¹⁰ Moreover, abiotic fully synthetic receptors commonly suffer from low solubility, aggregation propensity or undesired folding promoted by the presence of water (due to the well-known hydrophobic effect).¹¹ These facts make the molecular recognition process challenging in pure water. Additional issues source from the accurate control of important parameters in aqueous environments, such as the pH of the medium or the presence of competing salts (ionic strength), which could preclude the suitable host-guest matching by protonation-

^a Department of Biological Chemistry, Institute for Advanced Chemistry of Catalonia (IQAC), The Spanish National Research Council (CSIC) Jordi Girona 18-26, 08034, Barcelona (Spain).
E-mail: ignacio.alfonso@iqac.csic.es

† Footnotes relating to the title and/or authors should appear here. Supplementary Information available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x



deprotonation processes or shielding of the electrostatic interactions.³

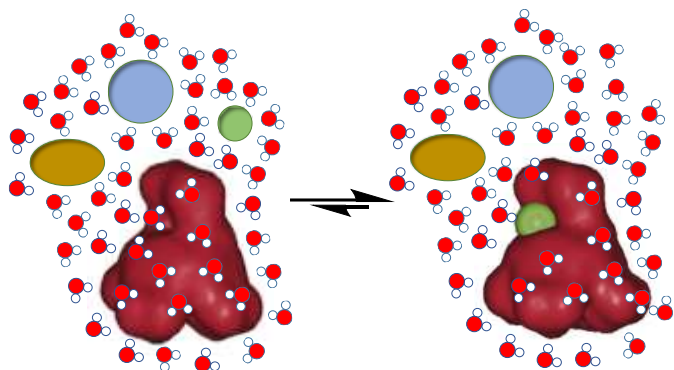


Fig. 1. Schematic representation of the challenges for biomolecular recognition: strength, selectivity and competition of the aqueous medium.

Apart from aqueous media, other environments are key for biological processes. For instance, cellular or organelle membranes are dynamic heterogeneous media with a polarity gradient that goes from an ion-rich surface to a hydrophobic core that resembles the polarity of aliphatic alkanes. Besides, these hydrophobic barriers are present in any type of chemical biology work, whether the molecular recognition occurs within biological membranes or in the aqueous cytosol. Thus, for a synthetic receptor or ligand to play a role inside the cell, this must cross the cell membrane taking advantage of the multiple mechanisms available.¹²

Another important hurdle in supramolecular chemical biology studies is the efficiency of the molecular recognition process, which is closely related to the effective concentration of the systems in the place of action. The binding constants needed for a molecular recognition useful in biological media must be in the same orders of magnitude of those already present in the corresponding biological networks. Despite strong host-guest systems reported in literature,¹³ typical binding processes in conventional supramolecular chemistry are in the range of millimolar values of the dissociation constants, which is often insufficient for the potency required in chemical biology.¹⁴ This handicap is additionally complicated by the fact that in biological systems, apart from the thermodynamic strength of the complexes, the exchange rate of the binding must be considered.¹⁵ The dynamic nature of the biological systems is closely connected with probably the challenge most difficult to achieve in supramolecular chemical biology: selectivity. Biological processes are held by a complex network of interacting species, crowded and confined within the cell volume. This makes necessary to design the corresponding supramolecular species able to bind the given target within a dense soup of competing species. High selectivity leads to specificity, which is a key factor in this scenario. For instance, high selectivity of recognition of a substrate as an inhibitor of a biological pathway reduces the potential appearance of side effects when developing hits for therapeutic applications. Conversely, in case of designing a putative supramolecular

sensor,^{16,17} specificity is fundamental for a diagnostic tool avoiding false positives or the absence of signal in real samples. Considering the complex process for designing supramolecular systems able to work in biological environments, the rate for success is often low, starting from tedious long syntheses with frustrating molecular recognition outcomes. In our own experience, it is advisable taking into account the inner complexity of biology as a designing conceptual parameter.^{18,19} In this feature article, examples using two main approaches will be discussed. In general, the more conventional design of receptors is time consuming but more predictable results can be obtained when starting from well-established moieties or frameworks. Alternative approaches using dynamic covalent chemistry^{20,21} have the advantage of less synthetic effort, since the target itself selects the best receptor. On the contrary, results are more difficult to predict and many control experiments must be planned to avoid chasing false positives. Thus, dynamic combinatorial chemistry (DCC)²² proposes the creation of a dynamic mixture of interconverting species (a Dynamic Combinatorial Library, DCL) made of building blocks joined by dynamic covalent bonds. The DCL is responsive to stimuli, and the presence of a given target biomolecule (a template) will shift the DCL to the preferred formation of the best binder (Fig. 2). This methodology allows the identification of receptors for either small molecules and ions, or larger biomacromolecules.²³ However, due to the dynamic and reversible nature of the chemical bonds joining the building blocks of the DCL, these must be either frozen or substituted by a robust and non-exchangeable isosters. For instance, imines are usually reduced to the corresponding amines and disulphides can be substituted by thioethers. This process can modify the observed selectivity or binding properties of the initially amplified members, meaning a potential drawback of the methodological approach.

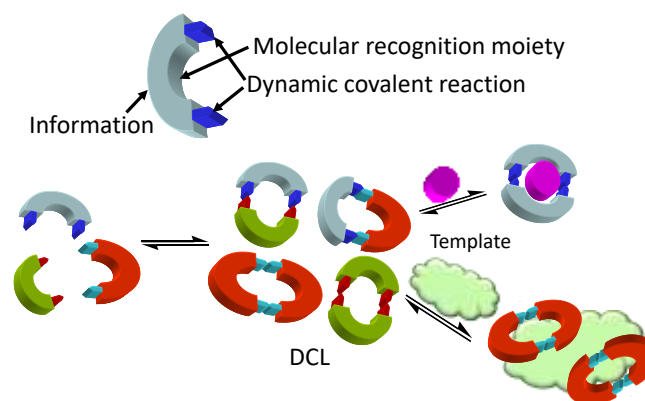


Fig. 2. Dynamic Combinatorial Chemistry (DCC) approach to the identification of hosts for either small molecules and ions or larger biomacromolecules, used as template.

Molecular recognition of ions

Ions play a fundamental role in many biological processes, such as the maintenance of cellular homeostasis or the membrane potential. Accordingly, its importance in the normal function of



the cell is related to serious diseases in case of failure. The ion imbalance mechanism can be exploited for the design and development of potential drugs.^{24–26} A prototypical example is the recognition and transport of chloride through lipid bilayers as a model for the cell and organelle membranes.²⁵ Recently, other ions have also attracted attention.^{27–30} This topic has become a very active research topic that combines anion molecular recognition approaches with membrane biophysical assays.³¹ The complexity of the systems is reflected in the fact that the process is not fully understood³² nowadays and relevant steps are needed in that direction.^{33,34} The development of efficient ion transporters is fundamental to propose molecular tools for the replacement of the activity of defective natural transporters, like for the treatment of cystic fibrosis.^{35–37} Moreover, the induced ion transport can be used to kill malignant cells as a mechanism of cancer chemotherapy.^{32,36,38} Several issues must be considered within this topic. First of all, putative transporter must be minimally soluble to be dispersed in aqueous medium, but hydrophobic enough to be efficiently transferred to the lipid bilayer and remain there. Moreover, the ion transport is a kinetic process for which the ion must be strongly complexed to be dehydrated but the ion-transporter complex should be labile enough to release the ion for an efficient turnover. Besides, for the process to be effective, the transporter must travel between the two membrane surfaces (a polar environment) by crossing a very hydrophobic environment. This makes the overall process a complex one that depends on many physicochemical parameters like the ion binding constant, lipophilicity, diffusiveness or conformational behaviour.³² Moreover, selectivity is an issue in this case, since lack of specificity is closely related to undesired side effects in case of biomedical applications. Very clever and elegant approaches to implement selectivity have been recently developed, such as the inclusion of photochemical or redox stimuli.³⁹

The effect of pH of the medium has been also proposed as a suitable stimulus,⁴⁰ since the increased metabolism of cancer cells produces an intracellular alkalization and an extracellular acidification, leading to an acidic microenvironment around solid tumours.⁴¹ Thus, receptors able to increase ion transport in slightly acidic pH values would be able to selectively kill cancer cells without affecting those healthy cells surrounded by neutral or slightly basic medium. Several groups have developed ion transporters with those pH-dependent transport ability,^{42–47} but requiring a pH-range much wider than that observed in cancer cell biology. Within this specific topic, we have been working for more than one decade with small tripodal pseudopeptidic cages that are able to strongly bind chloride anion when they are partially protonated (Fig. 3a).⁴⁸ Interestingly they showed a high selectivity for chloride versus the very similar fluoride and bromide anions, as demonstrated by ¹H NMR titration experiments in aqueous acetonitrile and by X-ray diffraction of single crystals in the solid state.⁴⁹ The selectivity is due to a size matching with the inner cavity of the cage that nicely hosts chloride but is not suitable for the larger bromide halide, while smaller fluoride allows water molecules to compete for the cage binding pocket. This size matching is

reflected on the strong binding constant ($K_a \approx 10^4 \text{ M}^{-1}$) of protonated cages towards chloride in aqueous acetonitrile, that is retained regardless the nature of the side chain, suggesting a conserved binding pocket for the anion, as confirmed from the crystal structures of several cages (Fig. 3b). Actually, the syntheses of the cages themselves were improved when chloride was added as template during the triple nucleophilic substitution reaction.^{48,49}

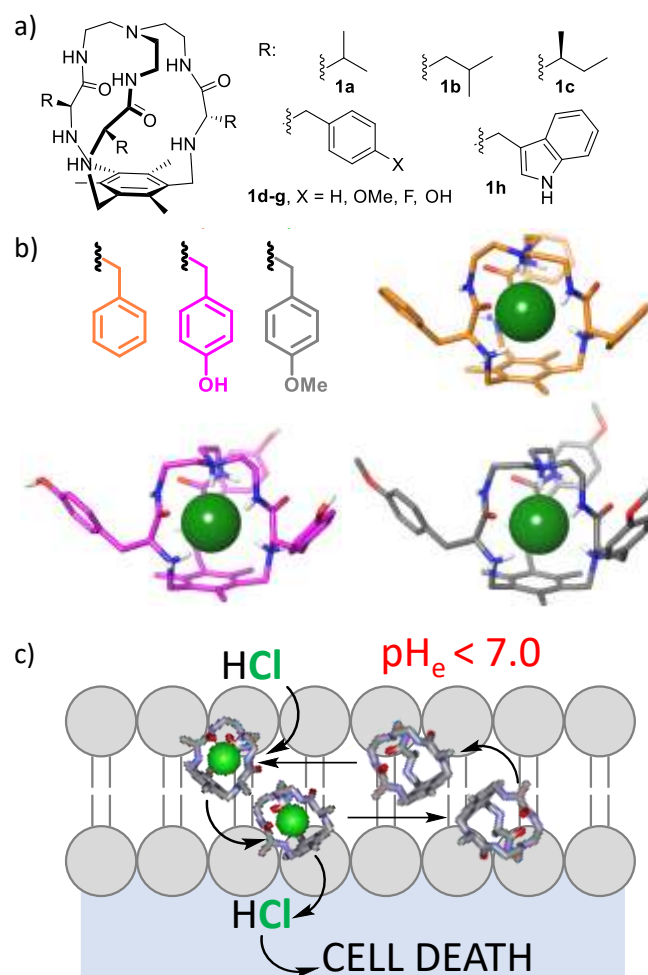


Fig. 3. (a) Chemical structures of the pseudopeptidic cages able to selectively bind chloride anion when protonated. (b) Solid state structures for the tetra chloride salt of selected cages showing the bound anion within the cage cavity as space filling green sphere (carbon colour code as in the depicted side chain, non-polar H atoms, crystallization solvent molecules and additional chloride anions are omitted for clarity). (c) Schematic representation of the mechanism for the H⁺/Cl⁻ symport mediated by the cages, which would lead to a pH-dependent cancer cell cytotoxicity.

The protonated cages transport chloride through lipid bilayers by a H⁺/Cl⁻ symport mechanism (Fig. 3c), with differential effectivity depending on the nature of the amino acid side chain decorating the external surface of the cages. The necessary protonation allowed us to identify some cages derived from aromatic amino acids that improved their chloride transport properties when a slightly acidic pH gradient is imposed, similar to the one expected around solid tumours.⁵⁰ Thus, the 4-F-Phe derived cage (**1f**) is a better HCl transporter at slightly acidic pH



values as demonstrated by Ion Selective Electrode and fluorescence measurements. Concomitantly, it is able to dissipate those pH gradients, therefore impacting on the overall ionic transmembrane imbalance. Satisfyingly, this property correlates with its pH-dependent cytotoxicity against human lung adenocarcinoma cells: a five-fold increase in cytotoxicity was observed for cells grown in slightly acidic pH values mimicking those found around solid tumours.⁵⁰ The intriguing effect of the fluorine atom must be related to the fine tuning of the corresponding physicochemical properties that modulate the activity of the cage as potential therapeutical ionophore in cancer chemotherapy.

Molecular recognition of small biomolecules

Recognition of small molecules in biomimetic environment is a hard task because their usually high hydration energy must be compensated by the interactions with the receptors. This is specially challenging when dealing with ionic⁵¹ or zwitterionic⁵² structures. In a recent work, Quesada and co-workers described a pincer-like receptor for lactate anion that is able to bind this hydroxy acid in competitive media.⁵³ Moreover, this receptor transport the substrate through lipid bilayers with a remarkable efficiency, meaning the first example of a transporter for this type of molecules. The recognition of amino acids is also very appealing for the development of sensors or ligands able to recognize these motives in water. For instance, receptors against *N*-methyl lysine⁵⁴ or *N*-acetyl lysine⁵⁵ have been reported, with a potential activity in the histone biology. Also the binding of proline and its transport through cell membranes has been recently described.⁵⁶

In our group, we have used an alternative DCC approach for the selective and efficient recognition of cysteine amino acid, both in the reduced and oxidized forms. Starting from a curiosity-generated DCL of pseudo-peptidic disulphides,⁵⁷ we identified the spontaneous assembling between a tripodal (**2**) and a bipodal (**3**) building block with Cys, leading to a heterotrimer stabilized by the recognition of the zwitterionic form of Cys and a favoured disulphide bond within a folded compact conformation (Fig. 4). The stability of the structure was established as dependent on parameters like pH or ionic strength, with a very good correlation with the proposed recognition motif.^{58,59} Considering the high specificity, we used that dynamic system to prepare a dynamic covalent mixture that renders a fluorescent response in the presence of Cys and cystine, by including the monopodal building block **4** in the mixture. The presence of Cys or cystine rearranges the initially complex DCL for the formation of the very stable heterotrimer, releasing the disulphide of thiol **4**, which exhibits a strong excimer emission in a folded conformation in aqueous media. The excimer/monomer emission ratio can be used to quantify the amino acid and its oxidized form even in the presence of other biologically relevant thiols or a large excess of other amino acids. Remarkably, this dynamic sensing system is able to detect the amino acid even in biological human fluids like urine, as a prospective diagnostic tool for cystinuria.⁶⁰

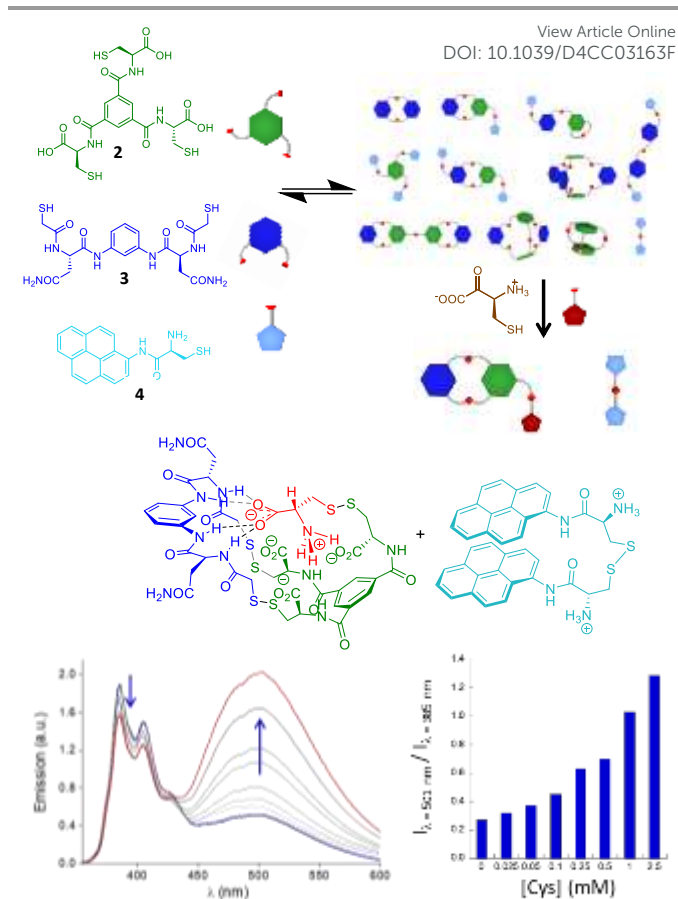


Fig. 4. DCL for Cys recognition and fluorescent sensing in biorelevant environment.

Among other small biomolecules, mono- and disaccharides are specially challenging, for several reasons. First, the polyhydroxylated structure mimics a cluster of water molecules, precluding desolvation of the substrate and binding to the host. Moreover, the large number of chiral centres produces a wide range of stereoisomers with very subtle structural differences, making selectivity a specially challenging goal. Within this aspect, the group of Davis has mastered the design of extremely efficient synthetic receptors for carbohydrates with a common structural motif, the so-called temple receptors (Fig. 5).⁶¹ Over the years, Davis' group has optimized their structure by tuning the binding epitopes complementary to the hydroxyl groups by H-bonding (red moieties in Fig. 5) and to the aliphatic core of the sugars by establishing CH- π interactions⁶² (blue in Fig. 5). Within this design, several hosts have been prepared. For instance, a prototypical example is macrocycle **5a**, which represents a simple and accessible synthetic lectin due to its easy preparation and similar behaviour to the natural carbohydrate-binding proteins. A combination of fluorescence, NMR and ITC rendered a modest $K_a = 56 \text{ M}^{-1}$, although the compound can be used to detect glucose in aqueous media. However, compound **5** shows moderate selectivity, which means a drawback to be used in biological environment. Actually, a structurally related host **5b** was described to be very strong binder of purine and pyrimidine bases with affinities rising up to 10^7 M^{-1} in aqueous buffer.⁶³



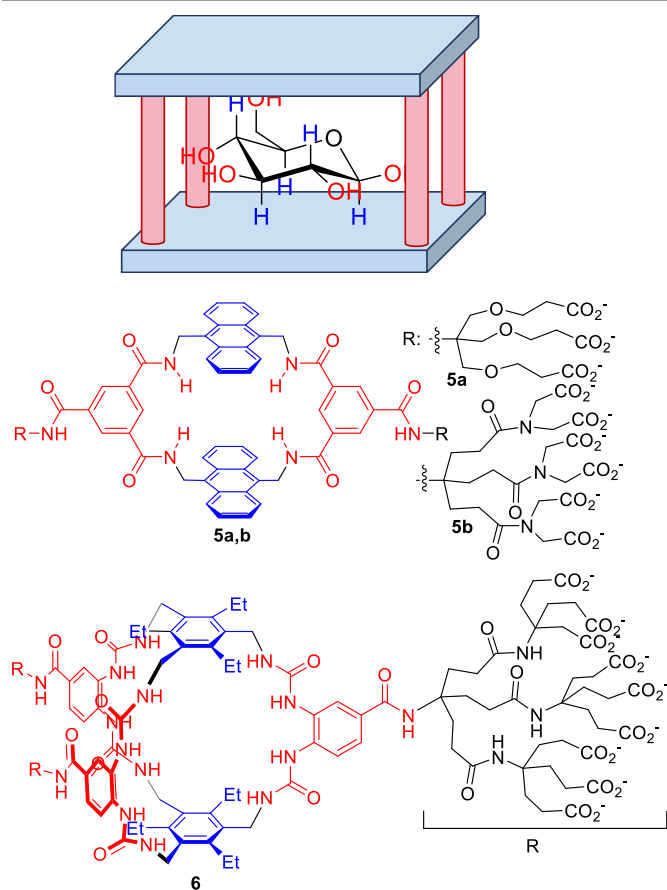


Fig. 5. General design of a temple receptor for saccharides and selected examples. The polar H-bonding columns are depicted in red while the aromatic non-polar roofs and floors are blue coloured. Peripheral solubilizing groups (R), anionic at neutral pH in water, are shown in black.

The same group prepared more elaborated generations of temple hosts for different types of carbohydrates, mainly by the extension of the aromatic floor and roof, or the increasing of the number of H-bonding columns.⁶¹ This systematic knowledge allowed them to optimize an extremely efficient receptor (**6** in Fig. 5) that is characterized by minimalistic aromatic CH- π recognition motives but a more elaborated H-bonding network to host the all-equatorial OH groups of glucose.⁶⁴ The measured binding constant towards β -D-glucose goes up to 18000 M^{-1} with a remarkable selectivity versus other simple carbohydrates and other biomolecules and drugs. As a matter of fact, the key macrocyclization step in the synthetic scheme of **6** can be improved by the presence of β -D-glucopyranoside, meaning an important template effect.^{65–67} More remarkably, the receptor is able to operate in cell culture media and in human blood serum, which clearly demonstrates the future utility of **6** in real world for glucose sensing⁶⁸ and monitoring or glucose-responsive insulin applications.

Saccharides have been used as templates also in DCC studies. Thus, a dynamic mixture of macrocyclic peptide disulfides was used for the molecular recognition of mono- and di-saccharides, with remarkable amplification of the macrocycles containing His and Tyr amino acids (Fig. 6).⁶⁹ Interestingly, two considerably different targets were possible to recognize, namely *N*-acetyl

neuraminic acid (NANA) and trehalose disaccharide. Further binding studies revealed the formation of complexes with 1:1 and 1:2 receptor:sugar stoichiometry.⁷⁰ Besides, parallel and antiparallel macrocyclic disulfides can be obtained with some differential binding behaviour. The His-containing macrocycles recognize one or two molecules of NANA, while the Tyr receptor binds just one molecule of trehalose. These facts would have been difficult to predict from molecular design, underscoring the potential of DCC for discovering unexpected receptors.

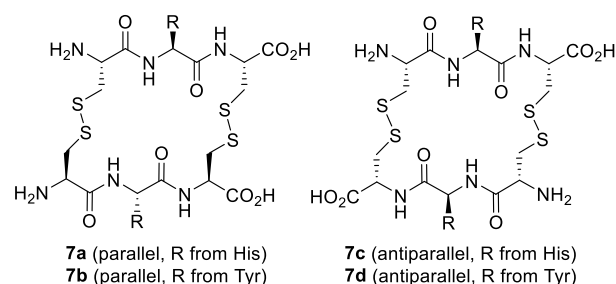


Fig. 6. Disulfide peptide macrocycles identified as efficient receptors for NANA and trehalose.

During a collaborative work, we have also contributed to the field of monosaccharide recognition by designing an extremely simple DCL of imines from isophthalaldehyde and several amines and amino acids in aqueous carbonate buffer at pH 10 (Fig. 7a).⁷¹ Different aldoses and ketoses were tested as templates of the DCL of imines, and *in situ* reduction allowed the identification and quantification of the amplified receptors, which were consequently synthesized at preparative scale, after reasonable structural simplifications (Fig. 7b). Isothermal titration calorimetry (ITC) confirmed that the observed amplifications correlate with the binding of the ligands to the sugar structures, being **8a,b** the most efficient ones. Actually, **8b** showed a remarkable affinity ($K_a \approx 1700 \text{ M}^{-1}$) and high selectivity for fructose over other monosaccharides (>50-fold) in pure water. Molecular modelling of the proposed complexes suggested that this selectivity arises from the specific H-bonding network within the anomeric moiety of β -D-fructopyranose form in addition to several CH- π interactions with the aromatic rings of the ligand (Fig. 7c). Despite this very simple host is far from finding practical applications, this study demonstrates the ability of DCC as a mechanism to discover unexpected binding motifs and systems for challenging biomolecules.

Molecular recognition of biomacromolecules

Molecular recognition of specific sequences in polypeptides is another biologically relevant but extremely difficult task.⁷² Apart from the general issues on biomolecular recognition, the secondary structure and conformational flexibility of polypeptides add additional complications on the design of specific hosts. Some relevant groups have proposed different receptors able to recognize sequences or solvent exposed regions of proteins and peptides.^{73–81} The general background in these receptors consist of a hydrophobic cavity surrounded by polar interactions, leading to the efficient binding of specific



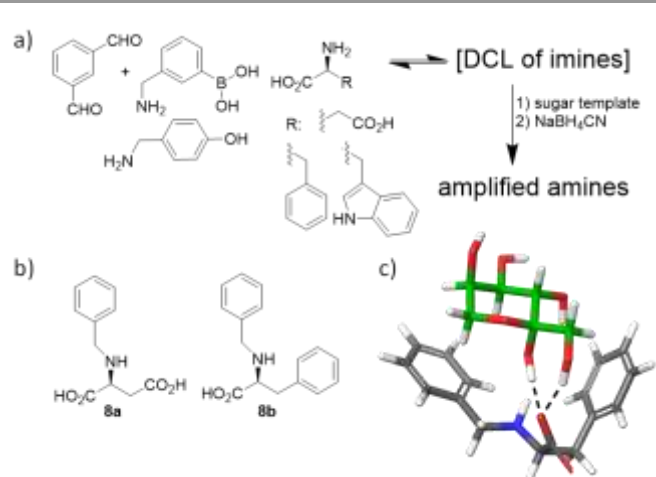


Fig. 7. (a) Sugar-templated DCL. (b) Simplified monosaccharide hosts thus identified. (c) proposed model for the interaction of **8b** with β -D-fructopyranose (green C-atoms).

sequences.^{54,75–77,82–84} In a long-term research project on the recognition of peptide sequences, we have designed large pseudopeptidic cages with a bigger cavity flanked by two aromatic rings and several polar groups (**9a-d**, Fig. 8a).⁸⁵ The modular design of the synthetic scheme allowed us to prepare

a large variety of cages decorated with different amino acid side chains. A systematic binding screening using fluorescence and NMR titration experiments, as well as ESI-MS competition assays identified di- and tri-peptide sequences bearing an aromatic amino acid (Phe or Tyr, but not Trp nor His) as suitable substrates.⁸⁶ Further structural (NMR, fluorescence and molecular modelling) studies combined with Ion Mobility Mass Spectrometry suggested the inclusion of the Tyr side chain within the inner cavity of the cage, stabilized by aryl-aryl and H-bonding interactions.^{87,88} Moreover, sequence selectivity was also achieved by secondary interactions between the external side chains of the pseudopeptidic cages and the amino acids surrounding the Tyr residue in the peptide substrates. Thus, electrostatic complementary contacts rendered stronger binding than those presenting residues with the same charge sign.⁸⁸ Remarkably, this recognition can be translated to polypeptides containing these binding motifs, as clearly reflected when using specific random peptide co-polymers containing Tyr and different number of either Glu or Lys residues.⁸⁹ Combination of fluorescence titrations with relaxation and diffusion-filtered NMR experiments demonstrated the selective and strong binding of large Tyr-containing polypeptides in buffered aqueous media (Fig. 8b).

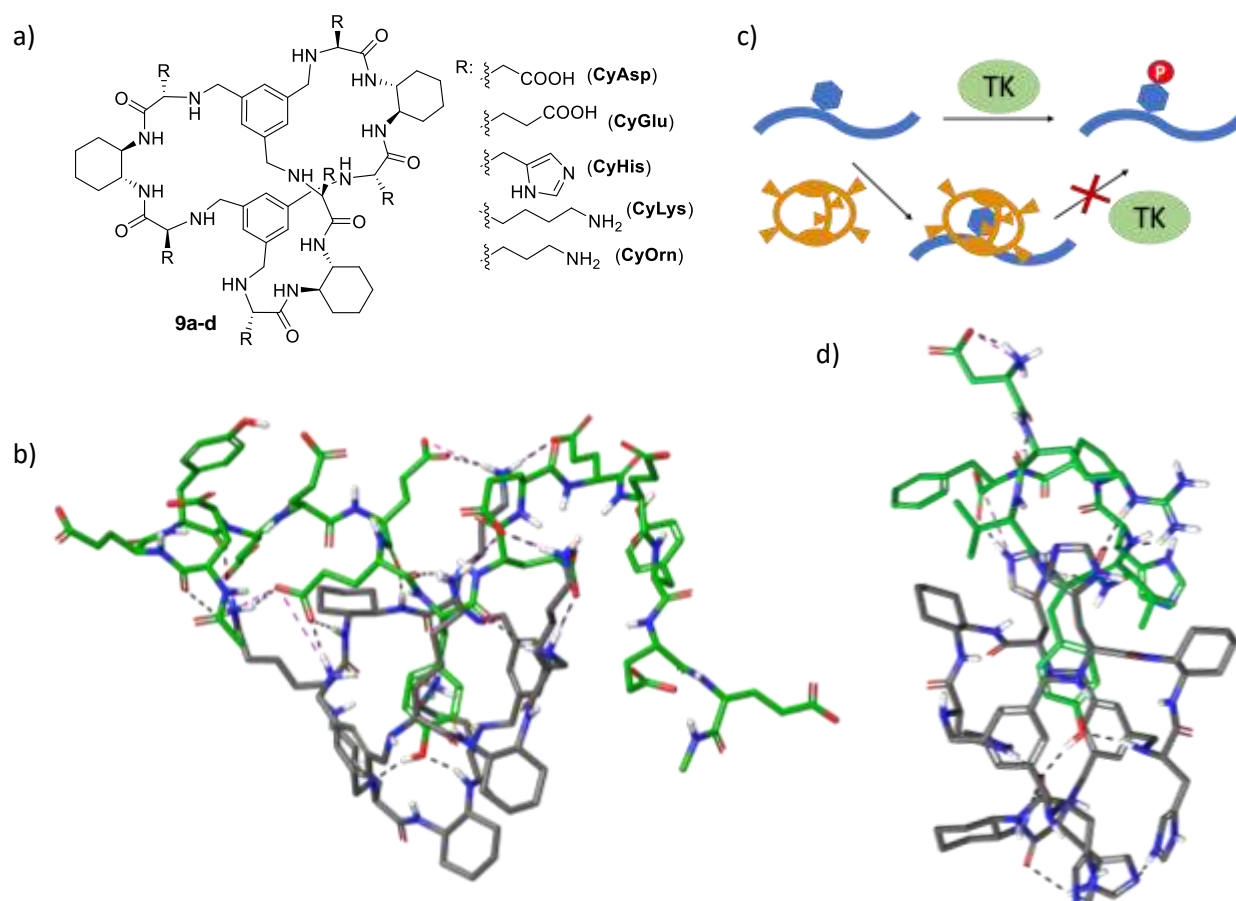


Fig. 8. (a) Chemical structures of large pseudopeptidic cages **9a-d** designed to bind Tyr aromatic amino acid side chains in peptides. (b) Molecular model for the interaction of CyLys with the Ac-EEYEEEEEEYEE-NH₂ peptide. (c) Schematic representation for the mechanism of inhibition of TK-mediated phosphorylation of Tyr-containing peptides (blue) mediated by the pseudopeptidic cages (orange). (d) Molecular model for the complex between CyHis and the Val5AngII peptide (sequence: DRVYVHPF). In (b) and (d), non-polar H-bonds are omitted, H-bonds and salt bridges are shown by black and pink dashed lines, respectively, and the peptide substrates are represented with green C-atoms. Both minimized structures show a Tyr residue included within the cage cavity with the phenolic OH setting H-bonds with different functions of the cage.



ARTICLE

The efficient binding of Tyr residues has important biological implications. Thus, Tyr phosphorylation by protein Tyr kinases (TK in Fig. 8c) is a key process in cell regulation and closely related to serious diseases.^{90,91} The efficient inclusion of Tyr side chain within the cage structure protects the residue from the action of the TK enzymes, as demonstrated by phosphorylation assays with different peptide sequences as substrates of Src TK.^{92,93} Satisfyingly, the inhibition of Tyr phosphorylation mediated by the cages correlates with their binding affinity towards the peptide substrates, as measured by fluorescence titration experiments.⁹³ Moreover, the cages show promising substrate selectivity depending on the peptide sequence close to the Tyr, even for peptides bearing additional aromatic amino acids (Fig. 8d).⁹³ Moreover, the generality of the strategy was demonstrated with a different TK enzymes. This TK-modulation allows protecting a defined Tyr-containing protein or peptide without affecting the activity of the TK enzyme towards a different substrate. Since a given TK is often implicated in several signalling pathways and in the modification of different proteins, this appealing approach has the potential to complement and improve the usually low specificity of available TK inhibitors.^{90,94}

Despite being major biomacromolecules with key functions in biological systems, the selective molecular recognition of polysaccharides remains a scarcely explored field. The group of Davis also reported a seminal work on the design of a temple receptor able to recognize cellulose, chitin and related biopolymers.⁹⁵ Thus, temple receptor **10** with extended pyrene rings and wider portals form very stable complexes with all equatorial β -pyranoside oligomers as confirmed by NMR and ITC titration experiments. Very remarkably, the threading of the substrates within the host cavity (Fig. 9) was demonstrated by a combination of detailed NMR experiments (including NOE) and molecular modelling. These threaded structures are retained with polymeric cellulose and chitosan leading to unique polypseudorotaxane complexes observable by Atomic Force Microscopy on mica surfaces. This unprecedented molecular recognition system could facilitate exploitation of cellulose by improving solubility of the corresponding fibres, or the manipulation of biotechnologically relevant chitosan for tissue engineering.

Another interesting group of polysaccharides are glycosaminoglycans (GAGs) that play an essential role in cell signalling and communication, being implicated in key serious pathological processes like cancer metastasis or viral infection.^{96,97} They are present in the extracellular matrix (ECM) either alone or conjugated with lipids and proteins, meaning the external face of mammalian cells.^{98,99}

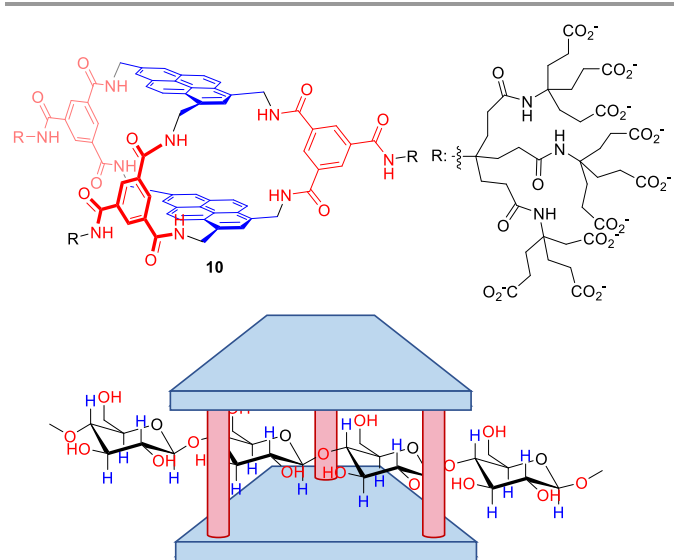


Fig. 9. Macrobicyclic receptor for the binding of β -pyranoside polysaccharides in water. The molecular recognition process occurs by threading the biopolymer through the host cavity in a pseudorotaxane structure.

GAGs are characterized by the repetition of highly anionic saccharides, leading to strongly hydrated and flexible polymers.¹⁰⁰ These facts make them extremely difficult targets for molecular recognition by design, as they lack of conventional binding pockets. Therefore, we hypothesized that GAGs could be the ideal templates for DCLs, since this approach does not require a detailed knowledge of the target structure. As a prototypical GAG, we selected heparin (see structure in Fig. 10a) which is the biopolymer with the highest negative charge density described so far. Heparin is a broadly used drug, mainly as anticoagulant^{101,102} but also employed in cancer¹⁰³ and infections treatment.¹⁰⁴ As a proof of concept, we selected a natural polyamine (spermine) that must be protonated in water at neutral pH¹⁰⁵ and thus it would interact with the sulphates and carboxylates of the GAG.^{106,107} Additional CH- π interactions were implemented with a mixture of aromatic aldehydes leading to a dynamic mixture of structurally varied imines.¹⁰⁸ *In situ* reductive amination of the DCL in the absence and presence of heparin rendered **11a** as an amplified member of the library. We prepared the ligand at preparative scale to confirm its strong binding to heparin at the low micromolar range, using a combination of ITC, NMR and fluorescence spectroscopy. The *in vitro* activity of the ligand was also tested with an enzymatic cascade reaction closely related to blood coagulation (Fig. 10c), which showed the ability of **11a** to revert the inhibitory effect of heparin. Very remarkably, a closely related ligand (**11d**) that was not amplified in the DCC experiments show weak binding



ARTICLE

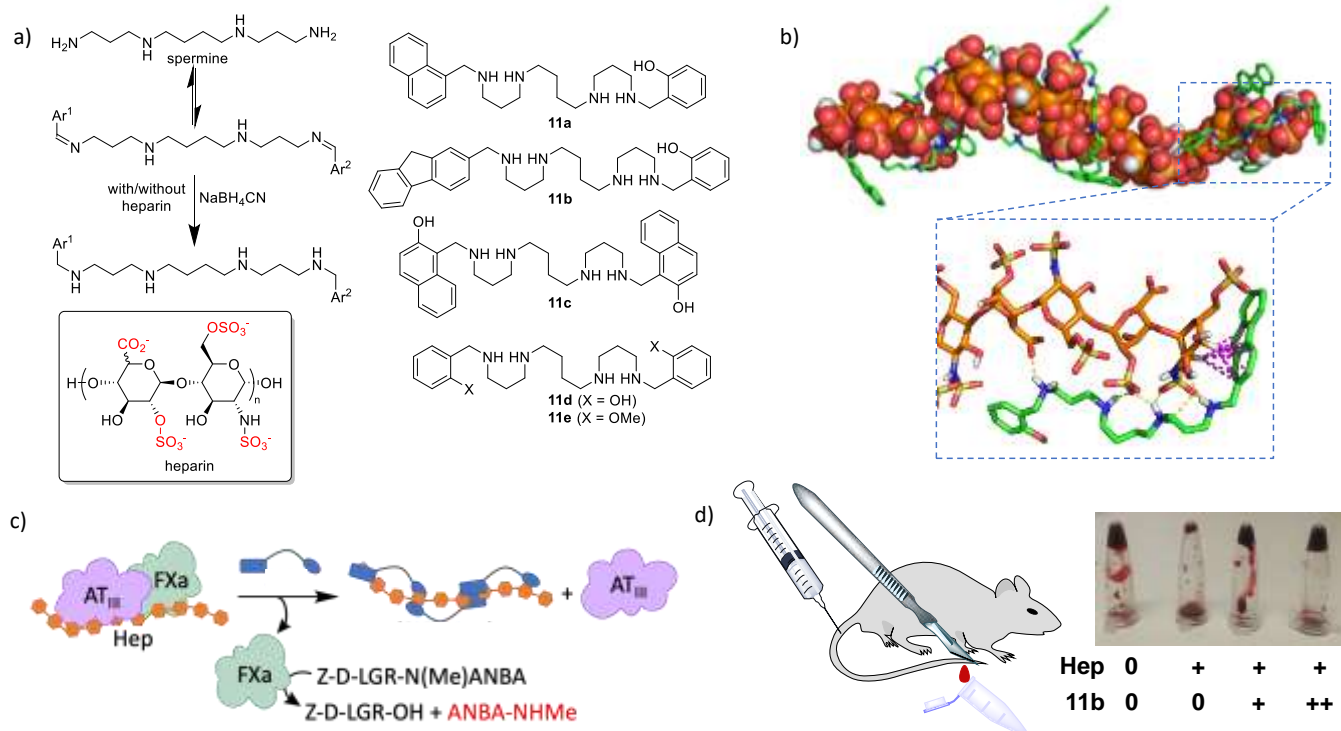


Fig 10. (a) DCL of imines from spermine and a mixture of aromatic aldehydes, by reductive amination reaction and using heparin as template. Some selected representative spermine structures there studied are also shown. (b) Illustrative snapshot from molecular dynamic simulations performed with a heparin oligomeric model (orange) and six molecules of compound **11b** (green). Non-polar H-atoms are omitted for clarity, H-bonds and CH- π interactions are shown in yellow and pink dashed lines, respectively. (c) Schematic representation for the enzymatic reaction used to test the *in vitro* activity of the identified ligands as reversal agents of heparin anticoagulant. In brief, the ternary complex [ATIII-Hep-FXa] inactivates the FXa protease, while strong heparin ligands dissociate this complex releasing FXa, which triggers the hydrolysis of a peptide substrate mimicking those related to the coagulation cascade. (d) The biological activity of the spermine derivatives in mice blood was additionally confirmed by *ex vivo* and *in vivo* experiments.

to heparin and no *in vitro* activity.¹⁰⁸ In a later study, we expanded the molecular diversity of the DCL by increasing the number and structural variety of the corresponding aldehydes. In this way, we identified two additional ligands (**11b** and **11c**) that were more amplified than **11a** in competition experiments.¹⁰⁹ Binding studies using Surface Plasmon Resonance (SPR) and fluorescence titrations rendered submicromolar affinity of **11b-c** towards different forms of heparin. Structural studies by NMR (including DOSY) and molecular dynamics simulations (Fig. 10b) confirmed the formation of stable complexes in water, by the polyamines wrapping around the GAG and establishing polar (coulombic contacts and H-bonds) and non-polar (CH- π) interactions. The corresponding enzymatic tests (Fig. 10c) of amplified and non-amplified members of the DCL served as a definitive validation of the screening protocol, since we found a very good correlation between the corresponding amplification factors and the *in vitro* activity. The most efficient ligands (**11b-c**) were additionally assayed as heparin reversal agents in more

challenging and meaningful environment. Thus, both *ex vivo* coagulation experiments with freshly extracted mice blood and *in vivo* tests using the tail transection assay showed a dose-response efficient activity as heparin antidotes (Figure 10d).¹⁰⁹ Accordingly, these works demonstrated how supramolecular principles can be used for the molecular recognition of challenging biopolymer targets, even with potential applications in real life as hits towards the development of future drugs.

Molecular recognition and self-assembling in specially challenging biological environments

When dealing with molecular recognition in chemical biology, the ultimate goal must be to characterize the supramolecular systems in living matter. This objective, despite being appealing and necessary for any type of real applications, is extremely challenging as living matter is molecularly complex and a



strongly competitive environment for non-covalent interactions.

In this context and as a step further in the development of GAGs ligands, we wanted to demonstrate the concept also with other structures apart from heparin. As mentioned before, GAGs form part of the ECM of mammalian cells, and their structure and composition strongly depend on the cell type.¹¹⁰ Moreover, in certain pathological processes like cancer metastasis¹¹¹ or host-pathogen infection,¹¹² the nature and composition of the ECM suffer from important changes, being the GAG composition a fingerprint for the characterization of the cell type or its progression stage in pathology. With this idea in mind, we hypothesized if the native ECM of living cells could be used as template in a similar DCL of imines against GAGs (Fig. 11).

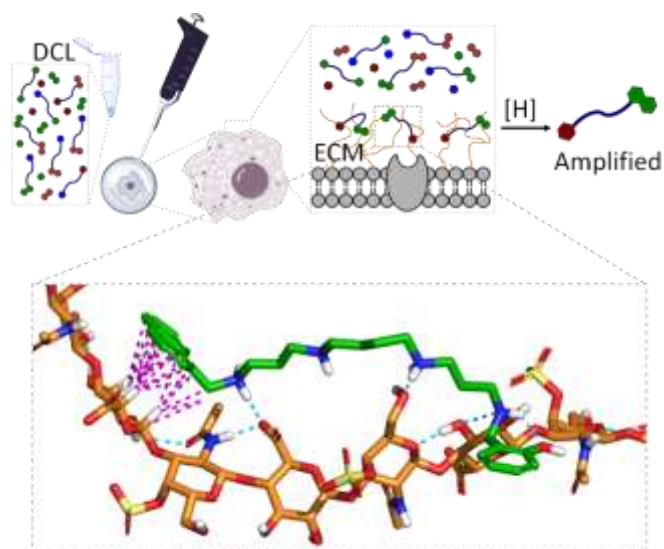


Fig. 11. Schematic representation of live-cell-templated DCC experiment targeting the GAGs of the ECM. The inset shows a model interaction between the amplified member of the library (**11a**) and chondroitin sulphate A (CDS-A), the main GAG in the ECM of A549 cells used for de DCC experiments. In the model, non-polar H atoms are omitted, CDS-A and **11a** are represented with orange and green C-atoms, respectively. On the other hand, H-bonds and CH- π interactions are depicted with blue and pink dashed lines, respectively.

Gratifyingly, the reductive amination reaction of a DCL generated from spermine and a small selection of aromatic aldehydes showed a strong amplification of **11a** in the presence of human lung adenocarcinoma (A549) cells.¹¹³ The amplification pattern varies with the cancer cell type (i.e. HeLa cells) suggesting that the DCC approach could be used as a cell-type fingerprint. Moreover, experiments performed in the presence of protamine (a small Arg-rich protein) as a competing binder, or with A549 cells grown with an inhibitor of ECM sulfation, suppressed the observed amplifications. These experiments identified GAGs as the binding motives for the DCL. On-cell NMR experiments with **11a** (amplified ligand) and **11e** (non-amplified ligand) confirmed their reversible non-covalent binding to the ECM, being much stronger for **11a**. Since chondroitin sulphate A (CDS-A) is the most abundant GAG in A549 cells, their strong binding to **11a** was confirmed by SPR and NMR, while illustrated by molecular dynamics simulations that revealed the proposed polar and non-polar interactions

(Fig. 11, inset). This work shows that living cells can be used in DCC and brings the supramolecular chemistry concepts to an especially relevant and meaningful environment for chemical biology.

Non-covalent self-assembly of designed building blocks inside living cells has been also used to target different organelles like nucleus or mitochondria, controlling cell fate and viability. Thus, short peptide sequences,^{114,115} DNA nanostructures¹¹⁶ or abiotic disulphides^{117,118} can be exploited in this regard. The key issue here is triggering the self-assembly to occur within the precise location and timing,¹¹⁹ using chemical^{117,118} or enzymatic^{114,115} stimuli (Fig. 12). There is a promising future of this type of systems for cancer treatment, while intracellular characterization of the assemblies still represents a challenge for which fluorescence imaging or in-cell NMR might play a key role.

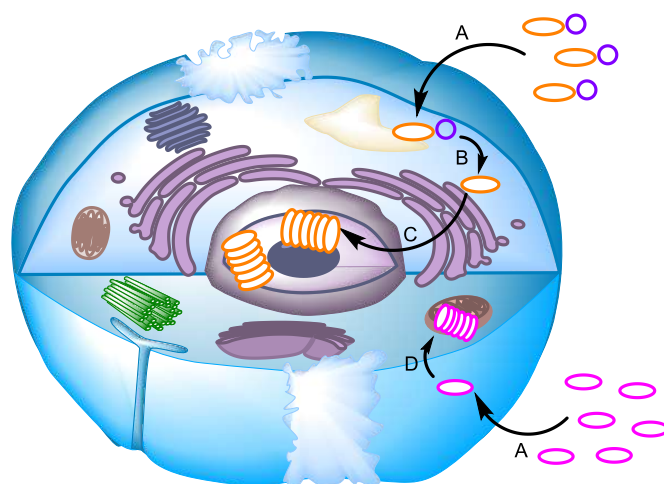


Fig. 12. Schematic representation of selected mechanisms for the self-assembly process inside a live cell. A chemical entity (orange oval) with a modifiable function (purple circle) enters the cell (A) and is transformed by a chemical or enzymatic reaction (B) to be transferred and aggregated in the nucleus (C). On the other hand, a species targeting mitochondria (pink oval) is internalized in the cell (A) and accumulated in this organelle, inducing aggregation due to a much higher local concentration (D).

As a final example of the elegant use of molecular recognition for the control of an extremely complex biological process, a recent contribution was reported in the field of bacterial infection. Thus, cationic pillarenes can be used to efficiently encapsulate chemical signals (homoserine lactones) for the quorum sensing of pathological bacteria, disrupting their intercommunication.^{120,121} This binding effect precludes biofilm formation and improves healing in open wound *in vivo* models. This inspiring supramolecular approach paves the way towards an alternative mechanism to fight antimicrobial resistance to conventional drugs, which is one of the main current concerns in human health.^{122,123}

Conclusions

The molecular recognition of biorelevant species is an essential topic of research in chemical biology. Thus, processes like the reading and transference of the genetic information, cell



communication, enzymatic activity or even the integrity of the cell rest on the establishment of non-covalent interactions between chemical entities. Since chemical biology focuses on the study, understanding and intervention of the biological processes from a chemical perspective, supramolecular chemistry has concomitantly influenced chemical biology. Despite their intrinsic connectivity, there are still important hurdles for classical supramolecular chemistry to step into the field of chemical biology. Efficiency, specificity and, specially, water compatibility of the supramolecular systems are essential in this regard. Also, temporal and spatial control of the supramolecular process to occur can be an issue in case of biological complex media. Different approaches for the design of biorelevant molecular recognition systems have been described in the literature, all of them having pros and cons. In our own experience, rational design usually renders more predictable outcomes while requiring higher synthetic efforts and sometimes leading to frustrating results. Dynamic covalent chemistry methodologies are interesting alternatives to speed up the identification of hits, although less predictable results and false positives are frequently obtained. Templated synthesis of designed receptors represents a somehow hybrid possibility as already illustrated in the syntheses of several hosts (i.e. **1a-h** or **6**). In any case, no methodology is perfect and keeping in mind all the accessible options is strongly recommendable.

Methodological connectivity between supramolecular chemistry and chemical biology is also evident when reviewing the experimental techniques or concepts supporting the hypotheses used in both disciplines. Moreover, the establishing of clear structure-activity relationships will benefit advancement in the joint multidisciplinary field.

The final transfer of the research to real-life applications requires testing the supramolecular systems in meaningful biological environments, which imposes additional hurdles. To that, sensitive assays in complex matrices like cells, organoids, tissues or even living animals must be developed. Regarding that, the early introduction of the matrix in the development of the molecular recognition systems is desirable, for which methodological alternatives like live-cell-templated DCC or the use of high-resolution imaging techniques are especially appealing.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

I wish to thank all the past and current members of the group, especially those who have contributed to the development of this chemistry and appear as co-authors in the corresponding cited references from our group. Far from being an exhaustive revision of the literature in this emerging field, I apologize beforehand to those authors who can feel excluded from the references cited in this Feature Article. Financial support from

the Spanish Research Agency within the Spanish Ministry of Science, Innovation and Universities (PID2021-123411NB-I00, MCIU/AEI/10.13039/501100011033, and FEDER/EU) is gratefully acknowledged.

Notes and references

- J.-M. Lehn, *Science* (80-), 2002, **295**, 2400–2403.
- J.-M. Lehn, *Science* (80-), 1993, **260**, 1762–1763.
- B. Smith, *Synthetic Receptors for Biomolecules*, The Royal Society of Chemistry, 2015.
- Nat. Chem. Biol.*, 2015, **11**, 378–379.
- K. L. Morrison and G. A. Weiss, *Nat. Chem. Biol.*, 2006, **2**, 3–6.
- K. Petkau-Milroy and L. Brunsveld, *Org. Biomol. Chem.*, 2013, **11**, 219–232.
- I. Insua and J. Montenegro, *Chem*, 2020, **6**, 1652–1682.
- L. M. Salonen, M. Ellermann and F. Diederich, *Angew. Chemie Int. Ed.*, 2011, **50**, 4808–4842.
- S. Mondal and B. Bagchi, *Curr. Opin. Struct. Biol.*, 2022, **77**, 102462.
- J. Dong and A. P. Davis, *Angew. Chemie Int. Ed.*, 2021, **60**, 8035–8048.
- N. B. Rego and A. J. Patel, *Annu. Rev. Condens. Matter Phys.*, 2022, **13**, 303–324.
- I. D. Alves, C.-Y. Jiao, S. Aubry, B. Aussedat, F. Burlina, G. Chassaing and S. Sagan, *Biochim. Biophys. Acta - Biomembr.*, 2010, **1798**, 2231–2239.
- S. Sarkar, P. Ballester, M. Spektor and E. A. Kataev, *Angew. Chemie Int. Ed.*, 2023, **62**, e202214705.
- P. L. Kastiris and A. M. J. J. Bonvin, *J. R. Soc. Interface*, 2013, **10**, 20120835.
- H.-X. Zhou, *Q. Rev. Biophys.*, 2010, **43**, 219–293.
- L. Motiei and D. Margulies, *Acc. Chem. Res.*, 2023, **56**, 1803–1814.
- E. V. Anslyn, *J. Org. Chem.*, 2007, **72**, 687–699.
- I. Alfonso, *Chem. Commun.*, 2016, **52**, 239–250.
- J. Solà, C. Jimeno and I. Alfonso, *Chem. Commun.*, 2020, **56**, 13273–13286.
- J.-M. Lehn and A. V. Eliseev, *Science* (80-), 2001, **291**, 2331 LP – 2332.
- O. Ramström and J.-M. Lehn, *Nat. Rev. Drug Discov.*, 2002, **1**, 26–36.
- P. T. Corbett, J. Leclaire, L. Vial, K. R. West, J.-L. Wietor, J. K. M. Sanders and S. Otto, *Chem. Rev.*, 2006, **106**, 3652–3711.
- A. Herrmann, *Chem. Soc. Rev.*, 2014, **43**, 1899–1933.
- I. Alfonso and R. Quesada, *Chem. Sci.*, 2013, **4**, 3009–3019.
- P. A. Gale, J. T. Davis and R. Quesada, *Chem. Soc. Rev.*, 2017, **46**, 2497–2519.
- P. A. Gale, R. Pérez-Tomás and R. Quesada, *Acc. Chem. Res.*, 2013, **46**, 2801–2813.
- L. Martínez-Crespo, S. H. Hewitt, N. A. De Simone, V. Šindelář, A. P. Davis, S. Butler and H. Valkenier, *Chem. – A Eur. J.*, 2021, **27**, 7367–7375.
- L. Martínez-Crespo and H. Valkenier, *Chempluschem*, 2022, **87**, e202200266.
- A. Cataldo, K. Norvaisa, L. Halgreen, S. E. Bodman, K. Bartik,



- S. J. Butler and H. Valkenier, *J. Am. Chem. Soc.*, 2023, **145**, 16310–16314.
- 30 L. Jing, E. Deplazes, J. K. Clegg and X. Wu, *Nat. Chem.*, 2024, **16**, 335–342.
- 31 R. Quesada, *Chem*, 2019, **5**, 1924–1926.
- 32 S.-H. Park, S.-H. Park, E. N. W. Howe, J. Y. Hyun, L.-J. Chen, I. Hwang, G. Vargas-Zuñiga, N. Busschaert, P. A. Gale, J. L. Sessler and I. Shin, *Chem*, 2019, **5**, 2079–2098.
- 33 X. Wu, J. R. Small, A. Cataldo, A. M. Withcombe, P. Turner and P. A. Gale, *Angew. Chemie Int. Ed.*, 2019, **58**, 15142–15147.
- 34 S. Cheung, D. Wu, H. C. Daly, N. Busschaert, M. Morgunova, J. C. Simpson, D. Scholz, P. A. Gale and D. F. O'Shea, *Chem*, 2018, **4**, 879–895.
- 35 X. Wu, E. N. W. Howe and P. A. Gale, *Acc. Chem. Res.*, 2018, **51**, 1870–1879.
- 36 N. Akhtar, O. Biswas and D. Manna, *Chem. Commun.*, 2020, **56**, 14137–14153.
- 37 N. Busschaert and P. A. Gale, *Angew. Chemie Int. Ed.*, 2013, **52**, 1374–1382.
- 38 X.-H. Yu, X.-Q. Hong, Q.-C. Mao and W.-H. Chen, *Eur. J. Med. Chem.*, 2019, **184**, 111782.
- 39 M. Ahmad, S. A. Gartland and M. J. Langton, *Angew. Chemie Int. Ed.*, 2023, **62**, e202308842.
- 40 N. Kumar and N. Madhavan, *Org. Biomol. Chem.*, 2023, **21**, 5892–5905.
- 41 K. M. Bailey, J. W. Wojtkowiak, A. I. Hashim and R. J. Gillies, in *Current Challenges in Personalized Cancer Medicine*, ed. K. S. M. B. T.-A. in P. Smalley, Academic Press, 2012, vol. 65, pp. 63–107.
- 42 E. N. W. Howe, N. Busschaert, X. Wu, S. N. Berry, J. Ho, M. E. Light, D. D. Czech, H. A. Klein, J. A. Kitchen and P. A. Gale, *J. Am. Chem. Soc.*, 2016, **138**, 8301–8308.
- 43 N. Busschaert, R. B. P. Elmes, D. D. Czech, X. Wu, I. L. Kirby, E. M. Peck, K. D. Hendzel, S. K. Shaw, B. Chan, B. D. Smith, K. A. Jolliffe and P. A. Gale, *Chem. Sci.*, 2014, **5**, 3617–3626.
- 44 R. B. P. Elmes, N. Busschaert, D. D. Czech, P. A. Gale and K. A. Jolliffe, *Chem. Commun.*, 2015, **51**, 10107–10110.
- 45 L. A. Marchetti, T. Krämer and R. B. P. Elmes, *Org. Biomol. Chem.*, 2022, **20**, 7056–7066.
- 46 A. Roy, D. Saha, P. S. Mandal, A. Mukherjee and P. Talukdar, *Chem. – A Eur. J.*, 2017, **23**, 1241–1247.
- 47 E. N. W. Howe, V.-V. T. Chang, X. Wu, M. Fares, W. Lewis, L. K. Macreadie and P. A. Gale, *Biochim. Biophys. Acta - Biomembr.*, 2022, **1864**, 183828.
- 48 I. Martí, J. Rubio, M. Bolte, M. I. Burguete, C. Vicent, R. Quesada, I. Alfonso and S. V. Luis, *Chem. – A Eur. J.*, 2012, **18**, 16728–16741.
- 49 I. Martí, M. Bolte, M. I. Burguete, C. Vicent, I. Alfonso and S. V. Luis, *Chem. – A Eur. J.*, 2014, **20**, 7458–7464.
- 50 L. Tapiá, Y. Pérez, M. Bolte, J. Casas, J. Solà, R. Quesada and I. Alfonso, *Angew. Chemie - Int. Ed.*, 2019, **58**, 12465–12468.
- 51 P. A. Gale, E. N. W. Howe and X. Wu, *Chem*, 2016, **1**, 351–422.
- 52 I. Alfonso and J. Solà, *Chem. – An Asian J.*, 2020, **15**, 986–994.
- D. Alonso-Carrillo, A. Arias-Betancur, I. Carreira-Barra, P. Fontova, V. Soto-Cerrato, M. García-Valverde, R. Pérez Tomás and R. Quesada, *iScience*, 2023, **26**, 107898.
- 54 J. E. Beaver and M. L. Waters, *ACS Chem. Biol.*, 2016, **11**, 643–653.
- 55 M. Orlandini, A. Pedrini, D. Marchetti, Y. Li, G. Aragay, E. Dalcanale, P. Ballester and R. Pinalli, *Chem. – A Eur. J.*, 2024, **30**, e202303715.
- 56 L. Martínez-Crespo, J. L. Sun-Wang, A. F. Sierra, G. Aragay, E. Errasti-Murugarren, P. Bartoccioni, M. Palacín and P. Ballester, *Chem*, 2020, **6**, 3054–3070.
- 57 J. Solà, M. Lafuente, J. Atcher and I. Alfonso, *Chem. Commun.*, 2014, **50**, 4564–4566.
- 58 M. Lafuente, J. Atcher, J. Solà and I. Alfonso, *Chem. – A Eur. J.*, 2015, **21**, 17002–17009.
- 59 M. Lafuente, I. Alfonso and J. Solà, *ChemSystemsChem*, 2019, **1**, 25–31.
- 60 M. Lafuente, J. Solà and I. Alfonso, *Angew. Chemie - Int. Ed.*, 2018, **57**, 8421–8424.
- 61 A. P. Davis, *Chem. Soc. Rev.*, 2020, **49**, 2531–2545.
- 62 J. L. Asensio, A. Ardá, F. J. Cañada and J. Jiménez-Barbero, *Acc. Chem. Res.*, 2013, **46**, 946–954.
- 63 D. Van Eker, S. K. Samanta and A. P. Davis, *Chem. Commun.*, 2020, **56**, 9268–9271.
- 64 R. A. Tromans, T. S. Carter, L. Chabanne, M. P. Crump, H. Li, J. V Matlock, M. G. Orchard and A. P. Davis, *Nat. Chem.*, 2019, **11**, 52–56.
- 65 S. Anderson, H. L. Anderson and J. K. M. Sanders, *Acc. Chem. Res.*, 1993, **26**, 469–475.
- 66 R. Vilar, *Angew. Chemie Int. Ed.*, 2003, **42**, 1460–1477.
- 67 S. J. Rowan, S. J. Cantrill, G. R. L. Cousins, J. K. M. Sanders and J. F. Stoddart, *Angew. Chemie Int. Ed.*, 2002, **41**, 898–952.
- 68 R. A. Tromans, S. K. Samanta, A. M. Chapman and A. P. Davis, *Chem. Sci.*, 2020, **11**, 3223–3227.
- 69 M. Rauschenberg, S. Bomke, U. Karst and B. J. Ravoo, *Angew. Chemie Int. Ed.*, 2010, **49**, 7340–7345.
- 70 M. Rauschenberg, S. Bandaru, M. P. Waller and B. J. Ravoo, *Chem. – A Eur. J.*, 2014, **20**, 2770–2782.
- 71 M. Alena-Rodriguez, M. Fernandez-Villamarin, I. Alfonso and P. M. Mendes, *Org. Biomol. Chem.*, 2024, **22**, 3854–3859.
- 72 M. W. Peczu and A. D. Hamilton, *Chem. Rev.*, 2000, **100**, 2479–2494.
- 73 R. E. McGovern, H. Fernandes, A. R. Khan, N. P. Power and P. B. Crowley, *Nat. Chem.*, 2012, **4**, 527–533.
- 74 P. B. Crowley, *Acc. Chem. Res.*, 2022, **55**, 2019–2032.
- 75 S. van Dun, C. Ottmann, L.-G. Milroy and L. Brunsveld, *J. Am. Chem. Soc.*, 2017, **139**, 13960–13968.
- 76 P. Suating, L. B. Kimberly, M. B. Ewe, S. L. Chang, J. M. Fontenot, P. R. Sultane, C. W. Bielawski, D. A. Decato, O. B. Berryman, A. B. Taylor and A. R. Urbach, *J. Am. Chem. Soc.*, 2024, **146**, 7649–7657.
- 77 P. Suating, M. B. Ewe, L. B. Kimberly, H. D. Arman, D. J. Wheritt and A. R. Urbach, *Chem. Sci.*, 2024, **15**, 5133–5142.
- 78 Z. Hirani, H. F. Taylor, E. F. Babcock, A. T. Bockus, C. D.



- Varnado, C. W. Bielawski and A. R. Urbach, *J. Am. Chem. Soc.*, 2018, **140**, 12263–12269. 103
- 79 L. C. Smith, D. G. Leach, B. E. Blaylock, O. A. Ali and A. R. Urbach, *J. Am. Chem. Soc.*, 2015, **137**, 3663–3669. 104
- 80 F.-G. Klärner and T. Schrader, *Acc. Chem. Res.*, 2013, **46**, 967–978. 105
- 81 T. Schrader, G. Bitan and F.-G. Klärner, *Chem. Commun.*, 2016, **52**, 11318–11334. 106
- 82 H. Wang, R. S. Dawber, P. Zhang, M. Walko, A. J. Wilson and X. Wang, *Chem. Sci.*, 2021, **12**, 5977–5993. 107
- 83 M.-H. Le, E. S. Taghvaei and T. Schrader, *Chem. Commun.*, 2022, **58**, 2954–2966. 108
- 84 H. Shahpasand-Kroner, I. Siddique, R. Malik, G. R. Linares, M. I. Ivanova, J. Ichida, T. Weil, J. Münch, E. Sanchez-Garcia, F.-G. Klärner, T. Schrader and G. Bitan, *Pharmacol. Rev.*, 2023, **75**, 263 LP – 308. 109
- 85 A. Moure, S. V. Luis and I. Alfonso, *Chem. - A Eur. J.*, 2012, **18**, 5496–5500. 110
- 86 E. Faggi, A. Moure, M. Bolte, C. Vicent, S. V. Luis and I. Alfonso, *J. Org. Chem.*, 2014, **79**, 4590–4601. 111
- 87 E. Faggi, C. Vicent, S. V. Luis and I. Alfonso, *Org. Biomol. Chem.*, 2015, **13**, 11721–11731. 112
- 88 L. Tapia, Y. Pérez, J. Solà, S. V. Luis, I. Alfonso and C. Vicent, *Analyst*, 2022, **147**, 5546–5556. 113
- 89 N. Solozabal, L. Tapia, J. Solà, Y. Pérez and I. Alfonso, *Bioconjug. Chem.*, 2023, **34**, 2345–2357. 114
- 90 M. D. Haskell, J. K. Slack, J. T. Parsons and S. J. Parsons, *Chem. Rev.*, 2001, **101**, 2425–2440. 115
- 91 F. H. Igney and P. H. Krammer, *Nat. Rev. Cancer*, 2002, **2**, 277–288. 116
- 92 E. Faggi, Y. Pérez, S. V. Luis and I. Alfonso, *Chem. Commun.*, 2016, **52**, 8142–8145. 117
- 93 L. Tapia, N. Solozabal, J. Solà, Y. Pérez, W. T. Miller and I. Alfonso, *Chem. - A Eur. J.*, 2021, **27**, 9542–9549. 118
- 94 V. C. Yan, H. E. Butterfield, A. H. Poral, M. J. Yan, K. L. Yang, C.-D. Pham and F. L. Muller, *Trends in Cancer*, 2020, **6**, 924–941. 119
- 95 T. J. Mooibroek, J. M. Casas-Solvas, R. L. Harniman, C. M. Renney, T. S. Carter, M. P. Crump and A. P. Davis, *Nat. Chem.*, 2016, **8**, 69–74. 120
- 96 A. Berdiaki, M. Neagu, E.-M. Giatagana, A. Kuskov, A. M. Tsatsakis, G. N. Tzanakakis and D. Nikitovic, *Biomolecules*, DOI:10.3390/biom11030395. 121
- 97 E. Kamhi, E. J. Joo, J. S. Dordick and R. J. Linhardt, *Biol. Rev.*, 2013, **88**, 928–943. 122
- 98 I. Capila and R. J. Linhardt, *Angew. Chemie Int. Ed.*, 2002, **41**, 390–412. 123
- 99 N. K. Karamanos, Z. Piperigkou, A. D. Theocharis, H. Watanabe, M. Franchi, S. Baud, S. Brézillon, M. Götte, A. Passi, D. Vigetti, S. Ricard-Blum, R. D. Sanderson, T. Neill and R. V. Iozzo, *Chem. Rev.*, 2018, **118**, 9152–9232. 124
- 100 S. Khan, J. Gor, B. Mulloy and S. J. Perkins, *J. Mol. Biol.*, 2010, **395**, 504–521. 125
- 101 N. S. Gandhi and R. L. Mancera, *Drug Discov. Today*, 2010, **15**, 1058–1069. 126
- 102 D. Imberti, W. Ageno, R. Manfredini, F. Fabbian, R. Salmi, R. Duce and M. Gallerani, *Thromb. Res.*, 2012, **129**, 418–425. 127
- C. Tieken and H. H. Versteeg, *Thromb. Res.*, 2016, **140**, 148–153. DOI: 10.1039/D4CC03163F 128
- C. C. La, L. E. Takeuchi, S. Abbina, S. Vappala, U. Abbasi and J. N. Kizhakkedathu, *Biomacromolecules*, 2020, **21**, 2595–2621. 129
- P. Gans, A. Sabatini and A. Vacca, *Talanta*, 1996, **43**, 1739–1753. 130
- C. Bazzicalupi, A. Bencini, A. Bianchi, M. Cecchi, B. Escuder, V. Fusi, E. Garcia-España, C. Giorgi, S. V. Luis, G. Maccagni, V. Marcelino, P. Paoletti and B. Valtancoli, *J. Am. Chem. Soc.*, 1999, **121**, 6807–6815. 131
- A. De Robertis, C. De Stefano, A. Gianguzza and S. Sammartano, *Talanta*, 1999, **48**, 119–126. 132
- M. Corredor, D. Carbajo, C. Domingo, Y. Pérez, J. Bujons, A. Messegueur and I. Alfonso, *Angew. Chemie - Int. Ed.*, 2018, **57**, 11973–11977. 133
- D. Carbajo, Y. Pérez, M. Guerra-Rebollo, E. Prats, J. Bujons and I. Alfonso, *J. Med. Chem.*, 2022, **65**, 4865–4877. 134
- N. Fujitani, J. Furukawa, K. Araki, T. Fujioka, Y. Takegawa, J. Piao, T. Nishioka, T. Tamura, T. Nikaido, M. Ito, Y. Nakamura and Y. Shinohara, *Proc. Natl. Acad. Sci.*, 2013, **110**, 2105–2110. 135
- A. Pudełko, G. Wisowski, K. Olczyk and E. M. Koźma, *FEBS J.*, 2019, **286**, 1815–1837. 136
- A. H. Bartlett and P. W. Park, *Expert Rev. Mol. Med.*, 2010, **12**, e5. 137
- D. Carbajo, Y. Pérez, J. Bujons and I. Alfonso, *Angew. Chemie - Int. Ed.*, 2020, **59**, 17202–17206. 138
- S. Liu, Q. Zhang, A. N. Shy, M. Yi, H. He, S. Lu and B. Xu, *J. Am. Chem. Soc.*, 2021, **143**, 15852–15862. 139
- S. Liu, Q. Zhang, H. He, M. Yi, W. Tan, J. Guo and B. Xu, *Angew. Chemie Int. Ed.*, 2022, **61**, e202210568. 140
- F. Li, Y. Liu, Y. Dong, Y. Chu, N. Song and D. Yang, *J. Am. Chem. Soc.*, 2022, **144**, 4667–4677. 141
- S. Kim, B. Jana, E. M. Go, J. E. Lee, S. Jin, E.-K. An, J. Hwang, Y. Sim, S. Son, D. Kim, C. Kim, J.-O. Jin, S. K. Kwak and J.-H. Ryu, *ACS Nano*, 2021, **15**, 14492–14508. 142
- S. Kim, J.-B. Chae, D. Kim, C.-W. Park, Y. Sim, H. Lee, G. Park, J. Lee, S. Hong, B. Jana, C. Kim, H. Chung and J.-H. Ryu, *J. Am. Chem. Soc.*, 2023, **145**, 21991–22008. 143
- M. T. Jeena, L. Palanikumar, E. M. Go, I. Kim, M. G. Kang, S. Lee, S. Park, H. Choi, C. Kim, S.-M. Jin, S. C. Bae, H. W. Rhee, E. Lee, S. K. Kwak and J.-H. Ryu, *Nat. Commun.*, 2017, **8**, 26. 144
- C. Jonkergouw, N. K. Beyeh, E. Osmekhina, K. Leskinen, S. M. Taimoory, D. Fedorov, E. Anaya-Plaza, M. A. Kostianen, J. F. Trant, R. H. A. Ras, P. Saavalainen and M. B. Linder, *Nat. Commun.*, 2023, **14**, 2141. 145
- O. I. V. Luotonen, E. Osmekhina, E. Anaya-Plaza, S. Kaabel, A. L. Harmat, M. Sammalkorpi, C. Jonkergouw, M. B. Linder and M. A. Kostianen, *Cell Reports Phys. Sci.*, 2024, **5**, 102089. 146
- L. S. J. Roope, R. D. Smith, K. B. Pouwels, J. Buchanan, L. Abel, P. Eibich, C. C. Butler, P. S. Tan, A. S. Walker, J. V. Robotham and S. Wordsworth, *Science (80-.)*, 2019, **364**, eaau4679. 147
- C. J. L. Murray, K. S. Ikuta, F. Sharara, L. Swetschinski, G. Robles Aguilar, A. Gray, C. Han, C. Bisignano, P. Rao, E. 148



Wool, S. C. Johnson, A. J. Browne, M. G. Chipeta, F. Fell, S. Hackett, G. Haines-Woodhouse, B. H. Kashef Hamadani, E. A. P. Kumaran, B. McManigal, S. Achalapong, R. Agarwal, S. Akech, S. Albertson, J. Amuasi, J. Andrews, A. Aravkin, E. Ashley, F.-X. Babin, F. Bailey, S. Baker, B. Basnyat, A. Bekker, R. Bender, J. A. Berkley, A. Bethou, J. Bielicki, S. Boonkasidecha, J. Bukosia, C. Carvalheiro, C. Castañeda-Orjuela, V. Chansamouth, S. Chaurasia, S. Chiurchiù, F. Chowdhury, R. Clotaire Donatien, A. J. Cook, B. Cooper, T. R. Cressey, E. Criollo-Mora, M. Cunningham, S. Darboe, N. P. J. Day, M. De Luca, K. Dokova, A. Dramowski, S. J. Dunachie, T. Duong Bich, T. Eckmanns, D. Eibach, A. Emami, N. Feasey, N. Fisher-Pearson, K. Forrest, C. Garcia, D. Garrett, P. Gastmeier, A. Z. Giref, R. C. Greer, V. Gupta, S. Haller, A. Haselbeck, S. I. Hay, M. Holm, S. Hopkins, Y. Hsia, K. C. Iregbu, J. Jacobs, D. Jarovsky, F. Javanmardi, A. W. J. Jenney, M. Khorana, S. Khusuwan, N. Kissoon, E. Kobeissi, T. Kostyanev, F. Krapp, R. Krumkamp, A. Kumar, H. H. Kyu, C. Lim, K. Lim, D. Limmathurotsakul, M. J. Loftus, M. Lunn, J. Ma, A. Manoharan, F. Marks, J. May, M. Mayxay, N. Mturi, T. Munera-Huertas, P. Musicha, L. A. Musila, M. M. Mussi-Pinhata, R. N. Naidu, T. Nakamura, R. Nanavati, S. Nangia, P. Newton, C. Ngoun, A. Novotney, D. Nwakanma, C. W. Obiero, T. J. Ochoa, A. Olivas-Martinez, P. Olliaro, E. Ooko, E. Ortiz-Brizuela, P. Ounchanum, G. D. Pak, J. L. Paredes, A. Y. Peleg, C. Perrone, T. Phe, K. Phommasone, N. Plakkal, A. Ponce-de-Leon, M. Raad, T. Ramdin, S. Rattanavong, A. Riddell, T. Roberts, J. V. Robotham, A. Roca, V. D. Rosenthal, K. E. Rudd, N. Russell, H. S. Sader, W. Saengchan, J. Schnall, J. A. G. Scott, S. Seekaew, M. Sharland, M. Shivamallappa, J. Sifuentes-Osornio, A. J. Simpson, N. Steenkeste, A. J. Stewardson, T. Stoeva, N. Tasak, A. Thaiprakong, G. Thwaites, C. Tigoi, C. Turner, P. Turner, H. R. van Doorn, S. Velaphi, A. Vongpradith, M. Vongsouvath, H. Vu, T. Walsh, J. L. Walson, S. Waner, T. Wangrangsimakul, P. Wannapinij, T. Wozniak, T. E. M. W. Young Sharma, K. C. Yu, P. Zheng, B. Sartorius, A. D. Lopez, A. Stergachis, C. Moore, C. Dolecek and M. Naghavi, *Lancet*, 2022, **399**, 629–655.

dynamic chemical systems and in general all aspects related to the molecular recognition of biorelevant species.

DOI: 10.1039/D4CC03163F



Ignacio Alfonso was initially trained as organic chemist at the University of Oviedo in Spain (PhD in 1999). He has worked in top-rated institutions in France (University of Strasbourg, short stay in 1997) and USA (The Scripps Research Institute, postdoc in 2000–2002). After being selected for the Ramon y Cajal tenure-track contract in 2004 (Universitat Jaume I, Castellón), in 2007 he got a permanent position at

IQAC-CSIC in Barcelona, where he created the Supramolecular Chemistry Group that he leads since then. His research interests are supramolecular chemistry, chemical biology, complex

Supramolecular Chemical Biology: Designed Receptors and Dynamic Chemical Systems

Ignacio Alfonso*^a

^a *Department of Biological Chemistry, Institute for Advanced Chemistry of Catalonia (IQAC), The Spanish National Research Council (CSIC)
Jordi Girona 18-26, 08034, Barcelona (Spain).
E-mail: ignacio.alfonso@iqac.csic.es*

This manuscript is submitted to be considered as Feature Article and, accordingly it does not contain original research. Therefore, no new data is needed to become available.

ChemComm Accepted Manuscript

