Arginine versus Lysine: Molecular Determinants of Cation–π Interactions in Biomolecular Condensates

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Abstract

Biomolecular condensates formed by intrinsically disordered regions of proteins are primarily stabilised by specific types of amino acids, usually labelled "stickers" for their ability to crosslink distinct polypeptide chains into droplet-spanning networks. Both aromatic and positively charged residues can play this role, but they differ in their interaction types and relative strengths. An outstanding problem is the quantification of these differences, since they determine the fate of multicomponent mixtures. Here we focus on the two main positively charged stickers, Arg and Lys, whose experimental behaviour in condensates shows a clear hierarchy: Arg is consistently observed to promote phase separation more effectively than Lys. We use alchemical transformations together with quantum chemical calculations to resolve the differences in their cation–π interaction strengths in different media. We find that, unlike the aromatic residues Phe and Tyr, Arg and Lys consistently maintain a hierarchy of interaction strengths across molecular environments, with Arg being favoured over Lys. While cation-π

interactions are important, the primary factor underlying this difference is the higher dehydration penalty of Lys. By contrast, the identity of the aromatic partner that forms the strongest interaction with a cation depends on the dielectric environment, in line with expectations from statistical potentials derived from protein structures. These results provide a molecular-level explanation for the distinct contributions of cationic and aromatic residues to condensate stability.

Introduction

In recent years, our understanding of cellular organisation has been transformed by the coming to prominence of membraneless organelles, formed primarily by proteins and nucleic acids.[1] These organelles can form both in the cytoplasm, such as P-bodies and stress granules, and in the nucleus, as in the case of nuclear speckles, Cajal bodies, or the nucleolus. The idea that membraneless organelles may arise through liquid–liquid phase separation of their constituents has renewed interest in the phase behaviour of biomolecules.

Much of our understanding of condensate formation is mediated by theories of polymers.[2] The "stickers and spacers" model borrowed from the theory of associative polymers[3–6] has been particularly successful as a framework for interpreting experiments on condensates, both in ordered[7] and disordered proteins.[5,8] In this model, different amino acid residues or groups thereof are assigned specific roles, either mediating the cross-links between interacting units ("stickers") or providing the necessary flexibility and fluidity to the resulting network ("spacers"). In the case of intrinsically disordered proteins (IDPs) or regions (IDRs), experiments have resolved a specific grammar for phase separation.[5] Aromatic amino acid residues (Phe, Tyr) and positively charged (Lys, Arg) residues appear prominently in the sticker category. Polar residues, like Ser, and Gly play the role of spacers. While all amino acid residues can form hydrogen bonds, the distinct chemical nature of different stickers allows them to form distinct types of interactions, including π-stacking, cation-π or CH-π, all of which are relevant in condensates.[5]

An important question to understand these condensates more fully is what is the relative strength of the different stickers. Experimentally, this problem has been approached via ingenious sequence perturbations in phase-separating proteins.[5,9–14] A key result is that interactions between Arg and Tyr govern condensation and that they are much stronger stickers than their counterparts Lys and Phe.[5] This suggests that the number of Arg and Tyr residues alone may predict the saturation concentration of a phase-separating protein,[5] although contributions from other residue types are also important.[15]

We have recently studied the molecular origin of the greater stickiness of Tyr relative to Phe in biomolecular condensates,[16] which may be surprising considering that Phe is broadly regarded as more hydrophobic than Tyr.[17] We employed molecular dynamics (MD) simulations of minimal model condensates[18] and quantum chemical calculations to demonstrate that the relative sticker strength is significantly influenced by the difference in transfer free energy, which depends on the environment.[16] Transferring stickers from water into the cores of folded proteins, with a very low dielectric constant,[19] is more favourable for Phe. However, in the case of condensates —with intermediate dielectric[20,21]— the balance tilts towards Tyr.[16]

Here we turn our attention to the positively charged residues Arg and Lys. As with Phe and Tyr, one might naively assume that these two residues are interchangeable, given their shared ability to engage in cation-π interactions and similarly high pK_{a} values in model compounds (12 and 10.4, respectively[22]). However, experiments on FUS,[5] Ddx4[23] or LAF-1,[12] where R→K mutants reduced or entirely suppressed phase separation, point to a greater sticker strength of Arg. The relative strengths of Arg and Lys as stickers have also been interrogated using computational approaches, including simulations with coarse-grained models[21] and atomistic force fields,[24–26] and computational chemistry calculations.[27] A consensus is emerging that while both share their role as cations, Arg interacts more strongly with aromatic partners like Phe and Tyr primarily due to the pseudo-aromatic character of the positively charged guanidinium group.[28]

In this work, we use a combination of alchemical transformations and DFT calculations to examine whether the context-dependent stickiness observed for Phe and Tyr applies also to the cation–π pairs formed with Arg and Lys. We find that, although Arg is consistently a stronger sticker than Lys, the dependency on the dielectric observed in our previous work determines whether Phe or Tyr form the most favourable cation-π pair. Our results highlight that, in addition to the interaction strengths of Lys and Arg within the condensate, a key distinction between both residues lies in their differing free energies of transfer from water.

Materials and Methods

Molecular simulations

Thermodynamic cycle for the estimation of free energy differences in transfer free energies

As an important contribution to sticker strength in biomolecular condensates,[16,29] we are interested in calculating the relative transfer free energy ($\Delta\Delta G_{\text{Transfer}}$) from water into a biomolecular condensate for a peptide bearing either Lys or Arg. Direct simulations to estimate the values of $\Delta G_{\text{Transfer}}$ are very challenging. Alternatively, we use the thermodynamic cycle in Figure 1, where we are representing the molecule of interest (GGXGG) in both chemical states (X=K and X=R) submerged in two different solvents (water and a model condensate). In the diagram, the transfer process is represented by the vertical arrows and associated with the free energy $\Delta G^{\text{X}}_{\text{Transfer}}$. Because we are interested in the difference in transfer free energies between Arg and Lys, we can instead calculate the free energies $\Delta G_{\text{K}\rightarrow\text{R}}$ in either water or the condensate associated with the horizontal branches, which involve an alchemical transformation. Because of the path-independence of free energy changes, we can write

$$\Delta\Delta G_{\text{Transfer}} = \Delta G^{\text{K}}_{\text{Transfer}} - \Delta G^{\text{R}}_{\text{Transfer}} = \Delta G^{\text{Condens}}_{\text{K}\rightarrow\text{R}} - \Delta G^{\text{H}_2\text{O}}_{\text{K}\rightarrow\text{R}} \tag{1}$$

Hence, using the values for $\Delta G_{\mathrm{K}\to\mathrm{R}}$ in water and in the condensate, which are easier to calculate, we can determine $\Delta\Delta G_{\mathrm{Transfer}}$.

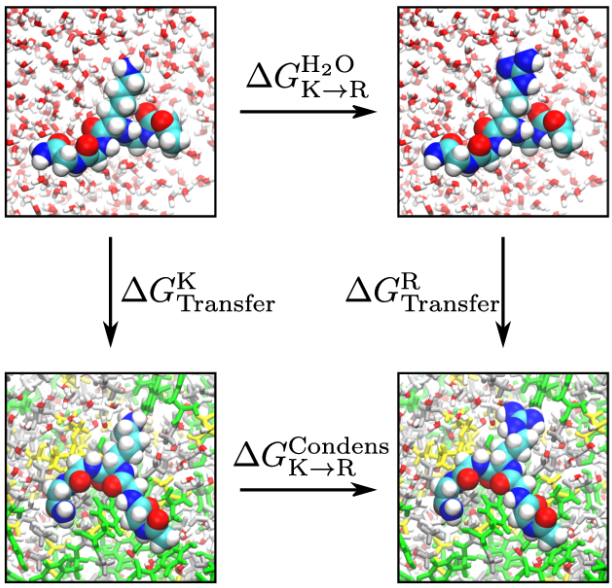

Figure 1: Thermodynamic cycle involving the transfer of short peptides with sequence GGXGG from water to condensates (vertical branches) and the alchemical transformation from X=Lys to X=Arg (horizontal branches). In the condensates, different colours indicate different constituent residues (Gly: white; Ser: yellow; Tyr: green).

Molecular models

We have run simulations of a short pentapeptide with sequence GGXGG with X=Arg or Lys capped at the N and C-termini in different media. All peptide structures were built using the LEaP program from Ambertools.[30] We used the Amber99SB⋆-ILDN force field, which includes corrections in backbone and sidechain torsions[31,32] as provided within the PMX software for the generation of hybrid topologies.[33] The three-point TIP3P model was used for simulations including water molecules.[34] As alternative solvents, we use a collection of organic molecules with different dielectrics using the GAFF parameters from the Free Solvation Database by Mobley and co-workers.[35]

Details of the simulations

We generated simulation boxes for the GGXGG peptide as before.[16] For simulations in pure water, we first inserted the fully extended structure of the peptide in an octahedral box, allowing at least 1 nm in each dimension. In the case of the organic solvents, we solvated the peptide using 500 molecules. Simulations of the peptide inside a condensate slab were performed by first inserting the GGXGG peptide in the center of a rectangular box of 10 nm×4 nm×4 nm and then surrounding it with a mixture of 300 copies of terminally capped Gly, Ser and either Phe or Tyr (which we term GSF and GSY condensates, respectively). This box was finally extended on both sides to reach a total length of 20 nm and filled with water molecules.

Due to the relevance of the electrostatic interactions of Arg/Lys to this study, we have tested different options for the neutralization of our simulation boxes. Whether ions are incorporated in the box can influence the results of the calculations, particularly when one considers non-homogeneous systems like our condensates in a slab geometry.[36] For this reason, we have carried out two batches of runs of condensate systems, one where a single Cl^- was added (termed "neutral") and one where, in addition to this anion, we include ions up to 0.15 M salt (termed "physiological"). We have followed the same approach in the case of water. For other pure solvents, we rely on the uniform background charge added by the Ewald method.

We have equilibrated all our simulation boxes using a similar simulation protocol. First, solvated boxes were energy-minimized using a steepest descent algorithm. Second, we run a short simulation in the NVT ensemble using the Berendsen thermostat.[37] Next, we equilibrated the density of the simulation box in a short NPT simulation using the Berendsen barostat. Finally, we run long production simulations of up to 5 μs using the Parrinello-Rahman barostat[38] (see details in Table S1 in the Supporting Information). A leap-frog stochastic dynamics integrator with a 2 fs time step was used in the simulations. Van der Waals interactions were switched at 1 nm, and long-range electrostatics were calculated using

the Particle-mesh Ewald method.[39] All the simulations were run using the Gromacs software package[40] (version 2024).

Analysis of the simulations

We have analysed the simulations using a combination of the Gromacs analysis programs,[40] in-house Python scripts that exploit tools from the MDtraj library[41] and the scripts from the PMX package for the estimation of free energy associated with the alchemical transformation from the non-equilibrium runs.[33,42,43] These are derived from the work performed in the forward and backward non-equilibrium transitions, $W = \int_{\lambda_0}^{\lambda_1} \left(\frac{\partial H}{\partial \lambda}\right) d\lambda$. We report free energy estimates from Bennet's Acceptance Ratio method[44] and refer the interested reader to the PMX papers for additional details.[33,42,43] Errors were determined via bootstrapping.

Quantum chemical calculations

We use the Gaussian 16 program to perform quantum mechanical calculations to study interaction energies of complexes formed by amino acid side chain analogues (see Figure 2B). We consider all atoms starting from the C^{β}, as in the past, Chipot and co-workers found that toluene was a better model than benzene for Phe sidechains in proteins.[45] We used Density Functional Theory (DFT) level, using the ωB97XD functional[46] and Pople's 6-311++G(d,p) basis set[47] for geometry optimization. This methodology was succesfully used by Jorgensen et al.[48] to describe cation-π interactions. To visualize the nature of the non-covalent interactions in the optimized dimers, we performed a Non-Covalent Interaction (NCI) Index analysis.[49] To recapitulate the effects of different solvents, we run the geometry optimizations using the Polarizable Continuum Model approach[50]. In a given solvent, the interaction energy was calculated according to

$$\Delta E_{\text{Int}}^{ij} = E^{ij} - \left(E^{i} + E^{j}\right) \tag{2}$$

where i and j specify the interacting monomers.

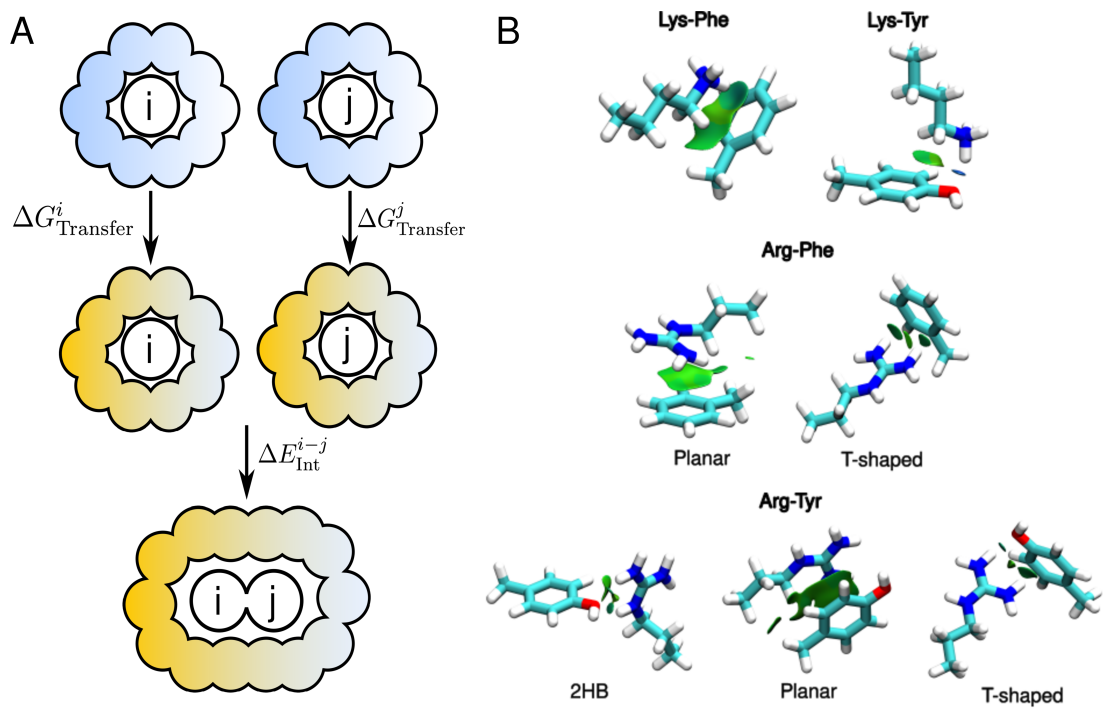

Figure 2: (A) Schematic of the process of contact formation for a pair of amino acid residues i and j. The first step corresponds to the transfer from water (blue), into a medium s (yellow), with an associated $\Delta G^{i/j}_{\text{Transfer}}$. The second step involves the contact formation within the medium s, characterised with an energy $\Delta E^{i-j}_{\text{Int}}$. (B) Structures of all the dimers considered in the QM calculations. The surfaces shown are generated with NCI (Non-Covalent Interaction) analysis. In the case of Arg, various configurations were calculated: i) coplanar (P), T-shaped (T) and dibridged hydrogen bonded (2HB) in the case of the Arg-Tyr dimer.

For a given monomer i, we calculate the free energy of transfer from water to a given solvent s using

$$\Delta G^{i}_{\text{Transfer}} = \Delta G^{i}(s) - \Delta G^{i}(\text{H}_2\text{O}) \tag{3}$$

The free energies in both water and the solvent s are estimated using the SMD solvation model[51], using B3LYP/6-31+G(d,p) level of theory[52,53]. As solvents, we have considered cyclohexane, benzene, toluene, n-octanol, 1-hexanol, acetone, methanol, and water. On the other hand, we have used water as a solvent by varying the dielectric value, similar to our previous publication,[16] in which this model was used in the context of aromatic-aromatic interactions. Combining the monomer transfer free energies with the dimer interaction energy

at a given solvent s (see Figure 2A), we obtain the contact energy for the i-j pair:

$$\Delta E^{ij}_{\text{contact}}(s) = \Delta G^{i}_{\text{transfer}}(s) + \Delta G^{j}_{\text{transfer}}(s) + \Delta E^{ij}_{\text{int}}(s) \tag{4}$$

Results and discussion

Alchemical transformations reproduce the experimental hierarchy of sticker strengths

We have first run long equilibrium simulations of the GGXGG peptide in the $\lambda = 0$ (X=K) and $\lambda = 1$ (X=R) states inside the ternary condensate slabs termed GSY and GSF. We show a representative snapshot of the GSY condensate in Figure 3A. The boxes take up to 500 ns to equilibrate, as inferred from the convergence of the solvent accessible surface area of the protein atoms ($SASA$) (see SI Figure S1). From that point, density profiles remain stable. In the case of the GSY condensate, the GGXGG peptide remains buried throughout most of the long simulation trajectories, although we see it escape in the box neutralised with just one Cl^- anion for the $\lambda = 0$ (see SI Figure S2B). We show the average density profiles for the equilibrated part of the simulation trajectories in physiological conditions in Figure 3B (see also SI Figure S2). In the case of the less protein-dense GSF condensate, the GGXGG peptide escapes from the condensate interior more readily and stays temporarily at the interface region (see SI Figure S3). These simulation results are consistent for both systems irrespective of the procedure used to neutralise the simulation box (see SI Figures S1-S3).

We selected equally spaced snapshots from the long simulation runs, excluding the first 500 ns of the trajectories and any configuration where the peptide was not buried within the condensate. Then we ran hundreds of fast, non-equilibrium switches from both end states. Using the statistics of $\left(\partial H/\partial\lambda\right)$ for all initial states, we estimate the values of $\Delta G^{\text{Condens}}_{\text{K}\to\text{R}}$ using the PMX workflow (see Methods and SI Figure S4). Switches were also run for simulations of

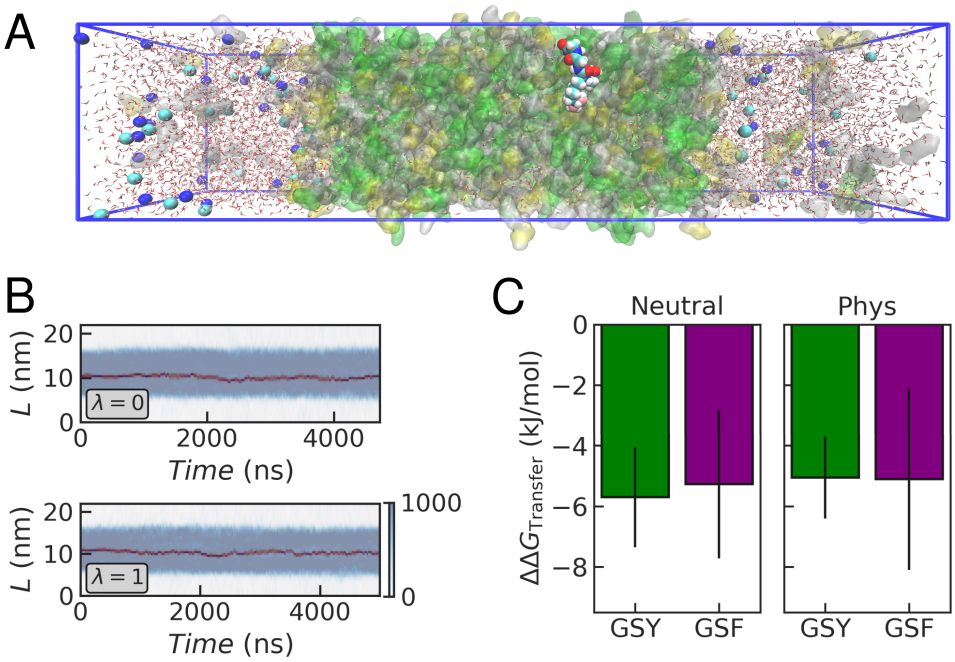

Figure 3: (A) Representative snapshot of the GGXGG peptide inserted in the GSY condensate in the slab simulation box at physiological salt. The peptide and Na^+ and Cl^- ions are shown as spheres. The condensate is shown as a transparent surface. (B) Density profiles as a function of time for the end states $\lambda = 0$ (X=K) and $\lambda = 1$ (X=R) in the GSY condensate at physiological salt. The total protein density is shown in blue, and density of the K/R residue is shown in dark red. Density is expressed in mg/mL. (C) Estimates of the transfer free energy difference at neutral and physiological ionic strength in the GSY and GSF condensates.

the peptide in both λ states in water matching the neutral or physiological neutralisation, in order to obtain $\Delta G^{\mathrm{H_2O}}_{\mathrm{K\to R}}$. Combining both free energy estimates, we obtain $\Delta\Delta G_{\mathrm{Transfer}}$ using Equation 1. We find that the value of $\Delta\Delta G_{\mathrm{Transfer}}$ is negative in both the GSF and GSY condensates (see Figure 3C). This indicates that Arg has a stronger propensity to be part of the model peptide condensates than Lys. The results are independent of the neutralisation conditions. The emerging hierarchy of sticker strength based on our calculations is: Arg-Tyr > Lys-Tyr and Arg-Phe > Lys-Phe. This result is consistent with a plethora of experimental and computational results that point to a higher stickiness in Arg.[5,11,12,21,23,25,54]

Several interesting comparisons can be drawn between the current results and those obtained for Tyr and Phe in our previous work.[16] For this comparison, we consider the "neutral" conditions, as no ions were added in the F→Y transformations. Although Tyr was found to be more "sticky" than Phe based on the negative $\Delta\Delta G_{\mathrm{Transfer}}$ of the F→Y transformation, the difference between aromatics was small ($\sim$ 1.5 kJ/mol). For the K→R transformation, the magnitude of the differences in transfer free energy is much greater ($\sim$ 5$\pm$2 kJ/mol). This is consistent with the generally more disruptive effect of R→K mutations, often capable of completely suppressing phase separation[12,23] and with the hierarchy of energy scales derived from atomistic potentials of mean force.[55]

Context independence of Arg and Lys transfer free energies

In our previous work, we reported that the hierarchy of sticker strengths between Tyr and Phe is context-dependent.[16] This effect is governed by the magnitude of the transfer free energy of the amino acid residue from water into a different environment. Tyr is favoured for polar media where it can form hydrogen bonds, such as the water-rich GSY and GSF condensates or acetone, whereas Phe is preferred in apolar solvents like cyclohexane or benzene. We found that the value of the dielectric constant ε of the media tracks this crossover. This is important because ε for condensates is around 40, roughly half that of water,[16,20,21] and it is much lower in the hydrophobic cores of folded proteins.[56]

In the case of Arg and Lys we have similarly interrogated the dependence of the relative transfer free energy on the target medium. We have performed the same type of alchemical transformations in multiple other solvents and estimated the value of $\Delta\Delta G_{\text{Transfer}}$ (see SI Figure S5). In this case, we find that irrespective of the solvent, $\Delta\Delta G_{\text{Transfer}}$ is always negative, and hence consistently favours Arg relative to Lys (see Figure 4A). There are, however, quantitative differences across solvents, although we cannot establish a systematic dependence of $\Delta\Delta G_{\text{Transfer}}$ on ε (see Figure 4B). Also, the numerical results for toluene, benzene and cyclohexane may be influenced by the difficulty of sampling the equilibrium distribution of peptide torsion angles in these solvents, which can affect the outcome of the non-equilibrium switching.[57] In any case, the results of the alchemical transformations suggest a robust trend for a more favourable transfer free energy of Arg across diverse target media.

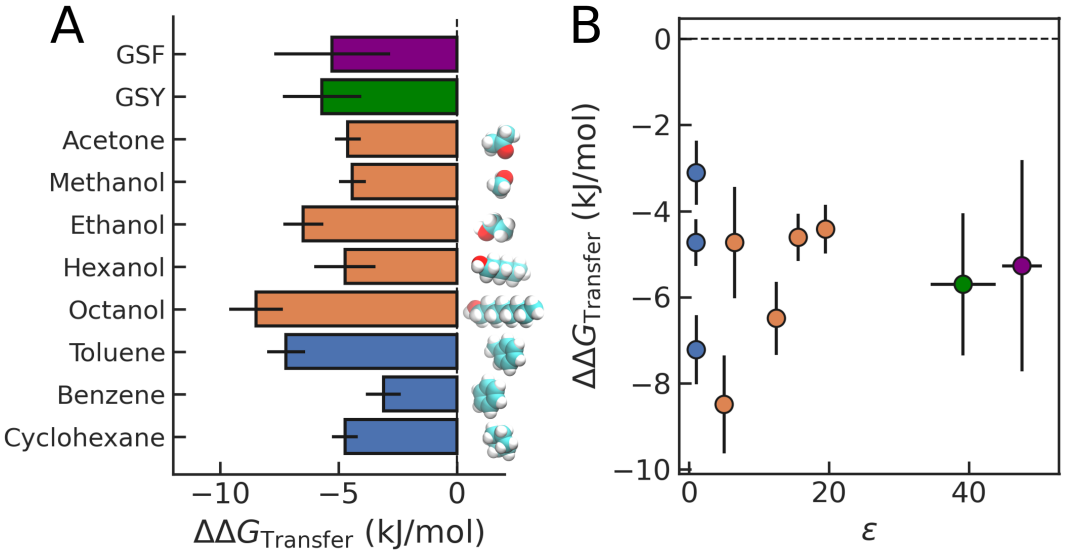

Figure 4: (A) Transfer free energy differences in different solvents from alchemical transformations. (B) Relationship between calculated dielectric constants and transfer free energy differences.

Interaction modes of Lysine and Arginine in peptide condensates

We next investigate the interactions formed by Arg and Lys inside the peptide condensates. For this, we study the contacts established during the long equilibrium runs by the sidechain of the alchemically transformed residue. We have inspected the values of inter-sidechain distances and orientations between vectors normal to the plane of both the aromatic ring of either Tyr or Phe and the Arg guanidinium group. We find a clear propensity to form π-stacked arrangements between the Tyr/Phe and Arg sidechains, corresponding to short distances and values close to 0 or π for the angle θ between normal planes to the aromatic ring and guanidinium group (see Figure 5 A, C). In the case of Lysine, there is no orientational preference relative to the aromatic ring, as expected. This trend is consistent for both GSY and GSF condensates (see representative snapshots for both condensates in Figure 5 B, D). This clear distinction supports a role of this type of interaction in the more favourable transfer free energy of Arg.

A potential limitation of our results is that the cation-π interaction can only approximately be reproduced by classical force fields. This is due to the lack of a proper representation of the polarizing effect of the cation over the π-electrons.[58–60] Recently, different approaches have been proposed to overcome these limitations, including an empirical correction to the Lennard-Jones potential[48] or ad-hoc modifications in the charges for bound states.[24] Another possibility is to resort to polarizable models to study the hierarchy of interactions.[26] Alternatively, here we resort to quantum mechanical calculations.

Contact energies from quantum chemical calculations

Our investigation of the interactions between positively charged and aromatic residues reveals an interplay of intrinsic forces and environmental influences. To gain insight into these effects, we performed quantum chemical calculations considering the side chains of Lys, Arg, Phe, and Tyr. We evaluated the interaction energies of all possible charged/aromatic dimers in various configurations, taking into account environmental conditions of different polarities.

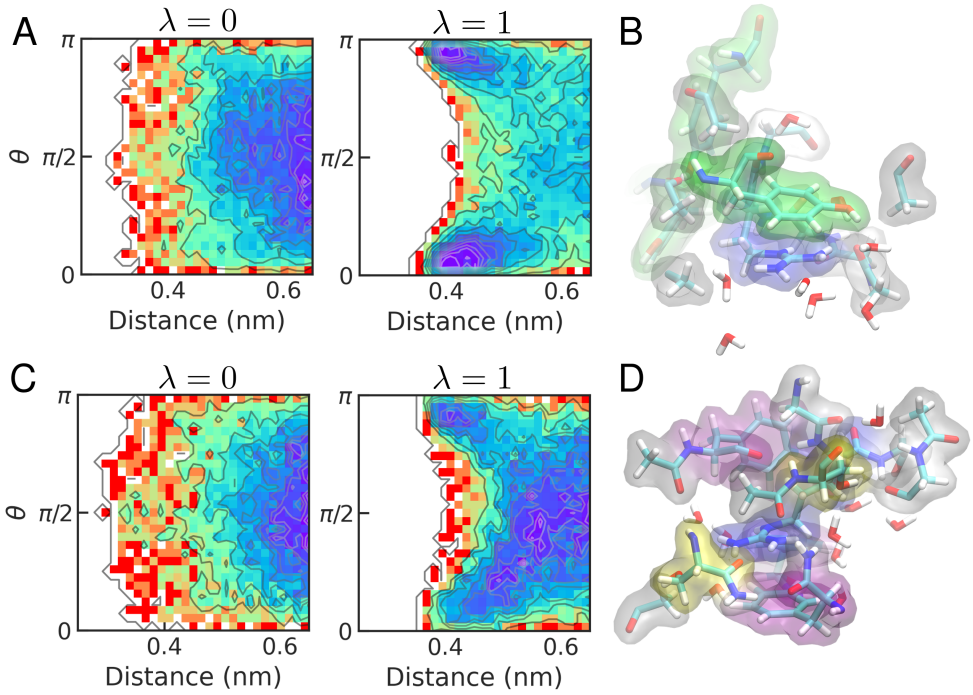

Figure 5: (A) Density plots of the distance and relative orientation of normal vectors between sidechain atoms of positively charged and aromatic residues in the GSY condensate. $\lambda = 0$ corresponds to X=K and $\lambda = 1$, to X=R. In the case of X=K, we use the dummy atoms in the dual topology to measure θ. (B) Representative snapshots of π-stacked arrangement of Tyr and Arg. (C-D) Same as A and B, respectively, for the GSF condensate.

To introduce environmental effects, two types of solvents were considered within a polarizable continuum model, as before:[16] i) solvents of different polarity and ii) water with a reduced dielectric constant, which may capture the effect of an increasingly restrained water solvent. Two realistic scenarios for a dielectric decrement are those of electrolyte solutions,[61] and water confinement within biomolecular condensates, where water molecules diffuse within a polymer network with mesh size in the range of a few nanometers.[62,63] In our calculations, we obtained the same qualitative results for both the different solvents and for water with a reduced dielectric constant. For brevity, we present the results for the latter case in the supplementary material (see SI Figure S6).

We first discuss the different geometrical arrangements considered for the Arg-containing complexes: i) T-shaped, ii) coplanar stacked conformations, and iii) dibridged hydrogen-bonded structures in the case of Arg-Tyr (see Figure 2B). These dimer structures are stabilized by different noncovalent interactions, characterized by noncovalent indices. Namely, we find wide areas corresponding to attractive van der Waals forces corresponding to $\pi - \pi$ and CH-π interactions (represented in green) and dense attractive areas corresponding to hydrogen bonds (in blue). When comparing T-shaped and coplanar stacked configurations, our results indicate that the preferred geometry depends on the dielectric environment (see Figure 6A). At very low dielectric regimes, T-shaped structures are favoured in both Arg-Phe and Arg-Tyr complexes, whereas at higher dielectrics, stacked conformations predominate, consistent with our observations from classical MD (see Figure 5).

Our results are consistent with earlier work by Chipot et al., which highlighted that Arg-containing cation-π complexes in the PDB predominantly adopt stacked geometries, in stark contrast to the perpendicular T-shaped motifs favored in the gas phase.[45] More recently, Calinski and Levy noted that although QM calculations support the stabilizing potential of π-stacked interactions, this need not necessarily translate into their prevalence in experimentally resolved protein structures.[27] This apparent discrepancy likely reflects the influence of the local environment, as interacting cation-π residue pairs are often situated in more hy-

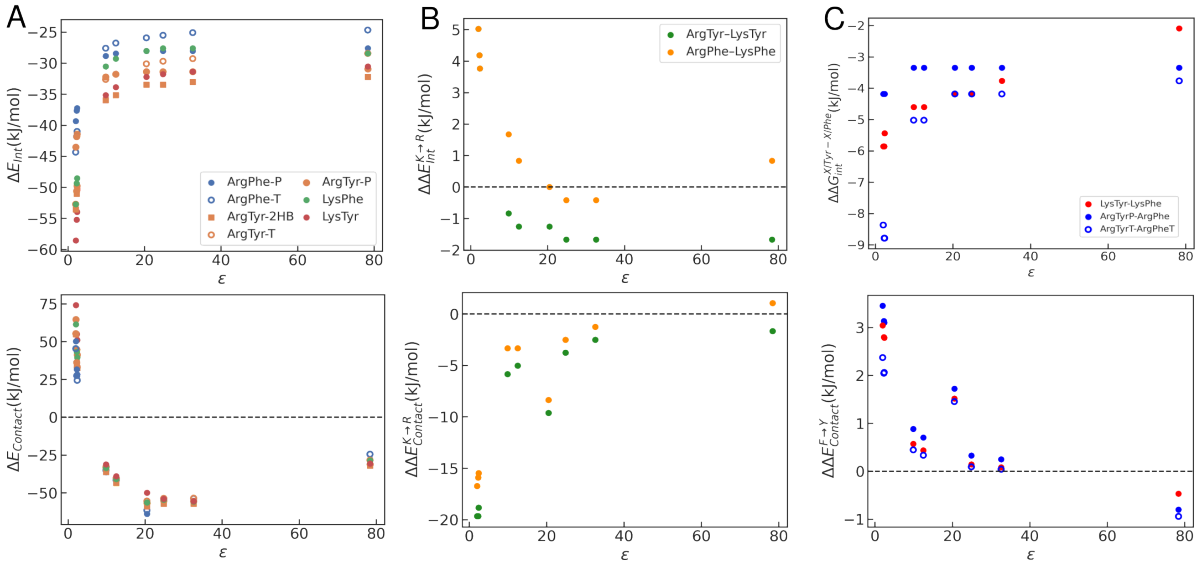

Figure 6: (A) ΔE_{Int} (top) and $\Delta E_{\text{Contact}}$ (bottom) energies for various configurations of the charged-aromatic residue dimers in various solvents as a function of the dielectric constant (ε). (B) $\Delta\Delta E_{\text{Int}}$ and $\Delta\Delta E_{\text{Contact}}$ energies for the Lys→Arg substitution in charged-aromatic dimers. We compare each Lys dimers with Phe and Tyr with the most stable conformation of the corresponding Arg pair at each dielectric value. (C) Same as B but for the Phe→Tyr substitution. $\Delta\Delta E$ values were calculated by comparing geometrically analogous conformations for each pair.

drophobic regions rather than being solvent-exposed. The existence of a solvent effect is further supported by the positive correlation between the frequency of π-stacked conformations and the number of water molecules in the vicinity of the interacting pair. Previous analyses show that π-stacked interactions are more frequent in solvent-exposed loops and turns[64] —and thus may be important stabilizers in disordered proteins— while protein interfaces also favour a coplanar arrangement of Arg's guanidinium with aromatic rings.[65] Consistent with these observations, our results indicate that Arg–Tyr pairs prefer stacked cation–π conformations at medium and high dielectrics, whereas T-shaped geometries are more stable only at very low dielectric values.

In addition to cation-π interactions, hydrogen bonds have also been shown to be highly stabilising in these types of dimers, allowing Arg to form multiple interactions simultaneously with Tyr's oxygen atom.[27] In our calculations, we find that the dibriged two hydrogen-bonded Arg-Tyr structure is the most stable Arg-Tyr dimer in a wide range of dielectrics. Although this propensity for hydrogen-bonded structures could be affected by the conformational restrictions of the peptide chain, in more 60% of the cases analysed by Calinsky et al. of the PDB structures, the cation-π contact was accompanied by hydrogen bonds with Arg.

Energetic analysis reveals the importance of the environment

So far, we have only considered interaction energies (ΔE_{int}), representing the direct attraction between residues in a given environment. Their values reveal that the interaction strength between charged and aromatic residues is significantly amplified in low-dielectric environments (Figure 6A, top). Interestingly, while Arg-Tyr consistently shows the strongest interaction at higher and medium dielectrics, Lys-Tyr emerges as the preferred dimer at very low dielectrics. This aligns well with previous reports on the dielectric dependence of the interaction strength,[66] and with the intuitive expectation that reduced dielectric screening enhances electrostatic forces.

However, to estimate the contact energies ($\Delta E_{\text{contact}}$), we must also take into account

the cost of transferring the individual residues to that environment from the situation where they are fully solvated by water (Figure 2A). When we inspect the trends in $\Delta E_{\text{contact}}$ for the different cation-π pairs (see Figure 6A, bottom), the picture fundamentally changes. Although contact formation remains favourable at intermediate and high dielectrics, at very low values of ε the contact energies become positive. This indicates that dimer formation is disfavoured despite the strong attractions. This destabilisation is primarily driven by the unfavourable transfer energies of the charged residues from a high to a low dielectric environment. This highlights the importance of considering monomer transfer energies in the overall outcome of dimer contact energies, as they can dominate over the specific interaction energy of a dimer.

Permutation of cationic and aromatic residues

To gain insight into the residue preference in cation-π interactions, we examined the relative interaction and contact energies after substituting Lys with Arg and Phe with Tyr (i.e. $\Delta\Delta E_{\text{int}}$ and $\Delta\Delta E_{\text{contact}}$, respectively, for K→R and F→Y). First we discuss the change in the cationic residue (see Figure 6B and supporting Figure S6 for the results in water). The value of $\Delta\Delta E_{\text{int}}^{\text{K}\rightarrow\text{R}}$ reveals an interesting crossover between Arg and Lys: at high dielectric values, Arg is preferred over Lys ($\Delta\Delta E_{\text{int}}^{\text{K}\rightarrow\text{R}} < 0$) regardless of the aromatic partner. However, as the dielectric drops, this preference flips and Lys becomes favoured ($\Delta\Delta E_{\text{int}}^{\text{K}\rightarrow\text{R}} > 0$). The presence of Tyr further enhances the preference for Arg at high dielectrics. Our findings are in agreement with the work of Kumar et al.,[66] who reported that cation-π interaction strength diminishes with increasing values of ε, and Arg forms stronger interactions than Lys in water, despite being less favourable in the gas phase. They reasoned that while Lys-arene interactions are predominantly electrostatic and severely weakened by dielectric screening, dispersion and electrostatics are more balanced in Arg-arene pairs, making them far less sensitive to screening effects.

The picture for the contact energy difference between Arg and Lys pairs is strikingly

different (see Figure 6B, bottom). Arg consistently remains the preferred charged residue throughout the dielectric range (i.e. $\Delta\Delta E^{\mathrm{K}\rightarrow\mathrm{R}}_{\mathrm{contact}} < 0$), an effect that is particularly pronounced at low-dielectrics. This finding is explained by the more favourable transfer free energy of Arg compared to that of Lys. This provides a clear mechanistic explanation for the relevance of Arg as a sticker in biomolecular condensates. Its intrinsic interaction is more robust to dielectric changes, and its transfer to diverse environments is thermodynamically more favourable.

Our calculations also allow us to evaluate the effect of the F→Y substitution. In Figure 6C, we show the differences in interaction energies ($\Delta\Delta E^{\mathrm{F}\rightarrow\mathrm{Y}}_{\mathrm{int}}$) and contact energies ($\Delta\Delta E^{\mathrm{F}\rightarrow\mathrm{Y}}_{\mathrm{contact}}$) between dimers of the aromatics with either Lys or Arg. The interaction energy difference consistently indicates a general preference for Tyr in all dielectrics ($\Delta\Delta E^{\mathrm{F}\rightarrow\mathrm{Y}}_{\mathrm{int}} < 0$) However, once again, trends in the contact energies are more subtle, showing a crossover in $\Delta\Delta E^{\mathrm{F}\rightarrow\mathrm{Y}}_{\mathrm{contact}}$ as the dielectric decreases. This finding mirrors our recent results on Phe and Tyr.[16] It is noteworthy that, although the general trends are similar when using the tuned water-dielectric model (Figure S6), the crossover appears at lower values of ε. This may be due to the capacity for the solvent to hydrogen bond with Tyr.

Hydration free energy and sticker strengths in charged residues

The different trends between the interaction and contact energies reveal that the transfer-free energy is a key factor underlying the preference for Arg over Lys in cation–aromatic contacts. In Figure 7, we analyse this contribution separately, now considering both cationic and neutral forms of the side-chains of Lys and Arg. First, we show the solvation free energies of Lys and Arg (ΔG_{Solv}, see Figure 7A), which is more negative for Lys than for Arg, reflecting the stronger localisation of the positive charge in Lys compared to Arg. When we instead consider the neutral forms of the amino acids, the trend reverses: Arg^0 becomes better solvated than Lys^0 in all solvents. These results are in agreement with experimental estimates of the hydration energy of charged residues and their neutral counterparts.[67–69]

For the charged Lys/Arg side chains, the differences in solvation free energies are strongly attenuated in low-dielectric environments such as toluene, cyclohexane, and benzene.

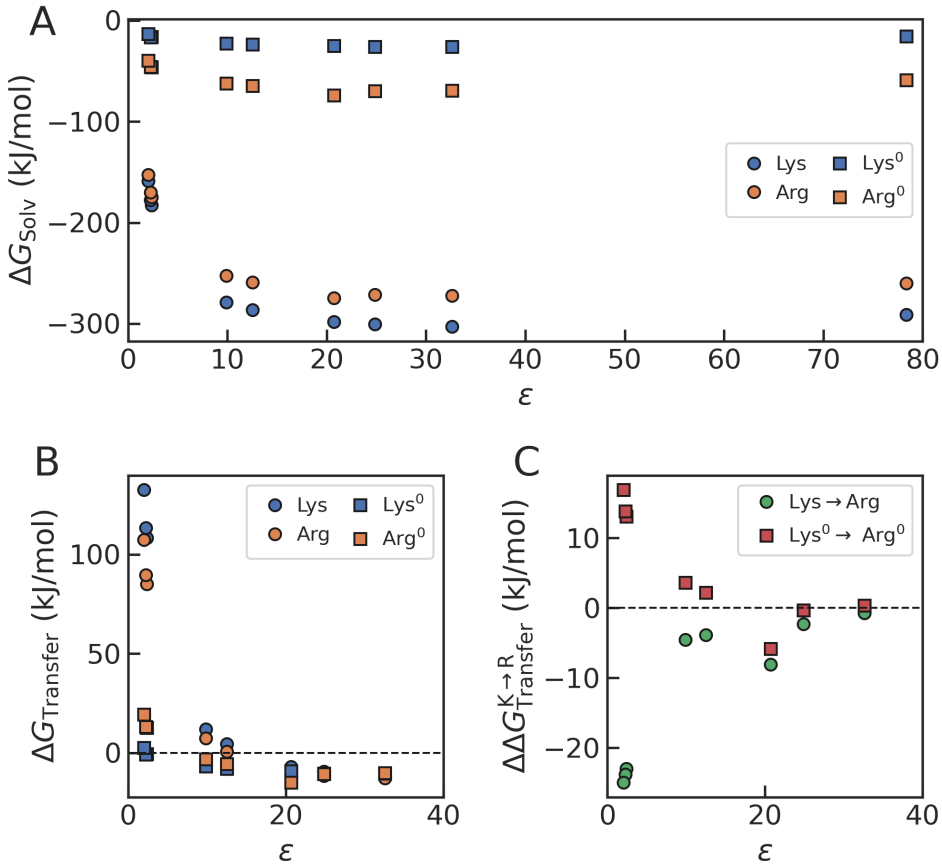

Figure 7: (A) Solvation free energy ΔG_{Solv} for charged and neutral Lys and Arg sidechain analogs at different solvents s as a function of their values of ε. (B) Transfer free energies ($\Delta G_{\mathrm{Transfer}}$) estimated from the difference between solvation and hydration free energies ($\Delta G^{\mathrm{solv}}(\varepsilon)$ and $\Delta G^{\mathrm{solv}}(\mathrm{H_2O})$). (C) Transfer free energy differences ($\Delta\Delta G^{\mathrm{K}\to\mathrm{R}}_{\mathrm{Transfer}}$) between between Arg and Lys.

From the solvation free energy in water and that of any other solvent s, we estimate the transfer free energy, i.e. $\Delta G_{\mathrm{Transfer}}(s) = \Delta G_{\mathrm{Solv}}(s) - \Delta G_{\mathrm{Solv}}(\mathrm{H_2O})$, which we show in Figure 7B. At low dielectrics, the values of the transfer free energies are large and positive for both Arg and Lys due to the penalty of removing the positive charge from water. This penalty is consistently higher for Lys than for Arg. Hence, the relative transfer free energies $\Delta\Delta G^{\mathrm{K}\to\mathrm{R}}_{\mathrm{Transfer}}$ are negative, approaching –25 kJ/mol in low-dielectric solvents (see Figure 7C). In alcohols,

the effect is less noticeable, with values of $\Delta\Delta G^{\mathrm{K}\rightarrow\mathrm{R}}_{\mathrm{Transfer}}$ between –1 and –5 kJ/mol. These results are in semi-quantitative agreement with the trends observed in Fig. 4 for alcohols and acetone, and in qualitative agreement with those for aliphatic solvents. In contrast, when we consider the neutral form of the side chains of Lys and Arg, an opposite trend emerges. In this case, the energetic penalty for transfer from water to less polar solvents is much smaller, and Lys is now preferred over Arg. Accordingly, $\Delta\Delta G^{\mathrm{K}\rightarrow\mathrm{R}}_{\mathrm{Transfer}}$ becomes positive and increases as the solvent polarity decreases.

Together, these results indicate that the "stickier" nature of Arg compared to Lys arises from the difference between the very localized positive charge in Lys, which promotes hydration, compared to the delocalized positive charge of the guanidinium group, which has a lower penalty to transfer to nonpolar environments. Our results confirm previous remarks by Fossat et al.,[70] who suggest that the differences in hydration free energy between Arg and Lys may explain their distinct roles as stickers. Our findings further indicate that charge neutralisation may play an important role in modulating the stickiness of amino acid residues.

Conclusions

We have used a combination of classical MD simulations and quantum chemical calculations to study the relative strengths of Arg and Lys in biomolecular condensates. We have found that, contrary to what was observed for Tyr and Phe, cationic stickers preserve their hierarchy of interaction strengths —with Arg being stronger than Lys— at a wide range of values of the dielectric constant. In this sense, the hierarchy of sticker strength for charged residues is not context-dependent. However, there are subtleties when we try to understand the molecular determinants of this hierarchy. Much attention has been paid to the strength of cation-π interactions and the pseudoaromatic character of Arg.[21,24–27] Our QM results show that the interactions become more favourable for Arg when the dielectric decreases, due

to the decreased screening of the electrostatics in apolar media. But Lys interactions also become stronger, and even exceed the interaction strengths of Arg-arene pairs. The main contribution to the favorability of Arg over Lys comes from the large penalty of dehydrating the latter residue, as proposed by Pappu and his co-workers.[70]

Additionally, we have studied which of the aromatics makes a better partner with the cationic. We find that this depends on the effective permittivity of the medium, as described previously.[16] The greater sticker strength of Phe-Arg/Lys pairs relative to Tyr-Arg/Lys at low dielectrics is consistent with knowledge-based potentials derived from statistics of contacts in protein structures,[71] where ε is known to be low.[19]

In conclusion, our results provide a comprehensive description of cation-π interactions under varying dielectric conditions. We reveal that monomer transfer energies are often the dominant factor in determining residue preferences, leading to significant crossovers that defy predictions based solely on intrinsic interaction strength. This has profound implications for understanding the behavior of residues in confined biological environments, particularly within biomolecular condensates, where dielectric properties are altered. Our findings not only corroborate established biological observations regarding the prevalence of Arg-Tyr interactions and the importance of solvation but also offer novel mechanistic insights into how changes in the dielectric environment can fundamentally reshape the very forces governing protein-protein interactions and phase separation.

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Supporting Information Available

Additional figures and tables providing supplementary data and analyses are available in the Supporting Information.

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