

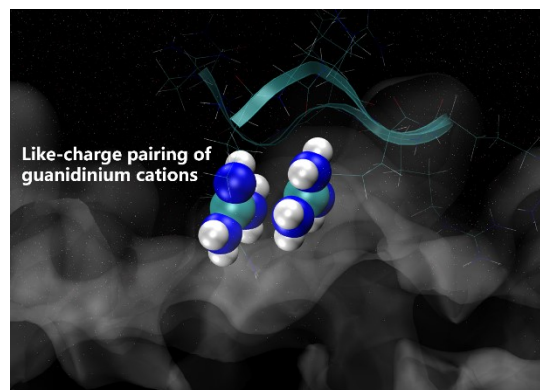
Arginine “Magic”: Guanidinium Like-Charge Ion Pairing from Aqueous Salts to Cell Penetrating Peptides

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Conspectus

It is a textbook knowledge that charges of the same polarity repel each other. For two monovalent ions in the gas phase at a close contact this repulsive interaction amounts to hundreds of kilojoules per mole. In aqueous solutions, however, this Coulomb repulsion is strongly attenuated by a factor equal to the dielectric constant of the medium. The residual repulsion, which now amounts only to units of kJ/mol, may be in principle offset by attractive interactions. Probably the smallest cationic pair, where a combination of dispersion and cavitation forces overwhelms the Coulomb repulsion, consists of two guanidinium ions in water. Indeed, by a combination of molecular dynamics with electronic structure calculations and electrophoretic as well as spectroscopic experiments we have demonstrated that aqueous guanidinium cations form (weakly) thermodynamically stable like-charge ion pairs.



The importance of pairing of guanidinium cations in aqueous solutions goes beyond a mere physical curiosity, since it has significant biochemical implications. Guanidinium chloride is known to be an efficient and flexible protein denaturant. This is due to the ability of the orientationally amphiphilic guanidinium

cations to disrupt various secondary structural motifs of proteins by pairing promiscuously with both hydrophobic and hydrophilic groups, including guanidinium-containing side chains of arginines.

The fact that the cationic guanidinium moiety forms the dominant part of the arginine side chain implies that the like-charge ion pairing may also play a role for interactions between peptides and proteins. Indeed, arginine-arginine pairing has been frequently found in structural protein databases. In particular, when strengthened by a presence of negatively charged glutamate, aspartate, or C terminal carboxylic groups, this binding motif helps to stabilize peptide or protein dimers and is also found in or near active sites of several enzymes.

The like-charge pairing of the guanidinium side chain groups may also hold the key to the understanding of the arginine “magic”, i.e., the extraordinary ability of arginine-rich polypeptides to passively penetrate across cellular membranes. Unlike polylysines, which are also highly cationic but lack the ease in crossing membranes, polyarginines do not exhibit mutual repulsion. Instead, they accumulate at the membrane, weaken it, and might eventually cross in a concerted, “train-like” manner. This behavior of arginine-rich cell penetrating peptides can be exploited when devising smart strategies how to deliver in a targeted way molecular cargos into the cell.

Introduction

The term arginine “magic”¹ poetically describes the unusual ability of positively charged arginine rich (Arg-rich) peptides to easily penetrate across cellular membranes. This makes arginine the primary choice as the building block of cell penetrating peptides.^{2–6} Arginine “magic” is related to the presence of the side chain of this amino acid containing the guanidinium cation (Gdm^+) group, which holds a number of fascinating chemical properties.

Gdm^+ is an intriguing chemical species composed of three amino groups bound to the central carbon atom, belonging thus to the class of planar Y-conjugated quasi-aromatic structures (Figure 1).⁷ Its pK_a value in water is 13.6, which renders it fully protonated in practically all biological contexts.⁸ Gdm^+ acts as a hydrogen bond donor interacting strongly with water only in the molecular plane, whereas its faces remain hydrophobic, as suggested by neutron diffraction techniques and molecular dynamics simulations.^{9,10} It is thus appropriate to call Gdm^+ an orientational amphiphile.¹¹ Hydration of Gdm^+ depends also on the local environment with small Gdm^+ water clusters exhibiting a stronger hydrogen bonding to water molecules than large Gdm^+ water clusters, as suggested by infrared photodissociation spectroscopy.¹²

The unique hydration pattern of Gdm^+ is also related to the fact that guanidinium salts serve as potent protein denaturants. In particular, the hydrophobic faces of Gdm^+ interact preferably with other planar aromatic amino acids such as tryptophan,¹³ destabilizing thus hydrophobic cores of proteins. At the same time, Gdm^+ forms strong hydrogen bonds with negatively charged amino acids, as well as with oxygen atoms in the protein backbone.¹⁴ As revealed also by 2D infrared spectroscopy, Gdm^+ in this way induces breaking of salt bridges in folded protein conformations,¹⁵ leading again to efficient protein denaturation.

Arguably the most striking molecular property of Gdm^+ is its ability to form contact like-charge ion pairs in water despite the obvious electrostatic repulsion.¹⁶ This unusual behavior has been predicted by molecular dynamics (MD) and Monte Carlo simulations.^{16–19} Not all experiments, notably dielectric relaxation spectroscopy,²⁰ directly confirm self-association of Gdm^+ cations. Quantitatively, the stability of cation-pairs relative to their ion- and water- separated forms was predicted by simulations as the experimental determination is still challenging.^{20,21}

Nevertheless, mounting experimental evidence shows that Gdm^+ like-charge pairing is a real effect^{22,23} being present in a number of different chemical environments, ranging from adenoviruses,²⁴ over antimicrobial polyelectrolytes,²⁵ to aqueous guanidinium salt solutions.²⁶ For completeness, we note that some degree of like-charge ion pairing has been computationally predicted (without direct experimental evidence) also for certain aqueous anions, in particular fluorides,²⁷ chlorides,²⁸ hydroxides,²⁹ and more complex oxoanions.³⁰

In this Account, we review results of computational and experimental studies aimed at unraveling “arginine magic” using a bottom-up approach. We start with computational studies of the simplest system, i. e., a Gdm^+ pair in aqueous solutions.^{16,31–33} Next, we present MD simulations and an analysis of the Protein Data Bank (PDB)³² together with electrophoretic mobility studies of short Arg-rich peptides in water.²² As a next step, we present results of small angle X-ray (SAXS) and NMR spectroscopies, as well as MD simulations, concerning aggregation of longer Arg-rich peptides in water.³⁴ Finally, we discuss the behavior of long Arg-rich peptides in the context of fluorescence spectroscopy measurements of liposomes and biomimetic control assays,³⁵ as well as similar studies on supported lipid bilayers³⁶ corroborated by molecular dynamics (MD) simulations of peptide/bilayer systems.^{35,36} The principal aim of these studies

has been to elucidate the molecular mechanism of self-aggregation of polyarginines as an important step in understanding the translocation process across cellular membranes.³⁷

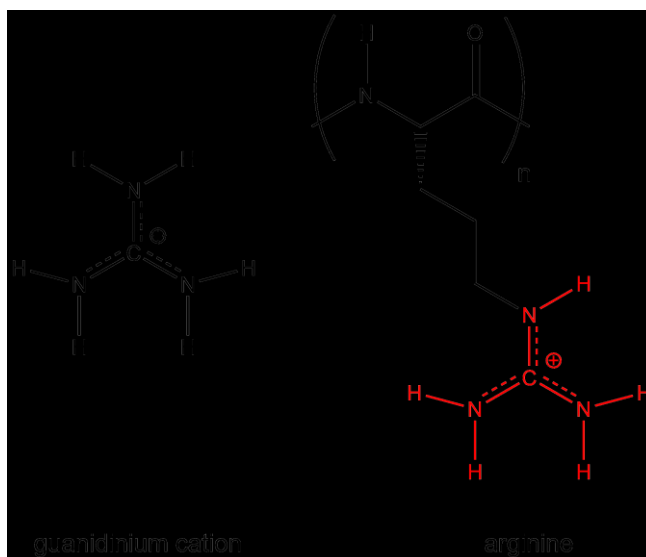


Figure 1. Chemical structures of the guanidinium cation and of the arginine amino acid in a polyarginine peptide chain.

Guanidinium like charge ion pairing in water

The simplest system exhibiting the occurrence of the like-charge ion pairing is an aqueous solution of a Gdm^+ salt. Based on MD simulations employing both non-polarizable and polarizable force fields, the like-charge pairing of Gdm^+ has been predicted to exist with the stabilization free energy of the guanidinium pair ranging from units to tens of kJ mol^{-1} , depending on the particular potential model.¹⁷ Quantum-chemical calculations with polarizable continuum solvent models have also shown the existence of the like-charged pair, with a stabilization energy in the range of 4 – 12 kJ mol^{-1} .^{18,19} The molecular origin of the association has been rationalized recently in terms of a combination of several effects – screening of the electrostatic repulsion by the high dielectric constant medium augmented with cavitation and dispersion stabilization, together with favorable quadrupole-quadrupole interactions.¹⁶ Although all of these effects are present also in other solvated ion pairs at a close contact, such as NO_3^- or NH_4^+ , it seems that only in the case of Gdm^+ the total free energy balance tips toward stabilizing the like-charge ion pair.¹⁶

Both force field MD simulations and quantum-chemical calculations with polarizable continuum models qualitatively captured the essence of the like-charge pairing. Nevertheless, we double-checked the stability of the Gdm^+ ion pair using a method free of potential artifacts arising from the use of empirical force-fields or the implicit description of water. To this end, we performed electronic structure calculations evaluating stabilities of a series of Gdm^+ dimers hydrated by an increasing number of water molecules. We showed that a Gdm^+ pair with 12 surrounding water molecules (Figure 2) is thermodynamically stable with a complexation energy of -4.6 kJ mol^{-1} at the BLYP-D/cc-pVDZ level of theory.³²

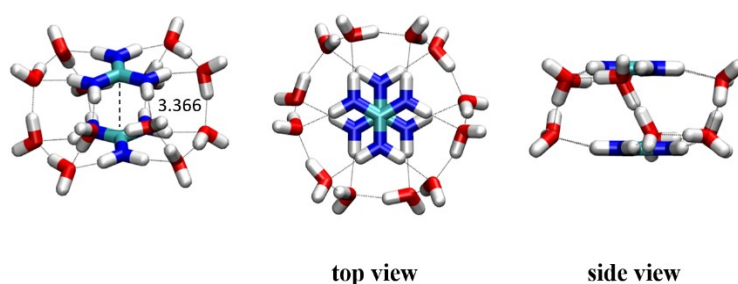


Figure 2. The optimized structure of the guanidinium dimer with twelve water molecules shown in different views, as obtained at the BLYP-D/aug-cc-pVDZ level of theory. The distance between central carbon atoms is given in Å. Hydrogen bonds are indicated with thin lines. Adapted with permission from Ref. 32. Copyright 2011 American Chemical Society.

Next, we utilized *ab initio* molecular dynamics (AIMD) simulations to verify the thermal stability of the Gdm^+ like-charge ion pair. We performed AIMD with the dispersion corrected BLYP-D density functional and the DZVP-MOLOPT-GTH basis set showing that the Gdm^+ pair surrounded by several solvation shells of explicit water molecules is thermodynamically stable. This is in contrast to control simulations of a hydrated NH_4^+ dimer, where no pairing occurs. In addition to the stacked Gdm^+ pair (Figure 3), we also observed a T-shaped structure of the dimer where two Gdm^+ ions are oriented perpendicularly to each other.³¹ We performed analysis of the electronic density of the systems, which revealed that the attractive interaction between Gdm^+ ions is dominantly of a van der Waals nature, whereas no such attraction exists in the case of the NH_4^+ dimer (Figure 3).

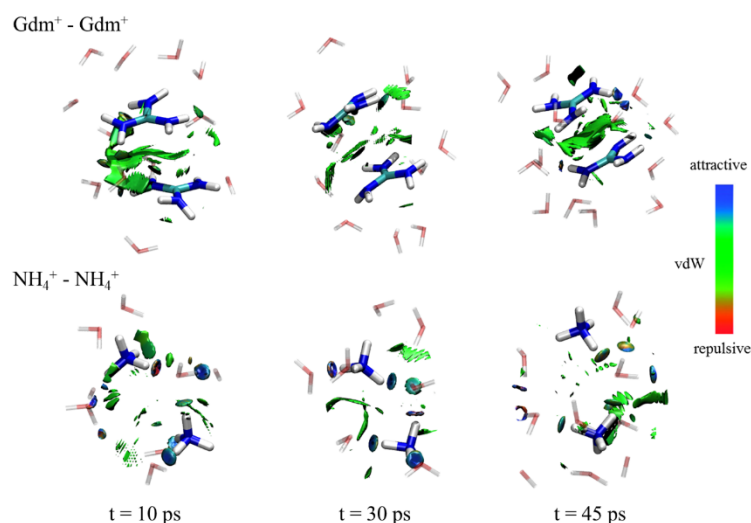


Figure 3. Selected snapshots from *ab initio* molecular dynamics simulations showing interactions between a pair of Gdm^+ cations (upper panel) or a pair of NH_4^+ cations (lower panel) in water. Gradient isosurfaces ($s = 0.6$ au) are

shown in color representing different types of interaction, ranging from attractive (blue) to repulsive (red) interactions. Adapted with permission from Ref. 31. Copyright 2012 American Chemical Society.

As a final task in establishing the stability of the like-charged Gdm⁺ pair, we also performed extensive AIMD simulations with umbrella sampling in order to extract the free energy of the stabilization. Figure 4 depicts a comparison between free energy profiles obtained by an empirical force field MD (blue line) and AIMD (red line), demonstrating that both computational approaches yield a thermodynamically stable Gdm⁺ ion pair. Namely, empirical force field MD predicts a weakly stabilized pair with a free energy minimum of ca. -1.5 kJ mol⁻¹, while (the necessarily less statistically converged) AIMD gives a deeper free energy minimum of about -5 kJ mol⁻¹. Also, AIMD predicts that T-type Gdm⁺ dimers are slightly more stable than stacked Gdm⁺ dimers.³³ Note that T-type dimers are not identified as stable in force field MD, thus showing the importance of explicit electronic structure treatment for a quantitative determination of the like-charged ion pair geometry and stability.

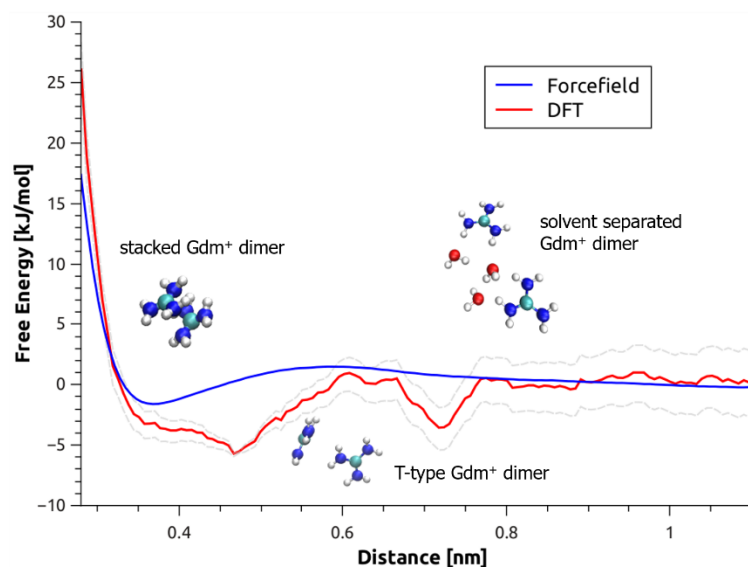


Figure 4. Free energy of stabilization of a Gdm⁺ pair in water obtained by the OPLS-AA empirical force field MD (blue) and *ab initio* MD with the BLYP-D/TZVP-GTH level of theory (red), together with selected snapshots from MD trajectories representing different interaction geometries. Standard deviations for *ab initio* MD simulations are indicated by grey lines. Adapted with permission from Ref. 33. Copyright 2016 American Chemical Society.

Short arginine-rich peptides in water

In the previous Chapter we showed that the computational evidence for the existence of Gdm⁺ like-charged ion pairs is qualitatively robust, regardless of the particular computational method used. In this

Chapter we extend the investigation of Gdm⁺ ion pairing to short aqueous peptides containing Arg amino acids. First, simulations of diarginines in water have demonstrated association of the side chains bearing Gdm⁺ ions, in stark contrast to dilysines which have not shown any sign of attractive interactions between side chains containing NH₄⁺ ions.¹⁶ The results from MD simulations are supported by a survey of the PDB database, which reveals a number of structures with a pair of arginines in a close contact, indicating that whenever two Gdm⁺ ions are sterically allowed to be in a vicinity of each other they easily form a like-charged ion pair.^{32,38–40} Also, the unusually large number of ligands containing Gdm⁺ groups interacting with Arg amino acids in receptors indicates a potential importance of the like-charge pairing in drug design as well.⁴¹

At the same time, Gdm⁺ ion pairing has also been demonstrated by a combination of capillary electrophoresis and MD simulations. Namely, in electrophoresis experiments it has been observed that addition of GdmCl to the solution of tetraarginine (Arg₄) leads to an increased mobility of Arg₄, while upon addition of NaCl no mobility enhancement has been observed (Table 1). In contrast, control experiments with tetralysine (Lys₄) have not shown any difference in mobilities of Lys₄ upon addition of GdmCl vs. NaCl.²² This effect has been rationalized with the help of MD simulations in terms of formation of the like-charged ion pair between Gdm⁺ ions in solution and the Gdm⁺ moieties in Arg₄ (Figure 5), which effectively increases the charge and thus the electrophoretic mobility of the peptide.

Table 1. Electrophoretic mobilities μ ($\times 10^{-9}$ m² V⁻¹ s⁻¹) in 50 mM GdmCl and NaCl aqueous salt solutions. Adapted with permission from Ref. 22. Copyright 2011 American Chemical Society.

	GdmCl	NaCl
Arg ₄	34.13 ± 0.06	31.89 ± 0.09
Lys ₄	35.31 ± 0.10	35.27 ± 0.06

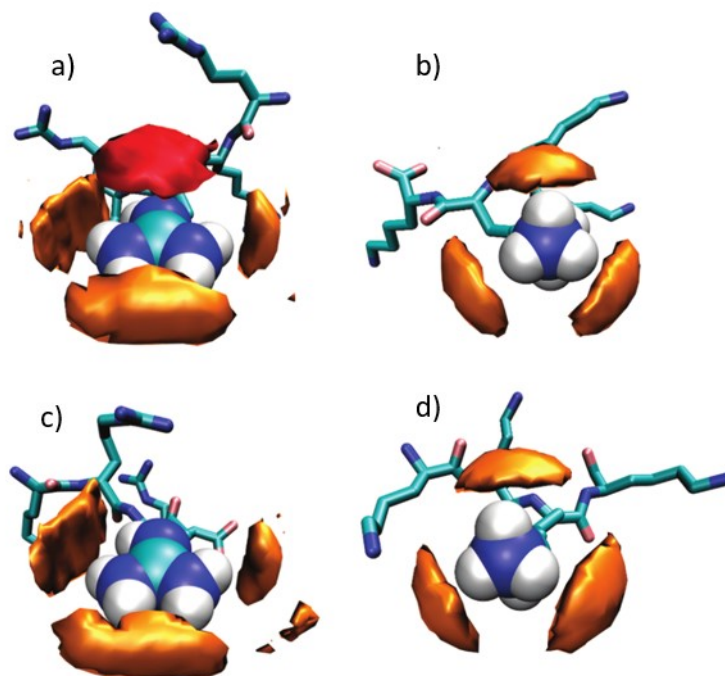


Figure 5. Distribution of Gdm⁺ cations (red), Na⁺ cations (not present in the vicinity of the side chains), and Cl⁻ anions (gold) around the side chains of (a) Arg₄ in GdmCl(aq), (b) Lys₄ in GdmCl(aq), (c) Arg₄ in NaCl(aq), and (d) Lys₄ in NaCl(aq). Adapted with permission from Ref. 22. Copyright 2011 American Chemical Society.

Long arginine-rich peptides in water

In this Chapter, we present the results concerning the behavior of longer Arg-rich peptides in aqueous solutions, in particular concerning the self-association of deca-arginines.³⁴ We have performed extensive MD simulations of Arg₁₀ and Lys₁₀ in aqueous NaCl solutions of increasing ionic strength. Figure 6a shows a typical MD snapshot of two Arg₁₀ peptides in a close contact. The mutual attraction of these two positively charged peptides is due to a combination of two factors. First, Gdm⁺ of the Arg residues of the two peptides interact with each other, forming like-charged ion pairs. In addition, Gdm⁺ of the ninth Arg residues also forms an intermolecular salt bridge with the negatively charged C-terminus of the other peptide. This newly discovered binding motif acts as an adhesive patch between the two polyarginines. Umbrella sampling simulations (Figure 6 b-d) have shown that this binding is present at varying ionic strengths, being most stable (-10 kJ mol⁻¹ at 298 K, Figure 6c) at a NaCl concentration of $c_s = 0.07$ mol dm⁻³. No such binding motif is present for a pair of Lys₁₀. Moreover, mutation in Arg₁₀ of the ninth Arg residue to Lys leads to disappearance of the free energy minimum (Figure 6e, red line), which points to the importance of the Gdm⁺ like-charge ion pairing in this binding motif. Interestingly, the association free energy between shorter peptides, in particular between Arg₄ peptides at $c_s = 0.07$ mol dm⁻³ (Figure 6f) is smaller than for the longer Arg₁₀ peptides at the same ionic strength (Figure 6c), suggesting that Arg residues contribute in an additive fashion to lowering of the mutual electrostatic repulsion between Arg-rich peptides. In contrast, interactions between Lys-rich peptides are purely repulsive; these control systems thus behave as classical Debye-Hückel (poly)electrolytes.

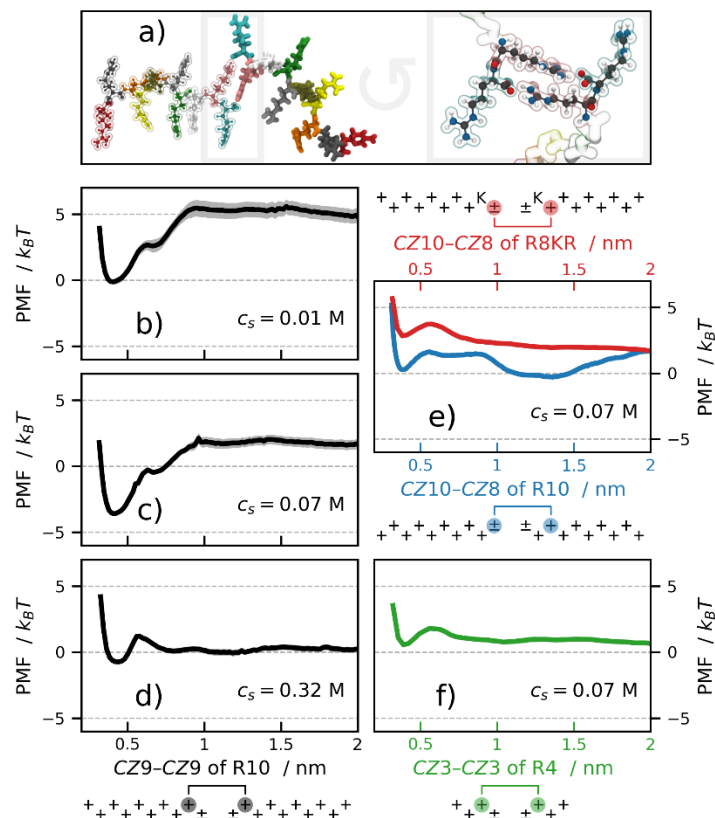


Figure 6. (a) Left, a snapshot from MD simulations illustrating the interaction between two Arg₁₀ peptides at a close separation; right, a close-up view at the ninth and tenth residues involved in the newly discovered binding motif; b)–d) potentials of mean force (PMFs) calculated from umbrella-sampling MD simulations for pairs of Arg₁₀ molecules at ionic strength, c_s , of 0.01 M (b), 0.07 M (c), and 0.32 M (d) as a function of the separation between the central Gdm⁺ carbon atoms of the ninth residues (CZ9–CZ9). (e) PMFs for pairs of Arg₈LysArg (R8KR, red line) and Arg₁₀ (R10, blue line) molecules as a function of the separation between the central Gdm⁺ carbon atoms of the eighth and tenth residues (CZ10–CZ8). (f) PMF for pairs of Arg₄ (R4) molecules as a function of CZ9–CZ9. Shaded areas along the PMFs represent standard deviations (SD) of bootstrapped free energy profiles. (b–f) Abscissa labels represent CZ9–CZ9 or CZ10–CZ8 separations between two peptides where +, ±, and K represent arginine, C-terminal arginine, and lysine residues, respectively. Adapted with permission from Ref. 34. Copyright 2017 National Academy of Sciences.

The above results obtained from MD simulations have been confirmed experimentally. Namely, SAXS experiments show a very different aggregation behavior of Arg₁₀ peptides compared to Lys₁₀. This is evidenced in the dependence of the normalized SAXS diffraction intensity $I(q)$ on the peptide concentration c_p , which differs significantly for Arg₁₀ vs. Lys₁₀ (Figure 7a-c). In particular, the increase of the normalized intensity maximum with increasing concentrations of Arg₁₀ in the low q -range corresponds to the increase in osmotic compressibility indicating an attraction between Arg₁₀ peptides, which is particularly pronounced for lower ionic strengths. These large and increasing (with peptide concentration) SAXS intensities for Arg₁₀ indicate that there is a fraction of Arg₁₀ which self-associate. In contrast (Figure 7d), we see a decreasing trends of normalized SAXS intensities with peptide concentration for Lys₁₀, as well as mutated peptides Arg₈LysArg and Lys₈ArgLys, demonstrating thus repulsion between these peptides as

expected for standard positively charged polyelectrolytes in water. Moreover, the experiments with mutated peptides point to the fact that the ninth Arg residue is pivotal for aggregation of Arg₁₀. As an additional experimental support of the self-association predicted by MD simulations, 2D ¹H-¹³C HSQC NMR spectra for Arg₁₀ (Figure 7e) exhibit changes in NMR chemical shifts with increasing peptide concentration, consistent with attractive intermolecular interaction between peptides due to like-charge ion pairing. In contrast, for Lys₁₀ the NMR chemical shifts at varying peptide concentrations almost perfectly overlap, indicating no attractive intermolecular interactions (Figure 7f). Finally, the computationally predicted binding motif responsible for self-association of two positively charged Arg₁₀ peptides, consisting of a Gdm⁺ like-charge ion pair and two salt bridges with the carboxylic group, is amply represented in the PDB database where 231 X-ray structures with at least one like-charged Gdm⁺ ion pair interacting with aspartate or glutamate residues have been identified.

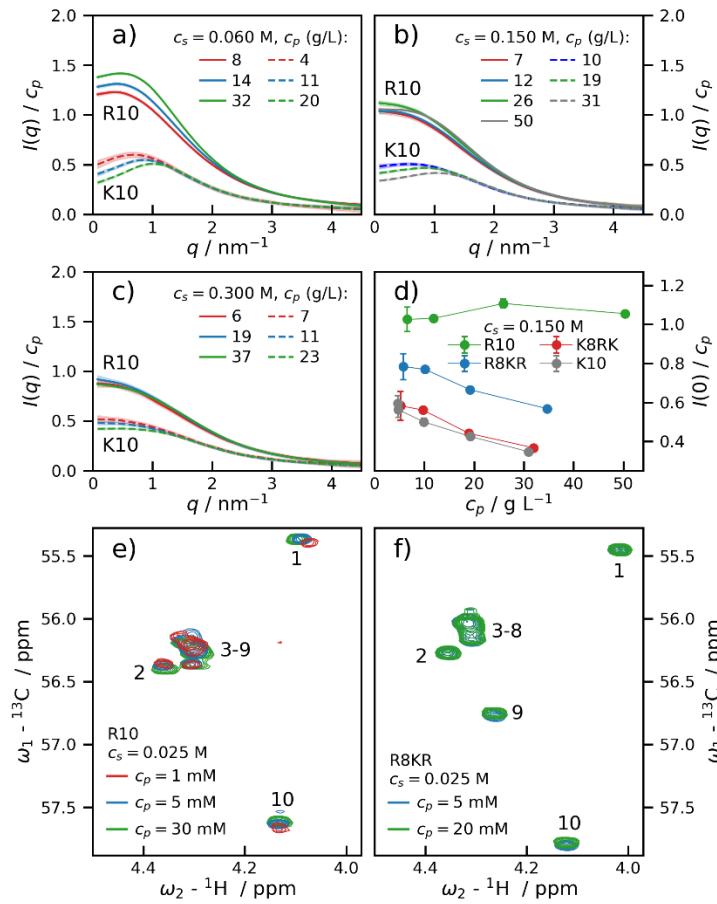


Figure 7. (a–c) Concentration-normalized SAXS intensities for Arg₁₀ (R10, solid lines) and Lys₁₀ (K10, dashed lines) at various peptide concentrations, c_p , in 0.020 M Tris buffer solutions of a 0.060 M (a), 0.150 M (b), and 0.300 M (c) ionic strength, c_s . (d) Extrapolated $I(0)/c_p$ values for samples of Arg₁₀ (R10), Lys₁₀ (K10), Arg₈LysArg (R8KR), and Lys₈ArgLys (K8RK) of increasing c_p at $c_s = 0.150\text{ M}$. (e and f) Regions corresponding to correlations between C α and H α atoms of 2D ¹H-¹³C HSQC NMR spectra of Arg₁₀ (R10, e) and Arg₈LysArg (R8KR, f) at various c_p , pH 5, and $c_s = 0.025\text{ M}$. Adapted with permission from Ref. 34. Copyright 2017 National Academy of Sciences.

Membrane aggregation and cell penetration of arginine-rich peptides

In the previous Chapters, we showed that like-charge Gdm⁺ pairing is operative also in polypeptides, as demonstrated for Arg₁₀ in aqueous solutions. As already mentioned in the Introduction, there is a vast number of studies dedicated to cell membrane penetration properties of Arg-rich oligo- or polypeptides, the so-called arginine “magic”. However, the molecular details of the onset of peptide penetration properties and the effects of the Arg-rich peptide self-aggregation are still not completely understood.⁴² What has been firmly established is the fact that in addition to the ATP fueled active process of endocytosis a passive ATP-free entry mechanism is operative as well.^{43,44} Computational studies of Arg-rich peptides have shown that the ease of the first step of this passive mechanism is related to the effectiveness of the peptide adsorption at the bilayer surface,⁴⁵ which is dependent on the size, shape, and chemical composition of the adsorbed species.⁴⁶

MD simulations of polyarginines at phospholipid bilayers have shown that in addition to adsorption, they also strongly self-aggregate at the bilayer surface in contrast to polylysines which show no sign of aggregation.^{35,36} This is illustrated in Figures 8c and 8d, where an Arg₉ dimer is formed at the POPC bilayer, as also evidenced by the analysis of the corresponding radial distribution functions with Gdm⁺ groups in a close contact (Figure 8a). In contrast, there are no close contacts between NH₄⁺ groups in Lys₉ peptides (Figure 8b). In addition to association and aggregation of Arg-rich peptides at phospholipid membranes, it has been suggested that formation of transient pores in the bilayer, which usually occurs spontaneously or upon lipid flip-flop process,⁴⁷ is also facilitated by the Gdm⁺ like-charge ion pairing in the bilayer core.³³ These pores are predicted to be strongly kinetically stabilized by Arg-rich peptides, while no such effect occurs for Lys-rich peptides.^{48–50}

Simulation results are supported by a number of experimental studies. For example, time fluorescence shift measurements have shown that longer polypeptides penetrate deeper than shorter peptides in the bilayer. Also, biomimetic color assays have demonstrated that polyarginines induce strong interactions in the membranes, in contrast to polylysines.³⁵ Experimental studies on supported lipid bilayers have also revealed that polyarginines bind two orders of magnitude more strongly to the bilayer than polylysines.³⁶ This is illustrated in Table 2, where dissociation constants K_D and Hill indexes of cooperativity n are presented for Arg₉ and Lys₉ at neutral POPC and negatively charged mixed POPC and POPG lipid bilayers. In the case of Arg₉, the Hill cooperativity index is close to unity at higher peptide concentrations at negatively charged bilayers, indicating effectively no mutual repulsion of positively charged Arg₉ peptides at the bilayer. Conversely, Lys₉ peptides have cooperativity values significantly smaller than one, which points to their mutual repulsion at the bilayer.

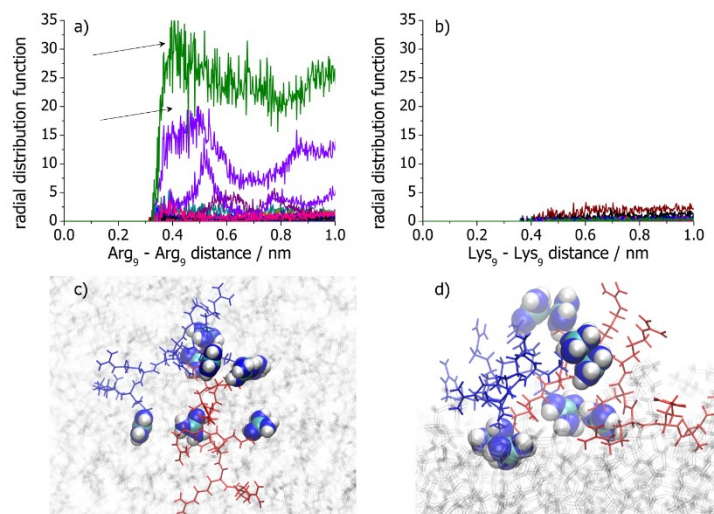


Figure 8. (a) Radial distribution functions (RDFs) between central carbon atoms of Gdm⁺ in different Arg₉ peptides at the POPC + POPG bilayer. The RDF peak marked by arrows denotes a direct contact pairs between central carbon atoms in Gdm⁺ moieties present in Arg₉-Arg₉ aggregates. (b) The same plot as (a) for the central nitrogen atoms of -NH₃⁺ moieties on different Lys₉ peptides. A snapshot of (c) a top-down view and (d) a side view at two individual peptides (in red and blue) forming an aggregate at the POPC + POPG system. The Gdm⁺ groups in close contact are shown in the van der Waals representation. Adapted with permission from Ref. 36. Copyright 2016 American Chemical Society.

Table 2. Apparent dissociation constants (K_D) and Hill coefficients of cooperativity n for Arg₉ and Lys₉ at POPC lipid bilayers with varying concentrations of POPG lipids with a *ortho*-rhodamine B probe (oRB). Adapted with permission from Ref. 36. Copyright 2016 American Chemical Society.

mol% POPG + mol% oRB	Lys ₉ K_D (μ M)	n	Arg ₉ K_D (μ M)	n
0 + 0	NA	NA	$70 \pm 19^{*†}$	0.75*
0 + 0.5	NA	NA	$0.89 \pm 0.38^*$	0.39*
5 + 0.5	$77 \pm 20^*$	0.12*	$0.46 \pm 0.047^*$	0.68*
10 + 0.5	55 ± 3	0.22	0.12 ± 0.016	0.73
20 + 0.5	2.5 ± 0.5	0.24	0.039 ± 0.005	1.0
30 + 0.5	2.1 ± 0.8	0.19	0.029 ± 0.007	1.0

*assay performed at pH 6.4; all others performed at pH 6.8

†assay performed with TAMRA dye labeling

Taken together, the combination of calculations and experimental results presented here points to the importance of membrane affinity and self-aggregation of Arg-rich peptide for their passive translocation across the bilayer. MD simulations also predict that Arg-rich peptides stabilize transient pores and aggregate inside in the bilayer.^{35,49} This is corroborated with several experimental studies showing deep penetration of long Arg-rich peptides, which are not mutually repulsive due to the Gdm⁺ like-charge ion pairing, despite the inevitable electrostatic repulsions.^{35,36} The present findings thus represent important pieces in the mosaic of the complex mechanism of passive cell penetration of Arg-rich peptides and thus help to provide a molecular interpretation of the empirical observations of arginine “magic”.

Conclusions and outlook

In this Account, we have presented in the broader context of arginine “magic” our work focused on the molecular understanding of the like-charge ion pairing of guanidinium cations, ranging from salt ions in water to polyarginine peptides in solution and at biological membranes. We have unraveled the molecular mechanisms of this counterintuitive, “Coulomb-defying” interaction. Using experimental techniques supported by extensive MD simulations, we have shown that like-charge Gdm⁺ pairing is responsible for

aggregation of Arg-rich peptides in different environments. Moreover, aggregation of Arg-rich peptides at biological membranes is likely one of the key ingredient in the molecular explanation of how Arg-rich cell penetrating peptides passively translocate to the cell interior.^{51–54} Our findings may also help to understand at a molecular level how the efficacy of cell penetrating peptides can be improved, for example, by methylation of arginines,^{55,56} or by imposing the steric constraints by peptide cyclization⁵⁷. Finally, we would be happy if the results presented here contribute in the future to a more rational design, based on molecular understanding, of cell penetrating peptides for efficient transport of therapeutic molecules or markers into the cell.

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Philip E. Mason

(*1972) is a 'hands on' scientific researcher. He started working on guanidinium in ~2000 at Cornell University, and now continues to work on similar systems at the Czech Academy of Science. He has popularized some of his research topics, such as the unexpected reason why alkali metals explode in water, on his youtube channel 'Thunderfoot'.

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Mikael Lund

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