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Katharina M. Fromm *et al.*

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# Alpha-helical folding of SilE models upon Ag(His)(Met) motif formation†

Valentin Chabert,<sup>id a</sup> Maggy Hologne,<sup>id b</sup> Olivier Sèneque,<sup>id c</sup> Olivier Walker<sup>id b</sup>  
and Katharina M. Fromm<sup>id \*a</sup>

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**The SilE protein is suspected to have a prominent role in Ag<sup>+</sup> detoxification of silver resistant bacteria. Using model peptides, we elucidated both qualitative and quantitative aspects of the Ag<sup>+</sup>-induced  $\alpha$ -helical structuring role of His- and Met-rich sequences of SilE, improving our understanding of its function within the Sil system.**

The bactericidal power of Ag<sup>+</sup> has been exploited for hundreds of years, and recent efforts have been made to develop new silver-based compounds able to tackle multidrug-resistant bacteria.<sup>1,2</sup> However, several Gram-negative bacteria are able to survive in silver contaminated media, thanks to intrinsic and/or acquired HME-RND (Heavy-Metal-Efflux Resistance-Nodulation-Division) efflux pumps.<sup>3,4</sup> The latter are the most common bacterial defence against toxic metal ions.<sup>5</sup> The Cus and Sil systems are part of the HME-RND family and have shown the capacity to make bacteria silver resistant.<sup>6–9</sup> While the Cus system is expressed in the context of chromosomal mutations, the Sil system is plasmid encoded. Besides the fact that they can be horizontally transferred, resistance plasmids have relatively low fitness costs in comparison with chromosomal resistance mutations.<sup>10</sup> As a consequence, plasmid-encoded resistances are deemed to be more widespread than mutational resistances.<sup>11</sup> Hence, the full understanding of the Sil system is of prime importance in the context of fighting silver resistant bacteria. Both systems function along similar lines and possess a common basis (CusCFBA and SilCFBA). However, this basis is not efficient enough to provide a significant silver tolerance, and needs to be complemented by a control of the cellular silver concentration.

Therefore, the CusCFBA transporter is associated to a deficiency in OmpC and OmpF porins, and the SilCFBA transporter is associated to three other proteins, SilP, SilE and SilG, of which only SilE is mandatory to confer the resistance.<sup>7</sup> Moreover, Randall *et al.* have shown that SilE could substitute the need for porin loss associated with the Cus system.<sup>7</sup> All this points out the prominent role of SilE in the bacterial resistance to Ag<sup>+</sup>. Nevertheless, the structure of the latter has never been solved and its mode of action is still debated. Previous studies have proposed histidine and methionine residues to be engaged in the coordination of Ag<sup>+</sup> ions by SilE *via* eight MX<sub>2</sub>H and HX<sub>2</sub>M motifs and one HXM motif, all individually able to bind a single Ag<sup>+</sup> ion with *K*<sub>d</sub> in the  $\mu$ M range.<sup>12–14</sup> However, the involvement of these motifs in Ag<sup>+</sup> coordination in the full-length SilE protein has never been proved, and Ag<sup>+</sup>/SilE binding affinities have not been reported to date. The mechanistic aspects of the Ag<sup>+</sup>-induced folding of the protein and its structural organization remained also unclear, making the understanding of its structure/function relationship difficult. Here we report qualitative and quantitative aspects of the Ag<sup>+</sup> coordination by structural motifs of SilE, through the characterization of the silver binding sites in terms of coordination numbers and geometries, binding affinities, and Ag<sup>+</sup>-induced structural folding. Based on our studies, the first structures of SilE key sequences have been solved and a non-cooperative Ag<sup>+</sup> binding is proposed for SilE.

The eight MX<sub>2</sub>H and HX<sub>2</sub>M motifs of SilE form two types of “twin” sequences, namely MX<sub>2</sub>HX<sub>6</sub>HX<sub>2</sub>M (type **A**) and HX<sub>2</sub>MX<sub>3</sub>HX<sub>2</sub>M (type **B**) (Fig. 1). Model peptides of these four sequences have been synthesized as N-terminal acetylated and C-terminal amidated peptides. The **B1** motif has been additionally studied as 14-amino acid peptide (**B1b**), to investigate the potential involvement of Met91 in Ag<sup>+</sup> coordination, and a sixth model peptide corresponding to the 28 C-terminal amino acids of SilE (**B2b**) has also been studied to examine the Ag<sup>+</sup> coordination of a model peptide containing three HX<sub>2</sub>M motifs. The different peptides were investigated by <sup>1</sup>H NMR titrations to determine their Ag<sup>+</sup>-binding capacity. The <sup>1</sup>H chemical shifts of both His and both Met residues (His-H<sup>δ2</sup>, His-H<sup>ε1</sup> and Met-H<sup>ε</sup>) are

<sup>a</sup> University of Fribourg, Department of Chemistry, Chemin du Musée 9, 1700 Fribourg, Switzerland. E-mail: katharina.fromm@unifr.ch

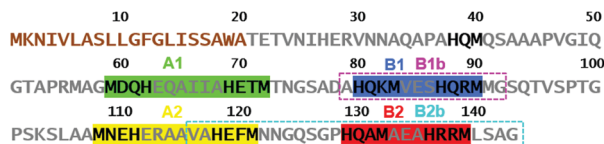
<sup>b</sup> Université de Lyon, CNRS, UCB Lyon 1, ENS-Lyon, Institut des Sciences Analytiques, UMR 5280, 5 rue de la Doua, 69100 Villeurbanne, France

<sup>c</sup> Université Grenoble Alpes, CNRS, CEA, BIG/LCBM (UMR 5249), 38000 Grenoble, France

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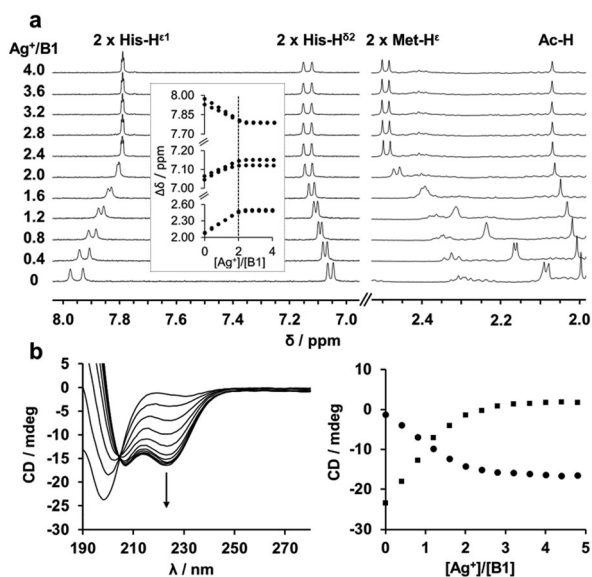


**Fig. 1** Amino acid sequence of SiLE. The first twenty amino acids (brown) are cleaved after the periplasm targeting. The His- and Met- containing regions hold two  $\text{MX}_2\text{HX}_6\text{HX}_2\text{M}$  motifs (**A1** and **A2**) and two  $\text{HX}_2\text{MX}_3\text{HX}_2\text{M}$  motifs (**B1** and **B2**) that are studied as structural model peptides of SiLE. **B1b** and **B2b** have been additionally studied as longer model peptides of **B1** and **B2** sequences, respectively.

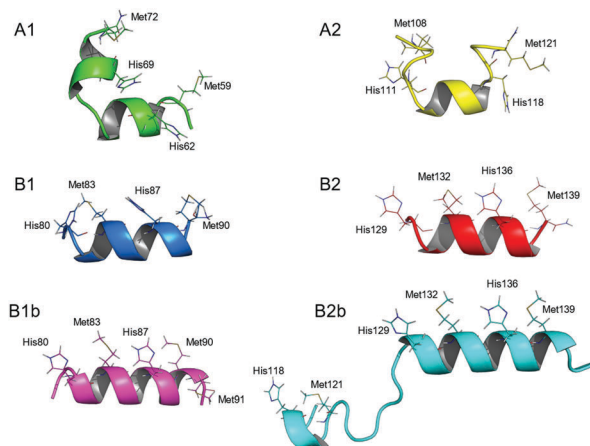
significantly affected by the addition of  $\text{Ag}^+$ , indicating their involvement in  $\text{Ag}^+$  coordination. The evolution of the chemical shift is clearly in agreement with the binding of two  $\text{Ag}^+$  ions per peptide with a plateau after 2 equiv. of  $\text{Ag}^+$  added (Fig. 2a and Fig. S1, ESI†). Similarly, the **B2b** model that harbors three  $\text{HX}_2\text{M}$  motifs can bind up to three silver ions (Fig. S1, ESI†). The behavior of **B1b**, which displays two His and three Met, is more complicated. It is described in detail in the ESI† (Fig. S1 and S2). This peptide can bind three  $\text{Ag}^+$  ions, the third of which has a lower affinity (mM range vs.  $\mu\text{M}$ , see below and ESI†). The monitoring of the chemical shifts reveals that the  $\text{HX}_2\text{M}$  motifs are the two primary binding sites. The third  $\text{Ag}^+$  ion may bind to Met90 and Met91. For all peptides, DOSY experiments were recorded in the presence or absence of  $\text{Ag}^+$  ions (Fig. S3–S8, ESI†) in order to compare the diffusion coefficients of the apo-peptides and their silver complexes. They indicate species of similar size suggesting the formation of silver complex species involving a single peptide, i.e.  $\text{Ag}_2\text{P}$  or  $\text{Ag}_3\text{P}$ . Thus, we can

conclude that all these peptides bind one  $\text{Ag}^+$  ion per  $\text{MX}_2\text{H}$  or  $\text{HX}_2\text{M}$  motif. From previous studies on  $\text{HX}_2\text{M}$  and  $\text{MX}_2\text{H}$  tetrapeptides, we can infer a His–Ag–Met coordination mode, but at this stage, His–Ag–His and Met–Ag–Met coordination modes cannot be ruled out. Further structural details about the  $\text{Ag}^+/\text{SiLE}$  interaction arise from circular dichroism (CD) and NMR. The two types of sequences ( $\text{MX}_2\text{HX}_6\text{HX}_2\text{M}$ , **A** and  $\text{HX}_2\text{MX}_3\text{HX}_2\text{M}$ , **B**), which exhibit random-coil conformations in their apo-form, adopt different structures in the presence of silver ions. CD experiments reveal the appearance of two minima at 207 nm and 223 nm for type **B** peptides, clearly indicating that they are folding into  $\alpha$ -helices upon  $\text{Ag}^+$  addition (Fig. 2b), in contrast to type **A** peptides, which exhibit a poor  $\alpha$ -helix signature evolution during  $\text{Ag}^+$  complexation and appear thus less structured in their holo-form (Fig. S9, ESI†).<sup>15</sup>

To gain deeper understanding of the coordination geometries and the structural features of the silver binding sequences of SiLE, the NMR solution structures of the different  $\text{Ag}^+/\text{SiLE}$ -derived peptide complexes have been solved (Fig. 3). As expected, the  $\text{Ag}^+$  binding to **B1** and **B2** induces a well-defined helical structure of the respective model peptides. On the other hand, **A1** and **A2** remain partly unstructured. In both cases, however, the orientation of His and Met side chains, which are alternating on the same side of the  $\alpha$ -helix in type **B** peptides, clearly indicates that each  $\text{Ag}^+$  ion is bound to one  $\text{HX}_2\text{M}$  or  $\text{MX}_2\text{H}$  motif, excluding His–Ag–His or Met–Ag–Met coordination geometries. Therefore, the 1 : 1  $\text{Ag}^+/\text{HX}_2\text{M}$  or  $\text{MX}_2\text{H}$  stoichiometry pointed out by both tetra- and poly-peptide model studies suggests a linear His–Ag–Met coordination mode, which is common for  $\text{Ag}^+$  ions. Moreover, **B1b** and **B2b** peptides adopt an  $\alpha$ -helical fold upon  $\text{Ag}^+$  binding similar to those of **B1** and **B2**, respectively, and their structures overlap to a large extent (Fig. S12, ESI†). In the case of **B1b**, the potential coordination of a third  $\text{Ag}^+$  ion by Met90 and Met91 (see above and ESI†) does not influence the folding of **B1b**. In the case of **B2b**, no NOE between the



**Fig. 2** **B1**/ $\text{Ag}^+$  NMR and CD titrations. (a) Histidine ( $\text{His-H}^{\epsilon 1}$  and  $\text{His-H}^{\epsilon 2}$ ) and methionine ( $\text{Met-H}^{\gamma}$ )  $^1\text{H}$  resonances shift by addition of  $\text{AgClO}_4$  (0 to 4 mM) to a solution of **B1** (1 mM) in HEPES buffer (20 mM, pD 7.8). The insert depicts the 2 : 1 stoichiometry of the  $\text{Ag}_2\text{B1}$  complex. (b)  $\alpha$ -Helix folding of **B1** (10  $\mu\text{M}$ , in  $\text{NH}_4\text{Ac}$  1 mM, pH 7.4) by addition of  $\text{AgClO}_4$  (0 to 48  $\mu\text{M}$ ) evidenced by the increase of the signature at 223 nm (left). Plot of the CD signal at 199 nm (square) and 223 nm (circle).



**Fig. 3** Lowest energy NMR solution structure of the six SiLE-derived peptides in presence of  $\text{Ag}^+$ . His and Met residues are represented by lines. Type **B** peptides fold into two  $\alpha$ -helices stretching from residues Gln81 to Arg89 and Gln130 to Arg138 whereas type **A** peptides own less defined secondary structure.



N-terminal HEFM motif and the two C-terminal motifs composing the **B2** sequence are observed, indicating that they bind different  $\text{Ag}^+$  ions.

As previously shown, isolated  $\text{MX}_2\text{H}$  and  $\text{HX}_2\text{M}$  motifs intrinsically bind silver ions with moderately strong affinities ( $\log K_{\text{ass}} = 5.3\text{--}6.6$ ).<sup>13</sup> However, it has to be determined whether the proximity of the binding sites and the  $\text{Ag}^+$ -induced  $\alpha$ -helix folding of the backbone affect the silver binding affinities of the various  $\text{MX}_2\text{H}$  or  $\text{HX}_2\text{M}$  motifs. Fluorescence competition titrations were chosen to determine the binding constants of the model peptides, requiring the design of a fluorescent probe. Inspired by the  $\text{MX}_2\text{H}$  and  $\text{HX}_2\text{M}$  motifs of SilE, a tryptophan containing tetrapeptide (HEWM) has been synthesized for this purpose, and its ability to bind  $\text{Ag}^+$  has been investigated (Fig. 4). A fluorescence titration of HEWM by  $\text{Ag}^+$  in HEPES buffer shows the formation of a 1:1 complex with a 50% quenching of the tryptophan emission upon  $\text{Ag}^+$  binding. <sup>1</sup>H NMR confirms the stoichiometry of the complex and the binding of both histidine and methionine to  $\text{Ag}^+$  (Fig. S13, ESI†).<sup>13</sup> Based on the difference of fluorescence intensity between the apo- and holo-forms of the probe, competition experiments were performed with four different tetrapeptide competitors of known  $\text{Ag}^+$  affinity (MDQH, MNEH, HEFM and HQAM)<sup>13</sup> in order to determine the association constant of the probe ( $K_{\text{ass}} = [\text{AgP}]/([\text{Ag}][\text{P}]$ ), yielding  $\log K_{\text{ass}} = 6.4 \pm 0.2$  (Fig. 4, Fig. S14–S16 and Table S3, ESI†).<sup>16</sup> Then, in order to determine the binding affinities of **A** and **B** peptides, similar experiments were performed with our six SilE-derived peptides using HEWM as competitive fluorescent probe (Fig. S17–S20 and Table S4, ESI†). The resulting binding constants are in the same order of magnitude as those obtained for the corresponding tetrapeptide complexes. For instance, the peptide **A1** binds two

Table 1 Binding constants ( $\log K_{\text{ass}}$ ) of 1:1 and 2:1  $\text{Ag}^+$ /peptide complexes

Model	$\log K_{\text{ass}}^a$	Model	$\log K_{\text{ass}}^b$
HQKM	$5.7 \pm 0.1$	B1	$\log K_1 = 6.2 \pm 0.3$
HQRM	$5.5 \pm 0.1$	B1	$\log K_2 = 5.1 \pm 0.5$
HQAM	$5.9 \pm 0.1$	B2	$\log K_1 = 6.5 \pm 0.3$
HRRM	$5.3 \pm 0.1$	B2	$\log K_2 = 5.3 \pm 0.4$
HETM	$6.4 \pm 0.1$	A1	$\log K_1 = 6.6 \pm 0.3$
MDQH	$5.8 \pm 0.1$	A1	$\log K_2 = 5.6 \pm 0.4$
HEFM	$6.6 \pm 0.1$	A2	$\log K_1 = 6.7 \pm 0.4$
MNEH	$5.4 \pm 0.1$	A2	$\log K_2 = 5.5 \pm 0.3$

<sup>a</sup>  $\log K_{\text{ass}}$  of  $\text{HX}_2\text{M}$  and  $\text{MX}_2\text{H}$  motifs studied as tetrapeptides from ref. 13. <sup>b</sup>  $\log K_{\text{ass}}$  of the model peptides determined in the herein described study.

$\text{Ag}^+$  ( $K_1 = [\text{AgP}]/([\text{Ag}][\text{P}]$  and  $K_2 = [\text{Ag}_2\text{P}]/([\text{Ag}][\text{AgP}])$  with  $\log K_1 = 6.6 \pm 0.3$  and  $\log K_2 = 5.6 \pm 0.4$ , while the two individual motifs composing the sequence, HETM and MDQH, bind one  $\text{Ag}^+$  with  $\log K = 6.4 \pm 0.1$  and  $5.8 \pm 0.1$ , respectively, when studied as tetrapeptides (Table 1). However, affinity constants of the trimetallic  $\text{Ag}_3\text{B1b}$  and  $\text{Ag}_3\text{B2b}$  complexes could not be extracted with this method, since it ends up with a too large standard deviation on the  $K_3$  value. Overall, with stepwise association constants ( $\log K_1$  and  $\log K_2$ ) between 5.1 and 6.7 ( $\pm 0.5$ ), the affinities of the herein described models for the two silver ions are in the same range as the intrinsic affinities of the  $\text{MX}_2\text{H}$  and  $\text{HX}_2\text{M}$  sites ( $\log K_{\text{ass}} = 5.3\text{--}6.6$ ).<sup>13</sup> Therefore, these similarities confirm the His–Ag–Met coordination mode in type **A** and **B** peptides. Moreover, the binding affinities of **B1** and **B2** being similar to those of **A1** and **A2**, no significant effect of the peptide structuration has been observed. Obviously, the presence of two silver binding sites in the model and the  $\text{Ag}^+$ -induced  $\alpha$ -helix folding of the peptide do not have a significant effect on the silver binding constants. Therefore, the four  $\text{MX}_2\text{HX}_6\text{HX}_2\text{M}$  and  $\text{HX}_2\text{MX}_3\text{HX}_2\text{M}$  sequences found in SilE are proposed to bind  $\text{Ag}^+$  in a non-cooperative binding mode.

In the context of accurate characterization of protein–metal interactions, the use of model peptides can help to reach qualitative and quantitative insights, which are not necessarily attainable by working with an entire protein. In this instance, while several research groups have investigated the role of SilE for two decades, no structural or numerical characterization of the metal centers has arisen. In contrast, the herein described work not only provides the structure of the four silver binding sequences of SilE, but also quantises the silver docking. As suggested by previous studies on  $\text{MX}_2\text{H}$  and  $\text{HX}_2\text{M}$  ultra-short model peptides of SilE, the latter seems to bind up to 8  $\text{Ag}^+$  ions *via* its  $\text{MX}_2\text{HX}_6\text{HX}_2\text{M}$  and  $\text{HX}_2\text{MX}_3\text{HX}_2\text{M}$  sequences, each of them binding two  $\text{Ag}^+$  ions. A ninth  $\text{Ag}^+$  ion could nevertheless be bound to the isolated HXM motif (H38–M40) with a similar affinity.<sup>13</sup> Asiani *et al.* suggested a complete folding of SilE after the binding of 6  $\text{Ag}^+$  ions (out of a total of 8  $\text{Ag}^+$  ions bound), and the presence of two core motifs (A77–M91 and E110–F120) which, when folded into  $\alpha$ -helices, should facilitate the folding of the rest of the protein.<sup>17</sup> The different backbone foldings between type **A** and type **B** sequences upon  $\text{Ag}^+$  complexation support this hypothesis. However, while the first core motif corresponds to the herein described **B1** peptide,

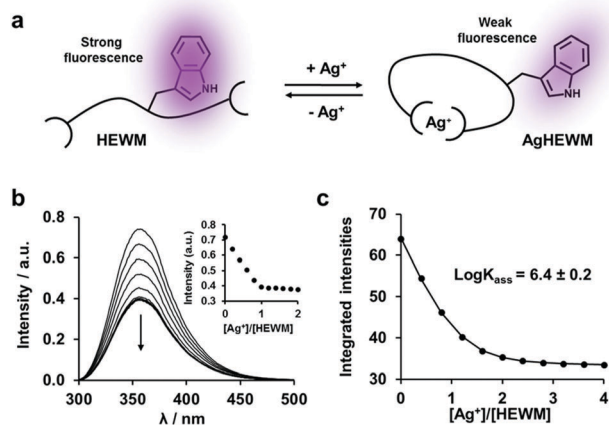


Fig. 4 Characterization of the fluorescent probe. (a) Principle of the probe. (b) Spectrofluorimetric titration of HEWM (200  $\mu\text{M}$ ) by  $\text{AgClO}_4$  (0 to 400  $\mu\text{M}$ ) in HEPES buffer (20 mM, pH 7.4), based on the tryptophan fluorescence ( $\lambda_{\text{ex}}$ : 280 nm) quench induced by  $\text{Ag}^+$  complexation. Insert depicts the 1:1 stoichiometry of the complex. (c) Plot of the variation of fluorescence integrated intensities by addition of  $\text{AgClO}_4$  (0 to 40  $\mu\text{M}$ ) to a solution of HEWM (10  $\mu\text{M}$ ) in competition with MDQH (10  $\mu\text{M}$ ), in a HEPES buffer (4 mM). The solid line corresponds to the fit obtained with Dynafit,<sup>16</sup> which yielded  $\log K_{\text{ass}} = 6.4 \pm 0.2$ .



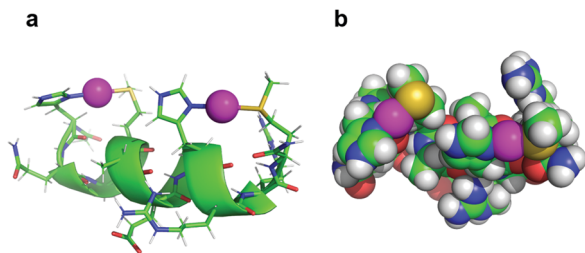


Fig. 5 Model of the coordination mode of two  $\text{Ag}^+$  by **B2**. The spacing of two amino acids between the histidine and methionine residues of the two  $\text{HX}_2\text{M}$  binding sites brings the two ligands onto the same side of the  $\alpha$ -helix, allowing a linear coordination of each  $\text{Ag}^+$  ion (purple spheres). The  $\text{His-N}^{\delta 1}\text{-Ag}^+$  and  $\text{Met}^{\text{S}6}\text{-Ag}^+$  distances used in the calculation were 2.1 and 2.5 Å respectively.<sup>18</sup>

which folds into a stable  $\alpha$ -helical structure upon  $\text{Ag}^+$  binding, the second core motif is a truncated version of the **A2** peptide, which does not adopt a well folded secondary structure in the presence of  $\text{Ag}^+$  (Fig. 3 and Fig. S9, ESI†). Furthermore, contrary to previous assumptions from Asiani *et al.* who assumed that each single  $\alpha$ -helix is unable to bind  $\text{Ag}^+$  by itself, each type **A** and type **B** sequence individually binds two  $\text{Ag}^+$  ions, even when histidine and methionine side chains are on the same side of an  $\alpha$ -helix (type **B**), as determined by  $^1\text{H}$  NMR.<sup>17</sup> Of particular interest is this alternation of histidine and methionine residues on the same face of the  $\alpha$ -helix in **B1** and **B2** peptides that clearly suggests the formation of His–Ag–Met motifs with linear geometry, which is common in  $\text{Ag}^+$  coordination chemistry. In order to assess if these peptides can accommodate linear His–Ag–Met coordination, the structure of **B2** was calculated once again using NMR-derived distance and dihedral angle restraints but forcing a linear His–N–Ag–S–Met geometry.<sup>18</sup> The obtained structure retains the helical fold of **B2** and displays no violation of NOE and dihedral constraints. It clearly establishes that this linear His–N–Ag–S–Met geometry is indeed possible within a  $\text{HX}_2\text{M}$  motif as part of an  $\alpha$ -helix (Fig. 5).

The plots of NMR and CD titrations of the herein described model peptides by  $\text{Ag}^+$  always adopt hyperbolic binding curves, suggesting a non-cooperative binding of  $\text{Ag}^+$  ions by the different binding sequences of SilE. Numerical values of stepwise affinity constants of the  $\text{Ag}^+$ /SilE-derived peptide complexes ( $6.2 \pm 0.3 < \log K_1 < 6.7 \pm 0.4$  and  $5.1 \pm 0.5 < \log K_2 < 5.6 \pm 0.4$ ) support this hypothesis. Most surprising, however, is that type **A** and type **B** peptides bind  $\text{Ag}^+$  with similar affinities, and that  $K_1$  and  $K_2$  are in the same range for all models, implying that the structuration of type **B** peptides is neither beneficial nor detrimental to silver binding in comparison to ultrashort  $\text{HX}_2\text{M}$  and  $\text{MX}_2\text{H}$  peptides. Indeed, the range of affinities is consistent with the previous hypothesis that SilE could buffer silver ions in case of high  $\text{Ag}^+$  overload, avoiding the saturation of the periplasmic adaptor SilB, in charge of the  $\text{Ag}^+$  externalization.<sup>13</sup> Moreover, while the interaction between  $\text{Ag}^+$  ions and the sensor kinase SilS has not been investigated to date, qualitative and quantitative data are available

for its homologue CusS. This homodimer protein possesses two different silver binding sites, of which one is more important for function, and is conserved between CusS and SilS.<sup>18</sup> The complexation of the four  $\text{Ag}^+$  ions by CusS, which is also mainly carried out by His and Met residues, is governed by an apparent  $K_d$  in the  $\mu\text{M}$  range.<sup>19</sup> Therefore, we propose that SilE could regulate the free  $\text{Ag}^+$  periplasmic concentration at a level to which enough  $\text{Ag}^+$  ions remain available to continuously derepress the expression of the *silCFBAGP* operon via SilRS, but not enough to overload SilB.

In conclusion, by means of model peptides, we qualitatively and quantitatively characterized the interaction between the different binding sequences of SilE and  $\text{Ag}^+$  ions. This study provides the first solution structures of the different silver centers found in SilE. When compared to other components of the Sil system, the characterization of the strength of  $\text{Ag}^+$ /SilE interactions supports the hypothesis that SilE buffers  $\text{Ag}^+$  ions in the  $\mu\text{M}$  range, and hence, sustains the  $\text{Ag}^+$  export and the *silCFBAGP* operon expression. In view of the different metal centers in the Sil and Cus systems, it is very likely that bacteria control the metal ion transfer between the different partners of the efflux systems with the number of histidine and methionine residues involved in metal coordination.

## Conflicts of interest

There are no conflicts to declare.

## Notes and references

- H. J. Klasen, *Burns*, 2000, **26**, 117.
- J. Liu, D. A. Sonshine, S. Shervani and R. H. Hurt, *ACS Nano*, 2010, **4**, 6903.
- K. Bridges, A. Kidson, E. J. Lowbury and M. D. Wilkins, *Br. Med. J.*, 1979, **1**, 446.
- S. L. Percival, P. G. Bowler and D. Russell, *J. Hosp. Infect.*, 2005, **60**, 1.
- S. Silver and L. T. Phung, *Annu. Rev. Microbiol.*, 1996, **50**, 753.
- S. Franke, G. Grass, C. Rensing and D. H. Nies, *J. Bacteriol.*, 2003, **185**, 3804.
- C. P. Randall, A. Gupta, N. Jackson, D. Busse and A. J. O'Neill, *J. Antimicrob. Chemother.*, 2015, **70**, 1037.
- A. Gupta, K. Matsui, J.-F. Lo and S. Silver, *Nat. Med.*, 1999, **5**, 183.
- E. Elkreui, C. P. Randall, N. Ooi, J. L. Cottell and A. J. O'Neill, *J. Antimicrob. Chemother.*, 2017, **72**, 3043.
- T. Vogwill and R. C. MacLean, *Evol. Appl.*, 2015, **8**, 284.
- S. Suzuki, T. Horinouchi and C. Furusawa, *Mol. Biosyst.*, 2016, **12**, 414.
- S. Silver, *FEMS Microbiol. Rev.*, 2003, **27**, 341.
- V. Chabert, M. Hologne, O. S  n  que, A. Crochet, O. Walker and K. M. Fromm, *Chem. Commun.*, 2017, **53**, 6105.
- M. Zimmermann, S. R. Udagedara, C. M. Sze, T. M. Ryan, G. J. Howlett, Z. Xiao and A. G. Wedd, *J. Inorg. Biochem.*, 2012, **115**, 186.
- N. Sreerama, S. Y. U. Venyaminov and R. W. Woody, *Protein Sci.*, 2008, **8**, 370.
- P. Kuzmi  , *Anal. Biochem.*, 1996, **237**, 260.
- K. R. Asiani, H. Williams, L. Bird, M. Jenner, M. S. Searle, J. L. Hobman, D. J. Scott and P. Sultanas, *Mol. Microbiol.*, 2016, **101**, 731.
- T. Affandi, A. V. Issaian and M. M. McEvoy, *Biochemistry*, 2016, **55**, 5296.
- S. A. Gudipaty and M. M. McEvoy, *Biochim. Biophys. Acta*, 2014, **1844**, 1656.

